Biodiversity mediates top-down control in eelgrass ecosystems: A global comparative-experimental approach

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Abstract

Nutrient pollution and reduced grazing each can stimulate algal blooms as shown by numerous experiments. But because experiments rarely incorporate natural variation in environmental factors and biodiversity, conditions determining the relative strength of bottom-up and top-down forcing remain unresolved. We factorially added nutrients and reduced grazing at 15 sites across the range of the marine foundation species eelgrass (*Zostera marina*) to quantify how top-down and bottom-up control interact with natural gradients in biodiversity and environmental forcing. Experiments confirmed modest top-down control of algae, whereas fertilization had no general effect. Unexpectedly, grazer and algal biomass were better predicted by cross-site variation in grazer and eelgrass diversity than by global environmental gradients. Moreover, these large-scale patterns corresponded strikingly with prior small-scale experiments. Our results link global and local evidence that biodiversity and top-down control strongly influence functioning of threatened seagrass ecosystems, and suggest that biodiversity is comparably important to global change stressors.

INTRODUCTION

Nutrient pollution and alteration of food webs by exploitation and invasion are two of the dominant human impacts on natural ecosystems. A wealth of evidence from controlled experiments confirms that each can be important in certain situations, fueling a long-running debate about the conditions favoring bottom-up versus top-down forcing and their management implications (Hunter & Price 1992; Cloern 2001; Heck & Valentine 2007; Gruner *et al.* 2008; Eriksson *et al.* 2009). Manipulative approaches have greatly advanced our understanding of how these processes shape experimental communities, but their implications for complex natural

systems are often unclear. By design such experiments hold constant many other environmental and human factors known to strongly influence ecosystem processes, raising the question of how important the manipulated factors are relative to other drivers. These include, in particular, the spatial variation in environment and biodiversity that are characteristic of wild ecosystems but that are rigorously controlled in most experiments. Resolving these issues poses formidable challenges, requiring new approaches that can integrate the power of experiments with observational data on those regional processes that cannot be manipulated (Grace 2006; Cardinale *et al.* 2012). One such hybrid approach involves a coordinated experimental network, in which simple controlled experiments are replicated across space, allowing for the incorporation of environmental context into the analysis. Such networks have made important advances, for example, in understanding nutrient dynamics in terrestrial grasslands (Borer *et al.* 2014).

The *Zostera* Experimental Network (*ZEN,* www.zenscience.org) seeks to understand how complex regional and local processes interact to mold community and ecosystem structure by focusing on communities associated with the marine foundation species, eelgrass (*Zostera marina*), which is distributed across a broad range of conditions and biogeographic provinces (Moore & Short 2006). Eelgrass creates habitat for productive and economically important communities along coasts and estuaries throughout the northern hemisphere, supporting assemblages of epifaunal herbivores and detritivores, including small crustaceans, gastropod mollusks, and polychaete worms. These mesograzers feed on micro- and macroalgae that grow on seagrass blades and are important conduits of production to higher trophic levels including commercial and recreational fisheries (Valentine & Duffy 2006). Seagrass dominance requires low biomass of competing algae, which is maintained by a combination of grazing and low

nutrient conditions. This balance can be upset by food-web perturbations that reduce grazing on algae, or by nutrient loading that stimulates algal accumulation, but the relative importance of these bottom-up and top-down factors in the dynamics of seagrass systems remains a subject of debate (Hughes *et al.* 2004; Heck & Valentine 2007).

Complementing the long interest in relative importance of top-down and bottom-up forcing, a parallel line of research has focused on biodiversity—including species and genetic richness—as an important driver of ecosystem structure and functioning. Numerous experiments have now converged on the conclusion that declining variety of species, genetic lineages, and functional types generally results in declining ecosystem productivity and stability across a range of systems (Cardinale *et al.* 2011; 2012). In eelgrass systems specifically, prior experiments implicate both genetic and species richness in mediating the key interaction between grazers and epiphytic algae in eelgrass beds: plots planted with genotypically diverse eelgrass recruited denser fauna (Hughes & Stachowicz 2004; Reusch *et al.* 2005), and plots seeded with species-rich grazer assemblages more effectively controlled epiphytic algae (Duffy *et al.* 2003; 2013). Yet it remains unclear how results from controlled experiments in mesocosms or single field sites translate to nature where many factors interact to drive productivity, trophic transfer, and other ecosystem processes (Srivastava & Vellend 2005).

Community functional composition and richness are potentially central in mediating the strength of top-down control and responses to changing resources (Strong 1992; Polis & Strong 1996; Oksanen & Oksanen 2000; Duffy *et al.* 2007). We sought to link these research traditions by testing whether the signature of biodiversity effects are apparent across larger scales, in naturally variable ecosystems, and specifically whether they influence the relative importance of top-down versus bottom-up forcing. To do so we conducted coordinated manipulations of

nutrients and grazing across 15 sites spanning the northern hemisphere (Fig. 1a, Table S1) and encompassing the global spectrum of environments supporting eelgrass, including a natural gradient in grazer species richness from 5 to 27 species (Fig. S1). Our experimental treatments simulated two major human influences on coastal ecosystems: nutrient pollution and fishing-induced food web changes that weaken top-down control (Heck & Valentine 2007; Duffy *et al.* 2013). Both types of perturbation have been demonstrated at various locations to cause blooms of epiphytic algae that compete with eelgrass, reducing its dominance (Duffy *et al.* 2013) and potentially tipping the system between states dominated by seagrass versus epiphytic algae (Reynolds *et al.* 2014). We evaluated how these bottom-up and top-down processes are influenced by environment and biodiversity, specifically eelgrass genetic and grazer species diversity, of the local community using path analysis (Grace 2006), by integrating experimental data with natural variation among communities and controlling statistically for cross-site variation in biodiversity, environmental, and potential anthropogenic drivers.

We used path analysis to test the following hypotheses (Fig. 2a). First we expected that grazing impacts on plants should increase with environmental temperature (O'Connor 2009), and with plant nitrogen content (Mattson 1980). Based on prior experimental work we hypothesized that biodiversity (grazer species richness) should increase resistance to perturbations, in this case experimental nutrient addition, and that increasing grazer richness should increase grazer biomass and grazing pressure on algae (Duffy *et al.* 2003). Human influence is likely to have strong impacts on structure and functioning of eelgrass systems through a variety of mechanisms but the specific expectations for grazer and algal biomass depend on food-web structure and the specific types of impact. Our comparative-experimental study demonstrated that grazer reduction has generally stronger effects on algal biomass in eelgrass ecosystems than does local nutrient

addition, and that grazer and algal biomass were better predicted by cross-site variation in biodiversity than by global environmental gradients.

MATERIALS AND METHODS

The ZEN field experimental module

In summer 2011 we conducted identical field experiments at 15 sites (Fig. 1a, Table S1) encompassing the geographic range of eelgrass (32-67° N) and a broad range in environmental conditions (Fig. S1). Each experiment was a 2-way factorial experiment crossing nutrient fertilization with crustacean mesograzer reduction. Forty plots were established at each site at depths between 0.5-3 m below mean low water. Each plot was defined by three PVC stakes forming a triangle ~50 cm on a side. Crustacean grazer reduction was accomplished by attaching a plaster block containing the degradable insecticide carbaryl (Poore *et al.* 2009; Whalen *et al.* 2013), hereafter deterrent, or alternatively a plaster control block to each stake. Fertilization was accomplished by attaching a mesh bag containing 300 g of slow-release fertilizer (Plantacote™, N:P:K = 14:14:14), or an empty mesh control bag, to one pole of each plot. Thus, there were ten plots of each of the four combinations of fertilizer (present, absent) and grazer deterrent (present, absent) at each site. Carbaryl effects on crustaceans extended ~60 cm from the source (Whalen *et al.* 2013). Plots were separated by > 2 m. The experiment was maintained for approximately four weeks at each site, a period long enough to see effects in pilot studies (Whalen *et al.* 2013).

At the end of the experiment we measured the abundance and species composition of epifauna, including mesograzers, in each plot by enclosing eelgrass and associated fauna in a 500 µm-mesh bag underwater, removing the fauna from the plants in the lab, and preserving in 70% ethanol. Epifaunal animals (excluding meiofauna) were identified and size-fractionated to

estimate biomass using empirical equations (Edgar 1990). Faunal biomass was standardized to above-ground plant biomass (mg fauna g plant^{-1}). A separate eelgrass shoot was removed from each plot and scraped to obtain microalgal biomass (μg chl *a* g dry *Zostera*$^{-1}$), which was measured spectrophotometrically or fluorometrically (Duffy *et al.* 2003). Biomasses of epiphytic algae, grazing crustaceans, and grazing gastropods constituted the response variables of primary interest. Eelgrass leaf nitrogen content was quantified in standardized young leaf material from five pooled shoots in each plot at the end of the experiment using a CHN analyzer.

Site characteristics

We collected data on several variables to assess how experimental effects varied across environmental context. At the site level, we measured water temperature throughout the experiment using HOBO Pendant ® temperature loggers, and salinity via refractometers. Eelgrass leaf % N was measured from ambient (control) plots and averaged across plots to obtain a proxy for site-level nutrient status (Burkholder *et al.* 2007). As a rough measure of anthropogenic influences, we obtained estimates of human population density near each site using the LandScan™ Global Population Database (Oak Ridge National Laboratory), implemented in National Geographic's Mapmaker Interactive (http://mapmaker.education.nationalgeographic.com); this tool presents human population density on a 5-point ordinal scale (bins) from <1 to >500 per cell.

We measured two aspects of biodiversity shown previously to influence eelgrass ecosystem properties in experiments. Eelgrass genotypic richness at a site was calculated as the sum of unique genotypes found among the 40 plots. Mesograzer species richness was estimated at the site level as the sum of all species recorded across the set of 40 plots. We chose to

aggregate diversity at the site level as we consider the list of species sampled at the site a better measure of the species potentially visiting a plot during the experiment than the single, final sampling point from that plot. To assess whether our richness estimates were affected by the number of individuals sampled, we employed fixed-coverage subsampling, a variant of rarefaction (Chao & Jost 2012). This approach extrapolates site-level estimates of richness based on sample 'completeness,' or the proportion of individuals in the community estimated to belong to species detected by sampling, and thus can be used to determine whether our efforts yielded richness estimates close to their asymptotic maximum.

All statistical analyses were conducted using R 3.03 (R Development Core Team 2013).

Eelgrass genetic analyses

Collection of eelgrass samples (40 ramets per site, i.e., one per plot), DNA extraction, microsatellite amplification and genotyping followed Olsen et al. (2013). Six microsatellite loci (CT2, CT35, CT12, CT17D, CT19, CT20) were scored (Reusch *et al.* 1999; Reusch 2000). The numbers of genets sampled at a site was distinguished with GENCLONE 2.0 (Arnaud-Haond & Khalid 2007), considering as identical only those genets with non-significant probabilities of identity by chance (*P*sex (F_{IS})). Genotypic richness, *R* (number of unique genets, *G*–1 divided by the number of sampled ramets, *N–1*) was also calculated with GENCLONE 2.0. Here we report values of genotypic richness per site (R).

Path analysis of combined experimental and observational data

We expressed hypotheses about the integrated functioning of the eelgrass ecosystem as graphical networks of interaction paths, and analyzed each as a set of linked equations using path analysis

(a variant of the broader field of structural equation models, but which uses only observed rather than latent variables, Figs. 2a, S3). An advantage of SEM for studying complex systems is that, by linking together component models for different response variables, it allows rigorous estimation of indirect effects and tests of the overall fit of a complex, causal network of influence (Grace 2006). Models were fit using data from the 40 plots at each of 15 sites for a total of 600 sampling units. Predictors included the following exogenous variables, i.e., whose variance arose outside the model: (1) experimental treatments (grazer deterrent, fertilization, their interaction, and unmanipulated control), (2) biogeographic variation (latitude, ocean [Atlantic vs. Pacific]), (3) abiotic forcing factors, including temperature, salinity, nutrient availability proxied as eelgrass % N (Burkholder *et al.* 2007), and (4) human population density. Endogenous variables are those whose variation the model seeks to explain (response variables), including in our case biodiversity (eelgrass genotypic richness, grazer species richness), and biomasses of crustacean grazers, gastropod grazers, and algae.

Preliminary exploration found that simple models with few explanatory variables had poor overall fit via D-separation tests (see below). Therefore we developed a set of candidate models by first removing paths from the "full" model (containing nearly all possible paths, Figure 2a, Table 1) to test their hypothesized importance. Next we used the best fitting models from this process and added interaction terms to test specific hypotheses about how bottom-up and top-down processes should vary with latitude, temperature, and biodiversity. Including the full model 1 incorporating all paths of interest, we fit 14 candidate models that tested the influence of the experimental and environmental variables listed above on biomasses of algae, crustacean and gastropod grazers (models 2-10), along with interactions motivated by theory (models 11-14, Table 1, Figure S3). To test the metabolic hypothesis that grazing impacts

(measured as effect of grazer deterrent on algae) should increase with environmental temperature, model 11 incorporated an interaction between grazer deterrent and temperature. We also tested the related hypothesis that grazer impacts should decrease with latitude by incorporating an interaction of deterrent effect and latitude (model 12). We tested the hypothesis that grazing is more effective where grazer richness is higher by incorporating an interactive effect of grazer richness and grazer deterrent on algae (model 13); the hypothesis is supported if the interaction is significant and positive. Finally, model 14 tested the hypothesis that grazer richness promotes resistance against nutrient loading; this hypothesis is supported if fertilization increases algal biomass less at sites with higher grazer richness (negative interaction of grazer richness and fertilization effect on algae). Because we used mean leaf % nitrogen of control plots as a proxy for site nutrient status in the path analyses, effects of experimental treatments on leaf % nitrogen were estimated separately across sites.

Our study design produced hierarchical (i.e., multilevel) data at two nested levels (40 plots within each of 15 sites, for a total of 600 plots), As a result the analysis proceeded in two phases. In the first phase, we sought to explain variation in the two endogenous variables measured at the site level, i.e., grazer species richness and eelgrass genotypic richness (all other endogenous variables were measured at the plot level). Because these two biodiversity variables were measured at the site level (taking 15, rather than 600, unique values), they had no hierarchical structure and were therefore modeled using standard multiple linear regressions. This analysis showed that the only significant predictor of grazer and eelgrass richness was latitude (compare models 1 and 2, Table 1), so the influence of latitude was retained as the sole predictor of grazer species richness and eelgrass genotypic richness in all subsequent models (models 3-14) that focused on the plot-level variables of algal and grazers biomasses.

In the second, main phase of analysis we sought to understand the drivers of plot-level biomasses of algae and crustacean and gastropod grazers, corresponding to the hypotheses described in Table 1. Because some predictor variables were measured at plot level (with 600 unique values) and others at the site level (with 15 unique values), we modeled the responses with linear mixed effects models, including a random effect modeling variation in intercepts among the 15 sites. In this phase we used Shipley's (2009) approach to estimating a multilevel path model using directional separation (D-sep) tests. This approach constructs the path model as a set of hierarchical linear mixed models, each of which was fit using restricted maximum likelihood with the *nlme* package (version 3.1-117), in R, and the overall path model (the SEM) was fit using the R package *piecewiseSEM* (Lefcheck & Duffy 2014). Prior to fitting the models we graphically examined distributions of all variables for outliers and severe departures from normality. Most biological variables were $\log_{10}$-transformed to improve normality. One path in the final model—connecting biomasses of crustacean and gastropods (Fig. 2b), was considered a correlated error rather than a directed causal path because the positive coefficient seemed best interpreted as reflecting parallel responses to unmeasured forcing variables.

We selected among candidate models using two criteria. First, goodness of fit was estimated for each path model using Shipley's (2009) test of directional separation (d-sep), which combines the significance of unrealized paths into a single, χ^2-distributed Fisher's *C* statistic. For each candidate model that passed this test of adequate fit ($P > 0.05$) we then computed an AIC value using Shipley's (2013) general approach to computing AIC in path analyses. Finally, AIC weights were calculated and compared to evaluate the relative support for each candidate model. We graphically assessed the validity of model assumptions by plotting the residuals against the fitted values of each component model.

Once a model was chosen, we compared the relative importance of its predictor variables using standardized path coefficients. We standardized coefficients to the relevant ranges of the component variables as recommended by Grace (2006). A raw coefficient β_{xy} expressing the effect of x on y is range-standardized as $\beta.range_{xy} = \beta_{xy} * (x_{max} - x_{min}) / (y_{max} - y_{min})$, where the max and min values represent the largest and smallest values of the variables recorded in the data set (see Fig. S1). This approach produces a dimensionless coefficient that is easily interpretable in the original units. For example a β.std value of -0.349 for effect of grazer richness on microalgal biomass means that microalgal biomass is expected to decline by 35% of its measured range as one moves across the entire measured range of grazer richness, when the influence of other variables is controlled for. Marginal R^2 values for endogenous variables were calculated from the best model (model 10) using an approach designed for hierarchical mixed models (Nakagawa & Schielzeth 2013).

Finally, to visualize key relationships among variables in our best model while accounting for the influence of other covariates, we generated partial correlation plots. The partial correlation between X and Y measures the association between X and Y when effects of all other variables in the model have been controlled statistically; it is visualized by plotting $r_{(X|others)}$ vs $r_{(Y|others)}$, where $r_{(X|others)}$ consists of the residuals of X from the linear model that regresses X on all variables included in the final model except for Y. Similarly, $r_{(Y|others)}$ are the residuals of the model that regresses Y on all variables except X. Where the model contains only site-level predictors the residuals used to calculate the partial correlation will take only as many values as there are sites (15 in our case) on the x axis (Fig. 3a,c), whereas when the model contains a predictor measured at the plot level (e.g., crustacean biomass) the residuals are not so constrained (Fig. 3b,d).

Meta-analysis of prior experiments

To explore parallels between the cross-site patterns found in our study and those of previous small-scale experiments, we extracted and summarized data from previous experiments manipulating biodiversity and/or nutrient fertilization in eelgrass systems (Table S3). We focused on experiments that manipulated eelgrass genotypic richness, species richness of eelgrass-associated mesograzers, and/or experiments that measured responses of both algae and grazers to nutrient fertilization (references are cited in Table S3). For comparison on a common scale, all values were standardized to a range of zero to one by dividing each value by the highest value within that study.

RESULTS

The coordinated experiment revealed stronger top-down control of microalgae by grazers than bottom-up control by fertilization, on average, across the geographic range of eelgrass (Fig. 1). This pattern of top-down control was also supported by path analysis showing negative covariation of algae and grazer assemblages across the 15 sites. Path analyses integrating the experimental (within-site) and observational (cross-site) data produced two very similar models, which compared with the other 12 models examined, had a combined AIC weight of 0.98 (Models 9 and10, Table 1, Figure 2b). Two additional models estimated to test the a priori hypotheses of interactions of grazer deterrent with temperature (model 11) and grazer richness (model 13) had poorer overall fit (each with AIC weight of 0.07) but significant interaction paths (Table S2b). All four models included a highly significant negative effect of deterrent on biomass of grazing crustaceans, and a concomitant positive, albeit weak effect on epiphytic

microalgae (Fig. 1b, Table S2a,b). In contrast, none of the best models included a significant effect of experimental fertilization on algal biomass (Fig. 1b, Table S2b), and removal of paths representing fertilization effects improved model fit (i.e., AIC, compare model 3 with 2, Table 1). The importance of top-down control of algae by mesograzers is further emphasized by the model (4) that omitted the path between deterrent and grazer biomass, which had by far the least explanatory power of any candidate model (delta AIC = 157). The absence of experimental fertilization effects on microalgae or grazers raises the question whether the treatment was adequate to raise local nutrient levels. A separate analysis suggests that it was, since experimental fertilization did increase average eelgrass nitrogen content ($P = 0.044$, Fig. S2)

The dominance of top-down versus bottom-up control of algae in the experiments was mirrored by patterns in the observational data, i.e., cross-site relationships among nutrient status, grazers, and algal biomass. Specifically, none of the best path models showed a significant path from eelgrass nitrogen content (a proxy for site nutrient status) to algal biomass, whereas all included a strong effect of this proxy for nutrient status on crustacean grazer biomass (Fig. 2b, Table S2). The latter result confirms that, as in the experiment, variation in site nutrient status was ecologically significant but did not affect local algal biomass, presumably due to efficient trophic transfer of the nitrogen through algae to grazers as a result of strong top-down control by grazers.

We tested four *a priori* hypotheses to explain geographic variation in top-down control by fitting interactions between grazing impact (estimated as deterrent effect on algae) and environmental factors or biodiversity. Model 11, testing the role of temperature on grazing, revealed a strong positive interactive effect of temperature and deterrent on algae ($P < 0.001$), suggesting that top-down control generally strengthened in warmer areas (Fig. 1c). Model 13,

testing how grazing varied with grazer richness, showed a tendency for higher grazing impact (i.e., more positive deterrent effect on algae) at sites with higher grazer richness ($P = 0.051$, Fig. 1c). Although the paths corresponding to these *a priori* hypotheses were supported, the models had considerably lower AIC support (AIC weight = 0.07 for each) compared with the best model 10. Model 14's hypothesis that more diverse grazer assemblages confer resistance to nutrient loading was not supported, as indicated by the poor fit of this model, which included a negative interaction between grazer richness and fertilization effect on algae (Table 1, Fig. 1c). Model 12, testing whether grazing impact declined with latitude, independent of covarying gradients in temperature, was also poorly supported (Table 1, Fig. 1c), suggesting that temperature alone is a better predictor of grazing impact than latitude.

Analysis of cross-site patterns indicated that top-down impacts on algal biomass were mediated primarily by variation in grazer species richness rather than by variation in grazer biomass (Fig. 2b). Indeed the best-supported path models revealed, surprisingly, that eelgrass genotypic richness and grazer species richness were equally strong predictors of algal and grazer biomass as were among-site differences in resource (nitrogen) status. The biodiversity variables also were stronger predictors than variation across the global range in temperature or salinity, neither of which significantly affected grazer or algal biomass (Fig. 2b, Table S2). A model (5) that omitted effects of biodiversity (grazer species richness and eelgrass genotypic richness) on algal and grazer biomass had an AIC weight near zero (Table 1). Both grazer species richness and eelgrass genotypic richness declined with increasing latitude, and the models show that these large-scale gradients in biodiversity were the dominant direct influences on grazer and algal biomass (Fig. 2b). Sites with more genotypically diverse eelgrass supported higher biomass of crustacean grazers (Fig. 2b, 3a) and sites with higher grazer species richness supported lower

algal biomass (Fig. 2b, 3b); indeed natural variation in grazer richness had an order of magnitude stronger effect on algae in the model than did variation in grazer biomass (Table S2). Finally, sites with more grazer species supported higher biomass of gastropod grazers (Fig. 2b).

When effects of biodiversity were isolated by controlling statistically for other factors using partial correlations, the strong influences of biodiversity emerging from cross-site comparison (100s to 1000s of km) in our analysis were strikingly similar to patterns documented in prior experiments comparing mesocosm or replicate field plots separated by meters to 10s of meters (Fig. 3a,b, Table S3). These cross-site comparisons also corroborate prior results showing that experimental fertilization tends to raise biomass of crustacean grazers but not of algae (Fig. 3c,d).

DISCUSSION

Our coordinated experiment showed that removing grazers had a considerably stronger average effect on microalgal biomass than did local addition of nutrients at sites across the global range of eelgrass ecosystems, and that the importance of grazers in controlling algae increased with temperature and with species richness of the grazer assemblage. Integrated analysis of experimental and observational data via path analysis revealed two surprising results. First, the influence of cross-site variation in biodiversity (eelgrass genotypes and mesograzer species richness) and environmental drivers generally overwhelmed experimental effects of grazer and nutrient manipulation across the global range. Eelgrass and grazer richness were as strong predictors of algal and grazer biomass as differences in resource (nitrogen) status, and were stronger predictors than temperature or salinity, neither of which affected biomass in the best models. Path analysis showed that latitude explained variation in biodiversity, which in turn

strongly influenced both plant and animal biomass. In contrast, the major environmental factor varying with latitude, temperature, had no main effect on algal and grazer biomass after controlling for grazer species richness (Fig. 2b, Table S2) although top-down control tended to be stronger at warmer sites (Fig. 1c).

Second, the strong influences of biodiversity in our global analysis were strikingly similar to results of prior small-scale experiments. This concordance addresses the common concern that biodiversity effects on functioning documented in experiments may derive from simplified conditions of questionable relevance to nature (Srivastava & Vellend 2005). Several points illustrate the match between our observational data, comparing sites around the globe, and prior small-scale experimental data. First, the higher crustacean biomass at sites with more genotypically diverse eelgrass (Fig. 3a) matches previous experimental findings of increased invertebrate abundance in genotypically diverse eelgrass (Fig. 3e). This can be due to greater eelgrass biomass in genotypically diverse plots (Hughes & Stachowicz 2004; Reusch *et al.* 2005), or because growth of individual herbivores is greater on diets of mixed genotypic composition (Tomas *et al.* 2011). Second, the lower algal biomass at sites with more grazer species (Fig. 3b) mirrors the stronger control of algal growth by diverse grazers in numerous eelgrass mesocosm experiments (Fig. 3f). Although we were unable to collect data on change in eelgrass biomass in our experiments, small scale experiments find that stronger grazing on algae does increase eelgrass growth (Hughes *et al.* 2004; Reynolds *et al.* 2014).

This concordance of patterns from previous experiments and from our global comparative analysis also extended to bottom-up control of grazer production. Nutrient status, proxied by eelgrass tissue nitrogen content (Burkholder *et al.* 2007), was strongly positively related to crustacean biomass but unrelated to algal biomass in comparisons across sites (Fig. 2b,

3c,d). Similarly, in prior experiments, fertilization consistently increased the biomass of eelgrass-associated grazers, but not of epiphytic algae (Moksnes *et al.* 2008; Spivak *et al.* 2009; Baden *et al.* 2010) (Fig. 3g,h). This pattern of fertilization "bypassing" plants to increase grazers suggests that nitrogen enhances algal productivity, but that (initially at least) it is immediately grazed and channeled into increasing herbivore biomass. Why then did experimental fertilization not increase crustacean biomass in our plots (Fig. 1b,c, Fig. 2b)? The most likely explanation is that these epifaunal crustaceans are sufficiently mobile that they dispersed from the small plots or were consumed by predators, resulting in no net accumulation of grazer biomass. In contrast, previous experiments used cages or mesocosms, which prevent the predation or export of nutrient-stimulated grazer production. Our results thus reinforce prior findings that moderate nutrient loading often fails to stimulate algal blooms when natural grazing pressure is maintained (Heck & Valentine 2007; Eriksson *et al.* 2009; Hughes *et al.* 2013) and that intact food webs may provide resistance against eutrophication in seagrass ecosystems.

The effects of biodiversity in our global analyses derive from comparisons among sites differing naturally in eelgrass and grazer richness, raising the possibility that these effects might not be causal. We consider this unlikely for several reasons. First, our results corroborate theory predicting that higher grazer richness both depresses producer biomass and increases grazer biomass (Holt & Loreau 2002). Second, the patterns we observed match those documented at smaller scales in multiple controlled experiments in eelgrass (Fig. 3, Table S3) and other systems (Cardinale *et al.* 2012). Third, the mechanisms involved should apply across scales: control of algae by diverse grazers involves both differences in feeding biology among species (complementarity) and dominance of strong grazer species (sampling effects) (Duffy *et al.* 2003; Best *et al.* 2013), both of which seem equally likely to operate at regional and small plot scales.

Fourth, the effects of biodiversity in our study emerged from path models that controlled statistically for many potentially confounding influences including latitude, temperature, and nutrients. Finally, a mechanistic link between grazer richness and top-down control is also consistent with the positive, albeit marginally significant, interactive effect of deterrent and grazer richness on algae (model 13, $P = 0.051$), indicating that grazing pressure tends to be stronger—and grazer deterrent is thus more effective at stimulating algal accumulation—at sites with high grazer richness.

It is also conceivable that the direction of causality might be opposite that of our predictions in some cases. Specifically, environmental conditions promoting higher abundance might also accumulate more species (rather than vice versa as we have hypothesized) because a larger random sample of individuals tends to contain more species from a given source pool. To test for such artifacts we employed a fixed-coverage subsampling approach (Chao & Jost 2012). This analysis revealed that observed richness ranged from 89-100% completeness across our 15 sites, with a mean of 98%, strongly suggesting that our estimates of grazer richness are robust and comparable among sites. Moreover, such sampling effects could not easily explain the negative relationship, across trophic levels, of grazer richness with algal biomass (Fig. 3), i.e., it is unclear how low algal biomass could promote high grazer richness via sampling effects. Nor is high grazer biomass likely to enhance eelgrass genotypic richness since most herbivores in our system do not consume eelgrass. Instead our results seem best explained by higher richness promoting more effective resource use as predicted by theory (Holt & Loreau 2002) and demonstrated in numerous experiments (Cardinale *et al.* 2012).

Our results have implications for coastal management, which has focused extensively on the threat posed by nutrient loading and associated algal blooms to seagrasses and other coastal

habitats (Cloern 2001; Burkholder *et al.* 2007). Surprisingly, we found little evidence that local fertilization increased epiphytic microalgae (Fig. 1b), and sites with higher nitrogen availability supported more grazing crustaceans but not more algae (Fig. 2b, 3c,d), despite wide variation in nutrient status (Fig. S1). Thus, while sustained nutrient loading can clearly have detrimental impacts on submerged vegetation (Cloern 2001; Burkholder *et al.* 2007), both our experimental results and our cross-site comparisons bolster the growing consensus that food web perturbations can have comparable or even greater impacts on estuarine producer biomass compared with moderate nutrient increases (Heck & Valentine 2007; Eriksson *et al.* 2009; Baden *et al.* 2010; Hughes *et al.* 2013).

To inform management effectively in an era of global change, ecology must move beyond demonstrating which processes occur to quantifying their importance and interactions under ambient conditions. Our comparative-experimental analysis shows that biodiversity is a strong predictor of fundamental ecosystem processes, including producer and consumer biomass accumulation, in naturally complex field ecosystems on a global scale and is comparable in importance to large-scale gradients in temperature, salinity, and nutrients. Moreover, these processes appear related to biodiversity at both genetic and species levels. The qualitative concordance of our results, and other recent large-scale observations (Frank *et al.* 2007; Paquette & Messier 2010; Mora *et al.* 2011; Maestre *et al.* 2012; Gamfeldt *et al.* 2013), with results of small-scale experiments supports the emerging conclusion that biodiversity is a fundamental controller of how ecosystems work, and is of comparable quantitative importance to major environmental drivers of metabolism, organismal fitness, and ecosystem processes (Cardinale *et al.* 2012; Hooper *et al.* 2012).

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Figure 1. Effects of experimental fertilization, grazer reduction, and their interactions with environment and biodiversity across the range of eelgrass. (a) Map of the 15 *Zostera* Experimental Network sites, with blue and green site codes (Table S1) identifying Atlantic (plus Baltic) and Pacific sites, respectively. (b) Mean (± s.e.m.) effects of grazer deterrent and fertilization on log biomasses of crustacean and gastropod grazers and epiphytic microalgae, estimated as partial regression coefficients from the full path model (1). (c) Influence of cross-site variation in temperature and grazer richness on grazing impact (Deterrent effect), estimated from interaction terms in SEM models 11 and 13 (Tables 1, S2b, Figure S3).

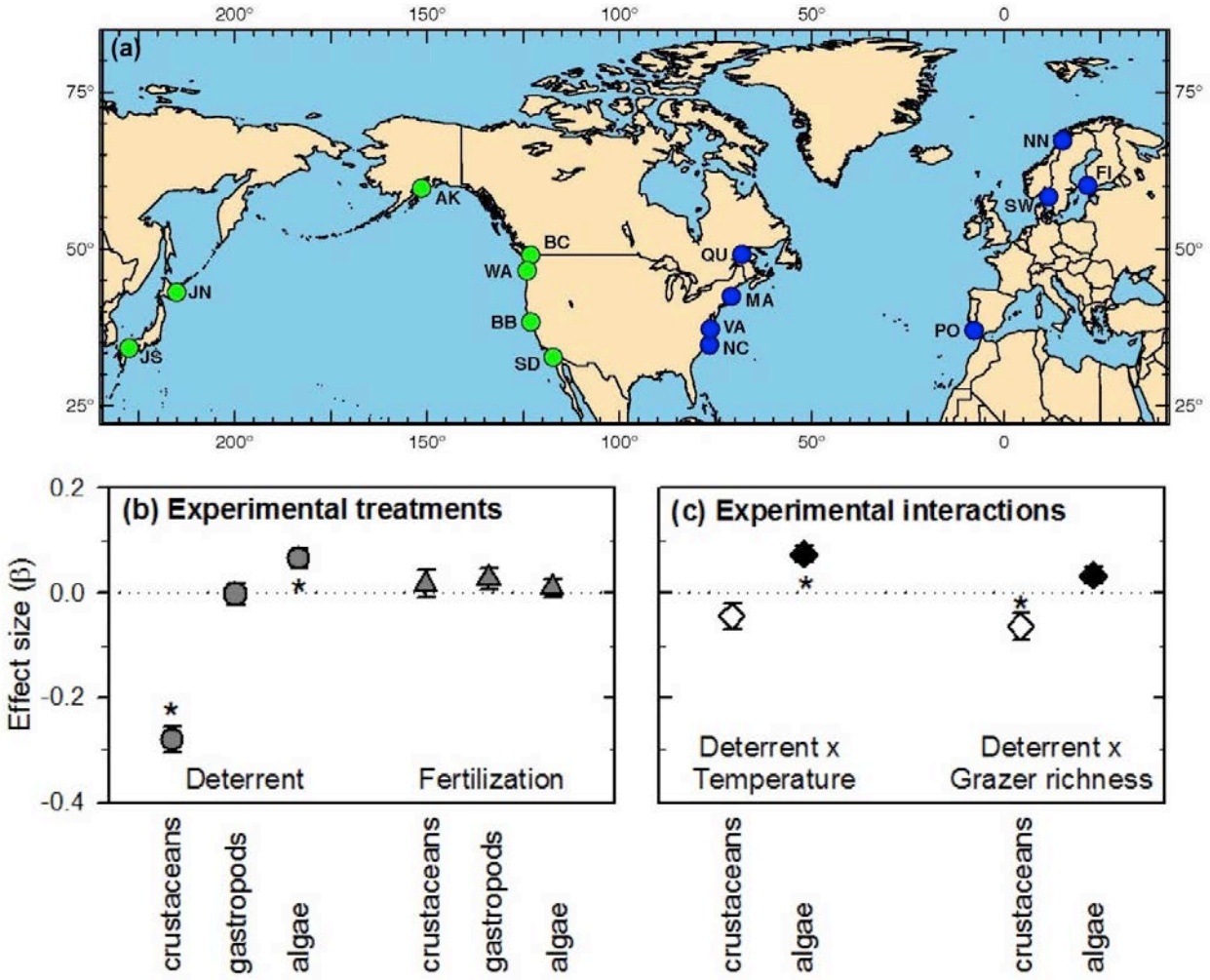

Figure 2. Path analysis of combined experimental and regional controls on grazer and algal biomass across the range of eelgrass. (a) Schematic representation of variables included in the model: those measured at site and plot levels are shown in in lower white and upper gray sections of panel, respectively. Experimental treatments are black. Biomasses of crustacean (Crust) and gastropod (Gast) grazers and algae were modeled as a function of temperature (Temp), salinity, site nutrient status (% N), latitude, ocean (Atlantic vs. Pacific), and richness of grazer species and eelgrass genotypes. (b) Best model 10 (AIC weight = 0.61, Table 1). Thickness of black (positive) and red (negative) paths is proportional to range-standardized path coefficient. Marginal R^2 values are shown for endogenous variables. The double-headed, dashed arrow represents a correlated error rather than a hypothesized directed causal path.

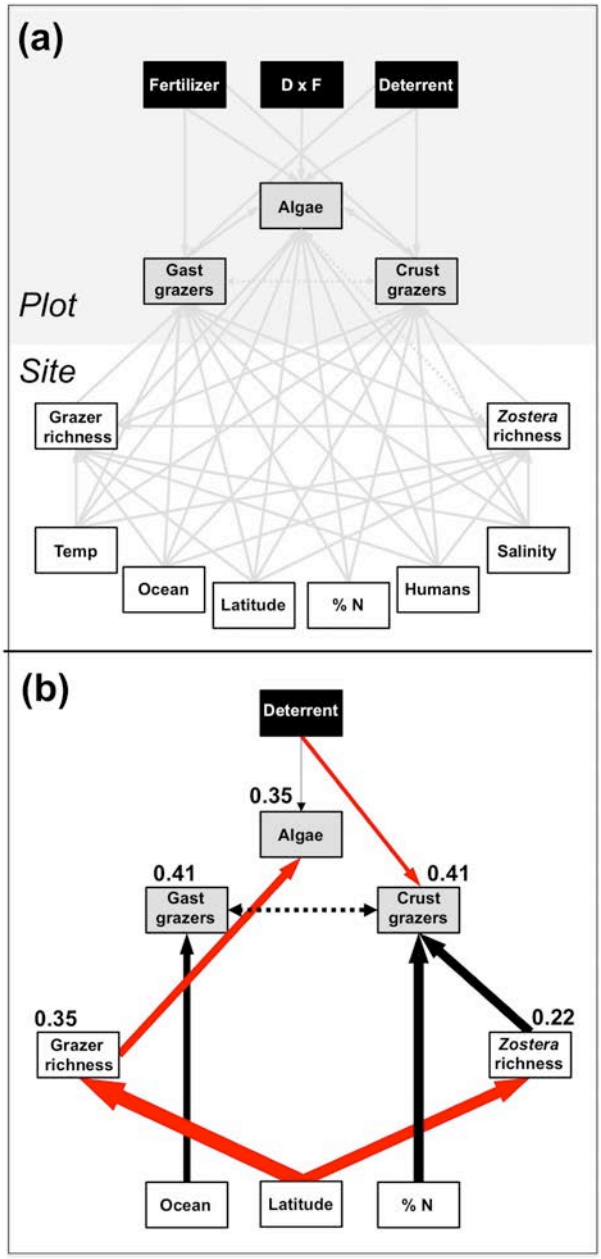

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Figure 3. Predictors of algal and grazer biomass in global comparisons (this study) and prior plot- or mesocosm-scale experiments. (a-d) Partial correlations from the best SEM (model 10, Fig. 2b, Table S2), i.e. influence of a predictor when other variables ("others") are controlled statistically. Gray symbols denote values (model residuals) for individual plots, larger symbols are site means. (e-h) Results of prior experiments. Values are standardized (see Methods) and bars show mean values across the studies summarized in Table S3 (L=low, H=high, A=ambient, F=fertilized).

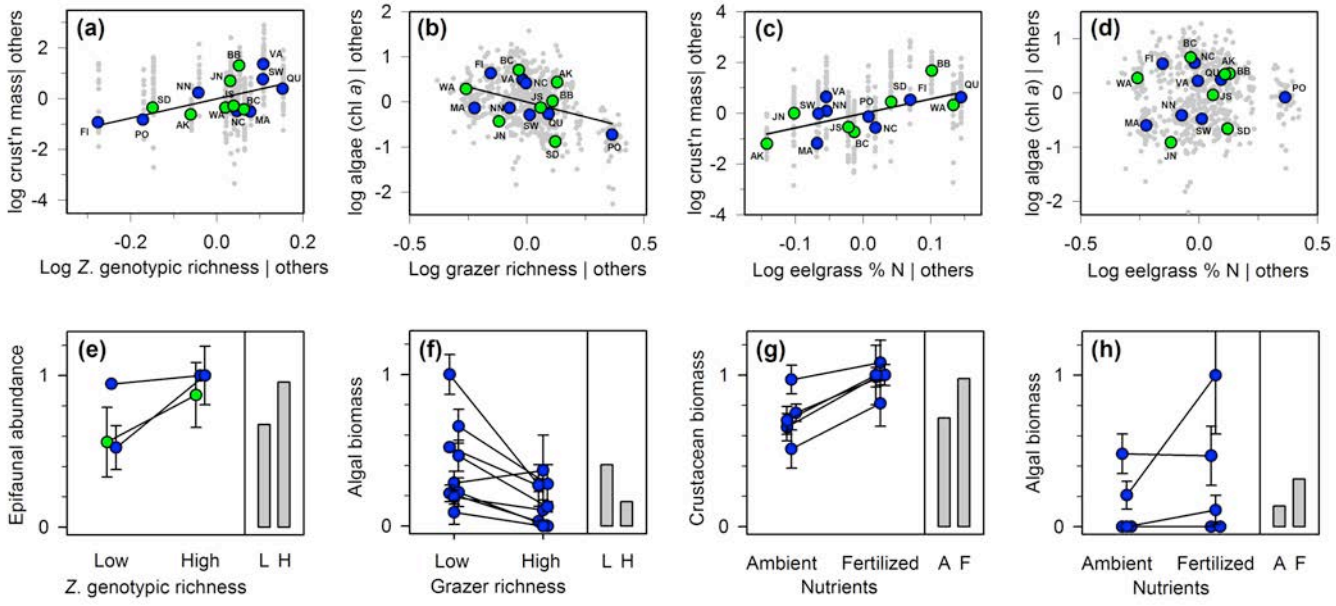

Table 1. Candidate structural equation models (see Figure S3 for structures). Fit was determined using D-separation tests. Models were compared via the Akaike Information Criterion (AIC), estimated from D-separation tests (Shipley 2013). Delta AIC is the difference in AIC score relative to the model with the lowest value (most parsimonious model) and AIC Weight (Wt) is the relative support for the model. Models in bold had AIC Weight $\geq$ 0.10 (see Materials and Methods for details).

Model	Explanation	Fit (P)	K	AIC	Delta AIC	AIC Wt
1	full model	0.83	60	122.8	47.3	0.00
2	Latitude model: grazer and eelgrass richness affected only by latitude	1.00	50	106.4	30.8	0.00
3	Latitude model minus experimental fertilization effects	0.96	44	101.4	25.8	0.00
4	Latitude model minus experimental grazer deterrent effects	0.00	44	233.2	157.6	0.00
5	Latitude model minus biodiversity effects on grazer or algal biomass	0.82	44	110.8	35.2	0.00
6	Latitude model minus salinity effects	1.00	47	102.8	27.2	0.00
7	Latitude model minus human population density effects	0.97	47	99.7	24.1	0.00
8	Latitude model minus fertilizer, salinity, human density effects	0.98	38	82.5	6.9	0.02
9	**Model 8 minus latitude effect on grazer or algal biomass**	**0.98**	**35**	**77.6**	**2.0**	**0.22**
10	**Pruned model: Model 9 minus deterrent effect on gastropod biomass**	**0.98**	**34**	**75.6**	**0.0**	**0.61**
11	**Pruned mode plus interaction: deterrent*temperature**	**0.99**	**36**	**79.8**	**4.2**	**0.07**
12	Pruned model plus interaction: deterrent*latitude	0.98	38	93.7	18.1	0.00
13	**Pruned mode plus interaction: deterrent*grazer richness**	**0.99**	**36**	**80.0**	**4.4**	**0.07**
14	Pruned mode plus interaction: fertilization*grazer richness	0.94	37	83.9	8.3	0.01

Table S1. Codes and locations of sites.

Code	Site	Principal Investigator	Latitude	Longitude
AK	Kachemak Bay, Alaska, USA	Iken	59.648	-151.436
BB	Bodega Bay, California, USA	Stachowicz	38.317	-123.033
BC	Vancouver, British Columbia, Canada	O'Connor	49.000	-123.100
FI	Ängsö Island, Finland	Boström	60.100	21.700
JN	Akkeshi-Ko Estuary, Hokkaido, Japan	Nakaoka	43.060	144.911
JS	Akiwan Bay, Hiroshima, Japan	Hori	34.178	132.550
MA	Nahant, Massachusetts, USA	Douglass	42.426	-70.919
NC	Beaufort, North Carolina, USA	Reynolds, Sotka	34.683	-76.600
NN	Misvaerfjord, Bodø, Norway	Olsen, Eriksson, Horeau	67.233	15.200
PO	Ria Formosa Lagoon, Portugal	Engelen	36.997	-7.829
QU	Pointe-Lebel, Quebec, Canada	Cusson	49.113	68.179
SD	San Diego Bay, CA, USA	Hovel	32.714	-117.226
SW	Gullmar Fjord, Sweden	Moksnes, Fredriksen	58.314	11.548
VA	Goodwin Islands, Gloucester Point, Virginia	Duffy, Reynolds	37.217	-76.383
WA	Willapa Bay, Washington, USA	Ruesink	46.500	-124.000

Table S2a. Parameter estimates (β) from the best-supported structural equation model (model 10, Figure 2b). Double-headed arrows indicate correlated errors, rather than directed paths, and are shown in the column for unstandardized estimate.

Response[1]		Predictor	unstandardized β		range-standardized β	
			estimate	SE	estimate	P
Log algal biomass	<--	Ocean	-0.340	0.416	-0.083	0.435
Log algal biomass	<--	Ave.Temperature	0.060	0.038	0.294	0.154
Log algal biomass	<--	log eelgrass % N	2.465	1.884	0.203	0.223
Log algal biomass	**<--**	**deterrent**	**0.098**	**0.027**	**0.024**	**0.000**
Log algal biomass	<--	Log crustacean biomass	-0.012	0.015	-0.012	0.446
Log algal biomass	<--	Log gastropod biomass	0.024	0.019	0.032	0.223
Log algal biomass	**<--**	**Log mesograzer richness**	**-1.945**	**0.852**	**-0.349**	**0.048**
Log algal biomass	<--	Log eelgrass genotypic richness	2.830	1.355	0.323	0.066
Log crustacean biomass	<--	Ocean	0.076	0.511	0.017	0.885
Log crustacean biomass	<--	Ave.Temperature	0.058	0.047	0.268	0.249
Log crustacean biomass	**<--**	**log eelgrass % N**	**5.706**	**2.318**	**0.439**	**0.036**
Log crustacean biomass	**<--**	**deterrent**	**-0.769**	**0.069**	**-0.176**	**0.000**
Log crustacean biomass	<--	Log mesograzer richness	-0.788	1.047	-0.132	0.471
Log crustacean biomass	**<--**	**Log eelgrass genotypic richness**	**3.803**	**1.667**	**0.406**	**0.049**
Log gastropod biomass	**<--**	**Ocean**	**-1.700**	**0.589**	**-0.311**	**0.018**
Log gastropod biomass	<--	Ave.Temperature	-0.037	0.054	-0.136	0.514
Log gastropod biomass	<--	log eelgrass % N	2.217	2.675	0.136	0.429
Log gastropod biomass	<--	Log mesograzer richness	2.580	1.209	0.346	0.062
Log gastropod biomass	<--	Log eelgrass genotypic richness	-1.819	1.924	-0.155	0.369
Log gastropod biomass	**<-->**	**Log crustacean biomass**	**0.201**	**NA**	**NA**	**<0.001**
Log mesograzer richness	**<--**	**Latitude**	**-0.014**	**0.004**	**-0.660**	**0.006**
Log eelgrass genotypic richnes	**<--**	**Latitude**	**-0.007**	**0.003**	**-0.503**	**0.041**

Table S2b. Parameter estimates for paths in models 9, 11, and 13 (Figure S3). Coefficients for other paths in these models are are identical to corresponding paths in model 10.

Response [1]		Predictor	unstandardized β		range-standardized β	
			estimate	SE	estimate	P
Model 11						
Log algal biomass	<--	Ocean	-0.341	0.416	-0.084	0.433
Log algal biomass	<--	Ave.Temperature	0.049	0.038	0.241	0.235
Log algal biomass	<--	log eelgrass % N	2.435	1.885	0.200	0.229
Log algal biomass	**<--**	**deterrent**	**-0.287**	**0.090**	**-0.070**	**0.002**
Log algal biomass	<--	Log crustacean biomass	-0.007	0.015	-0.007	0.665
Log algal biomass	<--	Log gastropod biomass	0.023	0.019	0.030	0.236
Log algal biomass	**<--**	**Log mesograzer richness**	**-1.938**	**0.852**	**-0.348**	**0.049**
Log algal biomass	<--	Log eelgrass genotypic richness	2.808	1.356	0.320	0.068
Log algal biomass	**<--**	**deterrent x temperature**	**0.021**	**0.005**	**0.102**	**0.000**
Log crustacean biomass	<--	deterrent x temperature	-0.023	0.013	-0.108	0.071
Model 13						
Log algal biomass	**<--**	Ocean	-0.339	0.416	-0.083	0.436
Log algal biomass	<--	Ave.Temperature	0.060	0.038	0.293	0.155
Log algal biomass	<--	log eelgrass % N	2.448	1.885	0.201	0.226
Log algal biomass	<--	deterrent	-0.134	0.122	0.033	0.273
Log algal biomass	<--	Log crustacean biomass	-0.009	0.015	-0.009	0.577
Log algal biomass	**<--**	Log gastropod biomass	0.024	0.019	0.032	0.217
Log algal biomass	**<--**	**Log mesograzer richness**	**-2.053**	**0.854**	**-0.368**	**0.040**
Log algal biomass	<--	Log eelgrass genotypic richness	2.817	1.356	0.322	0.068
Log algal biomass	<--	deterrent x mesograzer richness	0.220	0.113	0.040	0.051
Log crustacean biomass	**<--**	**deterrent x mesograzer richness**	**-0.802**	**0.310**	**-0.135**	**0.010**

[1] Units: Log algal biomass (chl a cm eelgrass^{-1}); Log crustacean and gastropod biomass (g eelgrass^{-1}); Log mesograzer richness (number of species site^{-1}); Log eelgrass genotypic richness (R)

Table S3. Summary of results from prior experiments manipulating diversity or nutrient loading in eelgrass systems shown in Figure 3.

MS Figure	Reference	Manipulated factor	Response variable	Source figure	low treatment response mean	low treatment response SEM	high treatment response mean	high treatment response SEM
3e	Hughes & Stachowicz 2004	eelgrass genotypic richness	epifaunal abundance	author	0.561	0.230	0.872	0.214
3e	Reusch et al 2005	eelgrass genotypic richness	epifaunal abundance	2d	0.945	0.010	1.000	0.010
3e	Reynolds et al 2012	eelgrass genetic diversity	epifaunal abundance	3c	0.525	0.145	1.000	0.193
3f	Duffy et al 2001	herbivore species richness	epiphytic algal biomass	1a	0.285	0.077	0.368	0.232
3f	Duffy et al 2003	herbivore species richness	total algal biomass	author	0.216	0.056	0.031	0.015
3f	Duffy et al 2005	herbivore species richness	total algal biomass	author	0.224	0.094	0.001	0.001
3f	France and Duffy 2006a	herbivore species richness	total algal biomass	3b	0.464	0.101	0.127	0.034
3f	France and Duffy 2006b	herbivore species richness	total algal biomass	3b	1.000	0.132	0.270	0.043
3f	Spivak et al 2009	herbivore species richness	epiphytic algal biomass	1c	0.091	0.079	0.000	0.000
3f	Blake and Duffy 2010	herbivore species richness	macroalgal biomass	2a	0.744	0.035	0.293	0.150
3f	Blake and Duffy 2012	herbivore species richness	macroalgal biomass	2b	0.659	0.112	0.278	0.128
3f	Eklöf et al 2012	herbivore species richness	macroalgal biomass	5	0.191	0.046	0.106	0.066
3g	Moksnes et al 2008	nutrients	amphipod abundance	1b	0.701	0.091	1.000	0.195
3g	Spivak et al 2009	nutrients	herbivore biomass	2a	0.512	0.126	0.813	0.151
3g	Baden et al 2010	nutrients	crustacean biomass	2	0.970	0.095	1.082	0.147
3g	Baden et al 2010	nutrients	crustacean biomass	2	0.751	0.056	1.000	0.069
3g	Baden et al 2010	nutrients	crustacean biomass	2	0.656	0.089	0.986	0.065
3h	Moksnes et al 2008	nutrients	macroalgae	1a	0.482	0.130	0.469	0.195
3h	Spivak et al 2009	nutrients	epiphytic algal biomass	1c	0.000	0.000	0.111	0.097
3h	Baden et al 2010	nutrients	algal biomass	2	0.208	0.092	1.000	0.387
3h	Baden et al 2010	nutrients	algal biomass	2	0.000	0.000	0.000	0.000
3h	Baden et al 2010	nutrients	algal biomass	2	0.000	0.000	0.000	0.000

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Table S3 (continued).

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Figure S1. Variation among sites in environmental and biological parameters. Histograms show the frequency of values across sites for each variable, with two-letter codes within histograms referring to sites listed in Table S1. Symbols at top of each panel show the median and interquartile range of values for Atlantic (blue) and Pacific (green) sites. Eelgrass %N, epiphytic algal (microalgal) biomass, and biomasses of crustacean and gastropod grazers are ambient values, i.e., means of samples from unmanipulated (control) plots at each site. Richness of eelgrass genotypes and grazer species represent site-level values and are summed across all plots at a site.

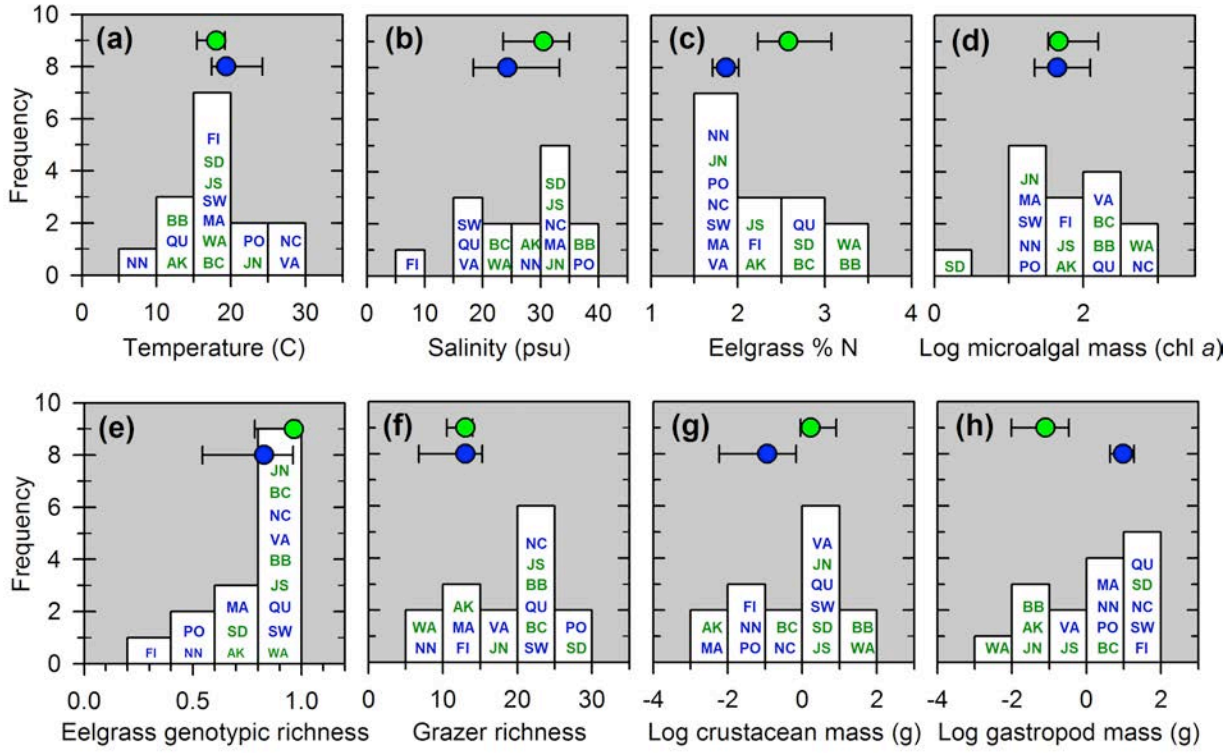

Figure S2. Effects of experimental grazer reduction (Deterrent), fertilization, and their interaction on biomass of grazers and epiphytic algae, and eelgrass leaf %N, at individual sites. Symbols show mean (± 1 s.e.m.) effects on biomasses of (a-c) crustacean grazers, (d-f) gastropod grazers, and (g-i) epiphytic microalgae (as chl *a*), and of (j-l) eelgrass leaf % N as coefficients from the linear models. Site codes and symbols as in Fig. 1. * P < 0.05 in 2-factor ANOVA estimated for that site.

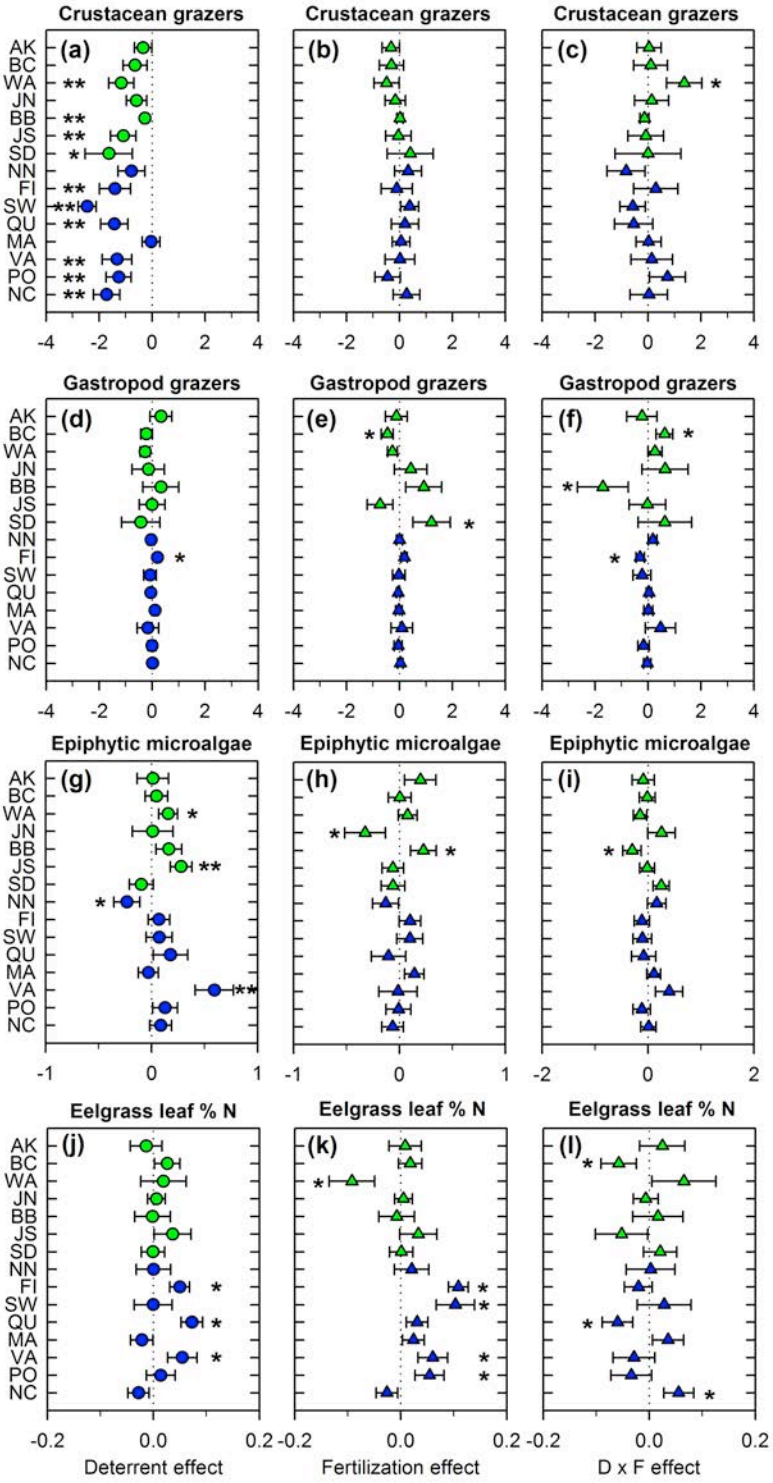

Figure S3 (part 1). Candidate structural equation models compared using AIC. See Materials and Methods for descriptions of models and Table 1 for model fits and AIC values.

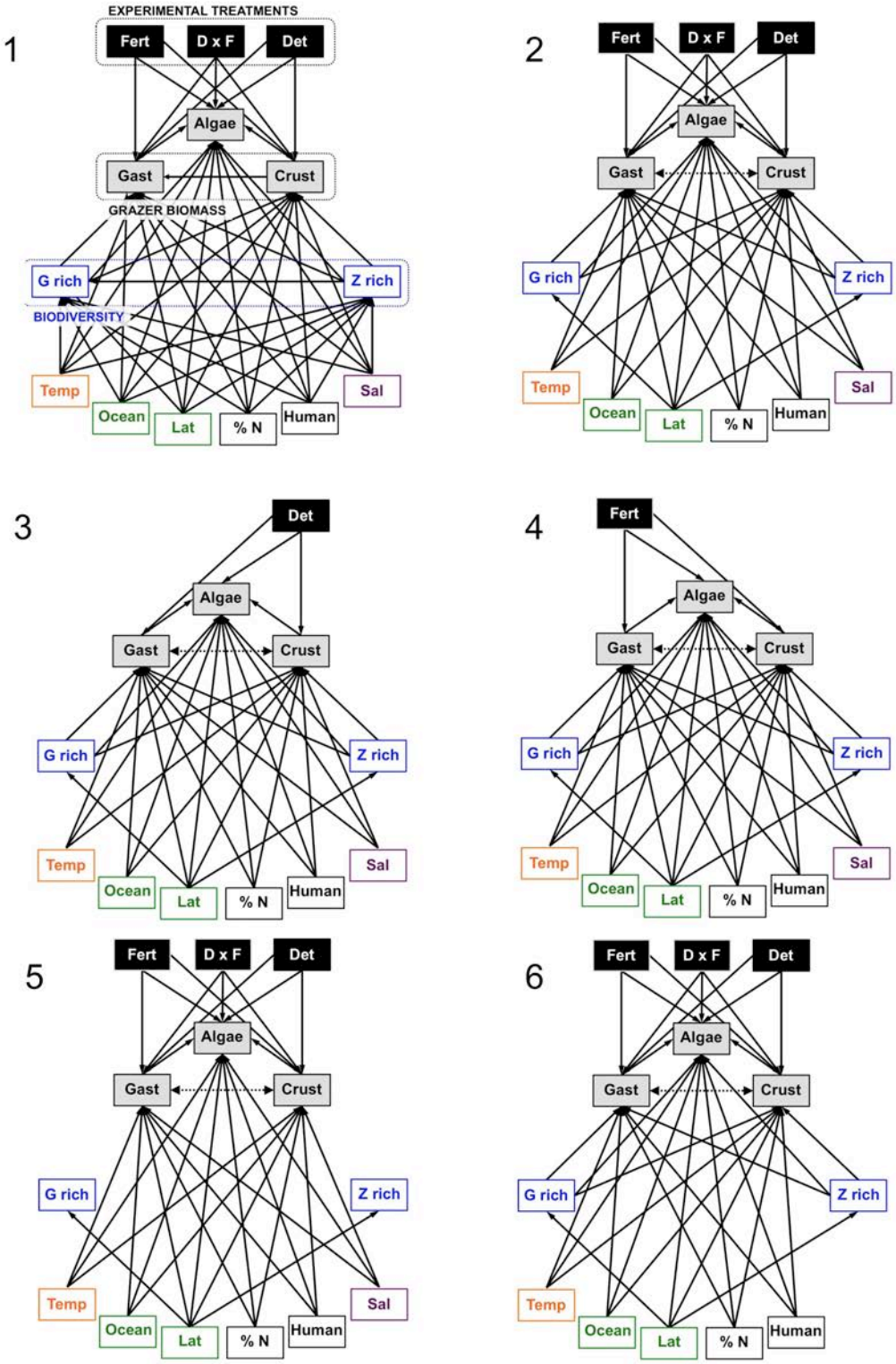

Figure S3 (part 2). Candidate structural equation models compared using AIC. See Materials and Methods for descriptions of models and Table 1 for model fits and AIC values.

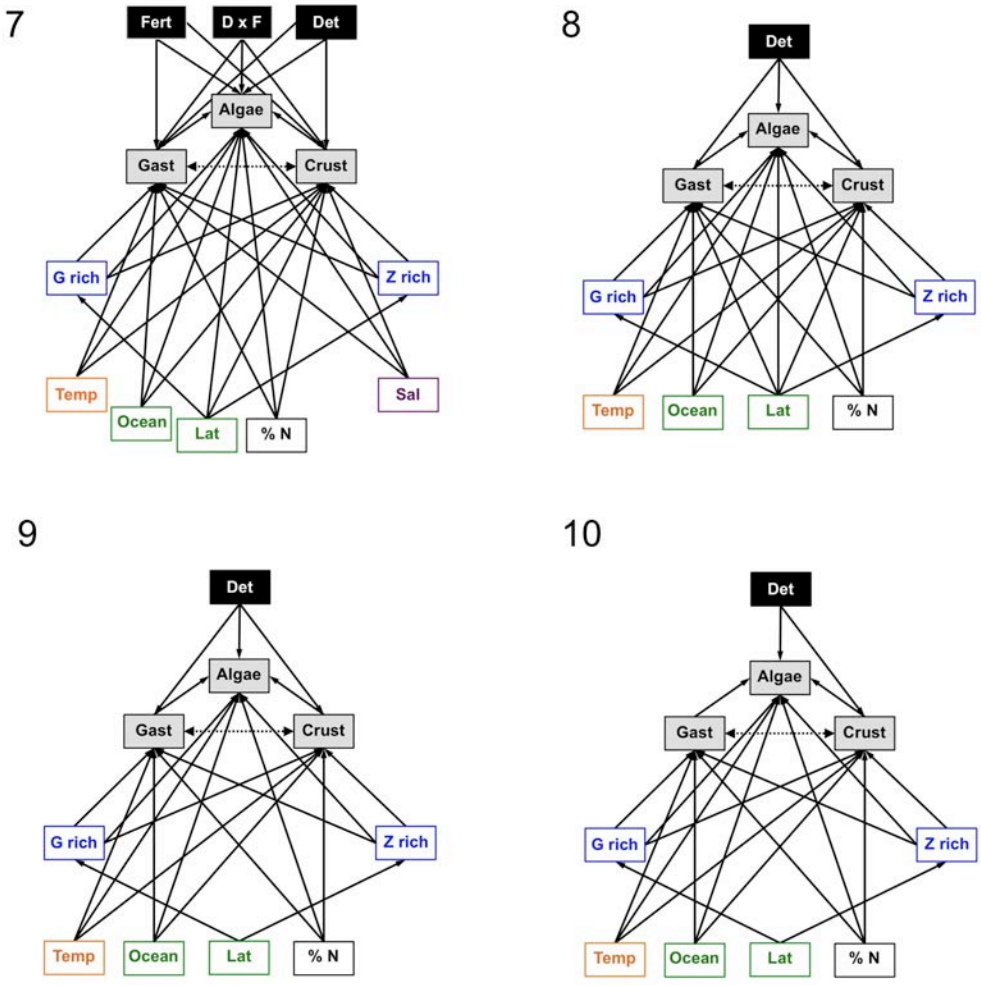

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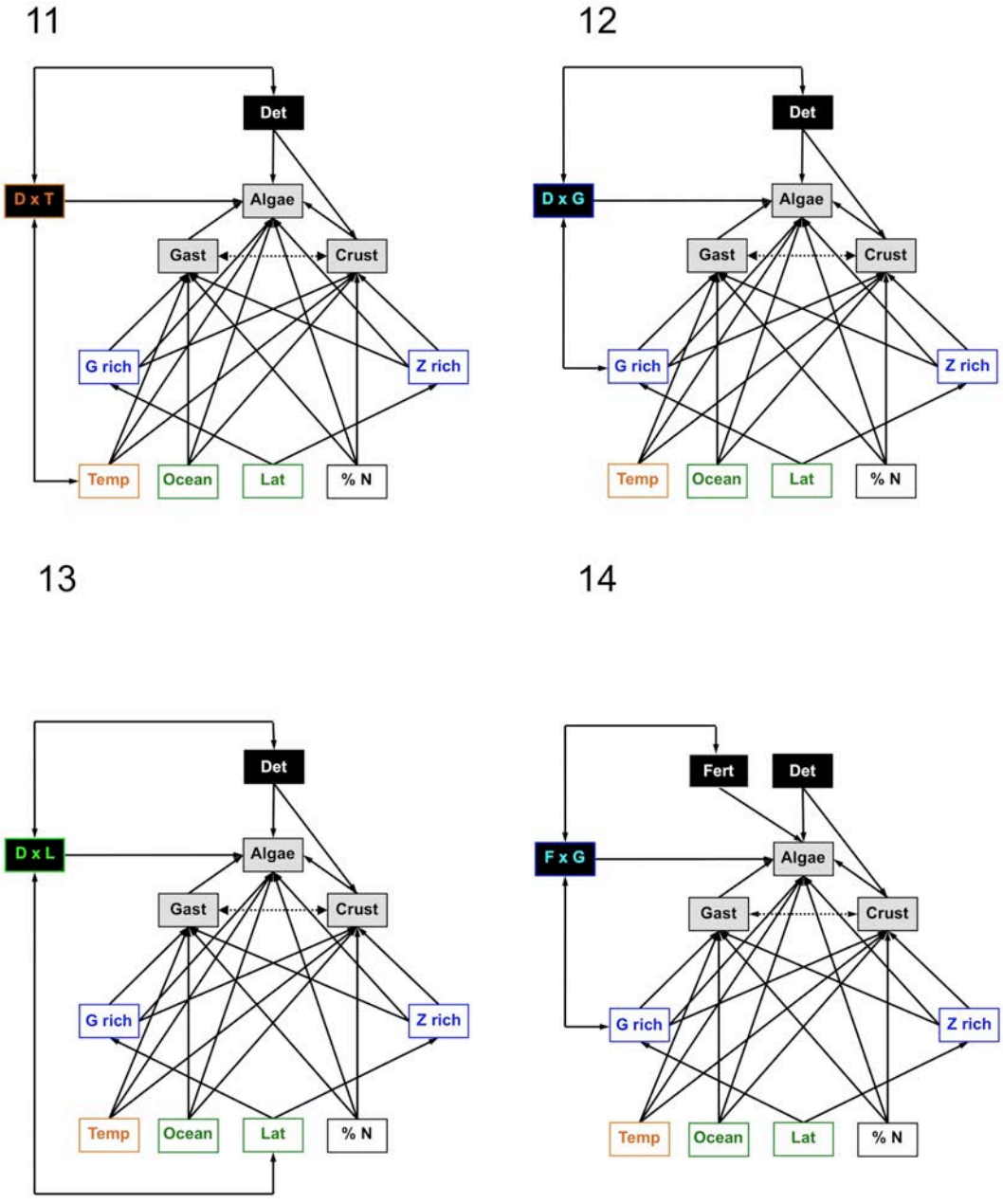
Figure S3 (part 3). Candidate structural equation models compared using AIC. See Materials and Methods for descriptions of models and Table 1 for model fits and AIC values.



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