BIOLOGICAL ECOSYSTEM TRAITS INFLUENCED BY STRUCTURAL CHANGES IN FOUNDATION SPECIES

by

VIRGINIA GRACE SCHUTTE

(Under the Direction of James E. Byers)

ABSTRACT

Foundation species are organisms that modify or create much of the biogenic habitat in an ecosystem. These types of organisms considerably affect biological ecosystem traits because changes that affect their structure or function will affect the entire biotic community associated with that engineered environment. Mangroves are an ideal foundation species to use when exploring structural changes in foundation species and subsequent shifts in ecosystem traits. Mangrove structure can be readily manipulated and simulated, mangrove trees grow quickly, and growth forms are plastic and highly structurally complex. In three studies, I link the habitat structure provided by the red mangrove *Rhizophora mangle* to biological aspects of its community: 1) *R. mangle* marine aerial roots influence species interactions in its attendant community, 2) anthropogenic nutrient pollution increases *R. mangle* canopy habitat but reduces subtidal root habitat, and 3) *R. mangle* subtidal roots and the epibionts that restructure that root habitat influence marine mangrove communities in the Caribbean.
R. mangle provides virtually the only hard substrate in a soft-sediment environment, requiring sessile benthic invertebrates to settle on mangrove roots in marine mangrove ecosystems. Mangrove root distance from the sea floor influences species interactions, indirectly determining sponge community composition on roots by denying or allowing sea star predators access to root-dwelling sponges.

In coastal areas with anthropogenically enhanced nitrogen levels, this extra nitrogen fertilizes trees. Fertilized trees favor canopy expansion at the expense of marine root growth, producing less marine root biomass over time.

Reduced root and root-dwelling epibiont structure shifts the abundance and diversity of attendant fish and benthic communities using this habitat. Roots and epibionts increase the capacity of marine root habitat to act as a refuge to fishes and they provide food to benthic organisms.

Together, these studies provide new perspectives on how specific elements of biogenecially engineered habitat can affect entire communities.

INDEX WORDS: ecosystem engineer, ecosystem services, red mangrove, marine sponge, epibiont, habitat, biodiversity, benthic ecology, Florida Keys, Bocas del Toro, Panama, Puerto Rico
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B.S., The University of North Carolina at Chapel Hill, 2007

A Dissertation Submitted to the Graduate Faculty of The University of Georgia in Partial Fulfillment of the Requirements for the Degree

DOCTOR OF PHILOSOPHY

ATHENS, GEORGIA

2014
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August 2014
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CHAPTER 1
INTRODUCTION AND LITERATURE REVIEW

Ecosystem engineers are organisms that shape the environment and resource availability (Jones et al. 1994). Engineers are inherently important because changes to the services they provide will affect communities associated with that engineered environment (Jones et al. 1997; Coleman & Williams 2002). Foundation species are autogenic engineers that modify or create the majority of the habitat in an ecosystem with their presence (Dayton 1972), such as trees in a forest, grasses on a plain, and corals on a reef (Jones et al. 1994; Byers et al. 2006; Cuddington et al. 2007).

Understanding how changes to foundation species’ structure consequently propagate throughout an ecosystem is essential to assessing mechanistic controls on ecosystem traits and creating effective management strategies. When foundation species are lost (such as clear-cutting a forest), the effects on the system can be immediate and drastic (e.g. Bell & Galzin 1984; Coleman & Williams 2002; Fahriq 2003). But we are just beginning to understand the details of how habitat structure and ecosystem traits are linked. How are the different components of foundation species affected by growth-altering factors? How are entire suites of species influenced by structural changes? How does foundation species density (rather than simple presence/absence) impact ecosystem traits? How do structure-modifying species dependent on engineered habitat modify habitat use themselves?
The research detailed here was conducted in Caribbean red mangrove (*Rhizophora mangle*) forests. Mangroves are woody plants that flourish intertidally in the tropics and they are an ideal foundation species to use in linking structural changes to ecosystem traits. To deal with the physical stresses of their marine habitat they have developed a variety of unusual root structures. Red mangroves (family Rhizophoraceae) in particular are spectacular structure creators, producing complex aerial roots that originate both from tree trunks and the canopy. In the Caribbean, red mangrove trees (*Rhizophora mangle*) growing on shorelines and island edges often lean out over the water. As aerial roots grow from these trees toward the sediment they enter the water column and can be permanently submerged, adding complexity to the study of these trees since they provide habitat structure in both terrestrial and marine ecosystems.

Mangroves lend themselves to scientific exploration of foundation species because their structure can be readily manipulated and simulated. Mangrove structure can be shaped with tools as simple as garden clippers and shears and structure can be approximated using PVC and dowel rods. Mangrove structure is also intuitively quantifiable; simply counting structural elements and measuring biomass are often the only techniques needed to describe structure in great detail. In addition, trees and their roots grow quickly. Mangrove roots can grow millimeters a day and changes in trees’ structural components can be captured in as little as 6 months, making many studies feasible that may not be with slower growing trees.

Mangroves serve as nursery grounds for commercially important species including crabs, shrimp, and fish (Nagelkerken *et al.* 2002; Nagelkerken *et al.* 2008), some of which are associated with seagrasses and coral reefs as adults (Kathiresan & Bingham 2001b; Mumby 2006). Despite their importance, a third of the world’s mangroves were lost in the last 50 years (Alongi 2002), and mangroves are currently declining at a rate of 1-2% each year (Ellison 2008).
This dissertation advances ecosystem engineering ecology and concretely contributes to conservation science (Fig. 1.1). It links the habitat structure provided by the red mangrove *Rhizophora mangle* to biological aspects of its ecosystem in three studies: 1) *R. mangle* marine habitat influences species interactions in its attendant community, 2) *R. mangle* marine habitat structure is influenced by enhanced nitrogen levels, and 3) *R. mangle* marine habitat and the epibionts that restructure that habitat influence marine mangrove biological communities in the Caribbean.

Chapter two describes the effects of *R. mangle* root growth into the sea floor on species interactions, which ultimately shapes root-dwelling epibiont communities. *R. mangle* roots provide nearly the only hard substrate in an otherwise soft-sediment environment, creating an obligate link between benthic organisms that require hard substrate to settle on and red mangrove root habitat. Sponges are a dominant feature of the subtidal epibiont community (Nagelkerken *et al.* 2008). Mangrove sponges’ dominant predators are benthic sea stars, which are confined to the sea floor. If a root comes within a critical distance of the sea floor, sea star predation on sponges dramatically increases. This habitat-influenced interaction may contribute to the restructuring of sponge epibiont communities. Sponge communities on suspended roots were nearly twice as diverse as those on grounded roots and palatable sponges were completely absent from grounded roots.

Marine *R. mangle* roots, however, are vulnerable to growth alterations. Chapter three examines the effects of eutrophication on *R. mangle* structure. Coastal nitrogen pollution acts as a fertilizer to mangrove trees, but mangroves can only take up terrestrial sources of nitrogen through their roots anchored in the mangrove peat. Experimental nutrient additions increased leaf production and reduced marine root production. Over time, eutrophication may change the
habitat provided by engineering *R. mangle*, shifting growth allocation away from marine habitat and into terrestrial habitat.

Marine *R. mangle* habitat is linked to the mangroves’ attendant community, where secondary engineers modify *R. mangle* root structure. Chapter four details an experiment in which marine aerial root habitat and its associated habitat-modifying epibionts were manipulated in three ways: subtidal roots and their epibionts were reduced by half, all subtidal roots and their epibionts were removed, and subtidal roots were left intact but all epibionts living on those roots were removed. Compared to the control, these treatments explored the influence of root and epibiont presence and density on associated fauna and physical ecosystem characteristics. In this system, root epibionts act as secondary engineers- they are dependent on the root habitat provided by *R. mangle* but themselves modify root habitat as they grow. Root reduction reduced fish abundances and epibiont reduction altered the mangrove fish community. Fish abundances also scaled with plot volume, which varied naturally.

These studies expand our knowledge of how engineered habitat traits influence communities dependent on engineered habitat. They also have direct conservation applications, providing details on how nutrient pollution influences marine mangrove structure and how this structure in turn influences subtidal Caribbean mangrove habitat quality.

**Literature Cited**


**Figure 1.1.** The following 3 chapters explore physical characteristics of the habitat created by the red mangrove, *Rhizophora mangle*, and how structural changes affect root-dwelling epibionts and fauna associated with *R. mangle’s* marine ecosystem.
CHAPTER 2

ENGINEERED HABITAT TRAIT DETERMINES PREDATION REFUGE STATUS
AND SUBSEQUENT COMMUNITY STRUCTURE

1 Schutte, V.G. and J.E. Byers. To be submitted to *Oecologia.*
Abstract

Ecosystem engineers create habitat that may vary in quality depending on its attributes. This variation may in turn affect species interactions among members of the engineer’s attendant community. We describe a habitat-creating engineer that produces two classes of the same habitat: one serves as a predation refuge and the other does not. Subtidal roots of the Caribbean red mangrove, *Rhizophora mangle*, are colonized by a vibrant epibiont community dominated by sponges. We experimentally demonstrate that roots touching the seafloor give benthic sea star predators access to their sponge prey living on the roots. In our study, half of sponges on grounded roots were eaten while sponges on suspended roots were unattacked. Correspondingly, in concomitant field surveys of mangrove root epibiont communities, we found palatable sponges only on suspended mangrove roots. Grounded roots were dominated by a single sponge that was never eaten in our experiment. Suspended roots had nearly twice the sponge species diversity and 1.5 times the sponge coverage of grounded roots, where sponges were exposed to sea star predation. This suggests that mangrove habitat traits allow sea star predators to dramatically restructure epibiotic sponge communities and demonstrates community-level ramifications of an engineered habitat trait.

**Key words:** threshold; state change; community structure; biodiversity; Everglades National Park; Florida Keys
Introduction

Ecosystem engineers are well known for habitat provisioning and for altering the abiotic environment, ultimately controlling resources for other species (Jones et al. 1994, 1997; Byers et al. 2006). But ecosystem engineers have more complex impacts as well, affecting interactions between members of their attendant communities (e.g. Miyashita & Takada 2007; Ransom 2012). Emphasis has recently been placed on studying species interactions in context (e.g. Zavaleta et al. 2001; Hay et al. 2004; Chamberlain et al. 2014), which should include the influence of engineers (as in Grabowski 2004; Hughes & Grabowski 2006).

Specific characteristics of engineered habitat may significantly affect species interactions by changing their context. We describe a case in which a single engineering individual produces habitat that either serves as a predation refuge or exposes community members to dramatically higher predation rates. Effects of this predatory access are pronounced - increased predation appears to almost completely restructure the benthic community dependent on that habitat.

The red mangrove, *Rhizophora mangle*, has aerial roots that originate from its branches and trunk and grow toward the sediment. *R. mangle* grows on Caribbean shorelines and island fringes, where portions of these aerial roots are often permanently submerged (Fig. 2.1). Epibionts such as algae, tunicates, bivalves, and sponges live on the intertidal and subtidal portions of mangrove roots (Rützler et al. 2000; Nagelkerken et al. 2008).

*R. mangle* roots are habitat for sponges in a soft sediment environment largely devoid of other suitable hard substrate. However, once a root grows into the seafloor, benthic organisms such as sea stars, a dominant mangrove sponge predator (Wulff 2006a), may be able to climb the root and eat these sponges. Through an experiment and community surveys, we addressed whether sponges living on mangrove roots are more vulnerable to sea star predation once the
root they live on has grown into the seafloor. We hypothesized that sponges living on roots grounded into the sediment would experience more predation by sea stars than sponges living on roots suspended in the water column. We also predicted that the sponge community on natural grounded roots would differ from the sponge community on suspended mangrove roots as a result of differential sea star access to these two types of roots.

**Methods**

*Predator susceptibility experiment.* In summer 2009, we worked beneath the canopy of *Rhizophora mangle* trees at the edge of a mangrove island north of Key Largo, just inside the southern border of the Everglades National Park (25.12876, -80.443811). To evaluate the effects of root position and thus sea star (*Echinaster sentus*) predator access on sponge fate, we manipulated root position by controlling whether a root was grounded in the sediment or suspended in the water column (Fig. S2.1). Each treatment was represented twice in each of ten blocks. Blocks were made using two rebar stakes to anchor either end of a clothesline (nylon string) with four roots strung on the line hanging down vertically between the stakes. We spaced two suspended and two grounded roots at least 30 cm apart and alternated them to increase apparatus stability. The minimum distance between suspended root tips and the sediment was 30cm. The first root type on each block was randomly selected. Sea stars on our experimental apparatus were unable to move between roots either on the clothesline or by reaching across neighboring roots, and so only had access to experimental sponges by climbing up roots from the seafloor. Blocks were set up parallel to the edge of the island (underneath the mangrove canopy and amongst natural, suspended mangrove roots).
For our experimental root material we used dead *R. mangle* root segments found above the high tide line, none of which had epibionts. We transferred three sponge pieces to each root, one individual of each of the three most abundant sponges (*Tedania ignis, Lissodendoryx isodictialis*, and *Chondrilla caribensis*) around Key Largo (Engel & Pawlik 2005). *T. ignis* is one of the most abundant mangrove sponges in the Caribbean (Wulff 2009 and references therein). Each of the three sponge individuals was randomly assigned an attachment location (upper, middle, or lower) on each root because the deepest sponges will necessarily be encountered first by climbing predators. Sponge pieces had a volume of approximately 50 mL each (measured by water displacement) and were collected from sponges living on roots outside the study area. Asexual reproduction through fragmentation is a common reproductive method for sponges (Wulff 1991) and the sponge pieces reattached to the experimental roots within 48 h of transplantation.

We photographed sponges approximately every three days for six weeks (sixteen observations total) to evaluate sea star predation. Sponge tissue loss could readily be attributed to a specific agent based on unique signatures left in sponge tissue (see Wulff 2006a). Diseases or unsuitable environmental conditions cause sponges to turn pale and then disintegrate. Fish leave crater-like bite marks in the surface of sponges. Limpets do not eat sponges but “bulldoze” through them as they move along the root surface, leaving behind a groove in sponge tissue near the mangrove root. Sea stars eat sponges by digesting sponge tissue externally, then ingesting that broken-down tissue. Portions of sponges consumed this way are translucent in contrast to the usual orange, blue, or gray coloration of *T. ignis, L. isodictialis*, and *C. caribensis*, respectively. Twenty percent of sponges were superficially damaged by fish (family Lutjanidae) and limpets (*Diodora cayenensis*) in isolated incidents and this damage quickly healed (see
Bingham & Young 1995; Wulff 2012). Observations during our experiment confirm that sponge condition was only impacted by sea star predators and was not affected by non-trophic factors. Frequent photographic sampling ensured that all sponge tissue damage could be matched with its cause before that damage signature was lost.

Once a sponge lost more than 50% of its tissue volume to sea star predation, determined by treating sponges as geometric shapes (sensu Wulff 2001), that sponge was classified as “majority consumed” and was removed from the experiment without replacement. Any sea stars eating the sponge were dropped to the seafloor beneath the root. Once sea stars began eating a sponge, they did not move until the sponge was consumed. This removal practice focused the experiment on sea star predation choice rather than consumption volume. Two predation events were excluded from analyses because large debris on the seafloor drifted into the experiment, allowing sea stars to have access they should have lacked in these treatments.

We counted the number of sea stars photographed on each sponge to quantify sea star location preferences. We also used haphazardly thrown quadrats to determine sea star abundances on the seafloor beneath our experiment.

Natural epibiont community surveys. To investigate how the mechanisms uncovered by the experiment may affect natural root communities, we documented natural sponge distributions at our site as a function of root position. We surveyed natural epibiont communities on the subtidal portion of ten haphazardly selected grounded roots and ten haphazardly selected suspended roots along the 14 m of island edge where our experiment was conducted. The percent coverage of all epibionts was determined photographically on the root surface facing away from the mangrove peat bank (since this was the only part of grounded roots logistically possible to survey accurately). Sponges were preliminarily identified to species using
photographic identification aides (Smithsonian Tropical Research Institute 2006; Zea et al. 2009). Species identities were confirmed using spicule plates except for Halichondria magniconulosa, which was only identified using photographic sponge guides.

To summarize and compare the sponge epibiont communities between the two root types, we first determined the percent cover of all sponge and non-sponge species on subtidal root lengths. We then calculated standard biodiversity statistics (discussed below) using only the percent cover of sponges. We did not include non-sponge species in these calculations because sponges dominate root epibiont communities on these roots (e.g. Kathiresan & Bingham 2001a; Engel & Pawlik 2005; Wulff 2012 and references therein) and the focus of this study was to describe sponge communities directly rather than to compare root epibiont communities in total. The average grounded root was 0.60 m long and the average suspended root was 0.92 m long (from the water’s surface to root tip, Fig. 2.1). Because species richness generally scales with area, to equalize the areas being compared we analyzed the portion of suspended roots from the mean tide level to the length of the average grounded root. This also allowed us to standardize for water depth. Although not an equal comparison, to see if the longer suspended roots harbored overall more diversity compared to grounded roots, we repeated our statistical calculations using the sponge community on the entire subtidal length of suspended roots.

We calculated several standard diversity metrics for each root type including species richness, evenness, and the Shannon Diversity Index. The Shannon Diversity Index takes both diversity and evenness into account and is higher for more diverse sponge communities. We compared communities using percent similarity, which is calculated using the abundance of each species in each community and is not sensitive to species richness differences between communities. Higher percent similarity values indicate more similar communities.
Results

*Predator susceptibility experiment.* Only sponges on grounded roots were attacked by sea stars, with a total of 34% of all grounded sponge pieces attacked and 0% of suspended sponges (Fig. 2.2). These rates are highly species specific as *Tedania ignis* and *Lissodendoryx isodictyalis* were the only two of our three experimental sponge species attacked by sea stars (Fig. 2.2). Nearly half (51%) of *T. ignis* and *L. isodictyalis* individuals in the grounded treatment were attacked. *T. ignis* and *L. isodictyalis* were preyed upon almost equally (with 58% and 45% individuals on grounded roots attacked, respectively). This is significantly different from *C. caribensis*, which was never attacked by sea stars.

Sea star distributions reinforced these predation patterns. We saw an average of 2.2 and 1.5 sea stars on each *T. ignis* and *L. isodictyalis* sponge individual, respectively, per sampling day versus just 0.1 sea stars on each *C. caribensis* sponge per sampling day. There were $86 \pm 85$ sea stars m$^{-2}$ (average plus standard deviation) on the seafloor beneath our experiment.

*Natural epibiont community surveys.* Collectively, we found a total of nine sponge species, but suspended roots had almost twice the species richness and much higher sponge coverage. In our equalized length comparison (see the Methods section for details of length equalization), seven sponge species covered 91.3% of subtidal suspended root length and four sponge species covered 63.2% of the subtidal root length on grounded roots (Fig. 2.3). Root length not occupied by sponges was primarily covered by turf algae on both types of roots. Suspended roots were dominated by massive, fleshy sponges while grounded roots were occupied by more encrusting sponge species (Fig. 2.4). Two of the three sponge species used in
our experiment (T. ignis and L. isodictyalis) were found only on suspended roots and the third species (C. caribensis) was found only on grounded roots.

Suspended and grounded R. mangle roots supported different sponge species assemblages (Table 2.1). Of the nine total species seen on both types of roots, five were found only on suspended roots, two were seen only on grounded roots, and two species were found on both types of roots (Fig. 2.3). Species evenness was lower for communities on grounded roots (0.49) than on suspended roots (0.77). The Shannon Diversity Index was 0.67 for grounded sponge communities and 1.50 for communities on suspended roots, a significant difference (paired t-test, t = 3.85, DF = 125, p < 0.01). Percent similarity between sponge communities on suspended and grounded roots was only 6.97%. Community statistics were virtually unchanged for our less conservative comparison between grounded roots and the (longer) whole length of suspended roots (Table S2.1 and Fig. S2.2).

**Discussion**

Mangrove root position relative to the seafloor significantly affected sea star predation rates on epibiotic sponges and perhaps subsequent community composition. The diversity and evenness of sponge epibionts on natural, suspended mangrove roots was high. In contrast, grounded roots had sharply reduced sponge diversity and were dominated by one unpalatable sponge, Chondrilla caribensis. The sponge species assemblages on the two types of roots were distinct from one another. Our experiment suggested that differential predation by sea stars on their highly favored prey species may help to drive these patterns. Specifically, Lissodendoryx
*isodictyalis* and *Tedania ignis* were exclusively preyed upon in our experiment and completely absent in surveys of natural grounded roots.

Epibiont sponges that live in this environment heavily colonize mangrove roots because subtidal mangrove ecosystems are typically devoid of alternative, predator-free settling surfaces. Highly palatable sponges on aerial roots are initially protected from benthic sea star predators, but the closer a root is to the sediment, the more likely it is that benthic sea star predators will gain access to these sponges. Roots need not anchor into the sediment to lose their status as refugia, since debris on the seafloor will form a bridge between the benthos and roots. This happened twice to experimental roots during this study and each time, sea stars quickly used the bridge to gain access to and attack palatable sponges on suspended roots.

Palatable sponges are not excluded altogether from the system by predation, however, because of inconsistent root accessibility and variable sea star abundances. Mangrove roots sometimes terminate growth above a critical height off the seafloor and may never grow close enough to the sediment to allow sea star predators access to palatable sponges. This is more common in deeper water, where the distance to the seafloor is greater. Additionally, *Echinaster sentus* and other sponge-eating sea stars (Wulff 2000, 2006b, a) are found throughout the Caribbean, but their local abundance can vary greatly. Underneath just the 14 m stretch of mangrove canopy used for this experiment, sea star abundances varied from 288 individuals m$^{-2}$ to 0 individuals m$^{-2}$.

Our survey results suggest that at our study site, sea star predation contributes to sponge community differences between suspended and anchored roots. It is possible that recruitment may affect these communities in addition to post-settlement factors, but both Farnsworth and Ellison (1996a) and Bingham (1992) found evidence that sponge settlement on mangrove roots is
patchy and affected more by larval retention times in the water column than by microhabitat variation between roots. In addition, there is evidence that sponge communities on subtidal mangrove roots in particular (Wulff 2000, 2004, 2005, 2009) and root epibiont communities more generally (Taylor et al. 1986, Perry 1988) may be influenced more by post-settlement processes than recruitment.

Sponges are obligate settlers on Rhizophora mangle subtidal roots in Caribbean mangrove ecosystems, where they thrive (Wulff 2012 and references therein). Their root habitat may terminate above the seafloor and serve as a refuge or it may anchor into the seafloor, giving sea star predators access to sponges. Our study suggests that where sea stars are abundant and depth is shallow, root position relative to the seafloor produces dramatic differences in epibiotic sponge communities. More broadly, the specific characteristics of structure produced by a habitat-creating engineer may significantly impact attendant species’ interactions, ultimately affecting community composition.

**Literature Cited**


**Figure 2.1.** Fringing red mangrove tree and typical marine mangrove habitat. For simplicity, we have depicted only the focal subtidal organisms of interest to this study. Diagram made with symbols courtesy of the Integration and Application Network, University of Maryland Center for Environmental Science (ian.umces.edu/symbols/).
Figure 2.2. Sea star predation rates on the sponges in our 6-week experiment.
Figure 2.3. Sponge communities constructed from cumulative percent coverage on natural roots at the study site. We compared equally long root segments of grounded and suspended roots. Sponge species are listed in alphabetical order from 1 – 9. Dark gray shading highlights the species used in our experiment and light gray shading highlights the sponge species seen on both suspended and grounded roots.
**Figure 2.4.** Sponge epibiont communities at our site in the Florida Keys. From left to right, the top row shows one grounded root with a typical less diverse sponge community, multiple grounded roots with an abundance of sea stars, one suspended root with a typical heterogeneous
sponge community, and multiple suspended roots that depict the increased sponge coverage on suspended roots in general. The bottom photo was taken at high tide and shows grounded roots on the left and suspended roots on the right. At this site, as in most subtidal red mangrove ecosystems in the Caribbean, *Rhizophora mangle* roots are virtually the only hard substrate available. Photo credits: VG Schutte.

**Table 2.1.** Equalized length biodiversity indices for sponge epibiont communities on grounded and suspended mangrove roots. Statistics in the equalized length analysis were calculated using only the portion of suspended roots equal to the length of the average grounded root. Indices are based on percent cover as a proportion of cumulative subtidal root length covered by sponges on each type of root. \( S = \) total species richness, \( E = \) evenness, \( H' = \) Shannon Diversity Index, \( PS = \) Percent Similarity.

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CHAPTER 3

NUTRIENT ENRICHMENT SHIFTS THE TYPE OF HABITAT PRODUCED BY AN ECOSYSTEM ENGINEER

\[2\] Schutte, V.G. and J.E. Byers. To be submitted to Marine Ecology Progress Series.
Abstract

Anthropogenic activities have significantly increased nitrogen inputs to the world’s tropical oceans, which may have especially pronounced effects on nitrogen limited coastal marine ecosystems. The habitat-creating red mangrove, *Rhizophora mangle*, is morphologically plastic and can increase growth rates with increased nutrient availability. However, the relative effect of nutrient enrichment on discrete habitats created by the tree has not been studied. *R. mangle* structure serves as habitat in both terrestrial and marine ecosystems. We hypothesized that additional nitrogen would change resource allocation within *R. mangle* on the edges of coastlines, increasing root production in the peat (to increase nitrogen acquisition) at the expense of marine root production. Marine roots cannot acquire nitrogen from the water column and so cannot increase nitrogen contributions to the tree in eutrophic areas, but they do provide key habitat for a number of important marine organisms. We fertilized the soil-like mangrove peat beneath mangrove trees on island edges in Jobos Bay, Puerto Rico with slow-release nitrogen plugs. Increased nitrogen levels in the peat did not increase subterranean root production in the peat. However, *R. mangle* leaves are produced in clumps at the ends of branches and fertilized trees had significantly more branches divide at their tips than control trees (35% compared to 6%, respectively), increasing the number of leaf clumps and leaf production points on fertilized trees. An increased number of leaves on a tree may allow the tree to increase carbon fixation. Enhanced nitrogen levels also decreased biomass production in marine roots by 61%. Thus, nutrient enrichment produced trees which shifted biomass production, increasing leaf production and decreasing marine root biomass production. Over time, differential structure production by
trees living in nitrogen enriched areas may negatively impact marine communities dependent on

*R. mangle*- engineered habitat.

**Key words:** nutrient pollution, eutrophication, nitrogen fertilization, foundation species, habitat, mangrove, tree root growth, canopy, Jobos Bay, Puerto Rico
Introduction

The world’s tropical oceans have historically been nutrient-poor (e.g. Corredor et al. 1999) but coastal areas may have high nutrient concentrations where they receive terrestrial runoff. Nitrogen is one of the most common pollutants entering the ocean from land-based sources and anthropogenic activities have doubled the global flux of nitrogen moving from land to sea since the mid-1900s (Vitousek et al. 1997; Galloway et al. 2003; Galloway et al. 2008; Howarth 2008). This influx, may especially affect nearshore, tropical marine ecosystems because they are often nitrogen limited (e.g. Nixon 1995; Corredor et al. 1999; Rabouille et al. 2001; Howarth et al. 2011).

Mangroves are the dominant intertidal habitat engineer (Jones et al. 1994; Jones et al. 1997) in tropical estuaries (Nittrouer et al. 1995). As they occupy the interface between land and sea, mangrove forests are often the first marine environment to receive terrestrially-derived nutrients as they enter tropical marine systems (Harrison & Pearce 2000). The red mangrove, Rhizophora mangle, fringes coastlines in the Caribbean, where it provides the structural basis for a unique marine habitat, attenuates physical stress to coastal areas from storms, and filters water moving offshore by slowing currents and causing siltation (Alongi & McKinnon 2005; Barbier et al. 2008; Das & Vincent 2009).

Despite the importance of the subtidal ecosystem created by R. mangle, our knowledge of how nutrient enrichment acts on R. mangle’s morphology is limited to the terrestrial portion of this tree (e.g. Feller et al. 2003; Lovelock et al. 2004; Feller et al. 2009). Fringing R. mangle trees (at the edges of islands or coastlines) are nitrogen limited (McKee et al. 2002; Feller et al. 2009) and they primarily assimilate nitrogen through roots anchored in mangrove peat (Wu et al.
2008; Pitt et al. 2009; Wickramasinghe et al. 2009), which is soil-like decaying plant matter that builds up underneath mangrove trees (McKee & Faulkner 2000; Lee et al. 2014).

When water moving offshore passes through the mangroves it is slowed by their roots, causing particles in the water column to settle onto and become incorporated into the mangrove peat. Nutrient pollutants have been shown to cycle through the mangrove forest this way without direct negative effects on the mangrove trees (Wong et al. 1997; Wickramasinghe et al. 2009), but *R. mangle’s* unique morphology (Fig. 3.1) may make it vulnerable to morphological changes caused by eutrophication. *R. mangle* exhibits preferential biomass investment in parts of the tree in the most favorable growth conditions (Farnsworth & Ellison 1996b). Unanchored marine roots cannot glean nutrients from the water column; the tree is dependent on roots in the peat for gathering nutrients. Seaward roots can receive nutrient contributions from mutualistic sponges (Ellison et al. 1996), but these resources are less versatile than those extracted from the peat—they are only locally retained in the marine root on which the sponge is located.

We hypothesized that elevated nitrogen levels will promote the growth of *R. mangle* roots physically anchored in the mangrove peat (where they have immediate access to terrestrially deposited nitrogen) at the expense of growth in marine aerial roots, which are not rooted (Fig. 3.1). We tested our hypothesis with a field experiment.

**Methods**

We selected 24 *Rhizophora mangle* trees of similar size, microlocation, and structural characteristics around the edges of the Cayos Caribe islands in the Jobos Bay National Estuarine Research Reserve (NERR), Puerto Rico (17.92922, -66.20516). Trees were approximately 7.0 m
high and 10.0 cm in diameter at breast height. Every tree grew at the edge of an island and had at least one root that grew from the canopy into the ocean water off the island’s edge, where it terminated above the seafloor at the start of this experiment. Trees were divided equally into 2 blocks and were assigned to a control group or a nutrient enrichment treatment group (n = 12) using a stratified random design. The experiment was installed in November 2010 and maintained and sampled every 6 months until May 2012 (i.e. three sampling periods after experimental setup), minimizing possible seasonal effects on results and allowing sufficient time for trees to respond to treatments.

We fertilized trees with urea (manufactured in Puerto Rico by Pan American Fertilizer) in order to mimic natural eutrophication dynamics. Urea readily dissolves in water and converts to ammonium, the preferred source of nitrogen for aquatic plant growth, through hydrolysis. In addition, while both ammonium and nitrate are important sources of nitrogen pollution (Camargo & Alonso 2006), wastewater discharged into mangroves in a representative field experiment (Wong et al. 1997) was composed of two orders of magnitude more ammonium than nitrate and more than an order of magnitude more ammonium was retained in mangrove peat than nitrate. We placed urea into dialysis tubing, which facilitates continual time-release of fertilizer rather than providing instantaneous nutrient pulses. Each dialysis tube was filled with 150 g of urea fertilizer and placed into 30 cm deep core holes in the peat (amount of urea based on proportional usage in Feller 1995). The peat removed when the core hole was created was used to fill in the hole on top of the tube. We placed rocks on top of this peat to ensure that everything we put into each hole would remain there in our absence. We marked fertilizing core locations with PVC guides inserted into the top 7.5 cm of the peat so that exact core locations could be used repeatedly, minimizing damage to tree roots. Tubes were replaced every six months (entire
fertilization procedure follows Feller 1995 and Feller 1996). Trees were fertilized with four tubes each. Each tube was placed approximately 15cm from where a root from an experimental tree entered the peat. Control trees were not fertilized but as a procedural control, 4 cores were taken from each tree every six months using the same procedure just described.

Even though the placement of our nutrient plugs targeted our experimental trees, it is possible that nutrients could have dissolved into the porewater and been flushed out of the experimental shorelines, or that non-targeted trees could have taken up our fertilizer. We used stable isotopes (Peterson & Fry 1987; Post 2002) in two ways to verify that our nutrient additions were effective at delivering nutrients to targeted trees.

First, we fertilized 5 R. mangle trees in Cayos Caribe but away from our experimental islands using the same fertilization techniques described above. However, the fertilizer that these trees received was $^{15}$N-labeled urea (manufactured by Sigma-Aldrich). Using isotopically labeled urea to fertilize our 24 experimental trees would have been cost prohibitive, but fertilizing five trees this way allowed us to trace urea-derived nitrogen through mangroves without reducing the number of replicate trees in our main experiment. After one year of fertilization with labeled urea, samples were taken from the leaves, branches, roots, and mangrove peat of target and nearby control trees.

Second, we collected samples from the leaves, branches, and roots of our experimental trees (as in Ellison et al. 1996) to determine their isotopic signature. Unlabeled urea fertilizer gives mangrove leaves a slightly depleted $\delta^{15}$N signature compared to unfertilized mangrove materials (around -3 ‰ instead of around 0 ‰, respectively, as reviewed in McKee et al. 2002), which obtain atmospheric nitrogen from nitrogen-fixing bacteria.
Both labeled and natural isotope samples were analyzed in the Analytical Chemistry Lab in the Odum School of Ecology at the University of Georgia.

Although Jobos Bay is an estuary, the river flowing into the bay is dry most of the time (called Río Seco, which means “dry river”) and groundwater discharge is the largest source of freshwater for the Bay (Dieppa et al. 2008). In addition, the Cayos Caribe islands where the experiment took place are 3.5 km offshore and are flushed by seawater pushed through the island canals by winds from the south. To verify that these conditions provided us with a location where our treatment would not be swamped by high ambient nutrient levels, we took 15 cm deep porewater samples before the experiment started. Samples were collected following the procedure in Lee et al. (2008) and were analyzed for porewater nitrogen concentrations in the Joye lab in the Marine Sciences Department at the University of Georgia. Ambient nutrient levels were extremely low for all nitrogen species in the mangrove peat around our experimental trees (e.g., porewater NH$_4$ = 4.20 ± 2.51 µM, mean and standard deviation, n = 48). This is comparable to other fringing mangrove zones located throughout the Caribbean and used as control sites in other nutrient enrichment experiments (e.g. Feller et al. 2003; Lovelock et al. 2005).

We tracked the trees’ morphological responses to treatments by monitoring: 1) the canopy, 2) peat-based terrestrial roots, and 3) marine aerial roots. All replicate samples discussed below were pooled at the tree level for analysis.

We measured the mass, area, length, and width of 30 leaves collected from each tree at the end of the experiment. *R. mangle* leaves are arranged in a clump at the end of each branch. The tree adds leaves to the branch tip (the outermost part of the clump away from the trunk) in pairs as leaves at the back of the clump (toward the tree trunk) die and fall from the tree. We
haphazardly chose three clumps on every tree and determined the number of new leaves produced in those clumps during the last year of this experiment (from 6 - 18 months). We used zip ties around branch tips to mark the leaves furthest from the trunk on the end of the branch, then counted the number of new leaves grown in front of our zip tie markers (away from the tree trunk) during the next sampling period. Monitoring leaf production elucidated the number of clumps that divided during our experiment, from one clump and therefore one leaf production point to more than one. We also quantified the total number of leaves at the end of the experiment in three additional haphazardly chosen clumps.

To monitor terrestrial root growth, we took three 30 cm cores, each 15 cm from where a root from an experimental tree entered the peat. Cores were located on the opposite side of each experimental root from our treatment cores described above (used to fertilize the tree or as a fertilizer procedural control). Core holes were filled in 2010 by peat taken from nearby islands and then dried in the sun for > 72 hours to kill any live root tissue. This “blank” peat was recovered in May 2012, at the conclusion of the experiment. As with our fertilizing cores, we marked blank core locations with PVC guides in order to accurately recover only new roots produced during this experiment. Recovered peat was broken up in a tub of water and live root tissue, which floats, was collected, dried, and weighed. This approach allowed us to quantify subterranean root growth that occurred solely during the course of the experiment (Symbula & Day Jr. 1988).

We had intended to measure growth in aboveground terrestrial roots as well, but tracking every terrestrial root associated with a single target tree proved to be infeasible. Individual roots growing in the tangled understory often fused or grew knots where they touched each other, making it impossible to determine in the field exactly which roots belonged to a target tree.
Complex root architecture in the understory also obscured new root tips, hindering photographic efforts to assess aboveground growth.

All aerial roots growing toward or into the water without any portion anchored in the peat were mapped in November 2010 (Fig. 3.2). Our measurements included aerial roots that terminated above the water in order to capture the response of all marine roots to our treatments, including roots that were destined to reach the water eventually (not just those that were already long enough to have done so). Mangrove roots elongate from the root tips only, so we tagged each live tip at the beginning of the experiment. Tagging tips rather than measuring entire root lengths greatly reduced measurement error since measured lengths were shorter and more manageable than they would have been otherwise. Root elongation and proliferation were measured every six months.

It is common for *Rhizophora mangle* root tips to die back from a variety of causes, then regrow from lateral meristems further up the root (as described in Gill & Tomlinson 1977). Seventeen percent of marine aerial roots terminating above the water had at least 1 point below which the root split (called a “junction” hereafter; Fig. 3.2), as did 100% of roots with the majority of their length below the water line. The average number of junctions on marine aerial roots was 0.2 for above-water roots and 6.7 in underwater roots. This constant growth, dieback, and splitting tend to produce very complex roots that may grow over time without ever getting closer to the bottom. None of the 199 roots identified as marine in the beginning of this experiment were touching the seafloor. Only 1 of these roots had grown into the sediment by the end of our experiment and this was because the root bifurcated so that one of the segments grew back toward the island edge and anchored into the peat there. So to quantify net growth including
elongation, dieback, and splitting, we harvested roots in May 2012 and determined the biomass grown from where root tips had been in November 2010, at the start of the experiment.

Long-term biomass trends will be determined not just from biomass gains on existing roots, however, but also from new root creation and from biomass losses. Mapping all marine aerial roots on all experimental trees in 2010 allowed us to determine how many new roots grew through the course of this experiment. We were unable to accurately quantify biomass lost if a root tip died back past its starting point (measured in November 2010) because tags marking root tips were lost when tips disappeared and we had no quantitative length or biomass information about the root above these tags. In these cases, we assumed a biomass change of 0 g. We classified root tips at the end of our 18 month experiment as: 1) retained (including moderate dieback and splitting- if a root tip died back and then split into two live tips, it was included in this count), 2) lost (dieback past the junction above the root tip), or new (created during the course of this experiment, growing either from the canopy as the beginning of a new root or laterally from an existing root).

Biomass data per marine aerial root tip were averaged at the tree level. We discuss average biomass change per tip rather than total biomass produced per tree in order to include the 0 g measurements from lost root tips. We calculated biomass changes in the average tree for each treatment to provide context for our per-tip data. The average tree in this experiment had 10 root tips in November 2010. Where \( x \) is the average biomass change per \( a \) percent of root tips that were retained through the course of this experiment, \( y \) is the average biomass change per \( b \) percent of root tips that were created during this experiment, and 0 g is our assumed biomass change per \( c \) percent of root tips that were lost:
(x g × 10(a/100)) + (y g × 10(b/100)) + (0 g × 10(c/100))

yields total biomass production for the average tree in each treatment during the course of this 18 month experiment.

Differences in tree growth metrics and isotopic signatures between control and fertilized trees were analyzed using t-tests when data were normally distributed and generalized linear models when they were not. In cases where a generalized linear model was appropriate, data had an overdispersed Poisson distribution (a negative binomial distribution), so a log link was used in our generalized linear models.

Results

Average per tree leaf morphology (area, length, width, and biomass) did not differ according to our treatments, although labeled fertilizer trials and ambient leaf isotopic signatures showed that leaves and to a lesser (non-significant) extent twigs received nutrients from peat-based fertilizer (Table 3.1 and Table S3.1). Leaves from fertilized trees also had a slightly higher percent N than leaves from control trees, but this difference was not significant (Table 3.1).

Each Rhizophora mangle branch ends in 1 clump of leaves. The average number of leaves in each clump per tree was not significantly different according to treatment, nor did the tree-level average number of new leaves produced per clump differ between treatments (Table S3.2). However, there was a difference in the number of branch tips and therefore leaf clumps produced per tree between our two treatments. An average of only 6% of clumps monitored per
control tree became more than one clump as the tree grew. The branches that divided split into an average of 2.5 ± 0.5 (standard error) clumps where there was formerly only one clump. The 6% of branches on control trees that divided was significantly different (neg. bin. GLM, $\theta = 2.04$, $Z_{20}^{DF} = 2.28$, estimate = 2.0, $P < 0.05$) from the 35% of clumps on fertilized trees on average that divided into 3.1 ± 0.3 clumps.

Terrestrial roots growing into our “blank” peat cores had more depleted average $\delta^{15}$N signatures, indicating nitrogen uptake from our urea fertilizer (Table S3.3). However, the average per tree biomass of mangrove material in these cores did not differ between treatments (Table S3.4).

Trees outside the experiment fertilized with labeled nitrogen echo the canopy and terrestrial root trends discussed so far (Fig. 3.3). The canopy and leaves, specifically, had the most enriched $\delta^{15}$N signatures, indicating that more urea-derived nitrogen was put into leaves than anywhere else sampled on the trees. Belowground roots and peat, meanwhile, had extremely low $\delta^{15}$N signatures that imply minimal investment of urea-derived nitrogen in these areas.

Biomass added to existing marine root tips averaged per tree was significantly different according to treatment (neg. bin. GLM, $\theta = 2.68$, $Z_{14}^{DF} = -2.75$, $P < 0.01$). Control trees added more than twice as much biomass to the average marine aerial root tip over the course of the experiment than fertilized trees (41.5 ± 5.3 g and 16.1 ± 4.4 g, respectively, with standard errors; Fig. 3.4).

An average of 33.6% of tags per tree were lost during the course of this experiment due to root dieback above the tags we used to monitor growth (Fig. 3.2). This proportion was very similar in control and fertilized trees, in which we lost 34.6% and 32.6% of tags, respectively. Control trees retained an average of 56.8% of root tips (ends of roots did not die back above the
tags we installed at the beginning of the experiment) and had only 9.6% new tips (created from the canopy and growing toward the water or appearing above tags on existing roots during the course of this experiment). Fertilized trees, on the other hand, had an average of 37.4% retained marine root tips and 30.0% new tips, indicating a higher marine root tip turnover rate. To provide context for these percentages and the per tip biomass data above, we calculated marine aerial root biomass production for the average control and fertilized tree (see the Methods section for calculation details). An average control tree with 10 marine aerial mangrove root tips at the beginning of this experiment would have gained 270 g during the course of this experiment, while the average 10-tip tree receiving nutrient additions would have gained only 128 g of marine aerial root biomass:

Control tree: \((42.2 \times 10^{(56.8/100)}) + (36.0 \times 10^{(9.6/100)}) + (0) = 274.3 \text{ g gain}\)

Fertilized tree: \((17.8 \times 10^{(37.4/100)}) + (20.5 \times 10^{(30.0/100)}) + (0) = 128.1 \text{ g gain}\)

**Discussion**

Our fertilization methods effectively boosted nitrogen available to targeted *Rhizophora mangle* trees. While isotopic signatures confirmed that our experimental trees took up this nitrogen, the nitrogen additions did not affect terrestrial root growth or leaf morphology during the course of this experiment. However, 1.5 years of nitrogen additions did produce trees that increased the number of leaf clumps and therefore leaf production points and decreased marine aerial root biomass production.
We expected that trees would allocate growth resources in order to increase their terrestrial growth when fertilized, perhaps at the expense of marine aerial root growth. Red mangroves are known to undergo dramatic transformations, even abandoning their main trunk, to grow toward favorable growth conditions (e.g. Ellison and Farnsworth 1996). Marine aerial roots did not grow into the sediment during this experiment and therefore could not take up nutrients for the tree. But why didn’t trees increase terrestrial root production when they received extra nutrients?

An increased number of leaves on a tree may increase the tree’s carbon fixation through photosynthesis. Previous studies have also shown that nutrient additions can cause mangrove trees to prioritize canopy growth (e.g. Breaux et al. 1995; Feller 1996; Wickramasinghe et al. 2009). \textit{R. mangle}’s documented morphological plasticity allows trees to grow toward conditions that increase photosynthesizing opportunities specifically (Farnsworth & Ellison 1996b). Other trees are also known to favor canopy growth over root growth with enhanced nutrient availability (Reich 2002 and references therein; but see variation as described in McCarthy & Enquist 2007). Fertilized trees in our experiment may have prioritized increasing carbon fixation opportunities rather than investing in growth toward the source of nutrients because it may not be necessary to grow more or bigger roots simply for nutrient transfer.

It is also possible that we may not have fully captured subterranean root growth. Our cores were placed on the opposite side of target roots from fertilizer in order to capture growth from our specific trees of interest, but these trees may have grown an abundance of roots toward the fertilizer plugs instead of increasing subterranean root growth in all directions.

Previous studies of mangrove responses to nutrient enrichment have shown (like this study) that additional nutrients increase mangrove growth rates (Corredor & Morell 1994; Feller
et al. 2009 and many additional similar references). This has led to the recommendation that mangroves could be substituted for or supplement inadequate municipal water treatment facilities in poor communities (Breaux et al. 1995; Wong et al. 1997; Wu et al. 2008; Wickramasinghe et al. 2009), since a major source of eutrophication is terrestrial runoff containing sewage and agricultural wastewaters (Nixon 1995; Camargo & Alonso 2006; Purvaja et al. 2008).

We show that when fringing, nitrogen limited R. mangle receive increased nitrogen inputs, they alter relative resource allocation to favor leaf production and reduce marine aerial root production. This means that over time, fringing R. mangle in nitrogen polluted areas may produce less marine habitat. Mangroves serve as nursery grounds for commercially important species such as crabs, shrimp, and fish (Nagelkerken et al. 2008), some of which are associated with seagrasses and coral reefs as adults (Kathiresan & Bingham 2001b; Mumby 2006). Subtidal R. mangle aerial roots increase fish densities and impact fish and benthic community composition (Schutte et al. [in submission by the time this manuscript is ready to go?], MacDonald and Wise 2013). Conservation strategies that call only for Caribbean mangroves to be used in wastewater treatment may produce faster growing trees, but if these are the only areas where mangroves are protected then marine mangrove habitat production may decline over time. A holistic approach that considers both the terrestrial and marine habitat produced by fringing R. mangle may be necessary to best protect both this habitat-creator and the communities dependent on its structure.
Literature Cited


Figure 3.1. Typical fringing Rhizophora mangle habitat in the Caribbean. A) Mangrove growth under normal conditions (without added nutrients) shown with two arrows each for growth in the canopy, in terrestrial roots, and in marine aerial roots. B) Resulting growth from experimentally increasing available nitrogen. Arrow sizes and colors are indicative of relative effects: large black arrows show increased canopy growth while smaller, white arrows show decreased marine aerial root growth. Diagram made with symbols courtesy of the Integration and Application Network, University of Maryland Center for Environmental Science (ian.umces.edu/symbols/).
Figure 3.2. Example root maps for two roots on tree number 11. Maps show relative placement of junctions, root tips, and tags used to monitor root growth and proliferation. “D” denotes root segments that were present but dead at the start of the experiment.
Figure 3.3. $^{15}$N signatures from trees outside our experiment that received $^{15}$N-labeled fertilizer. This labeled nitrogen allowed us to trace the path of nutrients taken up by the tree. More enriched $^{15}$N signatures identify the areas of the tree that received more nitrogen fertilizer.
Figure 3.4. Tree-level average *Rhizophora mangle* marine aerial root biomass production per root tip (with standard error) over the course of our 18-month experiment.
Table 3.1. Average canopy $\delta^{15}$N signatures and standard errors per tree pooled from 30 leaves and 3 twigs collected per tree. Leaf $\delta^{15}$N signatures from fertilized trees are significantly depleted (and closer to the $\delta^{15}$N signature of our urea fertilizer) than those from control trees (t-test, $t = -7.12$, DF = 16, $P < 0.01$) but twig $\delta^{15}$N signatures and leaf % N are not significantly different according to treatment.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Leaf $\delta^{15}$N (%)</th>
<th>Twig $\delta^{15}$N (%)</th>
<th>Leaf % N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.16 ± 0.20</td>
<td>-0.22 ± 1.26</td>
<td>73 ± 0.03</td>
</tr>
<tr>
<td>Fertilizer addition</td>
<td>-1.75 ± 0.21</td>
<td>1.06 ± 0.47</td>
<td>86 ± 0.04</td>
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</tbody>
</table>
CHAPTER 4

HIERARCHICAL ENGINEERING IN SUBTIDAL CARIBBEAN MANGROVE ECOSYSTEMS

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3 Schutte, V.G. and J.E. Byers. To be submitted to *Ecology.*
Abstract

Ecosystem engineers modify their environment, often creating unique habitat. The subtidal ecosystem created by the red mangrove, *Rhizophora mangle*, is engineered by the tree’s aerial roots that grow into the water. Epibionts that live on those roots act as secondary engineers— they are dependent on roots for substrate but as they grow, they significantly modify root structure. We experimentally manipulated the density and presence of *R. mangle* roots and epibionts in Bocas del Toro, Panama. We reduced roots and their epibionts by half, completely cleared plots of all subtidal roots and epibionts, and removed only subtidal epibionts while leaving roots intact. Root reductions and epibiont removal treatments shifted fish community composition, while complete root removal drastically reduced fish abundance and diversity. Larger plots had higher fish abundances and higher variation in community composition (plot volume varied naturally with mangrove canopy width and with depth to the seafloor), though plot volume was not correlated with the amount of root and epibiont habitat but rather with the amount of total space beneath the mangrove canopy. Benthic communities on the seafloor were primarily composed of brittle stars and sea stars, and also differed significantly among our treatments. Reductions in benthic individual abundances tracked experimental reductions in epibionts. Coupled with the observation that these echinoderms swarmed sponges and other material that fell to the seafloor from the mangrove roots above, these results suggest that epibionts are a source of food fall that attracts these benthic species. *R. mangle* creates key tropical marine habitat, supporting other marine ecosystems and fisheries. Epibionts tripled root width on average and account for two thirds of underwater biomass in our experimental location, where they shaped the spatial dimensions of habitat and, thus, habitat use. The influence of both
primary and secondary engineers on habitat structure and use in this system underscores the importance of understanding how engineers interact in hierarchically engineered systems.

**Key words:** foundation species; community structure; abundance; biodiversity; habitat; mangrove; fish; Bocas del Toro
Introduction

Ecosystem engineers shape the environment and resource availability (Jones et al. 1994). Engineers are inherently important because changes to the services they provide will affect communities associated with that engineered environment (Jones et al. 1997; Coleman & Williams 2002). Understanding how changes affecting these physical engineers consequently propagate throughout an ecosystem is essential to creating effective management strategies and assessing mechanistic controls on ecosystem functioning. Experimental linkages between engineered structure and ecosystem characteristics are becoming more common (e.g. Roberts & Ormond 1987; Skilleter & Warren 2000; Taylor et al. 2008), but we are just beginning to understand how secondary engineers impact ecosystems.

Secondary engineers are dependent on habitat created by primary engineers but add to and/or modify primary engineering effects. When these engineering effects increase resident community diversity, they are sometimes called facilitation cascades (e.g. Altieri et al. 2007; Altieri et al. 2010; Thomsen et al. 2010). For example, black mangroves in Australia are primary engineers because they create habitat structure. Algae that anchor themselves to black mangroves’ pneumatophore pencil roots are dependent on those roots for substrate but modify habitat structure, resulting in a higher density of mollusks (Bishop et al. 2012; Bishop et al. 2013). In this case, algae are secondary engineers and they are part of a facilitation cascade. Although it may be easy to list organisms that qualify as secondary engineers, there are only a handful of studies that quantify or even formally examine the influence of such organisms through an engineering lens. We do not yet have much information on their ecosystem-wide
influence, nor can we separate the conservation value of many of these engineers from the primary engineers they depend on.

The red mangrove, *Rhizophora mangle*, is a primary engineer at the foundation of a hierarchically engineered system. *R. mangle* grows on coastlines in the Caribbean, where its subtidal aerial roots provide the structural basis for a unique marine habitat (Fig. 4.1). Among the residents of this habitat are subtidal root-dwelling sponges, tunicates, bivalves, and other organisms (referred to generally as “epibionts” hereafter; Nagelkerken *et al.* 2008), referred to generally as “epibionts” hereafter. Mangrove epibionts are secondary engineers. They are almost completely dependent upon roots, which are nearly always the only hard substrate in an otherwise soft-bottom habitat. At the same time, root epibionts have a variety of morphologies that impact root habitat structure by increasing habitat surface area, rugosity, and structural heterogeneity.

Mangroves create habitat for commercially important species including crabs, shrimp, and fish, many of which live in seagrasses and coral reefs as adults (Kathiresan & Bingham 2001b; Mumby 2006; Nagelkerken *et al.* 2008). Many mangrove conservation and preservation programs are based on the premise that robust mangrove ecosystems are an essential component of a healthy tropical marine landscape. But, surprisingly, there is very little direct, experimental evidence elaborating on which elements of subtidal mangrove habitat are essential to maintaining habitat value. Most studies with similar objectives have been observational (e.g. Morrisey *et al.* 2003), have mimicked red mangrove structure instead of using the mangroves themselves (MacDonald *et al.* 2008), or they have experimentally demonstrated the effect of mangrove habitat on the abundance of a limited number of attendant species (e.g. Skilleter & Warren 2000; but see MacDonald & Weis 2013). In addition, most of the direct linkages made between
subtidal mangrove habitat and fishery stocks are from the Indo-Pacific (Manson et al. 2005), where different tidal regimes and mangrove morphologies support root epibiont communities that are less species rich and contribute less subtidal biomass than those in the Caribbean.

We conducted a field experiment in subtidal Caribbean red mangrove ecosystems. We independently manipulated the density of primary mangrove engineers and the presence/absence of secondary epibiont engineers. We hypothesized that root and epibiont structural reductions would change physical ecosystem characteristics and affect subtidal marine habitat quality. We further predicted that the effects of root and epibiont reductions would be similar and additive.

**Methods**

**Experiment details**

We conducted an experiment to address these hypotheses in Bocas del Toro, Panama, which has an abundance of small mangrove islands and undeveloped coastline where subtidal *Rhizophora mangle* roots provide the substrate for a dense and diverse epibiont community (Collin et al. 2005; Rocha et al. 2005; Wulff 2009). The average canopy extension over the water (measured perpendicular to the island edge; Fig. 4.1) where we set up our experiment was 2.3 m and average water depth at mean tide was 1.4 m. There was an average of 2.1 roots entering the water per m² of the water’s surface underneath the mangrove canopy.

We manipulated root and epibiont habitat complexity and measured how the ecosystem responded. We set up 48 plots in the water beneath the canopy of *R. mangle* forests fringing the coastlines of small mangrove islands. Half (24) of the plots were located around small islands just south of Isla Bastimentos (9.24563, -82.23880) and half (24) were located in a channel.
connecting Bocatorito to Bahía Almirante (9.26700, -82.17673). Plots were selected to have similar characteristics (we primarily considered exposure to wind and wave action, structure of mangrove peat, and subtidal root presence). Plots were 20 m in length as measured along island edges and included the subtidal ecosystem from the peat bank to the edge of the mangrove canopy (Fig. 4.1). Plots had naturally varying canopy widths (0.8 to 3.85 m) and depths (1.0 to 2.0 m), resulting in a range of plot volumes from 22.4 m$^3$ to 101.8 m$^3$ (60.8 ± 19.4 m$^3$, average and standard deviation). We assigned one of four treatments to each of the 24 plots in each of the 2 blocks (n = 12) using a stratified random scheme to ensure that treatment assignments were systematically interspersed within each block. These treatments were: 1) a control treatment in which no changes were made, 2) a root reduction treatment in which approximately half of the subtidal portions of roots (and their epibionts) were removed from a plot, 3) a root removal treatment in which all subtidal portions of R. mangle roots (and their epibionts) were cut away from a plot, and 4) an epibiont removal treatment in which as many root-dwelling organisms were removed from a plot as possible while leaving R. mangle roots intact.

The root reduction treatment was made by pairing roots based on their subtidal biomass including epibionts (e.g., a long root made structurally complex by large epibionts was not considered equal to a long, structurally simple root or a short, structurally complex root). One root in each pair was haphazardly selected to be removed from the plot. For the epibiont removal treatment, we used short-bladed hand saws to shave off the epibionts from roots, including sponges, tunicates, and bivalves. We made every effort to leave mangrove roots intact, carefully allowing roots to retain their original architecture. We were able to remove all but a small amount of epibionts on the roots, but the thin film of sponge tissue and occasional bivalves remaining on roots contributed very little to remaining structure. All removed root and epibiont
biomass was transported away from plots so it would not interfere with this experiment. We measured width every 10 cm along the entire subtidal root length of 20 intact roots from control plots and 20 shaved roots from epibiont removal plots to quantify epibiont contributions to subtidal architecture. To quantify the underwater biomass of epibionts versus their mangrove root habitat, we also haphazardly selected 44 roots located outside our experimental plots to be removed from the field and weighed them before and after epibiont removal.

We conducted post-manipulation surveys in all plots to evaluate ecosystem responses. This experiment was set up in late June 2011 and evaluated 1-year later in June and July 2012. This timeline allowed us to quantify persistent effects evident over a longer timescale than those possibly due to disturbance from setting up this experiment.

**Physical responses**

To assess how structure influences physical ecosystem traits in subtidal Caribbean mangrove ecosystems, we measured a number of descriptive ecosystem elements. We gathered data on irradiance, oxygen, temperature, salinity, turbidity, and wave energy in each plot. These data had overdispersed Poisson distributions and so were fit with a negative binomial generalized linear model (GLM) with a log link to evaluate statistical significance of predictor variables. These and all other statistical procedures described below were carried out in R version 3.1.0 (R Core Team 2014). All measurements collected over multiple days were averaged at the plot level for statistical analysis.

*Irradiance* (specifically, photosynthetically active radiation) was measured on five nonconsecutive days using a Li-Cor LI-193 Underwater Spherical Quantum Sensor. Irradiance measurements were averaged over 15 seconds. We measured irradiance just above the water’s
surface and at 50 cm below the water’s surface both in the center of each plot and just outside the canopy’s shadow. We used these four measurements to compare the percentage of ambient irradiance reaching the underwater portion of our plots (rather than absolute irradiance) so that variable cloud cover, canopy shade, and turbidity would not affect our analyses. We calculated the treatment effect (the influence of subtidal root and epibiont structure) on irradiance using the following formula:

$$\left(1 - \frac{c}{(a/b) \times d}\right) \times 100$$

where $a$ is irradiance above the water in the center of each plot, $b$ is irradiance above the water outside the canopy, $c$ is irradiance below the water in the center of each plot, and $d$ is irradiance below the water outside the canopy. Multiplying by 100 transforms this number from a proportion of change into a percentage of change. We also examined the effect of the canopy on irradiance (as the percent reaching the water’s surface under the mangrove canopy: $a/b \times 100$).

Before irradiance was statistically analyzed, we removed slightly negative numbers from the dataset (caused by converting irradiance values into percentages using the formula above) by adding 100 to the treatment effect percentages calculated using the formula above.

*Oxygen, temperature, and salinity* levels in the water were measured on each of three nonconsecutive days using a YSI Pro2030 handheld meter. We collected all measurements from 50 cm below the water’s surface in the center of each plot.

*Turbidity* of the water in each plot was measured on each of three days by collecting 1.5 L of water from 50 cm below the surface in the center of each plot. This water was filtered onto a pre-weighed and combusted Whatman GF/F 0.7 µm glass fiber filter, rinsed to remove salts, then
dried and re-weighed to determine the mass of total suspended solids in each sample. Each filter was then combusted in a muffle furnace at 500 °C for 12 h and weighed again to quantify suspended organic matter.

*Wave energy* was measured with dissolution blocks and erosion markers. Dissolution blocks were made using Die-Keen Type V dental plaster poured into a mold to have a cylindrical shape. The back and sides of the disc were painted with polyeurethane to ensure that weight loss due to dissolution would be linear over time. These cylinders were weighed and then hung for 58 days on the peat bank 50 cm below mean low tide in the center of each plot so that the exposed surface was vertical and parallel to the island’s edge. After collection, the discs were weighed again to determine total weight loss per plot.

To quantify erosion, stakes were driven into the peat bank horizontally 50 cm below mean low tide and also vertically on the seafloor beneath the center of each plot. Erosion was tracked by monitoring the height of the stakes over time.

**Biological responses**

To evaluate how structure influences habitat use in subtidal mangroves, we measured several attributes of the biological community. We gathered data on benthic and water column chlorophyll *a* levels, benthic organisms, and free-swimming species in each plot. All statistical procedures described below were carried out in R version 3.1.0 (R Core Team 2014). All measurements collected over multiple days were averaged at the plot level for statistical analysis.

*Chlorophyll* *a* concentrations in the water column were measured on each of three days by collecting 1.5 L of water from 50 cm below the surface in the center of each plot. This water was filtered onto a rinsed Whatman GF/F 0.7 µm glass fiber filter which was then submersed in
90% acetone and ground or sonicated to extract chlorophyll \( a \). The filter solution was allowed to steep for 24 h in the dark. After this period, centrifuging was used to separate pieces of the filter from the supernatant in ground samples. Chlorophyll \( a \) in solution was then measured using the nonacidification fluorometric method (Arar & Collins 1997; Collin et al. 2009).

Benthic chlorophyll \( a \) was measured by placing 3 2.5 cm x 2.5 cm uniformly-shaped slate tiles horizontally on a stake approximately 10 cm above the sediment’s surface (to avoid interference from benthic organism movement and sedimentation) in the center of each plot. Tiles were collected after 60 days in the field and were transported in and then scrubbed and rinsed with 90% acetone. Chlorophyll \( a \) in the resulting solution was then measured with a fluorometer.

We evaluated the influence of ecosystem components on chlorophyll \( a \) by fitting generalized linear models (GLMs) to the data. These data were Poisson distributed and overdispersed and so were fitted with a negative binomial GLM with a log link.

*Benthic community* biodiversity was assessed on each of three days and at the same time as seafloor percent coverage. We haphazardly threw a 25 cm x 25 cm \((0.0625 \text{ m}^2)\) quadrat 3 times in non-overlapping areas in the center of each plot to determine benthic biodiversity for both mobile and sessile organisms. Percent cover was estimated to the nearest 5%. We conducted these analyses during the day and at night, but will only discuss nocturnal results here. Most benthic organisms were much more active during the night than during the day so our nocturnal census numbers more accurately reflect habitat usage trends than the results of our diurnal benthic assemblage surveys.

*Free-swimming species* were evaluated using video transects recorded on each of three days. We approached the edge of each plot slowly to avoid disturbing the organisms there. Then
we swam just outside the edge of the canopy, recording all species visible in our study area and making sure to swim the length of each plot in the same amount of time (1.5 min). Videos were later analyzed to identify organisms to species using primarily Humann and DeLoach (2002a, b). We did not conduct free-swimming surveys at night because the number of species seen in the water column at night was extremely low (Hammerschlag & Serafy 2010) and surveys were logistically difficult to perform.

We used ordination (Pielou 1984; ter Braak 1987) to describe benthic and free-swimming communities surveyed in our plots. We took this approach in order to holistically evaluate community composition and statistically identify influential community members and independent variables. Ordination visually clusters samples (our 48 plots, in this case) according to similarity: the more similar one sample is to another, the closer they are together in the resultant ordination graph. Samples are plotted in n-dimensional space and then projected in 2 dimensions. When analyzing communities, the number of axes is usually the same as the number of species minus 1 or the number of samples minus 1, whichever is lower. The x axis in this 2-dimensional projection maximizes variation among samples and the y axis maximizes variation uncorrelated with the x axis. Species that are most correlated with these “first” axes are the ones that have the most influence on community position in the graph.

We first visualized our data using principal components analysis (PCA), which mapped our plots based solely on community composition data. We then used redundancy analysis (RDA), the constrained form of PCA, to classify species occurrences as dependent data. This type of analysis is called “constrained ordination” because it yields projections with axes constrained as a function of measured explanatory factors. Rather than generating plot location scores to maximize variance explained, as PCA does, RDA evaluates only the portion of
variation explained by a matrix of independent variables (which included factors like treatment and plot depth; Table S4.1) and maximizes the correlation between dependent and independent variable locations.

All ordination analyses were performed using the R package vegan (designed to perform ordination and community diversity analyses for ecologists; Oksanen et al. 2013) and all permutation functions were performed with 9999 permutations. We evaluated the influence of constraining (independent) variables using a permutation function. The model as a whole and individual model terms were considered significant if they explained more variation than that explained by at least 95% of random data permutations.

We also fit GLMs to the abundances of benthic and free-swimming individuals to better understand how significant independent variables influenced communities. Abundances were Poisson distributed but were overdispersed, so we used negative binomial GLMs with a log link to model these count data.

**Results**

Epibionts increased root widths from just 2.6 ± 1.6 cm to 6.8 ± 5.1 cm (averages ± standard deviations). Root epibionts account for 68% of subtidal marine mangrove habitat biomass, while roots make up the remaining 32% of subtidal biomass.
Physical responses
The descriptive ecosystem elements that we measured were not influenced by our treatments.

Irradiance was reduced an average of 20.3% ± 8.1% (mean and standard deviation) by the shade of the mangrove canopy. Removing biomass in root reduction and epibiont removal treatments decreased irradiance by 3.0% ± 30.6% and 2.4% ± 44.4%, respectively. This does not indicate a significant decrease in irradiance, however, it rather demonstrates the huge variability in irradiance both between and within plots. In contrast, our root removal treatment increased irradiance by an average of 27.8% ± 38.0%. None of these changes were significant, although irradiance was much higher in plots with roots removed.

The absolute differences in irradiance with root removal were low, however. Irradiance in the control treatment was an average of 201.6 µmol s\(^{-1}\) m\(^{-2}\) at 50 cm below the surface in the center of each plot, while plots in our root removal treatment had average irradiance of 264.3 µmol s\(^{-1}\) m\(^{-2}\). This difference between control and root removal plots pales in comparison to the variation seen in a single plot. For example, the highest measured irradiance in any plot was 1572 µmol s\(^{-1}\) m\(^{-2}\). This same plot, on a different day and at a different time, yielded an irradiance measurement of just 54.5 µmol s\(^{-1}\) m\(^{-2}\).

Oxygen, temperature, and salinity and turbidity did not vary according to our treatments (Tables S4.2 and S4.3) and was non-significantly associated with the within-block location of the plot being measured (Fig. S4.1). On average (with standard deviations), our plots were at 83.0 ± 10.2% oxygen saturation (5.3 ± 0.6 mg/L), 29.4 ± 0.8 °C, and had a salinity of 31.1 ± 0.4. Total suspended solids averaged 16.3 ± 19.2 mg/L and an average of 46.3 ± 30.9% of that mass was suspended organic matter.
Wave energy showed no significant trends according to treatment (Table S4.4) or within-block location. Long-term dissolution blocks showed an average loss of $8.1 \pm 0.7$ g/d. Very small changes in erosion marker height were always within the resolution associated with this method—no change was detected in the position of the island edge or in sediment height beneath the canopy.

Biological responses

In contrast, several of the biological responses we measured were affected by our structural manipulations.

*Benthic* community surveys were dominated by brittle stars, which accounted for 81.4% of the individuals we observed (Table S4.5). Maximum taxonomic richness in the 0.1875 m² area sampled from a single plot was 6 species, and brittle stars together with tunicates and sea stars comprised 92.2% of all benthic individuals surveyed. Our principal components analysis (PCA) confirmed that these few taxonomic groups dominate benthic communities. Brittle stars accounted for 96.6% of the total variance in our PCA and brittle stars and sea stars correlated strongly with PC1 and PC2, respectively (Fig. S4.2).

Depth was the component of our constrained model most strongly correlated with RDA1 (Fig. S4.3). Plot depth, plot width, and the amount of mud in surveyed quadrats significantly influenced benthic community composition ($P < 0.01$ and for depth and width and $P < 0.05$ for mud).

We fit a number of generalized linear models (GLMs) and selected the model with the lowest Akaike Information Criterion score to evaluate the independent variables driving benthic individual abundances (Table S4.6). Plot depth once again was a significant predictor and our
treatments significantly influenced benthic abundances as well, along with the amount of shell rubble in the quadrat (neg. bin. GLM, $\theta = 3.18$, $Z = 3.64$, $P < 0.01$ for shell rubble). Epibiont removal significantly lowered benthic abundances ($Z = -2.45$, $P < 0.05$; Fig. 4.2) and increasing plot depth significantly increased abundances ($Z = 4.17$, $P < 0.01$, Fig. S4.4).

All benthic community analysis model results are summarized in Table S4.7.

*Free-swimming* communities consisted of 24 fish species, 2 cnidarians, and 1 ctenophore in 21 total genera (Table S4.8). The rest of this section will only consider fishes, which accounted for 98% of the 693 total free-swimming individuals observed. Only one individual was seen of each of 7 fish species while there were 361 snapper (*Lutjanus apodus* and *Lutjanus griseus*) sightings. We combined sightings from these two snapper species because they often school together and move through a plot quickly, making it very difficult to tell them apart. Five of the 24 fish species accounted for 84% of all individual occurrences and 11 species accounted for 96% of all occurrences. Average species richness per plot was $4.3 \pm 1.9$ (mean and standard deviation). Species richness ranged from 0 to 10 and did not vary according to our treatments (Table S4.9).

PCA revealed that higher abundance species most influenced fish community composition (Fig. S4.5): PC1 was highly correlated with snapper (*Lutjanus* spp.) counts and explained 64.5% of total variance and PC2 closely tracked striped parrotfish (*Scarus iserti*) counts and explained 20.3% of total variance.

Treatment and plot width were most correlated with RDA1 and RDA2. Fish community composition was significantly influenced by our treatments and plot width ($P < 0.01$ for both; Fig. 4.3). Fish communities in our control and root removal plots were very different from those in plots that received root and epibiont reductions, both in the species that most seem to
influence their communities and in the amount of interspecific variation seen in these plots. Wider plots had more unusual fish communities.

Because our treatments significantly influenced fish communities, we performed a second RDA, substituting quantitative habitat description variables for our treatment designations in the model call (Table S4.2). This model did not fit the data as well (only 34.0% of variance was explained by constrained axes rather than the 41.3% that was explained by our previous RDA model) but it did identify the number of roots in a plot as the most significant influence on fish community ordination scores (P < 0.05). None of the other quantitative measures of treatment significantly influenced fish communities.

We used a negative binomial GLM to consider the effects of independent predictor variables on total fish abundance. We tested a number of models based on a priori expectations of what would affect fish abundances. The model with the best fit based on Akaike Information Criterion scores (Table S4.10) included only plot volume as a predictor for individual fish abundances per plot per day (neg. bin. GLM, θ = 2.21, Z = 2.71, P < 0.01; Fig. 4.4), although other models that included predictor variables such as treatment fit the data almost equally well.

All results from the models use to analyze counts of free-swimming individuals in plots are summarized in Table S4.7.

Chlorophyll a did not vary according to our treatments (Table S4.11) and instead was loosely correlated with within-block plot location, like some of the water quality descriptors discussed above. The chlorophyll a concentration measured in the water column of the average plot was 0.73 ± 0.10 µg L\(^{-1}\) while chlorophyll a on deployed tiles was 4.66 ± 1.25 µg cm\(^{-2}\).
Discussion

Local-scale habitat characteristics affect free-swimming and benthic communities

Free-swimming communities were comprised almost entirely of fish. Larger-bodied schooling fishes like snappers and striped parrotfish were most commonly observed in our plots, while solitary fishes were more rare. Fish communities in root reduction and epibiont removal plots overlapped with one another, but overlapped little with control and root removal plots. Removal plots had remarkably reduced variation in fish community composition compared to plots in other treatments (Fig. 4.3). It is possible that epibiont loss specifically rather than biomass loss generally was behind the similarities between fish communities in root reduction and epibiont removal plots. Epibiont losses have been previously reported to be a significant influence on marine mangrove fish communities (MacDonald et al. 2008; Nagelkerken et al. 2010; MacDonald & Weis 2013). Foureye butterflyfish, for example, are territorial (Neudecker & Lobel 1982; Gore 1983; Tricas 1989) and were consistently seen lurking around the same epibionts in our plots before our treatments were installed. When these epibiont territories were removed as part of this experiment, the fish disappeared with them.

Plot width also significantly influenced fish community composition and fish abundances significantly increased with increasing plot volume. The influence of plot size on fish communities was also not surprising, as plot dimensions have also been previously reported to affect fish abundances (although the effect was due to depth in MacDonald & Weis 2013).

However, plot size effects are not comparable to a species-area curve when it comes to root habitat as plot volume has no significant relationship with the number of roots in a plot (Fig. S4.6). That is, rather than larger plots having more root and epibiont habitat available, they have
approximately the same root and epibiont habitat available but there is presumably more open space around and between roots.

Removing epibionts from roots significantly changes the size of the nearest refuge and the distance to it, perhaps mimicking the structural changes felt by root reductions. This may be why these two treatments behave similarly in our fish community composition and abundance analyses (Fig. 4.4). Fish seem to be aware not only of the plot space occupied by habitat structure, but also of the relationship between this positive space and the negative “open water” around this habitat (similar to Nagelkerken et al. 2010). The influence of root epibiont removal and plot size on fish communities is consistent with reports that mangrove epibionts are used for shelter by at least some fish (Nagelkerken et al. 2000; Laegdsgaard & Johnson 2001; Verweij et al. 2006; Nagelkerken & Faunce 2008).

*Benthic* communities consisted mostly of brittle stars, so brittle star behavior dictated community response to the local habitat. Brittle stars and sea stars are scavengers and generalists and we frequently observed them swarming and feeding on root-dwelling epibionts that had fallen to the seafloor beneath mangrove roots. Our epibiont removal treatment significantly reduced the abundance of benthic organisms in our plots, perhaps because their source of food fall was removed.

The mechanism by which depth impacts benthic communities is less clear. Individual abundances increased with increasing plot depth, but only when deeper than about 1.3 m (4. S4). Perhaps the seafloor in plots shallower than this threshold is disturbed more often than in deeper plots by physical forces (e.g. boat wake or high wind events that cause rough waves) that move food fall from roots. Or perhaps water quality changes below this depth threshold, such that
temperature or other seawater properties become more favorable for hosting higher abundances of benthic organisms.

**Larger-scale factors affect other ecosystem characteristics**

*Irradiance* increased, though not significantly, in our root removal treatment. Differences in irradiance between treatments were not significant because of the high variation associated with measuring irradiance.

*Benthic chlorophyll* likely did not respond to our treatments because differences in irradiance were not biologically significant. At the extremes of the sun’s daily course, a given point below the mangrove canopy is likely to be either completely shaded from the island shelf or exposed to sunlight coming under the mangrove roots at an angle (Fig. 4.1). We measured light only during the middle of the day to promote consistency in measurement conditions between plots. Thus, the high variation in our measured irradiance values is likely conservative compared to what a photosynthesizing organism may deal with on a daily basis, meaning that ample sunlight is probably available to autotrophs during at least some portion of the day.

In addition, even the lower irradiance treatments may be on average above the saturation irradiance point for common autotrophs. This is reinforced by our percent cover data: the average plot had 60.5% cover of *Caulerpa verticillata*. Other *Caulerpa* species reach saturation irradiance around 200 µmol s\(^{-1}\) m\(^{-2}\) (e.g. Li *et al.* 2009) and the average irradiance in our plots ranged from 201.6 µmol s\(^{-1}\) m\(^{-2}\) in our control plots to 264.3 µmol s\(^{-1}\) m\(^{-2}\) in our root removal treatment.

*Oxygen, temperature, and salinity; turbidity;* and *water column chlorophyll* did not trend with treatment over the spatial scale that this experiment was conducted. Wind and specific
plot locations combined to flush the water column regularly in some plots while the water in other plots stagnated, producing a visible thermocline and halocline. Thus, temperature and salinity changed very little from plot to plot within a single block except for a few abrupt changes based on shoreline configuration (Farnsworth & Ellison 1996a; Fig. S4.1). Readings within each of our two blocks regardless of treatment were much more similar to one another than readings between blocks. Any treatment effects on these physical variables or on chlorophyll $a$ concentrations in the water column were completely overwhelmed by the scale of ocean currents in Bocas del Toro.

Wave energy effects, on the other hand, were masked by the temporal scale of this experiment. We saw no trends in the rate of change in our dissolution blocks, even though they were deployed for almost 2 months. It is possible that a signal may have emerged with the passage of a particularly large storm if our plots covered more area, but this experiment was not set up to evaluate such large-scale effects. We saw no changes in seafloor height or island peat bank structure during this 1-year experiment either. Mangrove islands go through a known pattern of succession in which organic matter accumulates beneath the mangrove trees and is captured by roots, building an island (Carlton 1974). This pattern of succession operates over a much longer timescale than was measured in this experiment.

Conclusions

Marine mangrove habitat is created by primary engineers (mangroves) and is modified by secondary engineers (sponges, bivalves, tunicates, and other subtidal root epibionts). To date, we have very little quantitative information about the effect of habitat-creating secondary engineers
on communities and ecosystems. In this study, root habitat creation and the habitat modification orchestrated by root epibionts appears to influence fish community composition.

The most abundant fish at our plots were snapper (*Lutjanus* spp.), which accounted for 52.1% of all fish occurrences. These are adult schooling fish that often traveled through plots in a group. This schooling behavior may compensate for structural dependence, allowing snapper to use plots regardless of structural features. Volume more significantly influenced fish abundances than treatment (Table S4.10), perhaps because snapper and other abundant schooling species (Table S4.8) may prefer larger plots that allow their school more space. Community composition, on the other hand, was most significantly influenced by treatment than plot size. Because community composition is more sensitive to the presence of less common species than abundance counts, the significant influence of treatment indicates that less common species may have been responding to structural differences between plots.

Current *Rhizophora mangle* management and restoration programs generally focus on mangroves only and do not address subtidal secondary engineers at all. For example, planting *R. mangle* mangrove seedlings on sandy beaches is certainly better than no restoration efforts, but an intertidal beach location with no island dropoff (as in Fig. 4.1) will be unable to sustain a subtidal secondary engineering community because mangrove roots there will not be permanently submerged. A third of the world’s mangroves were lost in the last 50 years (Alongi 2002), and mangroves are currently declining at a rate of 1-2% each year (Ellison 2008). In addition, less than half of the *R. mangle* forests we have seen in the Caribbean have lush epibiotic communities on roots. When mangroves are in very shallow water and their roots touch the ground, sponge epibiotic communities cover significantly less root surface area and have significantly lower species diversity (unpublished data), providing much less complex habitat.
To maintain the critical role of mangroves as marine nursery habitat in the Caribbean (Nagelkerken et al. 2002), we must consider secondary engineers in addition to mangroves given their role in affecting community composition.

Secondary engineers dependent upon primary engineers for their own existence may play essential roles in determining habitat quality in other systems as well.

**Literature Cited**


Figure 4.1. **Left panel:** Fringing *Rhizophora mangle* tree and typical marine mangrove habitat. For simplicity, we have depicted only the most common subtidal organisms of interest to this study. We left gaps in epibiont coverage on roots to better convey root complexity. Diagram made with symbols courtesy of the Integration and Application Network, University of Maryland Center for Environmental Science (ian.umces.edu/symbols/). **Right panel:** Underwater in one of our plots. The peat bank is to the left, delineated by the vertical dark line near the left side of the photo. Subtidal portions of aerial roots hang into the water to the right of the peat bank, and terminate above the seafloor, which is shell rubble (white) and algae (green). Green on the peat bank is algae, blue and orange objects on roots are sponges, and tan at the top of the leftmost root
is tunicates. The root furthest to the right highlights the difference between root structure with and without epibionts: the topmost portion of the root is exposed and is visible as a nearly unbroken curve from the water’s surface until the orange sponge, which dramatically increases root width and rugosity (photo credit: VG Schutte).

**Figure 4.2.** Treatment effects (shown with standard error) on benthic individual abundances (average number of individuals seen per 3-quadrat survey). Epibiont removal significantly
lowered abundances compared to the control. Treatment groupings (a, ab, and b) were determined using a Tukey multiple comparison test on our GLM results.

Figure 4.3. Redundancy analysis indicated that treatments (left panel) and plot width (right panel) significantly affected fish community composition. Both species identity and the variation in fish species present were affected by treatment. Numbers are plotted at the centroid of plot scores for each treatment and ellipses show 95% confidence intervals around these centroids.
**Figure 4.4.** Left panel: Fish abundances increased with increasing plot volume. Right panel: Treatment effects (shown with standard error) on fish individual abundances. Root removal significantly lowered abundances compared to the control. Treatment groupings (a, ab, and b) were determined using a Tukey multiple comparison test on our GLM results.
CHAPTER 5

CONCLUSIONS

*Rhizophora mangle* is a dominant coastal foundation species in the tropical Caribbean, where it performs a critical role in creating marine nursery habitat. Many tropical coastal species use the mangroves at some point in their life, including species in valuable coastal fisheries that feed about a billion people worldwide. Yet mangroves are disappearing at about the same rate as rain forests and coral reefs, primarily because of coastal development.

Because this is an anthropogenic problem, it has an anthropogenic solution. Mangroves are relatively robust and because of their fast growth rates, are projected to keep up with climate change better than many other species. Accurately linking elements of marine *R. mangle* habitat to ecosystem services will assist managers in targeting mangroves for protection.

*R. mangle* trees have extremely plastic growth forms. This dissertation documents consequences of this plasticity. Chapter two describes how naturally occurring plasticity indirectly controls benthic communities, chapter three documents a driver that causes differential habitat provisioning, and chapter four links both habitat provisioning and benthic community presence to habitat use by marine fauna.

Mangrove forests have been proposed to be used as a cheap alternative to sewage wastewater treatment in the tropics. Their complex structure naturally filters water as it moves offshore, slowing currents and allowing materials to settle out of the water column and into the peat, where it fertilizes mangroves. But this research shows that not all mangrove forests may be
well suited to filling this need. If management plans prioritize conservation through wastewater treatment, this will allow *R. mangle* to thrive while reducing marine mangrove habitat.

This research also documents how root epibionts (including sponges as well as oysters, tunicates, and many other species), play an important role in determining marine *R. mangle* habitat use by fishes. Management groups across the Caribbean do not typically include root epibionts in conservation plans, but many fringing *R. mangle* stands in the Caribbean lack a rich root epibiont community, perhaps in part because they are in shallow water. This work shows a shift in sponge community composition and abundance when roots anchor into the seafloor. Mangrove forest restoration by planting seedlings in intertidal beach locations is common, but intertidal mangrove roots cannot support subtidal epibiont communities like those studied here. Subtidal communities must be considered in conservation efforts in order to preserve the ecosystem services that mangroves provide.

Ecology is increasingly moving toward quantitative studies with a holistic focus on an entire community or ecosystem. This research advances our understanding of the interactions between foundation species and their attendant communities, including a demonstration of higher-order species interactions influenced by an engineer. It also details how an anthropogenic factor leads to differential habitat creation in this same engineer. Finally, it shows that this habitat creation influences attendant community use of that habitat, but use is also influenced by habitat-modifying secondary engineers.
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Figure S2.1. Experiment setup schematic. The four mangrove roots are stabilized by rebar stakes on each end of the block and two strands of nylon string across the top. Three sponge pieces are secured to each of the mangrove roots.
Figure S2.2. Whole-root sponge communities constructed from total percent coverage on natural roots at the study site. (Suspended roots are 55% longer than grounded roots on average. Sponge species are listed in alphabetical order from 1 – 9. Dark gray shading highlights the species used in our experiment and light gray shading highlights the sponge species seen on both suspended and grounded roots.

Table S2.1. Whole-root biodiversity indices for sponge epibiont communities on grounded and suspended (55% longer than grounded) mangrove roots. Indices in the whole-root analysis were calculated using percent cover as a proportion of total subtidal root length covered by sponges on each type of root. $S$ = total species richness, $E$ = evenness, $H'$ = Shannon Diversity Index, $PS =$ Percent Similarity.
CHAPTER 3 APPENDIX:

SUPPLEMENTARY TABLES

Table S3.1. Leaf area, length, width, and biomass (dry weight) averaged at the tree level and shown with standard errors. Per tree data were averaged from 30 leaves collected from each tree.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Area (cm)</th>
<th>Length (cm)</th>
<th>Width (cm)</th>
<th>Mass (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>25.49 ± 3.11</td>
<td>8.54 ± 0.58</td>
<td>3.61 ± 0.23</td>
<td>0.63 ± 0.05</td>
</tr>
<tr>
<td>Fertilizer addition</td>
<td>27.75 ± 2.27</td>
<td>8.94 ± 0.32</td>
<td>3.79 ± 0.20</td>
<td>0.69 ± 0.04</td>
</tr>
</tbody>
</table>

Table S3.2. Tree-level averages (with standard errors) for the number leaves per leaf clump and the number of new leaves produced per leaf clump. Per tree data were averaged from 3 clumps monitored on each tree.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Leaves in clump</th>
<th>New leaves in clump</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>7.5 ± 0.5</td>
<td>2.6 ± 0.5</td>
</tr>
<tr>
<td>Fertilizer addition</td>
<td>7.4 ± 0.7</td>
<td>4.3 ± 2.3</td>
</tr>
</tbody>
</table>
Table S3.3. Per tree average belowground root nitrogen isotopic signatures and standard errors (averages were pooled from 3 cores taken per tree). Belowground root from fertilized trees are significantly depleted (and closer to the $\delta^{15}$N signature of our urea fertilizer) than those from control trees (t-test, $t = -2.32$, DF = 8, $P < 0.05$).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Root $\delta^{15}$N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>-0.14 ± 0.32</td>
</tr>
<tr>
<td>Fertilizer addition</td>
<td>-1.82 ± 0.54</td>
</tr>
</tbody>
</table>

Table S3.4. Average (with standard error) belowground root biomass per tree (mass is dry weight and percentages are wet weight relative to the wet weight of the entire core). Values were averaged from 3 cores taken per tree.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mass (g)</th>
<th>Portion of core mass (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.29 ± 0.19</td>
<td>2.73 ± 1.23</td>
</tr>
<tr>
<td>Fertilizer addition</td>
<td>1.58 ± 0.30</td>
<td>2.98 ± 0.75</td>
</tr>
</tbody>
</table>
Figure S4.1. Water quality features of plots were non-significantly correlated with plot locations. Plot temperature is shown here as an example. Plots 1 – 24 are in block one, but plots 1 – 8 are clustered together in one bay while plots 9 – 24 are in a different bay. The same is true
of plots 25 – 48, which are all in block two but are distributed along the shoreline in three distinct clusters. These within-block clusters are better predictors of water quality features than our treatments were (Tables S3 and S4), although this relationship is not significant.

**Figure S4.2.** Ordination graph generated from principal components analysis and based on benthic communities. Organism names are plotted in the direction of plots with higher than expected numbers of those organisms, and their distance from the origin is generally indicative
of the strength of their influence on plot locations. Only the species most correlated with axes 1 and 2 are shown on this plot.

**Figure S4.3.** Plot depth significantly affected benthic community composition, with deeper plots having a higher number of brittle stars.
**Figure S4.4.** Benthic individual abundances significantly increased with plot depth (P < 0.05, negative binomial generalized linear model with a log link).
Figure S4.5. Ordination graph generated from principal components analysis and based on free-swimming communities. Species names are plotted in the direction of plots with higher than expected numbers of those species, and their distance from the origin is generally indicative of the strength of their influence on plot locations. Only the species most correlated with axes 1 and 2 are shown on this plot.
Figure S4.6. There was no significant relationship between plot volume and the number of subtidal roots in a plot ($P = 0.5$, negative binomial generalized linear model with a log link).
Figure S4.7. A juvenile mangrove snapper sheltering beside a large sponge embedded with bivalves and polychaetes and covered in brittle stars (photo credit: VG Schutte).
Table S4.1. Independent variables tested in models (see the Methods section for more details on the models used).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment¹:</td>
<td>Categorical</td>
</tr>
<tr>
<td>Number of roots per plot</td>
<td>Continuous</td>
</tr>
<tr>
<td>Number of tips per root</td>
<td>Continuous</td>
</tr>
<tr>
<td>Root width</td>
<td>Continuous</td>
</tr>
<tr>
<td>Root length to depth ratio</td>
<td>Continuous</td>
</tr>
<tr>
<td>Root volume to plot volume ratio</td>
<td>Continuous</td>
</tr>
<tr>
<td>Block</td>
<td>Categorical</td>
</tr>
<tr>
<td>Plot width</td>
<td>Continuous</td>
</tr>
<tr>
<td>Plot depth</td>
<td>Continuous</td>
</tr>
<tr>
<td>Plot volume</td>
<td>Continuous</td>
</tr>
<tr>
<td>Plot micro-location²</td>
<td>Categorical</td>
</tr>
<tr>
<td>Island edge variation³</td>
<td>Categorical</td>
</tr>
<tr>
<td>Benthic cover⁴:</td>
<td>Continuous</td>
</tr>
<tr>
<td>Mud</td>
<td></td>
</tr>
<tr>
<td>Shell debris</td>
<td></td>
</tr>
<tr>
<td><em>Caulerpa verticillata</em></td>
<td></td>
</tr>
<tr>
<td><em>Caulerpa racemosa</em></td>
<td></td>
</tr>
<tr>
<td><em>Caulerpa sertularioides</em></td>
<td></td>
</tr>
<tr>
<td><em>Thalassia testudinum</em></td>
<td></td>
</tr>
<tr>
<td><em>Penicillus capitatus</em></td>
<td></td>
</tr>
</tbody>
</table>

¹ Treatment sub-variables were only used in models after treatment was determined to significantly influence the response variable.

² Plots were classified based on the shoreline around them: corner (a plot bordering a channel cutting between 2 islands), intermediate (a plot in the corner of a bay where the shoreline changes from curving north-south to curving east-west), or straightaway (a plot on a shoreline that does not curve).

³ Plots were classified based on whether they had a consistently vertical island edge with continuous canopy cover or whether the island edge at some point in the plot had a shallower slope, pushing trees away from the island edge and creating a gap in canopy cover.

⁴ Percent coverage for all of these 7 elements in each plot was used only in models evaluating benthic community composition and abundance and not in models evaluating free-swimming communities.
Table S4.2. Average oxygen, temperature, and salinity measurements (with standard deviations), which were not significantly affected by treatment.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>$O_2$ (%)</th>
<th>$O_2$ (mg/L)</th>
<th>Temperature (° C)</th>
<th>Salinity (° C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>82.2 ± 9.8</td>
<td>5.3 ± 0.6</td>
<td>29.3 ± 0.9</td>
<td>31.1 ± 0.4</td>
</tr>
<tr>
<td>Root reduction</td>
<td>82.0 ± 11.5</td>
<td>5.3 ± 0.7</td>
<td>29.4 ± 0.9</td>
<td>31.1 ± 0.4</td>
</tr>
<tr>
<td>Root removal</td>
<td>84.7 ± 9.4</td>
<td>5.5 ± 0.6</td>
<td>29.4 ± 0.9</td>
<td>31.1 ± 0.3</td>
</tr>
<tr>
<td>Epibiont removal</td>
<td>82.8 ± 10.1</td>
<td>5.3 ± 0.6</td>
<td>29.4 ± 0.9</td>
<td>31.1 ± 0.4</td>
</tr>
</tbody>
</table>

Table S4.3. Average turbidity measurements (with standard deviations), which were not significantly affected by treatment.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Total suspended solids (mg/L)</th>
<th>Organic matter (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>16.3 ± 18.1</td>
<td>43.7 ± 31.5</td>
</tr>
<tr>
<td>Root reduction</td>
<td>13.1 ± 24.8</td>
<td>59.9 ± 26.5</td>
</tr>
<tr>
<td>Root removal</td>
<td>21.2 ± 20.5</td>
<td>37.6 ± 31.1</td>
</tr>
<tr>
<td>Epibiont removal</td>
<td>14.5 ± 14.0</td>
<td>45.1 ± 33.3</td>
</tr>
</tbody>
</table>

Table S4.4. Average weight change from dissolution blocks and sediment height change in erosion markers (with standard deviation), which were not significantly affected by treatment.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mass lost (g/d)</th>
<th>Height gained (cm/d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>8.0 ± 0.7</td>
<td>0.003 ± 0.004</td>
</tr>
<tr>
<td>Root reduction</td>
<td>8.3 ± 0.5</td>
<td>0.004 ± 0.003</td>
</tr>
<tr>
<td>Root removal</td>
<td>8.0 ± 0.9</td>
<td>0.003 ± 0.006</td>
</tr>
<tr>
<td>Epibiont removal</td>
<td>8.3 ± 0.6</td>
<td>0.007 ± 0.007</td>
</tr>
</tbody>
</table>
Table S4.5. Benthic organisms (% of total) seen at all plots, ordered by abundance. Note that we did not identify benthic individuals to species, but instead grouped them more broadly, to the lowest taxonomic unit possible (at least to Class).

<table>
<thead>
<tr>
<th>Taxonomic group</th>
<th>Common name</th>
<th>% of total individuals observed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Order Ophiurida</td>
<td>Brittle star</td>
<td>81.4</td>
</tr>
<tr>
<td>Ascidiacea</td>
<td>Tunicate</td>
<td>5.5</td>
</tr>
<tr>
<td>Class Asteroidea</td>
<td>Sea star</td>
<td>5.3</td>
</tr>
<tr>
<td>Order Decapoda</td>
<td>Shrimp</td>
<td>2.0</td>
</tr>
<tr>
<td>Species <em>Eupolymnia crassicornis</em></td>
<td>Spaghetti worm</td>
<td>1.6</td>
</tr>
<tr>
<td>Subclass Prosobranchia</td>
<td>Marine snail</td>
<td>1.4</td>
</tr>
<tr>
<td>Species <em>Sabellastarte magnifica</em></td>
<td>Feather duster worm</td>
<td>0.6</td>
</tr>
<tr>
<td>Class Anthozoa</td>
<td>Anemone</td>
<td>0.4</td>
</tr>
<tr>
<td>Species <em>Holothuria mexicana</em></td>
<td>Sea cucumber</td>
<td>0.4</td>
</tr>
<tr>
<td>Class Echinoidea</td>
<td>Sea urchin</td>
<td>0.4</td>
</tr>
<tr>
<td>Order Decapoda</td>
<td>Hermit crab</td>
<td>0.2</td>
</tr>
<tr>
<td>Species <em>Elysia crispata</em></td>
<td>Lettuce sea slug</td>
<td>0.2</td>
</tr>
<tr>
<td>Species <em>Stenorhynchus seticornis</em></td>
<td>Arrow crab</td>
<td>0.2</td>
</tr>
<tr>
<td>Species <em>Bartholomea annulata</em></td>
<td>Corkscrew anemone</td>
<td>0.2</td>
</tr>
</tbody>
</table>
Table S4.6. Model selection information for the negative binomial models fit to our invertebrate abundance data. Models are listed according to how well they fit the data. The first model in the list has the lowest AIC score and highest Akaike weight (normalized model likelihood), which indicates the best fit. We fit many more models than the 5 shown here for brevity: the full model including all terms listed in Table S4.1, a model that only included treatment as an explanatory variable, and the three models with the lowest AIC scores.

<table>
<thead>
<tr>
<th>Model terms</th>
<th>AIC</th>
<th>ΔAIC</th>
<th>wi</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plot depth, Shell debris, Treatment</td>
<td>372.6</td>
<td>0</td>
<td>0.42</td>
</tr>
<tr>
<td>Block, Plot depth, Shell debris, Treatment, Island edge variation</td>
<td>373</td>
<td>0.4</td>
<td>0.35</td>
</tr>
<tr>
<td>Block, Plot depth, Shell debris, Treatment, <em>Caulerpa verticillata</em></td>
<td>373.9</td>
<td>1.3</td>
<td>0.22</td>
</tr>
<tr>
<td>Full model (terms listed in Table S1)</td>
<td>380.4</td>
<td>7.8</td>
<td>0.01</td>
</tr>
<tr>
<td>Treatment</td>
<td>398.8</td>
<td>26.2</td>
<td>0.00</td>
</tr>
</tbody>
</table>
Table S4.7. Results of both ordination and generalized linear modeling of benthic and free-swimming communities (see the Methods and Results sections for more model details). Only significant variables are shown for each model.

<table>
<thead>
<tr>
<th>Response</th>
<th>Model</th>
<th>Model terms</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Benthic communities</strong></td>
<td>Ordination: redundancy analysis</td>
<td>permutation whole model fit</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td></td>
<td></td>
<td>plot depth</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td></td>
<td></td>
<td>plot width</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td></td>
<td></td>
<td>mud</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td></td>
<td>Generalized linear model</td>
<td>treatment</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td></td>
<td></td>
<td>plot depth</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td></td>
<td></td>
<td>shell rubble</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td><strong>Free-swimming communities</strong></td>
<td>Ordination: redundancy analysis</td>
<td>whole model fit</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td></td>
<td>treatment and environmental variables: permutation</td>
<td>treatment</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td></td>
<td></td>
<td>plot width</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td></td>
<td>quantitative measures of treatment: permutation</td>
<td>root number</td>
<td>&lt;0.15</td>
</tr>
<tr>
<td></td>
<td>Generalized linear model</td>
<td>plot volume</td>
<td>&lt; 0.01</td>
</tr>
</tbody>
</table>
Table S4.8. Total counts of all free-swimming species seen at all plots.

<table>
<thead>
<tr>
<th>Species</th>
<th>Common name</th>
<th>Total occurrences</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abudefduf saxatilis</td>
<td>Sergeant major</td>
<td>16</td>
</tr>
<tr>
<td>Acanthurus bahianus</td>
<td>Ocean surgeonfish</td>
<td>1</td>
</tr>
<tr>
<td>Anisotremus virginicus</td>
<td>Porkfish</td>
<td>5</td>
</tr>
<tr>
<td>Apogon binotatus</td>
<td>Barred cardinalfish</td>
<td>13</td>
</tr>
<tr>
<td>Carybdea sp.</td>
<td>Box jelly</td>
<td>7</td>
</tr>
<tr>
<td>Chaetodon capistratus</td>
<td>Foureye butterflyfish</td>
<td>76</td>
</tr>
<tr>
<td>Chloroscombrus chrysurus</td>
<td>Atlantic bumper</td>
<td>1</td>
</tr>
<tr>
<td>Eucinostomus melanopterus</td>
<td>Flagfin mojarra</td>
<td>3</td>
</tr>
<tr>
<td>Gerres cinereus</td>
<td>Yellowfin mojarra</td>
<td>10</td>
</tr>
<tr>
<td>Haemulon plumieri</td>
<td>White grunt</td>
<td>4</td>
</tr>
<tr>
<td>Haemulon sciurus</td>
<td>Bluestriped grunt</td>
<td>60</td>
</tr>
<tr>
<td>Hypoplectrus providencianus</td>
<td>Masked hamlet</td>
<td>1</td>
</tr>
<tr>
<td>Hypoplectrus puella</td>
<td>Barred hamlet</td>
<td>4</td>
</tr>
<tr>
<td>Lutjanus spp.:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lutjanus apodus</td>
<td>Schoolmaster</td>
<td></td>
</tr>
<tr>
<td>Lutjanus griseus</td>
<td>Gray snapper</td>
<td></td>
</tr>
<tr>
<td>361</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mnemiopsis mccradyi</td>
<td>Sea walnut</td>
<td>6</td>
</tr>
<tr>
<td>Nicholsina usta</td>
<td>Emerald parrotfish</td>
<td>1</td>
</tr>
<tr>
<td>Ocyurus chrysurus</td>
<td>Yellowtail snapper</td>
<td>1</td>
</tr>
<tr>
<td>Orchistoma pileus</td>
<td>Club medusa</td>
<td>1</td>
</tr>
<tr>
<td>Pomacanthus paru</td>
<td>French angelfish</td>
<td>4</td>
</tr>
<tr>
<td>Scarus guacamaia</td>
<td>Rainbow parrotfish</td>
<td>2</td>
</tr>
<tr>
<td>Scarus iserti</td>
<td>Striped parrotfish</td>
<td>41</td>
</tr>
<tr>
<td>Scarus taenioperus</td>
<td>Princess parrotfish</td>
<td>15</td>
</tr>
<tr>
<td>Sparisoma rubripinne</td>
<td>Yellowtail parrotfish</td>
<td>1</td>
</tr>
<tr>
<td>Sparisoma viride</td>
<td>Stoplight parrotfish</td>
<td>1</td>
</tr>
<tr>
<td>Sphyraena barracuda</td>
<td>Great barracuda</td>
<td>41</td>
</tr>
<tr>
<td>Tylosurus crocodilus</td>
<td>Houndfish</td>
<td>17</td>
</tr>
</tbody>
</table>
Table S4.9. Average number of species per plot (listed in Table S1) with standard deviation, which was not significantly affected by treatment.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Species Richness</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5.2 ± 1.9</td>
</tr>
<tr>
<td>Root reduction</td>
<td>3.9 ± 1.2</td>
</tr>
<tr>
<td>Root removal</td>
<td>3.4 ± 2.0</td>
</tr>
<tr>
<td>Epibiont removal</td>
<td>4.7 ± 1.8</td>
</tr>
</tbody>
</table>

Table S4.10. Model selection information for the negative binomial models fit to our invertebrate abundance data. A * between two terms denotes an interaction between them. Models are listed according to how well they fit the data. The first model in the list has the lowest AIC score and highest Akaike weight (normalized model likelihood), which indicates the best fit. We fit many more models than the 5 shown here for brevity: the full model including all terms listed in Table S1, a model that only included treatment as an explanatory variable, and the three models with the lowest AIC scores.

<table>
<thead>
<tr>
<th>Model terms</th>
<th>AIC</th>
<th>ΔAIC</th>
<th>wi</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plot volume</td>
<td>284.2</td>
<td>0</td>
<td>0.27</td>
</tr>
<tr>
<td>Treatment * Plot volume, Island edge variation</td>
<td>284.5</td>
<td>0.3</td>
<td>0.23</td>
</tr>
<tr>
<td>Treatment * Plot volume, Island edge variation, Plot depth, Block, Plot micro-location</td>
<td>284.7</td>
<td>0.5</td>
<td>0.21</td>
</tr>
<tr>
<td>Full model (terms listed in Table S4.1)</td>
<td>284.9</td>
<td>0.7</td>
<td>0.19</td>
</tr>
<tr>
<td>Treatment</td>
<td>285.9</td>
<td>1.7</td>
<td>0.11</td>
</tr>
</tbody>
</table>
Table S4.11. Average chlorophyll $a$ concentrations from the water column and from tiles deployed above the seafloor (with standard deviation), which were not significantly affected by treatment.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>chlorophyll $a$</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Water column (µg L$^{-1}$)</td>
<td>Tiles (µg cm$^{-2}$)</td>
</tr>
<tr>
<td>Control</td>
<td>8.0 ± 0.7</td>
<td>0.003 ± 0.004</td>
</tr>
<tr>
<td>Root reduction</td>
<td>8.3 ± 0.5</td>
<td>0.004 ± 0.003</td>
</tr>
<tr>
<td>Root removal</td>
<td>8.0 ± 0.9</td>
<td>0.003 ± 0.006</td>
</tr>
<tr>
<td>Epibiont removal</td>
<td>8.3 ± 0.6</td>
<td>0.007 ± 0.007</td>
</tr>
</tbody>
</table>