Multitrophic Functional Diversity Predicts Ecosystem Functioning in Experimental Assemblages of Estuarine Consumers

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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

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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

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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 variancecovariance 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: $\beta = –0.335 \, x \, 0.164 = –0.055$, 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 & Figures

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

Figure Legends

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 ($P \geq 0.05$).

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 ($P \geq 0.05$).

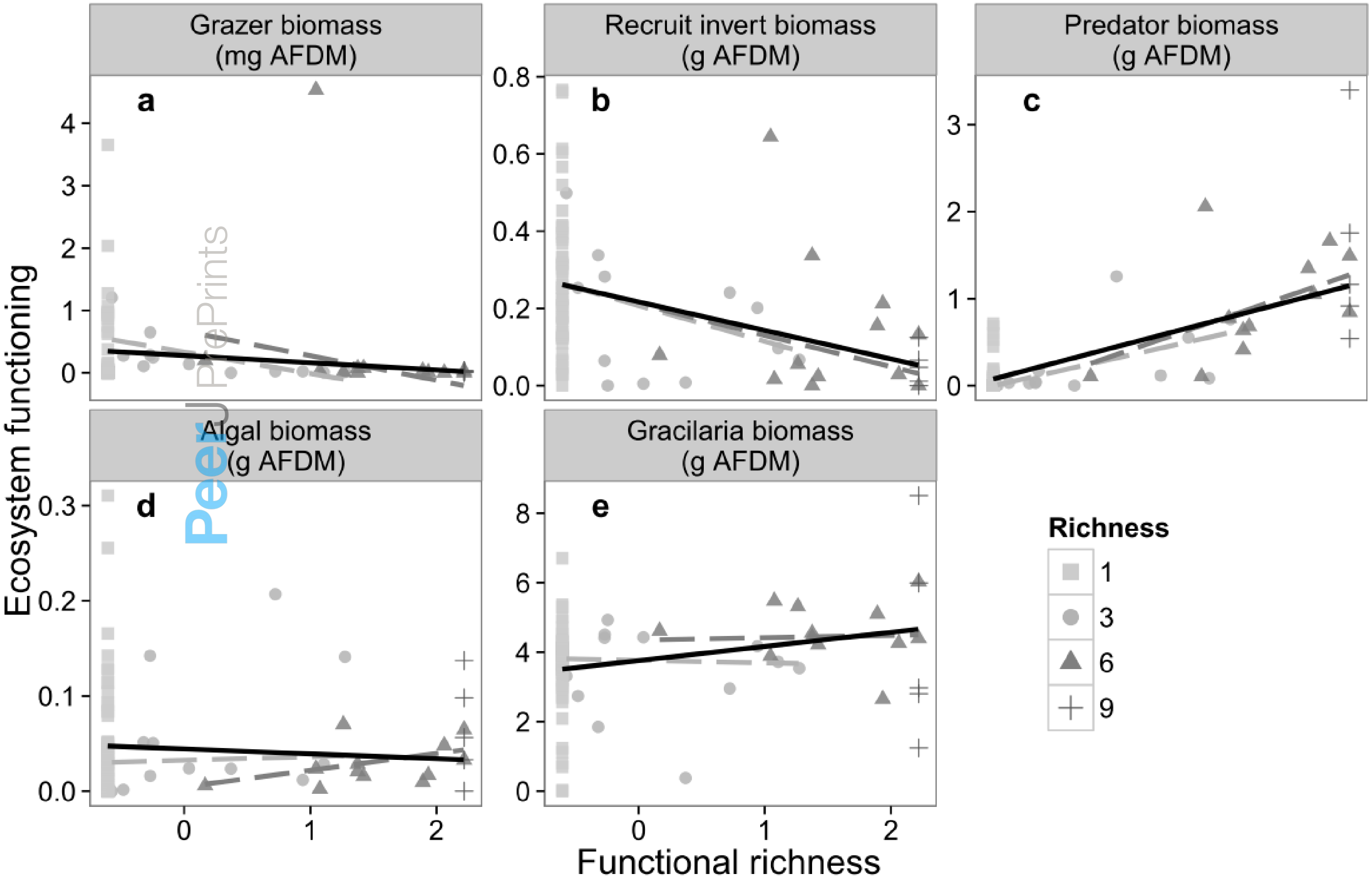

Figure 1

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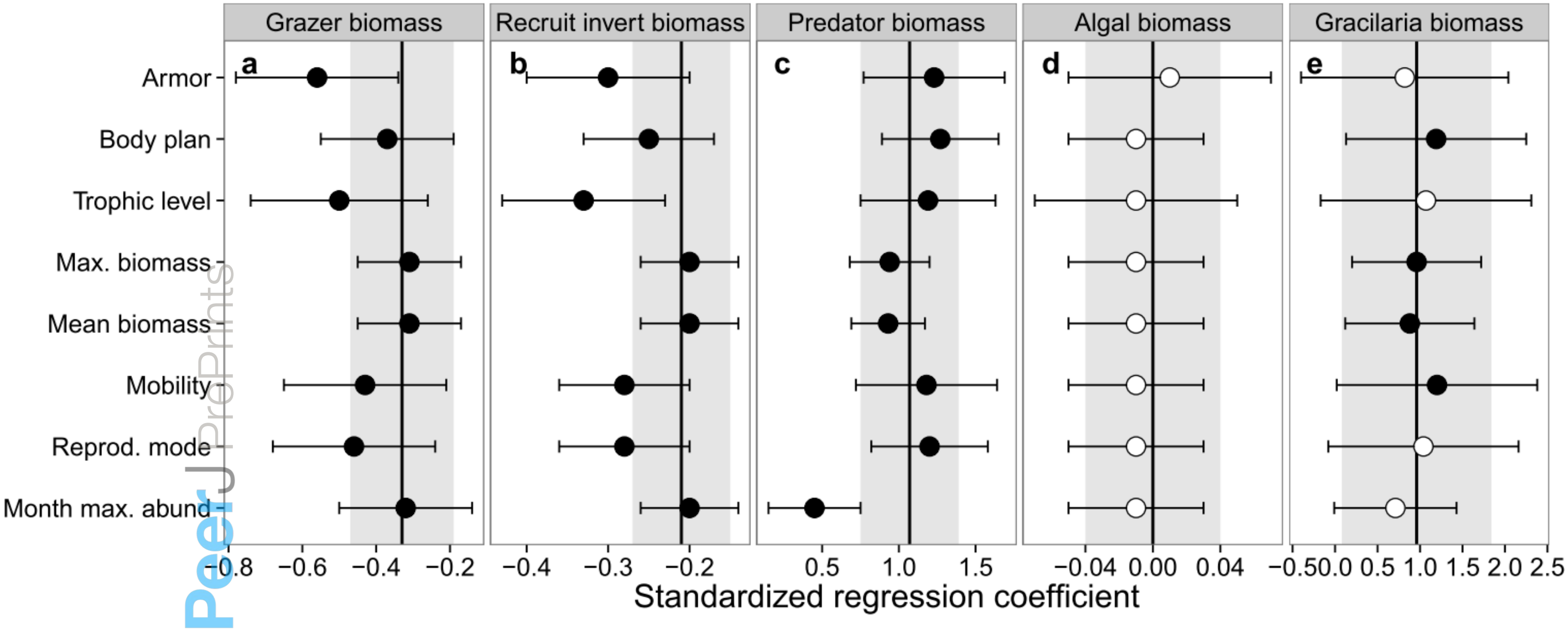

588 **Figure 2**

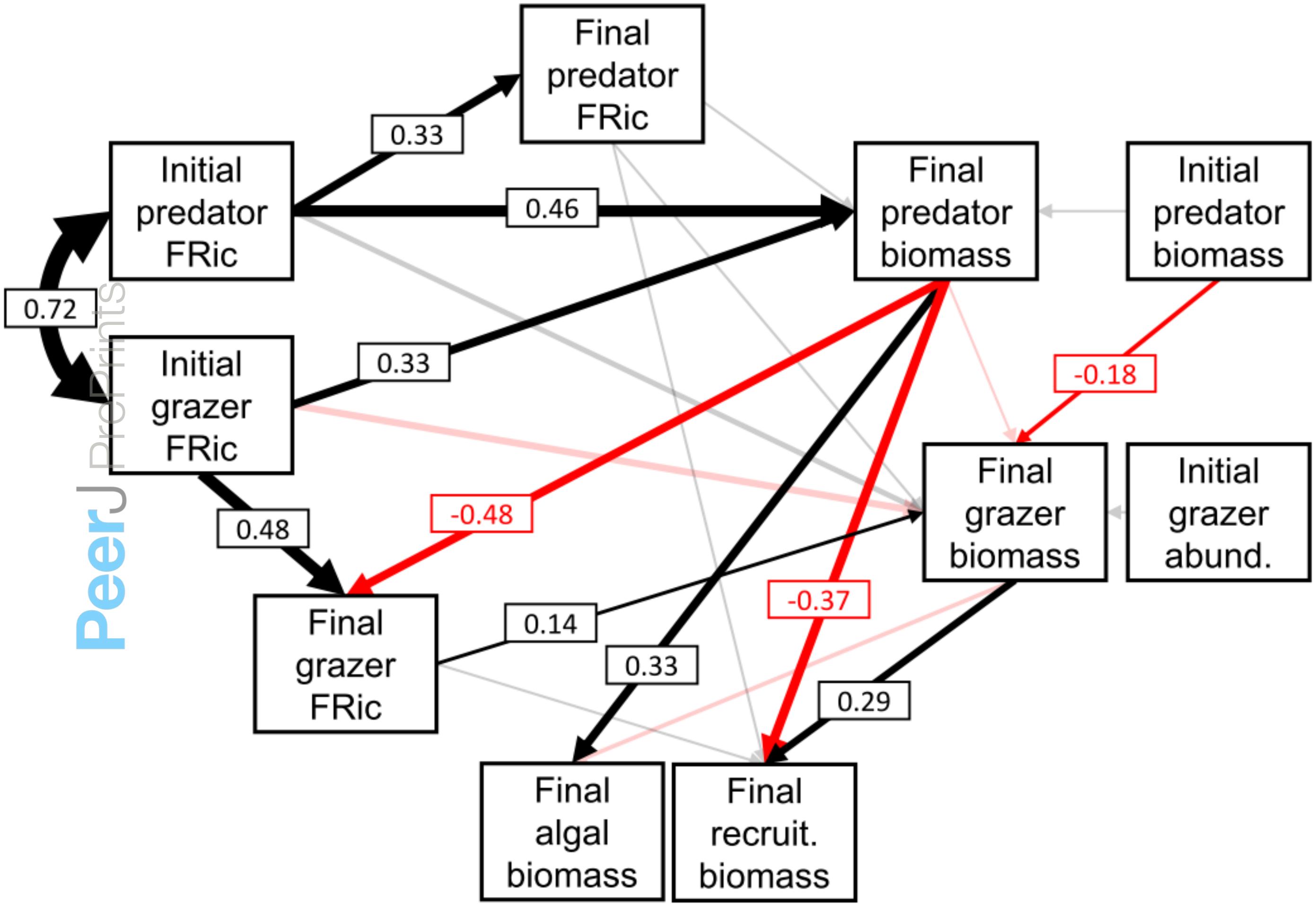
Final predator FRic
Initial predator FRic
0.33
0.46
Final predator biomass
Initial predator biomass
0.72
Initial grazer FRic
0.33
-0.18
Final grazer biomass
Initial grazer abund.
0.48
-0.48
Final grazer FRic
0.14
-0.37
0.33
0.29
Final algal biomass
Final recruit. biomass

Figure 3

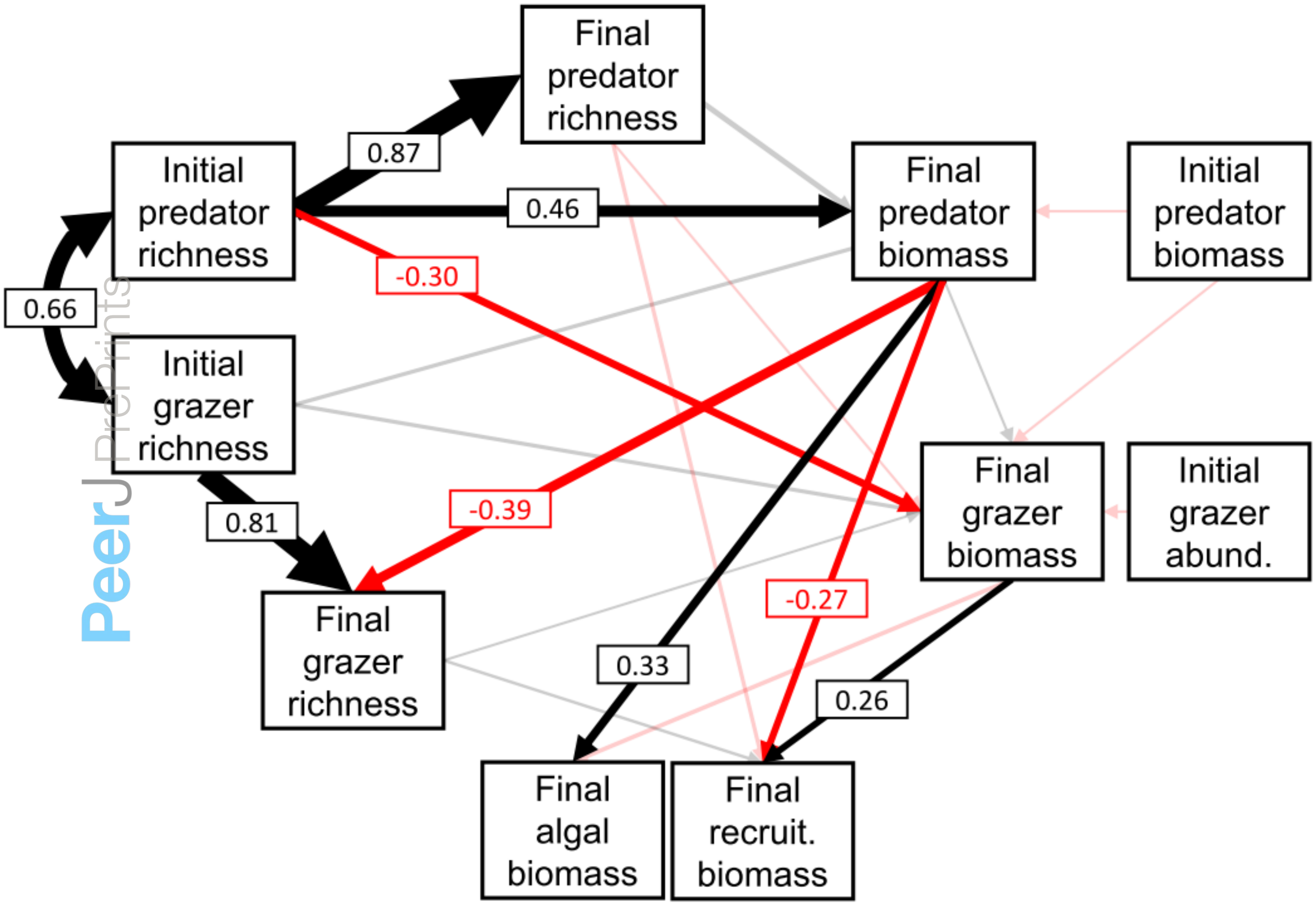
Final predator richness
Initial predator richness
0.87
0.46
Final predator biomass
Initial predator biomass
-0.30
0.66
Initial grazer richness
-0.39
0.81
Final grazer biomass
Initial grazer abund.
Final grazer richness
-0.27
0.33
0.26
Final algal biomass
Final recruit. biomass

Figure 4

Supplementary Tables and Figures

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

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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

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Table S3: The change in model likelihood (ΔAIC) after dropping a single trait and recalculating multivariate functional richness using the remaining seven traits ($\Delta AIC = AIC_{\text{All traits}} - AIC_{\text{1 trait removed}}$). 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

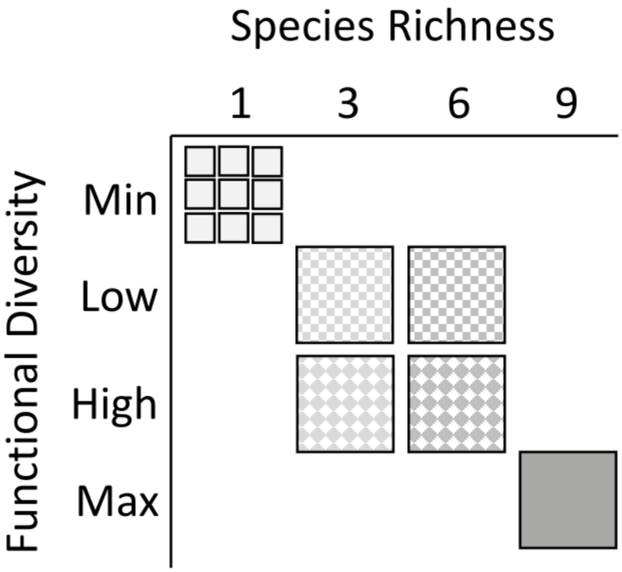


Figure S1: A schematic of the experimental design. We utilized four levels of species richness: 1, 3, 6, and 9. Each of the 9 species was represented in the single-species treatments (hence the 9 individual squares). All species were present in the 9-species mixture. For the 3- and 6-species treatments, 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).

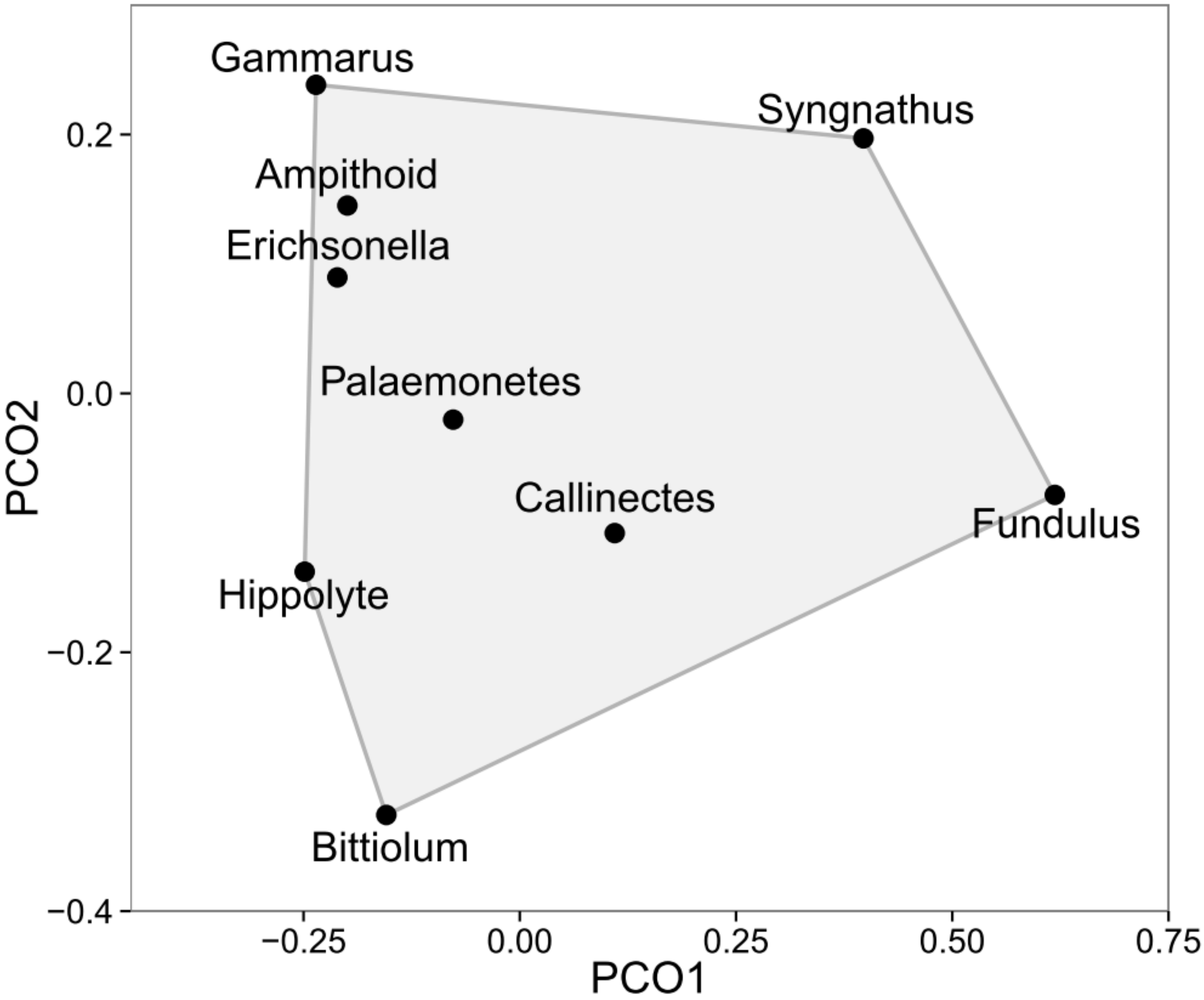


Figure S2: Principal coordinates analysis (PCO) collaping 8 functional traits into 2-dimensions. The convex hull (area of trait space encompassed by all 9 species) is given by the shaded polygon.

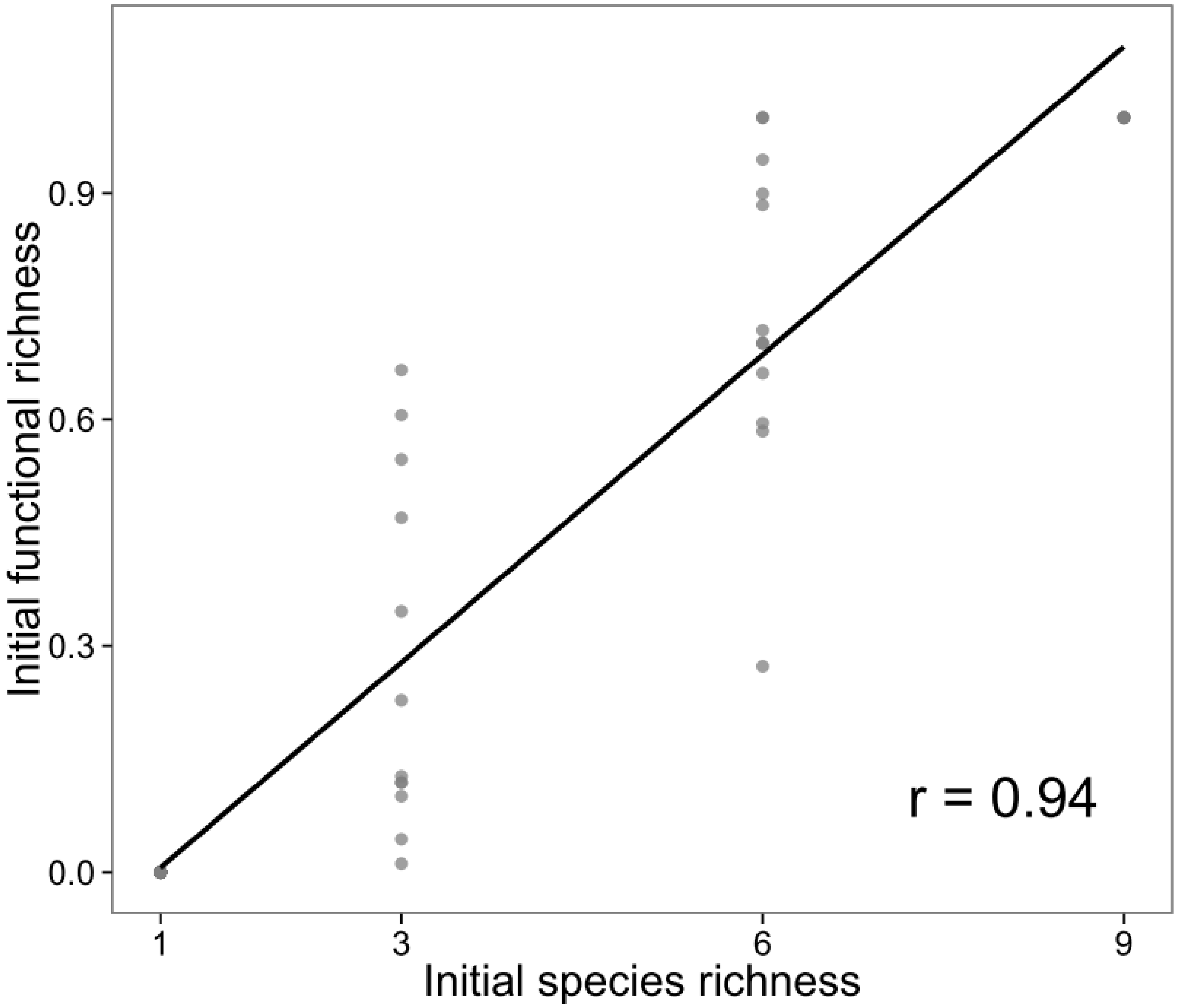


Figure S3: Plot of initial species richness against initial funciton richness (Pearson's correlation *r* = 0.94).

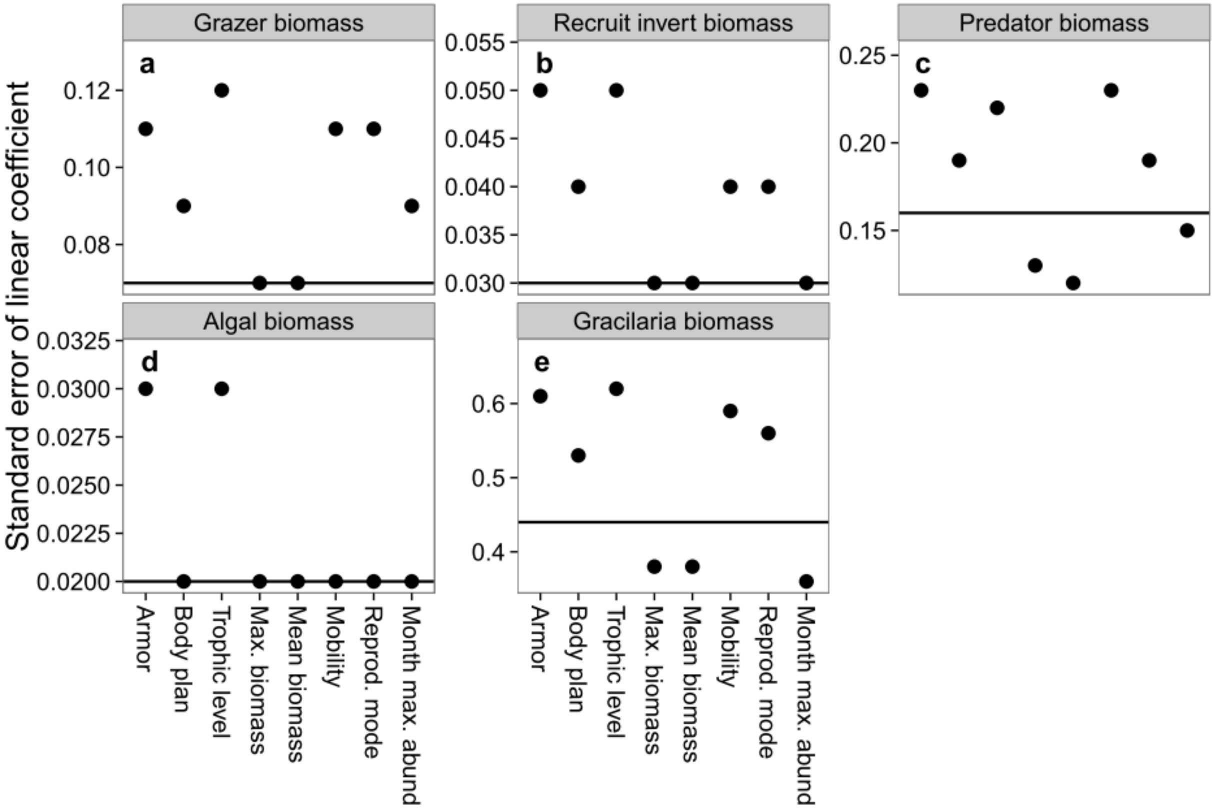


Figure S4: Standard errors of linear coefficients extracted from general linear mixed effects models regressing ecosystem responses against univariate and multivariate functional richness. Traits used in the univariate calculation of FD are listed on the x-axis, and the standard error of the multivariate 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.

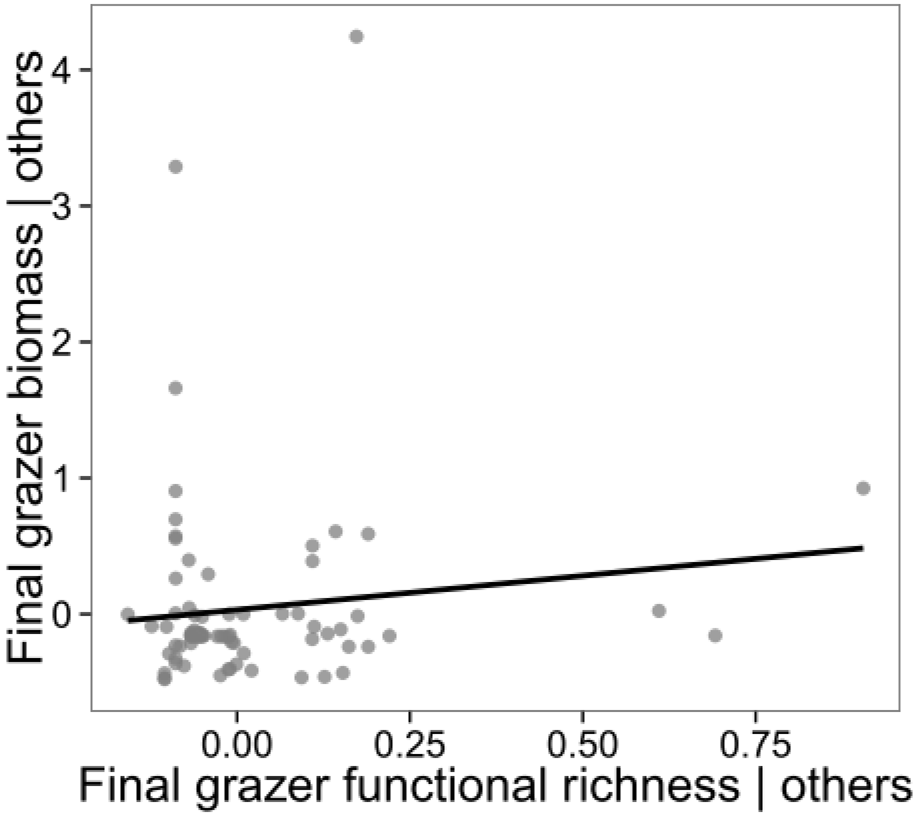


Figure S5: Partial correlation plot of final grazer functional richness against final grazer biomass (mg AFDM), after accounting for additional covariates. Points have been jittered to better illustrate density of points at 0 FD (i.e., single-species treatments).

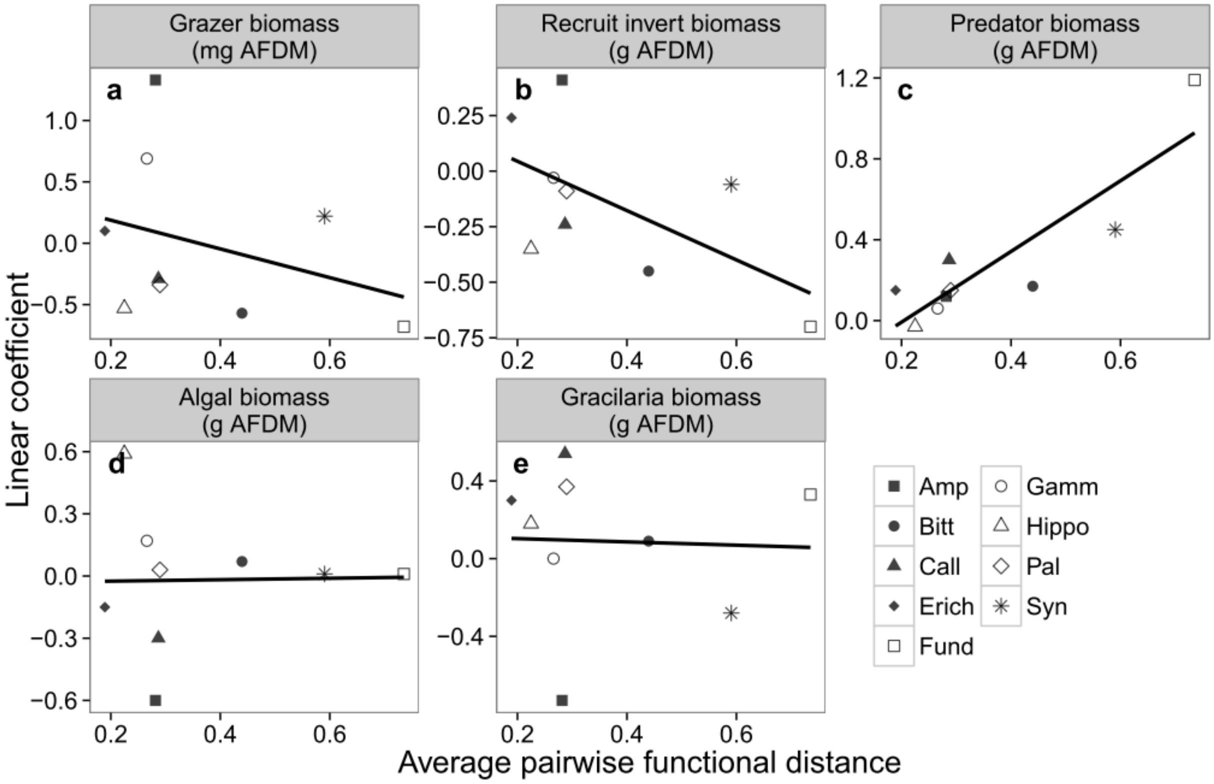
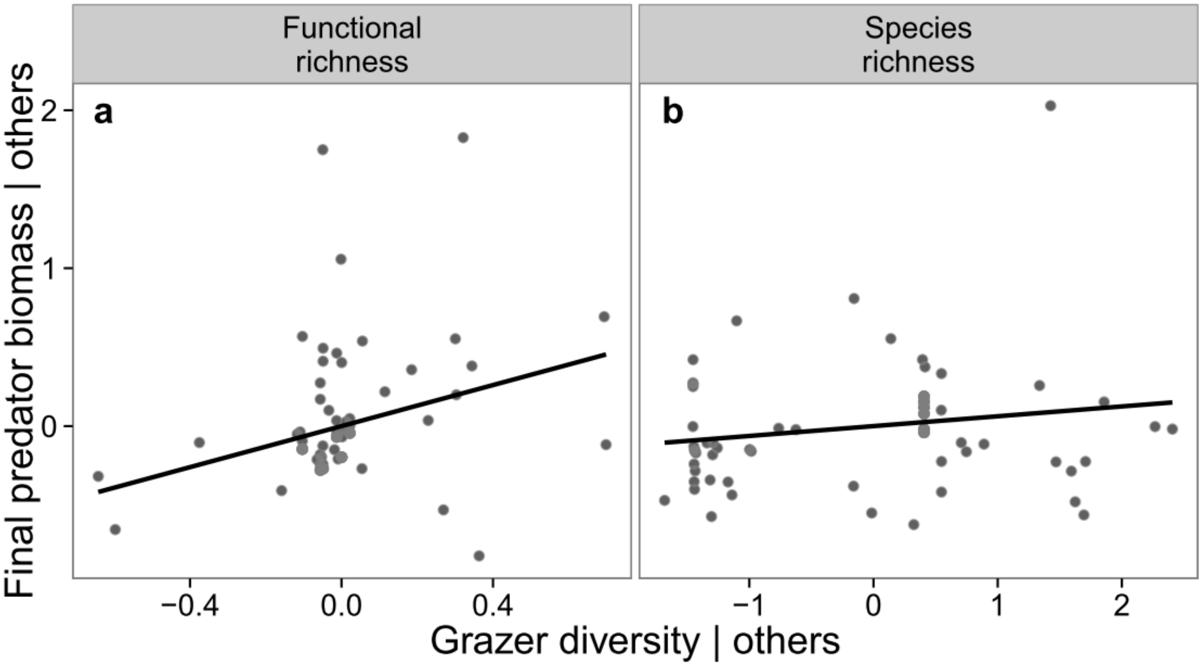


Figure S6: Average pairwise functional distance against effect sizes for each of the 9 species derived from a general linear mixed effects model regressing the presence/absence of each species against each ecosystem responses. Black lines represent predicted trends from a a simple linear regression.



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

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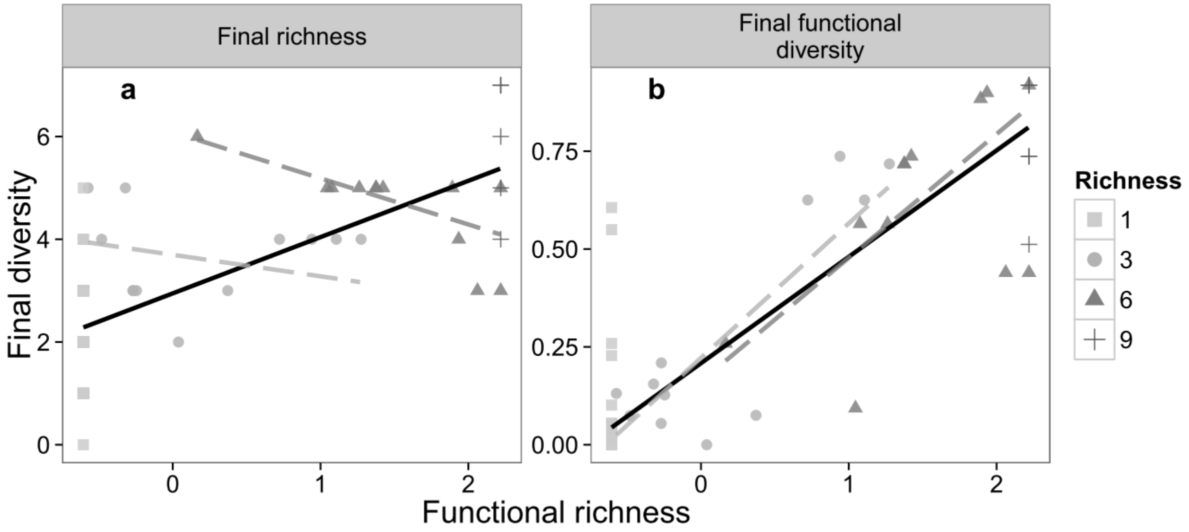

Figure S8: Scatterplot of initial FD (scaled by mean and variance) against **(a)** final species richness and **(b)** final FD of all stocked species. Shapes corresponds to the richness level (1, 3, 6, or 9). Grey lines represent predicted fits from a general linear mixed effects model for 3- (light 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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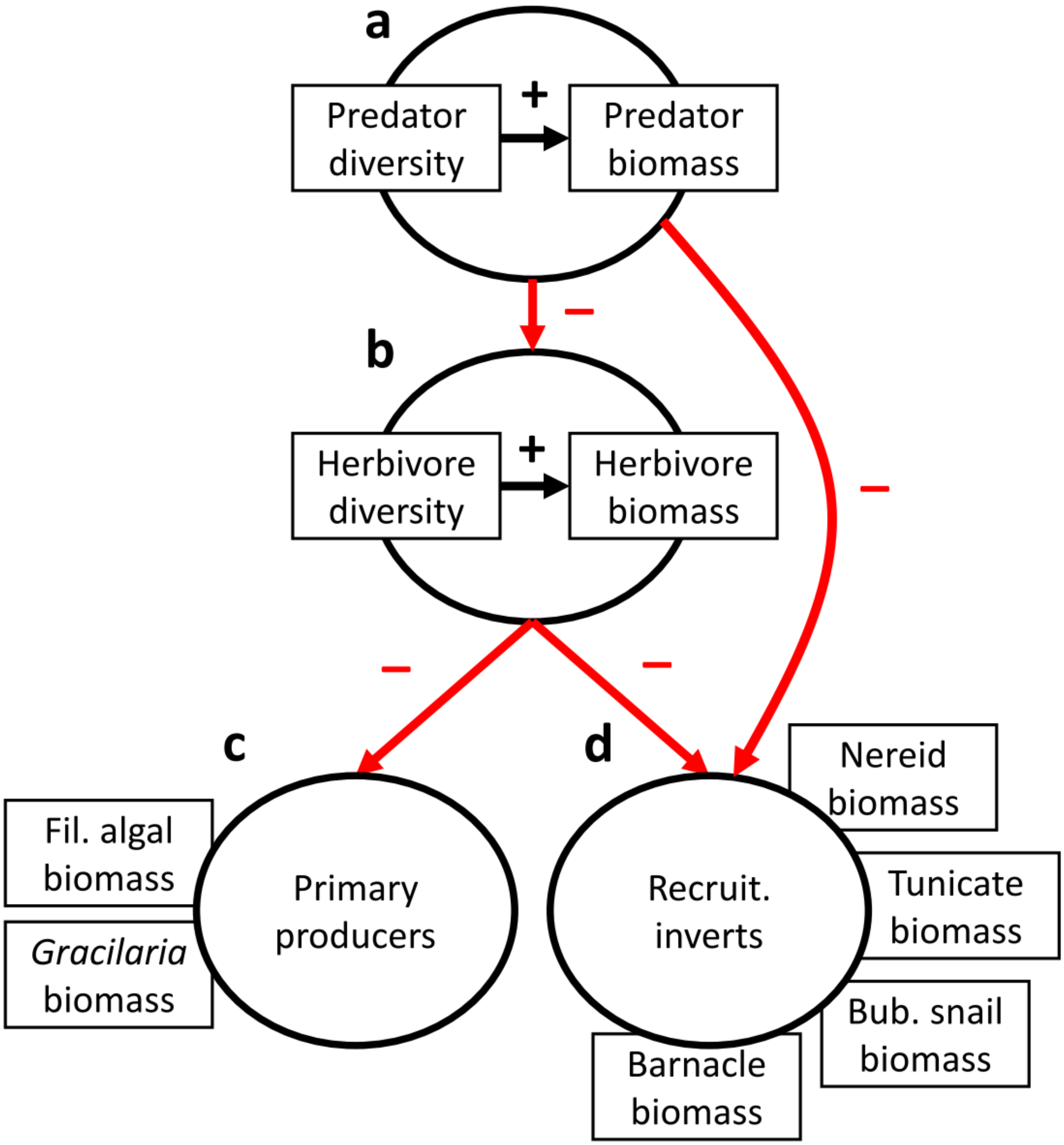

Figure A1: A conceptual 'meta-model' corresponding to a tri-trophic food web, with predators consuming herbivores, which in turn are consuming algal and invertebrate resources. Black arrows indicate hypothesized positive effects, while red arrows indicate hypothesized negative effects. Boxes surrounding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

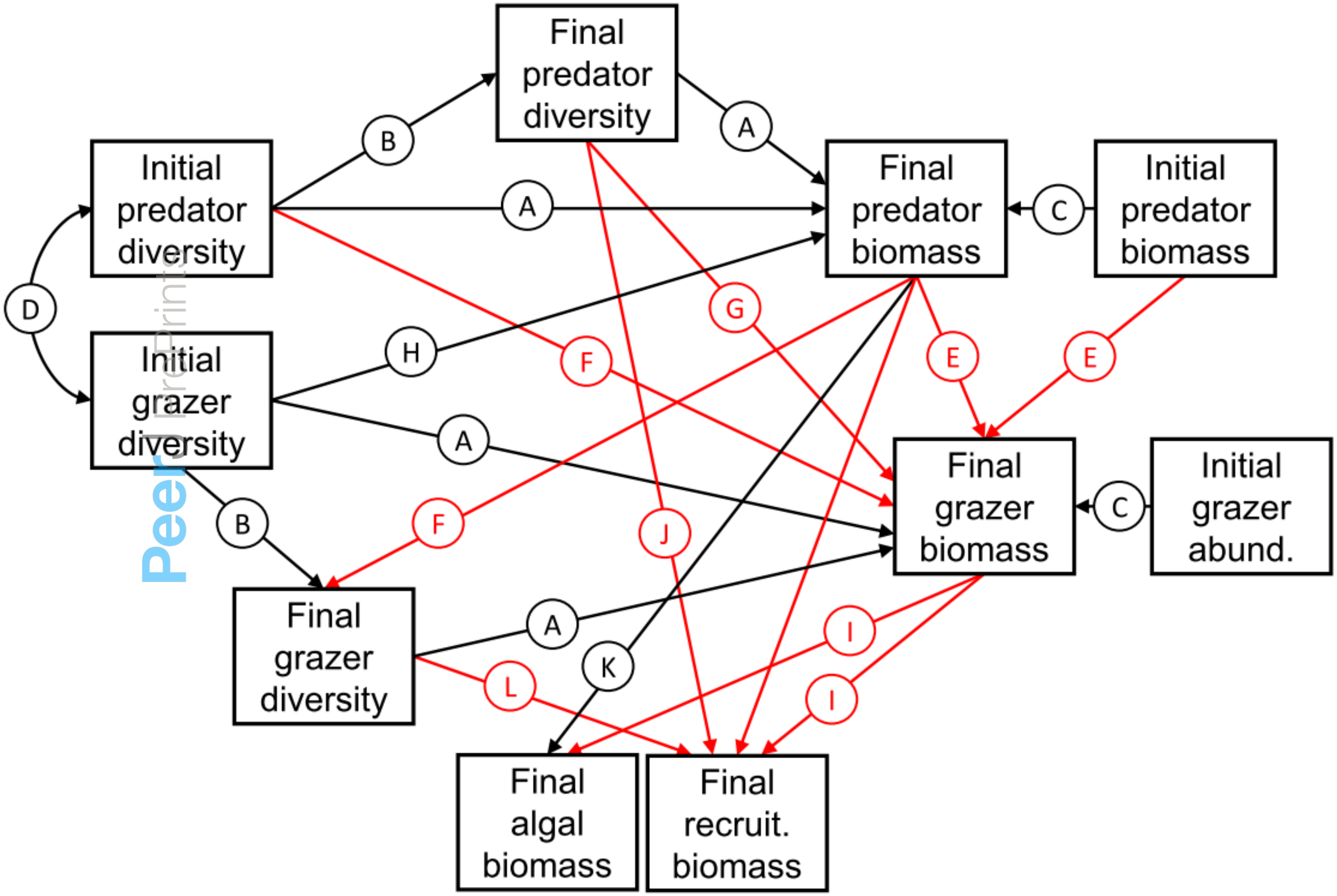

Figure A2: Hypothesized causal network relating variables measured during the experiment. Hypothesized positive relationships are given in black, and hypothesized negative relationships are given in red.