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The use of functional traits to elucidate the causes and consequences of biological diversity.

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The use of functional traits to elucidate the causes and consequences of biological diversity

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In Partial Fulfillment
Of the requirements for the Degree of
Doctor of Philosophy

By

Jonathan S. Lefcheck
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APPROVAL SHEET

This dissertation is submitted in partial fulfillment of
the requirements for the degree of
Doctor of Philosophy

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DEDICATION

To my wife-to-be, Katie May, and my parents, Steve and Debra.

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AUTHOR'S NOTE

The chapters that comprise this dissertation were written in manuscript format for scientific publication. At the time of writing, the citations for individual chapters are as follows:

CHAPTER 1.

Lefcheck, J.S., and J.E. Duffy. 2014. Multitrophic functional diversity predicts ecosystem functioning in experimental assemblages of estuarine consumers. *Ecology*. In revision.

CHAPTER 2.

Lefcheck, J.S., Stuart-Smith, R.S., Laumann, K.M., Duffy, J.E., and G.J. Edgar. Biotic mechanisms of reef fish community assembly change across global gradients.

CHAPTER 3.

Lefcheck, J.S., Richardson, J.P., and J.E. Duffy. Top-down versus bottom-up control of a temperate eelgrass bed: insights from a 15-year ecological survey.

ABSTRACT

The rapid and global rise in species extinctions has prompted much research into the causes and consequences of biodiversity loss. In the past two decades, efforts have expanded beyond characterizing diversity through numbers of species – or species richness – and integrated additional information on how species interact with one another and their environment via *functional traits*. Functional traits permit a more nuanced exploration of patterns in community structure and composition, and provide a mechanistic basis to link community diversity to ecosystem processes. In this dissertation, I apply functional traits to observational surveys and a small-scale experimental manipulation to understand and explain patterns in diversity, and to link functional diversity to ecosystem functioning. In all three cases, I show that functional traits yield substantial additional insight into ecological patterns and processes beyond what can be gained via richness alone.

In the first chapter, I use functional traits and two newly-derived phylogenies to understand the role of biotic interactions in determining how local communities of reef fishes assemble from the available pool of species. To address this question, I utilized data from the Reef Life Survey network, a global citizen science program that has conducted visual censuses of reef fish communities at nearly 2,000 sites across the globe. To rigorously disentangle the biotic and abiotic drivers of assembly, I aimed to factor out the effect of environment by grouping species based on their fine-scale habitat requirements, then tested for significant patterns in functional and phylogenetic diversity of local communities relative to the regional species pool. I found that most communities were functionally and phylogenetically clustered relative to the regional pool, meaning that species found in these communities were more functionally- and phylogenetically-similar than expected by chance. This clustering increased with increasing latitude independent of several major axes of environmental variation. I propose several non-mutually exclusive explanations for this pattern, including: (1) increased competition at higher latitudes, potentially driven by variability in resources; (2) higher mobility of fishes at high latitudes reducing trait and evolutionary composition at any given site relative to what could be observed there (i.e., high turnover), and; (3) reduced richness at high latitudes reducing the probability of capturing functionally and phylogenetically unique species. This chapter is one of the first studies to unite a macroecological perspective on assembly with functional biogeography across global gradients, particularly for vertebrates.

In the second chapter, I utilized data from a 15-year observational survey of an eelgrass *Zostera marina* L. bed in the York River Estuary, Chesapeake Bay, USA to test the relative strength of top-down and bottom-up control and the role of species richness and functional diversity in mediating trophic processes. I united biological data on eelgrass, microalgal epiphyte, and invertebrate grazer biomass, and predator abundances with physical data on temperature, light, turbidity, and nutrients using structural equation modeling. Across spring,

summer, and fall seasons, biological variables appeared to be largely controlled by temperature and turbidity. However, there was weaker but statistically significant evidence for top-down control in the spring and summer, changing over to bottom-up control in the fall. In contrast to evidence from small-scale experiments, there was no effect of diversity on ecosystem properties such as standing stock biomass of eelgrass, grazers, and predators, which may have been a consequence of the overall low diversity and high functional redundancy present in this system. This chapter reveals a small but significant role for biology in the face of strong, long-term natural variation in abiotic parameters in a temperate eelgrass bed.

In the third and final chapter, I experimentally manipulated functional trait diversity of estuarine mesograzers and predators within multiple levels of species richness to test the relative predictive ability of functional diversity and species richness on ecosystem functioning. I found that multivariate functional diversity based on 8 traits was a better predictor and explained more variation in standing stock biomass of predator, grazer, and recruiting invertebrates than did species richness. Aggregating across all 8 traits in a multivariate index of functional diversity improved prediction accuracy relative to any individual trait. I then used structural equation modeling to show that the positive effects of community-level functional diversity were a consequence of both predator and grazer functional diversity, although predator effects were much stronger. I also modeled the contributions of each individual species to show that different functions were driven by different species with unique combinations of traits, suggestive of functional complementarity. Together, these results suggest that functional diversity is a powerful alternative to species richness in predicting the ecosystem consequences of species loss. This chapter is one of the first studies to conduct an *a priori* manipulation of functional traits using consumers, and the first to manipulate traits across multiple levels of a realistic food web.

THE USE OF FUNCTIONAL TRAITS TO ELUCIDATE THE CAUSES AND CONSEQUENCES OF BIOLOGICAL DIVERSITY

FUNCTIONAL TRAITS: A PRIMER

The widespread and increasingly rapid extinction of species on planet Earth is a well-documented phenomenon (Millennium Ecosystem Assessment 2005), with some suggesting that the planet is in the midst of its sixth mass extinction (Barnosky et al. 2011). Such a pervasive decline in biological diversity raises the question: what are the consequences for human well-being? The answer requires that we first understand what exactly it is that we are losing. Traditionally, biodiversity has been quantified as the number of different species – or richness – and less often the equitability with which individuals are distributed among them – or evenness (Magurran and McGill 2011). Assuming that the study system is taxonomically resolved, this approach is relatively straightforward: count and identify the number of species and/or individuals.

A species-centric approach is, however, a very coarse and potentially misleading way of thinking about diversity, because it implicitly assumes that all species are equally distinct (Petchey and Gaston 2002). Yet, we implicitly understand that some species are clearly more similar than others, based on any number of identifiable morphological, behavioral, physiological, or ecological attributes. Consider an intertidal rockpool filled with six species of barnacles, and a nearby rockpool containing six dramatically different species: snails, crabs, urchins, and so on. Since both have the same species richness, a traditional ecologist would treat both communities as equally diverse. To the layperson, however, the distinction is easy: the barnacle-filled pooled is clearly 'less diverse,' because the barnacles are more similar to one another – they all possess hard calcareous plates, have similar body shapes and sizes, and feed in the same way – than are the diverse set of animals in the other tidepool (Leinster and

Cobbold 2012). These above observations clarify how similar (or dissimilar) co-occurring species are from one another, and it is only within the last two decades that ecologists have begun I earnest to integrate so-called 'functional traits' into more nuanced descriptors of diversity.

What Is A Functional Trait?

Over the last twenty years, the number of published papers reporting on 'functional traits' has increased exponentially (Cadotte et al. 2011). As a consequence, the literature is replete with conflated and increasingly specialized definitions of what a 'functional' trait is. Here, I briefly summarize the history of the term, followed by the definition I have chosen to use throughout this dissertation and why.

The functional trait concept appears to have evolved within the rich history of comparative plant ecology (Dansereau 1951). By the mid-1990s, functional traits were increasingly considered by researchers interested in understanding the relationship between plant morphological traits and ecosystem 'functions,' such as production and nutrient cycling (Hooper et al. 2005). McGill et al. (2006) later made the distinction between traits writ large, or well-defined attributes used to compare across species, and 'functional' traits specifically, used to indicate organismal effects on processes. Lavorel and Garnier (2002) co-opted the functioning-based definition under the guise of 'effect traits,' and also introduced 'response traits,' which define how organisms respond to, rather than shape, their environment. Violle et al. (2007) brought further specialization to the topic by proposing 10 separate categorizations for traits, only one of which they considered functional: "any trait which impacts fitness indirectly via its effects on [individual] growth, reproduction and survival" (p. 889). They argued that lumping together traits operating at different levels of organization (i.e., individuals, populations, ecosystems) could obscure the underlying mechanisms controlling community structure and functioning. Instead, they proposed a complicated hierarchy charting how traits influence individual responses to environmental conditions (response traits), translate to differences in individual fitness (functional traits), and ultimately drive aggregate ecosystem properties (effect traits).

While Violle et al. (2007) proposed a sound framework, it is restricted by its complexity. For instance, consider plant tolerance to grazing. They argue that it should not be considered a functional trait, as it requires the invocation of external forces (i.e., grazers) in its definition. Rather, grazing tolerance is a function of individual characteristics, such as plant lifespan, height (and thus accessibility), and the presence of certain anatomical features or defenses, all of which fit their definition of functional traits as they directly reflect potential individual fitness. Yet, if the goal of the analysis is to consider functional traits within the context of primary production, the use of individual traits should lead to the same conclusion as the response trait defined by external factors. Consider again the common plant trait of specific leaf area. This trait has implications for individual growth and fitness through its role in resource use and acquisition—a true functional trait, *sensu* Violle et al. (2007) —yet the degree of resource use has direct implications for ecosystem primary production, and thus it could also be thought of as a performance or effect trait. It also can denote how a plant would respond to changes in the environment, say via light reduction, and thus can also be considered a response trait. Thus, it becomes clear through these few examples that it should not matter precisely where in the hierarchy of Violle et al. (2007) traits fall, as long as one recognizes that they have some explanatory relationship to the pattern or process under investigation.

Consequently, I prefer the broader and more operational definition of functional traits proposed by Díaz et al. (2013), and modified slightly here with language from McGill et al. (2006):

> *Any morphological, biochemical, physiological, structural, phenological, life historical, or behavioral characteristic(s) that are expressed as measurable attributes of individual organisms, and which can be used to make comparisons across species.*

I will adhere to this definition throughout this dissertation.

Prevailing advice is that, "all traits are important for the function of interest and no traits are functionally informative" (p. 743, Petchey and Gaston 2002), although I believe there are some basic restrictions for what can be considered a useful functional trait within the context of any investigation. First, a functional trait should vary among at least a few species in the community, otherwise it does not provide any real insight (Petchey and Gaston 2006). Likewise, values of a functional trait should not be independent and equidistant for each species, in which case the trait simply reflects species richness (Schleuter et al. 2010). An informative functional trait should also have some realistic relationship to the pattern or process under investigation (Petchey and Gaston 2006). This relationship can either be hypothesized, in which case the analysis may be purely exploratory, or may have previous empirical support and is therefore confirmatory.

What Is Functional Diversity?

Functional diversity represents the range and value of organismal functional traits present in an ecological assemblage, which can be summarized in any number of metrics. Functional indices fall into three categories: functional richness, which reflects the total variation in functional traits encapsulated by a community, functional evenness, which reflects the equity with which traits values are distributed among individuals within a community, and functional dispersion, which reflects the degree to which species within a community differ from each other (Mason et al. 2005). A full review of the dozens of functional diversity indices proposed to date is beyond the scope of this introduction (reviewed in Schleuter et al. 2010), but the general advice is to utilize an index that reflects the goals of the analysis. For instance, if one is concerned only with maximizing the total functional diversity independent of relative abundances, or that information is unavailable, then an index of functional richness may suffice (Cornwell et al. 2006, Villéger et al. 2008). On the other hand, if one wishes to use an index that is not constrained to covary positively with species richness, and thus represents a mathematically (but perhaps not ecologically) independent component of diversity, one might instead use Rao's quadratic entropy (Rao 1982, Botta-Dukát 2005).

The idea of combining information from multiple traits into a single summary index of functional diversity is gaining traction in the literature, particularly with the advent of statistical techniques used to generate univariate summary statistics from multivariate trait data (e.g., Villéger et al. 2008). There are additional risks in trait selection when considering multivariate indices. For instance, correlations among traits can cause certain functional diversity indices to collapse to small values, making it more difficult to identify differences among communities or experimental treatments (Cornwell et al. 2006, Lefcheck et al. 2015). Trade-offs among traits may also obscure or nullify multivariate trends. For instance, Spasojevic and Suding (2012) noted that there were strong opposing trends in individual traits along an alpine tundra resource gradient, but because of trade-offs among the traits under high vs. low resources, these signals cancelled each other out in a multivariate index of functional diversity. Bellwood et al. (2006) found a low correlation between the degree of morphological specialization and diet specialization in tropical fishes. There was, however, a strong relationship between one particular anatomical trait and diet, but this signal was swamped by the overall weak effects of other characters in their multivariate analysis. It is difficult to anticipate when these kinds of trade-offs or weak relationships might occur. Thus, a common recommendation when using a multivariate index is to perform a thorough investigation of each trait individually, when possible (Ricotta and Moretti 2011, Lefcheck et al. 2015).

Thus, how many traits are sufficient? If the research question can be explained by a single trait, then one is enough. However, multiple traits can often be implicated in a single hypothesis. Explaining complex systems that are shaped by multiple factors all but requires multiple traits, particularly when considering the gamut of future scenarios generated by global change (Lefcheck et al. 2015). Additionally, certain highly informative traits may also be extremely difficult or costly to measure, but can be indirectly represented by a combination of multiple, less intensive traits (Petchey and Gaston 2006). As with highly variable traits, a pitfall of including too many traits is that, once combined, they simply reflect taxonomy and functional diversity more or less approximates species richness. However, recent analyses have uncovered significant discrepancies between species richness and indices derived from upwards of 20 functional traits (Devictor et al. 2010, Lefcheck et al. 2014, Granger et al. 2014), suggesting that this risk may never realistically be realized. However, caution should still be taken to avoid include irrelevant or redundant traits for the statistical reasons outlined above.

Why Functional Diversity?

The gathering of functional trait data can be costly in terms of time, money, and personnel. Why bother to collect that information when we have been getting along with species richness for close to a century? Functional diversity offers a number of benefits to ecological investigations compared to a traditional species-based approach. First, it purports to provide information on what organisms are doing, as opposed to their evolutionary history (i.e., their Latin names, Petchey and Gaston 2006). In this way, traits can be mechanistically linked to patterns or processes of interest (Díaz and Cabido 2001), and inferences are in principle generalizable to other organisms and systems that possess similar trait values, far more than citing the contributions of specific species (McGill et al. 2006). For instance, the ability to fix nitrogen has been shown to enhance total productivity more than species identity or richness across a number of nitrogen-limited grassland plant communities (Hooper and Vitousek 1997, Tilman et al. 1997, Cadotte et al. 2009). Furthermore, non-overlapping trait values can provide insight into niche differences among species, suggesting mechanisms such as resource partitioning that have often been invoked to explain the positive effect of species richness on ecosystem properties such as biomass production and resource use (Loreau & Hector 2001, Cardinale et al. 2007). For example, variation in habitat preferences allowed three species of intertidal grazers to access different resource pools, enhancing total resource depletion beyond what was observed for each species by itself (Griffin et al. 2009). Such niche differences can also shed light on the factors promoting species coexistence, and thus can be used to address potential mechanisms of community assembly and the maintenance of diversity in nature (Pavoine and Bonsall 2011, HilleRisLambers et al. 2012).

Functional diversity can also give insight into potential redundancy among species in their effects on ecosystem processes, which allows estimation of how many species can be lost before there are significant ecosystem consequences (Rosenfeld 2002). For example, Micheli and Halpern (2005) found strong positive relationships between functional diversity and species richness across a number of marine systems, implying low functional redundancy and thus high vulnerability of these communities to activities which remove species, such as overfishing. This aspect of functional diversity may also help aid in setting priorities for conservation and management, either by isolating new or unique areas of high diversity (Devictor et al. 2010, Stuart-Smith et al. 2013), or by identifying traits that contribute to beneficial ecosystem services

(Díaz et al. 2007). Finally, traits may allow us to better understand and predict community responses to global change, by linking traits that make species more or less vulnerable to warming, overexploitation, and other anthropogenic activities (Mouillot et al. 2013).

On a final note, there has been increasing interest in the substitution of phylogenetic data in place of functional traits, under the notion that genetic data capture the entirety of organismal variation, accounting for traits that were unmeasured or not considered (Srivastava et al. 2012). This may be the reason why several studies have uncovered equal or greater explanatory power for indices of phylogenetic than functional trait diversity (Cadotte et al. 2009, Flynn et al. 2011). Unlike functional traits however, phylogenetic data generally provide no mechanistic basis for the observed trends, perhaps outside of functional genomics in prokaryotes. For higher-order organisms, however, we may only reach a stage where the physiological and phenotypic consequences of nucleotide variation are sufficiently well-known across a variety of organisms to inform ecological processes in the very distant future. In the interim, functional traits provide a tractable alternative (Cadotte et al. 2011), but understanding where and when the descriptive power of functional vs. phylogenetic diversity diverge may help narrow the number and identity of certain functional phylogenetically-conserved traits.

Dissertation Rationale And Objectives

In this dissertation, I use functional traits to investigate the role of functional diversity in both generating patterns and driving processes in nature. Three chapters present the original research conducted as part of this dissertation.

Chapter 1 uses data from a global survey of reef fish communities collated by collaborators to understand the biotic drivers of community assembly across latitude. Reconciling how local communities are assembled from the available pool of species is a central question in ecology (Ricklefs 1987), and functional traits provide a mechanistic way to assess whether communities are composed of species with similar environmental tolerances, or species that possess traits which permit coexistence (HilleRisLambers et al. 2012). Previous approaches, however, may have confounded the role of environment vs. biology in evaluating community assembly (Mayfield and Levine 2010). Here, I use functional traits and data from two newly-derived phylogenetic trees with the goal of rigorously disentangling abiotic from biotic drivers of reef fish assembly, and to evaluate whether the importance of biological interactions changes between tropical and temperate regions, independent of co-occurring environmental gradients.

Chapter 2 utilizes data from a 15-year survey of a local eelgrass bed in the York River Estuary, Chesapeake Bay, USA to understand the role of biodiversity in mediating top-down and/or bottom-up control of ecosystem functioning in this important coastal system. Seagrasses are currently facing threats from increased nutrient loading and overfishing, both of which have the potential to alter the trophic structure and functioning of seagrass communities (Duffy 2006, Waycott et al. 2009). Despite numerous short-term experiments demonstrating strong top-down control of eelgrass by the presence and diversity of invertebrate grazers (reviewed in Valentine and Duffy 2006), we still have a limited understanding of whether this control will manifest in the face of long-term natural variation in the environment. In this chapter, I use structural equation modeling to unite physical and biological variables to evaluate the significance and direction of biological effects – particularly species and functional diversity – on ecosystem processes given a suite of environmental factors.

In Chapter 3, I report on experimental manipulations of functional trait diversity within multiple levels of species richness and across multiple trophic levels of an estuarine food web to understand the relative explanatory power of richness vs. functional traits in predicting ecosystem functioning. There is growing empirical evidence that functional trait diversity better predicts ecosystem functioning that species richness (Cadotte et al. 2009, Flynn et al. 2011, Gagic et al. 2015), but most of this evidence comes from *post hoc* analysis of existing richness manipulations. Moreover, there is relatively little knowledge of how functional traits may predict ecosystem functioning in multitrophic food webs (Reiss et al. 2009). In this chapter, I conduct an *a priori* factorial manipulation of richness and functional trait diversity across two levels of an estuarine food web, grazers and predators, and measure multiple ecosystem functions in experimental mesocosms.

For Chapters 2 and 3, I also provide a freely available and fully documented statistical package to construct structural equation models, *piecewiseSEM*, which can be used in the open-source software R (R Development Core Team 2014). This package greatly streamlines the process of building, evaluating, and interpreting complex structural equation models, and can be accessed from: https://github.com/jslefche/piecewiseSEM. In sum, my dissertation presents three analyses united by a functional trait approach, demonstrating the ability of functional traits to describe, explain, and predict ecological patterns and processes from the very small scale to the global scale.

Notes

Portions of this introduction appear in a slightly different form in the supplementary material of the following paper:

> Lefcheck, J. S., V. A. G. Bastazini, and J. N. Griffin. 2015. Choosing and using multiple traits in functional diversity research. *Environmental Conservation*. doi: 10.1017/S0376892914000307.

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CHAPTER 1.

BIOTIC MECHANISMS OF REEF FISH COMMUNITY ASSEMBLY CHANGE ACROSS LATITUDE

Abstract

There has been increasing interest in using functional and phylogenetic patterns to infer mechanisms of community assembly, but to date few studies have explained how these patterns co-vary across broad-scale gradients, particularly for animals. We used an unprecedented dataset on global reef fish assemblages, comprising 2,489 species and 1,702 sites, to rigorously disentangle the biotic drivers of community assembly across >100° of latitude. We show fish assemblages are more functionally and phylogenetically similar to the regional species pool (convergence) at higher latitudes, even after accounting for major sources of environmental variation. Since the analysis controlled statistically for environmental filtering, these results contradict most prior studies in suggesting stronger interspecific competition at high latitudes. This inference was supported by a meta-community analysis showing that species pairs were less likely to co-occur than expected by chance with increasing latitude. Further exploration revealed community abundance and human population size as potential drivers of functional and phylogentic convergence. In sum, multiple lines of evidence suggest that the negative effects of biotic interactions on community assembly increase between tropical and temperate zones, uniting community and macro-ecological predictions with functional biogeography.

Keywords: latitudinal diversity gradient, functional diversity, phylogenetic diversity, environmental filtering, limiting similarity, competitive exclusion

Introduction

Understanding what drives species composition at the local scale is a longstanding question in ecology (Macarthur and Levins 1967, Ricklefs 1987). Historically, local communities have been viewed as subsets of the regional species pool which must first tolerate local environmental conditions, then be sufficiently dissimilar in their ecological strategies to avoid competitive exclusion by one another (Weiher et al. 2011). This balance between environmental filtering and limiting similarity has often been inferred using functional traits, aspects of an organism's biology, physiology, life history and behavior that indicate how they interact with their environment and with other organisms (Díaz et al. 2013). Under strong environmental forcing, assemblages are expected to exhibit trait convergence relative to the larger species pool, as similar traits in principle reflect species' abilities to tolerate local conditions. Alternatively, under stronger competitive processes, assemblages are expected to exhibit trait divergence, which should theoretically limit the potential for interspecific competition (HilleRisLambers et al. 2012). Phylogenetic patterns have also been used to infer assembly mechanisms with similar expectations, under the assumption that traits contributing to physiological tolerances and/or relative competitive ability are phylogenetically conserved (Webb et al. 2002).

While traits and phylogeny have often been used to understand assembly processes within a particular region (Weiher et al. 2011), the same tools can be used in a biogeographic context to understand how the mechanisms driving assembly change from region to region across broad spatial gradients (Violle et al. 2014). For instance, there are clear ecological predictions relating the intensity of local competition to the well-described latitudinal gradient in species diversity. Specifically, tropical communities are expected to exhibit greater contemporary trait divergence as the result of stronger historical interactions (Vermeij 2005, Schemske et al. 2009), physiological specialization driven by low environmental variation (Janzen 1967), and/or increased rates of co-evolution (Stenseth 1984), all leading to niche partitioning.

Conversely, in temperate communities, increased environmental variation is predicted to drive adaptation towards 'fixed targets,' or trait values associated with generalism (Pianka 1966, Schemske 2002). For instance, mean body size has been shown to increase with latitude for many taxa, which is often implicated in temperature control – 'Bergmann's rule' (Ray 1960, Meiri and Dayan 2003). Body size has also been correlated with increased mobility in animals (Griffiths 2010), which may lead to increased connectivity among assemblages within temperate regions. Coupled with the overall lower richness at higher latitudes (Mittelbach et al. 2007), a higher degree of generalism and increased connectivity is expected to both homogenize the regional species pool, and decrease the probability of finding functionally or phylogenetically distinct species in any given community. Consequently, the temperate communities are likely to be more clustered relative to the regional pool, especially for traits related to habitat use and dispersal (Dynesius and Jansson 2000). Yet, to date, few studies have incorporated trait and/or phylogenetic data to evaluate the mechanisms driving community assembly across the latitudinal gradient, and all have focused on plants (Swenson et al. 2012, Lamanna et al. 2014, Qiao et al. 2015).

Recently, it has been proposed that biological interactions could lead to both trait divergence, in the event of competitive sorting and niche partitioning, or convergence, if species with certain competitively superior traits exclude competitively inferior species (Mayfield and Levine 2010, HilleRisLambers et al. 2012). This dichotomy is problematic as many studies have interpreted trait convergence as evidence for strong environmental filtering, when in reality it may instead indicate a significant biological component instead of or in addition to the environment (Kraft et al. 2014). One way to isolate the biotic effects on assembly from those associated with environmental filtering is to first control for the effects of environment before evaluating patterns in functional or phylogenetic diversity (de Bello et al. 2012). This approach relies on the careful definition of the regional species pool to only include species that have similar habitat requirements and environmental tolerances. Within a given pool of species with similar environmental associations, values of functional or phylogenetic diversity at the local level (Div_{comm}) can be compared to the regional pool (Div_{pool}) to determine if either of the two biotic mechanisms identified above are operating: niche partitioning leading to divergence ($Div_{comm} > Div_{pool}$), or weak competitor exclusion leading to convergence ($Div_{comm} < Div_{pool}$). In the absence of either, one would infer complete environmental control or stochastic processes ($Div_{comm} \approx Div_{pool}$). By grouping species based on shared environmental tolerances, this approach essentially asks if there is any biological signal driving community structure above and beyond what is explained by the environment.

The framework proposed above does not require the construction of complicated null models, where trait or phylogenetic distances are randomized among communities (de Bello et al. 2012), negating the selection and justification of an appropriate null model (Gotelli 2000). Moreover, null model approaches have recently been shown to poorly discriminate assembly rules under a variety of simulated scenarios, and instead are more reflective of the choice of metric (Mouchet et al. 2010) and the magnitude of the observed values of diversity (de Bello 2012). Under this new framework, significant divergence or convergence is instead detected by measuring the difference between the local diversity and the total regional diversity ($ES = Dcomm - Dpool$), which can be compared to the null expectation that the difference is zero using a simple and interpretable *t*-test. Additionally, these effect sizes can be used as responses in traditional modeling frameworks to more precisely quantify the drivers of local assembly, in contrast to other methods used to identify assembly processes, which yield insightful but largely qualitative inferences regarding assembly (Dray et al. 2014).

Marine fishes are the most diverse group of vertebrates on the planet, occupy a wide range of ecological roles, and are comparatively well-described and understood. Thus, they represent an ideal group with which to test how mechanisms of community assembly change across global gradients. Richness of marine fishes follows the classic inverse pattern with latitude (Rohde 1992, Tittensor et al. 2010), thus generating testable hypotheses with respect to the role of local competition. Further, a recent analysis revealed that functional trait diversity among marine fishes does not adhere to the same global pattern as richness (Stuart-Smith et al. 2013), suggesting that functional traits may provide further insight into the biological processes limiting local diversity. Here, we present an analysis of biotic drivers of local assembly using data from a global survey of reef fish communities by the Reef Life Survey program (RLS, www.reeflifesurvey.com) (Edgar and Stuart-Smith 2014). We calculated functional diversity using multiple functional traits representing differences in morphology, trophic ecology, behavior, and habitat use (Table S1), and phylogenetic diversity using a multi-gene tree proxy for phylogeny. We calculated the index of Rao's quadratic entropy (Rao's Q, 33) to quantify and compare the diversity of local communities to that of the regional species pool. We biogeographically defined each species pool using 'Marine Ecoregions of the World' (Spalding et al. 2007), and further partitioned them using fine-scale environmental data obtained during the surveys, including depth, temperature, nitrates, percent coral and algal cover, wave exposure, relief, slope, and current velocity.

Methods

Reef Life Survey Data

Fish community data were collected through the Reef Life Survey program, a global citizen-science initiative that combines visual censuses from scientific and skilled volunteer

SCUBA divers. Briefly, divers ran a 50-m transect across shallow reef habitats and visually recorded the identity and abundance of every species within 5-m on both sides and 5-m above the length of the transect. Further specifics about the survey methods, including diver training, quality control, data consistency, and management can be found in (Edgar and Stuart-Smith 2009, 2014, Stuart-Smith et al. 2013). In all, we used data from 4797 transects at 1986 sites in 77 ecoregions, spanning 133° of latitude and on every continent except Antarctica. For this analysis, surveys were aggregated at the site level.

Functional Traits And Phylogeny

In total, 2465 unique fish taxa were recorded from 175 families. Eight functional traits were scored for each (Table S1), mostly based on data obtained from FishBase (www.fishbase.org), although some were allocated based on expert knowledge (see supplementary material in Stuart-Smith et al. 2013). The species-by-trait matrix was converted into Gower's distance measure, which combines both continuous and categorical information into a single distance measure, using Podani's correction for ordinal data (Gower 1971, Podani 1999). Because the diversity metric used (mean pairwise distance, see below) can be maximized with fewer than all functional types if distances are not ultrametric (Pavoine et al. 2005), we used the Gower distances to construct a functional dendrogram using different clustering methods (Mouchet et al. 2008), and converted it to ultrametric using the function *clue* package in R (Hornik 2013). We then used the matrix 2-norm to identify the ultrametric dendrogram that best preserved the original distances (Mérigot et al. 2010), and standardized between [0,1] by dividing by the maximum distance across the entire matrix.

We also built two multi-gene trees as a proxy for phylogeny containing all species used in the analysis. First, we queried GenBank (www.ncbi.nlm.nih.gov/genbank) to assess coverage for commonly sequenced genes in fishes, and determined that four genes had sufficient coverage across all species (or their congeners) in our dataset: cytochrome oxidase subunit I (*COI*), 16s rRNA, 12s rRNA, and cytochrome b (*cytb*). The initial pulldown using the *taxize* package in R (Chamberlain et al. 2014) yielded sequences for 94% of the species, and additional sequences were collected manually. In a small number of cases, we substituted family-level relatives when sequences for species or congeners were not available (5.4% of species). No single gene covered all species, so we combined information from all four genes to create a multigene tree. We aligned the sequences using the program MAFFT version 7.145b (Katoh and Standley 2013). Alignments were then inspected visually and manually edited when necessary (to correct for inappropriately reduced gaps).

To build a multigene tree, we tested separate models of evolution for each gene using the program partitionFinder (Lanfear et al. 2014). All genes followed a GTR+G+I model. We combined the aligned sequences to create a single gappy supermatrix, which we then used to build two separate trees. To build a Bayesian tree, we used the program MrBayes version 3.2.2 (Ronquist and Huelsenbeck 2003), with 500,000 runs, retaining the top 25% of trees every 5,000 runs, and allowed model parameters to vary by gene. We also implemented family level constraints to assist with convergence, and utilized all compatible trees to build the single consensus tree used in subsequent analyses. To build a maximum-likelihood tree, we used the program RAxML (Stamatakis 2006), conducting 100 bootstrapped runs and also allowing the model parameters to vary by gene. Both trees used the basal *Myxine glutinosa* as the outgroup. To assist in convergence for both trees, we removed all identical congeners from the dataset, built the trees, and then reinserted those species with node lengths identical to those of their congeners. We converted the complete trees to ultrametric using a penalized likelihood approach (Kim and Sanderson 2008) implemented in the *ape* package in R (Paradis et al. 2004), rooted each tree, pruned the outgroup, and extracted the cophenetic distance matrix. As with the functional distance matrix, we scaled phylogenetic distances to the range [0,1] by dividing by the maximum distance across the entire matrix. All the code used to run each program (MAFFT, partitionFinder, mrbayes, and RAxML) are available in the supplementary R code, and both the functional and phylogenetic distance matrices are provided in Newick format.

Community Assembly

To test for biotic effects on community assembly, we implemented the framework described in (de Bello et al. 2012). This required the construction of regional species pools consisting of species with similar environmental and/or habitat requirements, which we first defined by tallying all species observed within a given 'Marine Ecoregion of the World' (MEOW, (Spalding et al. 2007)). We further restricted the pools based on site-level abiotic characteristics within each MEOW, including depth, temperature, nitrates, percent coral cover, percent algal cover, wave exposure, relief, slope, and current speed. We grouped sites that shared similar abiotic properties using *k*-means clustering. We determined the optimal number of 'sub-ecoregions' per MEOW by iteratively fitting to an increasing number of clusters, and comparing using the Duda-Hart test (Duda and Hart 1973). *k*-means clustering was conducted using the *fpc* package in R (Hennig 2014). Species pools that were based on fewer than three sites were discarded from any subsequent analyses (3% of all sites). This exercise produced 232 sub-ecoregions within 67 MEOWs, with an average of 6 sub-ecoregions within each MEOW.

For each local assemblage, which we considered to be a separate community, we calculated Rao's quadratic entropy (Rao's Q) and the mean pairwise distance (MPD) for both functional and phylogenetic distances. Rao's *Q* is the weighted average of pairwise dissimilarities for all individuals within a community (Botta-Dukát 2005). We additionally incorporated the correction for uneven sample size presented in the supplements of (de Bello et al. 2012). MPD is simply the average of all pairwise distances between species within a community, and does not take into account relative abundances or biomass (Pavoine and Bonsall 2011). Similarly, Rao's *Q* and MPD were calculated for each regional species pool. The diversity of the regional pool (Div_{pool}) was then subtracted from the diversity of each local community (Div_{comm}) to generate an effect size ($ES = Div_{comm} - Div_{pool}$). If the community has higher diversity than the regional pool ($Div_{comm} > Div_{pool}$, or $ES > 0$), then that local assemblage exhibits greater variation in functional traits or phylogenetic distance than the species pool (divergence), which is interpreted as limiting similarity or niche partitioning. If the community has lower diversity than the regional pool ($Div_{comm} < Div_{pool}$, or $ES < 0$), then the local assemblage exhibits less variation in traits or phylogeny than the species pool (convergence), which is interpreted as weak competitor exclusion. If there is no difference ($Div_{comm} \approx Div_{pool}$, or $ES = 0$) then the assemblage is simply a random subset of the regional pool (de Bello et al. 2012). We tested for significant differences from zero using a Student's *t*-test, with $\alpha = 0.05$. For comparisons of effect sizes between latitudinal zones, we used a Welch's *t*-test to account for unequal variances (Fig. 1), hence non-integer degrees of freedom reported in the text.

We used generalized linear mixed effects models (GLMMs) to predict the continuous variation in the above effect sizes with latitude, while accounting for additional covariates, including temperature, nitrates, salinity, and total community abundance and richness. Because we expected a hump-shapred relationship, we specified the latitude effect as a second-order polynomial. We allowed the intercept of the main effects to vary by the random effect of sub-ecoregion identified during the *k*-means clustering. Mixed models were constructed using the *nlme* package in R (Pinheiro et al. 2013). We extracted partial effects for the latitude effect and constructed 95% confidence intervals based on the variance of the fixed effects only using the *effects* package in R (Fox 2003).

We used gradient boosted models (GBM) to understand the relative importance of other drivers of assembly using the package *gbm* (Ridgeway 2014). GBMs are an ensemble learning technique similar to random forests that constructs many individual regression trees and generalizes across them (Friedman 2001). Unlike random forests, however, GBMs build the ensemble sequentially, fitting each subsequent tree to the residuals of the previous ('boosting'). In this way, additional trees in the ensemble focus on the classification errors from the earlier trees, yielding more and more accurate predictions as the ensemble is grown. Trees are added to the ensemble based on how much they minimize a loss function (decrease in predictive power)—in the case of regression trees, the loss function is the mean squared error. Overfitting is discouraged by modifying the 'learning rate', or how drastic the change in the predictions can be from one tree to the next, which is modified by a shrinkage parameter. A final prediction is generated at the end of the procedure by simply averaging across all trees in the ensemble. GBM is ideal for this type of analysis because of its strong predictive power for large or noisy datasets, particularly when predictors are on differing scales, non-normal, correlated, or interact with other variables (Elith et al. 2008).

We fit a GBM to both functional and phylogenetic indices in tropical and temperate zones, including the following predictors: total community richness and abundance, mean and standard deviation of temperate, nitrates, and salinity derived from the Bio-ORACLE dataset (Tyberghein et al. 2012), wave exposure, and an index of human population size derived from a year 2000 world-population density grid as in (Stuart-Smith et al. 2013). To further discourage overfitting, we used the *caret* package to test different ensemble sizes, interaction depths, and shrinkage levels to arrive at the optimal configuration that maximized the reduction in the loss function (Kuhn 2015). We drew inferences from a GBM of 5,000 trees, an interaction depth of 1, and shrinkage of 0.001 with 3-fold cross validation. We then derived estimates of relative influence for each predictor as the reduction of the squared error attributable to that variable (Ridgeway 2014).

Metacommunity Structure

To independently confirm the stronger role of competition in structuring temperate communities, we employed the metacommunity framework proposed in (Presley et al. 2010). The first part of this framework tests for coherence, or the degree to which species replace one another along a gradient of communities (Leibold and Mikkelson 2002). This was accomplished by using reciprocal averaging ordination to define the primary axis along which communities sort within a sub-ecoregion, and then generating a series of null models to test whether the pattern of species co-occurrences was significantly different than what was expected by chance (Presley et al. 2010). If species pairs have mutually exclusive distributions (i.e., 'forbidden combinations') and sort independently of other species pairs along this dimension, then the pattern can be interpreted as strong competitive exclusion, or 'negative coherence' (Connor and Simberloff 1979). Based on results from the community assembly analysis, we would expect negative coherence to become increasingly prevalent if strong interspecific competition is acting to structure local communities. If communities exhibit strong sorting along the primary ordination axis, or 'positive coherence,' then this result could be interpreted as turnover along a residual environmental gradient and further tests can be performed to identify the exact structure (i.e., 'turnover' and 'boundary clumping') (Leibold and Mikkelson 2002). The final possibility is that species do not sort predictably along the primary axis ('random'). To assess different patterns in metacommunity structure, we calculated coherence (Connor and Simberloff 1979) using the *metacom* package in R (Dallas 2014), modifying the functions to incorporate the new *commsim()* function in the *vegan* beta package to construct null models (see www.github.com/taddallas/metacom). We used a variant of the fixed-fixed model ('quasiswap') (Miklós and Podani 2004) suggested in (Ulrich and J. Gotelli 2007). All analyses, including those for community assembly and RF, were conducted in R version 3.1.1 (R Development Core Team 2015).

Results

Based on our comparison of local to regional diversity, we found evidence for significant functional convergence in both tropical ($t_{606} = -32.08$, $P < 0.001$) and temperate communities ($t_{1068} = -34.03$, $P < 0.001$) (Fig. 1a, Fig. S1). Functional convergence was, on average, twice as strong in temperate communities as in tropical ones, based on comparison of effect sizes ($ES_{Trop} = -0.045$ vs. $ES_{Temp} = -0.090$, Welch's $t_{1537.7} = 15.06$, $P < 0.001$). We also found significant phylogenetic convergence in tropical ($t_{606} = -6.94$, $P < 0.001$) and temperate communities ($t_{1068} = -29.02$, $P < 0.001$). In this case, there was a seven-fold increase in phylogenetic convergence across latitude ($ES_{Trop} = -0.006$ vs. $ES_{Temp} = -0.042$, Welch's $t_{1607.3} = 21.32$, $P < 0.001$), and tropical communities were very close to exhibiting a null pattern (Fig. 1b, Fig. S1). To visualize the increase in functional and phylogenetic clustering with increasing latitude, we continuously plotted effect sizes against latitude and fit a simple second-order polynomial (blue curves, Fig. 2).

Because these patterns might still be attributable to large-scale variation in abiotic parameters (as opposed to the fine-scale drivers used in our partitioning of regions), or to variation in sampling intensity, we employed a mixed model approach regressing the functional and phylogenetic effect sizes against a second-order polynomial of latitude, the mean temperature, nitrates (as a proxy for resource availability), and salinity, and total community richness and abundance as covariates. We then extracted and plotted the partial effect of latitude given all of these covariates (red lines, Fig. 2). Even after accounting for these major environmental gradients and potential differences in effort reflected in sampled richness and abundance, we found that there was still a significant decline in the effect size with increasing latitude for both functional and phylogenetic diversity ($P < 0.001$ for both linear and non-linear estimates of the latitude effect) (Fig. 2). In contrast with the results from the *t*-tests, the effect size was no different than zero for phylogenetic diversity in tropical communities, based on overlapping confidence intervals (Fig. 2b). Thus, there appear to be additional factors beyond variation in major sources of physiological stress (temperature and salinity), resources (nitrates), and the sampling intensity in driving the observed patterns in assembly with latitude.

To independently confirm the effect of latitude, we employed gradient boosted models (Friedman 2001) to rank the relative influence of latitude, environmental, and other variables on our index of assembly, including: the mean and variance in regional temperature, nitrates, and salinity, total community richness and abundance, and an index of human population density. Latitude was ranked among the most important predictors for both functional and phylogenetic assembly across temperate and tropical regions, and in several cases was the single most important predictor by a considerable margin (Fig. 3). Only richness was identified as having a stronger influence than latitude for phylogenetic assembly in temperate regions (Fig. 3c). Beyond latitude and richness, the next most important predictors were community abundance and an index of human population density, particularly in temperate regions. Partial dependency plots revealed a trend towards increasing convergence with increasing abundance in the tropics, and the opposite in temperate regions (Fig. S2a). Similarly, increased human population density generally drove functional and phylogenetic convergence, but this effect became negative quickly after a high threshold of density (Fig. S2b).

One possible explanation for the increasing functional and phylogenetic convergence with latitude is that we did not sufficiently account for important local environmental variation in reducing the regional species pool to species with similar abiotic tolerances. To test this, we used meta-community theory to test whether there were any non-random patterns in species distributions within regions (Presley et al. 2010). If any residual environmental variation remained within a region, then communities should sort predictably along it, with species (or groups of species) with similar preferences being replaced at discrete environmental boundaries (e.g., Gleasonian or Clementsian pattern, Leibold and Mikkelson 2002). Out of 122 regions along the entire latitudinal gradient, we identified only three that demonstrated non-random sorting along an environmental gradient (Fig. 4). The majority exhibited random sorting, and approximately one-sixth exhibited a checkerboard pattern. which is generated when pairs of species are never found together (so called 'forbidden combinations,' Leibold and Mikkelson 2002), and these pairs sort exclusively of one another within a region. This pattern is most often interpreted as strong interspecific competition (Presley et al. 2010), and the number of ecoregions demonstrating a checkerboard pattern increased with increasing latitude (Fig. 4).

We conducted several additional analyses to assess the robustness of these results. First, we repeated our analysis using a second distance-based index of diversity, the mean pairwise functional or phylogenetic distance among all species within an assemblage (MPD, Pavoine and Bonsall 2011), to ensure that these results were not influenced by our choice of metric. This index revealed identical patterns to the main analysis using Rao's *Q* (Fig. S3). Second, we calculated abundance- and biomass-weighted indices of Rao's *Q*, under the assumption that patterns of niche-partitioning or competition would more strongly manifest in patterns of relative abundance or biomass. All weighting schemes produced qualitatively similar results (Fig. S4), so we have chosen to present the equally-weighted values in the main text. Third, we constructed phylogenies using two different methods: Bayesian inference and maximum-likelihood (ML), and re-evaluated the phylogenetic patterns with latitude (Fig. S5).

Given the overall lower confidence intervals on the ML tree, we have chosen to present inferences from the Bayesian tree in the main text. Finally, we evaluated each trait individually (Table S1), to understand whether conflicting patterns among individual traits may have altered or weakened the overall trends presented in Figure 2a. We found that most traits followed the same pattern as multivariate functional diversity, except for diel activity pattern ('time') and habitat complexity requirements, which both showed an inverse parabolic but relatively weak relationship with latitude (Fig. S6). Similarly, patterns derived from the multivariate index of functional diversity (Fig. 2a) appear to be driven largely by traits relating to trophic ecology and water column position (Fig. S6).

Discussion

Overall, we found significant functional and phylogenetic convergence of local communities relative to the regional species pool (Fig. 1), and that this convergence increased with increasing latitude (Fig. 2). This trend appears to be independent of several major axes of environmental variation, richness, and potential differences in sampling intensity (Fig. 2, 3). One possible explanation for our results is that temperate communities are dominated by fishes with greater dispersal and/or lower site fidelity, leading to greater exchange of species among sites at higher latitudes and greater regional functional and phylogenetic homogenization, in turn minimizing differences among local assemblages and the regional species pool. We have two lines of evidence that indirectly address this prediction. First, the average size of individuals recorded on the surveys increased with increasing latitude, particularly in the well-sampled southern hemisphere (Fig. S9), which corresponds with previous trends observed in marine fishes (Ray 1960), and recent work has linked both latitude and body size in marine fishes to dispersal potential (Hillebrand 2004, Bradbury et al. 2008). Second, we scored a functional trait that partitions species based on whether they are benthic-associated or live in the water column and, if the latter, whether they are mobile or site-attached (Table S1). There was a slight increase in the frequency of species that were considered 'pelagic, site-attached' with increasing latitude (Fig. S10). This interpretation is compatible with existing theory linking high dispersal and larger range size of temperate species with lower rates of gene flow and speciation, which in turn reduces diversity at high latitudes (Janzen 1967, Dynesius and Jansson 2000, Jablonski and Roy 2003).

Another potential explanation for increasing convergence with latitude is that there exists some optimum set of traits (reflected in phylogeny) that leads to increased competitive success in temperate regions, excluding species that possess competitively inferior traits (Mayfield and Levine 2010, de Bello et al. 2012, HilleRisLambers et al. 2012). Our meta-community analysis would partially support this viewpoint, with potentially competing pairs of species co-occurring less often than would be expected by chance in many high latitude regions (Fig. 4). This explanation runs counter to existing theory implicating stronger interspecific competition in the tropics, with abiotic filtering operating more strongly at high latitudes (Pianka 1966, Mittelbach et al. 2007). However, a recent analysis of ant communities found that environmental filtering acted primarily at very large (continental) scales, with local interactions playing a stronger role as the pool was increasingly restricted to reflect more realistic dispersal scenarios (Lessard et al. 2012). Thus, our inferences may be a consequence of the scale at which we constructed our species pools to better reflect the potential for biological interactions.

Along those lines, we identified human population density as a potentially important driver of assembly behind latitude and species richness (Fig. 3). Partial effects plots revealed that higher population density is predicted to increasingly drive functional and phylogenetic convergence, particularly in temperate zones (Fig. S2). The ability of anthropogenic activities such as fishing and habitat degradation to reduce or alter the functional structure of fish communities is increasingly recognized (Villéger et al. 2010, Martins et al. 2012). These actions may facilitate convergence through the targeted removal of certain functional types or phylogenetic lineages. It may also increase competition by, for instance, removing larger predators. However, the influence of human population density was relatively weak compared to latitude (Fig. 3), suggesting that if it does play a role, it is probably minor. However, human density was measured on a coarser scale than other variables included in our analysis, and higher resolution estimates may shed additional light on this hypothesis.

It is also possible that our finding of greater convergence at higher latitudes as an artifact of low species richness at high latitudes (Fig. S7). For example, we observed relatively little change in functional diversity at the regional level across latitude (Fig. S8), and thus greater convergence at high latitudes could potentially have been a consequence of sampling fewer species from a relatively consistent functional pool. In other words, the probability of drawing a set of functionally dissimilar species is potentially much lower in species-poor temperate regions than in species-rich tropical ones. This is likely why richness emerged as having a significant influence on functional assembly in our gradient boosted models (Fig. 3a,b). However, we note that we specifically incorporated richness as a covariate for this reason in our analyses, and latitude still emerged as a significant predictor of functional patterns by quite a large margin (Fig. 3).

Finally, we must acknowledge that it is possible that we did not adequately account for the full suite of important environmental factors when defining the regional species pool, and thus the latitudinal trend still reflects some unmeasured environmental driver. We have taken several steps to ensure that this is not the case. As a first pass, we grouped species based on 'Marine Ecoregions of the World,' which are, "clearly distinct from adjacent systems, [determined] by the predominance of a small number of ecosystems and/or a distinct suite of oceanographic or topographic features," and are, "strongly cohesive units, sufficiently large to encompass ecological or life history processes for most species" (p. 575, Spalding et al. 2007). Within these ecoregions, we used fine-scale data to cluster sites based on a suite of environmental variables, and used the species observed across all sites within a cluster to construct the 'regional' pool. Because we used only observed species, we likely excluded many species that could potentially be at those sites but were absent or missed during the surveys ('dark diversity', Pärtel et al. 2011). However, the framework we employed has been shown to be insensitive to underestimates of the species pool (de Bello et al. 2012). There may have been, however, some unmeasured or unimportant environmental variables not included in our analysis. We have some assurance that this was not the case as our meta-community analysis revealed only a handful of regions where communities sorted along a detectable residual gradient (Fig. 4). Even so, recent work has shown that functional- and phylogenrtic-patterns are largely robust to changes in scale that would have been generated by our efforts to generate a realistic species pool (Münkemüller et al. 2014).

Another interesting outcome of our analysis is the greater proportional change in our index of phylogenetic versus functional community assembly across latitude (Fig. 1). This difference is consistent with the idea that phylogenetic distance may better reflect the entirety of functional variation among species than does a small suite of traits (Srivastava et al. 2012), and thus reflects unmeasured traits important in influencing biotic interactions. The application of phylogeny in lieu of functional traits may be especially relevant for coral reef fishes, for which functional differences between the numerous species may be finer than can be encompassed in large-scale studies. For example, herbivorous tropical fishes are covered within two coarse trophic groups in our analysis ('browsing' and 'scraping', Table S1), but they are known to demonstrate a wide variety of specialized feeding modes and diet preferences (Choat et al. 2002). This finer phenotypic variation may be better reflected in their genetic variation than feasibly covered in trait allocation for studies at scales large enough to provide generalizable results. Thus, our phylogenetic results may prove to be more robust until challenged with higher resolution trait data. A caveat to this idea is the differences we found in the mixed model inferences based on the tree recovered using Bayesian inference vs. maximum-likelihood (Fig. S5). There have been few investigations into how methodological choices made during phylogeny reconstruction—including gene(s) used, the model of evolution, and the incorporation of Bayesian priors—may influence inferences about ecological processes (Tucker and Cadotte 2013), and our analysis suggests that these may play a much larger role than previously suspected. However, the low confidence intervals on the ML tree and the difficulty with which it was converted into ultrametric distances preclude us from leaning too heavily on this line of inquiry, and suggest future explorations using a smaller, more amenable dataset.

On a similar note, while inferences from *t*-tests suggest phylogenetic clustering at low latitudes, confidence intervals on the predicted fits from mixed models overlap zero at tropical latitudes (Fig. 2b). This discrepancy between the two analyses is likely the result of incorporating both a hierarchical random structure and, more importantly, known environmental filters such as temperature, nitrates, and salinity. Thus, from both a statistical and biological standpoint, it may be more accurate to interpret the results as suggesting random assembly at low latitudes. In this case, our results are consistent with the 'lottery hypothesis' (Sale 1977), which posits that coral reef communities are not structured by resource partitioning, but by variation among species in their ability to colonize heterogeneous landscapes (Bode et al. 2011). Again, smaller body sizes in the tropics may support this idea by restricting species' ability to move between suitable habitats (Fig. S9). It is important to note that, under this scenario, species could in fact be under strong interspecific competition, but their success is instead determined by chance colonization (Shinen and Navarrete 2014). Such neutral dynamics would produce the seemingly random functional or phylogenetic structure of local tropical communities, but could obscure the true degree of biological interactions.

Many recent analyses of community assembly have found an overwhelming role for environmental filtering and a relatively minimal role for biology, an outcome which may lie with an insufficient ability to disentangle biological from environmental patterns driving functional and/or phylogenetic convergence (Kraft et al. 2014). Using both functional and phylogenetic approaches, we demonstrate that biotic interactions do play a role in structuring global reef fish communities above and beyond what is accounted for by several major axes of environmental variation. The results of this study provide some of the first truly global insights into the role of biological interactions in structuring communities above and beyond several known drivers of environmental filtering.

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Figures

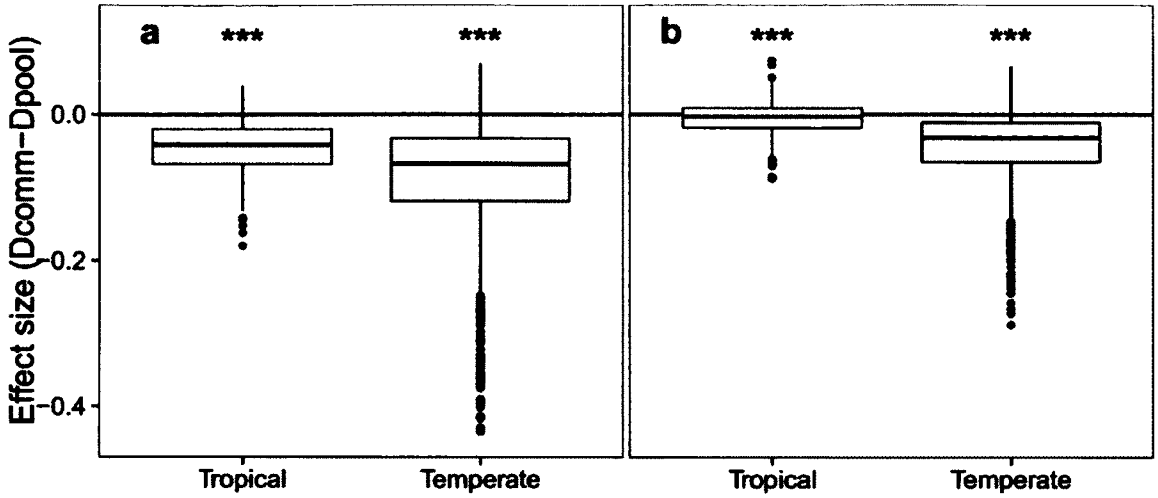

Figure 1: (a) Functional and (b) phylogenetic patterns of community assembly revealed significant convergence in both tropical and temperate regions. Effect sizes were calculated as functional or phylogenetic diversity of the regional species pool (Dpool) subtracted from the diversity of a local community (Dcomm). A mean effect size significantly less than 0 indicates functional or phylogenetic convergence (all mean effect sizes were significantly less than 0, *** = $P < 0.001$). Tropical communities were defined as those between -20 and 20° latitude, and temperate ones anything outside of the tropical range.

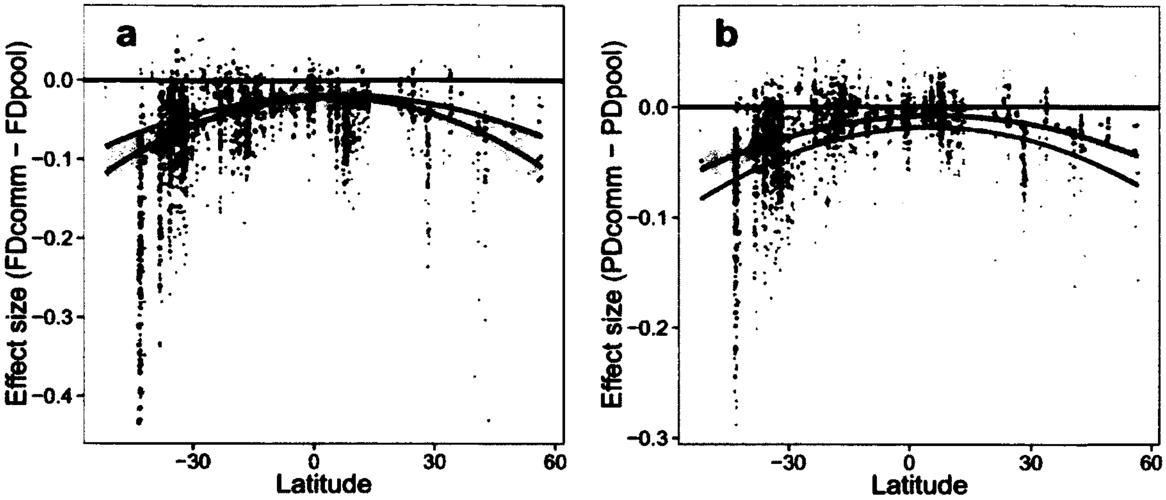

Figure 2: (a) Functional and (b) phylogenetic convergence increased with increased latitude. Effect sizes were calculated as functional or phylogenetic diversity of the regional species pool (Dpool) subtracted from the diversity of a local community (Dcomm). An effect size > 0 indicates functional or phylogenetic divergence, an effect size < 0 indicates convergence, and an effect size = 0 indicates stochastic processes or environmental filtering. Blue lines indicate predicted fits from a simple second-order polynomial regressing effect size against latitude. Red lines indicates predicted fits ± 95% confidence bands of the partial effects of latitude from a mixed model regressing effect size against the second-order polynomial of latitude plus additional environmental covariates (see description in main text).

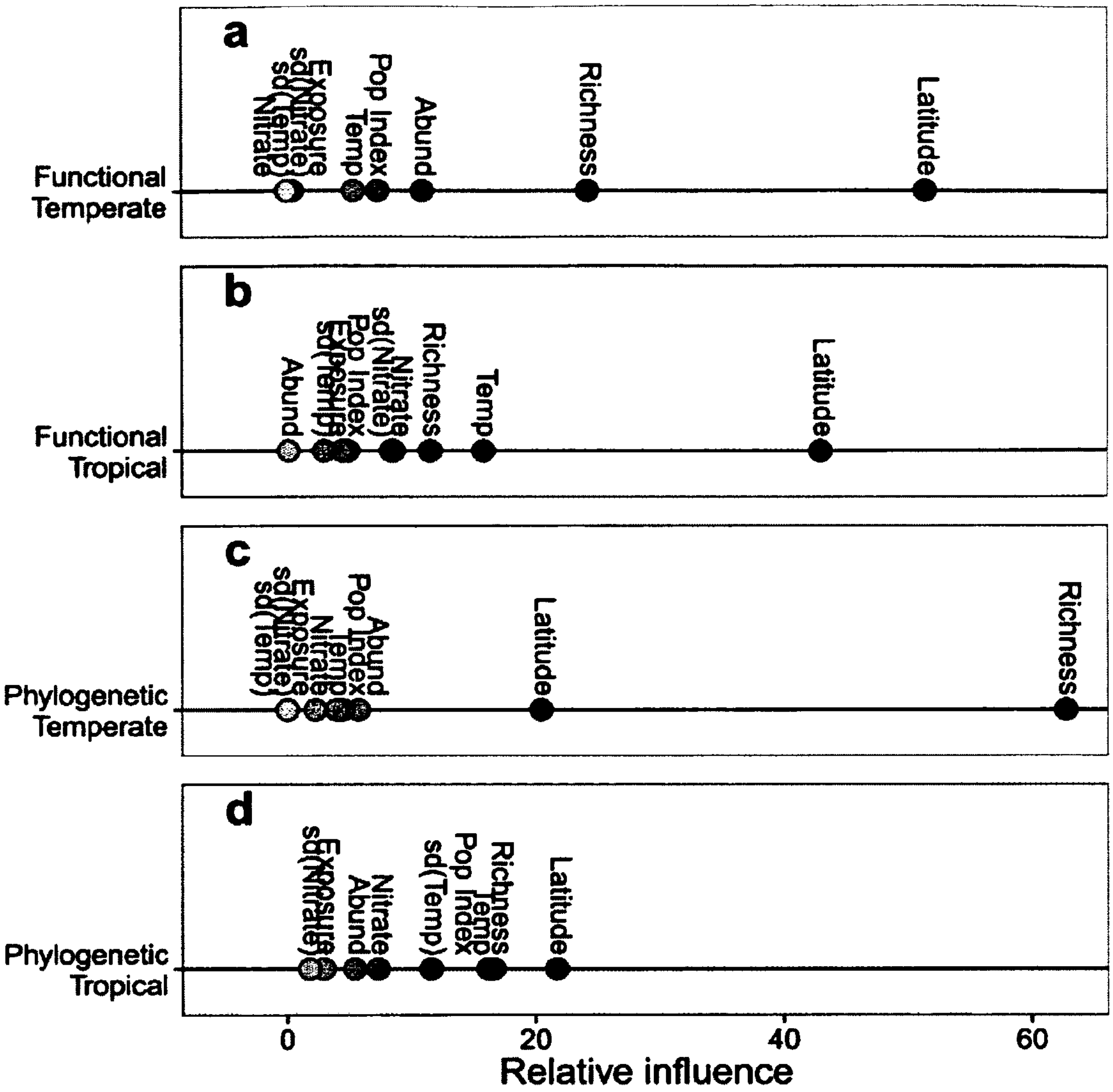

Figure 3: Gradient boosted models revealed richness, latitude, and human population index to be among the most important predictors of (a-b) functional and (c-d) phylogenetic patterns in community assembly. Temperate and tropical regions are defined as in Figure 1. The x-axis represents the mean percentage decrease in prediction accuracy across each tree in the random forest for the out-of-bag sample based on permutations of the variable of interest. Higher values denote a greater loss of accuracy, or greater importance, for that variable.

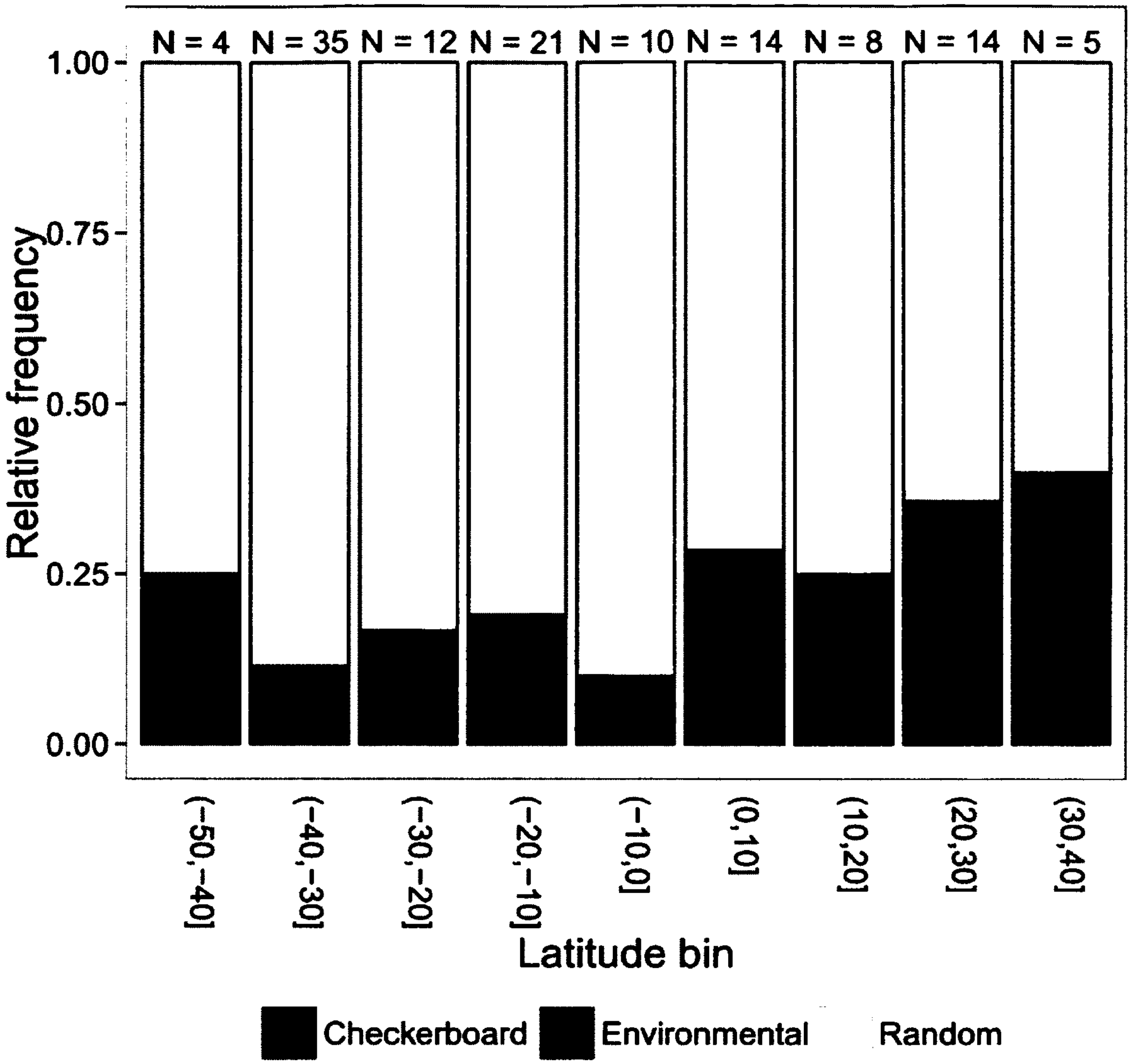

Figure 4: Metacommunity analysis revealed few regions structured along residual environmental gradients (light grey bars), instead exhibiting either random or checkerboard patterns, the latter of which can be interpreted as strong interspecific competition. The x-axis is latitude in 10° bins, and the y-axis is the proportional frequency of regions within each bin that exhibit random, checkerboard, or environmentally structured patterns. The number of regions (*N*) within each latitudinal bin (x-axis) are given above the bars.

Supplementary Tables

Table S1: Functional traits used in the analysis, and their expected effect on assembly mechanisms.

Trait	Values	Expected effect	Explanation
Maximum length	Continuous (cm)	Divergence	Resource partitioning
Trophic breadth	Number of prey phyla consumed (1-8)	Divergence	Resource partitioning
Trophic group	Browsing herbivore, scraping herbivore, benthic invertivore, planktivore, higher carnivore	Divergence	Resource partitioning
Water column position	Benthic, demersal, site-attached pelagic, roaming pelagic	Divergence/ convergence	Space and habitat partitioning
Diel activity pattern	Nocturnal, diurnal	Divergence	Resource partitioning, predator avoidance
Preferred substrate	Hard substrate, soft sediment	Convergence	Resource partitioning, predator avoidance
Habitat complexity requirements	Low, medium, high	Convergence	Space and habitat partitioning

Supplementary Figures

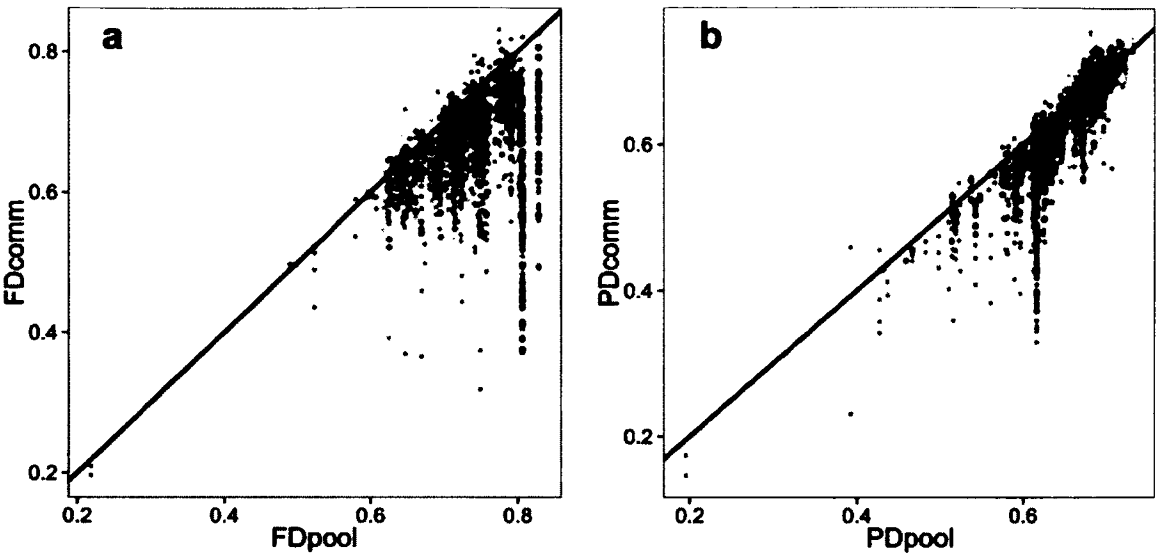

Figure S1: (a) Functional and (b) phylogenetic diversity of the regional species pool against local diversity. Individual points represent individual surveys. Red points indicate tropical sites and black ones temperate sites (defined as Figure 1 caption).

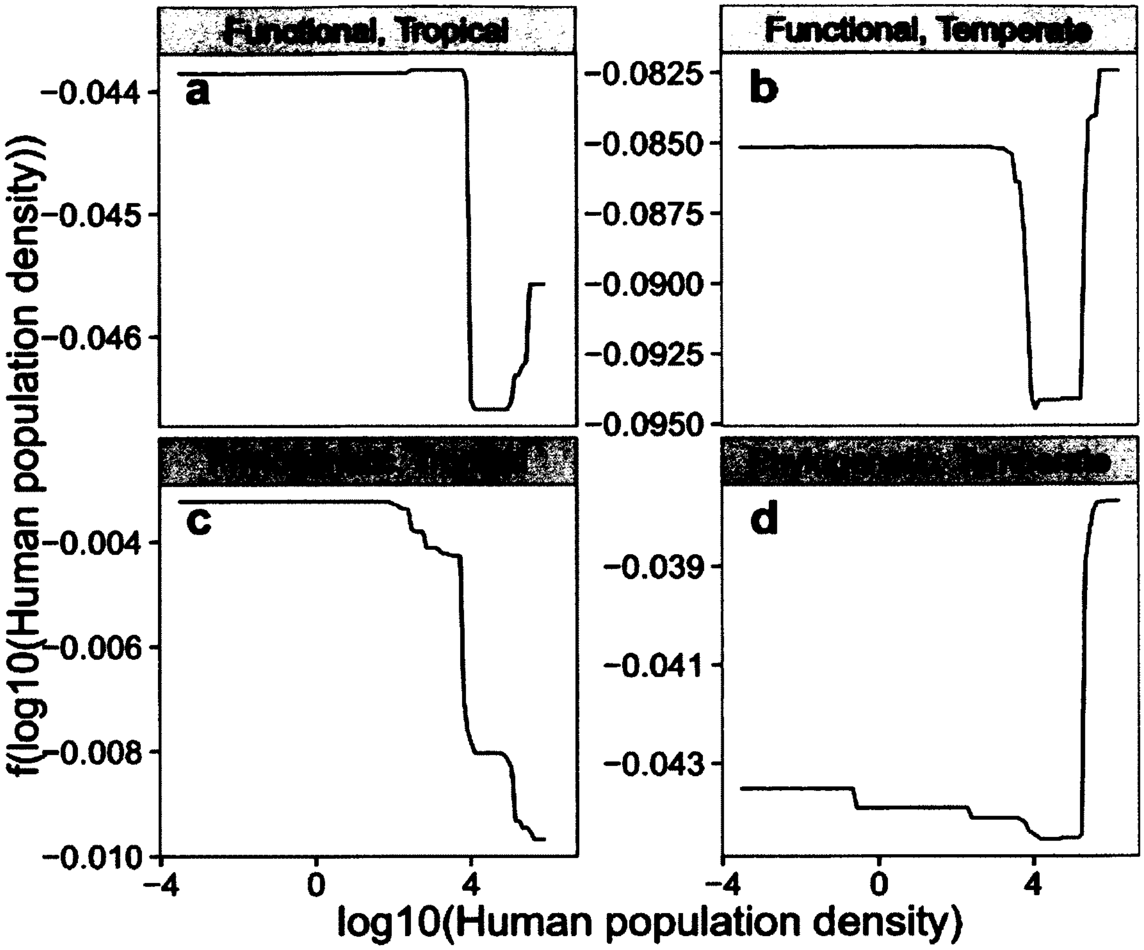

Figure S2: Partial dependency plot of log10-transformed index of human population size against (a-c) functional and (c-d) phylogenetic effect sizes. Upper panels (a, c) represent tropical sites, and lower panels (b, d) represent temperate ones (defined as Figure 1 caption).

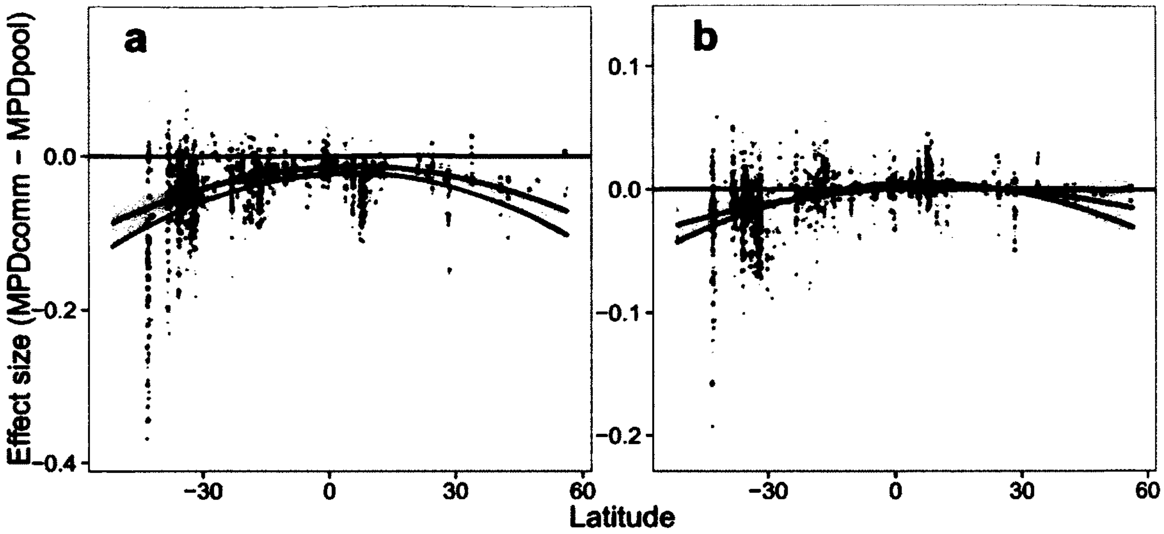

Figure S3: Effect sizes derived from the mean pairwise distance (MPD) index of (a) functional and (b) phylogenetic diversity. Interpretation is identical to Figure 2 in the main text, which reports the same effect sizes derived from a different index of diversity, Rao's Quadratic entropy.

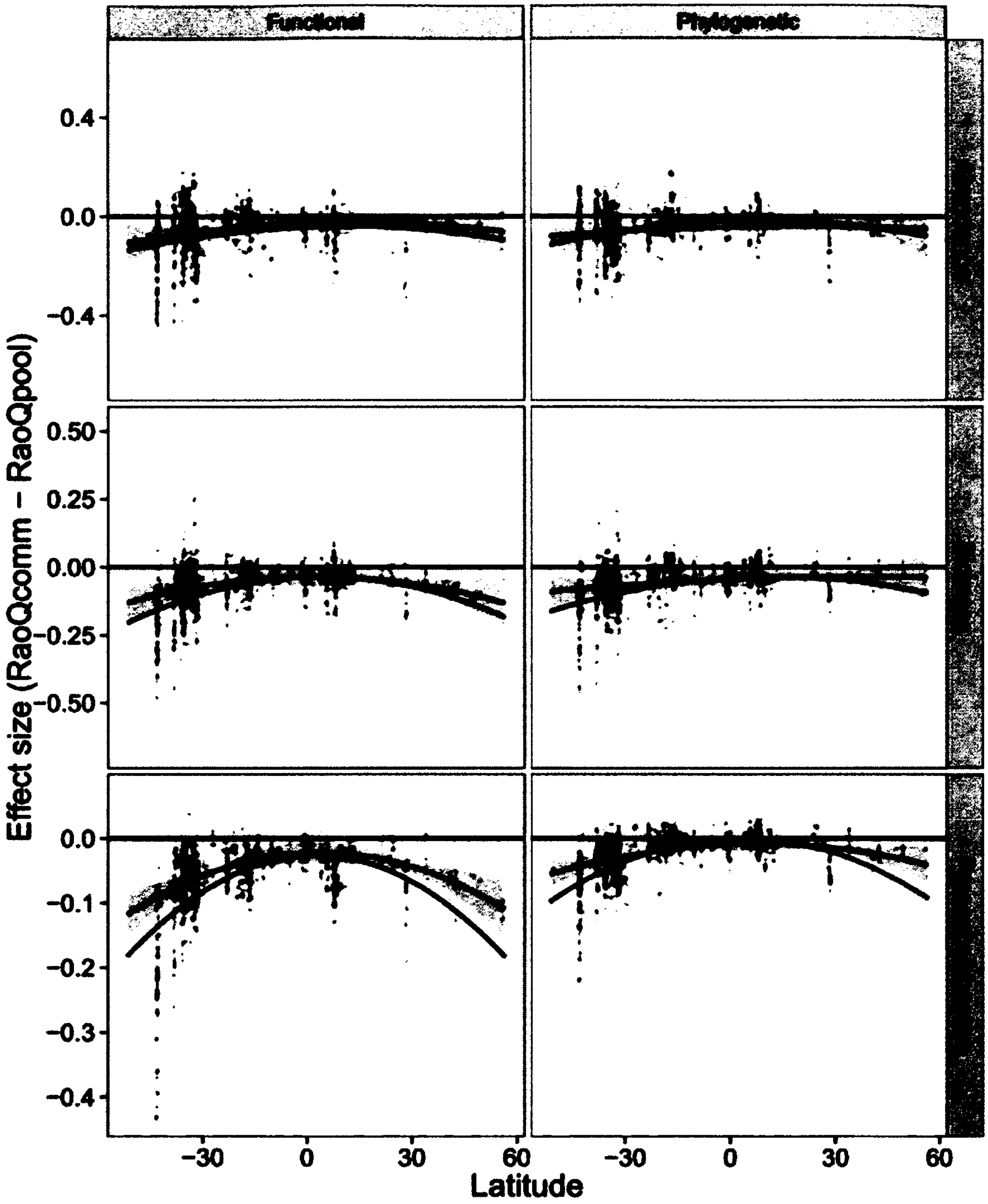

Figure S4: Abundance- (upper panels), biomass- (middle panels), and equally-weighted (lower panels) estimates of functional (left side) and phylogenetic (right side) effect sizes against latitude. Interpretation is identical to Figure 2 in the main text, which corresponds to the lowest set of panels.

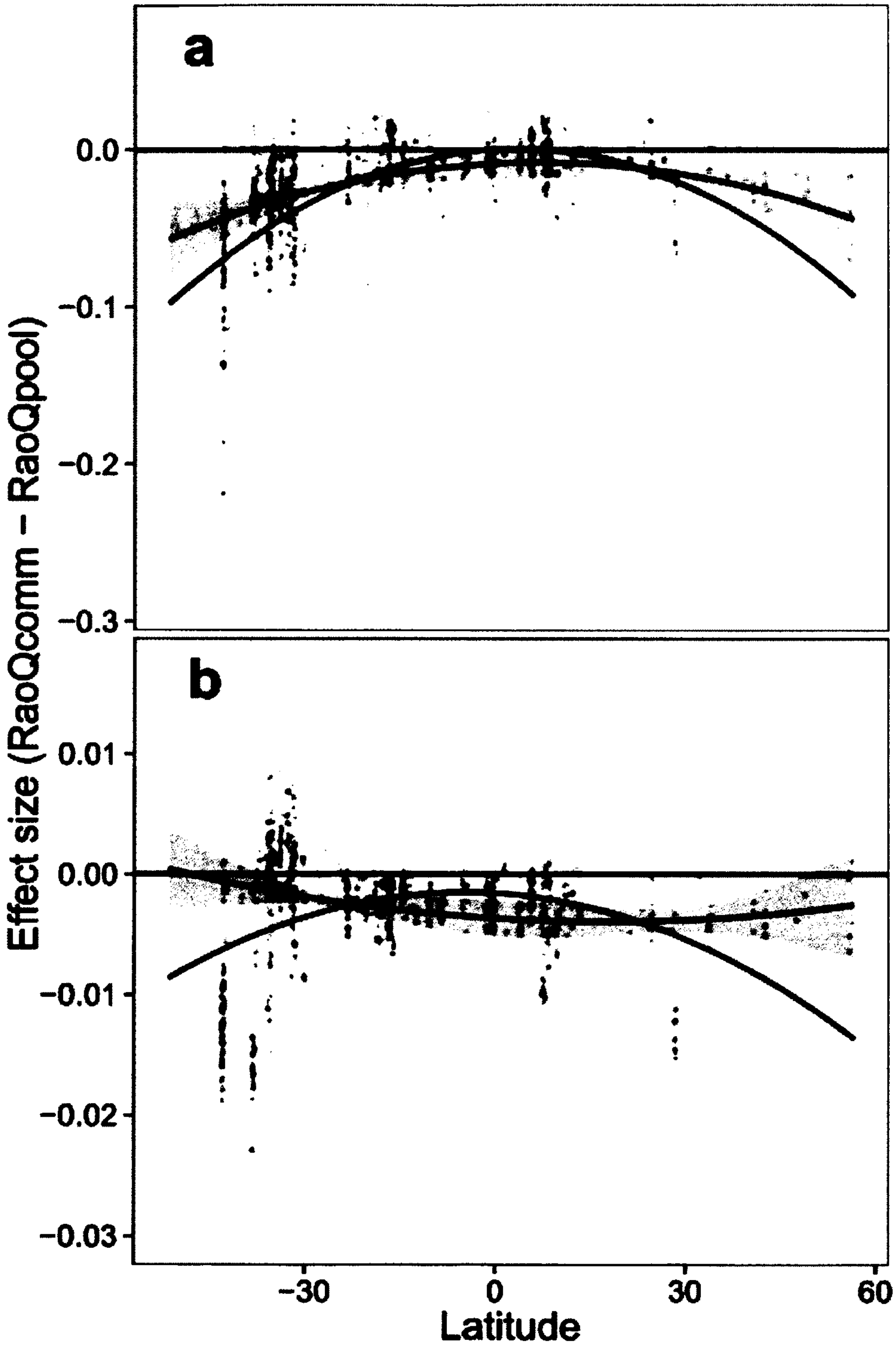

Figure S5: Estimates of phylogenetic effect sizes against latitude for phylogenies derived from (a) Bayesian approximation and (b) maximum-likelihood. Interpretation is identical to Figure 2b in the main text.

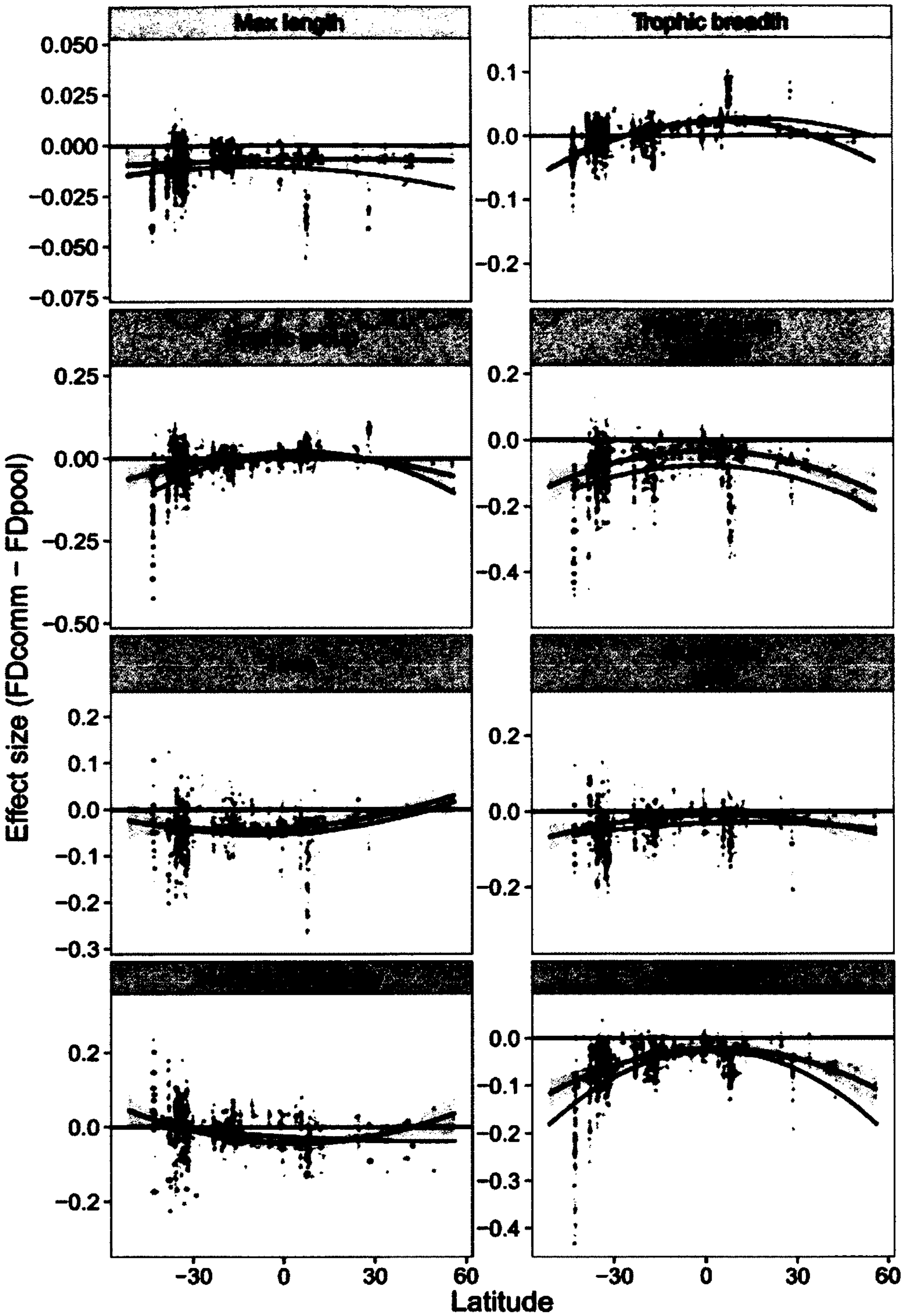

Figure S6: Estimates of functional effect sizes derived from individual traits (Table S1). Interpretation is identical to Figure 2a in the main text, which corresponds to the lower right panel.

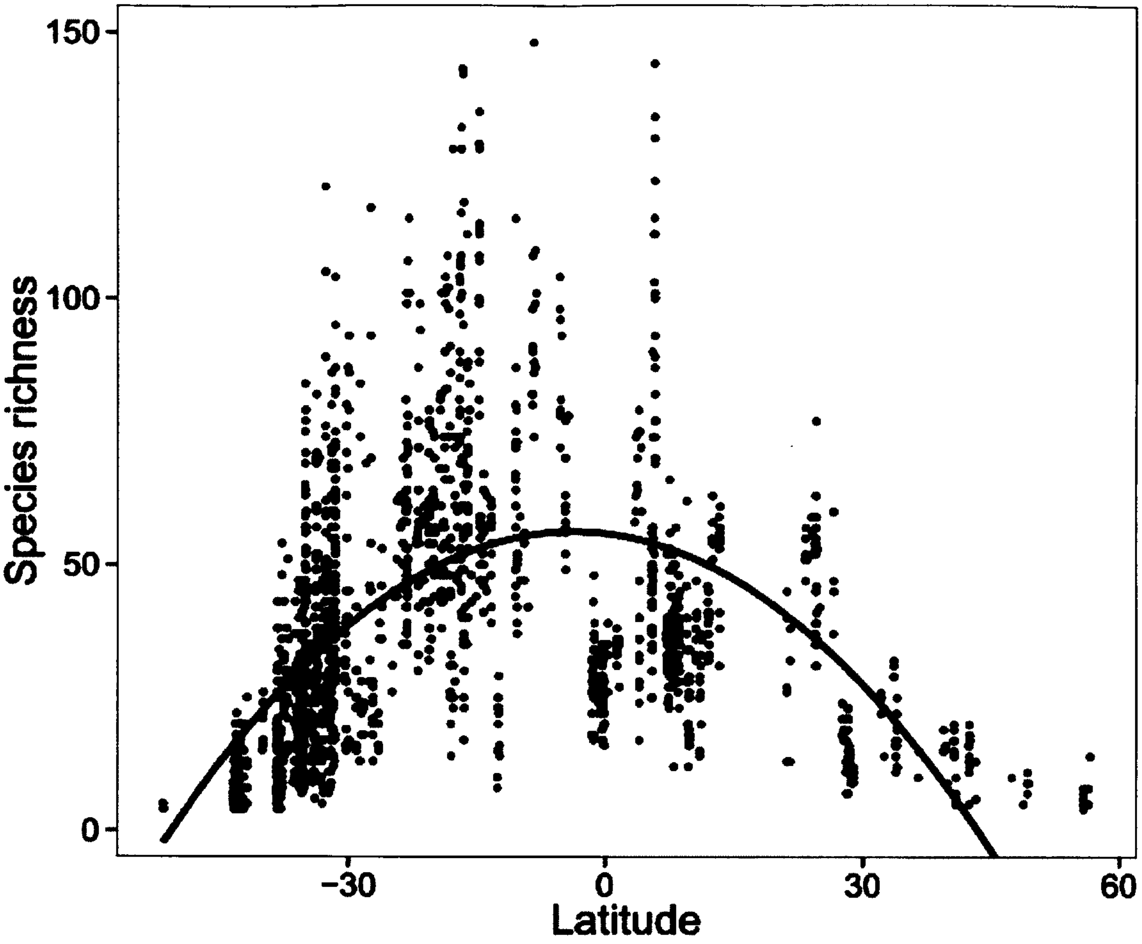

Figure S7: Species richness against latitude. Blue line is the predicted fit from a simple second-order polynomial regression.

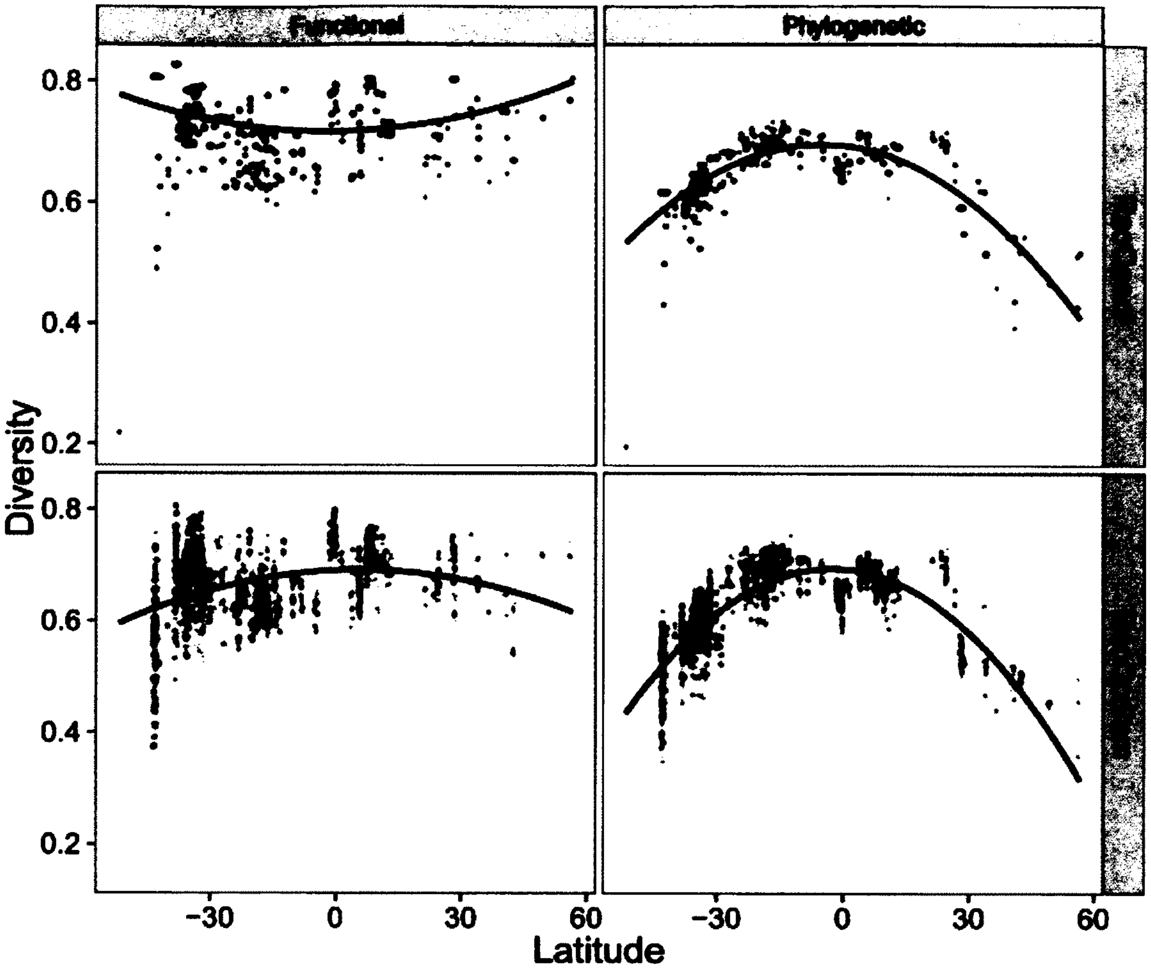

Figure S8: Plots of regional (upper panels) and local diversity (lower panels) for functional (left panels) and phylogenetic diversity (right panels). Blue line is the predicted fit from a simple second-order polynomial regression.

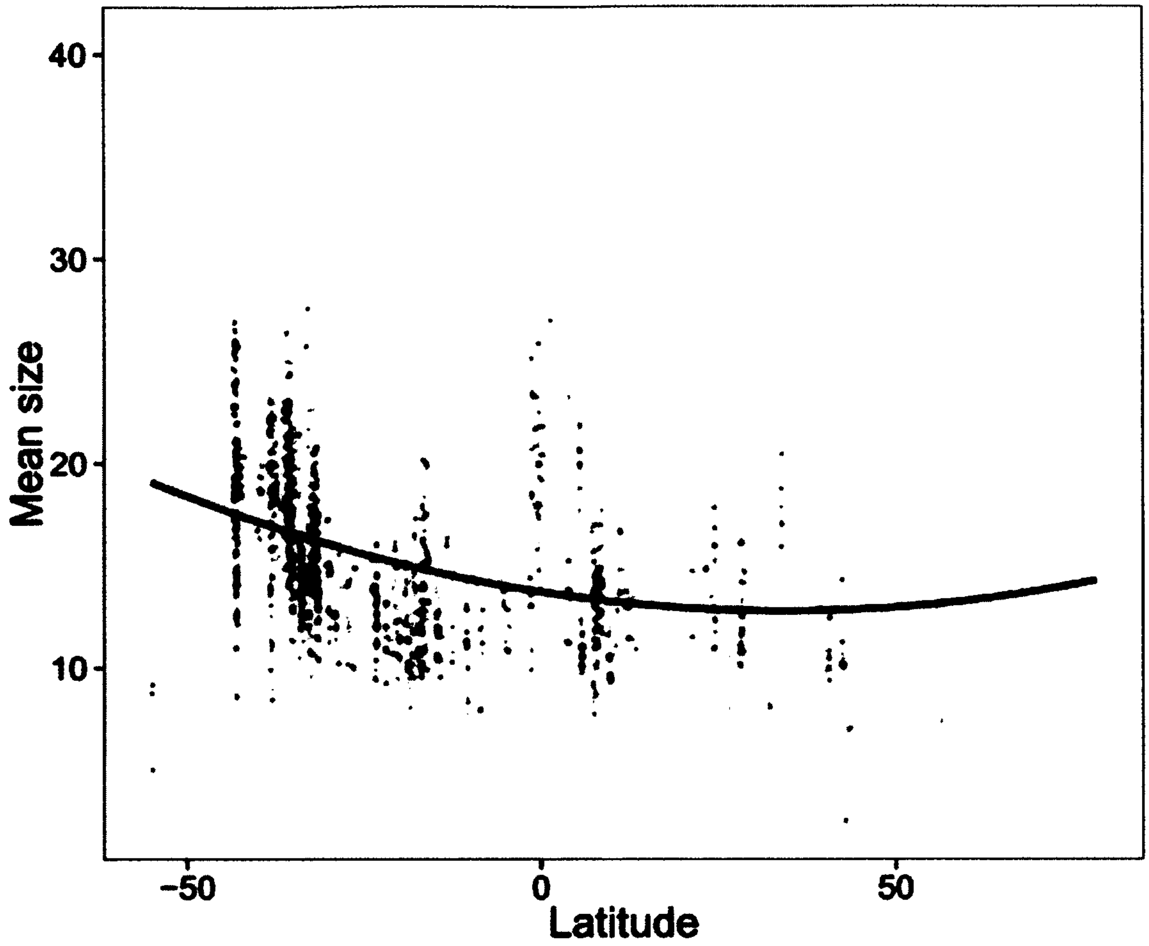

Figure S9: Mean body size per survey against latitude. Blue line is the predicted fit from a simple second-order polynomial regression.

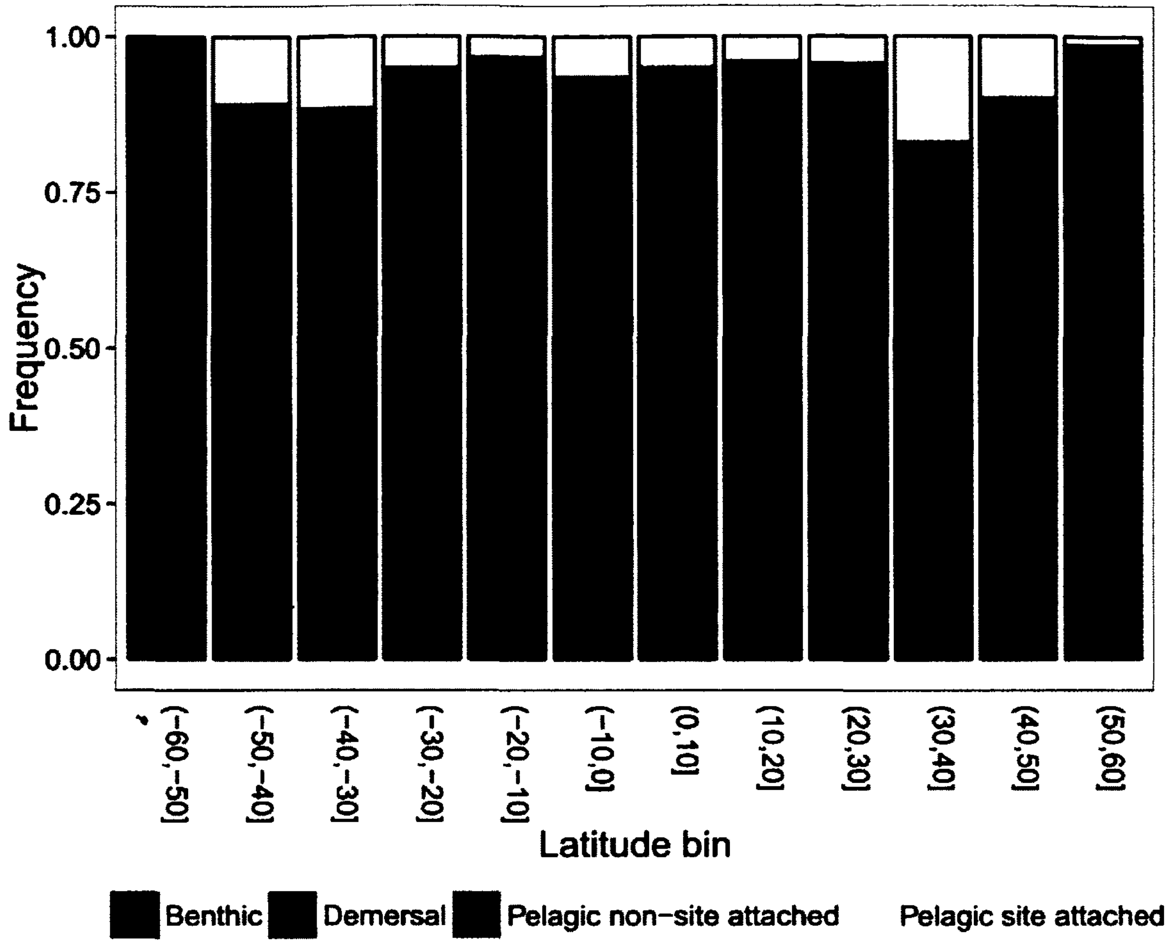

Figure S10: The frequency of species occupying different values of the functional trait 'water column position' against latitude.

CHAPTER 2.

TOP-DOWN VERSUS BOTTOM-UP CONTROL OF A TEMPERATE EELGRASS BED: INSIGHTS FROM A 15-YEAR ECOLOGICAL SURVEY

Abstract

The relative influence of top-down and bottom-up control of ecosystems has long been debated in ecology. Temperate eelgrass systems (*Zostera marina* L.) provide a model for the investigation of resource versus consumer control, especially in light of anthropogenic nutrient loading and overfishing of this ecosystem. We used data from a 15-year ecological survey of a local eelgrass bed in the York River Estuary, Chesapeake Bay to explore patterns in community abundance, biomass, and structure. We further integrated biological information with environmental data using structural equation modeling (SEM) to test the relative strengths of biotic vs. abiotic drivers of community properties. We identified strong seasonal turnover in species but not functional composition, especially for invertebrate mesograzers, implying a high degree of redundancy among grazers throughout the year. Through SEM, we also found evidence for alternating trophic control, with significant top-down effects of predators and grazers more evident in the spring and summer, respectively, and bottom-up effects of nutrients only apparent in the fall. Not surprisingly, this strongly seasonal system appears to be most controlled by variation in abiotic variables, principally temperature and light, whose influence changes seasonally. This long-term dataset provides insight into the role of biology in an important coastal ecosystem despite natural variation in environmental parameters.

Keywords: eelgrass, epifauna, grazers, predators, top-down, bottom-up, estuaries, structural equation modeling

Introduction

The role of top-down versus bottom-up control of community structure and function has been a longstanding question in ecology (Hairston et al. 1960, Power 1992, Strong 1992), leading to numerous studies to understand when, where, and why systems are under resource and/or consumer control (reviewed in Shurin et al. 2002, Gruner et al. 2008). Temperate seagrass systems, principally eelgrass *Zostera marina* L., have served as a model for investigations of top-down and bottom-up control (reviewed in Hughes et al. 2004, Valentine and Duffy 2006). These important coastal ecosystems are characterized by a numerous and diverse assemblage of epifaunal invertebrate 'mesograzers' that feed primarily on the epiphytic algae growing on seagrass blades (Orth et al. 1984, Valentine and Duffy 2006). This grazing can indirectly benefit the seagrass by removing the fouling epiphytes which block light and compete with the seagrass for nutrients (Orth and van Montfrans 1984, Orth et al. 1984, Duffy et al. 2015). However, increasing anthropogenic pressures in the form of coastal nutrient loading and overfishing may threaten this balance by spurring both epiphyte growth (eutrophication) and removing large predators, potentially freeing smaller predators to reduce grazer populations (Valentine and Duffy 2006, Duffy et al. 2015).

The dual threats of nutrient enrichment and grazer reduction have led to a number of experiments to identify which has more severe implications for eelgrass ecosystems. Generally, this question has been addressed using factorial manipulations crossing fertilization with either the inclusion or exclusion of predators using field cages. The results, however, have been mixed: some experiments have found evidence for stronger top-down control (Neckles et al. 1993, Heck Jr. et al. 2000, Moksnes et al. 2008, Lewis and Anderson 2012), while others for stronger bottom-up control (McGlathery 1995, Heck Jr. et al. 2006), and still others have found relatively equivalent (Douglass et al. 2007) or contrasting effects (Baden et al. 2010). More recent cageless exclusions of grazers in seagrass beds have supported stronger top-down influence (Cook et al. 2011, Whalen et al. 2013, Myers and Heck Jr. 2013, Reynolds et al. 2014, Duffy et al. 2015), while omitting many of the artifacts associated with cage exclusions (Douglass et al. 2007).

These experiments also reveal that there are contingencies relating to top-down vs. bottom-up control that have been uncovered over two decades of investigation. For instance, the presence of omnivorous grazers (Heck Jr. et al. 2000) and predators (McGlathery 1995, Heck Jr. et al. 2006) can circumvent trophic cascades by feeding directly on the seagrass itself. The degree of top-down control has also been shown to vary temporally and along environmental gradients. Neckles et al. (1993) found stronger top-down control during summer versus spring and fall as a consequence of higher ambient nutrient concentrations, lower temperatures, and lower grazer densities, and Whalen et al. (2013) showed that the effect of grazer reduction changed on the scale of weeks, commensurate with the sustained application of the experimental treatment and the natural senescence of *Z. marina* in this region. Finally, Baden et al. (2010) showed that control switched from top-down to bottom-up along an increasing salinity gradient. In sum, these results would suggest a pivotal but context-dependent role for top-down control in temperate seagrass beds, a conclusion also reached in a meta-analysis of 34 nutrient enrichment and/or grazer removal experiments by Hughes et al. (2004).

Recent experiments have built on this tradition by exploring how biological diversity and the degree of consumer control in seagrass systems. Duffy et al. (2001, 2003, 2005) showed positive effects of grazer species richness on grazer and, in some cases, eelgrass biomass, and negative effects on epiphyte biomass in experimental mesocosms. The effects of diversity appear to be robust to nutrient, temperature, and freshwater perturbations (Blake and Duffy 2010, 2012), and have recently been shown in *in situ* experiments conducted at 15 field sites across the northern hemisphere (Duffy et al. 2015). There is growing recognition, however, that species richness is a coarse measure which does not capture that some species vary widely in their ecological strategies, while others vary little. Seagrass fauna is no exception, and recent experimental evidence suggests that such ecological differences can be elucidated through the use of functional traits, aspects of the organism's biology, physiology, and life history that define how they interact with their environment and with each other (*sensu* Díaz et al. 2013). For example, Best and Stachowicz (2012) showed that several species of mesograzers vary in both their consumption rates and their susceptibility to predation, and only by incorporating that information were they able to sufficiently explain natural abundances of different grazers in local eelgrass beds. Similarly, complementarity in grazer consumption rates better explained variation in primary production than either species richness or phylogenetic diversity in experimental eelgrass mesocosms (Best et al. 2013). Lefcheck and Duffy (2015) showed that multiple functional traits better predicted standing stock biomass in an experimental estuarine food web than species richness. Together, these studies suggest that the diversity of functional traits may provide substantial additional insight into top-down control of seagrass ecosystems beyond what is gained through species richness.

While controlled experiments such as those summarized above are ideal for rigorously disentangling whether top-down control is possible, their simplified design and often short durations do not allow them to address the importance of top-down control in the face of long-term, natural variation in predators, resources, and the abiotic environment (Srivastava and Vellend 2005, but see Neckles et al. 1993). Temperate eelgrass beds can experience spatial and temporal variability in community properties (Nelson et al. 1982, Nakaoka et al. 2001, Douglass et al. 2010). Long-term monitoring of both environmental and biological variables is a relatively untapped resource with respect to this question (Gamfeldt et al. 2014, but see Jorgensen et al. 2007, Douglass et al. 2010), and advanced statistical tools, such as structural equation modeling, now exist that help to rigorously disentangle both the relative contributions and the directionality of complex relationships in natural systems (Grace et al. 2007).

Here, we utilize a 15-year observational dataset on a temperate eelgrass bed at Goodwin Islands in the York River Estuary, Chesapeake Bay, USA to evaluate the relative strength of top-down vs. bottom-up control on community properties, with a specific emphasis on the role of grazer diversity. The York River Estuary is a dynamic system characterized by intense seasonal variation in abiotic properties. Temperatures range annually from 0°C to in excess of 30°C, and freshwater pulses drive seasonal changes in salinity, turbidity, and water column nutrients (Moore et al. 1997, Douglass et al. 2010). Specifically, we wanted to: (1) characterize inter- and intra-annual patterns in eelgrass community properties and diversity; and (2) understand whether there was any signal of biological control in light of the natural variation in both abiotic forcing and nutrient resources, as found in controlled experiments (e.g., Duffy et al. 2003, 2005). This analysis builds substantially on the effort by Douglass et al. (2010), which reported on the first 8 of the 15 years of this dataset. Here, we incorporate 218 additional mesograzer samples, 542 additional epiphyte samples, 551 additional predator surveys, and 524 sediment core samples.

Methods

Location

We conducted surveys at the Goodwin Islands National Estuarine Research Reserve at the mouth of the York River Estuary, Chesapeake Bay, USA (37.22 N, -76.39 W) (Fig. 1). Goodwin Islands is a 3.15 km^2 salt-marsh archipelago that is fringed by intertidal flats and subtidal seagrass beds extending to 1-m mean-low-water depth. The seagrasses include the dominant eelgrass *Zostera marina*, with intermittent mixed stands of the widgeongrass *Ruppia maritima*.

The area is closed to development but remains open for commercial and recreational fishing. All community data were collected from a sheltered cove on the southeastern side of the island. Sampling was conducted once a month from approximately March to November, to coincide with the natural annual cycle of eelgrass growth and senescence in this region (Moore and Jarvis 2008). In all, 125 dates were sampled across 15 years.

Environmental Data

Goodwin Islands have been continuously monitored for water quality since 1995 by the Chesapeake Bay National Estuarine Research Reserve Systems (CBNERRS, http://www.vims.edu/cbnerr/monitoring/index.php). Water temperature, salinity, pH, dissolved oxygen, and turbidity are recorded at 15-minute intervals from a permanent monitoring station located on the southeastern shore of the island at approximately 1.5-m mean-low-water by a Yellow Springs Instruments (YSI) 6600 data sonde. Since 2002, these data are supplemented with monthly sampling of water column nutrient concentrations (nitrate, nitrite, ammonium, and phosphate) by CBNERRS staff. Additional meteorological data, including photosynthetically active radiation (PAR), have been continuously measured since 1998 at a separate permanent CBNERRS monitoring site by a Campbell Scientific UT-10 meteorological station, located approximately 35 km northwest of Goodwin Islands at Taskinas Creek. All data are publicly available at: http://cdmo.baruch.sc.edu/get/export.cfm. For all subsequent analyses, we utilized an average value of the environmental variables in the 30 days preceding the eelgrass sampling, with the exception of water column nutrients, which were averaged monthly according to the sampling protocol.

Biological Sampling Design

Community variables were sampled using two 50-m transects parallel to the shore, one at the innermost edge of the bed (inshore) and one at the outermost edge (offshore). A stratified random draw was used to select five sampling 'stations' along each transect in 10-m bins. Since 1998, the Marine Biodiversity Lab (MBL) at the Virginia Institute of Marine Science has conducted grab samples to characterize epifauna associated with grass canopy and dipnet sweeps to characterize small mobile predators at each sampling position. In 2001, the MBL began to take samples to estimate epiphytic chlorophyll-*a* (chl-a) on grass blades, and in 2004, they began to take sediment cores to quantify above- and belowground seagrass biomass. Thus, for all dates post-2004, 10 samples were taken (five for each transect) for each of: (1) epifaunal grab samples; (2) predator dip-net sweeps; (3) epiphytic chl-*a*; (4) biomass cores. Here, we present data for 814 epifaunal samples from Apr 1998 to May 2012, 1,112 predator sweeps from May 1998 to Nov 2013, 1,305 chl-a samples from Sept 2001 to Nov 2013, and 803 biomass cores from March 2004 to Nov 2013.

Seagrass Biomass

Biomass cores consisted of a 15-cm PVC tube inserted approximately 15-cm into the sediment. Cores were taken even if no apparent aboveground growth was present. In the lab, seagrasses were identified to species (*Z. marina* or *R. maritima*), and blades and rhizomes were separated, dried, and combusted at 450°C to obtain biomass in g AFDM.

Epiphytic Algal Biomass

At each station along the transect line, a single shoot (~5 blades) was collected. In the lab, all fouling material was scraped off both sides of each blade and collected on Whatman glass fiber filters. The surface area of the blades was then measured using a Li-Cor 3100 area meter. To estimate epiphytic algal biomass, we conducted chl-*a* extraction in 20-mL 90% acetone at –20°C for 24 hours, after which time the extract was passed through a 0.45-μm polytetrafluoroethylene membrane filter and absorbance was measured at 480, 510, 630, 647, and 750 nm using a Shimadzu UV-1601 spectrophotometer. Epiphytic algal biomass in μg was then estimated using the trichromatic equations for chl-*a* in Jeffrey and Humphrey (1975), and scaled to 2*the surface area in cm^2 of the grass blades.

Epifaunal Surveys

From 1998 to early 2004, epifaunal samples were taken with a 12-cm diameter, 50-cm long acrylic core tube. The tube was placed over a stand of seagrass with one end flush against the bottom. Scissors were used to cut the blades at the base of the tube, and the tube was inverted to flush the grass and associated fauna into a 500-µm mesh bag placed over the other end. Beginning in April 2004, epifaunal samples were taken using a grab sampler placed around the grass canopy, cut as before, and emptied in a 500-µm mesh bag (Virnstein and Howard 1987). The grab sampled a 20 x 20 cm area of the bottom, and previous analyses have shown no difference between the amount of aboveground biomass sampled between the core tube and the grab sampler ($P = 0.21$, Douglass et al. 2010). Epifaunal samples were immediately frozen at –20°C. At a later date, samples were thawed, and plant material and larger invertebrates separated out, dried at 60°C, and combusted at 450°C to obtain ash-free dry mass (AFDM). Smaller epifauna were passed through a series of nested sieves (8.0, 5.6, 4.0, 2.8, 2.0, 1.4, 1.0, 0.71, and 0.5-mm), identified to species, and counted. Abundances for each species in each sieve size class were used to estimate biomass in mg AFDM using the equations in Edgar (1990).

Predator Surveys

At each station along the transect, a 5-m subtransect line was run out perpendicular to the main transect. A dipnet with a 52-cm wide mouth was swept once along the subtransect to sample invertebrate and fish mesopredators living in the grass bed. At the end of the subtransect, all predators were identified to lowest possible taxon, usually species, counted, and released.

Community Diversity

To understand changes in species vs. functional diversity, we compiled data on six functional traits (Table S1). The traits included indicators of resource acquisition, growth and reproduction, defense, and habitat use, all of which have been proposed as key traits in marine invertebrates (Bremner et al. 2003, 2006a, 2006c, 2006b, Bremner 2008) and fishes (Villéger et al. 2010, Stuart-Smith et al. 2013, Lefcheck et al. 2014). The full trait data for all species are given in Table A1, Appendix A.

To quantify functional diversity, we chose a multivariate index of functional diversity: Rao's quadratic entropy (Rao's *Q*, Rao 1982). It is calculated as:

$$Q = \sum_{i=1}^{S-1} \sum_{j=i+1}^{S} d_{ij} p_i p_j \qquad (1)$$

where S is the number of species (richness), p_i is the relative abundance of species i, p_j is the relative abundance of species j, and d_{ij} is the functional distance between species i and j. Q is therefore the average difference between individuals in a sample weighted by their relative abundances. Rao's Q is a specialized case of general entropy, which can be used to derive many other diversity indices, including Shannon and Simpson diversity. When relative abundances are included, Rao's Q is the functional equivalent of Gini-Simpson (or inverse Simpson) diversity (Pavoine et al. 2004). However, presence-absence data can be used in place of relative abundances, in which case Rao's Q is the functional equivalent of species richness and is simply the average functional dissimilarity among all species present in the community, regardless of how abundant they are. To make the values of Rao's Q comparable to species richness, we conducted the transformation proposed in Jost (2006):

$$Q_{eff} = \frac{1}{(1-Q)} \tag{2}$$

This value represents the 'effective number' of species in the sample, if all species were equally abundant and maximally functionally distinct.

To derive functional distances to be used in the calculation of Rao's *Q* (Eq. 1), we calculated Gower distances (Gower 1971), using the correction from Podani (1999) to account for ordered categorical traits (Table 1). Because values of Rao's *Q* can be maximized when fewer than the maximum number of functional types are present unless distances are ultrametric (Pavoine et al. 2005), we employed hierarchical agglomerative clustering to produce an ultrametric functional dendrogram (*sensu* FD, Petchey and Gaston 2002). To account for the sensitivity of the dendrogram to the clustering algorithm used, we employed the approach by Mouchet et al. (2008) of using multiple algorithms and choosing the one that best preserves the original, non-ultrametric distances (Mérigot et al. 2010). Finally, we extracted the ultrametric distance matrix from the dendrogram and scaled by the maximum value so that all values were scaled between 0-1 before calculating Rao's *Q*.

Statistical Analyses

We calculated means and standard errors across all replicates (both inshore and offshore) for a given sampling date to examine temporal trends in community properties, including biomass, abundance, and diversity. For intra-annual trends, summary statistics were calculated across all years for a given month.

For certain environmental variables, such as turbidity, PAR, and water column nutrients, some dates were unmeasured or values were discarded due to low confidence in the reported values by the CBNERRs staff. To leverage the full power of the biological dataset, we used random forest (RF) analysis to impute missing environmental variables using the remaining environmental variables, month, and year. RF analysis is a machine-learning technique that builds a number of regression trees using a bootstrapped portion of the full data, challenges them with the unused data to determine how well they classify variables, then summarizes the classification scheme that is most represented across all trees in the forest (Breiman 2001). An RF approach is ideal because it inherently deals with non-normal relationships and correlations among predictors, which is often the case with environmental variables. After imputation, we summed the concentrations of ammonium, nitrates and nitrites, and phosphates to fully characterize water column nutrients. We conducted a similar random forest imputation procedure for several unobtainable values of the trait 'mean length' using the remaining traits as predictors. All random forests were conducted using the *randomForest* function in the *extendedForest* package in R (Liaw and Wiener 2002).

Values of seagrass biomass from the early epifaunal samples were reported only in units of dry mass, and not ash-free dry mass. We imputed the missing values of AFDM using a generalized additive mixed model (GAMM) constructed from the samples for which both DM and AFDM were reported. In this case, we used a GAMM to incorporate a potential non-linear relationship between DM and AFDM by modeling DM as non-parametric smoothing function (Wood 2006). We also allowed the linear slope of DM to vary by the random effect of sampling date. The GAMM was run using the *gamm* function in the *mgcv* package (Wood 2011).

We used non-metric multidimensional scaling (NMDS) to visualize community structure throughout the year. NMDS collapses multivariate community data into a reduced number of dimensions based on dissimilarity of species' rank abundances (Faith et al. 1987). We used Wisconsin transformation on the species-by-site matrix, then characterized community dissimilarity using Bray-Curtis distances. The distance matrix was further square-root transformed to reduce the influence of highly abundant species (Legendre and Gallagher 2001).

We conducted the NMDS using the function *metaMDS* in the *vegan* package (Oksanen et al. 2013). To statistically quantify differences among species composition through time, we conducted PERMANOVA, which partitions variance in the community dissimilarity matrix as a function of some predictors, in our case season, and uses random permutations of the data to assess significance (McArdle and Anderson 2001). We incorporated years as strata in the PERMANOVA to account for differences in the absolute dissimilarity among communities among years (to be expected, for instance, between pre- and post-die off years). We used the function *adonis* in the *vegan* package to conduct the PERMANOVA. Species were excluded from community analyses if they exhibited ≤10 individuals across the entire dataset.

Finally, we used structural equation modeling (SEM) to relate environmental and community variables to understand the drivers of seagrass ecosystem properties. SEM is a type of path analysis that combines relationships among multiple interconnected variables in a single, testable causal network (Grace 2006). A variant of SEM called piecewise (or directed acyclic) SEM constructs the causal network by piecing together a series of multiple regressions (Shipley 2000), rather than by simultaneously optimizing the observed and predicted variance-covariance matrix for the entire model (e.g.,Grace 2006). Recently, this approach has been generalized to incorporate non-random responses and hierarchical data in a mixed effects framework (Shipley 2009, 2013), hence making it the ideal approach for time series data, where samples are temporally nested as well as autocorrelated.

We invoked the hypothesis-testing mode of SEM, where models are constructed using *a priori* knowledge of the system. We constructed a single model linking environmental and biological variables, using predictions generated from prior published experiments and observations (Fig. S1). In particular, we were interested in comparing the magnitude of the top-down effect of grazers on epiphytes, and the corresponding bottom-up effect of nutrients. A further explanation of the rationale behind the model paths can be found in Appendix B. We also fit the same model for spring, summer, and fall to understand how the relative influence of the paths changed throughout the year.

For each of the individual regressions that make up the piecewise model, we constructed linear mixed effects models. We specified both a nested random structure of month within year, allowing only the intercept to vary, and an autoregressive 1 (AR1) correlation structure identical to the random structure to account for observed temporal autocorrelation. Model assumptions of homogeneity of variance and normality of errors were investigated visually, and we $\log_{10}$-transformed some variables to better meet test assumptions. Individual model fit was also assessed by calculating pseudo-R^2 values corresponding to the fixed effects only (marginal R^2) and fixed and random effects (conditional R^2) (*sensu* Nakagawa and Schielzeth 2012). R^2 values were calculated using the function *rsquared.glmm* (https://github.com/jslefche/rsquared.glmer). Where R^2 values are reported, we report the marginal value with the conditional value in parentheses. We set a threshold of $\alpha = 0.10$ to assess significance of individual paths to account for the tremendous intra-annual variation in both biotic and abiotic parameters observed in this dataset.

To assess the overall fit of the SEMs, we conducted tests of directed separation (*d*-separation) (Shipley 2000). This technique tests the significance of missing paths – paths that could have been included in the mode but were not – and uses the resulting *p*-values to construct a Fisher's *C*-statistic. This statistic is χ^2-distributed and can be compared to the null expectation with 2*k* degrees of freedom (where *k* is the number of missing paths) (Shipley 2009). Individual mixed effects models were constructed using the *lme* function in the *nlme* package (Pinheiro et al. 2013). Goodness-of-fit tests for piecewise SEM were conducted using the *piecewiseSEM* package (https://github.com/jslefche/piecewiseSEM). All analyses were conducted in R version 3.1.2 (R Development Core Team 2015).

Results

Environmental Variables

Over 15 years, we witnessed little interannual variability in environmental variables (error bars, Fig. 1). Temperature generally increased through the spring, peaked in the summer, and tapered off in the fall (Fig. 1a). Salinity increased marginally throughout the year but remained relatively constant around 18 parts per thousand (Fig. 1b). Turbidity increased through the spring and summer and peaked in September, dropping off quickly by November (Fig. 1c). PAR followed a similar pattern to temperature, peaking in the mid-summer (Fig. 1d). Finally, nutrient concentration – a composite index of nitrate, nitrite, ammonium, and phosphates – increased steadily throughout the year, peaking slightly in June and then again in November (Fig. 1e). Correlations among environmental variables was generally low, ranging from $r = -0.02$ between nutrients and both temperature and PAR, and $r = 0.53$ between temperature and turbidity.

Primary Producer Biomass

Eelgrass aboveground biomass generally increased during spring, peaked in June, and then declined rapidly, reaching its lowest point in late fall (Fig. 2a). This pattern corresponds with the well-described annual cycle of senescence in *Z. marina* in this region (Orth and Moore 1986, Douglass et al. 2010). Eelgrass belowground biomass generally tracked aboveground biomass, with a peak in early- to mid-summer, but spring and fall declines were less exaggerated than for aboveground biomass (Fig. 2b). Estimates of epiphyte biomass per unit area were lowest when eelgrass biomass was highest, and increased steadily throughout the year, peaking in November (Fig. 2c). This system experienced two previously documented die-off events in 2005 and 2010 in response to extreme temperatures (Moore and Jarvis 2008). Our dataset revealed that the die-off manifested in aboveground growth only (Fig. 3a), with belowground biomass largely unaffected, although we note that we did not distinguish between living and dead tissue, only the presence of rhizomes (Fig. 3b). We also found highest epiphyte loading per unit area during these times (Fig. 3c).

Epifaunal Surveys

Total mesograzer abundance and biomass per unit plant material tracked each other closely, increasing steadily throughout the year with a small dip in June (Fig. 2d,e). From year-to-year epifaunal biomass was idiosyncratic, but did noticeably spike during and immediately following the 2005 die-off event (Fig. 3d,e). Individual grazer species showed marked seasonal trends, with *C. penantis*, the gammaridean amphipod *Gammarus mucronatus*, and the ampithoid amphipod *Cymadusa compta* comprising the bulk of community biomass in the spring, the ampithoids *C. compta* and *Ampithoe longimana* and the amphipod *Elasmopus levis* in the summer, and the isopods *Erichsonella attenuata* and *Idotea balthica* in the fall (Fig. 4). Finally, the grazing gastropod *Bittiolum varium* showed no trends seasonally (Fig. 4b), although overall it comprised much less of total community biomass and abundance (Table 1). It did, however, show a marked increase in abundance following the 2010 die-off (Fig. S2). Other notable interannual trends include the disappearance of *I. balthica* for several years following the 2005 die-off, and the increase in *C. compta* following both die-offs (Fig. S2).

Predator Surveys

Total predator abundance peaked in the late spring to early summer (Fig. 2f). This trend was driven largely by high abundances of the grass shrimp *Palaemonetes* spp. in May and June (Fig. 5c). Abundance of pipefishes *Syngnathus* spp. peaked in July, and the sand shrimp *Crangon* *septimspinosa* peaked in September (Fig. 5b,d). Abundance of juveniles of the blue crab *Callinectes sapidus* was more variable, with peaks in late spring and fall (Fig. 5a). Over the 15 year dataset, predator abundances tended to be overall much lower after the winter of 2003 (Fig. 3f). Further investigation revealed that this was caused by declines in both *C. sapidus* and *C. septimspinosa* post-2003 (Fig. S3).

Community Composition

Non-metric multidimensional scaling revealed strong seasonal changes in total community composition based on relative abundances (Fig. 6). For grazers, spring communities were clearly distinct from summer and fall communities (Fig. 6a). PERMANOVA revealed that these seasonal transitions were highly significant ($F_{2,805} = 33.77$, $P < 0.001$). Predators demonstrated similar but more diffuse patterns, with spring communities being more distinct from summer and fall ones (Fig. 6b). Despite this, the seasonal differences in predator composition were also highly significant ($F_{2,1057} = 12.09$, $P < 0.001$). Stress – a measure of agreement between the multidimensional distance matrix and the two-dimensional reproduction – was between 0.1-0.2 for all NMDS analyses, implying that the above inferences are a fair representation of multivariate community composition in reduced dimensions.

Community Diversity

In all, 114 unique taxa of invertebrates and vertebrates were identified across all 15 years, although only 80 species were represented by >10 individuals. Of these, we identified 72 with sufficient taxonomic resolution (at least to family) and collected trait data for the calculation of functional diversity (Table S1). When we plotted the most abundant grazers in reduced trait space using principal coordinates analysis, we noted that the gammaridean and ampithoid amphipods, caprellid amphipods, and two isopods grouped together in three discrete clusters (Fig. 7a). The lone abundant gastropod grazer, *B. varium,* existed in its own section of trait space, reflecting its calcareous shell, small body size, and unique morphology compared to the crustaceous grazers (Table A1, Appendix A). In contrast, the most abundant predators were far less functionally redundant, with only the two shrimps, *Palaemonetes* spp. and *C. septemspinosa*, grouping together (Fig. 7b).

Across all sampling dates, the mean richness per epifaunal grab sample was 9.4 ± 0.2 (standard error of the mean), of which 5.2 ± 0.1 species were strictly classified as grazers (Table S1). The mean functional trait diversity of epifaunal grabs was overall much lower at 2.8 ± 0.03, and was 1.8 ± 0.01 for grazers. The mean predator richness per dip-net sweep was 2.5 ± 0.08, and the mean predator functional diversity was nearly equivalent at 2.1 ± 0.05. Mean species richness of grazers increased throughout the spring and peaked in July, with another lesser peak occurring in October. In contrast, grazer functional diversity was overall much lower, and remained fairly constant throughout the year (Fig. 8a). As such, there was only a moderate positive correlation between grazer species richness and functional diversity (Spearman rank correlation $r = 0.56$). As with grazer richness mean predator richness peaked in July with a smaller peak in October. However, predator functional diversity tracked predator richness much more closely than grazer diversity, although it was also overall lower, particularly in the summer (Fig. 8b). This equivalence is reflected in the strong correlation between predator richness and functional diversity ($r = 0.98$). Functional diversity appeared to be less variable through time, a pattern which can be better visualized by plotting the coefficient of variation (CV = mean/standard deviation). In addition to being fairly constant, grazer functional diversity was indeed much less variable based on examination of CVs (Fig. S4a). In contrast, predator functional diversity was only slightly less variable than species richness (Fig. S4b).

Top-Down vs. Bottom-Up Control

To test for seasonal differences top-down and bottom-up control, we fit the full structural equation model (SEM) described in Figure S1 to the spring, summer, and fall samples. The spring model was an acceptable fit to the data (C_{10}= 22.05, $P = 0.34$, $N = 200$, Fig. 9). The strongest driver of any property was the positive effect of temperature on predator abundance, based on comparison of standardized regression coefficients. Temperature also positively affected grazer abundance and eelgrass aboveground biomass. The second strongest effect was among grazer abundance and biomass, and grazer richness and functional diversity, reflecting the strong dependence of these properties on one another. Contrary to predictions from experiments, there was no relationship between grazer diversity and any ecosystem properties. With respect to trophic control, there was a moderate and significant top-down effect of predators on grazer abundance, but not biomass. Predator abundance also increased grazer functional diversity, albeit only slightly. There was also a negative effect of grazer abundance on eelgrass aboveground biomass, which was opposite to our expectation. Finally, there were no relationships between any physical or biological variables and estimates of epiphyte biomass, and no bottom-up effects of nutrient concentration on either epiphytes or eelgrass. To better facilitate comparisons between seasons, paths corresponding to top-down and bottom-up control, their standardized estimates, and significance are summarized in Table 2.

The model fit for the summer replicates was adequate ($C_{10} = 18.81$, $P = 0.53$, $N = 142$, Fig. 10). Once again, environmental variables, principally a negative effect of turbidity on predator abundance and temperature on eelgrass aboveground biomass, and a positive effect of temperature on grazer abundance, were the strongest effects observed in the SEM. The top-down effect of predators on grazer abundance was not significant (and thus is removed in Figure 10), but it did significantly reduce grazer functional diversity. In turn, grazer functional diversity actually decreased grazer biomass, implying an indirect positive effect of predators on grazer biomass. Grazer richness was unrelated to grazer biomass, but did have a weak but significant direct positive effect on epiphyte biomass. As with the spring model, there were no additional controls on epiphyte biomass (except grazer richness). There was, however, a direct positive effect of grazer biomass on aboveground eelgrass biomass that was not mediated through epiphytes. Also as in spring, there was no bottom-up effect of nutrients detected in this model (Table 2).

The fall model fit the data extremely well despite the low number of replicates (C_{10} =6.19, P = 1.00, N = 96, Fig. 11). Overall, there was much weaker environmental control in this model, with the strongest effects instead occurring among grazer abundance and both richness and biomass. There was once again a significant negative effect of predators on grazer abundance, although it was slightly weaker than in the spring (Fig. 9). There was also a significant but very weak positive effect of predators on grazer biomass. As with all models, there were no controls on epiphyte biomass. For the first time, there was a strong negative bottom-up effect of nutrient concentration on eelgrass biomass, and on grazer functional diversity. There were not, however, any significant top-down paths acting on eelgrass biomass, as in the spring model (Fig. 10).

Integrating across all dates and seasons and fit the same SEM (C_{10} = 13.04, P = 0.88, N = 438) revealed similar trends, with strong environmental forcing by temperature and PAR, strong relationships among grazer variables, except between diversity and biomass, moderate top-down control by predators on grazer abundance, and no biological control of epiphytes (Fig. S5). There was also a direct positive effect of grazer biomass on eelgrass aboveground biomass, but a 1.4*x* stronger bottom-up effect of nutrient concentration (Table 2). Thus, it appears that, on the whole, the stronger nutrient effect on eelgrass biomass later in the year negates the grazer effects earlier in the year. For all SEMs, we provide the full coefficient tables in Appendix C.

Discussion

Across 15 years of the survey data, we found alternating evidence for top-down versus bottom-up control of ecosystem properties in a temperate eelgrass bed. When biological control was present, however, it was almost always exceeded in magnitude by environmental effects, particularly temperature, PAR, and turbidity. This near-overwhelming effect of environment should come as no surprise given the tremendous variation in abiotic properties in the Chesapeake Bay (Douglass et al. 2010). In fact, this system has, "one of the most extreme annual temperature ranges known for the world's coastal ecosystems" (p. 3, Murdy et al. 2002). That we were able to recover any biological signal at all is actually quite remarkable in light of such immense environmental variation. Given the relative equivalence of the top-down and bottom-up models and the large effect sizes for abiotic versus biotic variables, is it fair to conclude that this system is a total consequence of environmental forcing and there is no role for biology?

The reality probably lies somewhere in between, with the levels of environmental variables determining the degree of biological control. For instance, we found that significant top-down control by predators on grazers was strongest in spring and fall and absent in the summer (Table 2). This pattern corresponds with minima in temperature and turbidity (Fig. 1a,c), as well as eelgrass biomass (Fig. 2a). Together, these results suggest that predators sampled by our gear may prefer to forage in periods of low stress and high visibility enhanced by low eelgrass density, a pattern which is mirrored by other, more mobile predators not captured by our dipnet sweeps, such as the silver perch *Bairdiella chrysoura* (Sobocinski et al. 2013). This pattern may also be a consequence of reduced eelgrass biomass during the spring and fall limiting the available habitat for grazers at the same time when less stressful environmental conditions encourage the abundance of predators (Fig. 2a,f). Unfortunately, SEM is currently unable to independently partition the reciprocal effects of grazers on eelgrass and eelgrass on grazers (Shipley 2000), although future developments may one day allow this kind of rigorous analysis (Jim Grace, *pers. comm.*). Another explanation is that high secondary production during the warm summer months replenished grazer populations faster than they were reduced by predators (Douglass et al. 2010). For example, the gammaridean amphipod *G. mucronatus* has been shown to reproduce in as few as three weeks (Fredette and Diaz 1986), translating to tremendous population growth for many of these epifaunal invertebrates at high temperatures (Fredette et al. 1990).

Top-down control by grazers on eelgrass was highest in the summer (Table 2). This pattern appears to be a consequence of reduced predator abundance (Fig. 2f), high temperatures increasing grazer abundance (Fig. 10), presumably through increased metabolic rates (Brown et al. 2004), and increasing resources (Fig. 2c). This result agrees with experimental evidence showing stronger grazer control in early summer (Neckles et al. 1993), and with other experimental manipulations conducted during this time period in this system (Douglass et al. 2007, Whalen et al. 2013, Reynolds et al. 2014, Duffy et al. 2015). It is important to note that we refer to the direct effect of grazers on eelgrass, which contradicts the prevailing paradigm that grazers reduce epiphytes, which then indirectly benefits seagrasses (Valentine and Duffy 2006). One explanation may be that grazers are so efficient at reducing epiphytes, that the relationship statistically manifest directly between grazers and eelgrass. It may also once again represent an untestable reciprocal relationship, with grazer biomass enhanced by the increase availability and complexity of eelgrass habitat, rather than grazers affecting eelgrass. However, given the litany of experimental and observational evidence focusing on the opposite relationship, we have chosen to report the effect as grazers affecting eelgrass.

There are two additional reasons why biological control might be more evident in the summer than in other parts of the year. First, the most abundant grazer in springtime was the caprellid amphipod *C. penantis* (Table 2, Fig. 4c). While it is known to ingest epiphytic microalgae (Caine 1974, Duffy 1990), and we classified it as a grazer (Table B1), *C. penantis* is also capable of suspension feeding. Although the exact conditions under which *C. penantis* employs one feeding mode versus the other are unknown, it is possible that even a small reliance on suspension feeding by this highly abundant grazer during the earlier months of the year may have failed to yield a significant relationship between epiphytes and grazers (Fig. 9). Second, epiphytic growth may be have been unable to overtake the rapid growth and turnover of eelgrass substrate during the early parts of the year (Fig. 2a), particularly with reduced water column nutrients and light (Fig. 1d,e). Thus, low epiphyte levels, particularly in spring, may be more a function of dilution per unit leaf area and decreased resources than any impact by grazers.

We only observed evidence for bottom-up control later in the year, with nutrient concentration having a direct negative effect on eelgrass aboveground biomass in the fall (Fig. 11). As with grazers, there was no direct effect of nutrients on epiphytes, suggesting that epiphyte growth is either efficiently transferred up the food web, or quickly depresses eelgrass biomass. There was, however, a significant positive effect of photosynthetically active radiation (PAR) on grazer biomass in the fall (Fig. 11), which is often implicated in primary production (e.g., Monteith 1972). Thus, PAR may be an additional proxy for resources and this path may be interpreted as a second bottom-up signal, this time on consumers. There was also a significant but negative effect of PAR on grazer abundance in the spring (Fig. 9). We suggest that this path may represent an indirect effect of PAR driving eelgrass growth diluting grazer abundance per unit plant material, which we were again unable to test due to the inability to model the reciprocal effect of grazers on eelgrass and vice versa. This hypothesis may also explain the negative effect of grazer abundance on eelgrass biomass during the same period (Fig. 9).

The stronger bottom-up effect of nutrients in the fall is consistent with previous experimental manipulations in this system (Neckles et al. 1993). The most likely explanation for this effect is the increasing nutrient concentration throughout the year (Fig. 1e), and the natural decline in eelgrass habitat in the fall (Fig. 2a) and increase in predation (Fig. 11) reducing the potential for top-down control by grazers. Previous experiments have also implicated light as the primary driver of eelgrass decline by mediating epiphyte growth (Moore and Wetzel 2000). In contrast, we did not find any predictable relationship between PAR (or any other environmental variable) and epiphytes, nor did we see any direct negative effects of PAR on eelgrass biomass that may be suggestive of this hypothesis.

We rarely observed a significant direct path between predator abundance and grazer biomass, only grazer abundance (Fig. 9, 10). Similar epifaunal invertebrate communities have been shown to be limited by 'resource ceilings,' where experimental assemblages converged on consistent values of secondary production regardless of habitat and composition (Edgar 1993). Under predation, the communities exhibited similar production but shifted their size structure towards many smaller individuals (Edgar and Aoki 1993). In our case, it is possible that predators reduced grazer abundances, but compensatory demographic responses prevented this effect from manifesting in observed biomass. A more nuanced investigation of grazer community size structure through time would shed light on this phenomenon, but, if true, would argue for a more resource-controlled view. We did see a positive effect of predator abundance on grazer biomass in the fall (Fig. 11). A likely explanation is that the predator community was dominated primarily by the sand shrimp *C. septemspinosa* in the fall (Fig. 5b), which is not known to be an important predator of epifauna and instead feeds on planktonic animals, primarily mysids (Price 1962), at the same time when eelgrass senescence inflated the grazers per unit plant habitat.

Contrary to experimental evidence, we found no role for species richness or functional diversity in predicting grazer biomass in this system (Fig. 9, 11), with the exception of the summer, where functional diversity was significantly decreased by predator abundance, and significantly reduced grazer biomass (Fig. 10). One possibility is that predators preferentially removed functionally unique grazers, reducing functional diversity. In turn, low functional diversity reduced complementarity, drove increased competition for resources among similar species, and ultimately reduced biomass production (Loreau 1998). A more likely explanation is that seasonal turnover in species composition yielded dominant species that were functionally similar, and that turnover corresponded with environmental conditions discouraging predator abundance, such as high temperatures and turbidity (Fig. 1, 9). Indeed, the two ampithoids *A. longimana* and *C. compta* and the amphipod *E. levis that* dominated community biomass during the summer also overlapped significantly in their functional traits (Fig. 4, 7a). This observation may also explain the lack of diversity effect at other points in the year. We witnessed very little variation in grazer functional diversity throughout the year (Fig. 8a, Fig. S4a), despite substantial turnover in community composition (Fig. 6a). Thus it may be that the high degree of functional redundancy among grazers buffered against predictable changes in grazer biomass independent of other factors, such as predators and environmental forcing. This may also explain the recovery of this system after the 2005 and 2010 die-off events, despite notable shifts in community structure (Fig. 2, Fig. S2). There was, paradoxically, a positive effect of grazer richness on epiphytes in summer (Fig. 10), although this could simply represent the community response to favorable conditions for epiphyte growth (Fig. 1), coupled with natural recruitment of new species to the system during this period (Fig. 8a).

The important question remains: why do experiments, several of which were conducted in this eelgrass bed (Douglass et al. 2007, Whalen et al. 2013, Reynolds et al. 2014), demonstrate stronger *in situ* effects of top-down control and diversity compared to this observational study? One explanation is time: these experiments are often conducted on the scale of weeks, which greatly restricts environmental variation. As shown in our SEMs, some aspect of environmental forcing was always a strong driver of ecosystem properties, regardless of seasons (Fig. 9-11, Fig. S5). However, this is a difficult hypothesis to evaluate as complex long-running experiments are often logistically unfeasible, and negative results are frequently unpublished. A complementary explanation is that experimental manipulations often decrease grazer community properties below what would occur naturally. This would certainly be the case in 'all-or-none' experiments where grazers are either present or absent (Douglass et al. 2007), but may also be true of *in situ* reductions that entirely exclude only certain taxonomic groups through the use of certain techniques (e.g., application of insecticides) (Whalen et al. 2013, Reynolds et al. 2014, Duffy et al. 2015). Even mesocosm experiments routinely employed only 3 species, on average (Gamfeldt et al. 2015). In contrast, we observed surprisingly little variation in grazer biomass within a given time point (Fig. 2e), and in grazer diversity throughout the year, holding around 5-6 species (Fig. 8). Experimentally inflating variance around grazer abundance, biomass, and/or diversity may increase the chances of observing an effect on epiphytes, but may render inferences artificial when compared to actual effects in nature. Finally, we targeted only a small subset of the predator community using our dipnet sweeps. Had we more fully sampled predators, we may have witnessed even stronger top-down control on grazers, given the known diets of other mobile fishes that frequent this bed (Orth and Heck Jr. 1980, Sobocinski et al. 2013).

Overall, this eelgrass community appears to be largely driven by abiotic variables, principally temperature and light. However, we did see comparatively weak but still significant biological control. Across all data points, there appeared to be strong bottom-up control through nutrients than top-down control by predators or grazers. However, when we parsed this effect by season, we found stronger top-down control earlier in the year switching over to stronger bottom-up control in the fall (Table 2). This approach emphasizes the dynamic nature of temperate estuaries, and the need for seasonal replication when designing long-term ecological surveys. We also observed high functional redundancy among the dominant grazer community, which may have explained the lack of significant diversity effects on grazer biomass after accounting for other constraints. Continued monitoring and processing of samples from this bed will provide valuable insight into the role of functional redundancy in sustaining this important coastal ecosystem in the face of increased temperatures, higher fishing pressure, and other coastal impacts.

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Tables

Table 1: Total abundance and biomass for epifaunal grazer species across all samples.

Species	Total biomass (mg AFDM)	Proportion of total biomass	Total abundance	Proportion of total abundance
Erichsonella attenuata	17931.43	0.46	7543	0.10
Caprella penantis	9148.33	0.23	31986	0.40
Gammarus mucronatus	4589.53	0.12	7915	0.10
Ampithoe longimana	2149.70	0.06	10263	0.13
Idotea balthica	2086.24	0.05	1389	0.02
Cymadusa compta	1004.96	0.03	3283	0.04
Elasmopus levis	684.80	0.02	4357	0.06
Bittiolum varium	533.08	0.01	2609	0.03
Paracaprella tenuis	280.87	0.01	4081	0.05
Ampithoe valida	279.36	0.01	698	0.01
Hippolyte pleuracanthus	270.10	0.01	3898	0.05
Caprella equilibra	111.29	0	913	0.01
Dulichiella appendiculata	28.33	0	86	0
Ericthonius rubricornis	9.65	0	31	0
Melita nitida	7.07	0	35	0
Microdeutopus anomalus	2.75	0	31	0
Erichthonius brasiliensis	2.21	0	11	0
Paracerceis caudata	1.71	0	17	0

Table 2: Comparison of standardized regression coefficients (+/- standard error) for key top-down and bottom-up processes across all dates, and by season. Crosses (†) indicate $\log_{10}$-transformed variables. Asterisks equal significant (* $P < 0.10$, ** $P < 0.01$, *** $P < 01$). Significant value are bolded.

Process	Predictor	Response	Spring	Summer	Fall	All dates
Top-down	Predator abundance†	Grazer abundance†	**-0.251 ± 0.080****	-0.116 ± 0.093	**-0.175 ± 0.070***	**-0.125 ± 0.047****
Top-down	Predator abundance†	Grazer biomass†	0.077 ± 0.068	-0.127 ± 0.089	**0.101 ± 0.051***	**0.074 ± 0.036***
Top-down	Grazer biomass†	Epiphyte biomass†	-0.001 ± 0.082	0.004 ± 0.051	-0.194 ± 0.098	-0.061 ± 0.043
Bottom-up	Nutrient concentration	Epiphyte biomass†	0.113 ± 0.216	-0.535 ± 0.356	-0.219 ± 0.364	0.194 ± 0.124
Top-down	Grazer biomass†	Eelgrass aboveground biomass†	0.159 ± 0.098	**0.229 ± 0.114***	0.246 ± 0.209	**0.184 ± 0.074***
Bottom-up	Nutrient concentration	Eelgrass aboveground biomass†	0.029 ± 0.141	-0.086 ± 0.240	**-0.357 ± 0.057***	**-0.274 ± 0.125***

Figures

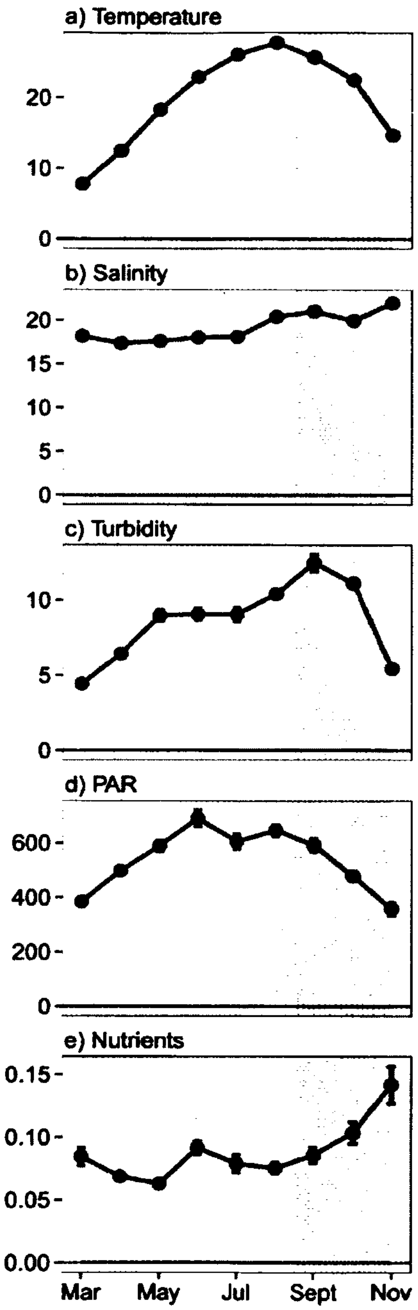

Figure 1: Intra-annual trends in four major environmental variables. Points represent mean +/- 1 standard error. Units for temperature are °C, salinity are PPT, turbidity is NTU, and nutrients represents the summed concentration of nitrate, nitrate, ammonium, and phosphate in mg/L.

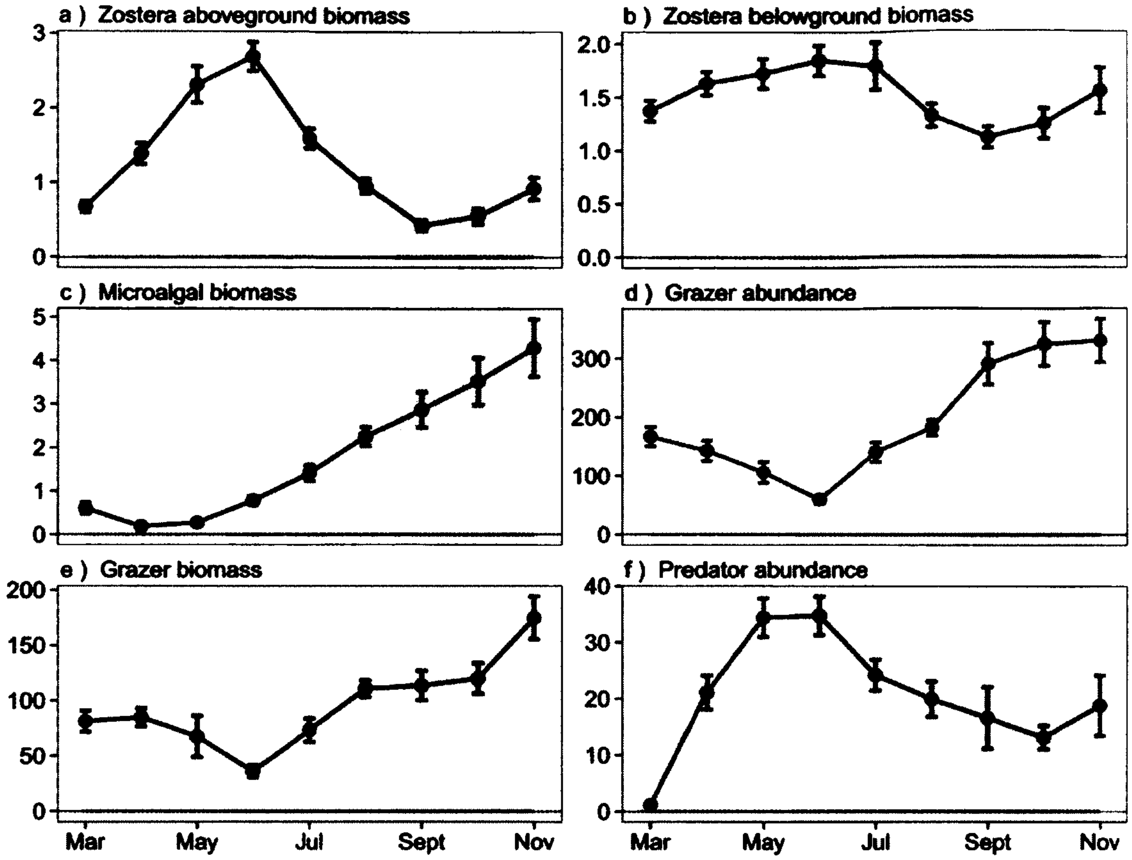

Figure 2: Seasonal trends in biological community variables. Points represent mean +/- 1 standard error. Units for **(a,b)** *Zostera* above- and belowground biomass are mg ash-free dry mass (AFDM); **(c)** microalgal biomass are µg cm^{-2}; **(d)** grazer abundance are number of individuals; **(e)** grazer biomass are mg AFDM, and; **(f)** predator abundance are number of individuals.

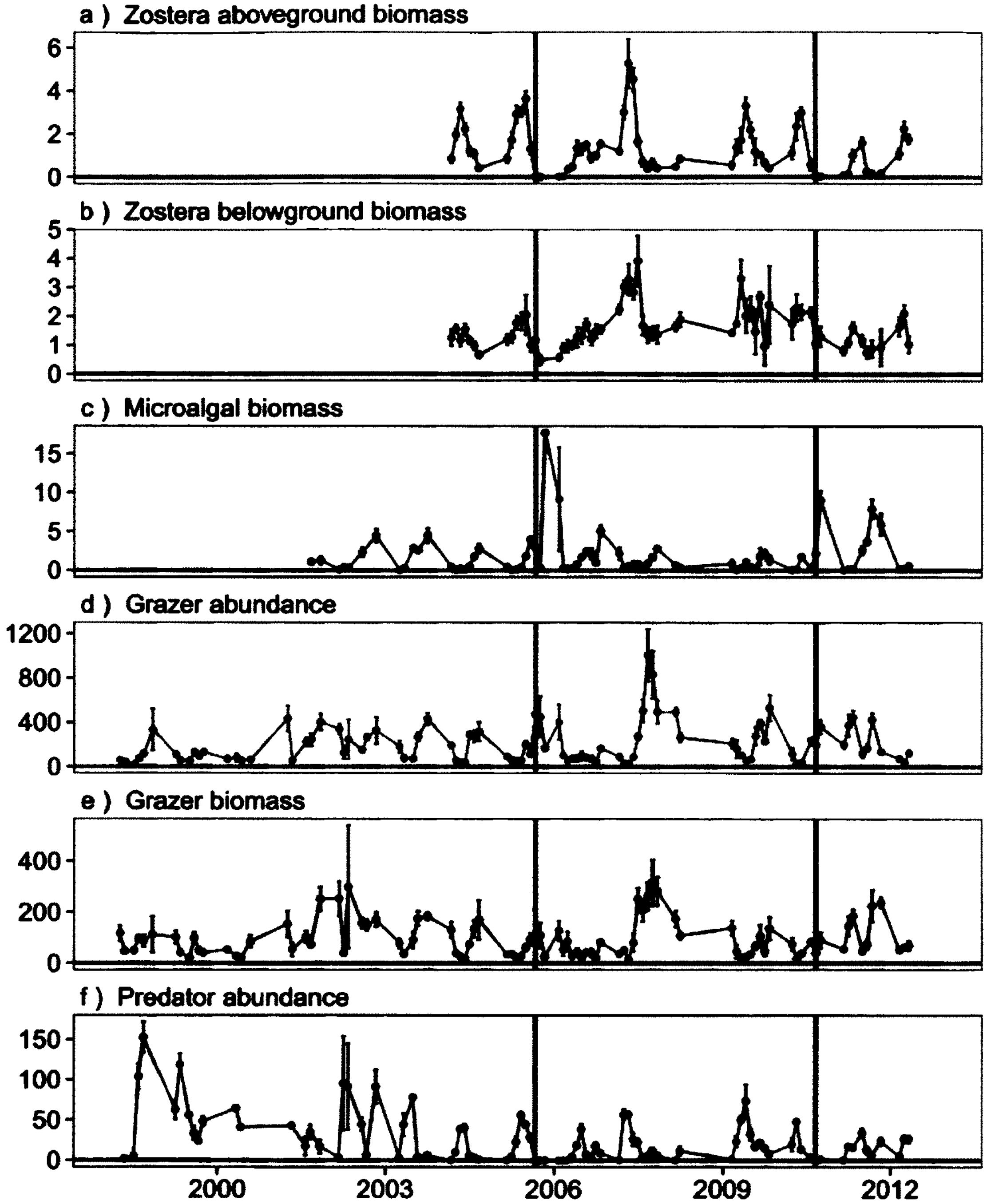

Figure 3: Interannual trends in biological community variables for the length of the survey. Points represent mean +/- 1 standard error. Units are the same as in Figure 2. Biomass cores for **(a,b)** *Zostera* above- and belowground biomass were not taken until spring 2004. Chlorophyll-*a* scraping to estimate **(c)** microalgal biomass were not taken until spring 2001.

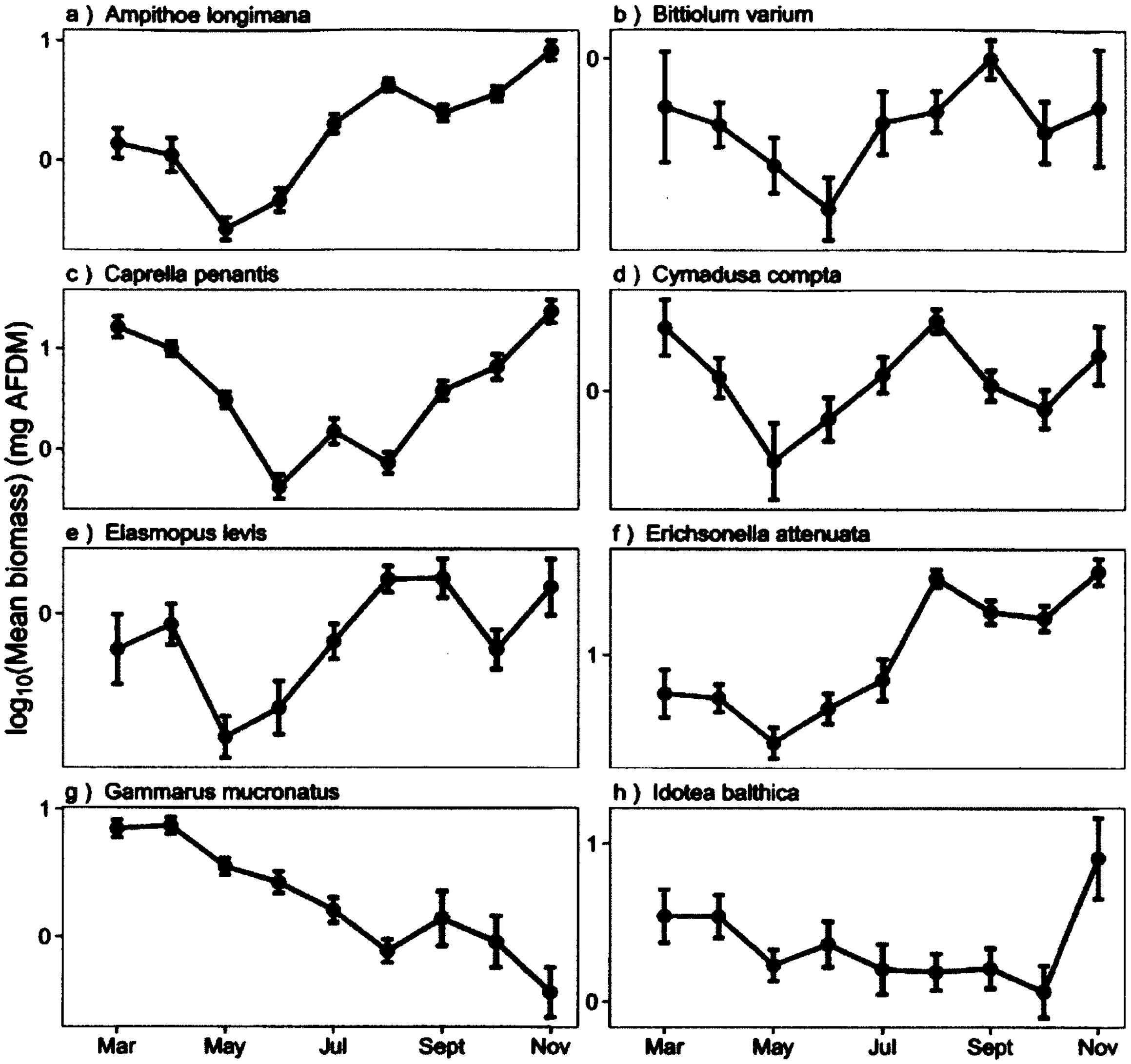

Figure 4: Seasonal trends in mesograzer estimated biomass (mg AFDM) for the eight most abundant mesograzers in the survey (Table 1). Points are $\log_{10}$-transformed mean per epifaunal grab sample +/- 1 standard error.

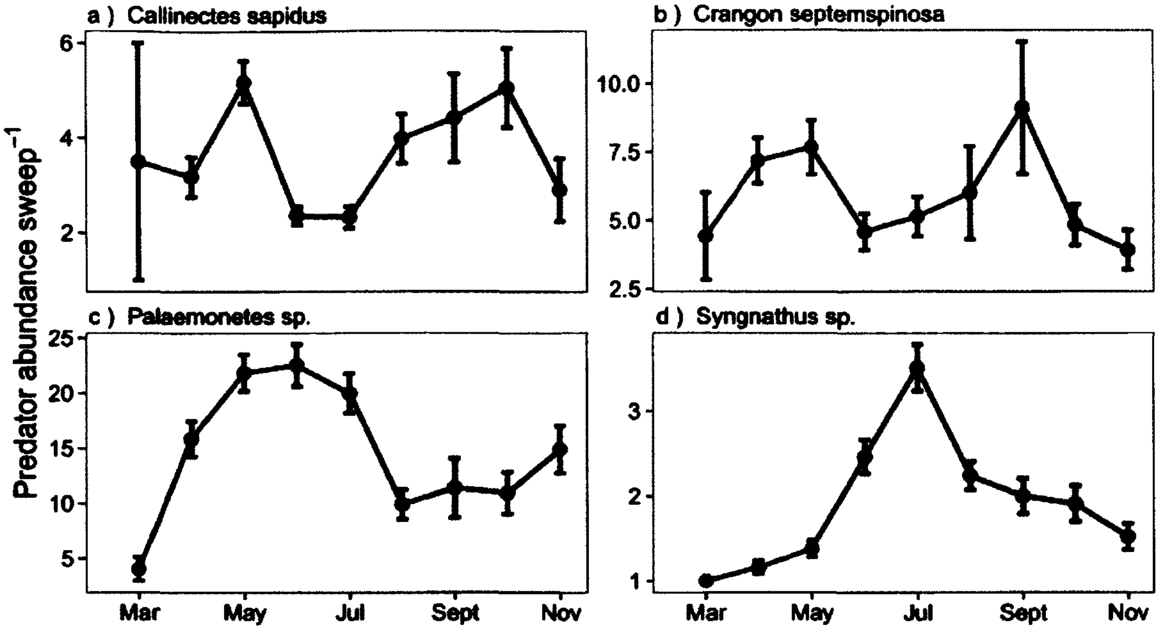

Figure 5: Seasonal trends in predator abundance for the four most abundant predators in the survey, representing >96% of cumulative abundance. Points are mean per dipnet sweep +/- 1 standard error.

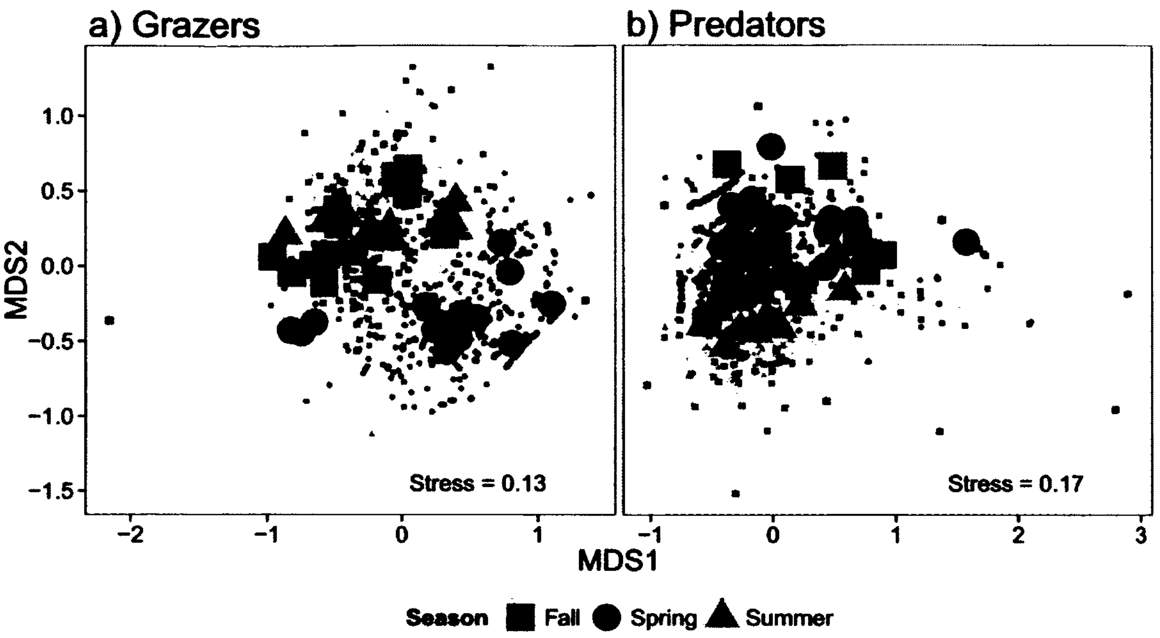

Figure 6: Non-metric multidimensional scaling of (a) grazer and (b) predator communities across all 15 years. Small points represent each sample. Large points represent the centroid for each year and season. Points are colored corresponding to season (blue = spring, yellow = summer, brown = fall). Stress (a measure of fit) is reported in the lower right of each panel.

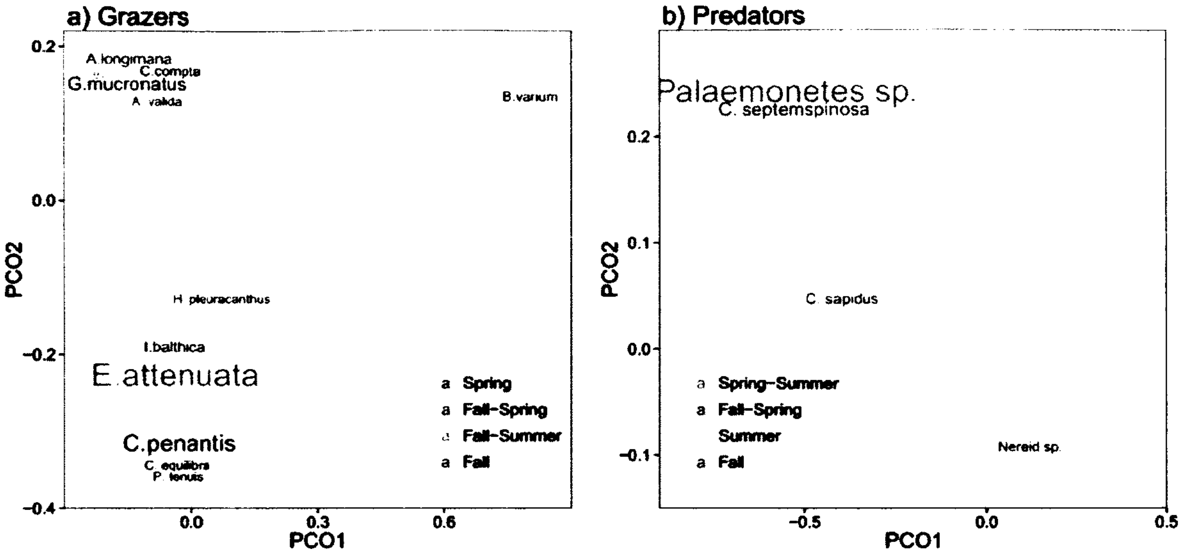

Figure 7: Principal coordinates analysis of (a) grazer and (b) predator functional traits. Labels correspond to the relative position of species in multidimensional trait space. Labels are sized corresponding to the proportional biomass of each species in the survey. Colors correspond to the season of maximum biomass. Where mean biomass was not significantly different between seasons, both seasons are reported.

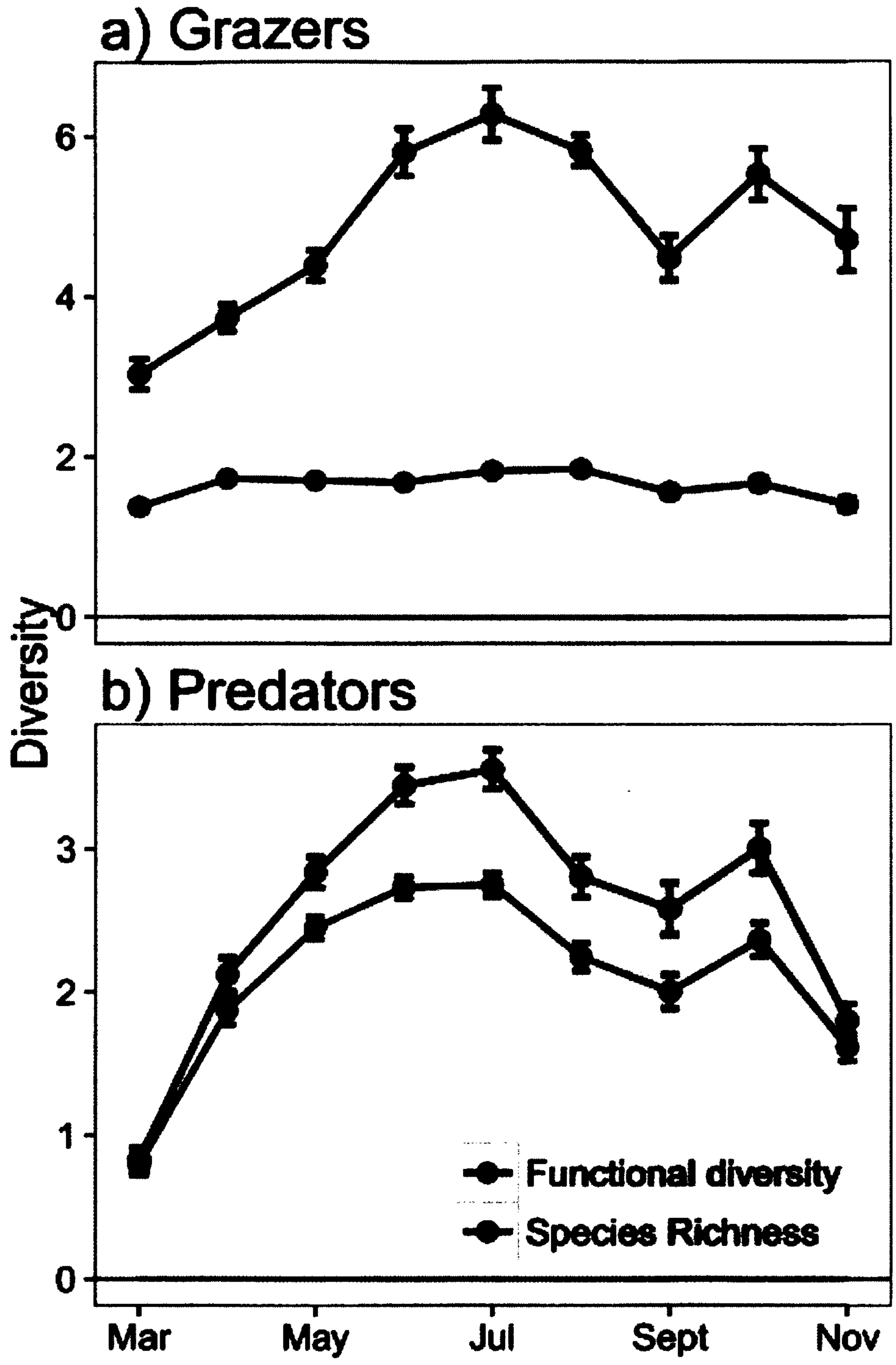

Figure 8: Seasonal trends in species richness and functional diversity of (a) grazers and (b) predators. Points represent mean +/- 1 standard error.

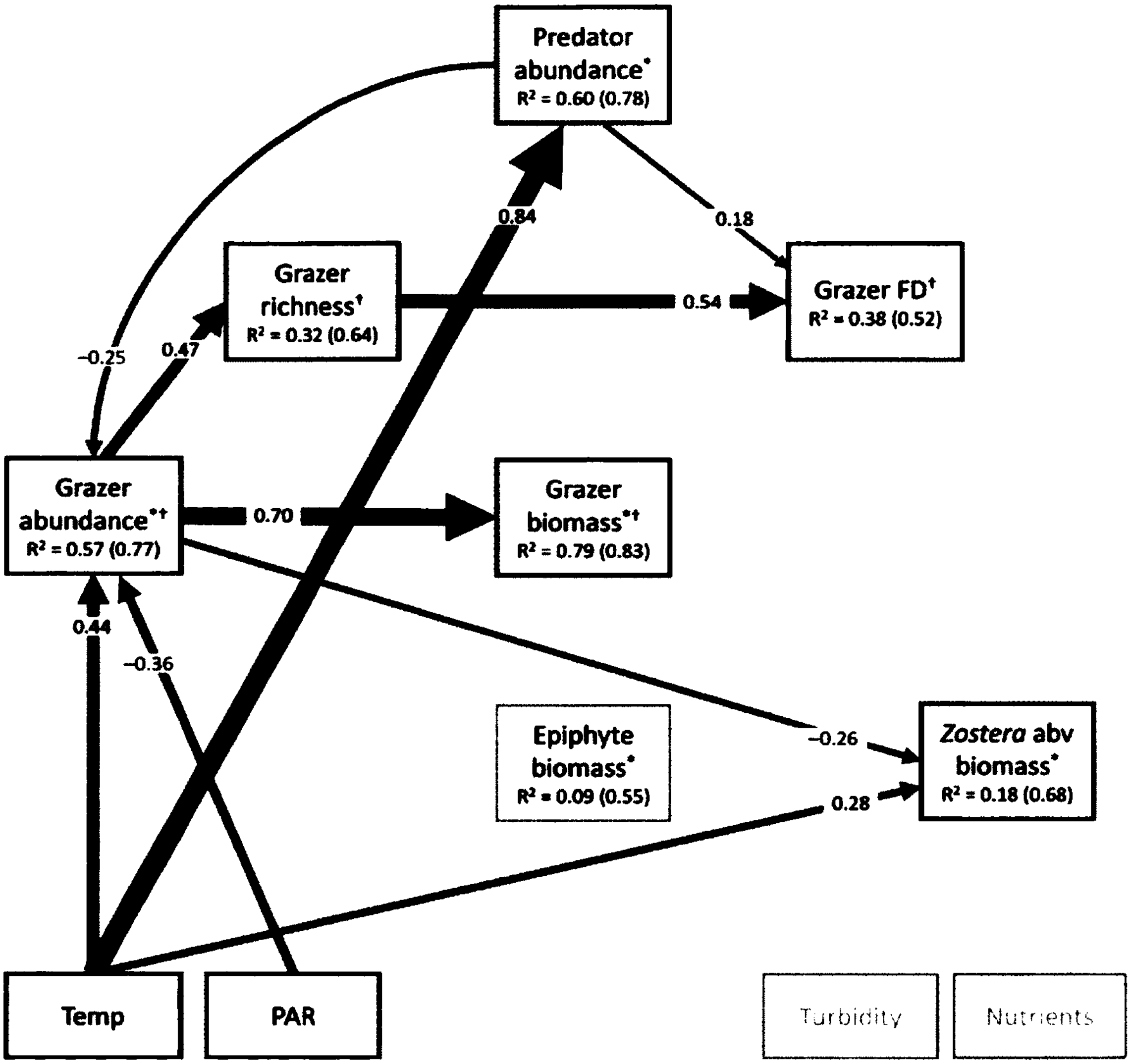

Figure 9: Structural equation model relating environmental and biological variables in spring across all years. Black arrows represent significant positive paths, while red arrows represent significant negative paths. Non-significant paths have been removed for clarity. Values represent standardized regression coefficients (centered and scaled prior to regression). Variables marked with an asterisk (*) have been $\log_{10}$-transformed to better meet the assumptions of linear regression. Variables marked with a cross (†) have additional significant paths from the covariate representing total plant material in each epifaunal grab, but this variable has been removed for clarity.

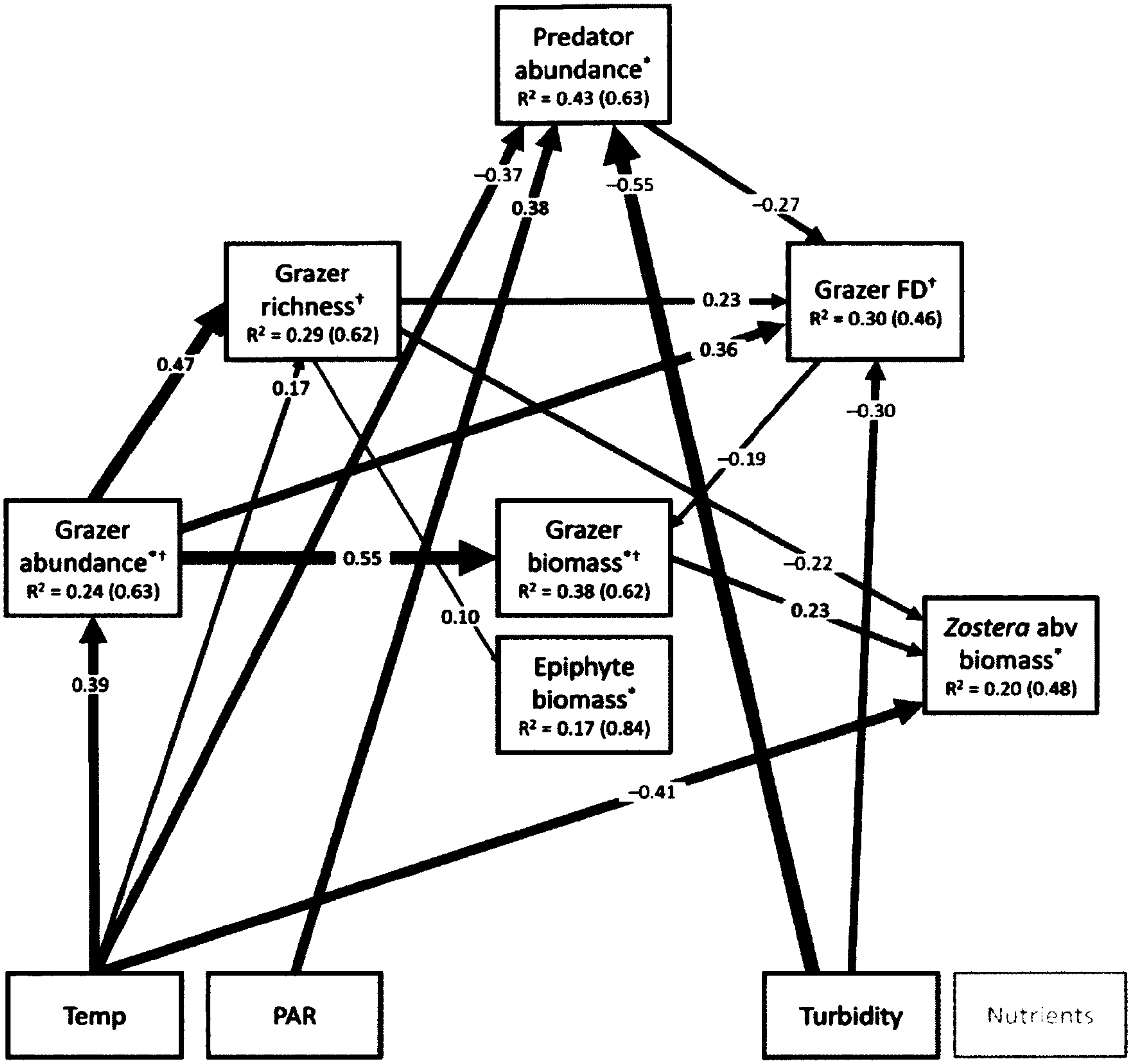

Figure 10: Structural equation model relating environmental and biological variables in summer across all years. Black arrows represent significant positive paths, while red arrows represent significant negative paths. Non-significant paths have been removed for clarity. Values represent standardized regression coefficients (centered and scaled prior to regression). Variables marked with an asterisk (*) have been $\log_{10}$-transformed to better meet the assumptions of linear regression. Variables marked with a cross (†) have additional significant paths from the covariate representing total plant material in each epifaunal grab, but this variable has been removed for clarity.

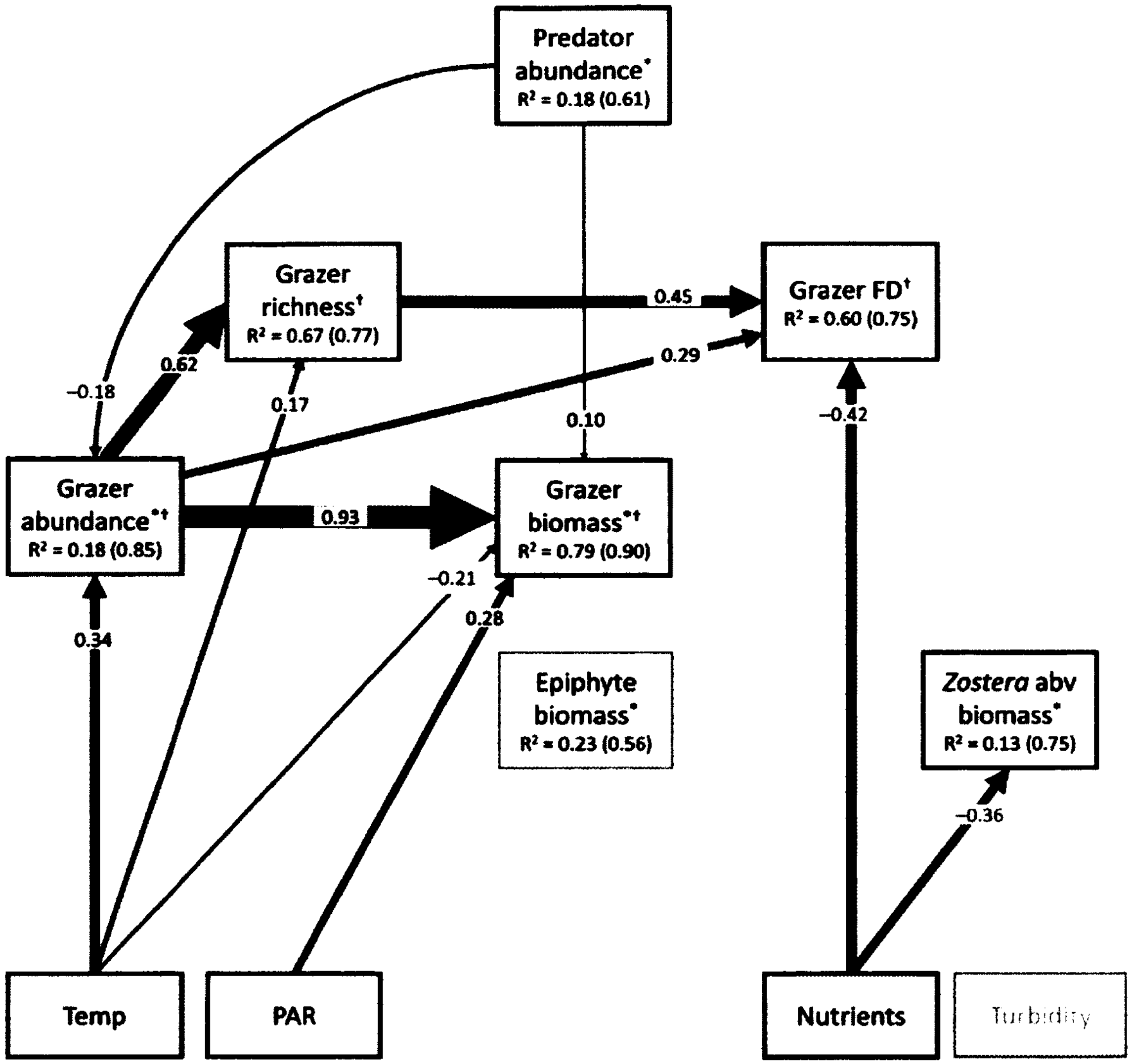

Figure 11: Structural equation model relating environmental and biological variables in fall across all years. Black arrows represent significant positive paths, while red arrows represent significant negative paths. Non-significant paths have been removed for clarity. Values represent standardized regression coefficients (centered and scaled prior to regression). Variables marked with an asterisk (*) have been $\log_{10}$-transformed to better meet the assumptions of linear regression. Variables marked with a cross (†) have additional significant paths from the covariate representing total plant material in each epifaunal grab, but this variable has been removed for clarity.

Supplementary Tables

Table S1: Functional traits used in the analysis, and their expected effect on assembly mechanisms.

Trait	Units	Description	Functional Interpretation	Reference
Defense	**None, shell (chitin), shell (calcium carbonate), spines**	**Describes the defense strategy of the organism.**	**Proxy for palatability and the likelihood of consumption, and energy transfer through the food web.**	**Bremner 2008[1]**
Body plan	Anguilliform, articulate (laterally-compressed), articulate (subcylindrical), articulate (ventrally-compressed), barrel, capped, conic, conic (elongate), conic (ovate), diffuse colonial, filiform, fusiform, globose, hinged (laterally-compressed), ventrally-compressed, worm-like (ventrally-compressed)	Describes the body shape of the organism.	Proxy for habitat preference and use; also for palatability and the likelihood of consumption, and energy transfer through the food web.	Bremner et al. 2003[2], Bremner et al. 2006a[3], Bremner et al. 2006b[4], Bremner 2008[1]
Feeding mode	**Detritivore, epiphytic grazer, filter feeder, omnivore, predator (epibionts), predator (generalist), predator (mollusks), predator (polychaetes), suspension feeder**	**Describes the trophic level of the organism.**	**Proxy for energy transfer through the food web and potential for interactions.**	**Bremner et al. 2003[2], Bremner et al. 2006a[3], Bremner et al. 2006b[4], Bremner 2008[1]**
Maximum length	Continuous (mm)	Describes the maximum attainable length of an organism.	Proxy for potential contribution to community production.	Bremner et al. 2003[2], Bremner et al. 2006a[3], Bremner et al. 2006b[4], Bremner 2008[1]
Mean length	**Continuous (mm)**	**Describes the typical length of an organism.**	**Proxy for average contribution to community production.**	**Villéger et eal. 2010[5]**
Development mode	Asexual/sexual (broadcast), sexual (broadcast), sexual (brooder/broadcast), sexual (brooder/direct development),	Describes the reproductive strategy of the organism.	A proxy for dispersal ability and recruitment.	Bremner et al. 2003[2], Bremner et al. 2006a[3],

sexual (brooder/planktonic), sexual (brooder/broadcast), sexual (direct development), sexual (oviparous)	Bremner et al. 2006b[4], Bremner 2008[1]

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Supplementary Figures

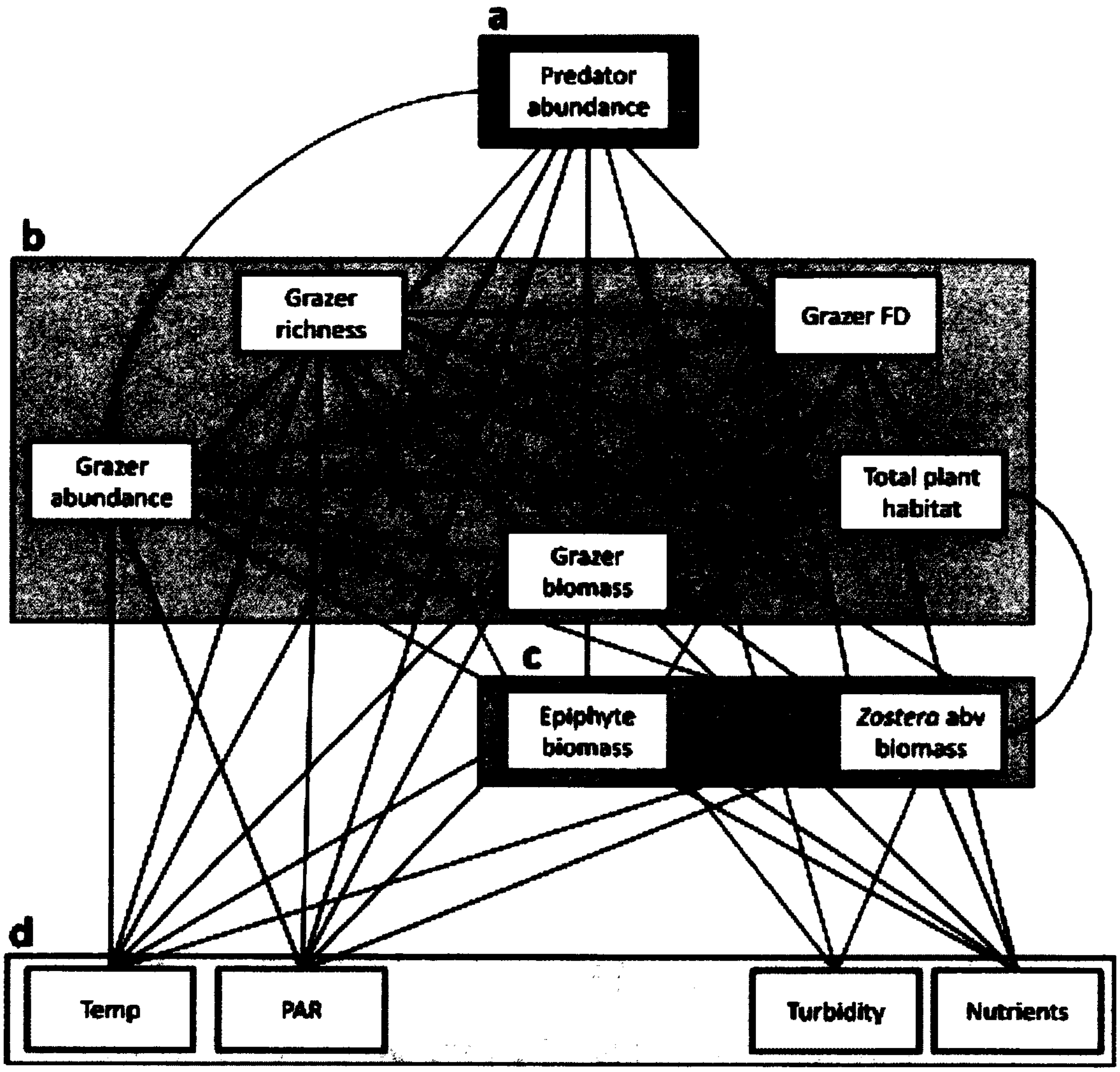

Figure S1: Structural equation model representing all paths fit for each of the models presented in the main text. Black arrows represent significant positive paths, while red arrows represent significant negative paths. Double-headed arrows represent correlations. Boxes correspond to discussion of the meta-model, Appendix A.

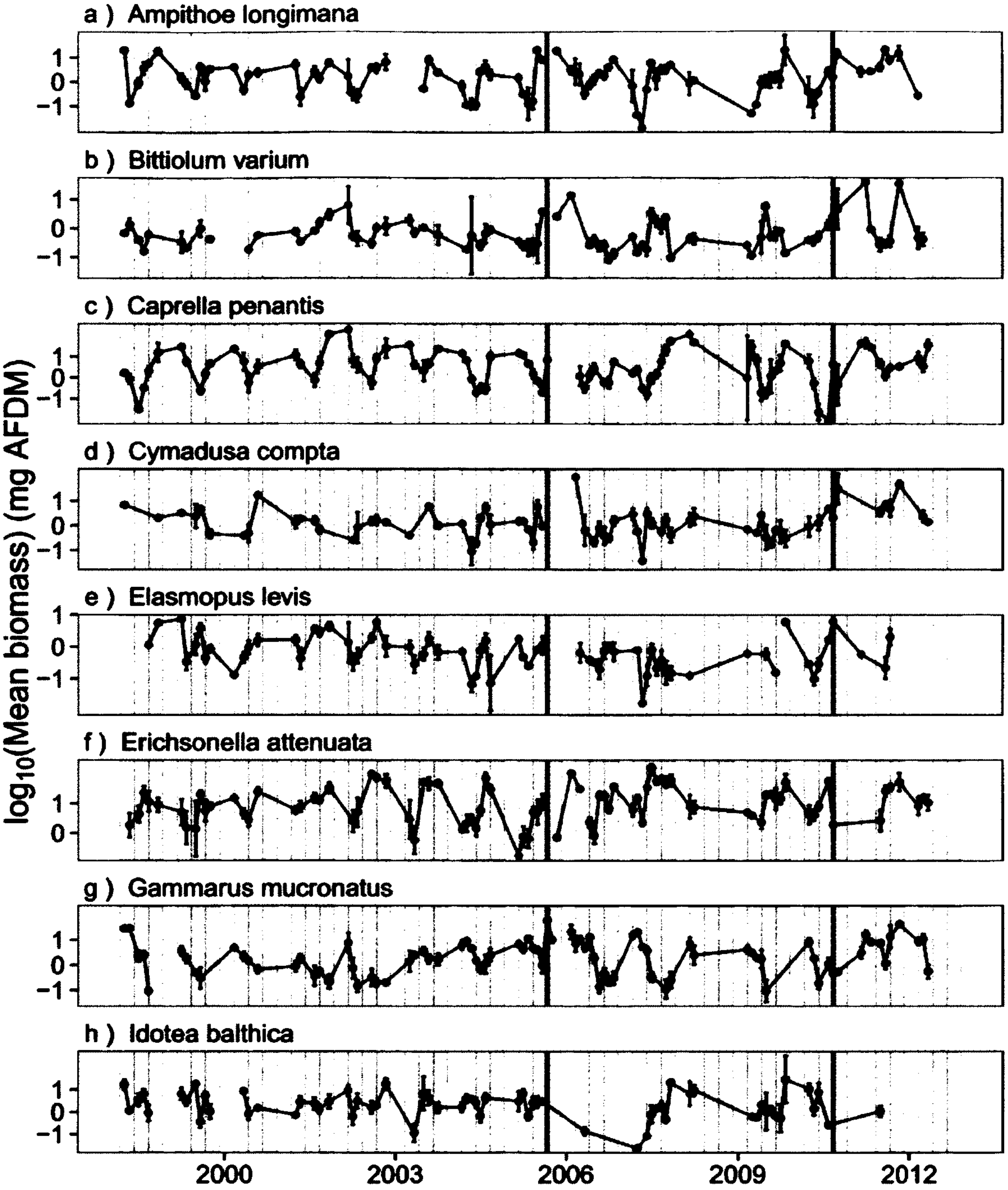

Figure S2: Interannual trends in mesograzer estimated biomass (mg AFDM) for the eight most abundant mesograzers in the survey (Table 2). Points are $\log_{10}$-transformed mean per epifaunal grab sample +/- 1 standard error.

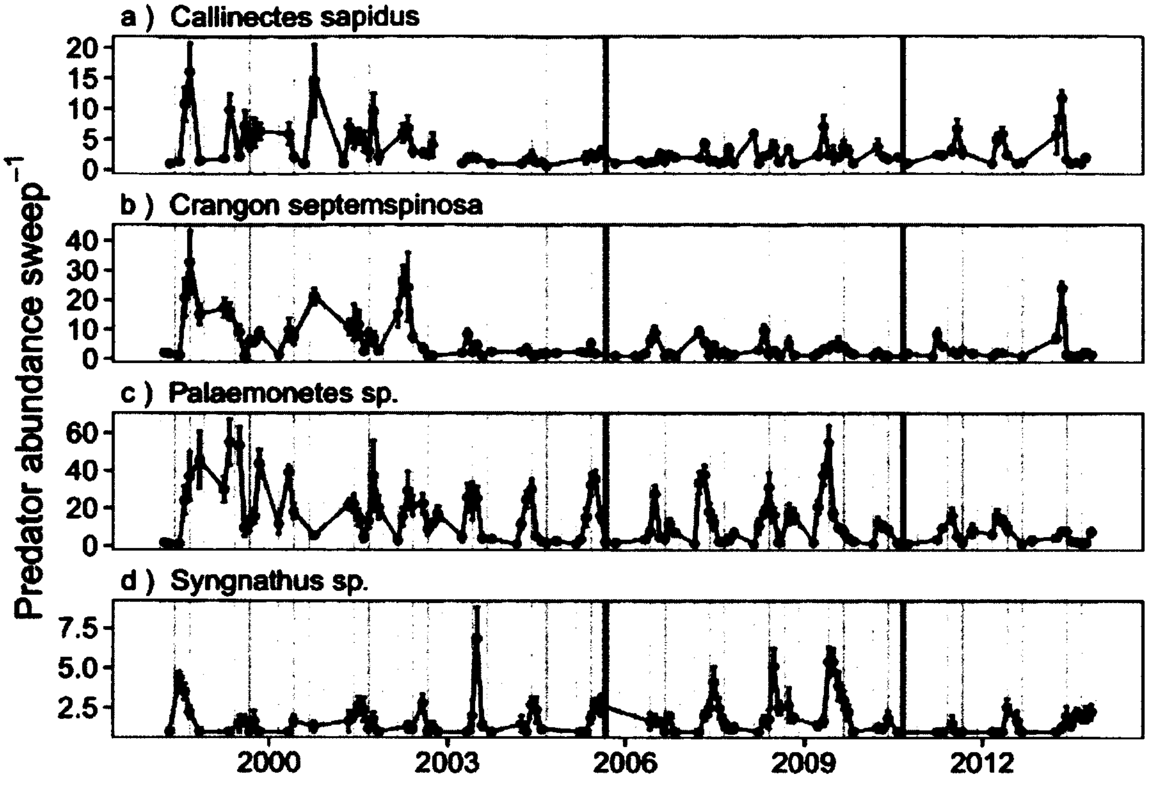

Figure S3: Interannual trends in predator abundance for the four most abundant predators in the survey, representing >96% of cumulative abundance. Points are mean per dipnet sweep +/- 1 standard error.

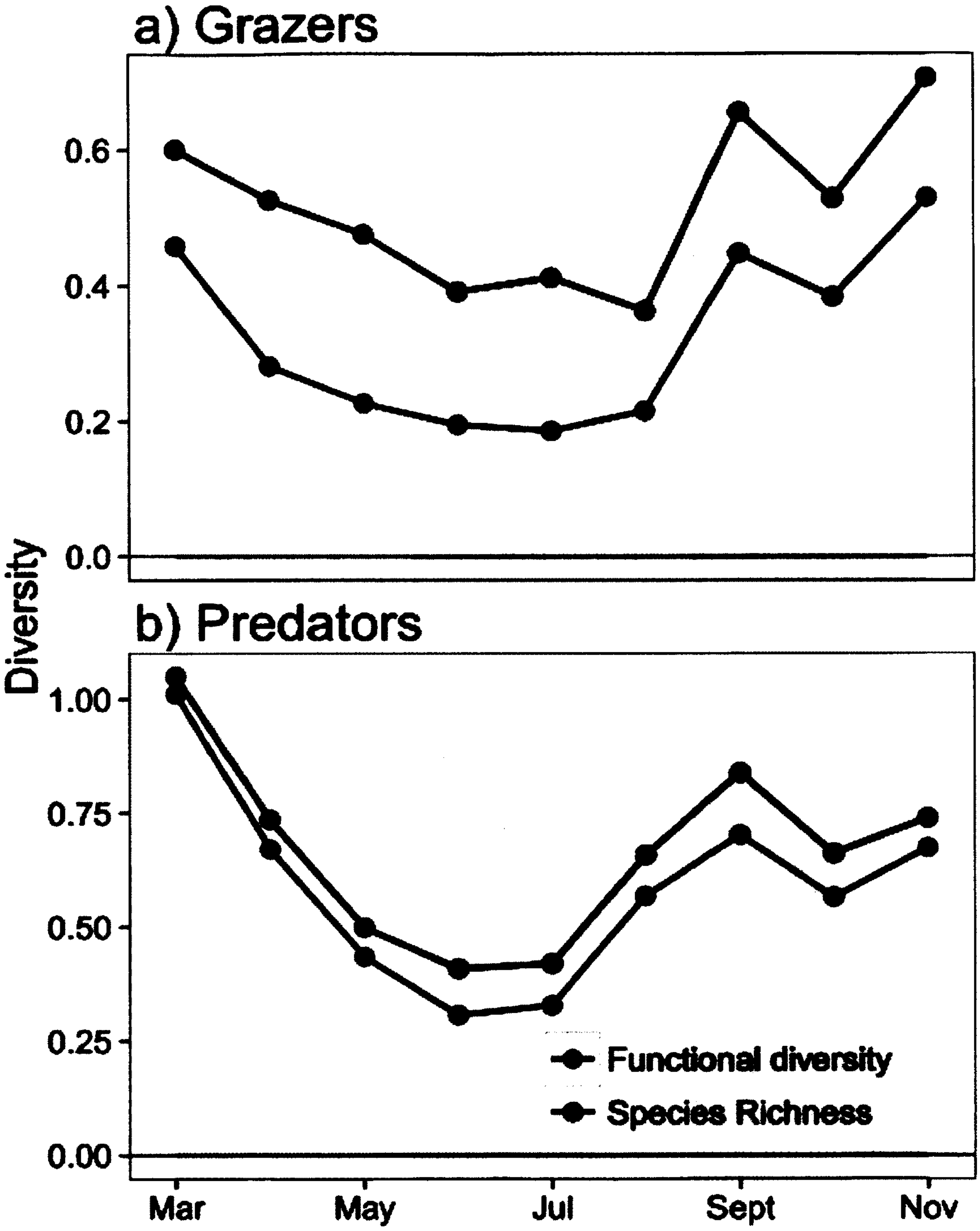

Figure S4: Seasonal trends in the coefficient of variation (CV = mean / standard deviation) for species richness and functional diversity of (a) grazers and (b) predators.

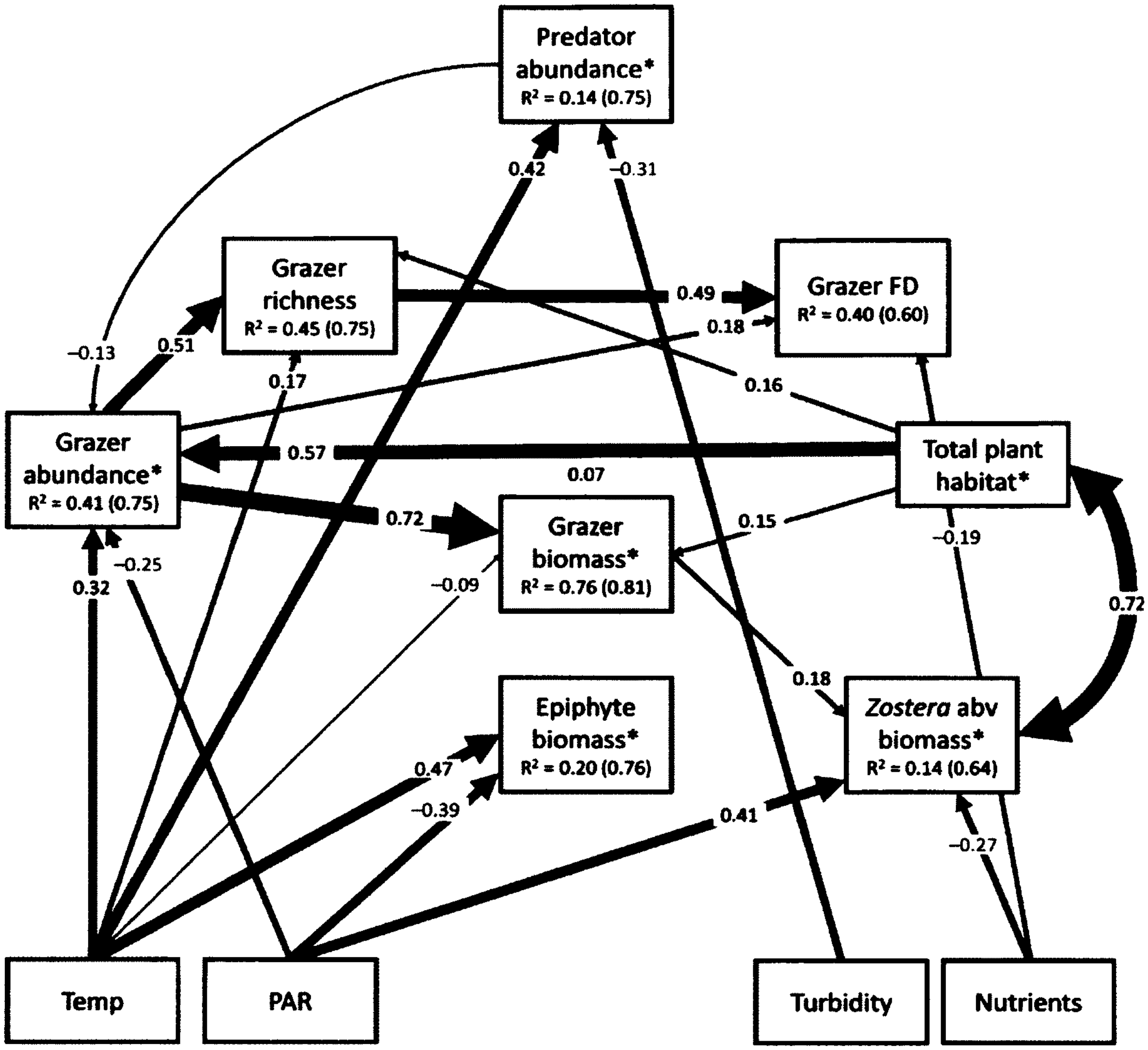

Figure S5: Structural equation model relating environmental and biological variables across all seasons and years. Black arrows represent significant positive paths, while red arrows represent significant negative paths. Non-significant paths have been removed for clarity. Values represent standardized regression coefficients (centered and scaled prior to regression). Variables marked with an asterisk (*) have been $\log_{10}$-transformed to better meet the assumptions of linear regression. Variables marked with a cross (†) have additional significant paths from the covariate representing total plant material in each epifaunal grab, but this variable has been removed for clarity.

Appendix A: Functional Trait Data

Table A1: Functional trait values used in the analysis of functional diversity.

Species	Defense	Body plan	Feeding mode	Max length mm	Mean length mm	Reproductive mode
Ameroculodes edwardsi	Shell - chitin	Articulate - laterally-compressed	Detritivore	9		Sexual - brooder / direct development
Ampelisca sp.	Shell - chitin	Articulate - laterally-compressed	Filter feeder	13.5	10	Sexual - brooder / direct development
Amphibalanus sp.	Shell - calcium carbonate	Barrel	Suspension feeder	20	10	Sexual - broadcast
Ampithoe longimana	Shell - chitin	Articulate - laterally-compressed	Grazer	20	11	Sexual - brooder / direct development
Ampithoe valida	Shell - chitin	Articulate - laterally-compressed	Grazer	20	12	Sexual - brooder / direct development
Anchoa mitchilli	None	Fusiform	Filter feeder	100	59	Sexual - broadcast
Anguilla rostrata	None	Anguilliform	Predator - generalist	1520	500	Sexual - broadcast
Astyris lunata	Shell - calcium carbonate	Conic - ovate	Predator - epibionts	5.8	2.5	Sexual - direct development
Bairdiella chrysoura	Spines	Fusiform	Predator - generalist	300	200	Sexual - oviparous
Bittiolum varium	Shell - calcium carbonate	Conic	Grazer	6.3	5	Sexual - oviparous
Blenniidae sp.	None	Fusiform	Predator - generalist	103		Sexual - oviparous
Boonea bisuturalis	Shell - calcium carbonate	Conic - ovate	Predator - mollusks	5.8	3.2	
Botryllus schlosseri	None	Diffuse colonial	Suspension feeder	100	10	Asexual / Sexual - broadcast
Bryozoa	Shell - calcium carbonate	Diffuse colonial	Suspension feeder	3	0.6	Asexual / Sexual - broadcast
Callinectes sapidus	Shell - chitin	Articulate - ventrally-compressed	Predator - generalist	250	145	Sexual - brooder / planktonic
Caprella equilibra	Shell - chitin	Articulate - subcylindrical	Suspension feeder	23		Sexual - brooder / direct development

Caprella penantis	Shell - chitin	Articulate - subcylindrical	Suspension feeder	16	13	Sexual - brooder / direct development
Corophium spp.	Shell - chitin	Articulate - laterally-compressed	Detritivore	11	4	Sexual - brooder / direct development
Crangon septemspinosa	Shell - chitin	Articulate - ventrally-compressed	Omnivore	70	32	Sexual - brooder / planktonic
Crepidula fornicata	Shell - calcium carbonate	Capped	Filter feeder	50	35	Sexual - brooder / planktonic
Cymadusa compta	Shell - chitin	Articulate - laterally-compressed	Grazer	20	11.5	Sexual - brooder / direct development
Doridella obscura	None	Capped	Omnivore	13	8	
Dulichiella appendiculata	Shell - chitin	Articulate - laterally-compressed	Grazer	25	16	Sexual - brooder / direct development
Edotia triloba	Shell - chitin	Articulate - ventrally-compressed	Detritivore	9		Sexual - brooder / direct development
Elasmopus levis	Shell - chitin	Articulate - laterally-compressed	Grazer	12	6	Sexual - brooder / direct development
Erichsonella attenuata	Shell - chitin	Articulate - ventrally-compressed	Grazer	13		Sexual - brooder / direct development
Erichthonius brasiliensis	Shell - chitin	Articulate - laterally-compressed	Detritivore	6		Sexual - brooder / direct development
Ericthonius rubricornis	Shell - chitin	Articulate - laterally-compressed	Detritivore	9		Sexual - brooder / direct development
Gammarus mucronatus	Shell - chitin	Articulate - laterally-compressed	Grazer	16	2.4	Sexual - brooder / direct development
Gemma gemma	Shell - calcium carbonate	Hinged - laterally-compressed	Filter feeder	5	2.5	Sexual - broadcast
Gobiesox strumosus	None	Fusiform	Predator - generalist	80		Sexual - oviparous
Gobiidae sp.	None	Fusiform	Predator - generalist	60	41	Sexual - oviparous
Gobiosoma bosc	None	Fusiform	Predator - generalist	60	41	Sexual - oviparous
Haminoea solitaria	Shell - calcium carbonate	Globose	Detritivore	19	13.5	Sexual - oviparous
Hippolyte pleuracanthus	Shell - chitin	Articulate - laterally-compressed	Omnivore	15		Sexual - oviparous

Hydrobia sp.	Shell - calcium carbonate	Globose	Grazer	6	3	Sexual - broadcast
Idotea balthica	Shell - chitin	Articulate - ventrally-compressed	Grazer	30	15	Sexual - brooder / direct development
Ilyanassa obsoleta	Shell - calcium carbonate	Conic	Detritivore	45	28	Sexual - oviparous
Japonactaeon punctostriatus	Shell - calcium carbonate	Barrel	Predator - polychaetes	7.5	5.3	
Lucania parva	None	Fusiform	Predator - generalist	50	37	Sexual - oviparous
Maldanidae sp.	None	Worm-like - ventrally-compressed	Detritivore	100		
Melita nitida	Shell - chitin	Articulate - laterally-compressed	Detritivore	15	4	Sexual - brooder / direct development
Mercenaria mercenaria	Shell - calcium carbonate	Hinged - laterally-compressed	Filter feeder	150	90	Sexual - broadcast
Microdeutopus anomalus	Shell - chitin	Articulate - laterally-compressed	Detritivore	11		Sexual - brooder / direct development
Micropogonias undulatus	Spines	Fusiform	Predator - generalist	550	300	Sexual - oviparous
Microprotopus raneyi	Shell - chitin	Articulate - laterally-compressed	Detritivore	5		Sexual - brooder / direct development
Molgula manhattensis	None	Globose	Filter feeder	40	25	Sexual - broadcast
Mulinia lateralis	Shell - calcium carbonate	Hinged - laterally-compressed	Filter feeder	20	15	Sexual - broadcast
Mya arenaria	Shell - calcium carbonate	Hinged - laterally-compressed	Filter feeder	152	26	Sexual - broadcast
Mysidae sp.	Shell - chitin	Articulate - laterally-compressed	Omnivore	17	13	Sexual - brooder / direct development
Nassarius vibex	Shell - calcium carbonate	Conic	Omnivore	20	15	Sexual - oviparous
Nereis sp.	None	Worm-like - ventrally-compressed	Omnivore	152		Sexual - broadcast
Pagurus annulipes	Shell - calcium carbonate	Conic	Omnivore	25	7.3	Sexual - oviparous

Palaemonetes pugio	Shell - chitin	Articulate - laterally-compressed	Omnivore	50	23.5	Sexual - brooder / broadcast
Palaemonetes sp.	Shell - chitin	Articulate - laterally-compressed	Omnivore	50	23.5	Sexual - brooder / broadcast
Palaemonetes vulgaris	Shell - chitin	Articulate - laterally-compressed	Omnivore	50	14	Sexual - brooder / broadcast
Panopeus herbstii	Shell - chitin	Articulate - ventrally-compressed	Omnivore	64	35	Sexual - brooder / broadcast
Paracaprella tenuis	Shell - chitin	Articulate - subcylindrical	Suspension feeder		10	Sexual - brooder / direct development
Paracerceis caudata	Shell - chitin	Articulate - ventrally-compressed	Omnivore	12		Sexual - brooder / direct development
Paralichthys dentatus	None	Ventrally-compressed	Predator - generalist	940		Sexual - oviparous
Percidae sp.	Spines	Fusiform	Predator - generalist	300	200	Sexual - oviparous
Pleuronectiformes sp.	None	Ventrally-compressed	Predator - generalist	940		Sexual - oviparous
Rhithropanopeus harrisii	Shell - chitin	Articulate - ventrally-compressed	Omnivore	21.3	8.41	Sexual - oviparous
Scianeid sp.	Spines	Filiform	Predator - generalist	550	300	Sexual - oviparous
Symphurus plagiusa	None	Ventrally-compressed	Predator - generalist	210		Sexual - oviparous
Syngnathus floridae	None	Filiform	Predator - generalist	250		Sexual - brooder / direct development
Syngnathus fuscus	None	Filiform	Predator - generalist	330	170	Sexual - brooder / direct development
Syngnathus sp.	None	Filiform	Predator - generalist	330	170	Sexual - brooder / direct development
Tanaidae sp.	Shell - chitin	Articulate - laterally-compressed	Detritivore	4	2	Sexual - brooder / direct development
Tozeuma sp.	Shell - chitin	Articulate - laterally-compressed	Omnivore	50		Sexual - oviparous
Triphora sp.	Shell - calcium carbonate	Conic - elongate	Omnivore	8		
Victorella pavida	Shell - calcium carbonate	Diffuse colonial	Suspension feeder	3	0.6	Asexual / Sexual - broadcast

Appendix B: Discussion of Structural Equation Models

To begin, we generated a 'meta-model' corresponding to our understanding of this system from numerous experiments and observations (Fig. A1). The meta-model relates suites of variables in general terms. For our purposes, we considered predators, grazers, primary producers, and environmental variables. Based on existing evidence summarized in Valentine and Duffy (2006), we expected predators to have a direct negative effect on grazers. Similarly, we expected grazers to have a direct negative effect on epiphytes. Thus, there should be an indirect positive effect of predators on epiphytes via a trophic cascade. We also expected epiphytes to negatively affect eelgrass biomass. Thus, we can extend the cascade to incorporate the indirect positive effect of grazers on eelgrass via the removal of epiphytes. Finally, we expected environment to have contrasting effects depending in the specific environmental variable, and the response. Thus we have chosen to represent it in the meta-model with grey arrows, to denote potentially positive or negative effects.

We populated the meta-model using the measured variables from the survey (Fig. A1, Fig. S1). For predators, we substituted a single variable corresponding to predator abundance. For grazers, we incorporated grazer abundance, biomass, richness, and functional diversity. The complex interplay among these variables deserve some attention. First, we included a path from grazer abundance to both grazer richness and functional diversity to account for potential sampling bias. In other words, as more individuals are sampled, it becomes more likely that a new species is discovered (Magurran and McGill 2011). One alternative is to create a rarefied index; however, these are not yet derived for our particular index of functional diversity (Chao et al. 2014). Thus we have instead chosen to model abundance as a covariate. Next, we expected richness to inform functional diversity, as the two must covary positively. For example, you cannot have more functional diversity than species diversity. Finally, we modeled the independent contributions of both grazer richness and functional diversity to biomass to test the hypothesis that greater diversity enhances biomass via increased functional complementarity (Loreau 1998).

For grazers, we also included the covariate of total plant habitat, which is the summed amount of planet material corresponding to eelgrass, widgeongrass *Ruppia maritima*, and any macroalgae in a given grab sample. These paths were intended to control for the amount of habitat and/or complexity increasing grazer properties. Because this was a methodological control vs. paths of actual biological interest, this variable and associated paths have been omitted from the graphics to aid in interpretability, but included in the actual statistical models. Thus for figures presented in the main text, the illustrated paths from and between grazer variables have had the effect of total plant habitat factored out.

We have modeled several top-down paths between predators and grazer properties. The most obvious are direct paths between predator abundance and both grazer abundance and biomass representing direct consumption. Of particular interest is the indirect path of predator abundance to grazer biomass mediated by grazer abundance, suggesting that predators can alter the size structure of the community but not the aggregate biomass (Edgar and Aoki 1993). We also had paths from predator abundance to grazer richness and functional diversity to test the hypothesis that increased diversity confers resistance to predation by increasing the probability of including a defended or unpalatable species (Duffy et al. 2007). In this case, we expected the effect to be stronger for functional diversity, as that better reflects the morphological differences among species that define defense or palatability. An alternative hypothesis is that these links are negative, implying that predators also reduce richness and functional diversity through the removal of individual species.

Next we modeled the top-down paths between grazer properties on primary producers. First, all grazer properties were connected with epiphytes. The obvious links are negative effects of grazer abundance and biomass reflecting direct consumption. However, we also included richness and functional diversity to test the hypothesis that increasing diversity increases the efficiency of resource consumption (Duffy et al. 2007). We chose not to model a path between predators and epiphytes as, unlike other systems (McGlathery 1995; Heck Jr. et al. 2000), major predators in this system are strictly carnivorous or are not heavily omnivorous (Douglass et al. 2011). We also chose to fit the direct effects between grazer propeties and eelgrass to reflect the potential for direct negative effects, as the isopod *Idotea balthica* and some ampithoid amphipods have been known to directly consume eelgrass (Duffy et al. 2005). This may be reflected in abundance or biomass (as the isopods tends to be larger), or richness (as higher diversity increases the likelihood of including a species that consumes eelgrass).

One of the paths of primary interest is the predicted negative effect of epiphytes on eelgrass biomass via shading and competition (Orth and van Montfrans 1984; Valentine and Duffy 2006). We also included a correlated error between total plant habitat obtained during the grab samples and eelgrass biomass derived from the core samples, since higher biomass of the cores reflected more plant material to sample with the grab. This correlated error is reported as the Pearson correlation in Figure S5.

Due to limitations in the statistical framework, we could not model several acyclic paths, i.e, A -> B -> C -> A (Shipley 2000; Shipley 2009). Thus, we could not, for instance, estimate the effect of eelgrass biomass (aka, habitat) on predator abundance. Similarly, we could not evaluate reciprocal relationships, i.e., A -> B, B -> A, since the directed effects cannot be independently partitioned. Thus, we have used *a priori* knowledge of the system to define hypotheses of interest. For example, we could equally assume the relationship between epiphytes and eelgrass to be reversed and positive, with increased eelgrass biomass provided more substrate for epiphytes. However, we have chosen to test the opposite hypothesis of epiphytes decreasing eelgrass, given its prevalence in the literature and direct role in evaluating the importance of epifaunal grazers (Valentine and Duffy 2006).

Finally, we hypothesized that most environmental variables would enhance ecosystem properties. For instance, temperature should increase metabolic rate and thus enhance predator and grazer abundance and/or biomass (Brown et al. 2004). The one exception is temperature and eelgrass, which actually may have a non-linear relationship, with temperature increasing eelgrass productivity up until a point after which it becomes physiologically stressful (Moore and Jarvis 2008). Due to statistical constraints, we have chosen not to model it as a non-linear predictor but instead anticipated that these threshold-responses would manifest across the three seasonal models (Fig. 9-11, main text). The one exception to the positive effects of environmental variables was turbidity, which we anticipated would reduce the foraging capacity of predators, decreasing their abundance as they migrate out of the bed, and primary producers, by reducing water column light availability. The pairwise correlations among environmental variables was sufficiently low that we feel comfortable including multiple variables in the same model, although we note that we did not explicitly test for collinearity. Finally, due to the overall low intra-annual variation in salinity (Fig. 1b), we have chosen not to include it in our SEM analysis.

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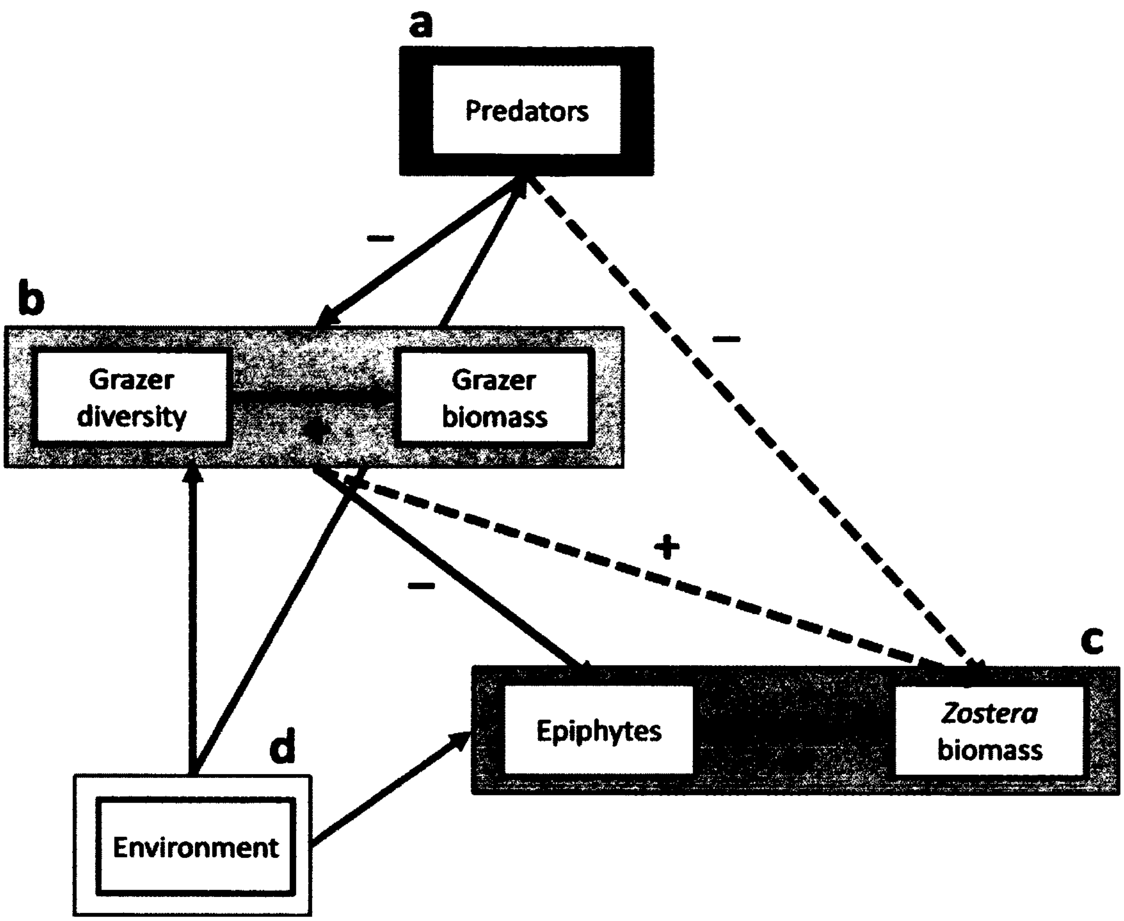

Figure A1: Meta-model linking (a-c) biotic and (d) abiotic variables. Black arrows indicate expected positive relationships, red arrows indicate expected negative relationships. Grey arrows indicate a mix of expected positive and negative effects, depending on the variables considered within the broad category represented in the figure. Dashed arrows denote indirect effects.

Appendix C: Coefficient tables for structural equation models

In this appendix, we report the standardized regression coefficients (variables scaled and centered prior to regression), their standard errors, and corresponding *P*-values for all models reported in the main text. Corresponding figures in the main text are noted.

Table C1: SEM coefficients from spring model (corresponding to paths in Figure 9, main text).

Response	Predictor	Estimate	Standard Error	*P*-value
Predator abundance	Mean Temperature	0.838	0.116	0
Predator abundance	Mean Turbidity	-0.258	0.148	0.104
Predator abundance	Mean PAR	0.064	0.125	0.62
Grazer species richness	Grazer abundance	0.471	0.096	0
Grazer species richness	Total plant habitat	0.224	0.115	0.053
Grazer species richness	Mean Temperature	0.142	0.111	0.204
Grazer species richness	Predator abundance	-0.158	0.161	0.342
Grazer species richness	Nutrient concentration	0.036	0.164	0.828
Grazer functional diversity	Grazer species richness	0.535	0.07	0
Grazer functional diversity	Grazer abundance	0.184	0.09	0.043
Grazer functional diversity	Nutrient concentration	-0.135	0.096	0.182
Grazer functional diversity	Mean Turbidity	-0.045	0.107	0.68
Grazer functional diversity	Predator abundance	-0.043	0.111	0.701
Grazer functional diversity	Total plant habitat	0.024	0.097	0.807
Grazer abundance	Total plant habitat	0.618	0.073	0
Grazer abundance	Mean Temperature	-0.251	0.08	0.002
Grazer abundance	Predator abundance	0.442	0.139	0.007
Grazer abundance	Mean PAR	-0.364	0.147	0.026
Grazer abundance	Nutrient concentration	-0.026	0.124	0.838
Grazer biomass	Grazer abundance	0.698	0.061	0
Grazer biomass	Total plant habitat	0.22	0.068	0.001
Grazer biomass	Predator abundance	0.068	0.043	0.118
Grazer biomass	Mean Temperature	0.077	0.068	0.257
Grazer biomass	Grazer functional diversity	-0.1	0.098	0.322
Grazer biomass	Nutrient concentration	0.011	0.049	0.816
Grazer biomass	Grazer species richness	0	0.074	0.997
Grazer biomass	Mean PAR	0	0.08	0.999
Epiphyte biomass	Mean Temperature	-0.204	0.214	0.357
Epiphyte biomass	Mean PAR	-0.205	0.219	0.366
Epiphyte biomass	Nutrient concentration	0.125	0.216	0.573

Epiphyte biomass	Grazer biomass	0.113	0.216	0.607
Epiphyte biomass	Grazer species richness	-0.015	0.069	0.832
Epiphyte biomass	Grazer functional diversity	0.003	0.079	0.974
Epiphyte biomass	Mean Turbidity	-0.001	0.082	0.995
Eelgrass aboveground biomass	Mean PAR	-0.262	0.112	0.02
Eelgrass aboveground biomass	Grazer biomass	0.159	0.098	0.107
Eelgrass aboveground biomass	Nutrient concentration	0.259	0.153	0.113
Eelgrass aboveground biomass	Mean Turbidity	0.109	0.069	0.117
Eelgrass aboveground biomass	Grazer functional diversity	0.14	0.117	0.253
Eelgrass aboveground biomass	Grazer abundance	0.136	0.19	0.486
Eelgrass aboveground biomass	Grazer species richness	-0.038	0.06	0.531
Eelgrass aboveground biomass	Epiphyte biomass	0.015	0.058	0.793
Eelgrass aboveground biomass	Mean Temperature	0.029	0.141	0.839

Table C2: SEM coefficients from summer model (corresponding to paths in Figure 10, main text).

Response	Predictor	Estimate	Standard Error	*P*-value
Predator abundance	Mean Temperature	-0.55	0.143	0.004
Predator abundance	Mean Turbidity	-0.367	0.14	0.028
Predator abundance	Mean PAR	0.38	0.156	0.037
Grazer species richness	Grazer abundance	0.465	0.087	0
Grazer species richness	Total plant habitat	0.182	0.101	0.076
Grazer species richness	Mean Temperature	-0.134	0.164	0.435
Grazer species richness	Predator abundance	-0.102	0.17	0.562
Grazer species richness	Nutrient concentration	0.018	0.096	0.853
Grazer functional diversity	Grazer species richness	0.356	0.101	0.001
Grazer functional diversity	Grazer abundance	-0.266	0.108	0.015
Grazer functional diversity	Nutrient concentration	0.23	0.094	0.016
Grazer functional diversity	Mean Turbidity	-0.302	0.122	0.033
Grazer functional diversity	Predator abundance	0.036	0.13	0.787
Grazer functional diversity	Total plant habitat	-0.03	0.111	0.791
Grazer abundance	Total plant habitat	0.587	0.08	0
Grazer abundance	Mean Temperature	0.39	0.16	0.038
Grazer abundance	Predator abundance	-0.116	0.093	0.217
Grazer abundance	Mean PAR	-0.062	0.287	0.834
Grazer abundance	Nutrient concentration	-0.04	0.249	0.875
Grazer biomass	Grazer abundance	0.548	0.089	0
Grazer biomass	Total plant habitat	-0.187	0.068	0.006
Grazer biomass	Predator abundance	0.239	0.09	0.009
Grazer biomass	Mean Temperature	-0.127	0.089	0.153
Grazer biomass	Grazer functional diversity	0.053	0.099	0.606
Grazer biomass	Nutrient concentration	0.027	0.076	0.719
Grazer biomass	Grazer species richness	-0.052	0.158	0.748
Grazer biomass	Mean PAR	0.019	0.206	0.93
Epiphyte biomass	Mean Temperature	0.103	0.053	0.056
Epiphyte biomass	Mean PAR	0.663	0.425	0.158
Epiphyte biomass	Nutrient concentration	-0.535	0.356	0.172
Epiphyte biomass	Grazer biomass	0.296	0.206	0.188
Epiphyte biomass	Grazer species richness	0.031	0.046	0.495
Epiphyte biomass	Grazer functional diversity	-0.117	0.259	0.664
Epiphyte biomass	Mean Turbidity	0.004	0.051	0.942
Eelgrass aboveground biomass	Mean PAR	-0.218	0.105	0.041
Eelgrass aboveground biomass	Grazer biomass	0.229	0.114	0.047
Eelgrass aboveground biomass	Nutrient concentration	-0.412	0.178	0.049
Eelgrass aboveground biomass	Mean Turbidity	0.11	0.133	0.412

Eelgrass aboveground biomass	Grazer functional diversity	-0.154	0.187	0.433
Eelgrass aboveground biomass	Grazer abundance	0.07	0.093	0.456
Eelgrass aboveground biomass	Grazer species richness	0.181	0.276	0.531
Eelgrass aboveground biomass	Epiphyte biomass	-0.086	0.24	0.731
Eelgrass aboveground biomass	Mean Temperature	0.025	0.128	0.846

Table C3: SEM coefficients from fall model (corresponding to paths in Figure 11, main text).

Response	Predictor	Estimate	Standard Error	*P*-value
Predator abundance	Mean Temperature	-0.731	0.543	0.227
Predator abundance	Mean Turbidity	0.469	0.625	0.482
Predator abundance	Mean PAR	-0.217	0.301	0.498
Grazer species richness	Grazer abundance	0.617	0.103	0
Grazer species richness	Total plant habitat	0.21	0.097	0.035
Grazer species richness	Mean Temperature	-0.106	0.099	0.317
Grazer species richness	Predator abundance	-0.08	0.106	0.475
Grazer species richness	Nutrient concentration	0.021	0.08	0.798
Grazer functional diversity	Grazer species richness	0.453	0.112	0
Grazer functional diversity	Grazer abundance	-0.422	0.113	0.007
Grazer functional diversity	Nutrient concentration	0.292	0.129	0.026
Grazer functional diversity	Mean Turbidity	0.176	0.11	0.154
Grazer functional diversity	Predator abundance	0.107	0.084	0.206
Grazer functional diversity	Total plant habitat	-0.032	0.11	0.775
Grazer abundance	Total plant habitat	-0.175	0.07	0.015
Grazer abundance	Mean Temperature	0.183	0.076	0.018
Grazer abundance	Predator abundance	-0.272	0.197	0.216
Grazer abundance	Mean PAR	-0.285	0.279	0.346
Grazer abundance	Nutrient concentration	-0.077	0.194	0.703
Grazer biomass	Grazer abundance	0.926	0.079	0
Grazer biomass	Total plant habitat	-0.214	0.072	0.025
Grazer biomass	Predator abundance	0.279	0.104	0.037
Grazer biomass	Mean Temperature	0.101	0.051	0.053
Grazer biomass	Grazer functional diversity	-0.097	0.067	0.152
Grazer biomass	Nutrient concentration	0.038	0.062	0.538
Grazer biomass	Grazer species richness	-0.008	0.069	0.905
Grazer biomass	Mean PAR	-0.007	0.073	0.932
Epiphyte biomass	Mean Temperature	-0.194	0.149	0.198
Epiphyte biomass	Mean PAR	-0.897	0.616	0.205
Epiphyte biomass	Nutrient concentration	0.129	0.136	0.343
Epiphyte biomass	Grazer biomass	-0.219	0.219	0.364
Epiphyte biomass	Grazer species richness	0.499	0.544	0.401
Epiphyte biomass	Grazer functional diversity	-0.036	0.158	0.82
Epiphyte biomass	Mean Turbidity	0.017	0.326	0.96
Eelgrass aboveground biomass	Mean PAR	-0.357	0.145	0.057
Eelgrass aboveground biomass	Grazer biomass	0.246	0.209	0.244
Eelgrass aboveground biomass	Nutrient concentration	0.089	0.076	0.244
Eelgrass aboveground biomass	Mean Turbidity	-0.149	0.132	0.263
Eelgrass aboveground biomass	Grazer functional diversity	-0.166	0.241	0.493

Eelgrass aboveground biomass	Grazer abundance	-0.101	0.197	0.631
Eelgrass aboveground biomass	Grazer species richness	-0.046	0.121	0.701
Eelgrass aboveground biomass	Epiphyte biomass	-0.044	0.345	0.904
Eelgrass aboveground biomass	Mean Temperature	-0.028	0.361	0.941

Table C4: SEM coefficients from the full model integrating across all years and months (corresponding to paths in Figure S5).

Response	Predictor	Estimate	Standard Error	*P*-value
Predator abundance	**Mean Temperature**	**0.421**	**0.135**	**0.003**
Predator abundance	Mean Turbidity	-0.309	0.148	0.042
Predator abundance	**Mean PAR**	**0.165**	**0.142**	**0.252**
Grazer species richness	Grazer abundance	0.511	0.054	0
Grazer species richness	**Total plant habitat**	**0.156**	**0.06**	**0.01**
Grazer species richness	Mean Temperature	0.171	0.08	0.037
Grazer species richness	**Predator abundance**	**0.062**	**0.053**	**0.244**
Grazer species richness	Nutrient concentration	0.017	0.093	0.858
Grazer functional diversity	**Grazer species richness**	**0.486**	**0.054**	**0**
Grazer functional diversity	Grazer abundance	0.175	0.065	0.007
Grazer functional diversity	**Nutrient concentration**	**-0.192**	**0.069**	**0.007**
Grazer functional diversity	Mean Turbidity	-0.073	0.07	0.3
Grazer functional diversity	**Predator abundance**	**0.049**	**0.057**	**0.39**
Grazer functional diversity	Total plant habitat	-0.024	0.067	0.717
Grazer abundance	**Total plant habitat**	**0.572**	**0.045**	**0**
Grazer abundance	Mean Temperature	0.324	0.085	0
Grazer abundance	**Predator abundance**	**-0.125**	**0.047**	**0.008**
Grazer abundance	Mean PAR	-0.248	0.116	0.037
Grazer abundance	**Nutrient concentration**	**-0.02**	**0.096**	**0.836**
Grazer biomass	Grazer abundance	0.716	0.041	0
Grazer biomass	**Total plant habitat**	**0.153**	**0.043**	**0**
Grazer biomass	Predator abundance	0.074	0.036	0.039
Grazer biomass	**Mean Temperature**	**-0.088**	**0.043**	**0.046**
Grazer biomass	Grazer functional diversity	0.018	0.03	0.549
Grazer biomass	**Nutrient concentration**	**-0.023**	**0.045**	**0.61**
Grazer biomass	Grazer species richness	0.018	0.036	0.613
Grazer biomass	**Mean PAR**	**0.006**	**0.053**	**0.905**
Epiphyte biomass	Mean Temperature	0.471	0.132	0.001
Epiphyte biomass	**Mean PAR**	**-0.392**	**0.148**	**0.011**
Epiphyte biomass	Nutrient concentration	0.194	0.124	0.124
Epiphyte biomass	**Grazer biomass**	**-0.061**	**0.043**	**0.162**
Epiphyte biomass	Grazer species richness	0.043	0.045	0.341
Epiphyte biomass	**Grazer functional diversity**	**0.013**	**0.037**	**0.715**
Epiphyte biomass	Mean Turbidity	0.048	0.139	0.73
Eelgrass aboveground biomass	**Mean PAR**	**0.413**	**0.152**	**0.009**
Eelgrass aboveground biomass	Grazer biomass	0.184	0.074	0.013
Eelgrass aboveground biomass	**Nutrient concentration**	**-0.274**	**0.125**	**0.034**

Eelgrass aboveground biomass	Mean Turbidity	-0.161	0.129	0.219
Eelgrass aboveground biomass	**Grazer functional diversity**	**0.032**	**0.047**	**0.503**
Eelgrass aboveground biomass	Grazer abundance	-0.057	0.085	0.508
Eelgrass aboveground biomass	**Grazer species richness**	**-0.034**	**0.06**	**0.567**
Eelgrass aboveground biomass	Epiphyte biomass	0.006	0.057	0.91
Eelgrass aboveground biomass	**Mean Temperature**	**-0.014**	**0.13**	**0.912**

CHAPTER 3.

FUNCTIONAL DIVERSITY PREDICTS ECOSYSTEM FUNCTIONING IN MULTITROPHIC ASSEMBLAGES OF ESTUARINE CONSUMERS

Abstract

The use of functional traits to explain how biodiversity affects ecosystem functioning has attracted intense interest, yet few studies have *a priori* manipulated functional diversity, especially in multitrophic communities. Here, we manipulated multivariate functional diversity of estuarine grazers and predators within two levels of species richness to test how species richness and functional diversity predicted ecosystem functioning in a multitrophic food web. Community functional diversity was a better predictor than species richness for the majority of ecosystem properties, based on general linear mixed effects models. Combining inferences from 8 traits into a single multivariate index increased prediction accuracy of these properties relative to any individual trait. Structural equation modeling revealed that functional diversity of both grazers and predators was important in driving final biomass within and between trophic levels, with stronger effects observed for predators. We also show that different species drove different ecosystem responses, with evidence for both sampling effects and complementarity. Our study extends experimental investigations of functional trait diversity to a multilevel food web, and demonstrates that functional diversity can be more accurate and effective than species richness in predicting community biomass in a food web context.

Keywords: biodiversity, functional diversity, ecosystem functioning, consumers, grazers, predators, estuaries

Introduction

Hundreds of experiments have shown that biodiversity generally enhances the functioning of ecosystems, including biomass production, efficiency of resource use, and nutrient cycling, yet there are many examples where diversity has had a neutral or even negative effect on functioning (Hooper et al. 2005, Cardinale et al. 2006, 2012, Lefcheck et al. 2013, Gamfeldt et al. 2015). A possible explanation for negative diversity effects is that the species used in these manipulations overlap sufficiently in their ecological strategies to prevent mechanisms like resource use partitioning from occurring (Hooper et al. 2005). One way to characterize the degree of overlap among species is to consider their functional traits, aspects of their morphology, physiology, phenology, and behavior that distinguish ecological differences among species. The variation in these traits across all species within an assemblage can be used to characterize functional trait diversity (hereafter FD).

There has been a great deal of interest in using FD to predict ecosystem functioning because traits not only account for potential functional redundancy (Rosenfeld 2002), but also provide a mechanistic link to observed diversity effects (Díaz and Cabido 2001). Recent investigations have integrated multiple traits in multivariate indices of FD, which have yielded varying support for the utility of FD as a predictor of ecosystem functioning, principally standing stock biomass (Petchey et al. 2004, Mouillot et al. 2011, Flynn et al. 2011, Gagic et al. 2015). However, most experimental studies utilizing multivariate FD have taken a *post hoc* approach by applying trait data to existing richness manipulations, principally of grassland plants. This approach can lead to ambiguous results if the replicates within and across levels of richness were not sufficiently varied in terms of their functional traits. Only a few studies have *a priori* manipulated multiple traits (e.g., Schittko et al. 2014), and two used only pairwise combinations of aquatic algae (Griffin et al. 2009, Shurin et al. 2014), which is not generally recognized as a diversity manipulation *per se* (Cardinale et al. 2006).

Much of biodiversity-ecosystem function research has been conducted with terrestrial plants, and an important challenge is understanding the consequences of changing diversity in complex natural food webs (Duffy et al. 2007, Reiss et al. 2009). Comparatively few studies have simultaneously manipulated the species richness of adjacent trophic levels (e.g., both predators and prey), and those that have done so generally found a strong role of consumer diversity for the structure and functioning of lower trophic levels (Fox 2004, Gamfeldt et al. 2005, Douglass et al. 2008, Bruno et al. 2008). This strong top-down effect of consumer diversity has often been shown to depend on feeding biology, specifically whether the consumers are omnivorous (Bruno and O'Connor 2005) or intra-guild predators (Finke and Denno 2004), or whether they vary in their per capita consumption rates (Straub and Snyder 2006) or resource preferences (O'Connor and Bruno 2007). While these studies suggested differences in feeding ecology among species as a potential explanation for their results, they did not directly manipulate resource acquisition strategies, but rather generally assumed that feeding diversity would be correlated with species richness. Of the three prior studies that *a priori* manipulated consumer traits within a single level of richness, two found variation in trophic ecology to be a strong predictor of resource depletion (Schmitz 2008, Best et al. 2013), while one found no effect (O'Connor and Bruno 2009).

In this study, we simultaneously manipulated eight functional traits of consumers both within and across multiple levels of species richness in experimental estuarine mesocosms. The consumers included naturally abundant herbivorous grazers and their predators, which allowed us to experimentally recreate a model estuarine food web. We expected multivariate FD to be a better predictor of ecosystem properties than species richness by capturing a wider range of variation in ecological strategies (Petchey and Gaston 2002). Further, we expected FD within a trophic level to enhance the biomass of that trophic level (Duffy et al. 2007), and for predator diversity to have a stronger top-down effect than the bottom-up effect of grazer diversity (Gamfeldt et al. 2005, Borer et al. 2006, O'Connor and Bruno 2007, Douglass et al. 2008).

Methods

Experimental Species

We defined a 9-species pool based on natural abundances of herbivores and their predators sampled over 15 years in the York River Estuary, Chesapeake Bay, USA (Douglass et al. 2010, Lefcheck 2015). The herbivores included three crustacean mesograzers: the amphipods *Gammarus mucronatus* and *Cymadusa compta* (potentially including a lesser incidental number of ampithoid amphipods, so referred to here as Ampithoid spp.), and the isopod *Erichsonella attenuata*. All three species are key grazers in the Chesapeake Bay and represent an important trophic link in local food webs (van Montfrans et al. 1984). We also used one gastropod, *Bittiolum varium*, a relatively small but seasonally abundant mesograzer (Duffy et al. 2003). The final herbivore was the shrimp *Hippolyte pleuracanthus*, whose diet is mainly micro- and macroalgae, but occasionally includes animal tissue (Douglass et al. 2011). The predators included the grass shrimp *Palaemonetes pugio* and juvenile blue crab *Callinectes sapidus* (30-50 mm carapace width), both of which are omnivorous (Douglass et al. 2011), as well as the pipefish *Syngnathus* sp. and mummichog *Fundulus heteroclitus*. Trophic guilds were assigned using existing stable isotope data (Douglass et al. 2011). For all of these species, we scored eight functional traits relating to morphology (defense, mobility, mean and maximum biomass, body plan), feeding habits (trophic level), and life history and phenology (reproductive mode, month of maximum abundance in the York River Estuary), with both direct and indirect consequences for ecosystem functioning (Table S1). All traits used in this study have been proposed to have a strong link to ecosystem function (Bremner et al. 2003).

Experimental Design

We employed a semi-nested design manipulating high and low multivariate FD within 3- and 6-species assemblages, along with each species by itself and all 9 species together (Fig. S1). To characterize FD, we chose the index of functional richness (Villéger et al. 2008). Functional richness quantifies the absolute volume of trait space occupied by all species within an assemblage. It is the volume of an *n*-dimensional polygon whose vertices are defined by the most functionally extreme species (Fig. S2). We chose functional richness as our index of FD because it does not take into account relative abundances. This behavior is ideal for our experiment, which combines large but rare predators with small but abundant grazers. Hereafter, when we refer to functional diversity (FD), we mean functional richness. Functional richness was calculated using the *dbFD* function in the *FD* package (Laliberté and Shipley 2011).

Within the two intermediate diversity levels, we generated every possible combination of 3- and 6-species. We calculated FD for each of these 168 combinations, and then randomly drew 6 replicates from the lower 25th percentile to represent 'low FD,' and 6 replicates from the upper 75th percentile to represent 'high FD,' for 3- and 6-species treatments respectively. We discarded and redrew any 3-species replicates that contained all predators, as we wanted to ensure resource availability for all multi-species replicates. Six additional replicates for each of the 9 single-species treatments and 9-species mixture yielded a total of *N* = 84 replicates. In each treatment, we equalized the initial biomass of the grazers at densities comparable to those observed in the field and used in previous mesocosm experiments with these organisms (Duffy et al. 2003, 2005). As a consequence of their large size and the logistical constraints on equalizing biomass, each predator simply stocked with a single individual in the treatments in which it appeared, and its initial weight recorded to include as a covariate in subsequent statistical analyses (see below).

In May 2012, experimental assemblages were created in 19-L mesocosm buckets placed in six flow-through seawater tanks. Water was passed through 150-µm mesh filters, which minimized the introduction of non-target species while permitting the passage of smaller invertebrate larvae (recruits) such as barnacles (*Balanus* spp.), bubble snails (*Haminoea solitaria*), polychaetes (*Nereis* sp.), and tunicates (*Mogula manhattensis*), as well as propagules of green and red filamentous algae. Mesocosms were arranged in a block design, with one replicate of each of the 14 treatments present in a single tank. Each mesocosm was filled with 1-kg of crushed oyster shell to provide a natural substrate, and 30-g wet weight of the macroalgae *Gracilaria* spp. (hereafter *Gracilaria*). *Gracilaria* is a common drift macroalgae in the Chesapeake Bay, and harbors a diverse epifaunal community (Parker et al. 2001). *Gracilaria* were defaunated in a diluted solution of the commercially available pesticide Sevin™ before being placed into the mesocosms for 72-h prior to introduction of any animals, after which time grazers were introduced into the experimental mesocosms, followed 48-h later by the predators. Twice a week, a pinch of freeze-dried krill was introduced into each mesocosm to prevent starvation of predators in monoculture.

The experiment was terminated after 3 weeks when we observed near total consumption of *Gracilaria* in some replicates. All algal and animal material was removed from the mesocosms and frozen, and predator wet weights were taken. Later, *Gracilaria*, recruiting red and green filamentous algae, predators, and recruiting invertebrates were thawed and identified to species, dried at 60°C until mass was stable, and then combusted to obtain final ash-free dry mass (AFDM) of each taxon. Smaller invertebrates, such as the stocked grazers and polychaetes, were isolated and passed through a series of stacked sieves, sorted to species, and counted. Abundance of each taxon in each sieve size was converted to an estimate of AFDM using the equations in Edgar (1990). Two replicates (one each of *C. sapidus* and *F. heteroclitus* monocultures) were discarded due to contamination by target species, and one replicate was lost during the experiment breakdown (9-species polyculture), leaving a total of $N = 81$ replicates for analysis.

Statistical Analysis

To quantify the relative contributions of initial species richness vs. FD in explaining ecosystem responses, we constructed general linear mixed effects models (GLMMs) regressing each response against species richness or FD, allowing the intercept to vary by the tank in which the mesocosm buckets were placed. For final predator biomass, an additional covariate of initial predator biomass was included in the model, since predator biomass could not be equalized at the start of the experiment. Species richness and FD were evaluated singly to avoid issues with multicollinearity. We selected the best model using AIC (Burnham and Anderson 2002). We also calculated marginal and conditional R^2 values (*sensu* Nakagawa and Schielzeth 2012)–corresponding to the variance explained by the fixed effect and the combined fixed and random effects, respectively—to gain a sense of the approximate variance in the response explained by each of the two predictors. We additionally fit regressions of each response against richness, FD, and their interaction, knowing that resulting *P*-values are likely to be inflated due to collinearity between richness and FD. All models were constructed in the R package *nlme* (Pinheiro et al. 2013). Model assumptions, including homogeneity of variance and normality of errors, were assessed graphically. Diversity indices were scaled by mean and variance to better meet model assumptions. Even so, for several responses, residuals were highly heteroscedastic. To resolve this issue, we modeled the variance using the function *varIdent*, using initial species richness levels as the stratum. Marginal and conditional R^2 values were calculated using the function by Lefcheck & Casallas (https://github.com/jslefche/rsquared.glmer).

As multivariate FD may obscure the potentially interacting contributions of individual traits (e.g., Spasojevic and Suding 2012), we conducted two additional analyses to assess and clarify the role of individual traits in explaining the observed patterns. First, we calculated the functional richness index separately for each individual trait, essentially representing the range of values encompassed by a particular assemblage for that trait. We then regressed these univariate FD values against each ecosystem response, as above. This procedure allowed us to quantify the contributions of individual traits, and determine whether trade-offs existed in the magnitude and direction of their individual effects. Second, we assessed the contribution of individual traits to the multivariate effect by conducting a jack-knifing procedure that removed a single trait, recalculated a multivariate FD from the remaining seven traits, and regressed this reduced jack-knifed index against each ecosystem response. We then re-fit the GLMMs to these jack-knifed indices and compared them to the GLMMs regressing the full multivariate index using Akaike's Information Criterion (AIC). The change in AIC score between the jack-knifed versus the full index of FD indicated whether any trait(s) had an inordinate influence on multivariate FD.

Because there was a potentially complex network of interactions among variables in the experiment, we conducted an additional analysis using piecewise structural equation modeling (SEM). Piecewise SEM combines information from multiple separate linear models into a single causal network (Shipley 2009). Because the individual models can incorporate various random structures, piecewise SEM is a powerful and flexible alternative to traditional variance-covariance based SEM. SEM also allowed us to decomposing the relative contributions of herbivore versus predator diversity on ecosystem responses, to test whether predators were wholly responsible for the significant community FD effects observed in our GLMMs. Following the recommendations of Grace (2006), we constructed a single causal network using knowledge of the system and ecological theory to define the paths of interest (outlined in Appendix A). We fit the component models as GLMMs (as above). We ran this model twice, substituting either species or functional richness for variables relating to herbivore or predator diversity. Overall fit was assessed using Shipley's test of d-separation, which yields a Fisher's *C* statistic that is χ^2 distributed (Shipley 2009). Species versus functional richness SEMs were compared using AIC (Shipley 2013). We used the open-source R package *piecewiseSEM* to conduct the piecewise SEM (https://github.com/jslefche/piecewiseSEM).

We further modeled the contribution of each individual species to understand whether species with different combinations of traits influenced different ecosystem functions. We constructed GLMMs regressing each response against the presence/absence of each species (e.g., Isbell et al. 2011). To understand whether the strongest effects were the result of extreme combinations of traits, we regressed the effect sizes from the GLMMs against functional distinctness, calculated as the average pairwise functional distance between a given species and all other species. Distances were derived from Gower's metric (Podani 1999), which unites both continuous and categorical trait information into a single continuous measure. All data and R code are provided as supplements. Vertebrates were handled according to IACUC standards (protocol 2012-05-11-7960 administered through The College of William & Mary).

Results

Multivariate functional diversity (FD) was a better predictor of and explained more variance in predator, grazer, and recruiting invertebrate biomass than species richness, based on comparison of model AIC values and marginal and conditional R^2 values (Table 1). Neither diversity index significantly predicted functions related to primary producers, explaining only 3-6% of the variance in recruiting algal and *Gracilaria* biomass. Despite the collinearity between initial species richness and FD (Fig. S3) leading to conservative *P*-values, models regressing the same responses in Table 1 against species richness, FD, and their interaction as predictors revealed identical trends to the model selection presented above (Table S2). Predicted fits extracted from the interaction models revealed a mild but significant decline in final grazer biomass with increasing FD (Fig. 1a), presumably due to the increasing frequency of predators with increasing FD. Recruiting invertebrate biomass also declined with increasing FD (Fig. 1b), also presumably indicating direct consumption by predators and omnivorous grazers (e.g., Duffy et al. 2003). Final predator biomass was higher in mesocosms with higher FD, even after accounting for differences in initial predator biomass (Fig. 1c). As found during the model selection procedure, there was no relationship between FD and either recruiting algal biomass (Fig. 1d) or final *Gracilaria* biomass (Fig. 1e).

Exploration of the effects of individual traits on final biomass responses revealed similar trends to multivariate FD (Fig. 2). Interestingly, the confidence intervals derived from multivariate FD tended to be narrower than for individual traits, particularly for armor, trophic level, mobility, and reproductive mode, suggesting that the composite index across multiple traits improved accuracy in predicting community biomass (Fig. 2, Fig. S4). These general trends were also conserved in our jack-knifing exercise, in which traits were individually removed and multivariate FD was calculated from the remaining pool of traits. The one exception was final predator biomass, which was more poorly predicted when either body plan, trophic level, or reproductive mode were left out, and better predicted when mobility and phenology (month of maximum abundance) were omitted (Table S3). Together, these results suggest that the inferences derived from multivariate FD were generally more robust than those for individual traits, and qualitatively corresponded with those derived from univariate FD.

To determine whether the overall effect of FD from the model fitting procedure persisted within each trophic level, we fit a structural equation model (SEM) decomposing community FD into independent herbivore and predator FD effects. We also fit the same model replacing FD with species richness. Overall, the FD SEM fit the data well ($C_{18} = 24.65$, $P = 0.924$, Fig. 3), and revealed that the strongest relationship in the experiment occurred between initial predator FD and final predator biomass ($\beta = 0.476$), after controlling for initial predator biomass. This relationship was still significant and similar in magnitude when considering only replicates that contained predators ($\beta = 0.418$, $P = 0.012$), to omit the extraneous influence of many replicates with 0 values for predator diversity. We also observed a positive but weaker relationship between final grazer FD and final grazer biomass ($\beta = 0.164$), even after the predator effects on grazer biomass were taken into account. This trend can be better visualized by extracting the partial correlations between final grazer FD and final grazer biomass, accounting for the other covariates in the SEM (Fig. S5). This relationship was still significant and even stronger when considering replicates that only contained grazers ($\beta = 0.353$, $P = 0.047$). In contrast, the SEM using species richness as the metric of diversity was a much poorer fit to the data than that using FD, but still adequate ($C_{18} = 39.49$, $P = 0.317$, Fig. 4). Indeed, comparison of AIC scores revealed that the SEM including FD was a much better than the one including species richness (AIC = 106.7 << 121.5 for FD and richness, respectively). The most striking difference between the two models was the lack of a significant effect of final grazer richness on grazer biomass in the richness SEM (Fig. 4). Additionally, the positive bottom-up path from grazer functional diversity to final predator biomass (Fig. 2) was absent in the richness SEM (Fig. 4).

In the SEM incorporating FD, the largest predator effects on final grazer biomass were mediated through grazer FD, as indicated by the lack of a significant direct path between final predator biomass and final grazer biomass (Fig. 3). The magnitude of this indirect effect is achieved by multiplying the two component paths: $\beta = -0.335 \times 0.164 = -0.055$, indicating a relatively weak but still significant reduction. Initial predator biomass weakly reduced final grazer biomass ($\beta = -0.180$), suggesting that grazer communities experienced rapid top-down control by predators, and only after prey communities had stabilized that a positive effect of grazer FD on grazer biomass was observed. In contrast, in the richness SEM, the primary top-down path manifested directly between initial predator richness and final grazer biomass (Fig. 4), although it was about 40% weaker than the corresponding effect of final predator biomass on grazer functional diversity ($\beta = 0.30$ vs. -0.48, Fig 3). All coefficients and their associated *P*-values are given in supplementary materials (Tables S4 and S5).

The individual contributions of each species to functioning revealed potential for complementarity across multiple functions (Table 2). As expected, most of the grazers positively and significantly contributed to final grazer biomass, with the exception of *E. attenuata*. Similarly, the two fishes *F. heteroclitus* and *Syngnathus* spp. both contributed positively to final predator biomass. The mummichog *F. heteroclitus* also significantly reduced recruiting invertebrate biomass, and Ampithoid spp. contributed significantly to reductions in final algal and *Gracilaria* biomass. Regression of the effect sizes in Table 2 against functional distinctness—calculated as the mean pairwise distance between a given species and all other species in multidimensional trait space—revealed that some functions were driven large-bodied, mobile predators, while others were driven by small-bodied, chitinous grazers (Fig. S6).

Discussion

In this study of an estuarine food web, we found that multivariate functional diversity better predicted standing stock biomass across multiple trophic levels than did species richness (Table 1). This result was a consequence of greater variation in FD than in richness (Fig. S3), confirming the superior utility of FD for capturing ecologically significant variation among members of an assemblage compared with the raw number of species. Further, structural equation modeling (SEM) revealed that this result was a consequence of functional diversity of both predators and grazers (Fig. 3), emphasizing the importance influence of multitrophic diversity on community structure and functioning (Strong 1992, Polis and Strong 1996).

Contrary to our predictions, we did not find a significant interaction between species richness and FD for most ecosystem responses, though initial species richness and functional diversity had antagonistic effects on final species richness (Table S2). This may be because of the high collinearity between species richness and functional diversity inflating standard errors of our model predictions ($r = 0.94$, Fig. S3). Despite this potential conservative bias, we were still able to isolate a significant positive effect of FD but not species richness. Thus, in our experiment, the effect of increasing FD on grazer, predator, and recruiting invertebrate biomass was not contingent on the level of species richness. One explanation may be our experimental design, which nested two levels of FD within only two levels of species richness (Fig. S1). There may have been too few levels of species richness, or too little variation among species' functional traits, to extract a clearer signal. Future manipulations may benefit from incorporating an even greater range of species richness and/or traits in investigation of diversity-function (Gamfeldt et al. 2015).

The stronger effects of predator FD compared relative to herbivores is consistent with both conceptual predictions relating to greater physiological, resource, and behavioral complexity with increasing trophic level (Duffy 2002), as well as experimental evidence (Griffin et al. 2013, Lefcheck et al. 2015). A possible explanation for the strong predator diversity effect in our experiment is that the predator species was more functionally distinct, on average, than the grazer species (mean functional distinctness ± S.E. for predators = 0.55 ± 0.06 vs. 0.45 ± 0.02 for grazers), enhancing the potential for resource complementarity among predators (e.g., Griffin et al. 2008). This distinctness, however, appears to be driven largely by *F. heteroclitus* (Fig. S6), which also happens to have the largest effects of all the predators on the various responses (Table 2). Thus, the stronger effect of predator diversity relative to herbivore diversity may best be interpreted as a 'sampling effect' (*sensu* Loreau 1998), driven by the presence of *F. heteroclitus*. This result speaks to the central role of *F. heteroclitus* in the food web dynamics of estuarine systems in the southeastern US (Kneib 1986).

In addition to positive effects of FD on biomass within trophic levels, we observed that initial grazer FD, but not initial species richness, increased final predator biomass (Fig. S7). These results confirm as expected that it is not just the total number of species, but also the functional identity of the grazers that are important in mediating predator-prey interactions. Our exploration of individual traits revealed that the predictive ability of multivariate FD decreased significantly when body plan and trophic level were left out of the index (Table S3), implying that variation in these traits was especially important in determining final predator biomass. It is not surprising that these traits come out as being particularly important, as they are central to classical habitat-based (Grinnell 1917) and resource-based definitions of ecological niches (Elton 1927). One possible mechanistic explanation is that body plan influenced susceptibility to predation. For instance, it is easy to envision how the long and slender body of the isopod *E.*

attenuata may exceed the gape limit of the pipefish *Syngnathus* spp., but could more easily be manipulated by the crab *C. sapidus*. Thus, differences in morphology may drive predator-specific selection of prey, and ultimately increase aggregate consumption across a variety of prey body types in a diverse assemblage.

Variation in trophic level may have been important in determining final predator biomass simply because high variation implies the presence more than one trophic level, i.e., predators and their prey. A related explanation for the positive effect of grazer functional diversity on predator biomass could be the 'balanced diet' hypothesis, where a diverse prey assemblage provides a more complete range of nutrients (Gamfeldt et al. 2005, Lefcheck et al. 2013). If prey species varied slightly in their positions within the food web, then they may be assimilating resources differently. For instance, the ampithoid amphipod complex was the only grazer to have a detectable negative effect on primary producers in our experiment by directly consuming algal species (Table 2), and previous experiments also documented distinct differences in diet between Ampithoid spp. and another amphipod grazer used in our experiment, *G. mucronatus* (Duffy and Harvilicz 2001). The positive effect of grazer functional diversity may thus indicate niche complementarity increasing aggregate biomass and/or nutritional value (Fig. 3, Fig. S5), leading to more prey for predators.

That the top-down effects of predators on grazers did not cascade to primary producers or recruiting invertebrate biomass was surprising, given both theoretical predictions (Strong 1992) and past experiments with these grazers (Duffy et al. 2003, 2005, O'Connor and Bruno 2007, Douglass et al. 2008). One possible explanation is that predators were simply so efficient at consuming grazers (Fig. 1a) that our model was unable to disentangle the indirect effects of predators removing grazers, and subsequent release of primary producers. Statistically, this would be manifested as the strong direct *positive* paths between predator biomass and final algal biomass in our SEMs (Figs. 3, 4). Similarly, while grazers can influence the recruiting invertebrates in mesocosms in the absence of predators (e.g., Duffy et al. 2003), their influence relative to larger predators was insignificant in our experiment (Table 2). This was also probably largely due to the rapid consumption of grazers (Fig. 1a). Thus, we observed a direct negative relationship between predator biomass and recruiting invertebrate biomass (Figs. 3, 4).

Our exploration of individual traits revealed that no individual trait was responsible for driving the patterns in multivariate FD. Rather, all traits showed generally similar trends to multivariate FD in influencing final biomass (Fig. 2). While this analysis confirmed that there were no strong trade-offs among individual traits that may have biased the multivariate trend, it also raises the question: why combine inferences from multiple traits at all? One answer is that the multivariate index generally had lower predicted standard errors (Fig. S4), thus improving prediction accuracy. In some cases, the multivariate index reduced standard errors on estimates of grazer and recruiting invertebrate biomass by up to 40%, particularly when considering only armor, body plan, and trophic level. This result also explains why models dropping these two traits generally had worse AIC scores when attempting to predict final predator biomass (Table S3). Thus, combining multiple traits enhanced the explanatory power of functional diversity, at least in our index of functional richness.

Richness and FD of species stocked in our mesocosms were lower at the end of the experiment than at the beginning (Fig. S8), highlighting the negative interactions among predators and grazers, and potentially among predators. For instance, blue crabs were lost in several replicates, leading to the overall non-significant effect of blue crabs on every ecosystem response (Table 2). The loss of *C. sapidus* corresponds with other experiments using this species (O'Connor and Bruno 2007, Douglass et al. 2008), and was partly due to crabs escaping the experimental mesocosms, and partly due to the death of crabs, as evidenced by empty carapaces found in the mesocosms at the end of the experiment. While there could have been antagonistic interactions among predators, all crabs were recovered from the polycultures, and virtually none from the monocultures. Cannibalism is not a likely explanation as predators were stocked individually in monoculture. This result contrasts those of Douglass et al. (2008), who found that crab growth and survival was highest in monoculture. They attributed this result to the presence of other predators modifying grazer composition to the detriment of blue crabs. The non-random pattern of crab loss across the treatments in this study suggests the opposite: that only the diverse assemblage provided the requisite resources for blue crab survival. This idea is bolstered by the finding that the 9-species mixture retained a higher number of stocked species (Fig. S8a).

Overall, this study empirically confirms that a focus on multiple functional traits can provide more accurate predictions regarding the functioning of whole food webs than single traits or species richness alone. Moreover, we show that functional diversity within multiple trophic levels (herbivores and predators) enhanced corresponding biomass even after accounting for the effects of adjacent trophic levels. This result suggests that conservation of diversity at multiple trophic levels, with a particular emphasis functionally diverse communities, can lead to enhanced community biomass.

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Tables

Table 1: AIC scores, marginal R^2_m, and conditional R^2_c values for competing models containing either species richness or functional diversity as a predictor of five ecosystem responses across three trophic levels. Models that are significantly better than the other at explaining the response based on lower AIC scores are bolded. Models predicting algal or *Gracilaria* biomass were approximately equivalent, and thus those rows have no bolded cells

	Species Richness			**Functional Diversity**		
Response	**AIC**	**R^2_m**	**R^2_c**	**AIC**	**R^2_m**	**R^2_c**
Final grazer biomass	133.1	0.106	0.107	**129.3**	**0.167**	**0.168**
Final predator biomass	31.4	0.479	0.479	**25.4**	**0.534**	**0.534**
Recruiting invertebrate biomass	-52.9	0.152	0.173	**-55.3**	**0.233**	**0.274**
Final algal biomass	-222.6	0.003	0.022	-222.3	0.000	0.018
Final *Gracilaria* biomass	288.5	0.063	0.063	288.9	0.059	0.059

Table 2: Standardized contributions of each individual species to ecosystem responses based on regressions of presence/absence of each species against a given response. Significant effects ($P < 0.05$) are bolded. Amp = Ampithoid spp., *Bitt* = *Bittiolum varium*, *Call* = *Callinectes sapidus*, *Erich* = *Erichsonella attenuate*, *Fund* = *Fundulus heteroclitus*, *Gamm* = *Gammarus mucronatus*, *Hippo* = *Hippolyte pleuracanthus*, *Pal* = *Palaemonetes pugio*, and *Syn* = *Syngnathus* spp.

Response	*Amp*	*Bitt*	*Call*	*Erich*	*Fund*	*Gamm*	*Hippo*	*Pal*	*Syn*
Final grazer biomass	**1.33**	**-0.57**	-0.29	0.10	**-0.68**	**0.69**	**-0.53**	-0.34	0.22
Final predator biomass	0.12	0.17	0.30	0.15	**1.19**	0.06	-0.03	0.15	**0.45**
Recruit invert biomass	0.41	-0.45	-0.24	0.24	**-0.70**	-0.03	-0.35	-0.09	-0.06
Final algal biomass	**-0.60**	0.07	-0.30	-0.15	0.01	0.17	**0.59**	0.03	0.01
Final *Gracilaria* biomass	**-0.73**	0.09	0.54	0.30	0.33	0.00	0.18	0.37	-0.28

Figures

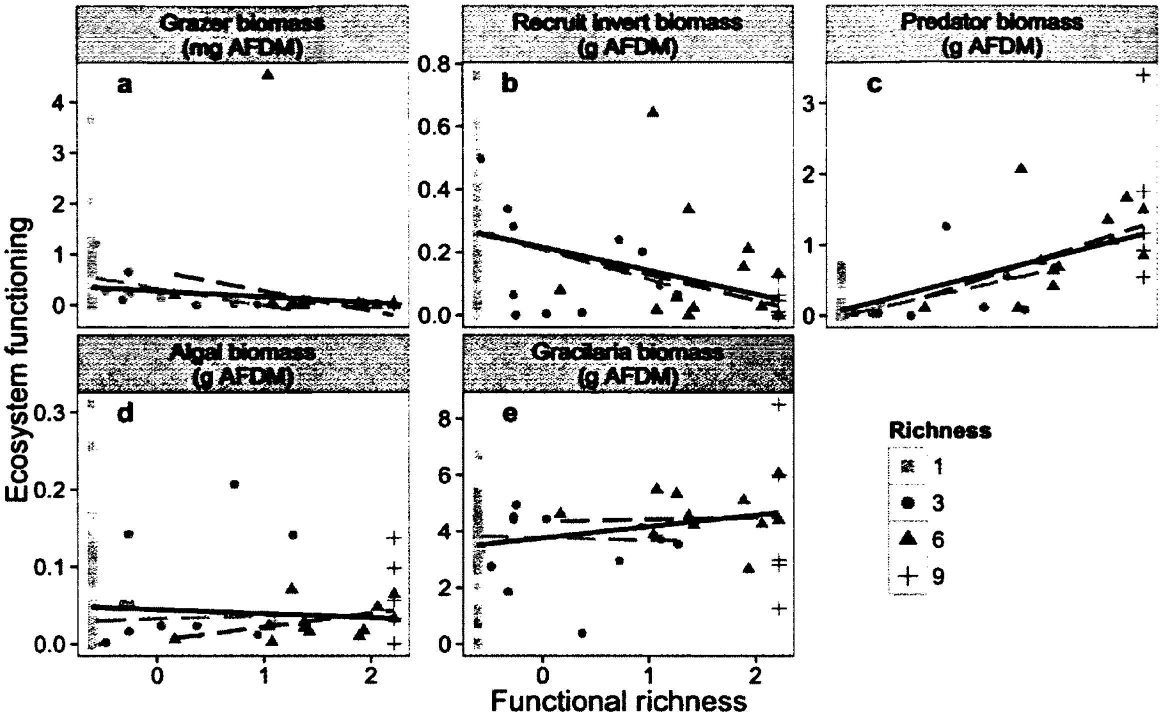

Figure 1: Scatterplot of initial functional richness against ecosystem responses. Shapes corresponds to the richness level (1, 3, 6, or 9). Grey lines represent predicted fits from a general linear mixed effects model for 3- (light grey) and 6-species (dark grey) treatment. The black line represents the overall trend from the same model. **(c)** The regression of final predator biomass again FD included initial predator biomass an additive covariate.

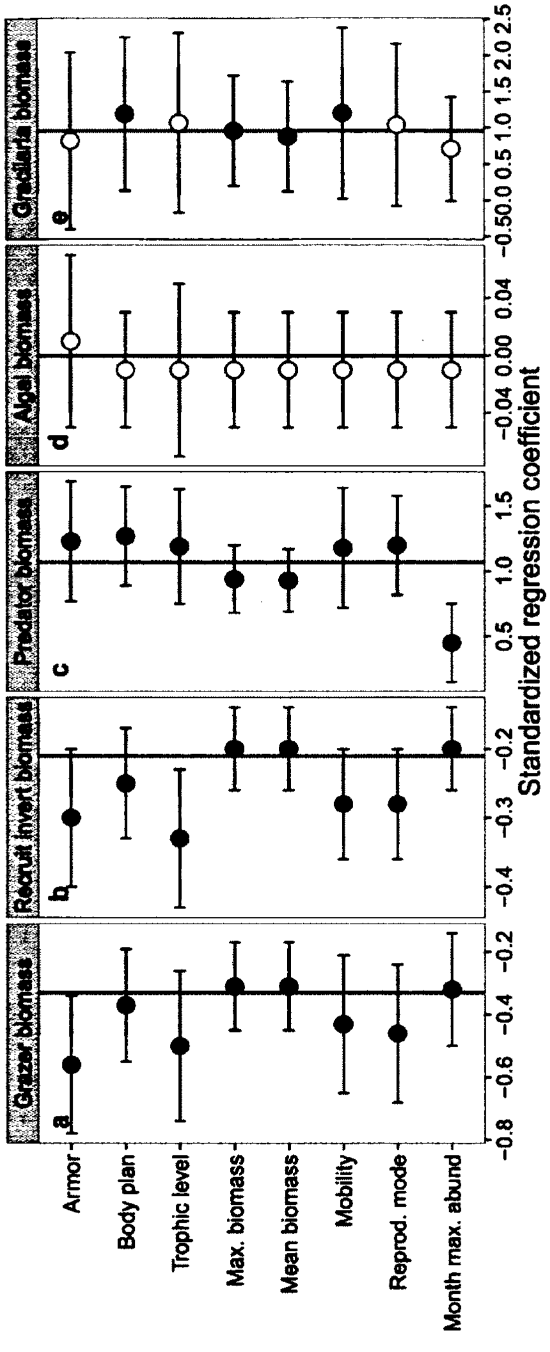

Figure 2: Standardized regression coefficients from models regressing ecosystem responses against functional richness calculated using each functional trait individually. Points are linear estimates ± 95% confidence intervals (2*SE). Black points indicate significance ($P < 0.05$), while white points are non-significant ($P \geq 0.05$). Vertical lines represent the linear estimates for the multivariate index of functional richness, and shaded areas indicate ±95% confidence intervals.

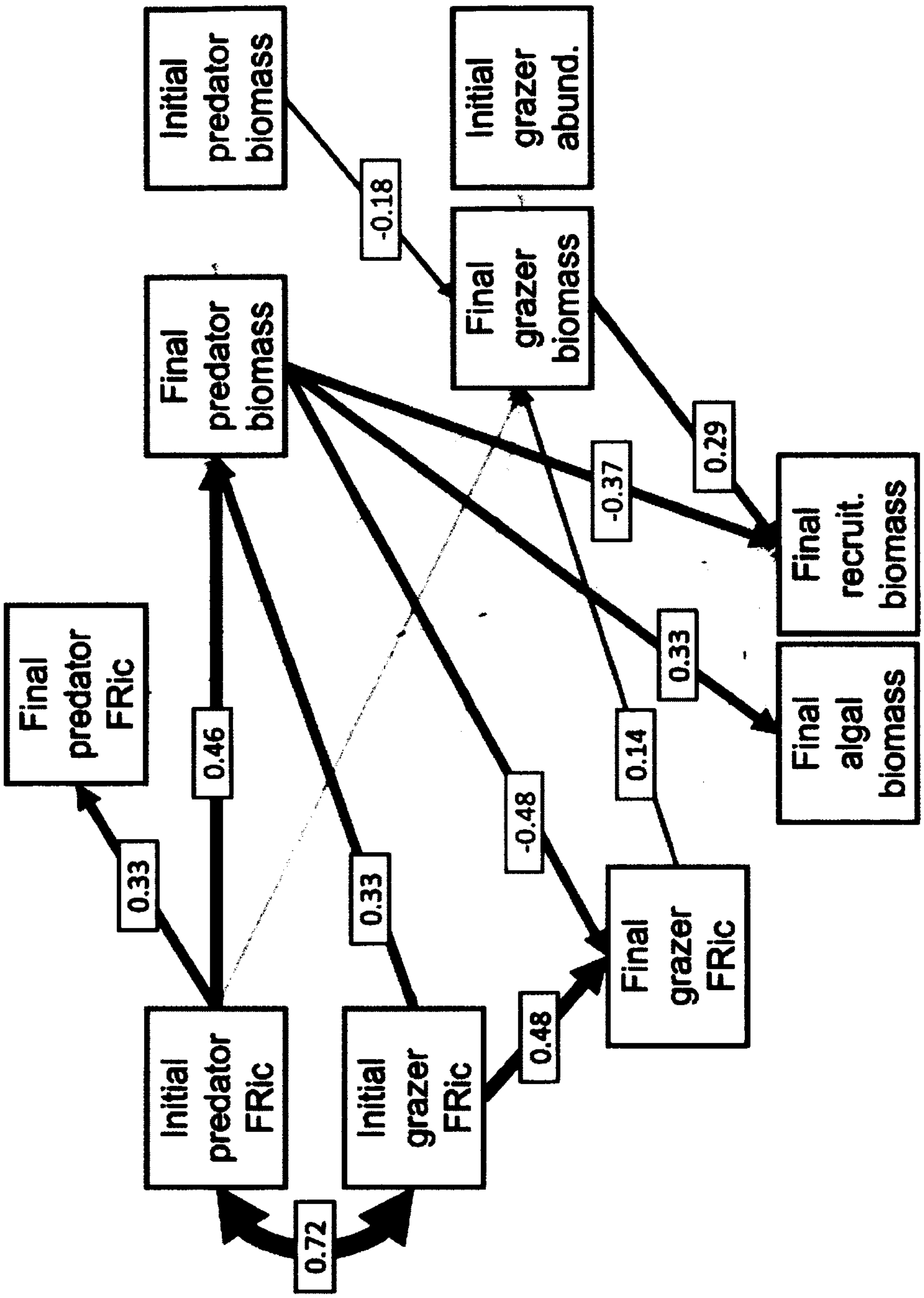

Figure 3: Structural equation model of herbivore and predator functional diversity (functional richness, FRic) as a predictor of community responses at the end of the experiment. Black arrows represent positive paths, and red arrows represent negative paths. Arrow width is proportional to the size of the effect, reported as the standardized effect size in the accompanying text box. Shaded lines represent non-significant paths (*P* ≥ 0.05).

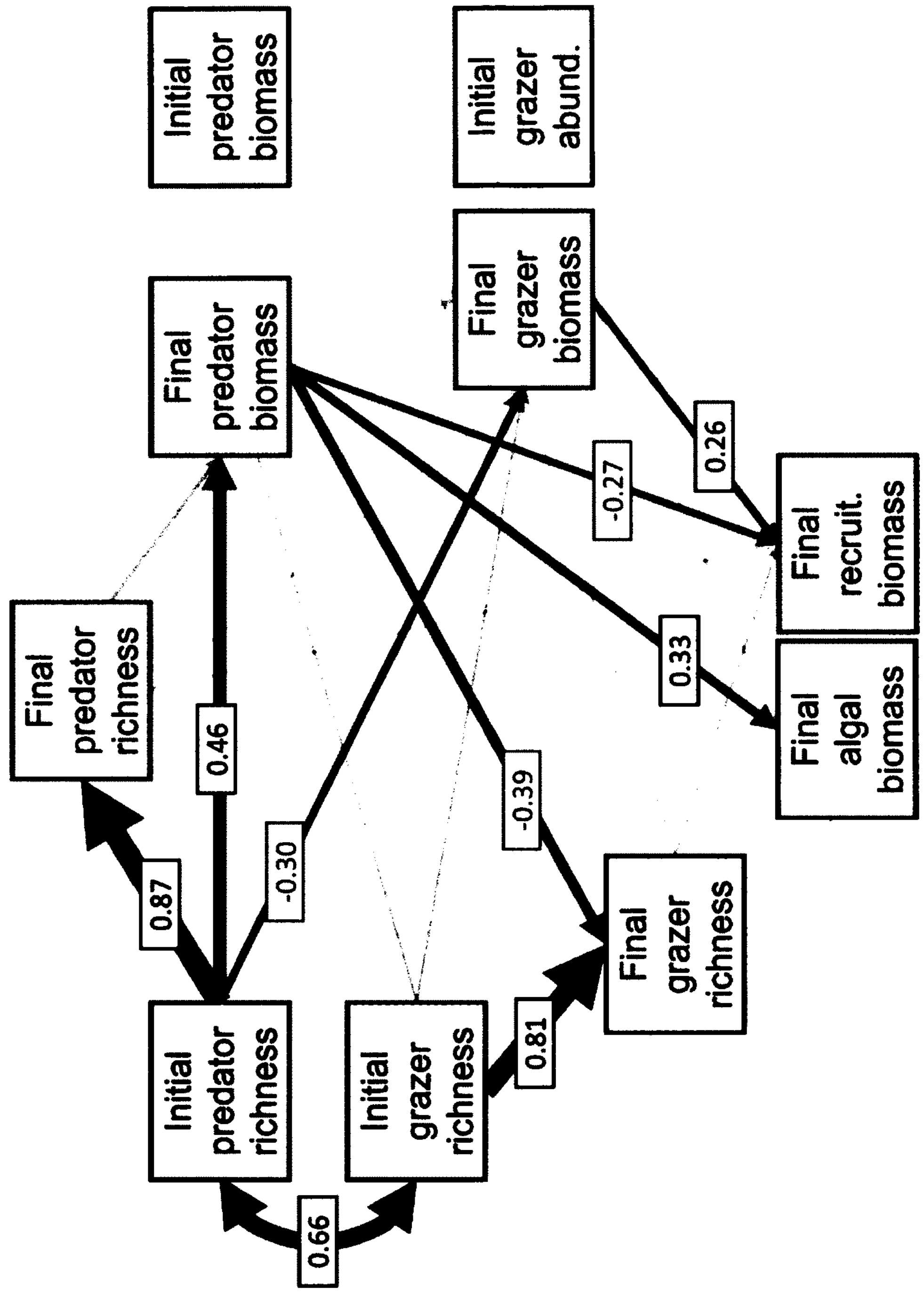

Figure 4: Structural equation model of herbivore and predator species richness as a predictor of community responses at the end of the experiment. Black arrows represent positive paths, and red arrows represent negative paths. Arrow width is proportional to the size of the effect, reported as the standardized effect size in the accompanying text box. Shaded lines represent non-significant paths ($P \geq 0.05$)

Supplemental Tables

Table S1: Functional traits measured for each species included in the experiment, along with their units and functional interpretation for ecosystem functioning.

Trait	Units	Functional Interpretation
Defense	Categorical: None, shell (chitin, calcium carbonate)	Palatability and likelihood of consumption and trophic transfer
Body plan	Categorical: Articulate (laterally-, ventrally-compressed, subcylindrical), shelled conic, filiform, fusiform	Habitat use and palatability
Trophic level	Categorical: Grazer, omnivore, predator	Resource use and trophic transfer
Maximum biomass	Continuous (mg)	Maximum contribution to community production
Mean biomass	Continuous (mg)	Average contribution to community production
Mobility	Categorical: Swimmer (low, high), tube-builder, crawler	Dispersal ability and potential for interactions (competition, predation, etc.)
Reproductive mode	Categorical: Direct, planktotrophic, ovoviviparous, oviparous	Dispersal ability, colonization potential, and population growth
Month of maximum abundance	Ordered (Jan, Feb, Mar, etc.)	Historical interactions with competitors and predators, resource use

Table S2: Standardized regression coefficients (scaled by mean and variance) from generalized linear mixed effects models regression ecosystem responses against species richness (S), functional diversity (FD), and their interaction (S x FD). Significant predictors are denoted in bold. Marginal R^2_m and conditional R^2_c values are also reported.

Response	S	FD	S x FD	R^2_m	R^2_c
Final grazer biomass	0.292	**-0.410**	-0.012	0.28	0.28
Final predator biomass	-0.145	**0.469**	0.088	0.68	0.68
Recruiting invertebrate biomass	0.014	**-0.118**	0.016	0.31	0.45
Final algal biomass	-0.022	-0.002	0.013	0.03	0.03
Final *Gracilaria* biomass	0.548	-0.211	0.161	0.16	0.16
Final species richness	**2.226**	**-0.602**	**-0.382**	0.63	0.63
Final functional diversity	-0.015	**0.291**	-0.021	0.75	0.75

Table S3: The change in model likelihood (ΔAIC) after dropping a single trait and recalculating multivariate functional richness using the remaining seven traits (ΔAIC = $\text{AIC}_{\text{All traits}}$ – $\text{AIC}_{\text{1 trait removed}}$). The trait removed is indicated in the column header: armor, body plan, trophic level, maximum biomass, mean biomass, reproductive mode, and month of maximum abundance. Bolded cells indicate models that were more than ±2 units difference in AIC scores. An increase in ΔAIC indicates a decrease in model likelihood (i.e., the model was less likely than the full model), and thus the trait had a stronger influence in predicting the response. Oppositely, a decrease in ΔAIC indicates an increase in model likelihood (i.e., the model was more likely than the full model), and thus the trait had a weaker or confounding influence in predicting the response.

Response	Armor	Body plan	Trophic level	Max. biomass	Mean biomass	Mobility	Reprod. mode	Month max. abund
Final grazer biomass	0.79	-0.51	-0.19	-0.01	-0.03	0.34	0.51	1.72
Final predator biomass	-0.86	**2.67**	**2.19**	1.05	1.92	**-4.29**	**3.72**	**-4.67**
Recruit invert biomass	-0.91	0.36	1.54	0.30	-0.09	0.63	1.22	1.47
Final algal biomass	-0.14	0.02	0.02	0.01	0.01	-0.04	0	-0.26
Final *Gracilaria* biomass	-0.87	0.77	0.49	0.29	0.12	0.22	0.09	-0.73

Table S4: Linear coefficients from the structural equation model decomposing independent predator and herbivore functional diversity effects given in Figure 3, main text.

Response	Predictor	Estimate	Standard error	*P*-value
Final grazer functional richness	Initial grazer functional richness	0.476	0.137	0.001
Final grazer functional richness	Final predator biomass	-0.335	0.137	0.017
Final predator functional richness	Initial predator functional richness	0.333	0.106	0.002
Final predator biomass	Initial predator functional richness	0.464	0.121	0.000
Final predator biomass	Initial grazer functional richness	0.327	0.118	0.007
Final predator biomass	Final predator functional richness	-0.083	0.086	0.338
Final predator biomass	Initial predator biomass	0.002	0.089	0.984
Final grazer biomass	Final grazer functional richness	0.142	0.050	0.006
Final grazer biomass	Initial predator biomass	-0.180	0.080	0.028
Final grazer biomass	Initial grazer functional richness	-0.267	0.138	0.057
Final grazer biomass	Initial predator functional richness	0.224	0.151	0.144
Final grazer biomass	Final predator functional richness	0.008	0.012	0.508
Final grazer biomass	Final predator biomass	-0.006	0.023	0.794
Final grazer biomass	Initial grazer abundance	0.005	0.082	0.956
Final primary producer biomass	Final predator biomass	0.333	0.106	0.002
Final primary producer biomass	Final grazer biomass	-0.168	0.106	0.116
Final recruiting invertebrate biomass	Final predator biomass	-0.370	0.098	0.000
Final recruiting invertebrate biomass	Final grazer biomass	0.292	0.095	0.003
Final recruiting invertebrate biomass	Final grazer functional richness	0.098	0.094	0.303
Final recruiting invertebrate biomass	Final predator functional richness	-0.019	0.096	0.846
Initial grazer functional richness	Initial predator funcitonal richness	0.715		0.000

Table S5: Linear coefficients from the structural equation model decomposing independent predator and herbivore species richness effects given in Figure 4, main text.

Response	Predictor	Estimate	Standard error	*P*-value
Final grazer species richness	Initial grazer species richness	0.810	0.106	0.000
Final grazer species richness	Final predator biomass	-0.389	0.107	0.001
Final predator species richness	Initial predator species richness	0.868	0.055	0.000
Final predator biomass	Initial predator species richness	0.457	0.165	0.007
Final predator biomass	Initial grazer species richness	0.154	0.096	0.115
Final predator biomass	Final predator species richness	0.217	0.146	0.141
Final predator biomass	Initial predator biomass	-0.004	0.081	0.964
Final grazer biomass	Initial predator species richness	-0.303	0.143	0.037
Final grazer biomass	Initial grazer species richness	0.145	0.149	0.335
Final grazer biomass	Final grazer species richness	0.059	0.079	0.457
Final grazer biomass	Initial predator biomass	-0.037	0.053	0.493
Final grazer biomass	Final predator biomass	0.005	0.015	0.756
Final grazer biomass	Initial grazer abundance	-0.028	0.111	0.803
Final grazer biomass	Final predator species richness	-0.003	0.039	0.932
Final primary producer biomass	Final predator biomass	0.333	0.106	0.002
Final primary producer biomass	Final grazer biomass	-0.168	0.106	0.116
Final recruiting invertebrate biomass	Final grazer biomass	0.263	0.098	0.009
Final recruiting invertebrate biomass	Final predator biomass	-0.275	0.135	0.045
Final recruiting invertebrate biomass	Final grazer species richness	0.120	0.098	0.228
Final recruiting invertebrate biomass	Final predator species richness	-0.163	0.137	0.237
Initial grazer species richness	Initial predator species richness	0.666		0.000

Supplemental Figures

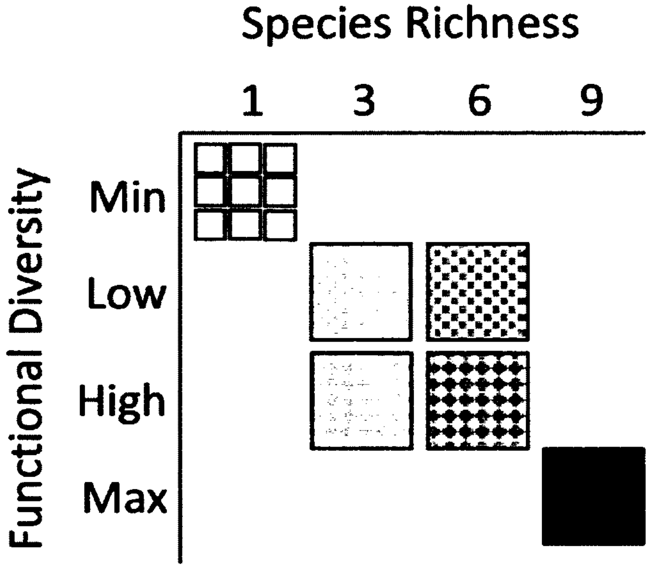

Figure S1: A schematic of the experimental design. We utilized four levels of species richness: 1, 3, 6, and 9. Each of the 9 species was represented in the single-species treatments (hence the 9 individual squares). All species were present in the 9-species mixture. For the 3- and 6-species treatments, we generated all possible combinations of species and calculated functional diversity (FD, as functional richness). We then randomly drew replicate assemblages from the lower 25th percentile to represent 'low FD,' and repeated this exercise for the upper 75th percentile to represent 'high FD.' The single species represented the minimum level of FD (FD = 0). The 9-species mixture represented the highest level of FD (maximum FD, visually depicted in reduced trait space in Figure S2).

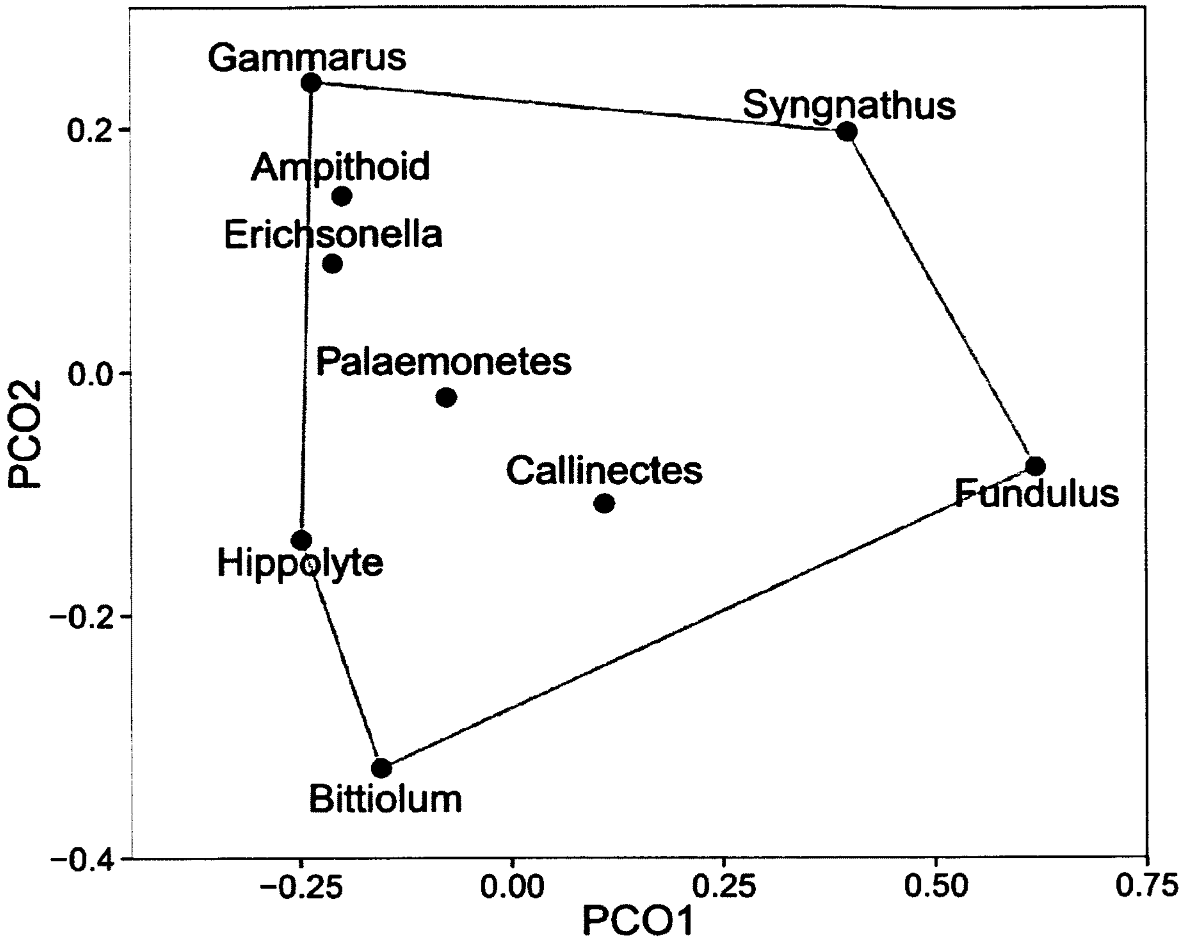

Figure S2: Principal coordinates analysis (PCO) collaping 8 functional traits into 2-dimensions. The convex hull (area of trait space encompassed by all 9 species) is given by the shaded polygon.

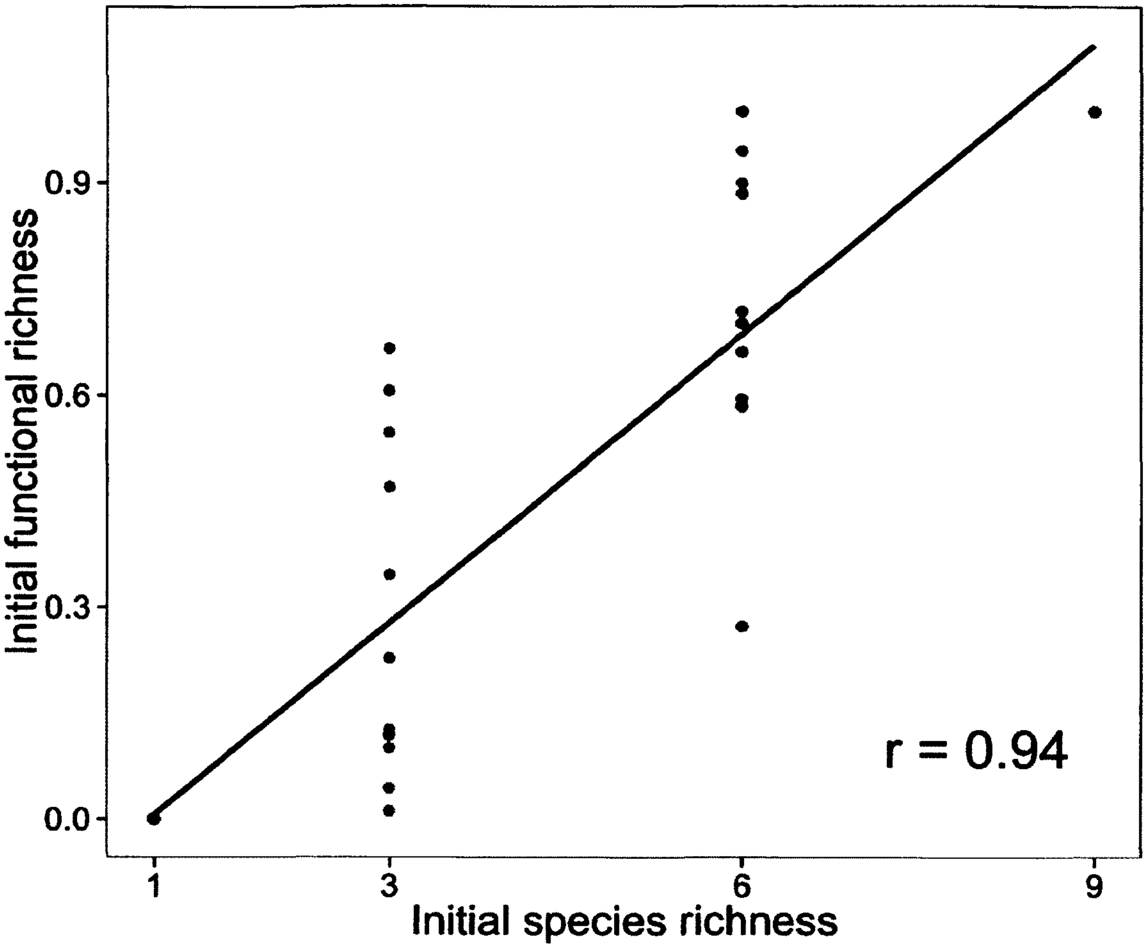

Figure S3: Plot of initial species richness against initial functional richness (Pearson's correlation *r* = 0.94).

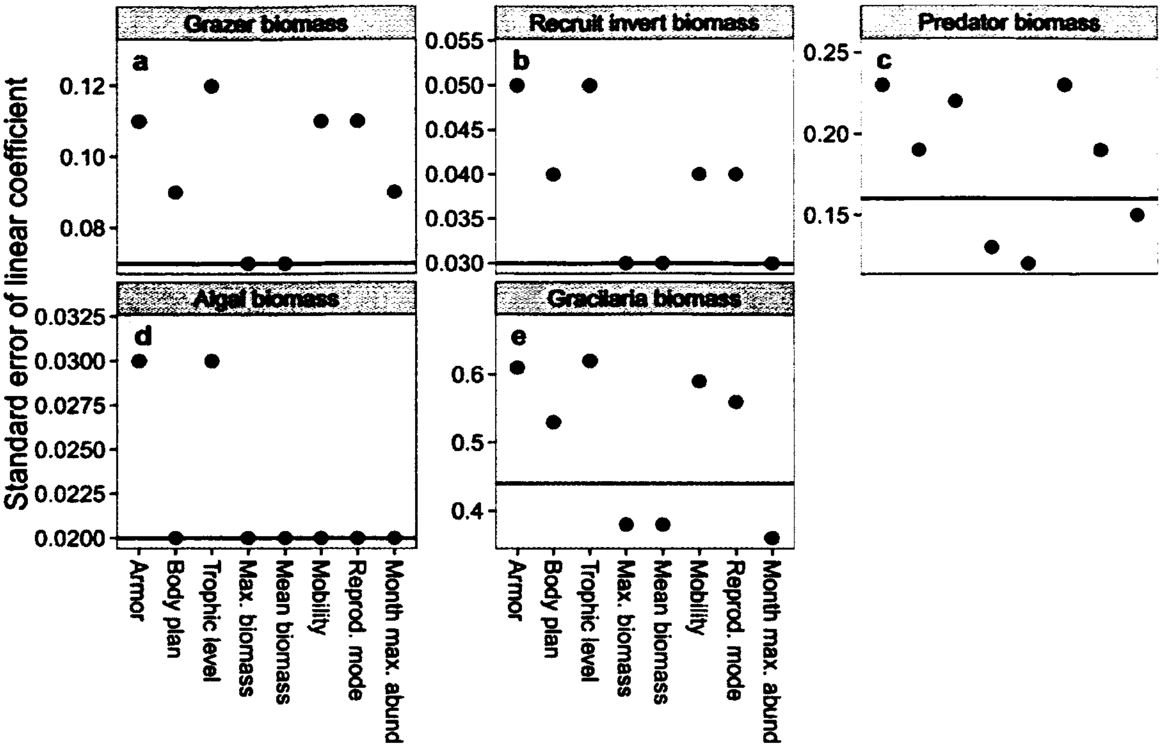

Figure S4: Standard errors of linear coefficients extracted from general linear mixed effects models regressing ecosystem responses against univariate and multivariate functional richness. Traits used in the univariate calculation of FD are listed on the x-axis, and the standard error of the multivariate FD estimate is given as the horizontal line. If points fall above this line, then variance around the univariate estimates was greater than around the multivariate one.

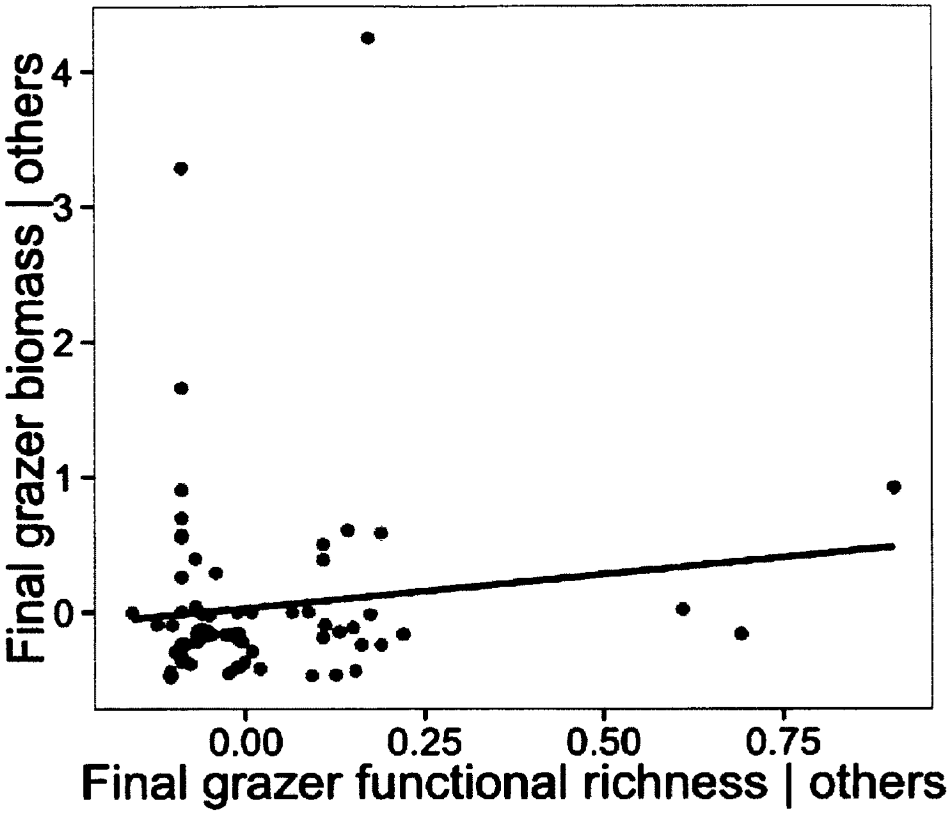

Figure S5: Partial correlation plot of final grazer functional richness against final grazer biomass (mg AFDM), after accounting for additional covariates. Points have been jittered to better illustrate density of points at 0 FD (i.e., single-species treatments).

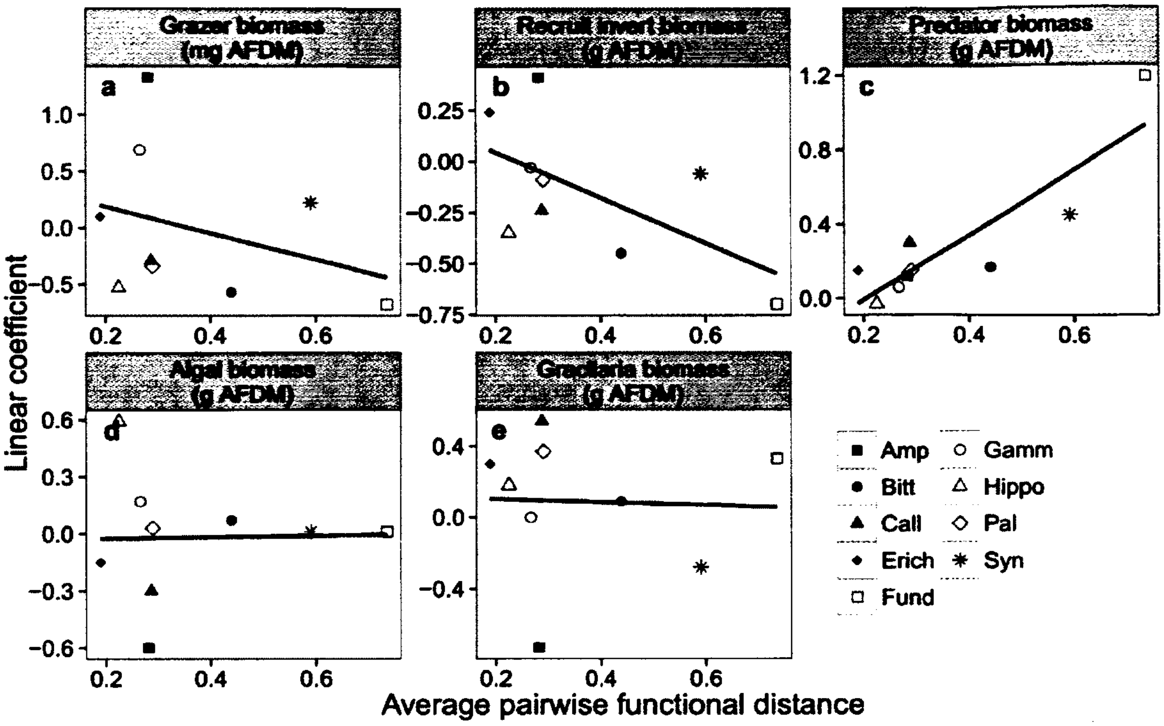

Figure S6: Average pairwise functional distance against effect sizes for each of the 9 species derived from a general linear mixed effects model regressing the presence/absence of each species against each ecosystem responses. Black lines represent predicted trends from a a simple linear regression.

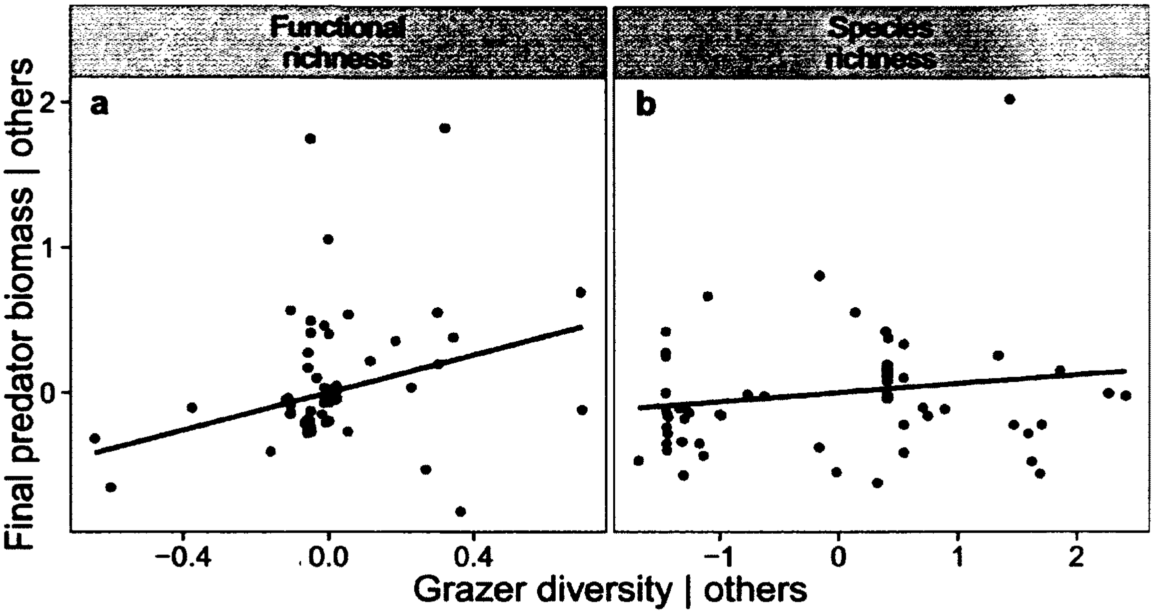

Figure S7: Partial effects plots of (a) initial grazer functional richness and (b) initial grazer species richness on final predator biomass, accounting for covariates (initial predator diversity, initial predator biomass, and final predator diversity).

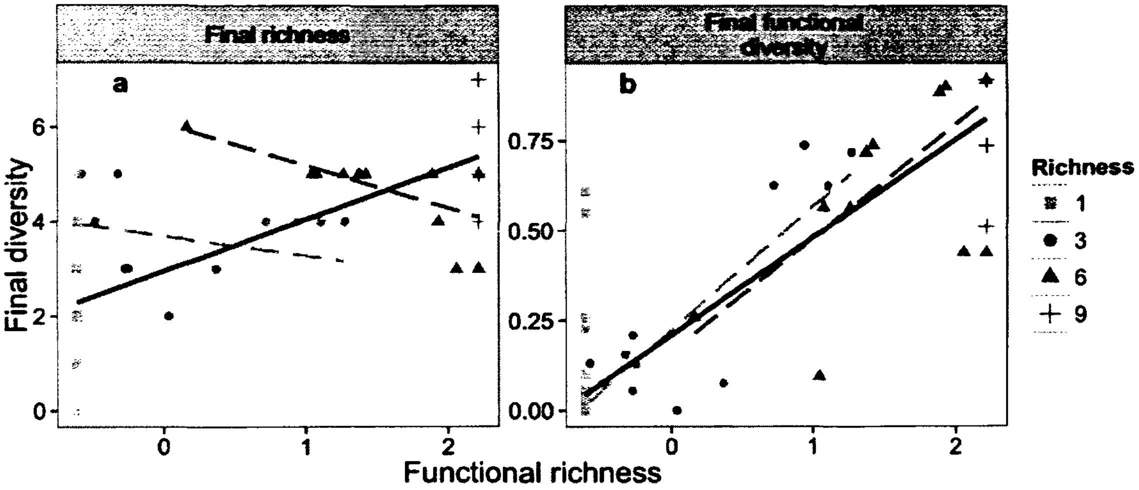

Figure S8: Scatterplot of initial FD (scaled by mean and variance) against (a) final species richness and (b) final FD of all stocked species. Shapes corresponds to the richness level (1, 3, 6, or 9). Grey lines represent predicted fits from a general linear mixed effects model for 3- (light grey) and 6-species (dark grey) treatments (Table S2). The black line represents the overall trend from the same model.

Appendix A: Discussion of Structural Equation Model

To begin, we generated a conceptual 'meta-model' (Fig. A1). This meta-model corresponded to a simple tri-trophic food web, with predators consuming herbivores, and herbivores consuming primary producers. Both predators and herbivores were predicted to consume recruiting invertebrates, as in past experiments with these organisms (Duffy and Harvilicz 2001, Duffy et al. 2003, 2005). Within each trophic level, we had the expectation that diversity would enhance biomass (see predictions below, Fig. A1a,b). We also created composite variables to represent the entirety of final primary producer biomass, which was a combination of: final *Gracilaria* spp. dry mass, and recruiting filamentous algal dry mass (Fig. A1c). And the entirety of recruiting invertebrate biomass, which was a combination of: Nereid spp. dry mass, tunicate (*Mogula manhattensis*) dry mass, bubble snail (*Haminoea solitaria*) dry mass, and barnacle (*Balanus* spp.) dry mass.

We populated this meta-model using variables measured during the experiment (Fig. A2). Here, we briefly describe the rationale behind each path. Letters correspond to the bubbles in Figure A2. In all cases, 'diversity' can mean either functional or species richness, depending on the model considered (see main text).

A) As in Figure A1, we expected predator or herbivore diversity (functional or species richness) to enhance corresponding final biomass (Loreau et al. 2001, Duffy 2002). By including paths from both initial and final diversity to the corresponding final biomass, we can account for loss of species within replicates over the course of the experiment.

B) We also expected initial predator or herbivore diversity to predict final predator diversity. In other words, we expected to find more species left if more species were initially stocked.

C) Because we could not incorporate predators in a substitutive design, we included initial predator biomass as a covariate in all paths leading to final predator biomass. Thus, the effects of, say, final predator diversity on final predator biomass accounted for differences in initial stocked biomass within each replicate. We also included a path from initial grazer abundance to final grazer biomass for the same reason, even though we equalized grazer biomass at the beginning of the experiment (this path ended up being highly non-significant in all models, confirming the efficacy of our substitutive design for grazers, Table S4, S5).

D) We also included a correlation between initial predator and herbivore diversity, to account for the fact that increasing diversity necessarily meant the inclusion of more grazers and predators. This has no bearing on the model estimates, but gives an indication of how the diversity of these two trophic levels scaled as assemblages were manipulated.

E) We expected both initial and final predator biomass to decrease final grazer biomass through direct consumption. Again, by incorporating paths from both initial and final predator biomass to grazer final biomass, we can account for changes in the predator community over the course of the experiment.

F) Similarly, we expected predators change the diversity of the grazer community through the removal of (functionally distinct) species (Duffy et al. 2005, Douglass et al. 2008).

G) We expected a more diverse predator assemblage to more efficiently consume grazers by employing a diversity of foraging strategies and capture mechanisms (reviewed in Duffy et al. 2007).

H) Along similar lines, we expected a more diverse prey assemblage to enhance final predator biomass (reviewed in Duffy et al. 2007).

I) We expected final grazer biomass to decrease both final algal biomass and final recruiting invertebrate biomass via direct consumption (Duffy and Harvilicz 2001, Duffy et al. 2003).

J) We expected a more diverse predator assemblage to more efficiently consume recruiting invertebrates, for the same reasons as path G.

K) The SEMs were always a poor fit unless a direct path between final predator biomass and final algal biomass was included. This path was always positive. In light of the lack of direct negative path between final grazer biomass and primary producers (path I, Fig. A2), we interpreted this efficient consumption of grazers by predators, leading to a direct statistical effect of predators on algal resources. Had predators been less efficient or grazer biomass less depressed, we may have been able to recover an indirect trophic cascade leading from predators to herbivores to primary producers.

L) Finally, we expected final grazer diversity to negatively affect final recruiting invertebrate biomass, as the invertebrates considered vary in their palatability to these small mesograzers (Duffy and Harvilicz 2001). Thus, only by including a variety of grazer species would we be able to see an effect on recruiting invertebrates as a whole.

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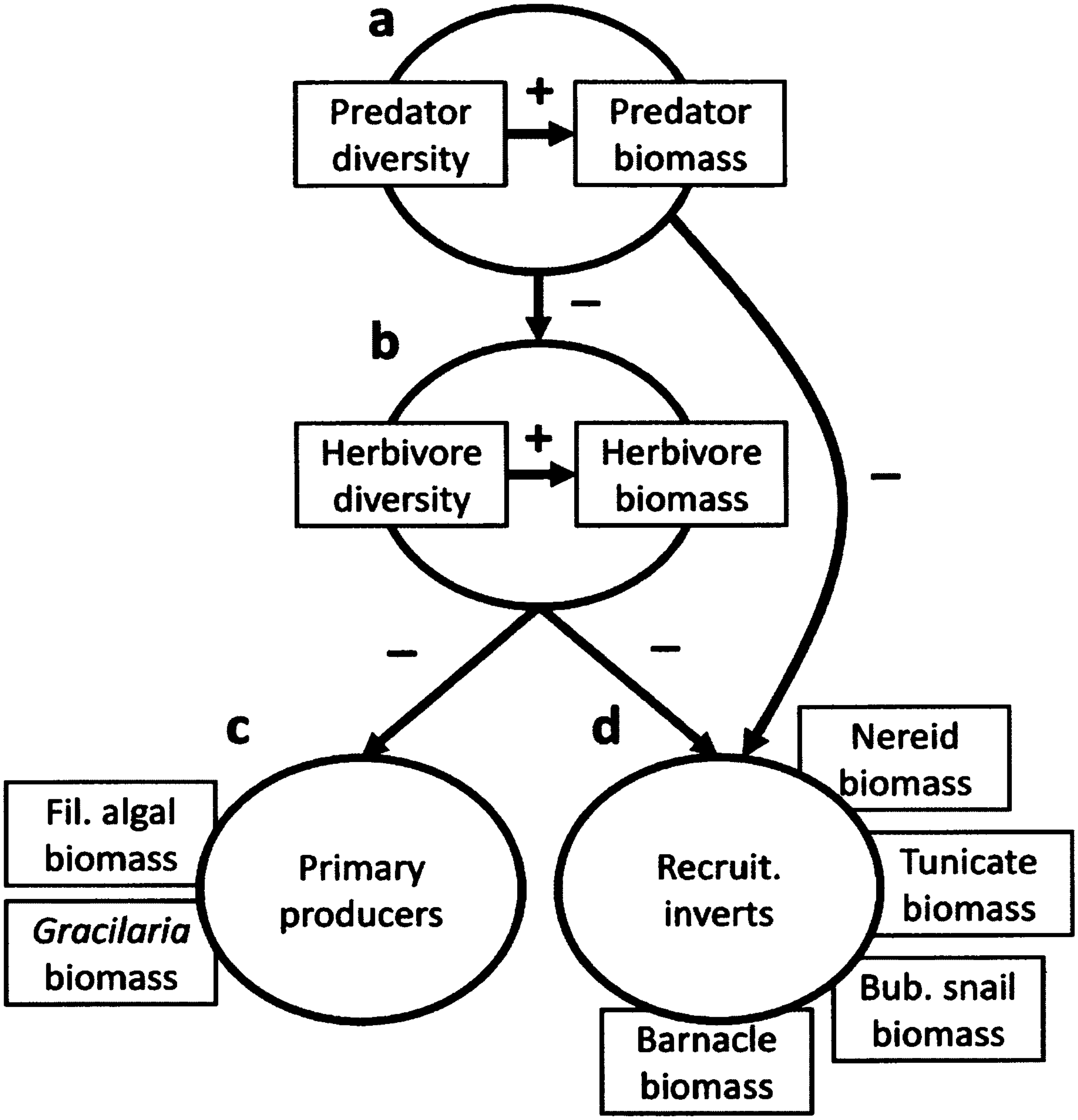

Figure A1: A conceptual 'meta-model' corresponding to a tri-trophic food web, with predators consuming herbivores, which in turn are consuming algal and invertebrate resources. Black arrows indicate hypothesized positive effects, while red arrows indicate hypothesized negative effects. Boxes surrounding circles **(c)** and **(d)** correspond to variables that were summed to create the composite response variable indicated in the circle.

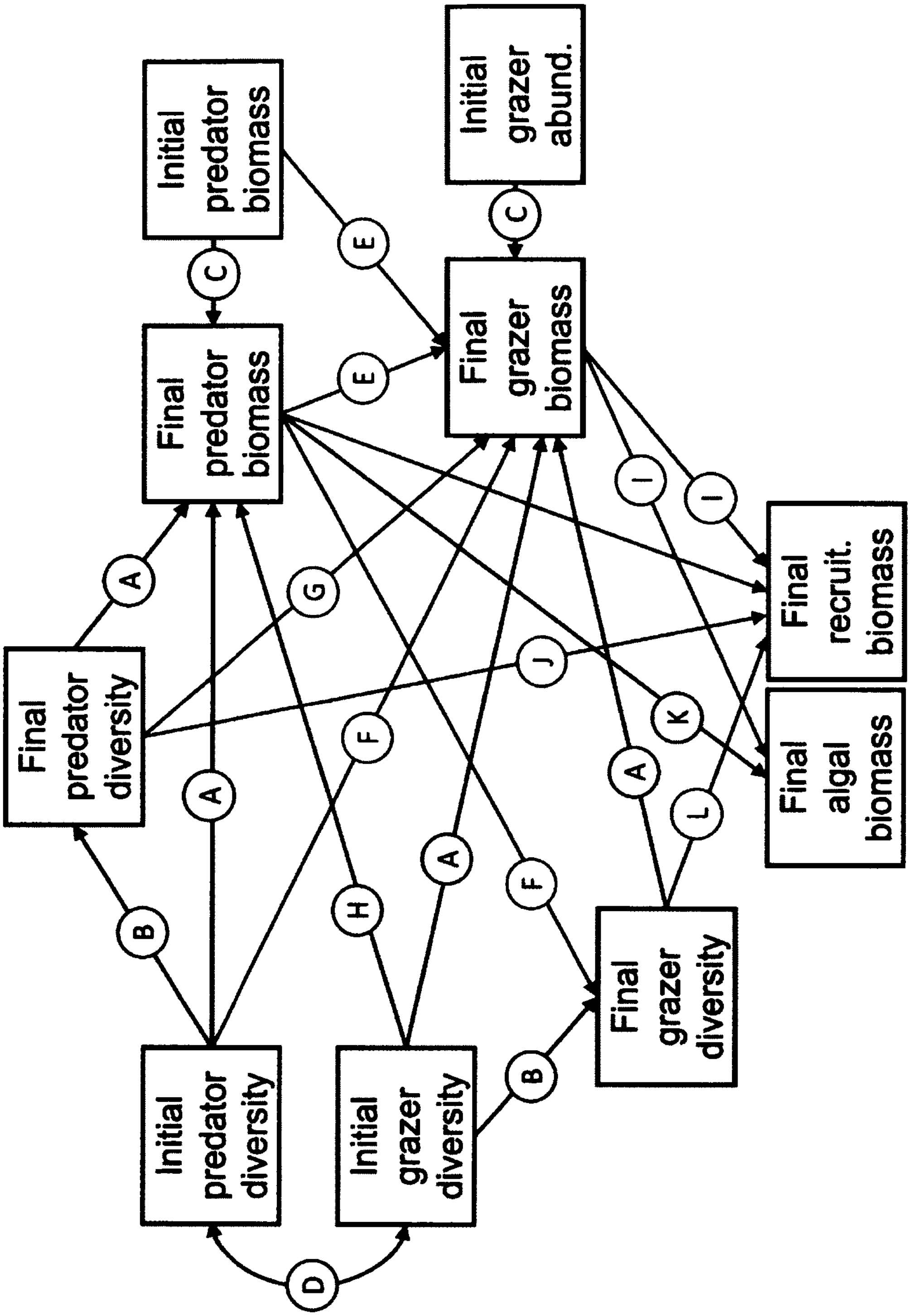

Figure A2: Hypothesized causal network relating variables measured during the experiment. Hypothesized positive relationships are given in black, and hypothesized negative relationships are given in red.

CONCLUDING REMARKS

In this dissertation, I provide three examples of how a functional trait approach can inform our understanding of both patterns and processes in the natural world. A central theme that emerges from Chapters 1 and 2 is that biology is important in driving community structure and function in addition to of in spite of abiotic variation. This was true through space, as in the analysis of global reef fish diversity, and time, as in the analysis of a local eelgrass bed. Recent meta-analyses have cited abiotic filters as driving a large proportion of the variation in community composition, and their influence was highest in marine and estuarine systems (Cottenie 2005, Soininen 2014). Similarly, in studies that directly compared biotic and abiotic drivers of assembly, nearly four-fifths (79%) reported evidence for environmental filtering (Kraft et al. 2014). Yet, I found that organisms have a detectable role in both shaping their own community, and influence processes in the environment in which they live, across two different systems. These findings support long-standing theoretical and conceptual predictions about the role of biology at large scales (Hillebrand 2004, Mittelbach et al. 2007, Poore et al. 2012), and shed light on important biotic processes that lead to the maintenance and stability of communities (Strong 1992, Shurin et al. 2006).

Another interesting outcome is that functional diversity played a large role in driving community biomass in mesocosms (Chapter 3), but did not have any detectable effect in similar communities in the observational dataset (Chapter 2). This finding raises the interesting and relevant question of whether small-scale experiments translate to the real world (Srivastava and

Vellend 2005, Cardinale et al. 2012). Compared to the hundreds of experimental manipulations of marine biodiversity (reviewed in Stachowicz et al. 2007, Gamfeldt et al. 2014), there have many fewer studies that have explicitly tested the correlation between diversity and functioning using observational datasets (Emmerson et al. 2001, Stachowicz et al. 2002, Dunstan and Johnson 2004, Hughes and Stachowicz 2004, Stachowicz and Byrnes 2006, Worm et al. 2006, Danovaro et al. 2008, Godbold and Solan 2009, Ptacnik et al. 2008, Mora et al. 2009, Duffy et al. 2015). While these studies have generally found a positive effect of observed diversity on various ecosystem functions (Stachowicz et al. 2007), they are far from a general test.

It is also difficult to infer causative relationships from observational datasets, particularly in the face of complex interactions with environmental drivers (Godbold et al. 2009). Structural equation modeling and other modern statistical tools help to alleviate this issue (Grace et al. 2007), but they also assume that abiotic data are available and collected on the scale relevant to the biological data. We were lucky to have long-term local monitoring of abiotic properties for the analysis in Chapter 3. However, technological advancements and improvements in remote monitoring are already reducing the effort needed to collect high-resolution environmental data, and will facilitate the coupling of such data with biological surveys to better explore when, where, and why diversity effects manifest in nature. The emergence of large-scale research networks (e.g., Duffy et al. 2015) that integrate experimental approaches with natural gradients in diversity and abiotic variables may also bring some resolution to this question by directly testing causality while incorporating more realistic variation in both species composition and richness and the environment.

Across all three chapters, key functional traits that discriminated among species and influenced observed patterns and effects were feeding ecology and body size. That traits related to resource acquisition predict coexistence and functioning is no surprise, given that they form the basis for one of the oldest and most venerable concepts in ecology, the feeding niche (Elton 1927). Similarly, body size correlates with trophic level and also represents potential habitat use, another fundamental niche axis (Grinnell 1917, Hutchinson 1957). Yet, I observed functional complementary among epifaunal grazers that were considered equivalent based on the traits used in my analysis (Table 2, Chapter 3). Thus, the question remains: what traits beyond those used here discriminate among closely-related species, particularly grazers in eelgrass systems? Specific diet preference and predator susceptibility are obvious candidates, based on other work involving amphipods (Best and Stachowicz 2012, Cothran et al. 2013), and dispersal mode is certainly important in determining fish community structure and diversity across heterogeneous landscapes (Riginos et al. 2014), but such traits require rigorous experimentation to obtain, or may not even be known for poorly described species. In an increasingly time- and resource-strapped world, are there tractable alternatives that give qualitatively similar insights? Such a question can only be answered through continued experimentation with the goal of identifying useful and realistic functional traits across a variety of organisms, particularly animals.

It will also be important to understand which traits are important under what scenarios. For instance, I observed no effect of functional diversity on filamentous algae in the mesocosms, but perhaps that was because there were other abundant resources available, or because top-down control by predators reduced species with traits that allowed them to capitalize on this particular resource. This idea is particularly relevant under future scenarios of global change, and whether traits can be used to predict community structure and function in the face of numerous anthropogenic stressors (Mouillot et al. 2013). Finally, one of the most attractive qualities of functional traits is that they are, in principle, generalizable to other taxa and systems (McGill et al. 2006). Yet there has been very little progress made on identifying a core set of functional traits that can be broadly applied across animal groups. Here, we propose a few traits

– trophic guild and breadth, and body size – that are measurable across invertebrates and vertebrates and appear to have some general utility in describing patterns and processes. The use of coordinated research networks conducting parallelized trait manipulations may be one way to shed further light on this issue (e.g., Wahl et al. 2011).

In sum, this dissertation presents evidence ranging from buckets to the entire biosphere that functional traits can: (1) provide mechanistic insight into the shifting role of biological interactions in how local reef fish communities are assembled from the regional species pool across the globe; (2) shed light on the functional role of epifaunal invertebrate grazers in eelgrass systems, both through time and in the face of considerable abiotic variation; and (3) better explain ecosystem functioning than species richness in multitrophic experimental assemblages.

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