NUTRIENT DYNAMICS IN COASTAL ECOSYSTEMS OF THE BAHAMAS

by

JACOB EDWARD ALLGEIER

(Under the Direction of Amy D. Rosemond)

ABSTRACT

This dissertation explores the relative importance of two macronutrients, nitrogen (N) and phosphorus (P), for primary production in coastal ecosystems of The Bahamas and contextualizes these findings with respect to nutrient supply from a widely underappreciated source of nutrients, fish excretion. I used observational and experimental approaches to examine the spatial variation of nutrient limitation of seagrasses and benthic algae within and across mangrove estuaries representing a gradient of habitat degradation, i.e., ecosystem fragmentation. In Bahamian mangrove ecosystems, primary production is strongly nutrient limited and that while production is always fundamentally limited by both nutrients, the degree of limitation is typically decreased due to ecosystem fragmentation. To further explore the relative degree of nutrient limitation across sites I developed the Interaction Effect Index (IEI) to quantify the degree to which benthic algae across these mangrove ecosystems respond to dual versus single nutrient enrichment. These results demonstrated that Bahamian mangrove estuaries are among the most nutrient poor marine environments. I then applied this metric to 653 multiple nutrient enrichment studies across terrestrial, freshwater and marine ecosystems using a meta-analytic approach. Findings from this study suggest that the vast majority of ecosystem types were characterized by antagonistic responses to dual enrichment, suggesting weak colimitation and that one nutrient is likely more limiting that the other. However, when ecosystems tended to be strongly colimited, the magnitude of limitation was often great. Finally, I quantified nutrient supply rates from 144 Caribbean fish species to assess the relative importance of a widely underappreciated nutrient source in coastal ecosystems. This research explored which community characteristics explain the supply and storage of nutrients by fish across different ecosystem types (i.e., seagrass beds, mangroves, coral reefs). Findings suggest a strong relationship between species richness and nutrient supply and storage, but that dominant species tend to have disproportionate effects on these processes. Simulation modes supported these findings but indicated that the relative importance of dominant species decreased in communities with greater species richness. Collectively, these findings provide a thorough assessment of how nutrients limit primary production, and the role of fishes in provisioning these nutrients in tropical coastal ecosystems.

INDEX WORDS: fish, nutrients, coastal ecosystems, consumer mediated nutrient cycling, biogeochemistry, stoichiometry

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DEDICATION

I dedicate this to my family: Momma, Poppa, Jesse, Jackson, Maxwell and, of course Grandmother. They are the best. None of this could have been done without them.

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CHAPTER 1

INTRODUCTION AN LITERATURE REVIEW[1]

The cyclic interplay of nutrients between biogeochemical and biological processes is a fundamental determinant of ecosystem function. In nutrient limited ecosystems, where nutrient demand is greater than supply and nutrients are the primary limiting factor for production at the base of the food web, the efficiency at which nutrients are cycled between ecosystem compartments is proportional to the degree of nutrient limitation (Vitousek 1982; DeAngelis 1989). As such, when the geochemical, or allochthonous, supply of nutrients is minimized the role of consumers as the primary source, through nutrient remineralization (i.e. excretion), of bioavailable nutrients for basal resource production (autotrophic or microbial) becomes increasingly important. Thus, in nutrient limited ecosystems, the structure (storage of nutrients within trophic levels) and function (degree of recycling within trophic levels) of the food web is inextricably linked to ecosystem functioning (i.e. basal resource production).

Biogeochemical pathways that determine the availability of nutrients provide a basis for understanding key factors that constrain primary production (Hecky & Kilham 1988; Elser *et al.* 2007; 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 (Smith 1984; Howarth *et al.* 1988; Fourqurean & Zieman 2002). 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 (Smith 1984; Howarth *et al.* 1988;

Fourqurean & Zieman 2002). 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 (Smith 1984; Smith & Atkinson 1984; Howarth *et al.* 1988; Fourqurean & Zieman 2002; Howarth & Marino 2006; Paerl 2009).

Various characteristics of The Bahamas suggest that the typical model of nutrient pathways 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 & 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. As such, alternative models may be needed to explain patterns of nutrient limitation in systems with these characteristics.

Human activities are drastically altering nutrient dynamics and the functioning of ecosystems through physical alteration of landscapes as well as altering community composition and population sizes of biota through over-harvesting practices (Vitousek *et al.* 1997; Chapin 2000; Lotze *et al.* 2006). Such alterations can have significant feedbacks on ecosystem processes through both top-down (altered consumer communities) and bottom-up (nutrient supply and storage) pathways (Worm 2002; Hillebrand *et al.* 2007). While the traditional top-down model focuses on the conspicuous implications of this phenomenon such as increase prey densities after predator release, both through direct and indirect (e.g., trophic cascades) pathways, the less apparent bottom-up effects may also be highly influential on food web structure and function especially in nutrient limited ecosystems (Menge *et al.* 1997; Vanni & Layne 1997; Vanni *et al.* 1997).

Coastal ecosystems are among the most degraded ecosystem types in the world (Halpern *et al.* 2008), with habitat degradation, nutrient pollution (i.e. eutrophication) and overexploitation of species (Lotze *et al.* 2006; Barbier *et al.* 2008) being the most prolific stressors affecting the health and function of these ecosystems. Habitat degradation, including development, coastal canalization and ecosystem fragmentation (i.e., typically occurring when roads are constructed across wetlands to access the coast), is among the most widespread sources of impairment throughout the Caribbean (Barbier *et al.* 2008). Nutrient loading and mobilization from agricultural, industrial and municipal sources (Lotze *et al.* 2006; Diaz & Rosenberg 2008) has greatly exacerbated the incidence of toxic algal blooms, hypoxia, fishery die-offs and associated human health problems along coasts (Rabalais *et al.* 2002; Dodds 2006b). Further, these stressors have been shown to drastically alter the community composition and structure of the dominant primary producer communities. These community shifts often include the overgrowth of other algal species and shading via pelagic and benthic algal blooms, both of which are predicted to result in the net decrease of primary production (Deegan *et al.* 2002; Ferdie & Fourqurean 2004; Armitage *et al.* 2005; Armitage *et al.* 2006). The predicted ecological response is altered nutrient and energy pathways resulting in inefficient trophic transfer of energy and ecosystem functioning (Armitage & Fong 2004; Gil *et al.* 2006).

Likewise, in coastal ecosystems the overexploitation of commercial species has garnered much attention as marine fisheries become increasingly depleted and the associated negative repercussions of cascading top-down effects of overfishing becomes more apparent (Pauly *et al.*

1998). In addition to top-down controls on the abundance of prey organisms, fishes fill other functional roles in ecosystems. Namely, through the physiological processes of consumption, assimilation and regeneration, fishes recycle nutrients into forms that are readily available for biological uptake by heterotrophic microorganisms and primary producers (Sterner & Elser 2002). This consumer-mediated nutrient supply may contribute a substantial proportion of ecosystem nutrient demand under conditions of low ambient nutrient concentrations and/or high fish biomass (Vanni 2002; McIntyre *et al.* 2008). For example, the average fish community across a suite of coral reefs in the Florida Keys has been shown to exceed all other sources of N to these reefs, including anthropogenic sources, oceanic upwelling and gyres, N fixation and influx by Florida Bay waters, by over 25 times (Burkepile *et al.* 2013).

Fish have historically represented one of the largest pools of biomass in coastal ecosystems (Jackson *et al.* 2001). Among the various ecosystem types within sub-tropical and tropical coasts, coral reefs are widely recognized for their important ecological and economic roles. Coral reefs are often likened to tropical rainforest because of their high species diversity (Connell 1978), nutrient poor conditions, high levels of productivity and tight nutrient cycling (Chapin *et al.* 2011). In this sense the fish communities on a coral reef may also be likened to the tree community in a rainforest; whereby both communities are primary nutrient vectors, facilitating critical nutrient pathways through processes of storage and cycling (DeAngelis 1989; Chapin *et al.* 2011). Yet, unlike tropical trees, fish have traditionally been largely overlooked in discussions about nutrient cycling in coastal ecosystems. As such, if fish communities are a critical driver of nutrient pathways in these coastal ecosystems, further understanding of these processes will provide novel insight to nutrient dynamics, and needed perspectives to further the discussion on the role of nutrients for subtropical/tropical coastal marine ecology.

The collective body of this dissertation seeks to accomplish three primary goals: (1) to assess the degree to which nutrients limit primary production in Bahamian coastal ecosystems, (2) to extending these findings and assess the generality of nutrient limitation across all ecosystem types and latitudinal zones, and finally (3) improve our understandings of the role of fishes in supplying critical nutrients to the three dominant types of coastal ecosystems: seagrass beds, mangroves and coral reefs. The Bahamas offers a useful location to test this hypothesis for two reasons: (1) it spans gradients from drastically impacted by humans (i.e., nutrient pollution or overfishing) to relatively pristine (i.e., Andros island is widely considered to harbor some of the most healthy ecosystems in the Caribbean), (2) it is characterized by nutrient poor environments with high fish densities, thus providing two of the most important factors needed for the role of consumers in nutrient dynamics to be important. As such, this dissertation provides a linear progress of understanding the demand and supply of nutrients within Bahamian coastal ecosystems.

The four chapters within this dissertation are as follows:

Chapter 1: Small-scale variation in nutrient limitation and seagrass nutrient content in Bahamian mangrove wetlands.

Chapter 2: Significant nutrient co-limitation across a diversity of mangrove-dominated estuaries in the Bahamas.

These two chapters are complementary in that one evaluates natural variation in nutrient limitation in tidal creeks (Chapter 1) and the other focuses on variation due to ecosystem fragmentation (Chapter 2). Both consisted of two studies in 2008 and 2009 using nutrient diffusing substrates and in chapter one ambient nutrient concentrations of seagrasses. These papers provided important information regarding if and to what extent nutrients limit primary product in these coastal ecosystems, and thus provide an important first step in developing a general understanding of the role of consumers in nutrient processes.

Findings from these studies showed strong nutrient limitation of both benthic microalgae, and seagrass, two of the most dominant producer groups in Bahamian coastal ecosystems in terms of production and biomass, respectively. Further, comparing two measures of nutrient limitation with values from other measures around the world, findings from these studies place The Bahamas as among the most nutrient limited ecosystems globally.

Chapter 3: The frequency and magnitude of non-additive responses to multiple nutrient enrichment.

This study was a follow-up to chapter 2, whereby I created a metric to quantify the magnitude of non-additive effects of algal production associated with single nutrient enrichment (N or P) and simultaneous nutrient enrichment (NP). This metric was applied to 653 experiments that included enrichment of N, P and NP across terrestrial, marine and freshwater ecosystem types. Findings from this research demonstrated that synergistic responses were not as frequent as previously expected, but when they occurred they tended to do so with great magnitude.

Chapter 4: Biogeochemical implications of regional biodiversity loss across coastal marine ecosystems.

This study applies extensive fish nutrient supply and storage processes to a large dataset of fish surveys across 172 communities in six ecosystem types (four categories of coral reefs, mangroves and seagrass). Hierarchical statistical models were used and strong positive relationships between all ecosystem functions and species richness were found. Simulation models were then applied to test how robust these communities were to various scenarios of species loss across the different ecosystem types, i.e., the insurance hypothesis. This study highlights the importance of biodiversity, but that in addition to species richness the maintenance of biomass in coastal fish communities is critical for the maintenance of ecosystem function.

In sum, this dissertation demonstrates the importance of nutrients for Bahamian coastal ecosystem function, and underscores the need to incorporate consumers into models of nutrient dynamics in coastal marine ecosystems.

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CHAPTER 2

VARIATION IN NUTRIENT LIMITATION AND SEAGRASS NUTRIENT CONTENT IN BAHAMIAN TIDAL CREEK ECOSYSTEMS[1]

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[1] Jacob E. Allgeier, Amy D. Rosemond, and Craig A. Layman. 2011. Journal of Experimental Marine Biology and Ecology. 2011. 407: 330-336 Reprinted here with permission of the publisher

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 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.

Introduction

Biogeochemical pathways that determine the availability of nutrients provide a basis for understanding key factors that constrain primary production (Hecky & Kilham 1988; Elser *et al.* 2007; 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 (Smith 1984; Howarth *et al.* 1988; Fourqurean & Zieman 2002). 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 (Smith 1984; Howarth *et al.* 1988; Fourqurean & Zieman 2002). 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 (Smith 1984; Smith & Atkinson 1984; Howarth *et al.* 1988; Fourqurean & Zieman 2002; Howarth & Marino 2006; Paerl 2009).

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 & 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, 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.

Methods

Site Description

The study was conducted between March 5-29, 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, ~0.74 km^2) (Fig. 2). These creek systems are typically characterized by a relatively narrow creek mouth (Fog. 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 (<1 m at low tide) wetlands with red 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 are 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. (2007), Valentine-Rose et al. (2007), Allgeier et al. (2010) and Hammerschlag-Peyer et al. (2010).

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 & Smith 1983; Duarte 1990, 1992; Fourqurean & 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 (Tank & Dodds 2003; Tank *et al.* 2007; Allgeier *et al.* 2010). 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 (Johnson *et al.* 2006; Layman 2007; Allgeier *et al.* 2010). 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 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 hours 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 (~5cm diameter x ~5cm 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, and 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 regularly spaced along a transect from the mouth to the terminal end of the tidal creek (Fig. 1). The three creeks measured 600, 1,300 and 1,900 meters, 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 (µg 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 hours 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.

Data Analysis

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 & 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 & 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.

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 & 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 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 & 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 (Davidson and Howarth 2008, Allgeier et al. 2011). This interplay continues until either another factor becomes limiting or a saturation state is reached (Davidson and Howarth 2008, Allgeier et al. 2011). This interaction is often more prevalent (i.e., greater producer response to dual nutrient enrichment) under conditions of low ambient nutrient concentrations.

Results

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 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 suggests 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 JC this relationship was positive but only marginally significant (Fig. 3).

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).

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) (Duarte ,1990, 1992, Allgeier et al. 2010). 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 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 (Smith 1984; Smith & Atkinson 1984; Howarth *et al.* 1988; Fourqurean & Zieman 2002; Howarth & Marino 2006; Paerl 2009).

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}. However, 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. Yet, 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 & 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 thus 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. 1993, Vanni 2002). As such, the presence of animals may provide a consistent pulse 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 (Rypel *et al.* 2007; Hammerschlag-Peyer & Layman *in press*). 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 (Capone 1979, Atkinson and Smith 1984, 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 particularly 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 systems (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.* 2007a). 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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Table 2.1

Mean values (± 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**	**TDP**	**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

Table 2.2

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**	**RR_{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 2.3

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***	***Fvalue***	***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***	***Fvalue***	***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		

Table 2.4

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		

Figure Legends

Figure 2.1

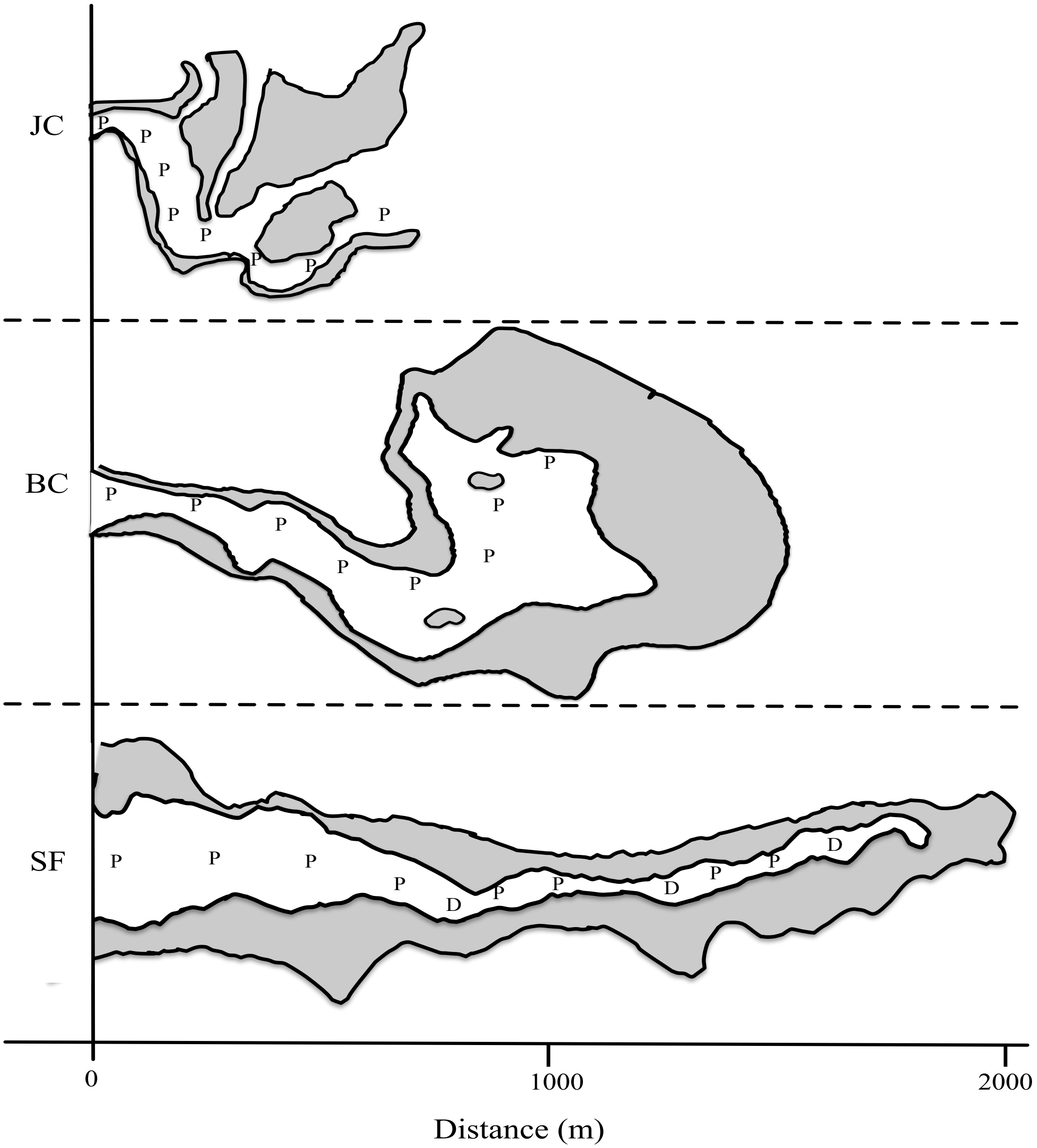

Figure 2.1

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).

Figure 2.2

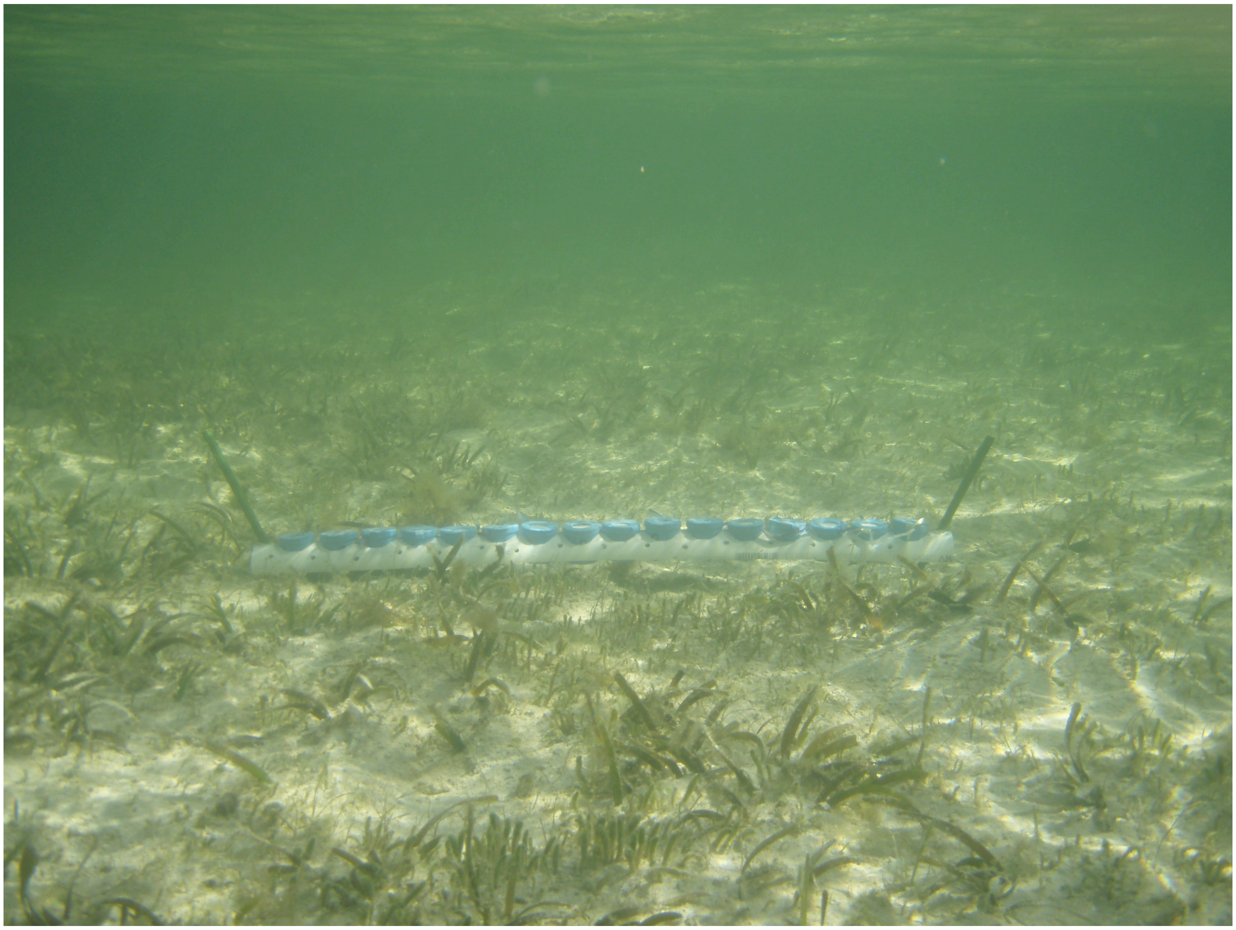

Figure 2.2

Nutrient diffusing substrates during deployment.

Figure 2.3

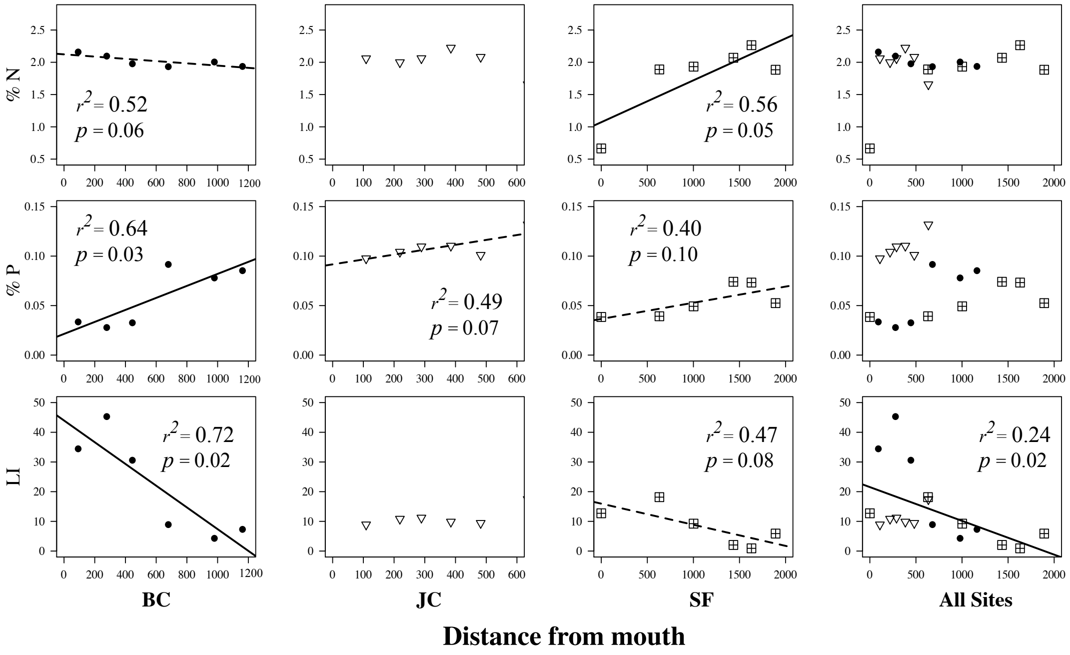

Figure 2.3

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, dashed lines indicate 0.05<*P*<0.1.

Figure 2.4

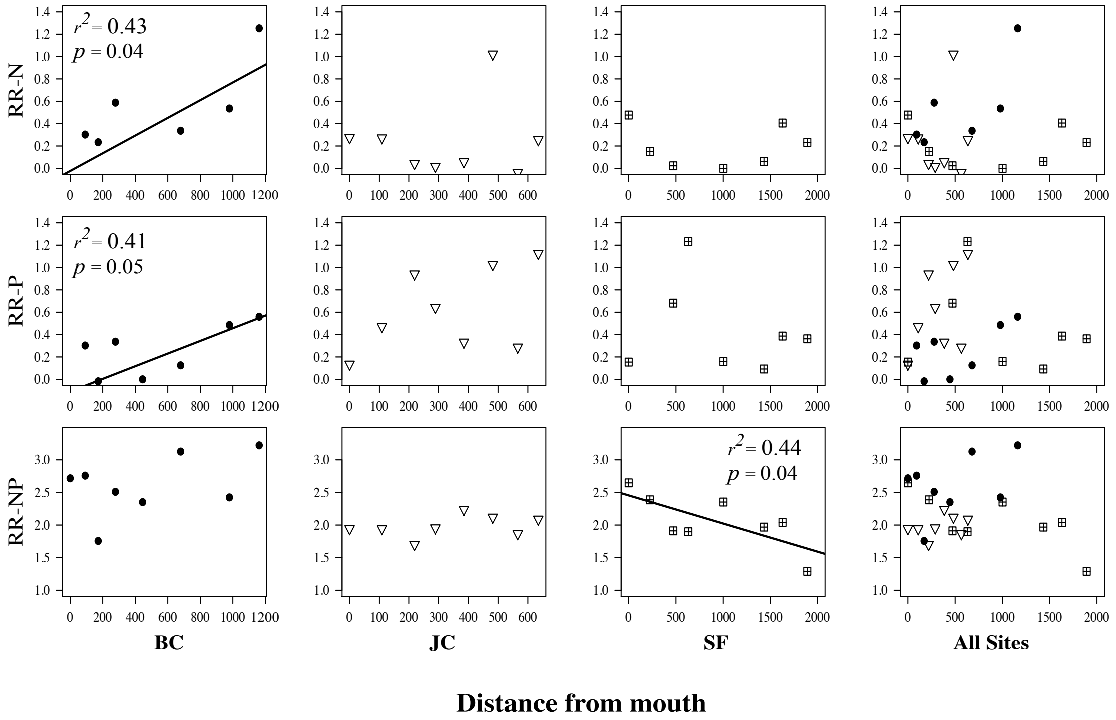

Figure 2.4

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 $p<0.05$.

CHAPTER 3

SYNERGISTIC NUTRIENT COLIMITATION ACROSS A GRADIENT OF ECOSYSTEM FRAGEMENTATION IN SUBTROPICAL MANGROVE-DOMINATED WETLANDS [1]

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Acknowledgments

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Abstract

We examined benthic algal response to nutrient enrichment by nitrogen (N), phosphorus (P), and N + P in mangrove wetlands of The Bahamas, to test the hypothesis that human impacts (fragmentation) on these ecosystems altered nutrient limitation, thereby changing the frequency and/or magnitude at which ecological synergies occurred. Fragmentation occurred due to road construction, resulting in reduced hydrological connectivity between the wetlands and marine environment. Strong, persistent, and synergistic nutrient co-limitation occurred in both pristine and fragmented estuaries. Ecosystem fragmentation did not alter the biomass response to dual nutrient enrichment, but did alter the relative magnitude of the non-additive response. That is, synergistic responses were less extreme in fragmented systems. This was supported by the strong, negative relationship between ambient algal biomass (a surrogate for background productivity) and the strength of synergistic responses ($R^2 = 0.69$ and 0.79, year 1 and 2, respectively). Bahamian coastal ecosystems exhibited the greatest synergistic responses reported for a marine ecosystem, suggesting that the benthic algal community associated with Bahamian wetlands are among the most nutrient limited marine ecosystems. Our findings provide a case study illustrating how altered nutrient dynamics associated with land-use change may decrease the frequency and/or magnitude of synergistic responses to nutrients in aquatic ecosystems.

Introduction

Coastal environments are among the most anthropogenically altered ecosystems on Earth (Lotze *et al.* 2006). Of the multiple stressors affecting these systems, two of the most conspicuous are: altered hydrologic connectivity (Pringle 2001), and eutrophication (Lotze *et al.* 2006; Diaz & Rosenberg 2008). Hydrologic connectivity affects transport of nutrients, energy, and organisms, and can be increased or decreased as a result of human modification of landscapes (Pringle 2001). Eutrophication of coastal zones results from the downstream flux of nutrient loading and mobilization from agricultural, industrial and municipal sources (Lotze *et al.* 2006; Diaz & Rosenberg 2008). A consequence of both of these stressors is altered nutrient availability, which has numerous implications for the structure and function of estuarine ecosystems (Dodds 2006a; Conley *et al.* 2009; Paerl 2009). Yet, because coastal ecosystems have been so widely affected by these stressors, our understanding of how these systems functioned before human intervention is often unclear, presenting a dilemma for long-term conservation and restoration initiatives (Jackson et al. 2001; Paerl 2009).

In any ecosystem, determining which nutrient(s) most limit primary production is at the core of understanding nutrient dynamics and ecosystem function (Hecky & Kilham 1988; Elser *et al.* 2007; Tank *et al.* 2007). Recent research has demonstrated that ecosystems tend to be nutrient co-limited, i.e., primary producers respond greater to enrichment by multiple nutrients (typically N and P together) than by either nutrient independently (Elser *et al.* 2007). The commonality of nutrient co-limitation has led to the idea that synergistic responses to multiple nutrient enrichment are also common (Elser et al. 2007; Davidson and Howarth 2007). In this case, a synergism is defined as a non-additive form of co-limitation that occurs when the producer response to dual nutrient enrichment is greater than that predicted by the sum response to both nutrients independently (e.g., when N and P together > [N alone + P alone]).

Synergisms generally result from oscillating nutrient limitation, whereby the ambient availability of nutrients is minimal, and given an increased supply of one nutrient, limitation shifts towards limitation by the other (Davidson & Howarth 2007). Thus, limitation oscillates between nutrients until either production is maximized or a tertiary factor becomes limiting (Arrigo 2005; Davidson & Howarth 2007). Synergistic responses to multiple nutrient enrichment are predicted to occur most strongly in ecosystems that are extremely nutrient limited. But anthropogenic perturbations generally enhance the availability and supply rates of nutrients (Halpern *et al.* 2008; Conley *et al.* 2009; Paerl 2009). Therefore, it is logical to hypothesize that human modification of nutrient availability may decrease the frequency and/or magnitude at which ecological synergies occur.

Coastal waters of the subtropics and tropics in areas with low human population densities tend to be relatively nutrient poor, especially in the Caribbean (Lapointe & Clark 1992; Rivkin & Anderson 1997; Koch & Madden 2001). Yet land-use change associated with increasing population densities alters nutrient availability (Lapointe 1997). One of the most prevalent forms of land-use change in the region is physical fragmentation of coastal ecosystems via road construction (Layman *et al.* 2004; Layman *et al.* 2007). Fragmentation generally occurs when roads bisect mangrove wetlands, causing a physical barrier between the associated wetland and the ocean. Fragmentation, via reduced hydrologic connectivity (i.e., tidal flushing), is predicted to affect nutrient availability in two primary ways: 1) enhanced N availability via atmospheric fixation because nitrogen-fixing cyanobacteria tend to proliferate in systems with minimal tidal flushing (decreasing N limitation, and thus potentially increasing P limitation) (Smith 1984;

Howarth *et al.* 1988; Howarth & Marino 2006), and 2) decreased P availability because a primary source of P to coastal ecosystems is from oceanic upwelling (Smith 1984; Howarth *et al.* 1988; Howarth & Marino 2006). As such, fragmentation provides an ecosystem-scale context to independently test the relative importance of changes in hydrologic connectivity on nutrient dynamics because it is predicted to alter nutrient dynamics, but it is not directly (in this case) associated with anthropogenic nutrient loading.

Here we used single nutrient (N and P) and dual nutrient (N+P, herein NP) enrichment experiments across unaltered and fragmented wetlands in subtropical mangrove ecosystems of The Bahamas to address two objectives: 1) to quantify nutrient limitation of benthic algae and determine whether response to N, P, or dual nutrient enrichment was affected by fragmentation, and 2) to quantify the presence and strength of non-additive responses to dual nutrient enrichment in fragmented and unfragmented systems. We also compared our findings to published studies examining dual nutrient limitation from other marine ecosystems. Our specific hypotheses were that: 1) benthic algae would be co-limited by N and P and would exhibit strong synergistic responses to dual nutrient enrichment, and 2) ecosystem fragmentation would reduce synergistic responses to dual nutrient enrichment.

Methods

Site description

The study was conducted in mangrove-dominated wetlands on Abaco Island, Bahamas, locally known as 'tidal creeks'. Bahamian tidal creeks receive little freshwater input because of small watershed size (essentially having no freshwater rivers or streams on any of the islands), little topographic relief, and porous calcium carbonate geology. Reduced salinities in some systems (*see* Table 1) are due to direct rainwater input. Creeks are typically characterized by a relatively narrow creek mouth that is the primary hydrologic conduit for tidal exchange (~0.8 meter tidal range). The creeks typically broaden with increased distance from the mouth, grading into expanses of shallow (<1 m at low tide) wetlands with red mangrove (*Rhizophora mangle*) as the primary emergent vegetation. All of our study creeks, including those that were fragmented, 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. Ambient nutrient concentrations are extremely low in unaltered sites (Table 1).

Our study was conducted in three unaltered (unfragmented) tidal creeks and four fragmented tidal creeks, during the months of February and March in 2008 and 2009. The unfragmented and fragmented sites ranged from ~0.12 to ~0.47 and ~0.001 to ~0.3 km^2, respectively, with a maximum mean depth of ~1.13 meters at high tide. Tidal oscillations are reduced by >90% in all of the fragmented sites used in this study (Layman et al. 2007). Ambient water nutrients in fragmented sites are slightly higher than in unfragmented sites (Table 1). More detail on these systems can be found in Layman et al. (2007) and Valentine-Rose et al. (2007*a,b*).

Experimental design

Nutrient diffusing substrates (NDS) have been widely used to determine nutrient limitation for primary production in freshwater ecosystems (Tank & Dodds 2003; Tank *et al.* 2007). Here we used four NDS treatments: N (0.5 mol L^{-1} NH_4Cl), P (0.5 mol L^{-1} KH_2PO_4), N+P (0.5 mol L^{-1} NH_4Cl + 0.5 mol L^{-1} KH_2PO_4), and a control (agar only) (Tank et al. 2007). Each treatment was incubated at each plot for 24-26 days. NDS experiments are short-term enrichments that are colonized by epiphytic and epibenthic (herein benthic) algae. The treatment that elicits the largest algal response (measured as μg cm^{-2} chlorphyll *a* (Chl *a*)), indicates the nutrient that is most limiting. 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 of estuarine and wetland food webs (Johnson *et al.* 2006; Layman 2007; Valentine-Rose *et al.* 2007a). Because they represent an important component of the primary producer pool (Armitage *et al.* 2005; Armitage *et al.* 2006), benthic algae are a useful proxy to measure nutrient limitation in these systems.

In year 1, eight and four plots of NDS were placed in each unfragmented and fragmented site, respectively (24 total plots in unfragmented sites and 16 total plots in fragmented sites). Plots were regularly spaced along a linear transect from the mouth to the terminal end of the tidal creek (i.e., there were different distances among plots because the size of creek systems varied). Each plot contained four replicates of each of the four treatments (16 individual nutrient diffusing assays plot^{-1}; 384 assays in unfragmented sites and 256 assays in fragmented sites). In year 2, two plots were placed in each site (unfragmented and fragmented) following the same design used in year 1 with the exception of one unfragmented creek that had three plots (seven plots with 96 assays in unfragmented and eight plots with 128 assays in fragmented sites). Chl *a* values from multiple replicates of a nutrient treatment were considered subsamples and averaged within each plot.

NDS experiments were collected after 24-26 days, placed in foil, transported on ice and frozen for analysis following the protocol of Tank et al. (2007). All experiments at a given site were collected on the same day. Chl *a* content (μg cm^{-2} Chl *a*) of each sample was determined spectrophotometrically (Shimadzu 2100) for pheopigment corrected Chl *a* (APHA 1995). Water nutrient samples were taken in year 1 from each plot when the plates were retrieved at the end of the experiment. All samples were prefiltered through a 0.45 Whatman nylon membrane filter and all but NH_4, were frozen until analysis. The water samples were analyzed for NH_4 within 12 hours of collection using a fluorometric method, following Holmes et al. (1999) as modified by Taylor (2007). The concentration of total dissolved phosphorus (TDP) was determined using the persulfate digestion method and the concentrations of soluble reactive phosphorus (SRP) and NO_3^- were determined with continuous flow colorimetry.

Data analysis

Differences among nutrient treatment effects in fragmented and unfragmented sites (Table 2) were analyzed with a three-factor analysis of co-variance (ANCOVA) using the Generalized Linear Model (GLM) procedure in SAS version 9.1 (SAS Institute Inc., Cary, NC USA) with α set at 0.05. Distance from the plot to the mouth of the tidal creek served as a covariate to account for the effect of location relative to potential sources of nutrients (e.g., the ocean as a source of P). Planned comparisons of three-factor joint means were made using the LSMEANS test with a Bonferroni adjustment in SAS (Milken & Johnson 1992). Chl *a* values were log transformed to meet assumptions of normality and homoscedasticity. In year 2, one fragmented site (f3) was not included in the analysis because it was lost during a storm event.

To determine the relative frequency and magnitude of non-additive responses to multiple nutrient enrichment we applied the Interaction Effect Index (IEI) (J. E. Allgeier unpubl.) to our data. The IEI provides a relative measure of the degree of non-additivity in response to multiple factors (in this case dual nutrient enrichment), by incorporating all response terms.

$$\text{IEI} = \ln \left[\text{response NP} / (\text{response N} + \text{response P}) \right] \qquad (1)$$

where response NP is the primary producer biomass reported for NP treatments and response N and response P are primary producer biomass reported for N and P treatments, respectively. IEI values close to zero, either positive or negative, are functionally additive (i.e., response NP = response N + response P). As IEI increases or decreases, the magnitude of the non-additive effect becomes more synergistic or antagonistic, respectively (J. E. Allgeier unpubl.). We applied the IEI to all published experiments from marine systems (*n*=105) that reported biomass per unit area or volume response to enrichment by N, P, and NP (J. E. Allgeier unpubl.). The raw data, initially reported in Elser et al. (2007), were obtained via the National Center for Ecological Analysis and Synthesis (http://knb.ecoinformatics.org/knb/metacat?action=read&qformat=nceas&docid=nceas.347).

To test for the effects of fragmentation on the degree of synergy, we conducted two tests. We examined the difference in mean IEI values for fragmented and unfragmented sites using a *t*-test. We expected synergies to be strongest in ecosystems with the lowest ambient production. If fragmentation enhanced the ambient production of an ecosystem, synergies would be expected to be weaker than they would otherwise be in a relatively unimpaired ecosystem. That is, the magnitude of the IEI value would be negatively correlated with ecosystem production. We used least squares regression of the mean response to the control treatment for each site and the mean IEI value for each site to test for this relationship (*n*=7 and 6 in year 1 and 2, respectively). To verify that the relative size of the creek was not also an important variable to consider in our analysis, we regressed creek surface area to volume ratio (SA:V) with IEI. Because the relationship was not significant for either year ($p = 0.91$ and $p = 0.32$ for 2008 and 2009, respectively), creek size was not further considered. *T*-test and least squares regression analysis were conducted in R software (R Development Core Team 2008).

Results

Significant interactions between nutrient treatments and levels of fragmentation in both years necessitated multiple comparisons of joint treatment means rather than the interpretation of simple main effects (Table 2). NP treatments elicited the greatest algal responses in all fragmented and unfragmented sites in both years (Figs. 1, 2). Single nutrient treatments (N or P alone) did not differ significantly from controls or from one another in unfragmented sites in either year (Fig. 2). At fragmented sites in year 1, algal biomass of N treatments was significantly higher than controls and P treatments, which did not differ significantly from each other. However, neither single nutrient treatment differed from the control in fragmented sites in year 2 (Fig. 2). In year 1, algal biomass on controls and on treatments of N alone differed significantly between fragmented and unfragmented sites, but in year 2, only single nutrient treatments of N differed between fragmented and unfragmented sites (Fig. 2). The effect of distance was significant in year 1 alone (Table 2). The lack of a significant effect of distance in the second year is likely due to decreased sample size.

Strong synergistic responses to dual nutrient enrichment were found at every site for both years (Fig. 3). The mean IEI values for unfragmented sites were 1.19 ± 0.12 (± 1 SE) and 2.60 ± 0.16 in year 1 and 2, respectively, representing a 228% and 1246% increase in biomass response above that predicted from an additive model (response NP = response N + response P). The mean IEI values for fragmented sites were 0.69 ± 0.18 and 1.64 ± 0.23 in year 1 and year 2, respectively, and represented 95% and 416% increases in biomass above additivity. Our findings from year 2 experiments represent five of the highest IEI values recorded for any marine ecosystem.

The Chl *a* response within the control treatment, representing a measure of ambient algal production, was negatively correlated to IEI values ($R^2 = 0.79$, $p < 0.001$ and $R^2 = 0.69$, $p < 0.05$, in year 1 and year 2, respectively; Fig. 4a,c). *T*-tests revealed that IEI values from fragmented sites were significantly different than those from unfragmented sites in year 2 (*t*-test, t_6= 3.4, df = 6, *p* = 0.01), but not in year 1 (*t*-test, t_7= 1.3, df = 7, *p* = 0.11; Fig. 4b,d).

Discussion

Our findings demonstrate strong and persistent nutrient co-limitation and synergisms in both unaltered and anthropogenically altered Bahamian estuaries. Specifically, over two years (7 sites, 55 experimental units and 880 total assays) all experiments indicated N+P co-limitation, and all sites demonstrated synergistic responses to dual nutrient enrichment. The classic model of biogeochemical supply of nutrients to coastal ecosystems predicts two primary inputs: 1) down-stream transport from terrestrial ecosystems, supplying nutrients at concentrations of high N relative to P, and 2) tidal flushing that delivers mobilized nutrients at concentrations of low N relative to P from oceanic upwelling (Smith 1984; Howarth *et al.* 1988). Our findings of substantial nutrient co-limitation and strong synergistic responses to dual nutrient enrichment suggest that both of these mechanisms may play a relatively small role in delivery of nutrients to Bahamian coastal ecosystems. This is highlighted by the fact that even though fragmentation was found to alter nutrient availability and to increase ambient algal production (*see* below), nutrient co-limitation was still prevalent and strong in every study site.

Our findings regarding the implications of ecosystem fragmentation for nutrient limitation did not support our a priori predictions. We expected that reduced tidal flushing would decrease N-limitation and concomitantly NP-limitation because of two underlying mechanisms. First, we expected enhanced N supply rates because waters that do not experience significant tidal flushing tend to promote the presence of N-fixing microbes and algae (e.g., cyanobacteria) (Howarth & Marino 2006). Second, because fragmentation drastically decreased tidal flushing, and thus would reduce a potential source of P from incoming seawater (given the potential of nearby coastal upwelling), we predicted an increase in P limitation. But our study demonstrated that the largest effect of fragmentation was enhanced N limitation while P and NP limitation remained relatively similar to unfragmented sites.

Our on-going research suggests that fragmented sites undergo substantial daily oscillations in dissolved oxygen concentration, often reaching anoxic conditions at night (C. A. Layman unpubl.). Low oxygen levels may release bound P from sediments, which would enhance algal production and facilitate a shift towards N limitation (Wetzel 2001). Thus, P inputs to the ecosystem would be from the benthos (from sediment-bound P release), where the NDS experiments were conducted. Conversely, our findings of greater availability of NH_4^+ in fragmented sites (*see* Table 1), suggests that the system is also receiving N inputs at the air-water interface (the part of the water column from which samples were taken), potentially from N-fixing microbes such as cyanobacteria. Because fragmented sites can be stratified due to lack of tidal flow, nutrient limitation may vary in upper and lower parts of the water column. Irregardless of the mechanism, it is clear that the relatively greater ambient nutrient availability in fragmented sites facilitated enhanced algal production (control mean Chl *a* (μg cm^{-2}) year 1: 0.93 ± 0.09 and 0.47 ± 0.03; year 2: 0.89 ± 0.23 and 0.35 ± 0.04, fragmented and unfragmented, respectively).

The IEI provides a quantitative measure of how primary producers in a particular system respond to dual nutrient enrichment. We found strong synergistic responses across all sites for both years (Fig. 3). Our findings from year 1 represent the third highest IEI values (i.e., greatest synergistic effect) recorded for all experiments reported in the literature up to 2007, and 5 of the 15 values we recorded from year 2 were greater than all published marine experiments up to

2007. The maximum IEI values 2.38 (year 1) and 3.00 (year 2) demonstrated an increase of 980% and 1909% above additivity. Likewise, of the total 54 experiments reported in our study, only one (from a fragmented site) had an IEI value that was lower than the average IEI value for all marine studies (IEI = -0.02 ± 0.1) (Fig. 3). These data emphasize the nature of nutrient limitation in Bahamian coastal ecosystems and highlight them as among the most nutrient-limited marine ecosystems yet recorded.

While the magnitude of the response to the NP treatment was similar between fragmented and unfragmented sites (Fig. 2), the IEI values deviated significantly (in one of the two years examined) (Fig. 4d). This deviation is due to the relatively large response to single nutrient enrichment found in fragmented sites. For example, in unfragmented sites the response to the NP treatment was 457% and 957% greater than that of the P treatment (the greatest single nutrient response) in year 1 and year 2, respectively. In comparison, the NP treatment in fragmented sites was only 211% and 294% greater than that of the N treatment (the greatest single nutrient treatment) in year 1 and year 2, respectively.

The disparity in IEI values between unfragmented and fragmented sites supports the hypothesis that human activity may be altering the relative frequency and/or magnitude at which synergisms occur. The IEI compares the *net* response to enrichment by single and dual nutrients because each value is not control-corrected. Thus, the IEI value is inclusive of the background productivity of the ecosystem and quantifies the net ecosystem response to enrichment as opposed to simply the effect size of the specific experiment. The negative relationship between the Chl *a* response to the control treatment and the IEI value suggests that as an ecosystem increases in ambient algal production (e.g., via anthropogenic alteration) the relative strength of synergistic effects decrease (Fig. 4a). Though fragmentation does not represent direct anthropogenic nutrient loading, it is indicative of the alterations in nutrient cycling that are likely associated with this kind of land use change.

Our findings provide a case study illustrating how altered nutrient dynamics associated with land-use change may be decreasing the frequency and/or magnitude of synergies in aquatic ecosystems. As has been stressed in the ecological literature, anthropogenic impacts to ecosystems have drastically altered the 'ecological baseline' and have skewed our understanding of basic ecological processes and mechanisms (Jackson et al. 2001). In this same context, physical alterations of coastlines may be skewing our understanding of nutrient dynamics in the pre-human era (Jackson *et al.* 2001; Knowlton & Jackson 2008). Basic understanding of how unaltered systems function is imperative if we are to fully grasp the ecological repercussions of the human footprint.

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Table 3.1

Ambient water nutrient concentrations (μg L^{-1}) and salinity measured in March 2008 (year 1) for all unfragmented (uf1-3) and fragmented (f1-4) sites. Values are averaged from *n*=8 and *n*=4 samples taken at each NDS plot in each of the unfragmented and fragmented sites respectively. 'bd' indicates below detection limits for the analytical equipment used. SRP and TDP are abbreviations for soluble reactive phosphorus and total dissolved phosphorus.

Site	SRP	TDP	NO_3^--N	NH_4^+-N	Salinity
uf 1	0.9 ± 0.9	4.3 ± 3.3	3.0 ± 1.3	4.9 ± 0.5	34 ± 0
uf 2	bd	2.9 ± 1.6	3.03 ± 1.4	5.2 ± 0.3	34 ± 0
uf 3	0.9 ± 0.5	bd	0.3 ± 0.9	8.1 ± 0.9	40 ± 0
unfrag means	0.60 ± 0.3	2.4 ± 1.27	2.13 ± 0.91	6.06 ± 1.03	36 ± 2
f 1	2.7 ± 2.7	4.5 ± 1.9	0.5 ± 0.25	14.3 ± 0.7	37 ± 0
f 2	bd	4.8 ± 0.3	6.2 ± 0.5	41.8 ± 4	40 ± 0
f 3	3.8 ± 1.6	5.8 ± 1.9	0.6 ± 0	12.7 ± 2.3	3 ± 0
f 4	bd	0.4 ± 0	3.2 ± 1.4	22.7 ± 5.2	10 ± 0
frag means	1.61 ± 0.96	3.76 ± 1.26	2.61 ± 1.35	22.86 ± 6.67	22.5 ± 9.37

Table 3.2

ANCOVA results for chlorophyll *a* response measured in nutrient treatments (N, P, and NP). For the ANCOVA, 'distance' of plot from the mouth of the tidal creek was used as a covariate. For the ANCOVA, the predictor variable 'Frag' indicates if the treatment was from a fragmented or unfragmented site.

Year 1

Treatment	Df	Sum of squares	*F*-value	*P*-value
Frag	1	11.952	53.751	<0.001
N	1	57.046	256.556	<0.001
P	1	31.341	140.952	<0.001
N x P	1	20.852	93.778	<0.001
Frag x N	1	1.292	5.810	0.017
Frag x P	1	4.868	21.891	<0.001
Frag x N x P	1	0.380	1.708	0.193
Distance	1	1.504	6.763	0.010
Error	151	33.575		

Year 2

Treatment	Df	Sum of squares	*F*-value	*P*-value
Frag	1	0.585	5.644	0.022
N	1	10.704	103.265	<0.001
P	1	6.832	65.909	<0.001
N x P	1	5.230	50.453	<0.001
Frag x N	1	0.025	0.239	0.627
Frag x P	1	0.576	5.556	0.023
Frag x N x P	1	0.212	2.042	0.160
Distance	1	0.028	0.266	0.609
Error	43	4.457		

Figures

Figure 3.1

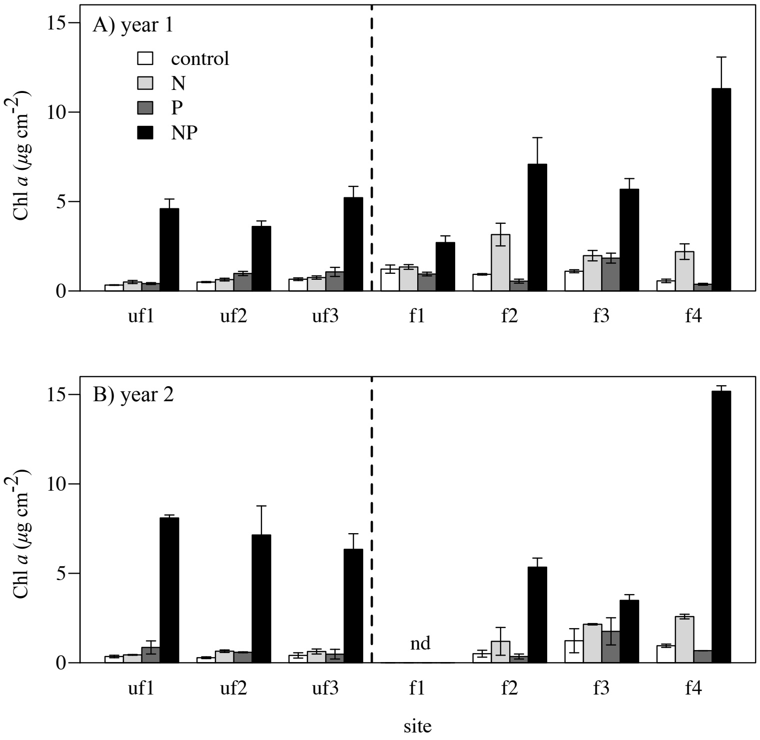

Figure 3.1

Mean Chl *a* (μg cm^{-2}) response to nutrient diffusing substrate (NDS) across all unfragmented (uf 1-3) and fragmented (f 1-4) sites for year 1 and year 2. Treatments are: control (Cont), nitrogen (N), phosphorus (P), and nitrogen plus phosphorus (NP). Error bars indicate standard error based on 4 replicates per value. 'nd' indicates no data, as these experiments were lost in a storm.

Figure 3.2

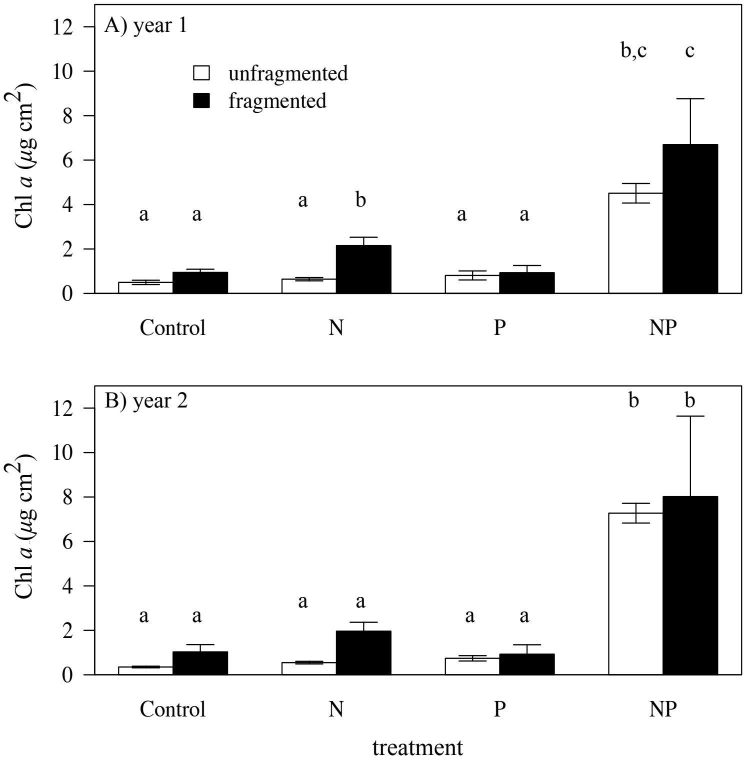

Figure 3.2

Comparison of mean Chl *a* (μg cm^{-2}) response to nutrient diffusing substrate (NDS) between unfragmented (white) and fragmented (black) estuaries. Treatments are control (Cont), nitrogen (N), phosphorus (P), and nitrogen plus phosphorus (NP) for year one and year 2. Error bars indicate standard error. Letters above columns indicate significant differences in joint mean comparisons (Bonferroni corrected, $p<0.05$).

Figure 3.3

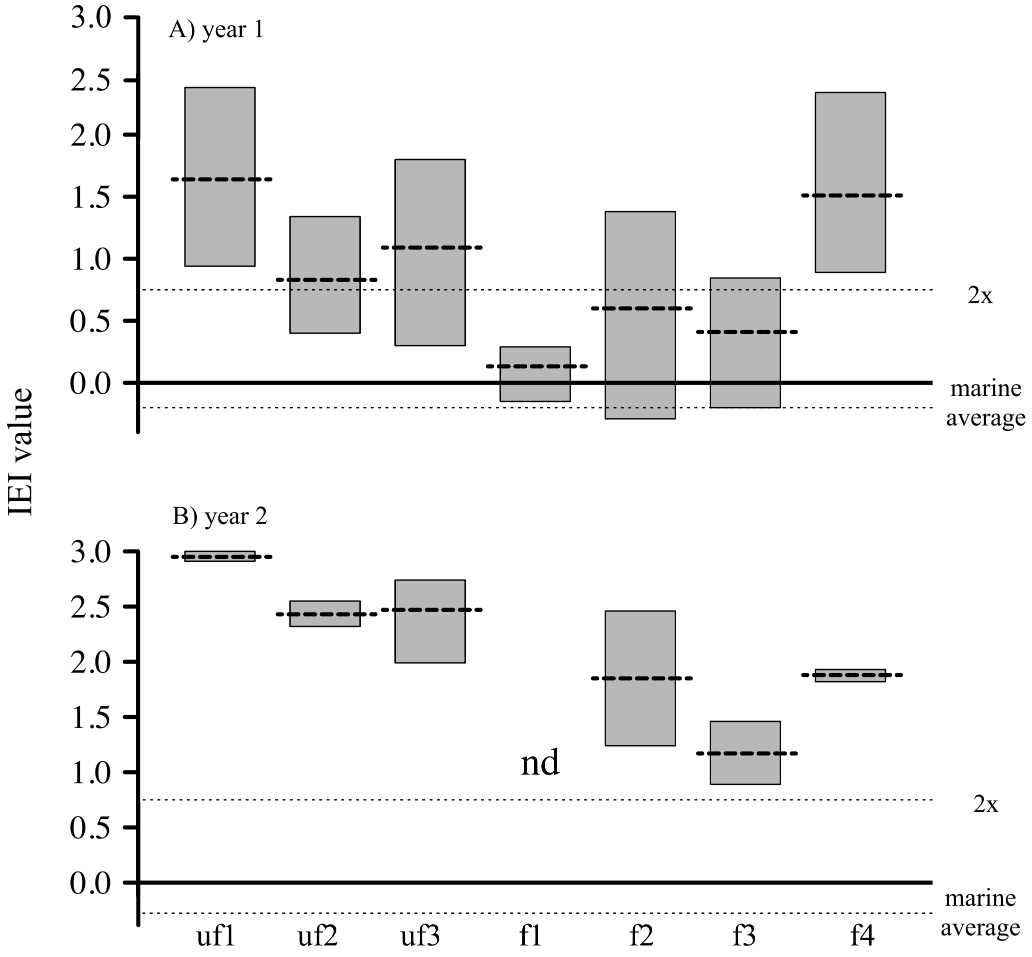

Figure 3.3

Comparisons of IEI values for unfragmented (uf 1-3) and fragmented (f 1-4) sites for (A) year 1 and (B) year 2. The gray bar represents the total range of values per sites. The dashed black line on each bar represents the mean value per site. The line indicated by '2x' delineates a synergistic non-additive IEI value that is approximately twice as large as additivity. The line indicated as 'Marine Avg.' represents the mean IEI value (-0.02 ± 0.10) for 105 published marine studies (J. E. Allgeier unpublished). Raw data for marine studies were obtained via the public data repository of the National Center for Ecological Analysis and Synthesis.

Figure 3.4.

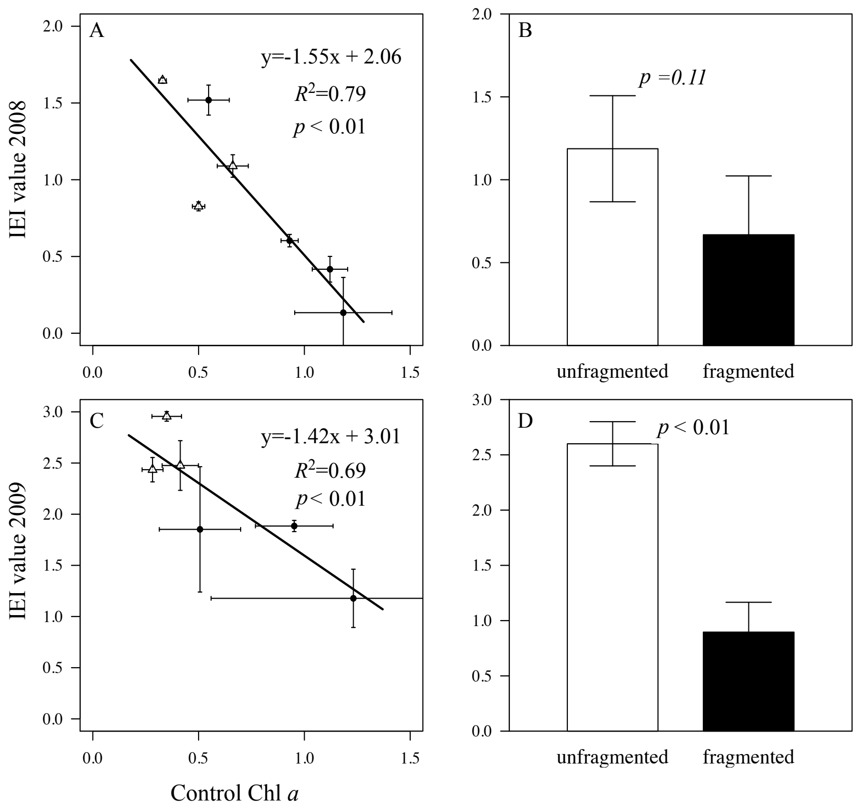

Figure 3.4

(A,C) Linear regression between the average chlorophyll *a* (Chl *a*) values for control treatments at a given site and the respective IEI values for that site for year 1 and 2. Chl *a* values are a way to represent ambient algal production and thus are a useful proxy for baseline ecosystem productivity. Closed circles indicate fragmented sites (n=4; n=3 in year 2) and open triangles indicate unfragmented sites (n=3) averaged for years 1 and 2. (B,D) Average IEI values for all unfragmented and fragmented sites in years 1 and 2.

CHAPTER 4

THE FREQUENCY AND MAGNITUDE OF NON-ADDITIVE RESPONSES TO MULTIPLE NUTRIENT ENRICHMENT

Abstract

Anthropogenic eutrophication is among the greatest threats to ecosystem functioning globally, often occurring via enrichment of both nitrogen (N) and phosphorus (P). As such, recent attention has focused on the implications of non-additive responses to dual nutrient enrichment and the inherent difficulty associated with predicting their combined effects. We used a simple metric to quantify the frequency and magnitude of non-additive responses to enrichment by N, P and N+P in 653 experiments conducted across multiple ecosystem types and locations. Non-additive responses were found to be common in all systems. Freshwater ecosystems and temperate latitudes tended to have frequent synergistic responses to dual nutrient enrichment, i.e., the response was greater than predicted by an additive model. Terrestrial and arctic systems were dominated by antagonistic responses (responses to N+P that were less than additive). The mean of all experiments was synergistic because despite being less common, synergistic responses were generally of greater magnitude than antagonistic ones. Our study highlights the ubiquity of non-additive effects in response to dual nutrient enrichment and further elucidates complex ways ecosystems respond to human impacts. Our results suggest how alternative nutrient limitation scenarios can be used to guide approaches to conservation and management of nutrient loading to ecosystems. This review provides the first published summary of non-additive responses by primary producers.

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[1] Jacob E. Allgeier, Amy D. Rosemond, and Craig A. Layman. 2011. Journal of Applied Ecology. 2011. 48,96-101
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Introduction

The ecological impacts of excessive nutrient loading are substantial, driving losses of ecosystem services worldwide (Vitousek *et al.* 1997; Smith & Schindler 2009) and stimulating debate over how to most effectively regulate anthropogenic nutrient inputs (Conley *et al.* 2009). At the crux of the debate is whether controlling nitrogen (N), phosphorus (P), or both, should frame conservation initiatives (Carpenter 2008; Conley *et al.* 2009). The underpinning research that has informed this debate is generally based on quantifying the primary producer response to enrichment by these key nutrients. Most notably, measuring the production response to multiple nutrients, i.e. both N and P, has received much attention because many anthropogenic stressors tend to alter concentrations of both nutrients simultaneously (Sala & Knowlton 2006; Halpern *et al.* 2008).

A recent study by Elser et al. (2007) demonstrated the prevalence of nutrient co-limitation across ecosystems. Co-limitation of nutrients (i.e., N and P) occurs when the primary producer response is greater to simultaneous enrichment by both nutrients (N+P) than to enrichment by either nutrient individually (sensu Elser et al., 2007). Some interpretations of these findings have suggested that they likewise imply a dominance of synergy in ecosystems, assuming that co-limitation is necessarily synergistic (Davidson and Howarth, 2007; Elser et al., 2007). However, a synergism only occurs when the response is greater than additive, whereas co-limitation can also be an equal to or less than additive response. Understanding these different outcomes forms the basis of our ability to predict how an ecosystem will respond to nutrient enrichment and, therefore, our ability to develop effective management strategies.

We developed a simple metric to quantify the relative response to additions of both N and P in plant production to: (1) quantitatively assess the generalities of non-additive responses to nutrient enrichment and, (2) distinguish different types of co-limitation across ecosystem types and latitudinal gradients. We also test the hypothesis that the distribution of these data are consistent with null distributions based on random values. Our results suggest how alternative nutrient limitation scenarios can be used to guide approaches to conservation and management of nutrient loading to ecosystems.

Methods

We developed the Interaction Effect Index (IEI) to quantify the response of primary producers to N and P additions:

(Eq 1) $$\text{IEI} = \ln \left[\textit{response}\ \text{NP} \,/\, (\textit{response}\ \text{N} + \textit{response}\ \text{P}) \right]$$

Where *response NP* is the primary producer biomass (and in some cases the change in mass) reported for N+P treatments (hereafter NP) and *response N* and *response P* are primary producer biomass responses in those treatments, respectively. Taking the natural log of the quotient proportionally centers the IEI values around zero. For example, an IEI value generated from an experiment where *response* NP is two times greater than *response* N + *response* P is equal to the absolute value of an experiment where *response* N + *response* P is two times greater than *response* NP.

We applied the IEI to 653 experiments from marine, freshwater and terrestrial ecosystems that tested for primary producer responses to enrichment in all three treatments: N, P and NP (compiled in Elser *et al.* 2007; obtained via the National Center for Ecological Analysis and Synthesis). Experiments that used the metric of biomass per unit area or volume were included, but proxy variables for biomass were also allowed (e.g., chlorophyll *a* concentration, ash-free dry mass, carbon mass, biovolume, percent cover; Elser et al. 2007). We included only studies that reported mean community-level biomass responses to nutrient enrichment. Thus, the only single species responses that were included were drawn from communities dominated by single species. One hundred twenty-nine studies were conducted in laboratory settings, the rest of the experiments were conducted *in situ*. A total of 39 of the 653 experiments included additional manipulations (e.g., grazer exclusion), but only data from unmanipulated controls (e.g., grazers at natural densities) were included. Because of the nature of our categories, all experiments were classified simultaneously in two categories (based on ecosystem type and latitudinal zone)

A simple prediction regarding dual nutrient enrichment is that NP response would be equal to the sum of individual N and P responses (i.e., an additive response, Fig. 1b). Our metric provides a continuous measure to assess the relative departure from additivity. IEI values close to zero, either positive or negative, can be characterized by additive co-limitation (AD) (Fig. 1b). As IEI increases or decreases, the non-additive effect becomes more pronounced and can be classified into one of three response categories: synergistic co-limitation (SC), antagonistic co-limitation (AC), and absolute antagonism (AA) (Fig. 1a,c,d).

Co-limitation implies that the producer is limited by both nutrients (Arrigo 2005; Davidson & Howarth 2007), and is demonstrated when the response to both nutrients is greater than either nutrient individually (*sensu* Elser et al. 2007). Synergistic co-limitation (SC) results when there is a positive non-additive response whereby NP response is greater than the sum of N and P responses (Fig. 1a). Antagonistic co-limitation (AC) is a less than additive response that occurs when NP response is less than the sum of N and P responses, but is still greater than response to either single nutrient. Absolute antagonisms (AA) occur where NP response is less than at least one of the single nutrient enrichments. The relative strength of the non-additive effect (i.e. SC, AC, AA) increases as the IEI value deviates from zero, either positively (SC) or negatively (AC, AA).

The term nutrient co-limitation has been subject to various interpretations and requires specific clarification (Arrigo 2005; Elser *et al.* 2007; Lewis & Wurtsbaugh 2008). According to Liebig's law of the minimum, only one nutrient can functionally limit primary production at a given point in time. But with dual nutrient enrichment, an individual (or producer assemblage with similar physiological nutrient demands) may oscillate between single nutrient limitation of two nutrients (here N and P). In this case, the supply of one nutrient is sufficient to shift demand toward that of the other, next most limiting nutrient. This interplay continues until either another factor becomes limiting or a saturation state is reached (Davidson and Howarth 2008). As such, over the course of time, e.g., an experimental time period, an individual producer (or producer assemblage) may be considered functionally co-limited, even though a single factor may always be limiting at any instantaneous point.

We test the hypotheses that the distribution of the data from each category (e.g., freshwater) was consistent with null distributions based on random numbers. To do this we compared the distribution of a given category (e.g. marine; *n*=105) with distribution of a randomly sampled dataset of the same size, using Kolmogorov-Smirnov tests for 1000 permutations. Then we pooled the p-values from these model runs to determine the proportion of models runs that fall within a 95% confidence interval of a significant p-value. The dataset of artificial IEI values from which the null distributions for each category was sampled, was generated by randomizing the three response variables (N, P and NP) from the original dataset and recalculating IEI values based on these numbers. Each null distribution for each permutation was then sampled from this dataset.

Results

Synergistic co-limitation (SC), antagonistic co-limitation (AC) and absolute antagonism (AA) occurred in all ecosystem types and latitudinal zones (Fig. 2). When comparing the frequency of each response for all experiments combined, 37% were SC, 40% were AC and 23% were AA (Fig. 2). Across all six subcategories (marine, freshwater, terrestrial, arctic, temperate, and tropical), SC occurred more frequently in all but terrestrial and arctic ecosystems, in which AC occurred 64% and 71% of the time, respectively (Fig. 2). AA occurred more frequently than SC in arctic (8% SC, 21% AA) and terrestrial systems (18% SC and 18% AA) but never occurred more frequently than AC (Fig. 2). Across all categories, SC occurred substantially less frequently than antagonistic responses (i.e. AC and AA combined).

A study that incorporates multiple experimental units can be considered additive if the mean of all experiments does not significantly differ from zero (i.e., the 95% confidence intervals overlap zero). Because of the complex nature of our dataset, applying such confidence intervals to individual studies was inappropriate, thus to provide perspective as to the number of studies that were characterized by values close to additive (i.e., zero), we chose an arbitrary positive and negative interval of 10% from perfect additivity (0.095>IEI>-0.095). Under these conditions, we found only 5% of experiments yielded additive responses (AD). Extending the interval to 15% (0.139>IEI>-0.139), the frequency of such responses increased to only 11%.

All experiments combined reflect a mean SC response, (IEI = 0.12, $P < 0.001$ for *t*-test of IEI=0). Freshwater, temperate and tropical sub-categories had mean net SC IEI values ($P <$ 0.005 for *t*-test of IEI=0 for freshwater and temperate; tropical did not differ from zero, $P = 0.43$, (see Appendix S1 in Supporting Information)). Marine, terrestrial and arctic sub-categories had mean AC IEI values ($P < 0.001$ for *t*-test of IEI=0 for terrestrial and arctic, marine did not differ from zero $P = 0.83$, (Appendix S1)) (red lines, Fig. 3). SC values were on average of greater magnitude than AC or AA values in most subcategories (colored bars Fig. 3). Freshwater ecosystems had the greatest mean SC value (IEI = 1.23 ± 0.07, NP responses 3.4x greater than additivity). Tropical and marine systems demonstrated the lowest IEI values (IEI = -0.88 ± 0.5, -0.92 ± 0.12; NP responses 2.4x and 2.5x less than additivity, respectively). Terrestrial ecosystems and arctic latitudes were the categories that had greater absolute mean AA than SC values.

The highest IEI value (IEI=5.01; NP response 150x greater than additivity) was from a benthic freshwater stream (Chessman *et al.* 1992). However, of the top 50 highest IEI values, all but two (both in benthic freshwater environments) experiments were conducted in pelagic freshwater and marine environments. The lowest IEI value (IEI= -2.81; NP response 16x less than additivity) was conducted on the benthos of a temperate marine estuary (Taylor *et al.* 1995). Unlike the positive IEI values, the lower IEI values were not dominated by experiments from any category. A bimodal trend is apparent in freshwater, marine, temperate and tropical categories, whereby there is a secondary mode centered around IEI ≈ 2 (Fig. 2). Examination of the data showed that this trend was strongly driven by a single set of experiments in temperate lakes (62 of the 82 studies) (Maberly *et al.* 2002). Of the 82 experiments that fall within the range of $1.5 < \text{IEI} < 2.5$, we found that all but two were conducted in the pelagic zone of freshwater or marine environments, emphasizing that pelagic environments may tend towards relatively strong synergistic response to dual nutrient enrichment.

Comparing the distribution of the data within each category with that of a randomly generated null distribution allows inference as to the probability that these data were the product of random organization of data, or some underlying mechanism driving these trends. Over 99% of random permutations of the dataset differed from the true distribution of values from all the experiments combined. With the exception of marine and tropical categories, >95% of the random permutations of the dataset differed from the true distribution of values in every category. These findings provide evidence that the distribution of these data is a product of underlying patterns that emerge from each subcategory.

Discussion

Synergies have garnered much attention in the ecological literature, often under the assumption that they occur frequently and with great magnitude (Myers 1995; Sala & Knowlton 2006; Halpern *et al.* 2008). Our findings provide more detail to this broad generalization. Though synergistic responses (SC) were often demonstrated, they occurred less frequently than antagonistic responses (the combination of AC and AA). However, where they occurred, SC tended to be of greater magnitude than antagonisms, as is supported by the bimodal distribution of the data with the second mode occurring approximately around 2. Thus, although the distribution of experiments is skewed towards negative IEI values (Fig. 2), the overall mean IEI is positive.

The presumed mechanism for synergisms results from primary production that is limited by both nutrients to such a degree that little production occurs under enrichment by a single nutrient. SC is generally a result of oscillating nutrient limitation, whereby ambient availability of nutrients is minimal, and given supply of one nutrient, limitation shifts towards limitation by the other (Davidson & Howarth 2007). Thus, limitation oscillates between nutrients (if supply rate of both nutrients is constant relative to demand) until either production is maximized or another factor becomes limiting. These conditions are often prevalent in extremely nutrient poor ecosystems (Arrigo *et al.* 2005).

Antagonistic co-limitation (AC), the most common response type, can be explained by a third (or additional) limiting factor. Other micronutrients (e.g. iron, magnesium, molybdate), as well as physical factors (e.g. light, water), can limit production (Howarth *et al.* 1988; Arrigo 2005; Davidson & Howarth 2007). Thus, stimulating production beyond a certain level may incur limitation by a resource(s) besides N or P. Another mechanism may derive from physiological and/or environmentally-related limitations (e.g., maximum physical size, disturbance or grazing), whereby the upper bound of community or individual primary production is constrained in mass or size irrespective of nutrient resources (Rosemond 1993).

An additional plausible mechanism for AC may occur if increased supply of one nutrient concomitantly decreases the need for another. An example is the requirement of N for the anabolism of phosphatase enzymes which can be used to process organic P at low availability of inorganic or bioavailable P (Chrost 1991). In this case, enrichment of N can enhance net primary production (via increased production of phosphatase, and thus increased access to inorganic P). However, under conditions of enrichment with N and P, the availability of inorganic P can simultaneously inhibit production of phosphatase resulting in potentially similar or only slightly higher production than with N additions alone. The net response to NP would then be less than additive, but still greater than the response to N or P alone (Ivancic *et al.* 2009; Rees *et al.* 2009; Scott *et al.* 2009).

Absolute antagonisms (AA), perhaps the most counterintuitive response, were the most infrequent response category. The effect of grazing could lead to AA, whereby the grazer could selectively feed on the resource with the highest production rate, or potentially with the highest nutrient content from enrichment (DeMott *et al.* 1998; Heck *et al.* 2006). However, there are multiple examples that suggest that antagonisms could simply be experimental artifacts. For example, Taylor et al. (1997) reports a strong AA response (IEI=-2.81) by eelgrass to enrichment by NP. The enrichment study was conducted in mesocosms where, under enriched conditions, phytoplankton, which were growing simultaneously with eelgrass, responded synergistically to nutrient addition (Taylor *et al.* 1995). This experiment was characterized by a large algal bloom, causing light limitation and thus reducing seagrass biomass. These findings are consistent with the widely predicted response of seagrass to nutrient enrichment at an ecosystem scale (Deegan *et al.* 2002), and arose due to complex interactions involving two different producer assemblages.

The experiments compiled in this study measured the biomass response to enrichment by communities (e.g., a stand of a single tree species) as well as entire assemblages of different producer species (e.g., a phytoplankton assemblage). The differences in response to nutrient enrichment between an individual species and a community of different species can be substantial. For example, a diverse assemblage of producers likely consists of organisms with varying physiological requirements (e.g. N limited *or* P limited) and growth potential (e.g. greater size/growth rate). As such, under various magnitudes and time duration of nutrient enrichment, differential non-additive responses may be expected, and knowledge of the existing community is required to fully understand the mechanisms behind these responses. These differences may help explain the disparity in findings between terrestrial and aquatic (freshwater and marine together) systems, whereby aquatic systems are characterized by a greater range in IEI values with notably greater frequency and magnitude of SC. Many aquatic studies were conducted on assemblages of producers, whereas the majority of studies conducted on monocultures were from terrestrial ecosystems. These findings are consistent with the fact that pelagic environments with mixed species assemblages (e.g., phytoplankton) tend to be particularly susceptible to large production responses (e.g., algal blooms) from multiple nutrient enrichment (Conley *et al*. 2009).

Distributional trends that emerge from these data appear to be the product of underlying ecological patterns as opposed to randomness within the data. Yet, isolating specific factors that determine the frequency of the type of non-additive effects are difficult given the biological complexity associated with interaction of multiple nutrients (i.e., species life history, physical conditions etc.). A notable finding from our study was the dominance of antagonistic responses (AC and AA combined) in terrestrial and arctic sub-categories. One explanation for terrestrial ecosystems may be that the growth rate and generation time of terrestrial producers is substantially greater than for producers in aquatic systems due to the greater requirement of structural and supporting tissue (Cebrian 1999; Chapin 2002). Thus, even given adequate experimental time frames, physiological constraints may hinder synergistic responses. Consistent with this observation, the strongest synergistic effects tended to occur in aquatic ecosystems, particularly in the pelagic zone, occurring among more speciose assemblages with relatively minimal structural physiological demands (see Appendix S2 in Supporting Information). As for arctic regions, a less than additive response to nutrient enrichment may reflect the fact that producer growth rates are positively correlated with temperature and thus temperature could be a physical factor mitigating synergistic responses (Chapin 2002). However, despite the similarity in frequency of response types between terrestrial and arctic sub-categories, arctic experiments were primarily conducted in freshwater ecosystems (S2), illustrating the complex nature of non-additive responses to multiple nutrients.

Our findings have important implications for management of nutrient loading to aquatic ecosystems. The prevalence of non-additive effects across all systems suggests that when possible, both nutrients should be controlled in conservation and management because the ecological repercussion of simultaneous nutrient enrichment is relatively unpredictable. This is particularly relevant in ecosystems where IEI is close to zero as they are often characterized by a relatively large response to at least one, but more often both, nutrients individually (Fig. 1b,c). As the IEI value deviates from zero, positively or negatively, it may indicate the potential for effective control of nutrient loading by focusing on the single most limiting nutrient. For example, a large IEI value (i.e. a synergistic response) generally indicates that both nutrients are critical for enhancement of production, thus controlling the single most limiting nutrient (in the case of Fig. 1a, P is most important to control) may be effective to mitigate unwanted ecosystem responses. Likewise, an extremely negative IEI value (i.e. absolute antagonism) generally indicates that only one nutrient is significantly limiting and thus suggests that controlling the loading rate of this most limiting nutrient may provide a significant reduction in ecosystem-scale responses.

In a perfect world, all stressors that negatively affect ecosystems would be carefully managed. Yet, conservation efforts are constrained by cost, time and societal will to manage ecosystems. Our findings show frequent and strong non-additive responses to nutrient enrichment across ecosystem types and locations. We emphasize that a single conservation model for mitigating nutrients is not appropriate and stress that future efforts need to account for the complex nature of dual nutrient limitation. We further highlight the importance of incorporating all treatments (N, P and NP) into enrichment experiments in conjunction with quantitatively assessing the nature of the interaction on a system-specific basis. These data are critical for building effective predictive models needed to inform conservation and management decision-making regarding nutrient control.

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Figures

Figure 1.

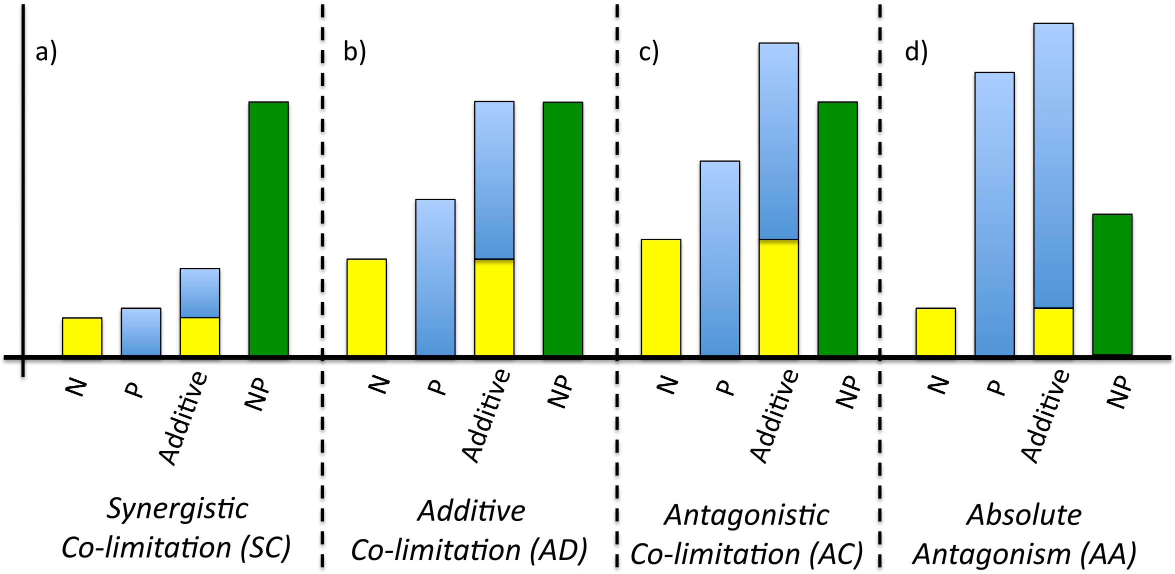

Figure 4.1 A conceptual diagram of possible responses from enrichment by N, P and NP. An additive response is indicated in each panel by summing the individual N (yellow) and P (blue) responses. a) Synergistic co-limitation (SC) such that the biomass or production response to dual enrichment (NP) is greater than the additive response of both single nutrient treatments (N and P alone). b) Additive co-limitation (AC) whereby the response to NP is equal to that of the sum of N alone and P alone. c) Antagonistic co-limitation (AC), whereby the response to NP is greater than that of either N or P alone, but not their sum. d) Absolute antagonism (AA) whereby NP results in less biomass or production than either N or P alone.

Figure 2.

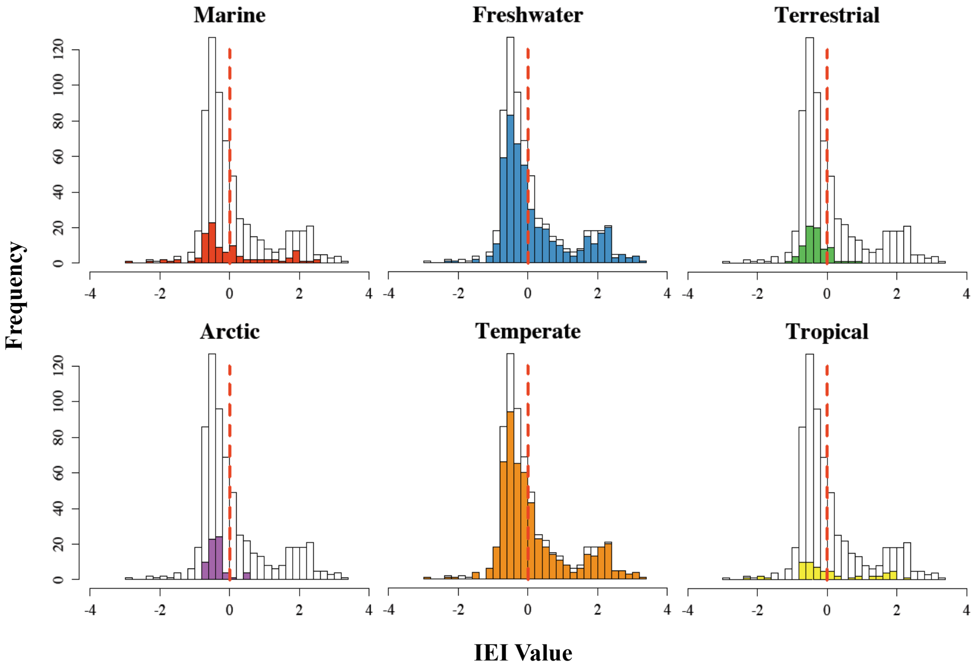

Figure 4.2 Frequency of IEI values within each sub-category. In each plot the white background bars indicate the frequency of IEI values for all experiments combined. A positive value represents synergistic co-limitation, a negative value indicates either antagonistic co-limitation or absolute antagonism, and zero represents additive co-limitation.

Figure 3.

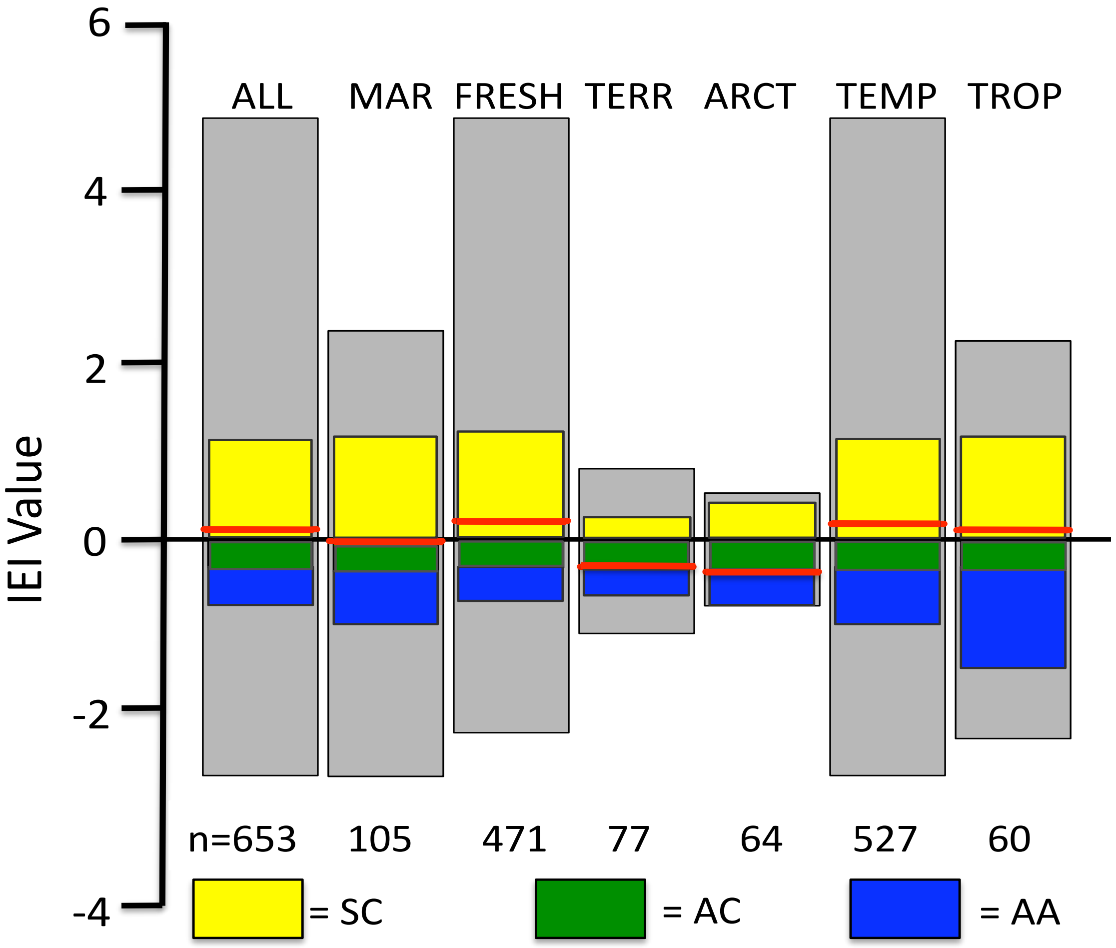

Figure 4.3 Full range of all values (gray bars) and mean values for each response type (colored bars; e.g., SC) for different ecosystem types and latitudinal zones. Positive and negative values as in Fig. 2. The red line indicates the net mean IEI value for the respective category. For context, an absolute IEI value of 0.69 or 1.09 indicate a 100% or 200% increase or decrease from additivity, respectively. The colored bars indicate mean values for each category: yellow bars for SC (synergistic co-limitation), green for AC (antagonistic co-limitation), and blue for AA (absolute antagonism). Categories include fundamental ecosystem types (MAR = marine,

FRESH = freshwater, and TERR = terrestrial) and well as categories based on latitudinal zones (ARCT = arctic (latitudes >66.5°), TEMP = temperate (latitudes 23.5°- 66.5°) and TROP (latitudes 23.5°N to 23.5°S)).

CHAPTER 5

BIOGEOCHEMICAL IMPLICATIONS OF REGIONAL BIODIVERSITY LOSS ACROSS COASTAL ECOSYSTEMS[1]

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[1] Jacob E. Allgeier, Craig A. Layman, Peter J. Mumby, and Amy D. Rosemond, to be submitted to Mbuzi Keechaa

Abstract

A major societal concern is that biodiversity loss will hinder our ability to meet the rising demand for ecosystem services. Predicting consequences of species loss is difficult, especially because the role of biodiversity for ecosystem functioning across large spatial scales of environmental variability remains poorly understood. We quantified five biogeochemical processes, and an aggregate measure of multifunctionality, in species-rich fish communities across a biogeographic region including coral reef, mangrove and seagrass ecosystems. Species richness strongly predicted ecosystem processes for all ecosystems. Biodiversity stabilized ecosystem processes under multiple scenarios of species loss, providing robust support for the insurance hypothesis. Multifunctionality required the most species to maintain stability. Few species had disproportionate effects on ecosystem processes, but exclusively in communities with low species richness. As biodiversity declines continue, ecosystem processes will become more reliant on fewer species. As such, ecosystems once stabilized by complementarity will be at increased risk for collapse with even small additional declines in species richness.

Main Text

A primary rationale for conservation of biodiversity is that species loss may undermine our ability to meet the rising demand for ecosystem services (e.g., food security) for growing human populations (Loreau *et al.* 2001; Cardinale *et al.* 2012; Naeem *et al.* 2012). Small-scale experiments and theory suggest that as species richness increases, ecological functions provided by communities are stabilized by species responses to environmental fluctuations, i.e., the "insurance hypothesis" (Yachi & Loreau 1999). While the nature of this relationship and the mechanisms by which it is governed (e.g., species dominance versus complementarity) have been well studied in model communities, our understanding of this phenomenon across large spatial scales of environmental variability and gradients of biodiversity remains poor (Duffy 2009), particularly in species-rich communities (e.g., tropical rainforests and coral reefs).

Coastal marine ecosystems, including coral reefs, mangroves and seagrass beds, are among the most species-rich and productive ecosystems in the world. Despite yielding critical ecosystem services for society, they are among the most heavily impacted by humans (Worm *et al.* 2006), e.g., declines in coastal fisheries that support human populations living along coastlines (McGranahan *et al.* 2007). While the implications of such declines have been widely studied from a food web (i.e., top-down) perspective, the role of fishes in mediating biogeochemical pathways (Vanni 2002), a critical ecosystem function (Naeem *et al.* 2012), is increasingly being recognized. Namely, through stoichiometric processes of assimilation (storage in biomass) and regeneration (excretion), fishes are among the largest pools (Maranger *et al.* 2008) and fluxes (Allgeier *et al.* 2013) of nutrients in oligotrophic coastal waters, regulating energy and material pathways within these energetically efficient ecosystems (Deangelis 1980). These stoichiometric processes are governed by traits that are unique to each species, and thus provide a framework to understand mechanisms through which biodiversity mediates ecosystem processes (Vanni *et al.* 2002).

Using two extensive empirical datasets, we modeled five ecosystem processes and an aggregate measure of ecosystem function, multifunctionality (*M*)(Maestre *et al.* 2012), across fish communities in six ecosystem types with relatively low human impact throughout the Northern Antillies (The Bahamas archipelago and Turks and Caicos) (~10-100km scales)(Fig. 1a). We tested the hypothesis that species richness is positively related to ecosystem function (Fig. 1b) and that species and functional group complementarity govern this effect (Fig. 1b*ii,iii*). We then applied probabilistic simulation models to empirical fish communities to explore the implications of species loss, under three scenarios of community disassembly, for all ecosystem processes. In doing so, we tested the hypothesis that insurance effects strengthen with increased species richness and community structure (Fig.1c,d). Our findings demonstrate positive biodiversity effects and provide support for the insurance hypothesis in species-rich coastal ecosystems (Fig. 1b,d), with complementarity among species being a dominant driver of these relationships. Moreover, community structure contributes to the strength and magnitude of insurance effects for most, but not all, ecosystem processes. These findings provide novel, empirically-based, insight into the role of biodiversity in maintaining ecosystem function in species-rich communities that span regional spatial gradients and multiple ecosystem types.

We quantified biogeochemical processes of nutrient storage and supply for nitrogen (N) and phosphorus (P), and an aggregate measure of multifunctionality, *M,* (herein collectively “ecosystem processes”), in fish communities across multiple coastal ecosystem types. Species-level models of nutrient supply and storage (N, P, and carbon (C)) were constructed by informing empirical field measurements of nutrient excretion from 665 individual fish (79 species, 46 genera and 26 families) with bioenergetics models (for 28 families and parameterized by ~1,600 stoichiometric explicit values), in a Bayesian framework (Fig 1a)(SOM). *M* was calculated as the average z-score of each process following Maestre *et al.* (2012)(Maestre *et al.* 2012)(SOM). These data were modeled onto each individual fish (n= 71,729), within all communities (n=172, within 82 sites), to quantify species-level and aggregate ecosystem processes (SOM). Surveys across all ecosystem types include a total of 158 species, of which we account for 144 with our species-specific biogeochemical models, including 99.4% of all biomass found within surveys.

Using hierarchical linear mixed effects models and information theory (Akaike information criterion; AIC_c) (Burnham & Anderson 2002) we found strong positive support for species richness as a predictor of ecosystem processes across all ecosystem types (Table 1)(SOM). Species diversity (i.e., Simpson's index; SD) was negatively related to all responses, indicating certain species have disproportionate roles (i.e., species dominance) in aggregate ecosystem processes (Table 1); however, SD was not always retained in top models for *M* (SOM – Table S1). Functional guild (i.e., trophic guilds; FG) diversity was only retained in the top models for nutrient supply of P and storage of C and N, for which the effect was positive. The effect of FG diversity was negative for *M* suggesting that certain trophic guilds have disproportionate effects on aggregate ecosystem function. Though other aspects of community structure were important parameters, e.g. maximum body size per species within each community (Lmax), these aspects are explored in a complementary study; here we focus only on aspects relating to species diversity. These findings suggest a positive, saturating, effect of species richness on ecosystem processes in species-rich coastal fish communities (i.e., grey line Fig. 1b*i*), but that species dominance (i.e., a negative diversity relationship; black line Fig. 1b*ii*) is also an important driver of ecosystem processes. There was a significant effect of ecosystem (random intercept only) in all models, suggesting inter-ecosystem variability in the mean ecosystem response, but not in the overall direction or magnitude of change.

We used probabilistic simulations to model the implications of species loss for aggregate ecosystem processes, and explored the nature of the insurance hypotheses across fish communities associated with each ecosystem type (Fig. 1c). Simulations were parameterized by the non-random assembly of species within each ecosystem type and for the entire region. We modeled species loss under three scenarios: *i*) random removal with no replacement (herein no-replacement), *ii*) random removal with compensation for biomass (herein Biomass), and *iii*) random removal with compensation for biomass and maintenance of trophic structure (herein Biomass+FG) (Fig. 1c*i*-*iii,* respectively; SOM). These scenarios allowed us to test the importance of various levels of community structure in mediating ecosystem processes and determining the relative strength of insurance effects (SOM).

Bifurcations in model variance structure emerged in the seagrass bed communities under the Biomass+FG model scenarios, and strongly so under the no-replacement scenarios (Fig. 2). Bifurcations indicate strongly disproportionate effects on ecosystem processes by single species (i.e., species dominance), whereby when this species is lost, a fundamental shift in aggregate ecosystem process occurs (Fig. 1c*i*). While bifurcations might be mitigated by the compensatory dynamics of the simulations, e.g., the maintenance of community biomass, the general paucity of their occurrence suggests species complementarity, not species dominance, may be driving richness effects across these ecosystems for most scenarios of species loss (SOM – Fig. 2,3). Exceptions are found in the communities with the lowest species richness, i.e., seagrass beds, or within the processes that are most strongly driven by species-specific traits as opposed to biomass, e.g., P supply and storage (Fig. 2; SOM Figs. 1-3). For example, total N supply by seagrass fish communities (n=45 species, the lowest richness) was dominated by a single species of *Belonidae* (49%). Interestingly, the only model scenario that produced indications of bifurcations for *M* in any ecosystem was the Biomass+FG (Fig. 2), suggesting that there is less complementarity among functional groups than among species when considering multiple simultaneous processes, a finding that is consistent with the negative FG diversity effect with *M* in our statistical models.

The collective variance across individual simulation models captures the relative strength of insurance effects (Tilman 1999; Cottingham *et al.* 2001). Strong insurance effects are found within communities with strong species complementarity, whereby stabilization in ecosystem processes is maintained by differences in species' responses to environmental fluctuations (Yachi & Loreau 1999). In our analysis, insurance effects were indicated for each process by negative slopes in the relationship between model variance (measured as the normalized root mean squared error, NRMSE) and maximum species richness within each ecosystem and the entire region (Fig. 3; SOM). Shallower slopes indicate stronger insurance effects and greater ecosystem stability (Fig. 1d; Fig. 3a; SOM Table S2)(Ives *et al.* 1999; Cottingham *et al.* 2001). Our results provide strong support for the insurance hypothesis in these species-rich fish communities. When contrasting the two scenarios of species loss that maintain community biomass (Biomass only and the Biomass+FG) with the no-replacement scenarios, slopes decreased for all three storage processes, suggesting that the maintenance of community biomass helps stabilize the storage of nutrients by fish communities (Fig. 3b). There was no effect for either supply process or *M* (Fig. 3b), indicating these processes are more strongly regulated by species-specific traits than community biomass. Slopes typically did not differ between the

Biomass only and Biomass+FG scenarios (P storage was the exception), demonstrating that additional maintenance of trophic structure does not generally enhance insurance effects and that the relative degree of species complementarity is not substantially affected by the trophic role of species. The general significance of these findings is that while trophic complexity has been widely cited for its importance for food web stability in aquatic food webs (Pauly *et al.* 1998), this study shows that maintenance of community trophic structure (apart from maintaining biomass) is generally not important for the regulation of biogeochemcial pathways.

The mean variance for each model represents the relative degree of species complementarity under the different extinction scenarios, whereby the greater the variance the weaker the complementarity among species, and thus potential for weaker insurance effects. Similar to findings from the slope of these relationships, complementarity was only enhanced with maintenance of community biomass for storage processes, but for no process did additionally controlling for trophic structure significantly affect the degree of complementarity (Fig 3b). *M* appears to be more strongly regulated by species-specific traits than by biomass or trophic structure, as it was the only process in which complementarity decreased with greater regulation of community composition (Fig. 3). These findings collectively demonstrate that: (1) community biomass is an extremely important regulating factor for biogeochemical processes, but its relative importance can vary depending on the ecosystem process; (2) additional maintenance of community trophic structure does not increase species complementarity or strengthen insurance effects (consistent with the weak role of FG diversity in our statistical models); and (3) insurance effects are generally weak for *M* when biomass and trophic structure is maintained, demonstrating the importance of species-specific traits, and thus species identity.

Biodiversity-ecosystem function research often relies on small-scale experiments that capture limited gradients of species richness, or meta-analyses and global scale analyses that may overlook important ecosystem-specific details. Our study provides unique opportunities to test basic tenets of biodiversity-ecosystem function theory across regional scales of environmental heterogeneity and large gradients of biodiversity. Consistent with previous research, we found strong positive effects of species richness on ecosystem processes and evidence of insurance effects across ecosystems and various levels of species richness. However, the strength and magnitude of insurance effects vary across processes and, critically, are contingent on the scenario of species loss. Further, our findings that high numbers of species are required to maintain multifunctionality is consistent with previous research (Cardinale *et al.* 2012; Maestre *et al.* 2012), suggesting that our typical measures of ecosystem function are potentially underestimating the net effects of species loss for aggregate ecosystem processes. We extend this work to show the insurance hypothesis is supported for multifunctionality, though somewhat weakly relative to independent processes. In contrast to independent processes, retaining trophic structure decreases insurance effects for multifunctionality. Previous research has shown key species often exert disproportionate control over ecosystem processes, i.e., species dominance (Cardinale *et al.* 2006); however, in our study, complementarity was more important in stabilizing ecosystem function. The role of species dominance appeared to be relegated to ecosystems with relatively few species (e.g., seagrass beds, richness=45), and under scenarios of species loss when trophic structure was retained.

Ecological baselines have shifted significantly over decades and centuries, precluding our understanding of “pristine” ecosystems (Knowlton & Jackson 2008). Our research from species-rich marine ecosystems that are relatively unimpacted by human activity offers novel insight of the role of species complementarity in ecological communities. From a conservation perspective, if ecosystem processes depend on only one (or few) species, then under scenarios of further species loss, e.g. sustained overharvesting of species, the likelihood of a significant disruption in the provisioning of these processes by the community is substantially greater. As such, as ecological baselines continue to shift, ecosystems that once had strong species complementarity may face scenarios whereby the role of species dominance becomes increasingly important. Thus, under continued biodiversity loss, ecosystems will face increasing risks for wholesale decreases in the provisioning of important services.

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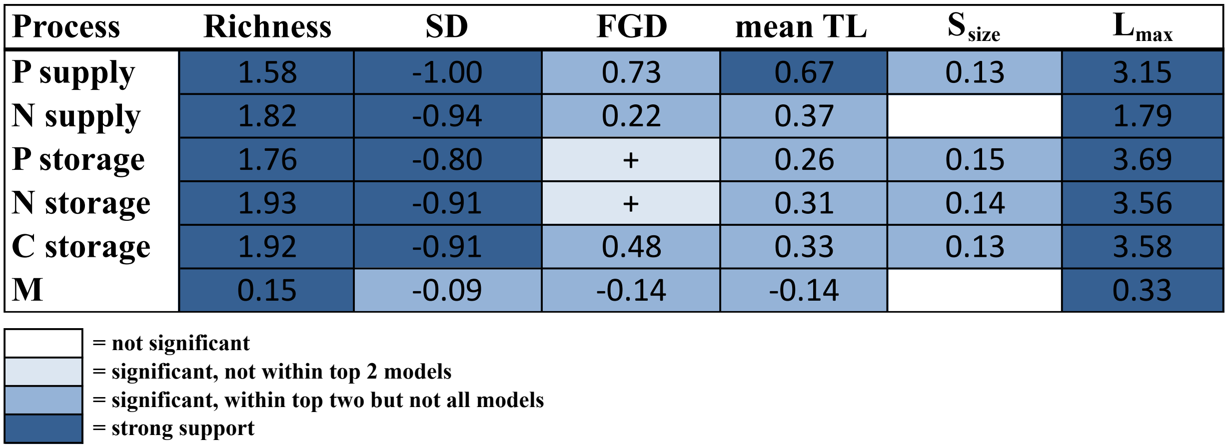
Table 5.1. Results from hierarchical mixed effects models exploring the relationship between aggregate nitrogen (N) and phosphorous (P) supply, and N, P and carbon (C) storage of nutrients and mutlifunctionality (*M*) and six independent variables of community assembly. Variables include: Species Richness (Richness), the number of species within a community; Species Diversity (SD) and Functional Group Diversity (FGD), both measured by the reciprocal Simpsons' Diversity Index at the species level and functional group level based on discrete trophic delineations (SOM), respectively; mean trophic level (mean TL) and mean maximum size per species within the community (L_{max}) (SOM); skewness of the size frequency distribution of the community (S_{size}). Color shade indicates relative amount of support for that given parameter within the model, whereby strong support refers to when the parameter was retained in all models throughout the model selection process with significance defined by p-value < 0.05. The sign of the values associated with each parameter (even when estimate is not provided) indicates the direction of change.

Table 1.



Process	Richness	SD	FGD	mean TL	S_{size}	L_{max}
P supply	1.58	-1.00	0.73	0.67	0.13	3.15
N supply	1.82	-0.94	0.22	0.37		1.79
P storage	1.76	-0.80	+	0.26	0.15	3.69
N storage	1.93	-0.91	+	0.31	0.14	3.56
C storage	1.92	-0.91	0.48	0.33	0.13	3.58
M	0.15	-0.09	-0.14	-0.14		0.33

⬜ **= not significant**
░ **= significant, not within top 2 models**
▒ **= significant, within top two but not all models**
█ **= strong support**

Figure 1.

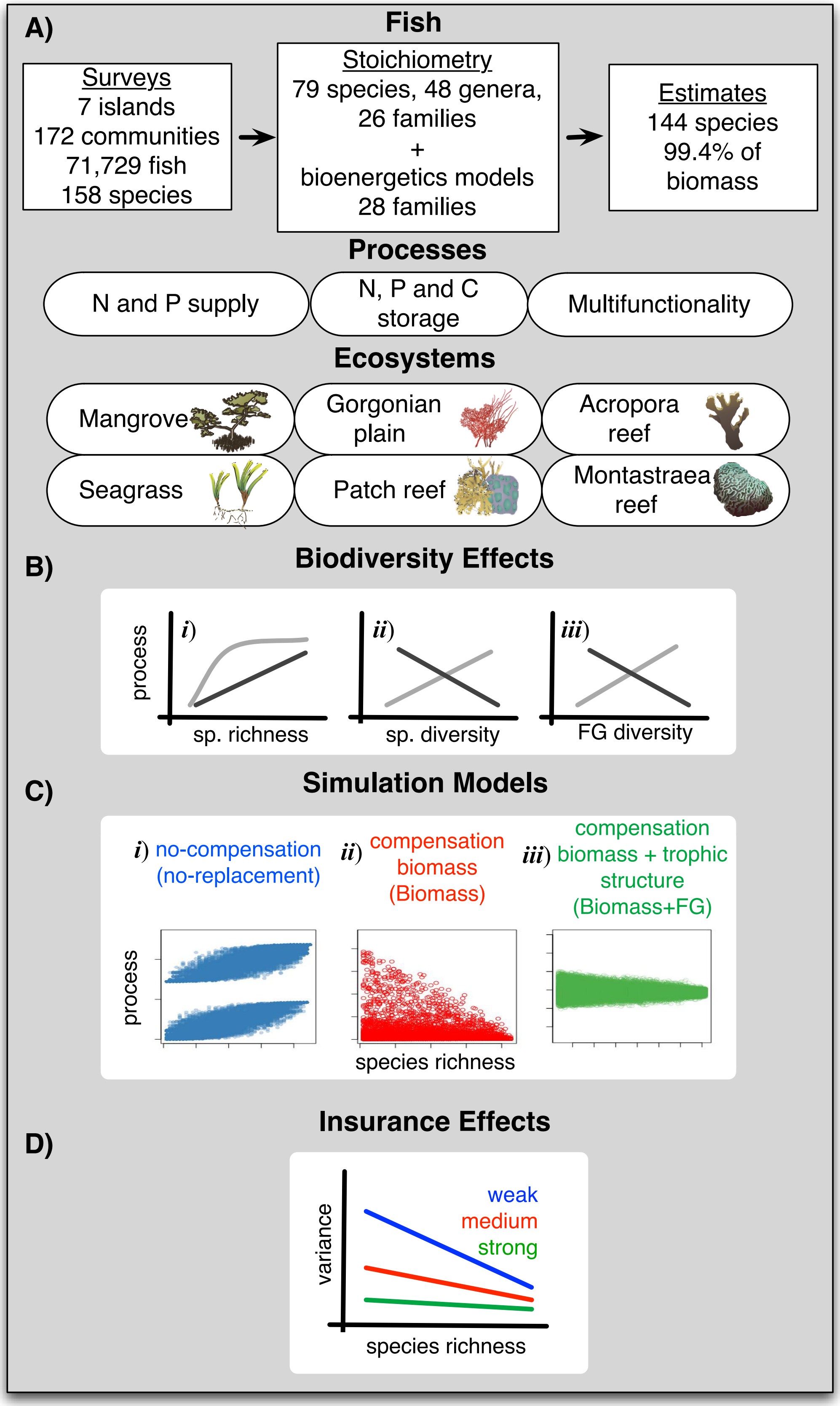

A)
Fish
Surveys
7 islands
172 communities
71,729 fish
158 species
Stoichiometry
79 species, 48 genera,
26 families
+
bioenergetics models
28 families
Estimates
144 species
99.4% of
biomass
Processes
N and P supply
N, P and C storage
Multifunctionality
Ecosystems
Mangrove
Gorgonian plain
Acropora reef
Seagrass
Patch reef
Montastraea reef
B)
Biodiversity Effects
process
i)
sp. richness
ii)
sp. diversity
iii)
FG diversity
C)
Simulation Models
i) no-compensation (no-replacement)
ii) compensation biomass (Biomass)
iii) compensation biomass + trophic structure (Biomass+FG)
process
species richness
D)
Insurance Effects
variance
weak
medium
strong
species richness

Figure 5.1. Hierarchical conceptual model of research and hypotheses. A) Empirical data used in statistical models to test for Biodiversity Effects, including the empirical survey dataset, stoichiometric dataset and the total species and percent biomass accounted for by our study. B) Hypotheses for Biodiversity Effects: *i*) Species richness has either a positive saturating non-linear, or positive linear, relationship with ecosystem processes; *ii*) Species dominance, i.e., the degree to which individual species have disproportionate influence on ecosystem processes - here quantified with diversity indices (e.g., Simpson's Diversity Index), may be either positively or negatively related to ecosystem process; *iii*) Functional Group (FG) Dominance, here calculated using diversity indices for FGs, may be either positively or negatively related to ecosystem processes. C) Three scenarios of species loss used in simulation models in this study: *i*) no replacement, i.e., random removal of species without replacement; *ii*) Biomass, i.e., random removal with replacement to maintain total community biomass; and *iii*) Biomass+FG, i.e., random removal with replacement to maintain total community biomass and trophic structure. D) Hypothesized strength of insurance effects for the models, as indicated by color.

Figure 2.

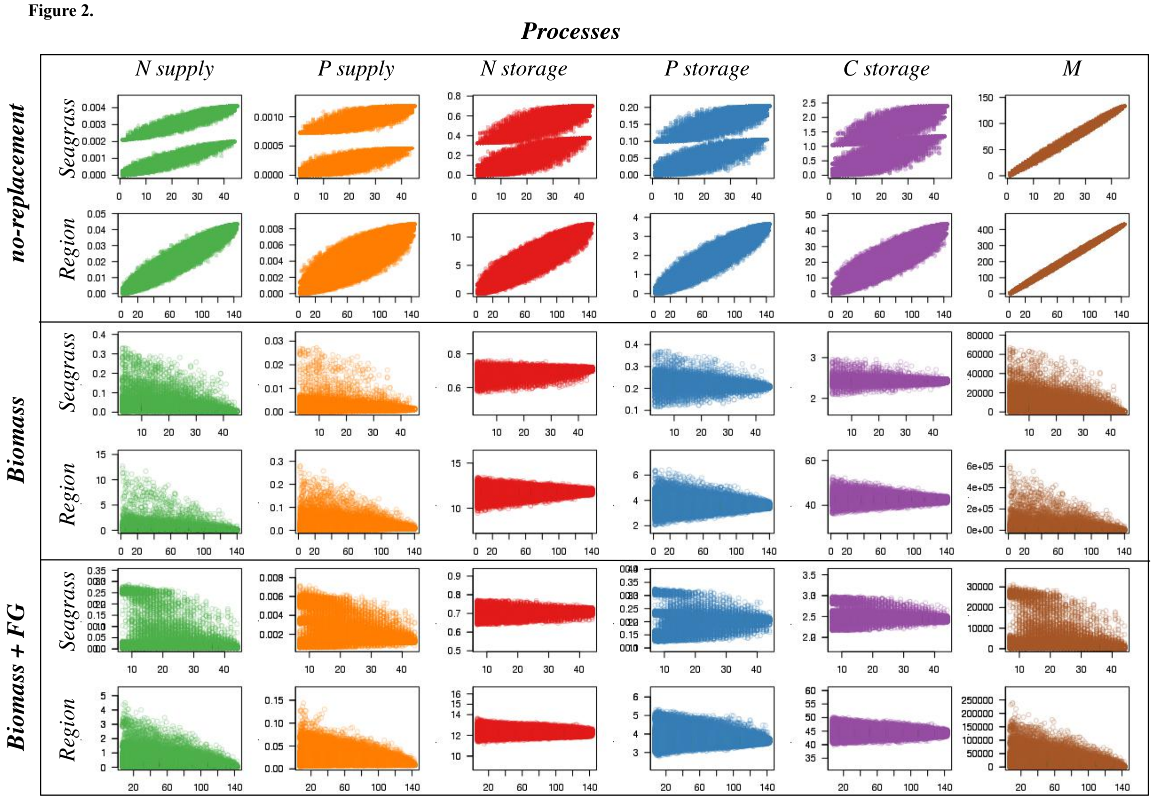


Figure 5.2. Results from probabilistic simulation models for the three different scenarios of species loss within a given community: No replacement, Biomass and Biomass+FG. Only fish communities associated with (*i*) seagrass ecosystems and (*ii*) the entire region are shown for simplicity (for all others see SOM). For each bi-plot, the y-axis is the rate (g m^{-2} day^{-1}) for each of the five processes and *M*. Each data point within a graph indicates the aggregate process rate for each simulated community with its corresponding species richness (x-axis).

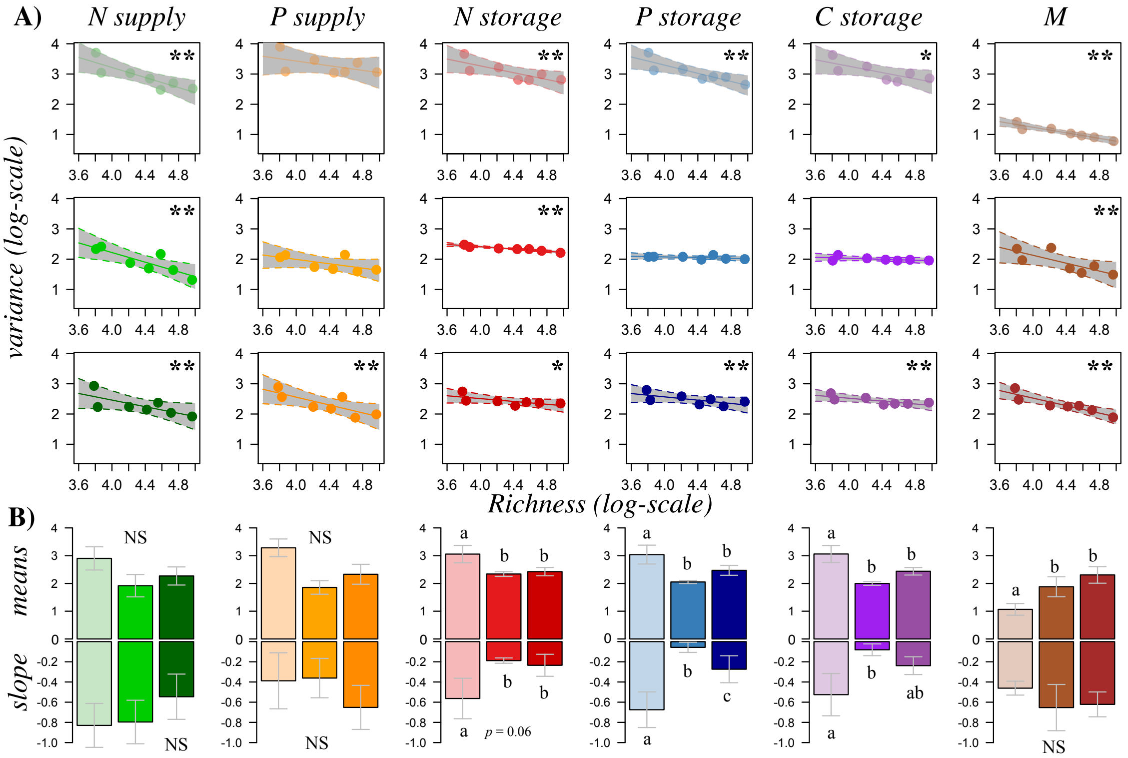


Figure 5.3. A) Relationship between model variance structure (normalized root mean square error; NRMSE, log-scale) and species richness (Richness, log-scale) for each fish community associated with each ecosystem type and across the entire region (* indicates *p*-value < 0.1; ** indicates *p*-value < 0.05; gray bands indicate 95% confidence intervals). B) Barplots of the mean value of the variance for each regression (top) and the slope for each regression (bottom). Error bars indicate SD of the mean and different letters indicate significant differences between bars within each individual plot. Each color is associated with a given ecosystem process or *M*. Color shade indicates the type of simulation model: light = no replacement, medium = Biomass and dark = Biomass +FG.

CHAPTER 6

CONCLUSION

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. My dissertation provides novel insight towards a understanding nutrient dynamics within ecosystems that deviate from the more traditional model. Specifically, I quantify nutrient limitation of two of the most dominant primary producer types in terms of biomass and productivity, seagrass and benthic microalgae, respectively, and provide evidence that fishes are among the most important sources of nutrients within these coastal ecosystems. Because The Bahamas is characteristic of many other island-types, particularly in the Pacific Ocean, findings from this dissertation can be extended beyond the study system and may provide useful information for management of these imperiled ecosystems throughout the world.

Compiling seagrass nutrient content along a spatial transect of three coastal mangrove-lined tidal creeks 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. Experimental results demonstrated mixed support for increasing and decreasing nutrient limitation of benthic micro algae along this spatial gradient. Findings showed 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. These findings suggest that, various site-specific biological factors such as consumer nutrient recycling may be more important than large-scale hydrologic factors in driving trends of nutrient availability in coastal ecosystems under strong nutrient constraints and suggest that even minor changes in nutrient loading rates can have significant implications for primary production in subtropical oligotrophic systems.

To extend these findings nutrient enrichment experiments were conducted in mangrove wetlands of The Bahamas, to test the hypothesis that human impacts (fragmentation) on these ecosystems altered nutrient limitation. Fragmentation occurred due to road construction, resulting in reduced hydrological connectivity between the wetlands and marine environment. Strong, persistent, and synergistic nutrient co-limitation, as measured by the Interaction Effect Index (IEI), occurred in both relatively pristine and fragmented estuaries with synergistic responses being less extreme in fragmented systems. This was supported by a strong, negative relationship between ambient algal biomass and the strength of synergistic responses across sites. Bahamian coastal ecosystems exhibited the greatest synergistic responses reported for a marine ecosystem, suggesting that the benthic algal community associated with Bahamian wetlands are among the most nutrient limited marine ecosystems. These findings provide a case study illustrating how altered nutrient dynamics associated with land-use change may decrease the frequency and/or magnitude of synergistic responses to nutrients in aquatic ecosystems.

The global comparisons from the third chapter were extended to all published studies across terrestrial, marine and freshwater ecosystems (n=653) to assess the generality of non-additive responses to dual nutrient enrichment. The IEI allows understanding of the specific type and degree of non-additive effect that occurs under conditions of single and dual nutrient enrichment. In this paper we outlined different types of non-additive responses (e.g., synergistic, antagonistic etc.) and proposed the IEI as a metric to be used to specify not only the type of response, but the degree to which it occurs. Applying this metric to studies around the world, non-additive responses were found to be common in all systems. Freshwater ecosystems and temperate latitudes tended to have frequent synergistic responses to dual nutrient enrichment, i.e., the response was greater than predicted by an additive model, whereas terrestrial and arctic systems were dominated by antagonistic responses (responses to N+P that were less than additive). However, we found that synergistic responses were less common than antagonistic responses but that when they occurred they tended to be of greater magnitude. This study highlights the ubiquity of non-additive effects in response to dual nutrient enrichment and further elucidates complex ways ecosystems respond to human impacts. In context of the dissertation research, these findings suggest that the role of consumer nutrient supply could be extremely important across many ecosystem types.

The final chapter of this dissertation focused on understanding how biodiversity within fish communities mediates biogeochemical pathways in these ecosystems. A major societal concern is that biodiversity loss will hinder our ability to meet the rising demand for ecosystem services, but predicting consequences of species loss is difficult, especially because the role of biodiversity for ecosystem functioning across large spatial scales of environmental variability has been poorly studied. Five biogeochemical processes, and an aggregate measure of multifunctionality were quantified in species-rich fish communities across a biogeographic region including coral reef, mangrove and seagrass ecosystems. Results show that species richness was a strong positive predictor of ecosystem processes for all ecosystems. Extending these findings to further test the applicability of the insurance hypothesis in these systems, we found robust support for the insurance hypothesis and that biodiversity stabilized ecosystem processes under three different scenarios of species loss. Findings also showed that multifunctionality required the most species to maintain stability, suggesting that typical measures of ecosystem function are potentially underestimating the net effects of species loss. Finally, we show that a few species did have disproportionate effects on ecosystem processes, but exclusively in communities with low species richness, i.e., seagrass ecosystems. Findings from this study suggest that as biodiversity declines continue, ecosystem processes will become more reliant on fewer species. As such, ecosystems once stabilized by complementarity will be at increased risk for collapse with even small additional declines in species richness.

In 1983, Meyer *et al.* documented for the first time the effect of fish excretion on coral development. In this paper they note that other animals, namely birds, have been implicated as contributing substantial inputs of nutrients to coral reefs, but fish, despite the long-standing knowledge of important schooling behavior have been overlooked. My dissertation extends this important research to show that fishes not only provide nutrients to coral reefs and other coastal ecosystems at the patch scale as demonstrated by Meyer and others (Holbrook *et al.* 2008; Roopin *et al.* 2008; Roopin & Chadwick 2009), but also at the ecosystem-scale. Additionally, this dissertation contextualizes the importance of fish nutrient cycling by improving our understanding of the degree to which production within different autotrophic groups are limited by nutrients. Collectively, this body of research places fishes as an extremely important source of nutrients in coastal ecosystems and highlights the role of biodiversity in maintaining these important ecological processes.

While this dissertation provides support of the overarching hypothesis: *that fishes facilitate important nutrient pathways in coastal marine ecosystems*; it does not provide any direct evidence that primary production can actually be enhanced through these processes. However, complementary studies to this research have provided experimental support for this hypothesis. Namely, Allgeier et al. 2013 and Layman et al. 2013 used artificial reefs of variable sizes to test how aggregating fishes on these reefs affected seagrass, and benthic microalgae production. Further, additional analyses have been conducted to understand the aspects of community composition for facilitating the various biogeochemical pathways within seagrass, mangrove and coral reef ecosystems (Allgeier et al. *in prep*). Specifically, it was found that the quantity of individual nutrients supplied and stored by a community can vary substantially among ecosystem types, even across different coral-dominated ecosystems, and that the quantity of these nutrients is impressive when compared to other nutrient supply rates such as input estimates of anthropogenic sources of N to the Mississippi River basin. Moreover, the ratio at which these nutrients are supplied and stored is remarkably consistent despite the variability in supply of individual nutrients and in community structure across ecosystems. These studies provide further, compelling evidence that the role of fishes in mediating nutrient dynamics needs to be interjected in models of coastal marine ecosystem function.

The erosion of coastal ecosystem function by various anthropogenic stressors is among the greatest threats to society because of the vast services these ecosystems provide. This dissertation seeks to elucidate an alternative approach from which to consider ecological processes and thus to understand human impacts on these ecosystems. Specifically, through observation, experimentation and quantitative techniques, including methodologies previously applied only in freshwater ecosystems and novel quantitative tools, this dissertation compiles a diverse suite of approaches to provide a holistic understanding of ecosystem-level nutrient demand by producers and supply via consumers. It further highlights the role of two specific stressors, nutrient pollution (eutrophication) and overfishing, in altering nutrient pathways, enhancing our understanding of the ecological implications of these stressors for ecologically and economically important coastal marine ecosystems.

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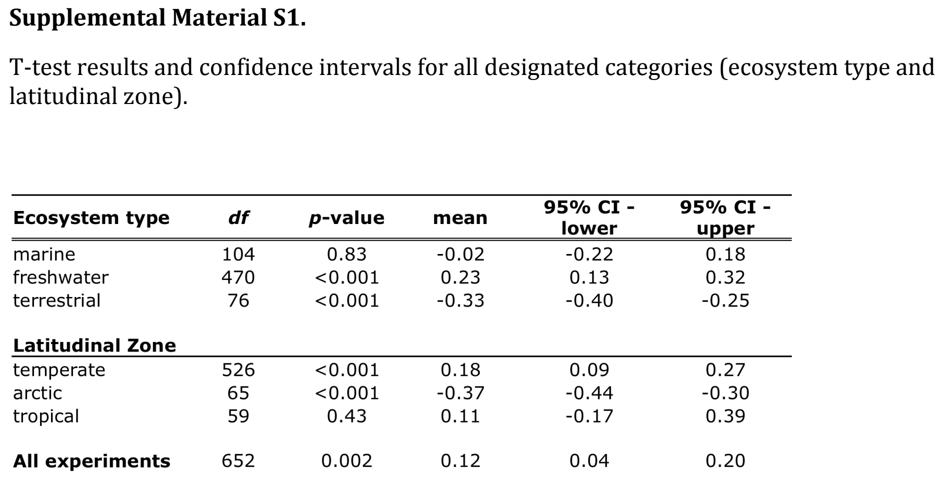
Appendix Chapter 2

Site/Contrast	*p*-value
BC	
BC:n-BC:c	0.66
BC:p-BC:n	0.99
BC:p-BC:c	0.99
BC:p-BC:np	<0.001
BC:np-BC:n	<0.001
BC:np-BC:c	<0.001
JC	
JC:p-JC:n	0.55
JC:p-JC:c	0.03
JC:n-JC:c	0.96
JC:p-JC:np	<0.001
JC:np-JC:n	<0.001
JC:np-JC:c	<0.001
SF	
SF:n-SF:c	0.99
SF:p-SF:c	0.77
SF:p-SF:n	0.99
SF:np-SF:c	<0.001
SF:np-SF:n	<0.001
SF:p-SF:np	<0.001

Table S1. Results from Tukey HSD post hoc analysis of ANCOVA (Table 4). Barracuda Creek (BC), Jungle Creek (JC) and Sucking Fish Creek (SF) indicate the site and the lowercase letter indicates the treatment (n= Nitrogen, p= Phosphorus, NP= Nitrogen+Phosphorus, c= Control).

Appendix Chapter 4.



Supplemental Material S1.

T-test results and confidence intervals for all designated categories (ecosystem type and latitudinal zone).

Ecosystem type	***df***	***p*-value**	**mean**	**95% CI - lower**	**95% CI - upper**
marine	104	0.83	-0.02	-0.22	0.18
freshwater	470	<0.001	0.23	0.13	0.32
terrestrial	76	<0.001	-0.33	-0.40	-0.25
Latitudinal Zone					
temperate	526	<0.001	0.18	0.09	0.27
arctic	65	<0.001	-0.37	-0.44	-0.30
tropical	59	0.43	0.11	-0.17	0.39
All experiments	652	0.002	0.12	0.04	0.20

Table S1. T-test and confidence intervals for all designated categories

Supplemental Material *S2*

Frequency of IEI values for each latitudinal zone (arctic, temperate, tropical) within each ecosystem type (freshwater, marine, terrestrial). In each plot the white background bars indicate the frequency of IEI values for all experiments within that given ecosystem type (e.g. the first row the white bars indicate the IEI values for all experiments in freshwater ecosystems).

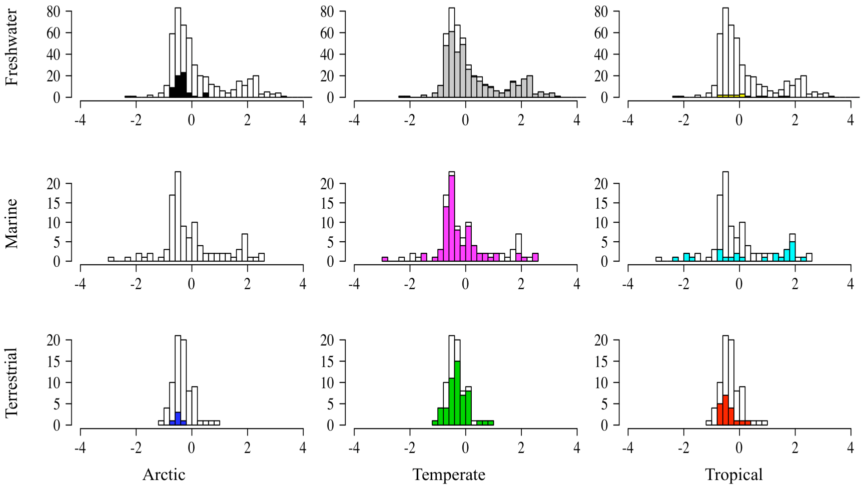


Figure. S1. The frequency of IEI values for each latitudinal zone with each ecosystem type. In each plot the white background bars indicate the frequency of IEI values for all experiments within that given ecosystem type.

Appendix – Chapter 5.

Nutrient Excretion – empirical estimates

Fish were captured using hook and line or traps. Excretion rates were determined *in situ* following the methodologies of Schaus *et al*. (1997)(Schaus 1997) as modified by Whiles et al. (2009)(Whiles *et al.* 2009). Fish were incubated in bags containing a known volume (depending on fish size ranging 1.2-8 L) of prefiltered (0.7 μm pore size Gelman GFF) seawater for ~30 minutes. Many factors can affect excretion rates by fishes (Vanni 2002; Whiles *et al.* 2009). This time interval was chosen based on the recommendations made by Whiles et al. (2009) and our own experimental time trials (n=64 trials for 22 species) assessing excretion rates ever 5- 15 minutes for up to 2 hours for a suite of sizes classes. All bags were placed together in a holding tank of water at similar ambient temperature (20-23°C). Excretion rates ($\mu g \cdot h^{-1}$) were calculated based on the difference between the dissolved nutrient concentrations (soluble reactive phosphorus (SRP-P) and ammonium (NH_4-N)) before and after the fish were incubated in the water. Values were control corrected through the use of multiple (typically n=6) identical control incubation bags without fish. Water samples (filtered with 0.45 μm Whatman nylon membrane filters) were immediately placed on ice and, within 10 hours, analyzed for NH_4 using the methodologies of Taylor *et al*. (2007)(Taylor *et al.* 2007), and were frozen for transport to the Odum School of Ecology (UGA) for SRP analysis using the persulfate digestion method.

Each fish used for excretion experiments was weighed for wet mass and measured to standard length. Fish were identified, and dissected to remove stomach contents, and then frozen for transport to Odum School of Ecology. Samples were lyophilized to a consistent dry weight then ground to a powder with a ball mill grinder. Larger individuals required blending to homogeneity before mill grinding. Ground samples were analyzed for %C and N content with a

CHN Carlo-Erba elemental analyzer (NA1500) CN Analyzer, and for %P using dry oxidation-acid hydrolysis extraction followed by a colorometric analysis (Aplkem RF300). Elemental content was calculated on a dry weight basis. The University of Georgia's Institutional Animal Care and Use Committee approved protocols for the capture and handling of fish (AUP # A2009-10003-0).

Bioenergetics models

Bioenergetics modeling allows for nutrient excretion rates of an organism to be estimated using a mass balance approach given *a priori* knowledge of the natural history (e.g., diet, feeding activity), physiology (e.g., stoichiometry of predator and prey, assimilation efficiency of nutrients, consumption rates, energy density of prey) and environmental conditions (temperature) (Schreck & Moyle 1990; Hanson *et al.* 1997). Excretion estimates for nitrogen and phosphorus were modeled at the genus-level given that we had sufficient parameter estimates at this taxonomic resolution. In all but one case (Labridae *Lachnolaimus maximus*), we chose to not to model at the species level because, although parameter estimates were available, we felt that this level of resolution was beyond the applicability of the models. As such, we generated 29 genus-level models, 12 family-level models and 1 species-level model. This provided us with the ability to model excretion rates for all but one family present in the survey data (n=28).

Stoichiometry data for each family/genera were determined by averaging the percent nutrient content for species within the given level of taxonomic classification. Use of parameters for closely related species may increase error in model estimates (Hansen *et al.* 1993; Ney 1993); however empirical work suggests that variation in excretion rates vary little within families but widely among families (Vanni *et al.* 2002). Energy densities of prey items were obtained from Cummins and Wuycheck (1981) (1981). Assimilation efficiencies, which have been shown to have only marginal influence on model estimates (Hood *et al.* 2005) were assumed to be 80% for N and 70% for P based on literature recommendations (Schreck & Moyle 1990; Hanson *et al.* 1997). The growth rate of an animal has been shown to be a particularly influential parameter in bioenergetics (Hood *et al.* 2005). As such, published growth rate values were found for each taxon of interest. Other parameter estimates were obtained from literature values specific to the given taxonomic level. Models were parameterized from thousands of diet data collected by the authors (J.E. Allgeier *unpublished*)(Layman & Silliman 2002; Layman *et al.* 2007; Hammerschlag-Peyer & Layman 2010; Layman & Allgeier 2012).

We used Fish Bioenergetics 3.0 software (Hanson *et al.* 1997), to determine consumption rates for the dominant feeding guilds present in our datasets. For example, we groups were parameterized as follows: predators – consuming a diet of other fishes (e.g., Carangidae), predator-insectivores – consuming a mixed diet of vertebrate and invertebrate prey (e.g., Lutjanidae), insectivores – consuming almost exclusively invertebrate prey items (e.g., Labridae) or herbivores – consuming >90% primary producer material (e.g., Scaridae). To do this we chose the taxon per feeding guild for which we had the best parameter estimates (e.g., Lutjanidae >100 individuals, thousands of diet data, etc.) and used the software to calculate consumption rates based on energetic demands of the taxon. These consumption rates were then used for all families within that particular guild, holding this parameter constant and allowing other important estimates (e.g., body stoichiometry, prey stoichiometry and growth rate) to have influence over the model. Bioenergetics models were run using R software (R Core Development Team). To account for inherent error that occurs when parameterizing such models, we propagated uncertainty associated with diet content and consumption rates, two of the most influential parameters for bioenergetics models (Schreck & Moyle 1990; Hanson *et al.*

1997; Schindler 1997), through the models using Monte Carlo simulations. Specifically, a normal distribution of values was created for each parameter with a standard deviation of 5% of the maximum potential value of that parameter (in both cases the parameters represent a proportion, so the standard deviation was 0.05). For each model run, random draws were taken from within these distributions 500-10,000 times, depending on the mean size of the fish within that family. The number of draws within this range did not change the outcome of the model.

Field and Laboratory methods for bioenergetics models

Fish and invertebrate specimens for predator and prey nutrient analysis were caught using hook and line, traps or netting under permits provided by The Bahamas Department of Marine Resources and following guidelines under University of Georgia Animal Care and Use Protocols (AUP # A2009-10003-0). Gut passages were cleared, either through live captivity without feeding or via dissection. Macroalgal samples were hand collected, rinsed with clean seawater, and cleaned of all epiphytes. Samples were frozen and transported to the lab for processing. Samples were dried to a constant weight in a lyophilizer and ground to a fine powder, using a ball mill grinder. Large samples were first ground in a blender when necessary. Ground samples were analyzed for %C and N content with a CHN Carlo-Erba elemental analyzer (NA1500) CN Analyzer, and for %P using dry oxidation-acid hydrolysis extraction followed by a colorometric analysis (Aplkem RF300). Elemental content was calculated on a dry weight basis. For crustaceans, samples were first acidified to remove inorganic carbon. See Allgeier et al. 2013 (Allgeier *et al.* 2013) and Burkepile et al. *in press (Burkepile et al. 2013)* for further details on bioenergetics models.

Bayesian models for nutrient supply

Bioenergetics models were used to inform empirical estimates of fish excretion through Bayesian linear regression models. Specifically, we first ran Bayesian simple linear regression models for all output data generated by the bioenergetics models. This provided credible intervals with which to determine prior distributions used to inform the empirical estimates (McCarthy 2007). There were 6 families of fish for which we did not have empirical excretion estimates so the bioenergetics model estimates for these families were used in a similar Bayesian regression with uninformed priors. All models were run with three chains for 50,000 iterations with a burn-in period of 1000. In total, we were able to estimate excretion rates for 144 species of fish, representing 99.4% of the biomass of fishes within our surveys and accounting for the vast majority of fishes in the greater Caribbean (Munro 1983). Because we were unable to empirically estimate excretion rates for each of the 144 species of fish and thus at times use the same model for different species within the same genera, our study if anything, underestimates species-specific excretion rates. Thus our analysis is conservative with respect to the role that individual species affect the ecosystem functions examined. Data for excretion models were not transformed and assumptions of normality were met. Bayesian analysis was run using the "rjags" package in R(team 2012).

Ecosystem-level modeling for biogeochemical pathways

Fish survey data was used to scale biogeochemical pathways to the ecosystem. Fish lengths were converted to biomass (g) using length-weight regressions from Mumby et al. 2006 (Mumby *et al.* 2006) and J.E. Allgeier *unpublished*. These values were then converted to nutrient supply per individual (g day^{-1}) by applying the linear regression equation from the

Bayesian models for nutrient supply, or conversion to nutrient storage (g) by multiplying the biomass by the percent nutrient content for that species.

Fish nutrient supply is expected to be variable as it is a function of body size, organism identity and diet (Schreck & Moyle 1990; Hanson *et al.* 1997; Vanni *et al.* 2002). As such, we used Monte Carlo simulations to model uncertainty into our estimates of fish nutrient supply for each individual fish within the dataset. We ran 1000 iterations whereby we randomly pulled values from a normal distribution (mean ± SD) of the parameter estimates for the slope and the intercept calculated from the Bayesian models (Robert & Casella 2010). In doing so we were able to create a range of values that represent a realistic probable distribution of rates of nutrient supply for N and P for nearly every fish in our survey. We applied the same methodology to calculate nutrient storage, whereas in this case we used the SD associated with our stoichiometric estimates for body nutrient content at each taxonomic level appropriate.

We eliminated sharks and rays from our analysis and did not include any fish from the family Scombridae (mackerel, 1 species), as the goal of our analysis was to focus on fishes that are typically considered resident within a give site. As such, of the 158 species present in our surveys, we were able to account for 144, representing a total of 99.4% of the total biomass.

Multifunctionality (*M*)

Multifunctionality was calculated following Maestre et al. 2012 (Maestre *et al.* 2012), whereby we calculated the average of the Z scores for each ecosystem process of interest. Z scores were calculated from log transformed normalized data:

$$\text{Z score} = [\,x - \mu\,] / \sigma$$

Where x is the site-level ecosystem process, μ is the mean value for all sites, and σ is the standard deviation of all sites. This index was chosen over other transformations used in the multifunctionality literature for three primary reasons: 1) this index follows a normal distribution (Kolomgorov-Smirnof test, df= 81, D = 0.20, *p*-value = 0.18 when used to evaluate community-level multifunctionality in mixed effects model, Kolomgorov-Smirnof test, df = 143, D = 0.07, *p*-value = 0.79, when used at the individual species level for simulation models) and thus is applicable to the modeling approach we used herein, 2) all of our response variables were positively correlated, and 3) z scores do not constrain the variability found in the raw data (Maestre et al. 2012). We also applied this metric in our simulation models by quantifying a z score for each species within a given community (as determined from survey data). Because this metric centers values around zero (and thus creates negative values) and we were specifically interested in using this metric for quantifying aggregate community effects, we added 3 (a value that is greater than the lowest negative value) to each z score. In doing so, we did not alter the net value or distribution of the metric, but instead simply shifted all values to be centered around three.

Hierarchical Mixed Effects Models

We used hierarchical mixed effects models to explore the relationship between the aggregate supply, and storage of nutrients and multifunctionality (*M*) and community assembly. To do so we ran six separate models, one for each of the ecosystem processes of interest and one for *M*. All models included the same six parameters: Species Richness (SR), Species Diversity (SD), Functional Group Diversity (FGD), mean Trophic Level (TL), mean Maximum Size of individuals in the community (L_{max}) and skewness of the size frequency distribution of the community (S_{size}). SR was a simple measure of the number of species within a community. SD and FGD were both measured by the reciprocal Simpsons' Diversity Index ($SD = 1 / \Sigma(P_i^2)$, where P_i is the abundance of species *i* divided by the total richness at that site)(Simpson 1949) at the species level and functional group level, respectively, whereby the greater the number the higher the diversity within the community. Functional group classifications were based on discrete trophic delineations following Newman et al. (2006)(Newman *et al.* 2006) (i.e., piscivore, piscivore-invertivore, macroinvertivore, microinvertivore, herbivore, omnivore, planktivore). Much ecological research has relied on classifications based on discrete trophic levels, and while recently developed continuous measures have merit (Naeem 2003), we chose this more traditional metric as it has proven to be a useful ecological level of organization in previous research (Naeem 2003; Floeter *et al.* 2004; Micheli & Halpern 2005). TL and L_{max} were calculated following (Nicholson & Jennings 2004) and trophic level values from (Harborne *et al.* 2006) were used. S_{size} was calculated by determining the skewness of the size frequency distribution of the community(Joanes & Gill 1998), whereby the further the value deviates from zero, either positively or negatively, the more small or large individuals dominate the community, respectively. There were six response variables of interest: N and P supply, C, N and P storage and *M.* In all cases, the response variable represented an aggregate value of all species contributions within a given fish community.

We modeled data from 172 communities across 82 sites within 6 different ecosystems (*Acropora* reef, Gorgonian Plains, Mangroves, *Montastraea* reef, Patch Reef, Seagrass) across 7 different islands in The Bahamas and Turks and Caicos. Fish community surveys consisted of multiple, (typically 8-10 transects) which were averaged per area following Mumby et al. 2006 (Joanes & Gill 1998) and Harborne et al. 2010 (Harborne *et al.* 2008). Fish communities were then averaged at the site level to account for variability that may exist within sites. Site and ecosystem were held as random effects in all models to control for the confounding effects that may be present due to site or ecosystem differences. In all cases, both random effects were for the intercept only as the random slope, or random intercept and slope, models were always significantly different ($p > 0.001$) and selected against using Akaike's information criterion (Zuur *et al.* 2009). Models were run using the "lme4" package in R (team 2012). All response variables, as well as SR, SD, FGD and L_{max}, were log transformed and, in all cases, model assumptions of normality and homogeneity of variance were met. Because the calculations for SD inherently includes SR, collinearity would be expected and to an extent did occur. However, these variables were never correlated more than $r = 0.51$, across all models, satisfying standard permissibility of collinearity (Gelman & Hill 2007). We additionally tested this by calculating variance inflation factors, a simple diagnostic for collinearity, for each model and in all cases the models met proper assumptions (Heiberger & Holland 2003).

We further tested possibilities of relationships in the data structure that may confound our overall findings and found no significant relationships. For example, there was no significant relationship between the total area surveyed per site (1020 – 2800 m^2) and any predictor variable (p-value > 0.1).

To examine the relative importance of the different variables for ecosystem processes and *M*, we used a multi-model inference approach (Burnham & Anderson 2002; Johnson & Omland 2004). This approach uses information theory to assess the probability that a given model most appropriately describes the data *(39)*. We calculated AIC_c, a value that corrects for the number of terms in the model, whereby the lowest AIC_c value constitutes the model with the best fit to the data *(39)*. For each model we also calculated the ΔAIC_c, representing the difference in AIC_c between each model. Values above seven indicate that a model has a poor fit relative to the best model and values below two indicate that models are indistinguishable *(39)*. We also calculated

Akaike weights (w_i), a parameter that provides further evidence for the best explanatory model *(19)* (Table S1).

Simulation models

We conducted three types of simulations of community disassembly: 1) random removal without replacement (no-replacement models)(Figure S1), 2) removal with compensation of biomass by any remaining species (Biomass models)(Figure S2), 3) removal with compensation of biomass constrained within FG, such that in every simulation the proportion of biomass for each FG remained relatively constant (Biomass+FG models)(Figure S3). In models which biomass was held relatively constant (Solan *et al.* 2004; McIntyre *et al.* 2007), for each species removed from the community we compensated by increasing the abundance of other species remaining in the community until a relatively constant total biomass was met. We allowed community biomass to vary within ±2.5% of the total (e.g., for the entire region 114 g m^{-2} ± 2.5%). For the Biomass+FG models, an additional constraint required the trophic structure of the community to remain relatively static, whereby at each level of species richness, the initial percentage of the biomass that each functional group (i.e., piscivore, piscivore-invertivore, macroinvertivore, microinvertivore, herbivore, omnivore, planktivore) occupied of the total community biomass was maintained. That is, if an herbivore species was removed, a member of that functional group had to be increased to fulfill the loss of that species' biomass. In doing so, we were able to decouple the relative importance of biomass and trophic structure for a species' influence on ecosystem processes and *M*. This is important because if species were completely complimentary among FGs, then it would be expected that ecosystem processes would be static if biomass within FG were maintained.

All simulations were initially parameterized by using the non-random communities associated with each ecosystem type (*Acropora* reef = 68 sp., Gorgonian Plains = 98 sp., Mangroves = 48 sp., *Montastraea* reef = 114 sp., Patch Reef = 86 sp., Seagrass = 45 sp., Entire region = 144 sp.) and the entire coastal ecosystem, calculated in our surveys. In doing so, we explored the prevalence of species complementarity at the level of trophic group or the entire fish community with each ecosystem the entire region. For each level of species richness we simulated 500 combinations of communities (created from random draws of species until that given level of richness was achieved) to create distinct communities. For each community, the aggregate sum of each ecosystem process and *M* was calculated. For simulations within ecosystems and the entire region, we used the average data for each species relative to the specific ecosystem and the entire region, respectively. To investigate the variance associated with the response axis for each simulation, we calculated the normalized root mean squared error (NRMSE) [100 * RMSE / max(response) – min(response)] (Moore *et al.* 2010). This normalizing statistic is similar to the coefficient of variance in that is often used to characterize variance (Tilman *et al.* 1998), but it calculates variance for the entire model. See Table S2 for model statistics. A generalized linear model was run to assess differences in model slopes and mean variance for each model (see Table S3 for statistics).

	Intercept	SR	SD	FGD	TL	Lmax	Ssize	df	logLik	AICc	ΔAICc	*w*i
P supply	-22.14	1.576	-1.001	0.7351	0.6762	3.151	0.1626	10	-63.555	150.2	0	0.569
	-22.34	1.642	-0.837		0.7163	3.231	0.1556	9	-65.435	151.4	1.16	0.318
	-21.52	1.761	-1.062	0.6669	0.7592	2.762		9	-67.097	154.7	4.48	0.06
	-21.7	1.82	-0.9174		0.7931	2.84		8	-68.522	155	4.81	0.051
N supply	-15.4	1.822	-0.9379		0.3783	1.789		8	-58.574	135.1	0	0.489
	-15.38	1.799	-0.9863	0.2221	0.3762	1.769		9	-58.384	137.3	2.15	0.167
	-15.43	1.811	-0.933		0.3711	1.81	0.01267	9	-58.546	137.6	2.47	0.142
	-14.5	1.684	-0.8403			2.023		7	-61.567	138.6	3.53	0.084
	-15.42	1.779	-0.9845	0.2507	0.3643	1.8	0.02042	10	-58.313	139.7	4.6	0.049
	-14.65	1.662	-0.8337			2.08	0.03898	8	-61.318	140.6	5.49	0.031
	-14.47	1.672	-0.8832	0.168		2.003		8	-61.47	140.9	5.79	0.027
	-14.65	1.64	-0.8927	0.2374		2.06	0.04631	9	-61.132	142.8	7.64	0.011
P storage	-17.57	1.758	-0.8011			3.687	0.1511	8	-73.291	164.6	0	0.288
	-18.28	1.897	-0.8703		0.259	3.521	0.1371	9	-72.247	165	0.44	0.232
	-17.67	1.756	-0.9382	0.4734		3.653	0.1648	9	-72.832	166.2	1.61	0.129
	-17.68	2.008	-0.9249		0.3162	3.211		8	-74.241	166.5	1.9	0.112
	-18.39	2.011	-1.176	0.7186	0.3128	3.313	0.1474	10	-71.944	167	2.43	0.085
	-16.8	1.844	-0.8322			3.408		7	-75.751	167	2.46	0.084
	-17.73	2.026	-1.016	0.2941	0.3265	3.163		9	-74.066	168.6	4.08	0.038
	-16.8	1.846	-0.896	0.2176		3.378		8	-75.651	169.3	4.72	0.027
	-17.54	1.296				3.675	0.1631	7	-79.603	174.7	10.17	0.002
	-17.49	1.372		-0.4249		3.713	0.1483	8	-79.105	176.2	11.63	0.001
	-17.92	1.343			0.1322	3.604	0.1584	8	-79.345	176.7	12.11	0.001
N storage	-17.45	1.933	-0.915		0.3057	3.558	0.1375	9	-70.134	160.8	0	0.277
	-16.75	1.78	-0.8491			3.8	0.1573	8	-71.866	161.7	0.94	0.173
	-17.28	1.898	-1.046	0.5314	0.2783	3.478	0.1423	10	-69.312	161.7	0.96	0.172
	-16.68	1.756	-1.013	0.6273		3.7	0.164	9	-70.745	162	1.22	0.15
	-16.87	2.088	-0.9913		0.3709	3.211		8	-72.306	162.6	1.82	0.112
	-16.76	2.073	-1.111	0.4618	0.3573	3.134		9	-71.716	163.9	3.16	0.057
	-15.86	1.873	-0.8739			3.47		7	-74.451	164.4	3.65	0.045
	-15.65	1.992	-1.196	0.6566		3.244		8	-74.501	167	6.21	0.012
	-16.76	1.295				3.799	0.1684	7	-78.609	172.7	11.96	0.001
C storage	-16.24	1.927	-0.9112		0.3314	3.548	0.131	9	-69.487	159.5	0	0.314
	-16.09	1.896	-1.029	0.4767	0.3069	3.478	0.1353	10	-68.813	160.7	1.25	0.168
	-15.71	2.088	-0.9916		0.3995	3.212		8	-71.501	161	1.5	0.148
	-15.52	1.766	-0.8462			3.822	0.1551	8	-71.567	161.1	1.63	0.139
	-15.42	1.74	-0.9946	0.5835		3.718	0.1586	9	-70.579	161.7	2.18	0.105
	-15.59	2.062	-1.092	0.4144	0.3805	3.149		9	-71.009	162.5	3.04	0.069
	-14.64	1.863	-0.8696			3.489		7	-74.121	163.8	4.28	0.037
	-14.65	1.88	-1.037	0.5301		3.413		8	-73.593	165.2	5.68	0.018
	-15.52	1.285				3.819	0.1661	7	-78.257	172	12.55	0.001
M	-0.672	-0.1604		-0.08603	0.3449	0.1522		8	56.667	-95.4	0	0.305
	-0.5879	-0.1669			0.3128	0.1106		7	54.69	-93.9	1.51	0.143
	-0.6608	-0.1526	-0.07981	-0.07063	0.3331	0.1638		9	57.156	-93.9	1.53	0.142
	-0.6445	-0.1464	-0.1174		0.3116	0.1403		8	55.883	-93.9	1.57	0.139
	-0.5981	-0.164		-0.08607	0.3217	0.157	-0.008692	9	56.86	-93.3	2.12	0.106
	-0.625	-0.1471	-0.09254	-0.07104	0.3109	0.1753	-0.01149	10	57.542	-92.1	3.33	0.058
	-0.6093	-0.1409	-0.1301		0.2897	0.1515	-0.01127	9	56.243	-92.1	3.35	0.057
	-0.5627	-0.1649			0.2995	0.1155	-0.006887	8	54.826	-91.8	3.68	0.048

Table S1. Complete model results for hierarchical mixed effects models. Parameter acronyms are as described in the text.

Model	**Process**	**R^2**	***p*-value**
No replacement	N supply	0.69	0.01
	P supply	0.14	0.22
	N storage	0.54	0.04
	P storage	0.69	0.02
	C storage	0.47	0.05
	M	0.88	0.001
Biomass	N supply	0.68	0.01
	P supply	0.28	0.12
	N storage	0.89	0.001
	P storage	0.04	0.31
	C storage	0.13	0.22
	M	0.54	0.04
Biomass+FG	N supply	0.45	0.06
	P supply	0.57	0.03
	N storage	0.38	0.08
	P storage	0.35	<0.1
	C storage	0.51	0.04
	M	0.8	0.004

Table S2. Model results for individual linear models of log(NRMSE) vs. log(Richness).

N Supply	**Estimate**	**SE**	**T-value**	**p-value**
Intercept	5.402	0.969	5.574	0.000
NR - B	1.133	1.368	0.828	0.420
BF - B	-0.758	1.356	-0.559	0.584
BF - NR	-1.891	1.353	-1.397	0.183
Slope	-0.796	0.221	-3.608	0.003
Slope NR - B	-0.034	0.311	-0.109	0.915
Slope BF - B	0.250	0.309	0.808	0.431
Slope BF - NR	0.284	0.309	0.920	0.372
P Supply				
Intercept	3.436	1.030	3.337	0.005
NR - B	1.546	1.453	1.063	0.304
BF - B	1.730	1.440	1.201	0.248
BF - NR	0.184	1.438	0.128	0.900
Slope	-0.361	0.234	-1.541	0.144
Slope NR -B	-0.027	0.331	-0.082	0.936
Slope BF - B	-0.291	0.329	-0.884	0.390
Slope BF - NR	-0.264	0.328	-0.804	0.434
N Storage				
Intercept	3.163	0.586	5.396	0.000
NR - B	2.359	0.827	2.851	0.012
BF - B	0.285	0.820	0.348	0.733
BF - NR	-2.074	0.818	-2.534	0.023
Slope	-0.188	0.133	-1.410	0.179
Slope NR -B	-0.375	0.188	-1.993	0.065
Slope BF - B	-0.047	0.187	-0.249	0.807
Slope BF - NR	0.329	0.187	1.760	0.099
P Storage				
Intercept	2.302	0.580	3.967	0.001
NR - B	3.690	0.819	4.506	0.000
BF - B	1.360	0.812	1.675	0.115
BF - NR	-2.330	0.810	-2.877	0.012
Slope	-0.057	0.132	-0.431	0.673
Slope NR -B	-0.618	0.186	-3.315	0.005
Slope BF - B	-0.217	0.185	-1.169	0.261
Slope BF - NR	0.401	0.185	2.171	0.046
C Storage				
Intercept	2.352	0.598	3.935	0.001
NR - B	3.007	0.844	3.564	0.003
BF - B	1.122	0.836	1.341	0.200
BF - NR	-1.886	0.835	-2.259	0.039
Slope	-0.081	0.136	-0.594	0.561
Slope NR -B	-0.444	0.192	-2.314	0.035
Slope BF - B	-0.157	0.191	-0.824	0.423
Slope BF - NR	0.287	0.190	1.509	0.152
M				
Intercept	4.740	0.684	6.931	0.000
NR - B	-1.656	0.965	-1.715	0.107
BF - B	0.272	0.957	0.284	0.780
BF - NR	-0.160	0.218	-0.736	0.473
Slope	-0.653	0.156	-4.196	0.001
Slope NR -B	0.192	0.220	0.874	0.396
Slope BF - B	0.032	0.218	0.145	0.887
Slope BF - NR	-0.160	0.218	-0.736	0.473

Table S3. Statistics for generalized linear model and pairwise comparisons of model slopes and means. NR = no replacement models, B = Biomass models and BF = Biomass + FG models.

Figure S1.

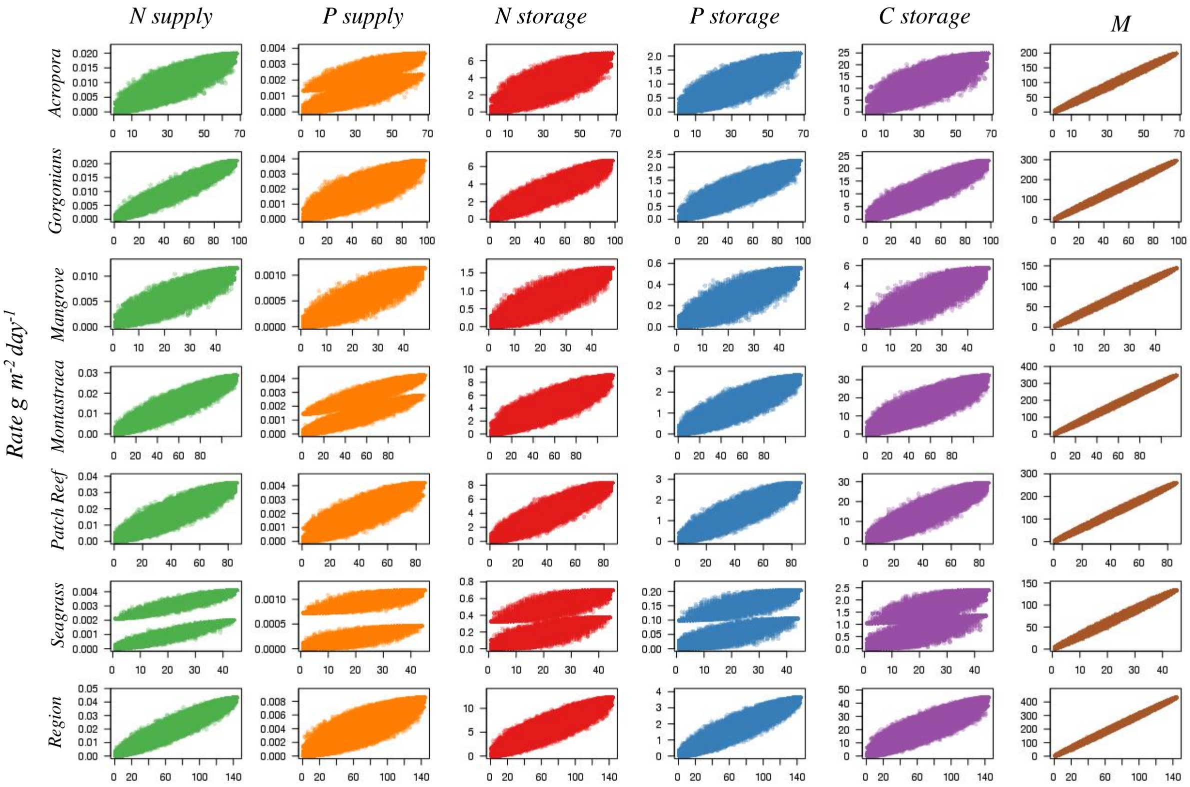


Figure S1. Model results for no replacement simulations.

Figure S2.

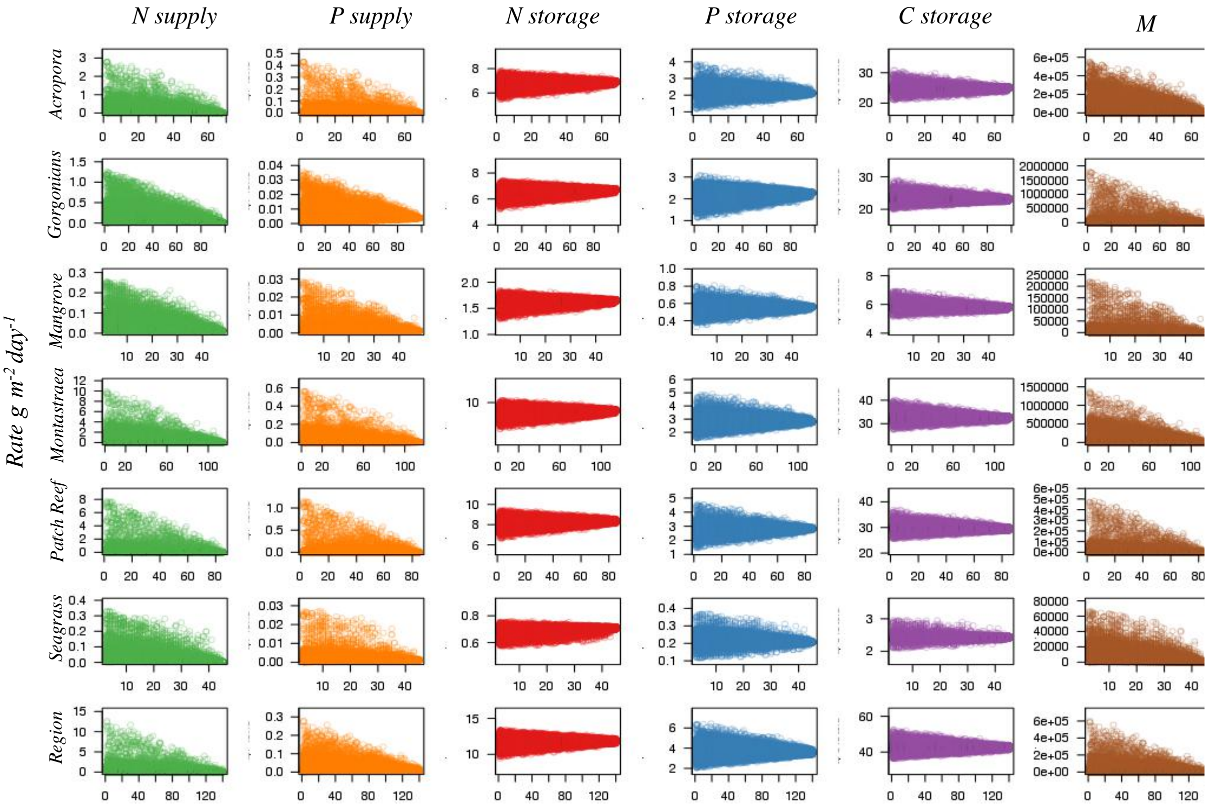


Figure S2. Model output for simulations with compensation for Biomass only.

Figure S3.

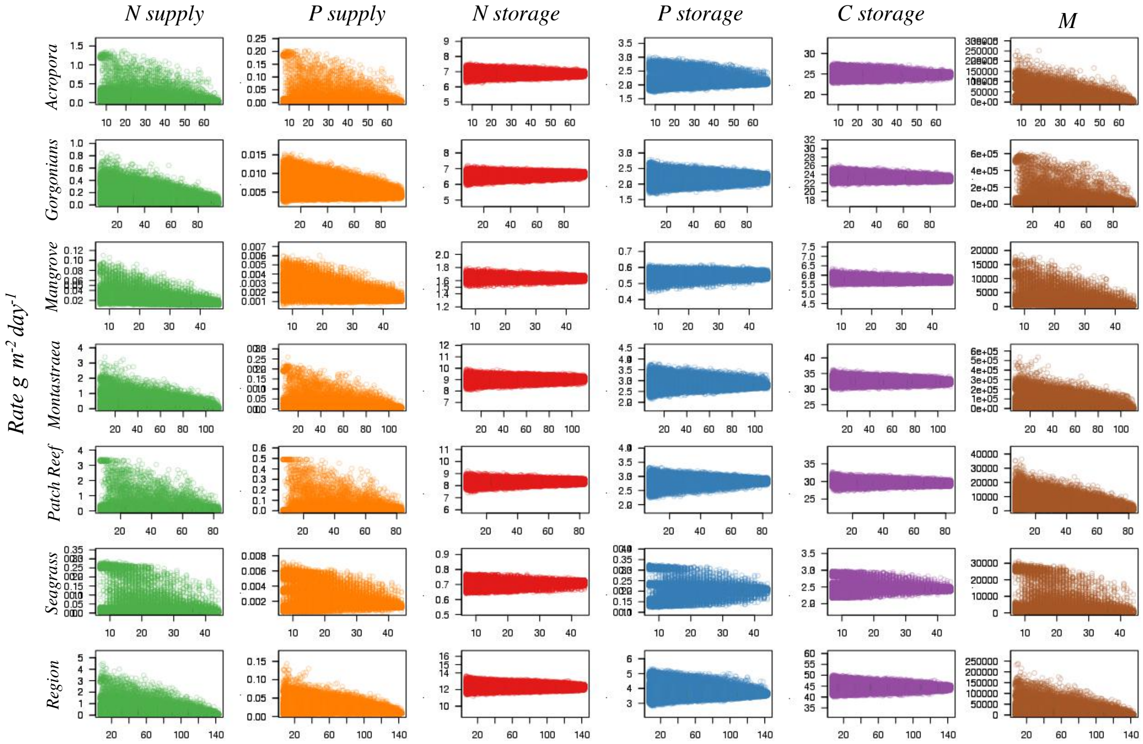


Figure S3. Model output for simulations with compensation for Biomass+FG.