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

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## Abstract

The use of functional traits to explain biodiversity effects on ecosystem functioning has attracted intense recent interest, yet very few *a priori* manipulations of functional diversity have been attempted to date, especially from a food web perspective. Here, we simultaneously manipulated multiple functional traits of estuarine grazers and predators within multiple levels of species richness to test whether species richness or functional diversity is a better predictor of ecosystem functioning in multitrophic estuarine food webs. Community functional diversity better predicted the majority of ecosystem responses based on results from generalized linear mixed effects models. Structural equation modeling revealed that this outcome was independently attributable to functional diversity of both trophic levels, with stronger effects observed for predators. Functional complementarity was also important, as species with different combinations of traits influenced different ecosystem functions. Our study is the first to extend experimental investigations of functional diversity to a multilevel food web, and demonstrates that functional diversity is more effective than species richness in predicting ecosystem functioning 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 (Cardinale et al. 2012). However, there are many examples where diversity has a neutral or negative effect, suggesting that the positive diversity effect is not universal (Cardinale et al. 2012, Lefcheck et al. 2013, Gamfeldt et al. 2014). One possible explanation for the frequency of neutral and negative diversity effects is that the species used in these manipulations overlap sufficiently in their ecological strategies to prevent the mechanisms behind positive diversity effects, 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 can be used to more precisely 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 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 (Petchey et al. 2004, Mouillot et al. 2011, Flynn et al. 2011). However, most of the studies utilizing multivariate FD have taken a *post hoc* approach by applying trait data to existing richness manipulations, principally of grassland plants. Only a handful of studies have scored multiple traits prior to manipulation (e.g., Schittko et al. 2014), of which two used only pairwise combinations of aquatic algae (Griffin et al. 2009, Shurin et al. 2014).

Much of biodiversity-ecosystem function research has been conducted with terrestrial primary producers, and an important challenge is understanding the consequences of changing diversity in complex natural food webs (Duffy et al. 2007, Montoya et al. 2009). Few studies have simultaneously manipulated the diversity of adjacent trophic levels (e.g., both predators and prey), and those that have 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 relationship depends on the feeding biology of the consumers, specifically whether they 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). Functional diversity provide a rigorous framework for understanding the specific mechanisms underlying diversity effects, and which trait(s) promote complementarity or permit one or few species to dominate





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63 the system. To date, only two studies have experimentally manipulated a single consumer trait within a
64 single level of richness, and these found univariate FD to be a strong predictor of ecosystem functioning
65 (Schmitz 2008, Best et al. 2013).

66 In this study, we simultaneously manipulated eight functional traits of consumers both within
67 multiple levels of species richness in experimental estuarine mesocosms. The consumers included
68 naturally abundant herbivorous grazers and their predators, which allowed us to experimentally
69 recreate a model estuarine food web. We expected FD to be a better predictor of ecosystem functioning
70 than species richness (Petchey and Gaston 2002). Further, we expected FD within a trophic level to
71 enhance the biomass of that trophic level (Duffy et al. 2007), and for predator diversity to have a
72 stronger top-down effect than the bottom-up effect of grazer diversity, as found previously (Gamfeldt et
73 al. 2005, O'Connor and Bruno 2007, Douglass et al. 2008).

74 ## Methods

75 *Experimental Species*

76 We defined a 9-species pool based on historical abundances of herbivores and their predators in
77 the York River Estuary, Chesapeake Bay, USA (Douglass et al. 2010). The herbivores included three
78 crustacean mesograzers: the amphipods *Gammarus mucronatus* and *Cymadusa compta* (potentially
79 including a lesser incidental number of ampithoid amphipods, so referred to here as Ampithoid spp.),
80 and the isopod *Erichsonella attenuata*. All three species are key grazers in the Chesapeake Bay and
81 represent an important trophic link in local food webs (van Montfrans et al. 1984). We also used one
82 gastropod, *Bittiolum varium*, a relatively small but seasonally abundant mesograzer (Duffy et al. 2003).
83 The final herbivore was the shrimp *Hippolyte pleuracanthus*, whose diet is mainly micro- and
84 macroalgae, but occasionally includes animal tissue (Douglass et al. 2011). The predators included the
85 grass shrimp *Palaemonetes pugio*, juvenile blue crab *Callinectes sapidus* (30-50 mm carapace width),

pipefish *Syngnathus* sp., and mummichog *Fundulus heteroclitus*, all of which are abundant mesopredators in the Chesapeake Bay (Douglass et al. 2010). 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 body size, feeding habits, and phenology, 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 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.

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 25$^{th}$ percentile to represent 'low FD,' and 6 replicates from the upper 75$^{th}$ percentile to represent 'high FD,' for 3- and 6-species treatments. 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 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.

In May 2012, experimental assemblages were created in 19-L mesocosm buckets placed in six flow-through seawater tanks. Filtering minimized the introduction of non-target species while permitting the passage of smaller invertebrate larvae (hereafter, 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 plant 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).





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*Statistical Analysis*

To quantify the relative contributions of initial species richness vs. FD in explaining ecosystem responses, we constructed generalized linear mixed effects models (GLMMs) regressing each response against species richness or FD, allowing the intercept to vary by the random block term. For certain responses, such as final predator biomass, additional covariates, such as initial predator biomass, were included in the models, since these 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 conditional and marginal $R^2$ values (*sensu* Nakagawa and Schielzeth 2012)–corresponding to the variance explained by the fixed effect and the fixed and random effects, respectively—to gain a sense of the approximate variance in the response explained by each of the two predictors. We also 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. In several cases, residuals were highly heteroscedastic. To resolve this issue, we modeled the variance using the function *varIdent*, using initial species richness levels as the stratum.

To decompose the relative contributions of herbivore versus predator FD to ecosystem functioning, we fit a piecewise structural equation model (SEM), which combines information from multiple separate linear models into a single causal network (Shipley 2009). Because the individual models can incorporate non-normal distributions, hierarchical nesting, and random effects, piecewise SEM is a powerful and flexible alternative to traditional variance-covariance based SEM. We fit the component models as GLMMs as above. We constructed a single SEM using knowledge of the system and ecological theory to define the paths of interest. Overall model fit was assessed using Shipley's test of d-separation, which yields a Fisher's *C* statistic that can be compared to a $\chi^2$-distribution (Shipley

2009). We provide a fully documented R package, *piecewiseSEM*, to conduct piecewise structural equation modeling (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, including functions from the *piecewiseSEM* package, are provided as supplements.

## Results

Initial 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). The two diversity indices were equally poor at predicting functions related to primary producers, explaining between 3-6% of the variance in the recruiting algal and *Gracilaria* biomass. Despite the collinearity between initial species richness and FD (Fig. S3) leading to conservative *P*-values, models regressing each response 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), and a much stronger negative effect on recruiting invertebrate biomass (Fig. 1b). There was a strong positive relationship between initial FD and final predator biomass after accounting for initial predator biomass (Fig. 1c). As found



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during the model selection procedure, there was no relationship between FD and either recruiting algal biomass (Fig. 1d) or final *Gracilaria* biomass (Fig. 1e).

To determine whether the significant trends from the model fitting procedure were due entirely to the presence and/or diversity of predators, we fit a structural equation model (SEM) decomposing the independent herbivore and predator FD effects. Overall, the SEM fit the data extremely well ($C$ = 22.19, $P$ = 0.965). The significant paths and standardized regression coefficients are presented in Figure 2, and the full model, including non-significant paths, is given in Figure S4. The SEM confirmed that the strongest relationship in the experiment occurred between initial predator FD and final predator biomass (β = 0.476). This relationship was still significant and approximately equal in magnitude when considering only replicates that contained predators (β = 0.469). We also observed a positive but weaker relationship between final grazer FD and final grazer biomass (β = 0.164), even after the predator effects on grazer biomass were taken into account. Indeed, this trend can be clearly seen by extracting the partial correlations between final grazer FD and final grazer biomass, accounting for the other covariates in the SEM (Fig. S5). Predator effects on final grazer biomass were mediated through grazer FD, as indicated by the lack of a direct path between final predator biomass and final grazer biomass (Fig. 2). The magnitude of this indirect effect is achieved by multiplying the two component paths: β = –0.335 *x* 0.164 = –0.055, indicating a relatively weak but still significant reduction. In contrast, initial predator biomass had a direct negative effect on final grazer biomass (β = –0.298), suggesting that grazer communities experienced rapid top-down control by predators, and perhaps only after prey communities had stabilized that a positive effect of grazer FD on grazer biomass was observed.

The individual contributions of each species to functioning revealed strong potential for functional complementarity (Table 2). As expected, all of the grazers contributed significantly to final grazer biomass, and all predators to final predator biomass. The one exception was *C. sapidus*, whose contribution to final predator biomass was non-significant. *Fundulus heteroclitus* had a strong negative

effect on recruiting invertebrate biomass, and Ampithoid spp. contributed significantly to final algal and *Gracilaria* biomass. Regression of the effect size 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 by species with extreme combinations of traits, principally predators, while others were driven by functionally average species (Fig. S6).

## Discussion

In this study of an estuarine food web, we found that multivariate functional diversity was a better predictor of standing stock biomass across multiple trophic levels than species richness. SEM revealed this result was a consequence of functional diversity of both predators and grazers (Fig. 2). The stronger FD effects compared with herbivores is consistent with both conceptual predictions (Duffy 2002) and experimental evidence (reviewed in Griffin et al. 2013). A likely explanation for the strong predator diversity effect in our experiment is that predators were more functionally distinct, on average, than the grazer community (average functional distinctness for predators = 0.57 vs. 0.45 for grazers). Thus, niche partitioning was potentially stronger among predator species than grazer species (e.g., Griffin et al. 2008). A potential alternative explanation is that we confined predators and their prey in a small space, artificially inflating encounter rates and overall predation, although the interactions observed in the mesocosms reconcile with the known diets and interactions of these organisms (Douglass et al. 2011).

In addition to positive effects of FD on biomass within trophic levels, we observed that initial grazer FD increased final predator biomass. This result is potentially consistent with the 'balanced diet' hypothesis, where a diverse prey assemblage provides a more complete range of nutrients (Gamfeldt et al. 2005). The functionally diverse grazer assemblages were likely consuming and incorporating resources differently. For instance, Ampithoid spp. was the only grazer to have a strong negative effect

on primary producers in our experiment, and previous experiments have also presented strong evidence for resource partitioning between Ampithoid spp. and *G. mucronatus* (Duffy and Harvilicz 2001). It may also indicate niche complementarity among grazers, leading to an increase in aggregate biomass (Duffy et al. 2003) and thus food for predators (Duffy et al. 2005). Both scenarios are potentially reflected in the indirect effect of final predator biomass on final grazer biomass that was mediated through grazer FD (Fig. 2). That this effect did not cascade to primary producers or recruiting invertebrate biomass was surprising, given both theoretical predictions (Duffy et al. 2007) and past experiments with these grazers (Duffy et al. 2003, 2005, Douglass et al. 2008). One possible explanation is that predators preferentially consumed Ampithoid spp., resulting in the strong negative effect of predators on final grazer FD. Thus, by removing the strongest interactor with primary producers, predators appear to have a direct positive effect on plant biomass. This hypothesized effect would not be evident in our model since we did not include grazer species composition as a predictor variable.

Overall richness and FD were lower at the end of the experiment than at the beginning (Fig. S7a,b), 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 3). This trend corresponds with other experiments using this species (see O'Connor and Bruno 2007). The loss was partially due to crabs escaping the experimental mesocosms, and partially 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) 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. S7a).

We were unable to directly investigate the relative contributions of individual traits, as the calculation of functional richness requires ≥2 traits (Villéger et al. 2008). Instead, we regressed the effect of each species on ecosystem functioning against their functional distinctness (Fig. S6). Species with the strongest effects were generally predators, like *F. heteroclitus* and *Syngnathus* spp., which occupied functional extremes, possessing large bodies, minimal defenses, and high mobility. In contrast, the grazing amphipod Ampithoid spp. was functionally very similar to the two other grazers (Fig. S2), but had the strongest effects on primary producer biomass and final grazer biomass (Table 2). Thus, it appears there are subtle differences among herbivores in terms of their diets and resistance to predation that may explain, for instance, the significant positive effect of initial grazer FD on final predator biomass but not final grazer FD (Fig. 2).

Overall, this study empirically confirms that a focus on traits known or suspected to be mechanistically related to resource use can provide greater insight into the functioning of whole food webs than 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 at adjacent trophic levels. This result suggest that conservation of diversity at multiple trophic levels, with a particular emphasis on the variety of organismal traits, can lead to enhanced ecosystem functioning.

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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.

| Response | Species Richness | | | Functional Diversity | | |
|---|---|---|---|---|---|---|
| | AIC | $R^2_m$ | $R^2_c$ | AIC | $R^2_m$ | $R^2_c$ |
| Final grazer biomass | 133.1 | 0.103 | 0.104 | **130.5** | **0.145** | **0.146** |
| Final predator biomass | 31.4 | 0.473 | 0.473 | **21.5** | **0.636** | **0.636** |
| Recruiting invertebrate biomass | -52.9 | 0.153 | 0.193 | **-55.0** | **0.233** | **0.316** |
| Final algal biomass | -222.6 | 0.003 | 0.040 | -222.4 | 0.001 | 0.037 |
| Final *Gracilaria* biomass | 288.5 | 0.061 | 0.061 | 288.9 | 0.057 | 0.057 |



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371 **Table 2:** Standardized contributions of each individual species to ecosystem functions. Only significant

372 effects ($P < 0.05$) are shown. Amp = Ampithoid spp., *Bitt* = *Bittiolum varium*, *Call* = *Callinectes sapidus*,

373 *Erich* = *Erichsonella attenuate*, *Fund* = *Fundulus heteroclitus*, *Gamm* = *Gammarus mucronatus*, *Hippo* =

374 *Hippolyte pleuracanthus*, *Pal* = *Palaemonetes pugio*, and *Syn* = *Syngnathus* spp.

375

| **Response** | Amp | *Bitt* | *Call* | *Erich* | *Fund* | *Gamm* | *Hippo* | *Pal* | *Syn* |
|---|---|---|---|---|---|---|---|---|---|
| Final grazer biomass | 1.33 | -0.57 | | | -0.68 | 0.69 | -0.53 | | |
| Final predator biomass | | | | | 1.19 | | | | 0.45 |
| Recruit invert biomass | | | | | -0.70 | | | | |
| Final algal biomass | -0.60 | | | | | | 0.59 | | |
| Final *Gracilaria* biomass | -0.73 | | | | | | | | |





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## Figure Legends

**Figure 1:** Scatterplot of initial functional richness (scaled by mean and variance) against ecosystem functions. Shading corresponds to the richness level (1, 3, 6, or 9). Grey lines represent predicted fits from a generalized 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.

**Figure 2:** Structural equation model of functional diversity as a predictor of ecosystem and 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 in the accompanying box. Path coefficients report standardized effect sizes. Only significant paths are shown ($P < 0.05$). Non-significant paths and variables are given in Figure S4, supplementary text.





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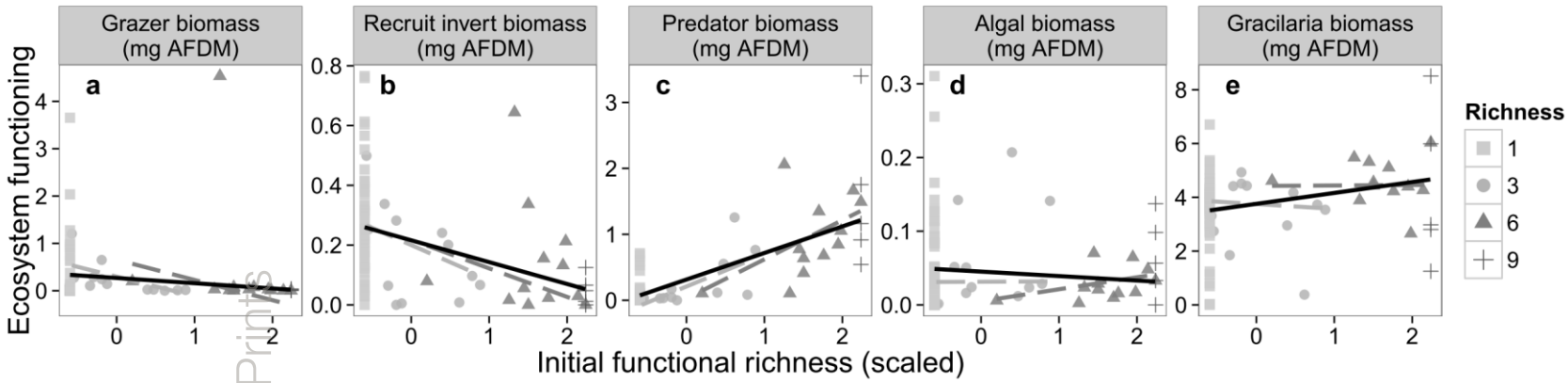


386

387 **Figure 1**





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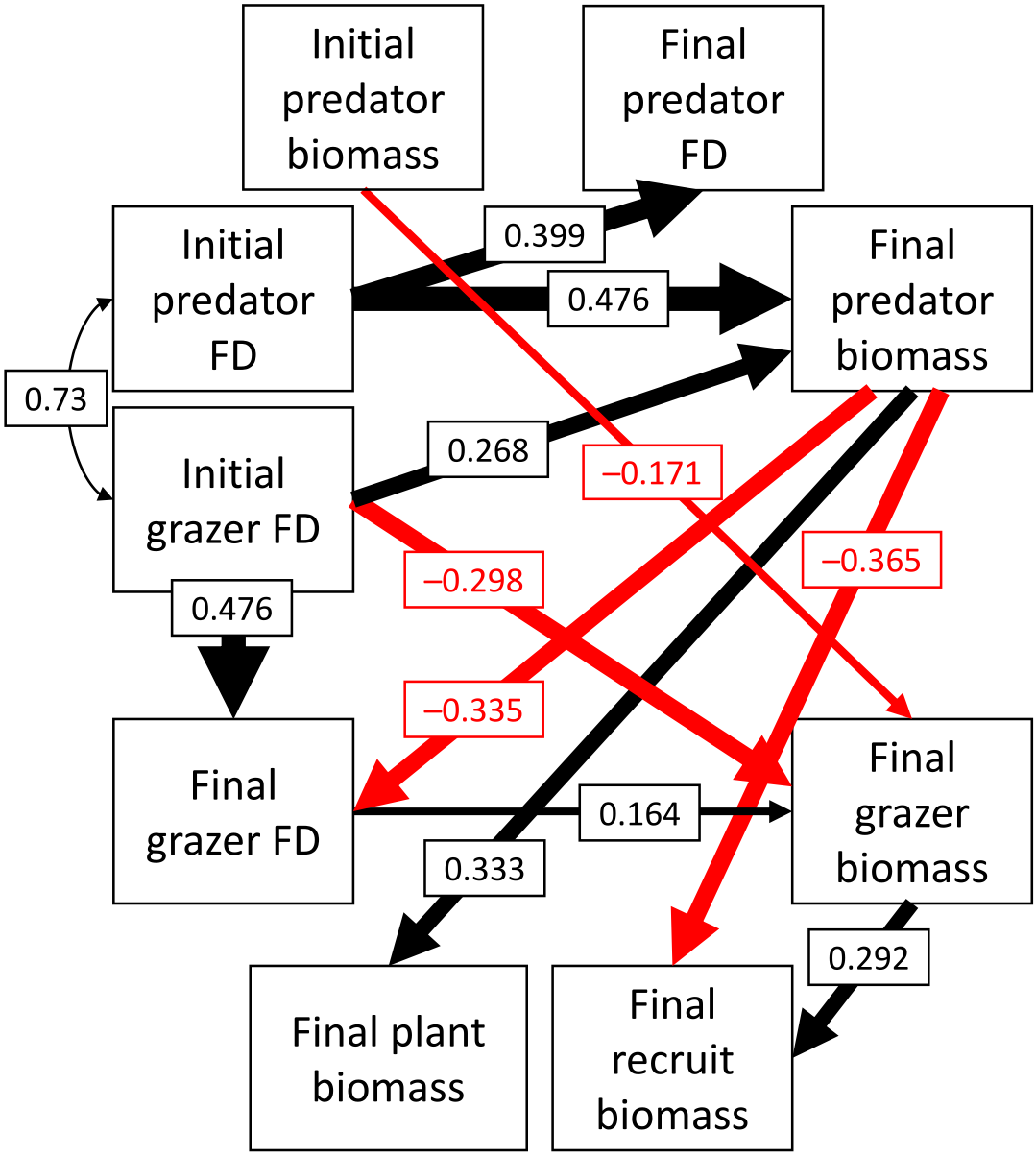


388

389 **Figure 2**

## Supplemental Methods

Functional richness was calculated using the *dbFD* function in the *FD* package (Laliberté & Shipley, 2011). Marginal and conditional $R^2$ values were calculated using the function by Lefcheck & Casallas (https://github.com/jslefche/rsquared.glmer). Vertebrates were handled according to IACUC standards (protocol 2012-05-11-7960 administered through The College of William & Mary).

In addition to biomass estimates, we sought to measure an ecosystem-level response that might reflect the integrated contributions across both herbivores and predators. Three times during the experiment, we quantified net ecosystem respiration (mmol $O_2$ $L^{-1}$ $d^{-1}$ $m^{-2}$) by taking dissolved oxygen (DO) measurements using a YSI Data Sonde. We turned off the water supply and stirred each mesocosm to disrupt any stratification. We then took 3 measurements in succession, waited approximately 1 hour, and took another 3 measurements. We then calculated the slope of the changes in DO concentration against time and divided by the area of the bucket to obtain $O_2$ flux. We repeated these measurements during the daytime (12:00-13:00 h) and the following nighttime (0:00-01:00 h) and scaled the hourly rates to 14 h of daylight and 10 h of darkness (Spivak, Canuel, Duffy, & Richardson, 2007). Due to time and equipment constraints, we were only able to measure one replicate of each treatment (one block) at a time. Thus, over the course of the experiment we were only able to measure 3 replicates of each treatment, for a total of $N$ = 32 replicates.

## Supplemental Results & Discussion

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).

411 Contrary to our predictions, we did not find a significant interaction between species richness
412 and FD for almost all ecosystem responses, though initial species richness and functional diversity had
413 antagonistic effects on final species richness (Table S2). This may be because of the high degree of
414 collinearity between the two predictors inflating standard errors of our model predictions ($r$ = 0.95, Fig.
415 S3). Despite this potential conservative bias, we were still able to isolate a significant positive effect of
416 FD but not species richness. Thus, in our experiment, the effect of increasing FD was not contingent on
417 the level of species richness. One explanation may be our experimental design, which nested two levels
418 of FD within only two levels of species richness (Fig. S1). There may have been too few levels of species
419 richness, or too little variation among species' functional traits, to extract a clearer signal. Future
420 manipulations should look towards incorporating an even greater range of species richness in
421 investigation of diversity-function (Gamfeldt et al., 2014).

422 Neither species richness nor FD were particularly good predictors of net oxygen flux, daytime or
423 nighttime (Table S3). Generalized linear mixed effects models explained between 0-10% of the variation
424 in these responses. There was no relationship between net oxygen flux, daytime or nighttime, and either
425 species richness or FD (Fig. S6c,d). Modeling species-specific contributions to oxygen flux yielded some
426 of the strongest effects observed for any response (Table S4). The presence of *H. pleuracanthus* strongly
427 decreased daytime oxygen flux, while *B. varium* strongly decreased and *G. mucronatus* strongly
428 increased nighttime oxygen flux. However, there is no strong mechanistic explanation for these results.
429 The herbivore *H. pleuracanthus* was shown to increase final algal biomass (Table S4), making it
430 somewhat paradoxical that it would decrease daytime oxygen flux, as this is the time when primary
431 producers would be photosynthesizing most. Similarly, *B. varium* and *G. mucronatus* represent some of
432 the smallest grazers, making it unlikely that they would respire much during the night relative to the
433 larger predators. Moreover, *G. mucronatus* at least achieved quite high biomass in monoculture,
434 although its effect was to decrease nighttime oxygen flux. These results are likely due to both the





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435 inherent variability in the mesocosms, the precision of the YSI instrument, and the low number of
436 replicates measured during the experiment. As such, we have elected to present these data in the
437 supplements and instead focus on the fully replicated response variables relating to standing stock
438 biomass in the main text.

## 439 Supplemental References


440 Gamfeldt, L., Lefcheck, J. S., Byrnes, J. E. K., Cardinale, B. J., Emmett Duffy, J., & Griffin, J. N. (2014).
441 Marine biodiversity and ecosystem functioning: what's known and what's next. *Oikos*, n/a–n/a.
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446 sediment organic matter composition in an experimental seagrass ecosystem. *Limnology and*
447 *Oceanography*, *52*(6), 2595–2607. Retrieved from http://www.jstor.org/stable/10.2307/4502405






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448 **Table S1:** Functional traits measured for each species included in the experiment, along with their units
449 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 |

450





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**Table S2:** Scaled coefficients 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 |
| $O_2$ flux (day) | -1.398 | 1.129 | 0.210 | 0.10 | 0.10 |
| $O_2$ flux (night) | -0.089 | 0.186 | 0.062 | 0.00 | 0.01 |
| 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:** 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 various ecosystem responses. Models that are significantly better than the other at explaining the response based on lower AIC scores are bolded. The first five rows are identical to Table 1 (main text); the next 4 rows report model results for additional variables relating to dissolved oxygen and final community diversity (see supplemental methods). Models predicting algal or *Gracilaria* biomass and $O_2$ flux were approximately equivalent, and thus those rows have no bolded cells.

| Response | Species Richness | | | Functional Diversity | | |
|---|---|---|---|---|---|---|
| | AIC | $R^2_m$ | $R^2_c$ | AIC | $R^2_m$ | $R^2_c$ |
| Final grazer biomass | 133.1 | 0.103 | 0.104 | **130.5** | **0.145** | **0.146** |
| Final predator biomass | 31.4 | 0.473 | 0.473 | **21.5** | **0.636** | **0.636** |
| Recruiting invertebrate biomass | -52.9 | 0.153 | 0.193 | **-55.0** | **0.233** | **0.316** |
| Final algal biomass | -222.6 | 0.003 | 0.040 | -222.4 | 0.001 | 0.037 |
| Final *Gracilaria* biomass | 288.5 | 0.061 | 0.061 | 288.9 | 0.057 | 0.057 |
| $O_2$ flux (day) | 205.3 | 0.014 | 0.016 | 204.1 | 0.046 | 0.049 |
| $O_2$ flux (night) | 183.2 | 0.004 | 0.004 | 182.7 | 0.017 | 0.017 |
| Final species richness | **233.6** | **0.586** | **0.589** | 252.6 | 0.478 | 0.478 |
| Final functional diversity | -69.6 | 0.699 | 0.699 | **-97.2** | **0.786** | **0.786** |



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465 **Table S4:** Contributions of each individual species to ecosystem function. Bolded cell indicate significant
466 effects ($P < 0.05$). Differs from Table 2 (main text) in that all effect sizes are reported, not just significant
467 effects, and includes additional responses relating to final diversity and $O_2$ flux.

| Response | *Amp* | *Bitt* | *Call* | *Erich* | *Fund* | *Gamm* | *Hippo* | *Pal* | *Syp* |
|---|---|---|---|---|---|---|---|---|---|
| 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 |
| $O_2$ flux (day) | -0.18 | -0.60 | 0.19 | -0.3 | 0.59 | 0.69 | **-0.87** | 0.18 | 0.49 |
| $O_2$ flux (night) | -0.60 | **-0.98** | 0.57 | 0.28 | 0.58 | **0.72** | -0.10 | -0.51 | -0.21 |
| Final species richness | **0.51** | **0.39** | 0.07 | **0.83** | 0.05 | 0.27 | 0.22 | **0.48** | 0.08 |
| Final functional diversity | 0.19 | **0.41** | 0.17 | **0.36** | **0.85** | 0.14 | 0.15 | **0.19** | **0.61** |





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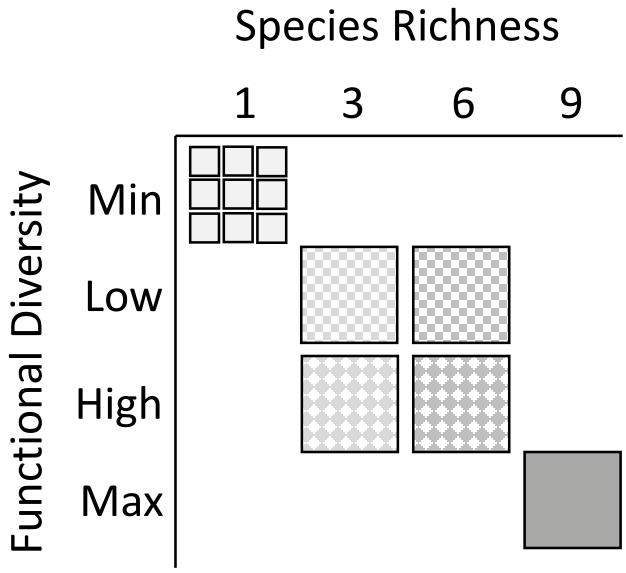
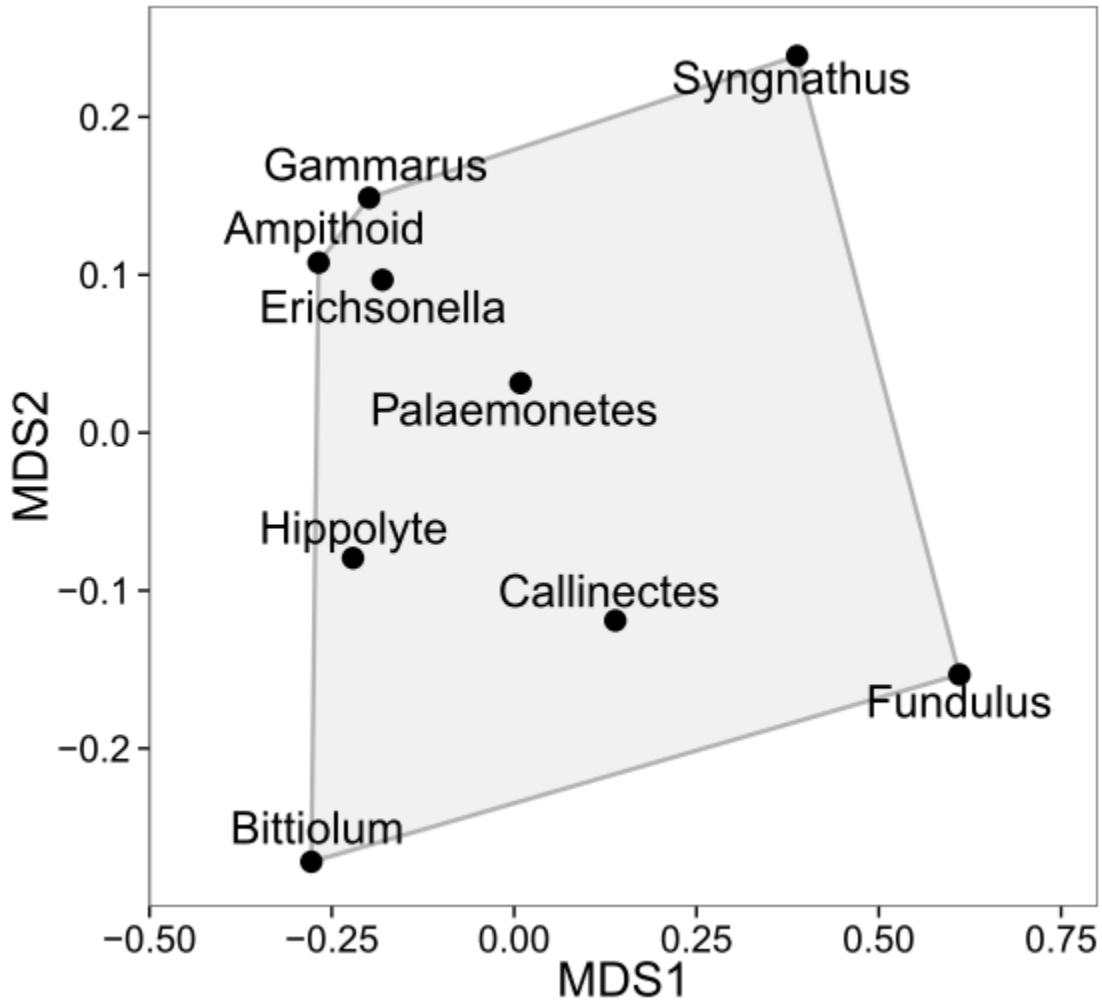



**Figure S1:** A schematic of the experimental design. We utilized four levels of species richness: 1, 3, 6, and 9. Each of the 9 species was represented in the single-species treatments (hence the 9 individual squares). All species were present in the 9-species mixture. For the 3- and 6-species treatments, we generated all possible combinations of species and calculated FD. We then randomly drew replicate assemblages from the lower 25$^{th}$ percentile to represent 'low FD,' and repeated this exercise for the upper 75$^{th}$ 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).




479

480 **Figure S2:** Non-metric multidimensional scaling plot collaping 8 functional traits into two dimensions.

481 The convex hull (area of trait space encompassed by all 8 species) is given by the shaded polygon.





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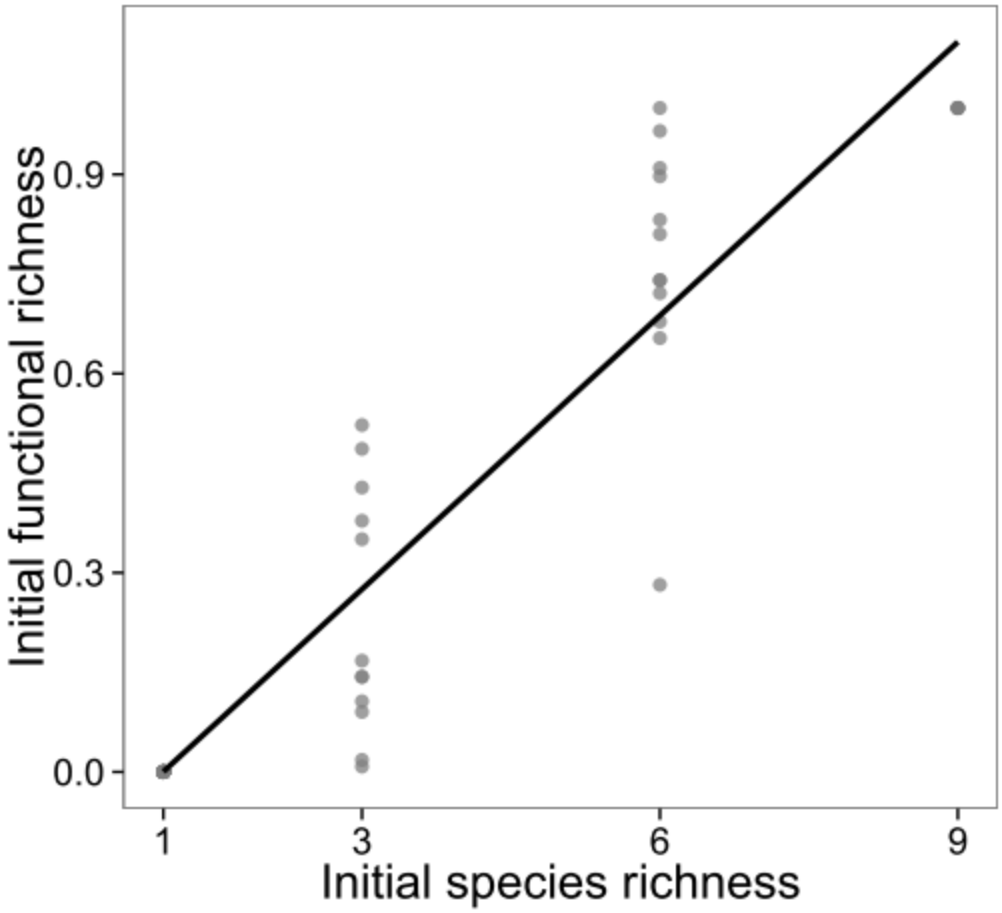
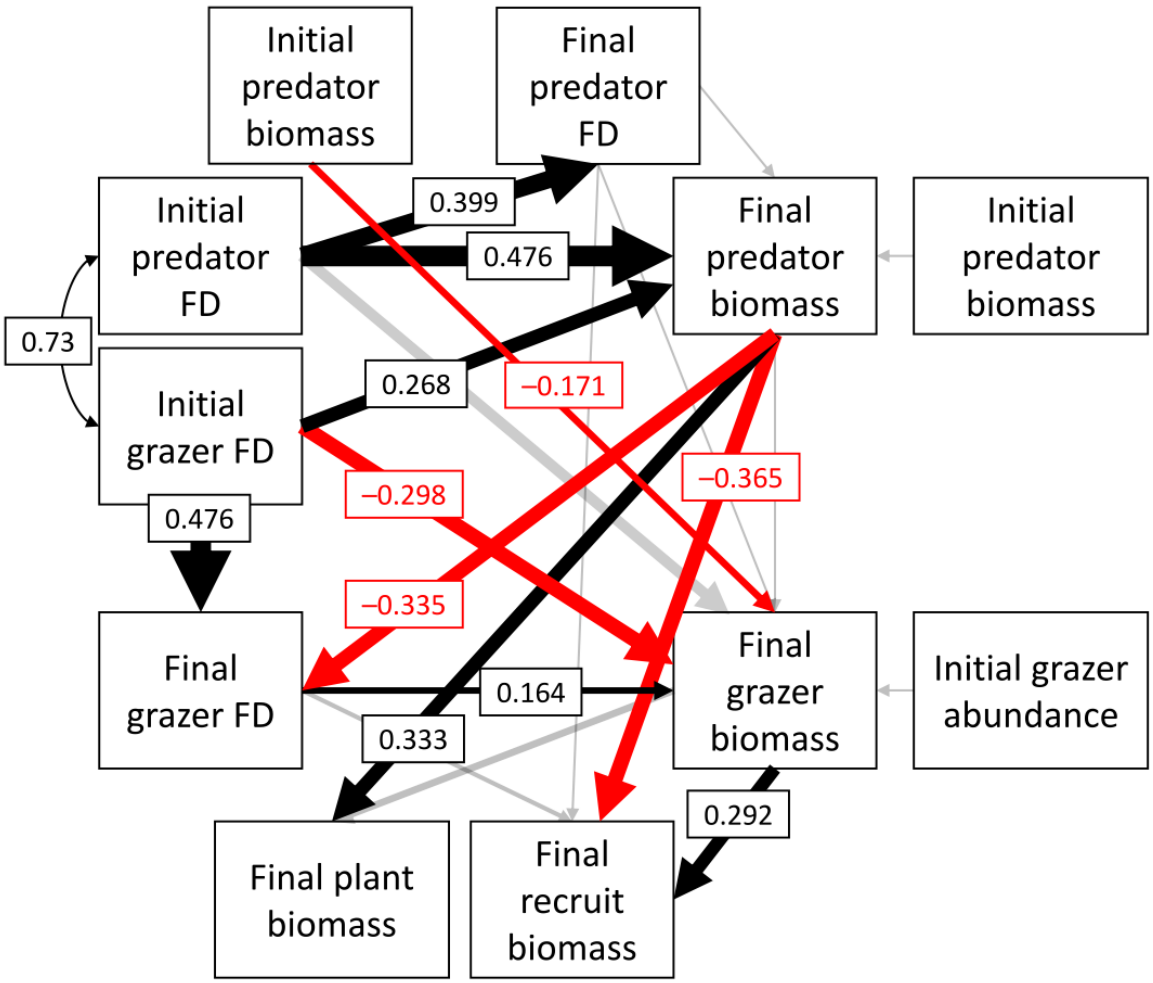



482

483 **Figure S3**: Plot of initial species richness against initial funciton richness (Pearson's correlation $r = 0.95$).




484

485 **Figure S4:** Structural equation model of functional diversity as a predictor of ecosystem and community
486 responses at the end of the experiment. Black arrows represent positive paths, and red arrows
487 represent negative paths. Arrow width is proportional to the size of the effect, reported in the
488 accompanying box. Path coefficients report standardized effect sizes. Transparent arrows represent
489 paths that were included in the model but were not significant ($P \geq 0.05$). Significant paths are reported
490 as in Figure 2, main text.

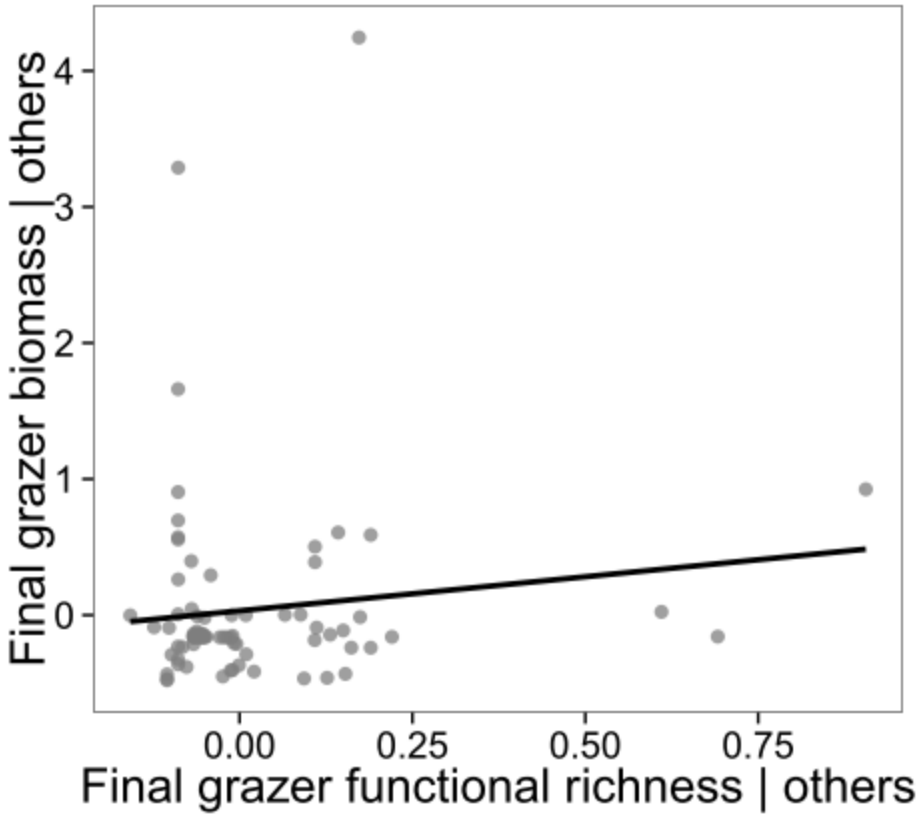
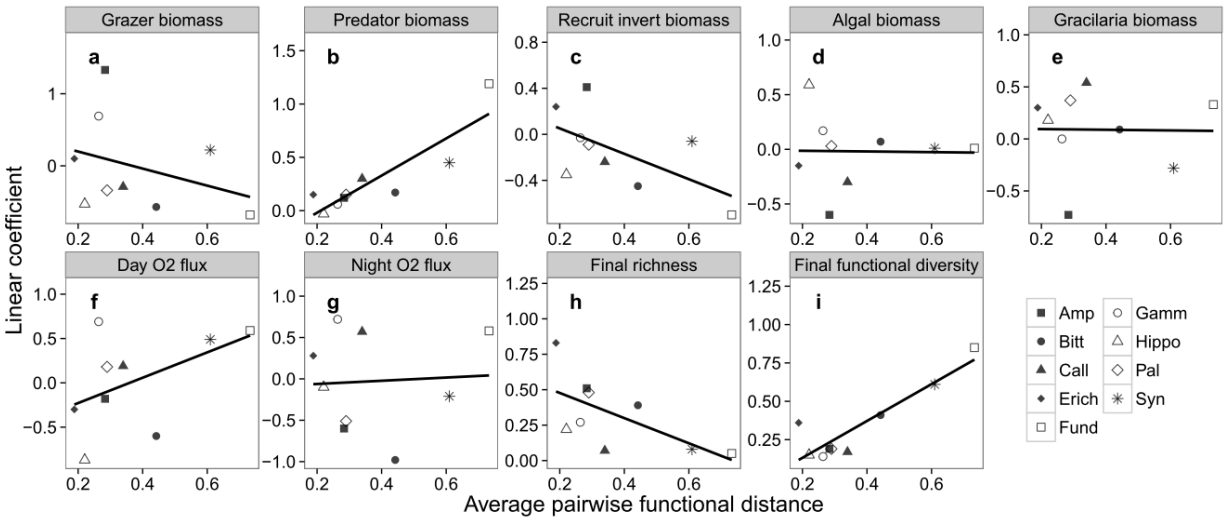



**Figure S5:** Partial correlation plot of final grazer functional richness against final grazer biomass, after accounting for the covariates in Figure S1.




494

495 **Figure S6:** Average pairwise functional distance against effect sizes for each of the 9 species derived

496 from a generalized linear mixed effects model. Black lines represent predicted fits from a linear model.





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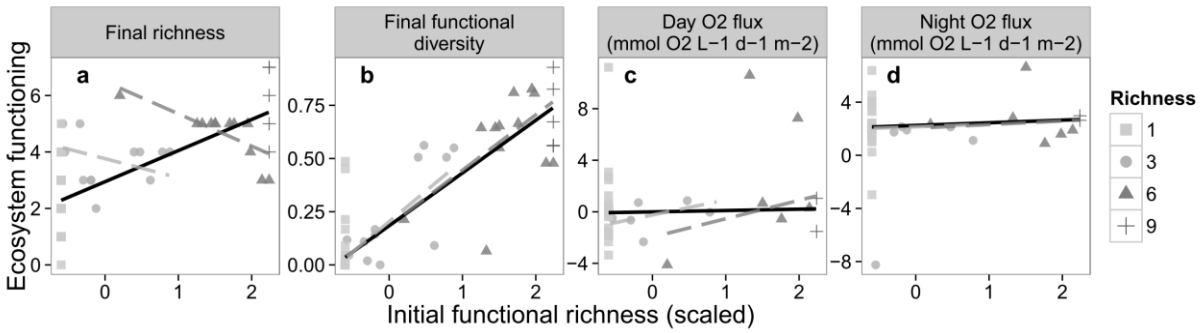


497

498 **Figure S7:** Scatterplot of initial FD (scaled by mean and variance) against **(a)** final species richness, **(b)**
499 final FD, and **(c)** daytime and **(d)** nighttime dissolved oxygen flux. Shading corresponds to the richness
500 level (1, 3, 6, or 9). Grey lines represent predicted fits from a generalized linear mixed effects model for
501 3- (light grey) and 6-species (dark grey) treatments (Table S2). The black line represents the overall trend
502 from the same model.





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