

Unexpected resilience of a seagrass system exposed to global stressors

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Abstract

Despite a growing interest in identifying tipping points in response to environmental change, our understanding of the ecological mechanisms underlying nonlinear ecosystem dynamics is limited. Ecosystems governed by strong species interactions can provide important insight into how nonlinear relationships between organisms and their environment propagate through ecosystems, and the potential for environmentally mediated species interactions to drive or protect against sudden ecosystem shifts. Here, we experimentally determine the functional relationships (i.e., the shapes of the relationships between predictor and response variables) of a seagrass assemblage with well-defined species interactions to ocean acidification (enrichment of CO₂) in isolation and in combination with nutrient loading. We demonstrate that the effect of ocean acidification on grazer biomass (*Phyllaplysia taylori* and *Idotea resicata*) was quadratic, with the peak of grazer biomass at mid-pH levels. Algal grazing was negatively affected by nutrients, potentially due to low grazer affinity for macroalgae (*Ulva intestinalis*), as recruitment of both macroalgae and diatoms were favored in elevated nutrient conditions. This led to an exponential increase in macroalgal and epiphyte biomass with ocean acidification, regardless of nutrient concentration. When left unchecked, algae can cause declines in seagrass productivity and persistence through shading and competition. Despite quadratic and exponential functional relationships to stressors that could cause a nonlinear decrease in seagrass biomass, productivity of our model seagrass—the eelgrass (*Zostera marina*)—remained highly resilient to increasing acidification. These results suggest that important species interactions governing ecosystem dynamics may shift with environmental change, and ecosystem state may be decoupled from ecological responses at lower levels of organization.

KEYWORDS

ecological threshold, eutrophication, nutrient loading, ocean acidification, resistance, species interaction, tipping point, *Zostera*

1 | INTRODUCTION

Many biological processes exhibit nonlinear relationships with their environment (e.g., performance curves), where small changes in environmental drivers can cause large shifts in organismal performance and function (Huey & Stevenson, 1979; Zhu, Chiariello, Tobeck,

Fukami, & Field, 2016). If environment-organism relationships are nonlinear, the gradual nature of environmental change may give the appearance of resilience prior to an ecological regime shift, also known as an ecological threshold (Andersen, Carstensen, Hernández-García, & Duarte, 2009; Connell & Ghedini, 2015; Doak & Morris, 2010; Holling, 1973; Scheffer, Carpenter, Foley, Folke, & Walker,

2001). This can be important for management and conservation because it can be difficult to bring a system back to its original state if an alternative stable state exists once an ecological threshold is surpassed (Scheffer & Carpenter, 2003). To assess potential ecological thresholds, critical biological responses need to be tested across the full spectrum of environmental change. Most environmental change research still uses factorial designs that compare current and future scenarios (Alsterberg, Eklöf, Gamfeldt, Havenhand, & Sundbäck, 2013; Crain, Kroeker, & Halpern, 2008; Kroeker et al., 2013), which could fail to identify nonlinear dynamics.

Each component of the ecosystem may exhibit nonlinear responses to single and multiple stressors; however, it is unclear whether responses at lower levels of organization (e.g., organismal physiology or population) relate to the functional relationship at higher levels of organization (e.g., species interactions or ecosystem dynamics). Here, we use the term 'functional relationship' to describe the mathematical relationships between a predictor and response variable (e.g., quadratic vs. linear relationships). A nonlinear response in a strong biotic interaction could translate to an ecological threshold in ecosystem properties. Alternatively, nonlinear responses of populations with strong ecological linkages could be buffered by other species (e.g., functional redundancy, density compensation (Gonzalez & Loreau, 2009; Reich et al., 2012), or species interactions (Connell & Ghedini, 2015)), resulting in ecosystem stability despite ecological thresholds at higher levels of organization. Our empirical evidence of how nonlinear dynamics propagate through an ecosystem, however, is limited.

Here, we use an ecosystem with well-described species interactions governing community dynamics to examine the effects of two drivers of global change, operating on local (nutrient enrichment) and regional to global (ocean acidification) scales, on key components of the system. In temperate seagrass ecosystems, elevated nutrient loading can lead to an overgrowth of epiphytic or ephemeral algae and a decline in seagrass performance and abundance (Burkholder, Tomasko, & Touchette, 2007; Valiela et al., 1997), sometimes resulting in an ecological regime shift (Andersen et al., 2009) from seagrass to ephemeral macroalgae. However, some functionally important grazers (e.g., small crustaceans and snails) can limit competitive algae, especially algal epiphytes on seagrass, thereby facilitating the persistence or recovery of seagrass (Duffy et al., 2015; Hughes et al., 2013; Williams & Ruckelshaus, 1993). These mesograzers differ from macrograzers (i.e., turtles, dugongs, manatees, geese, fish, and sea urchins) because the latter can consume large quantities of seagrass (Burkholder, Heithaus, Fourqurean, Wirsing, & Dill, 2013; Prado, Romero, & Alcoverro, 2010). Enrichment of CO₂ and bicarbonate (a product of ocean acidification) can increase productivity in both non-calcareous algae (Campbell & Fourqurean, 2014; Cornwall et al., 2012) and seagrasses (Zimmerman, Hill, & Gallegos, 2015); however, the balance between algal and seagrass productivity is likely to depend on the regulation of algae by grazers (Alsterberg et al., 2013) and their physiological responses to ocean acidification.

Using a mesocosm experiment, we aimed to: (1) elucidate the functional relationships between global change (ocean acidification)

and key components of a temperate seagrass ecosystem, which consists of the habitat-forming foundation species (*Zostera marina*) (Bruno, Stachowicz, & Bertness, 2003), their ephemeral algal competitors, and functionally important grazers that consume algal competitors (Hughes, Bando, Rodriguez, Williams, & Davis, 2004; Orth & Van Montfrans, 1984; Williams & Ruckelshaus, 1993), and (2) determine how an additional stressor (nutrient enrichment) affects the functional relationships between global change and the components of the ecosystem. A better understanding of the functional relationships between components of the ecosystem, ocean acidification, and nutrients can provide insight regarding potential resilience (linear increases in both eelgrass with decreasing pH), phase shifts (linear decreases in eelgrass and increases in algae with decreasing pH), or ecological thresholds (nonlinear changes with decreasing pH; Andersen et al., 2009), although we do not attempt to quantify particular ecological thresholds.

We hypothesized that grazer responses would have a quadratic relationship with pH (Figure 1a,b). With minor changes in seawater pH, we predict that grazer biomass could increase in response to increasing food availability when the conditions are not physiologically stressful. At more moderate decreases in pH, we predict there could be a window for trophic compensation, where increasing energetic demands associated with physiological responses (Melnzer et al., 2011) are compensated by increasing consumption (Connell & Ghedini, 2015). As acidification becomes more severe, we predicted a steep decrease in these responses would eventually occur due to the inability of grazers to adequately compensate for increasing physiological stress.

Second, we hypothesized that both macro- and epiphytic algal growth would be promoted with acidification due to an enrichment of CO₂ (Koch, Bowes, Ross, & Zhang, 2013), but that increased grazing by consumers would limit this effect at higher pH values (Figure 1c,d). At the point where grazers could no longer maintain homeostasis and trophic compensation fails (Connell & Ghedini, 2015), we predicted there would be an increase in epiphytic and macroalgal abundance with decreasing seawater pH. Thus, a steep decrease in grazing rates would trigger an exponential increase in algal biomass. At this level of organization (algal-grazer interactions), we predicted that nutrient additions would shift the exponential rise in biomass toward higher pH by promoting algal growth. Alternatively, decreasing pH could also decrease the calcareous algal epiphytes growing on seagrass leaves (Kroeker et al., 2013; Martin et al., 2008; Nogueira et al., 2016) and thus dampen the effect of increased nutrients and increased carbon dioxide on epiphyte biomass.

Third, we hypothesized that seagrass growth and survival would respond positively to ocean acidification until the negative effects of algal growth on seagrass performance outpace the benefits of acidification on seagrass physiology (Figure 1e). We predicted this would create a quadratic relationship between seagrass growth and seawater pH, with peak biomass occurring at mid pH levels. These predictions are based on an exponential response in algal biomass triggering a quadratic response in seagrass growth and survival. We

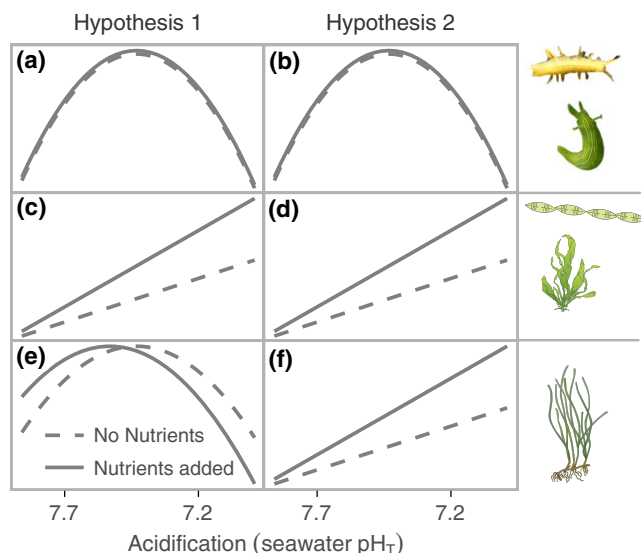


FIGURE 1 Hypotheses of seagrass community responses to changes in pH under nutrient enriched conditions for epiphyte grazers (a, b), noncalcareous algae (c, d), and eelgrass (e, f). In Hypothesis 1, we predicted that moderate decreases in pH could trigger trophic compensation and increased growth of grazers, before more severe decreases in pH become too stressful for compensation (a, b). The failure of trophic compensation could trigger an increase in algal biomass, as noncalcareous algae respond to the combined direct (CO_2 enrichment) and indirect (reduced grazing pressure) effects of ocean acidification (c, d). While seagrasses are expected to benefit from CO_2 enrichment, we hypothesized that the predicted increase in algal biomass with decreasing pH could cause decreases in seagrass biomass at lower pH levels, as the driver shifted from direct (CO_2 enrichment) to indirect effects (increased algal shading) with continued acidification (e). We further hypothesized that nutrient loading could shift both the intercept and slope of the functional relationships (see text for detailed hypotheses). Alternatively, we hypothesized that the indirect effects of altered species interactions with ocean acidification would not affect functional relationships (f). Vertical axes represent natural (e.g. for change in mass) or log-transformed (e.g. for length) response variable values. Image credits: Steve Lonhart (NOAA MBNMS), Tracy Saxby (IAN), and Brent Hughes

hypothesized that nutrient additions would cause seagrass to decline at a higher pH due to increased algal growth. Alternatively, we hypothesized that algae (Figure 1d) and seagrass (Figure 1f) could respond linearly if they can compensate for nonlinear dynamics of consumers (algal consumption) or exponential increases in competition (algal shading of eelgrass).

2 | MATERIALS AND METHODS

To test for functional relationships between environmental stressors and seagrass communities we used a mesocosm experiment that consisted of fourteen 200 L mesocosms at the University of California Santa Cruz's Long Marine Laboratory. Mesocosms were paired and randomly assigned one of seven treatments (pH 6.95–7.90), and

then each pair was randomly assigned two nutrient treatments (ambient and enriched; Fig. S1). To manipulate carbonate chemistry, header barrels receiving fresh sand-filtered seawater were mixed with acidified seawater using a custom-built system with pH sensors, controllers and relays. The pH in the header barrels was continuously measured by sensors, which were connected to controllers with preprogrammed set points (Honeywell Inc, Durafets and UDA controllers) to encapsulate current pH conditions in northeast Pacific estuaries (pH 7.4 – 8.0) and below (minimum pH = 7.0), which allowed us to better determine functional relationships and the potential for lower pH under future scenarios (Feely et al., 2010). When the pH in each header barrel reached values above the set pH point, the controllers triggered solenoid valves to release pre-equilibrated low pH water (pH 5.7–6.0) that was created by bubbling pure CO_2 in a separate recirculating seawater mesocosm. The treatment water from the seven independent header barrels then flowed to experimental mesocosms at consistent rates ($1.75 \text{ l/min} \pm 0.57 \text{ SD}$), where temperature, salinity, and light (water bottom = $802 \mu\text{m}^2 \text{ s} \pm 55 \text{ SD}$) were held nearly constant. Half of the mesocosms were enriched with 100 g Osmocote™ slow-release fertilizer (N:P:K = 19:6:12; Scotts, Columbus, OH, USA) to represent nutrient loads in local eelgrass beds (Hughes et al., 2013). We deployed nutrient containers at the bottom of nutrient enriched mesocosms in 50 ml centrifuge tubes drilled with holes to ensure release, yet preventing nutrients from flushing out of the mesocosm. We replenished nutrients every 7 days.

2.1 | Community interaction experiment

In late July 2015, we collected terminal shoots of the seagrass (*Zostera marina*, hereafter eelgrass) and rhizomes, and the two most conspicuous grazer species from Elkhorn Slough, CA (Hughes et al., 2013, 2016)—a gastropod seahare (*Phyllaplysia taylori*) that feeds exclusively on eelgrass algal epiphytes and a crustacean isopod (*Idotea resecata*) that primarily feeds on algae, but can consume eelgrass as well (Best & Stachowicz, 2012; Martinez-Crego, Arteaga, Tomas, & Santos, 2016; Reynolds, Carr, & Boyer, 2012). Seahares are exclusively found on eelgrass and consume epiphytic algae growing on eelgrass (Morris, Abbott, & Haderlie, 1980), whereas *I. resecata* is more of a generalist herbivore that consumes both epiphytic algae and nonepiphytic macroalgae (Hughes et al., 2016; Morris et al., 1980; Williams & Ruckelshaus, 1993), and can consume eelgrass when algae is limited (Duffy, Macdonald, Rhode, & Parker, 2001). Terminal shoots were collected because they enabled us to (1) better standardize shoot and rhizome biomass (terminal shoots tend to have the greatest biomass compared to lateral shoots), and (2) minimize the collection and transplantation of clones which can bias results. Collecting one shoot every 30 m^2 , also decreased the chances of selecting clones since most clones are found within 1 m in the field (Williams, 2001). Eelgrass shoots were cleaned and trimmed to 20 cm (to standardize eelgrass biomass and remove the majority of epiphytes), and rhizomes were cut to 7 cm. We transplanted shoots ($n = 25$) in five clusters of five shoots (mean total

eelgrass mass = 143.8 g FW/mesocosm \pm 7.4 g SD) in sterilized sand (10 cm deep). Each mesocosm was seeded with an equal density of grazers ($n = 5$ seahares and $n = 8$ isopods), with size classes (0.5–2.5 cm total length) typically observed in the field (Hughes et al., 2013). Grazer biomass was similar among the mesocosms (3.25 g FW/mesocosm \pm 0.40 g SD). Grazer sizes were evenly distributed across each mesocosm for seahares (1.89 cm \pm 0.29 SD) and isopods (1.75 cm \pm 0.12 SD). We gradually reduced the pH treatments for 4 days to the preprogrammed set points before adding nutrients.

We monitored the pH and temperature in header barrels every 15 s with in situ sensors (Honeywell Durafet) and twice daily in our mesocosms using handheld sensors (Yellow Springs Instruments). We collected discrete water samples in the mesocosms every other day and measured them for pH and total alkalinity. Spectrophotometric pH was measured with a spectrophotometer (Shimadzu, UV-1800) and total alkalinity was measured using closed cell titration on triplicate samples (Metrohm, 905 Titrando). All pH values are reported on the total scale. We collected weekly seawater samples and measured them for nitrate and phosphate (μM) using a Lachat Quickchem 8000 Flow Injection Analyzer. We scrubbed the mesocosm walls daily to remove fouling.

We harvested and weighed all eelgrass, algal epiphytes, macroalgae, grazers, and detritus 24 days after the initiation of the experiment. Algal epiphytes were wiped clean using predried and preweighed cosmetic facial pads (CVS™ Exfoliating Cotton Rounds). We counted live shoots and measured shoot and rhizome length and biomass. We measured and reweighed each grazer before returning seahares to the mesocosms for the grazing trial. All species were dried at 60°C to determine dry weight at the conclusion of the experiment.

2.2 | Algal recruitment and grazing experiment

Following the initial experiment, we ran a second experiment testing for grazer effects on algae recruitment using seahares that were exposed to global stressors from the initial 24-days experiment. Whereas the previous experiment allowed us to quantify changes in grazer and epiphyte biomass as emergent effects, this experiment allowed us to explicitly quantify grazing rates of individuals acclimated to the carbonate chemistry conditions. Petri dishes were placed in each mesocosm to culture epiphytic algae (i.e., diatoms and macroalgae) that naturally recruited from the seawater system. After 5 days, we dried the petri dishes (45°C) and determined starting biomass. We then placed a single *P. taylori* in a petri dish with seawater and algae cultured from its respective mesocosm. After six hours, the petri dishes were dried and weighed. We estimated the percent cover of diatoms and *Ulva* recruits using microscopy with a 49 point (7 \times 7) grid. We used the mean of three fields of view for each dish and 2–3 petri dishes for each treatment. High seahare mortality in the initial experiment precluded the inclusion of the 7.6 pH treatment. Isopods were not analyzed in the second experiment for grazing trials because of low replication due to mortality in the initial 24-days experiment.

2.3 | Statistical analyses

We fit regression models to assess the evidence for effects of pH and nutrient addition on each response variable (Table S1). To isolate potential linear and quadratic effects of pH on each response variable, we fit models with orthogonal 2nd order polynomials of pH. We modeled the effect of adding nutrients as a categorical (0/1) predictor, and in a separate set of models we also considered an interaction between nutrients and the linear pH response. For continuous response variables that could take on positive or negative values (e.g. change in mass), we fit linear regression models. For response variables that could only take on positive values (e.g. length) we fit linear regression models to log-transformed response variables. This ensured that predictions remained positive when exponentiated and changed the interpretation of slope coefficients to be multiplicative. In a small number of instances where one of the response variables included zeros, we added half the next smallest value observed before log transforming the response. For response variables representing counts (e.g. shoot mortality), we fit negative binomial generalized linear models (GLMs) with a log link using the NB2 parameterization (Hilbe, 2011) where the variance is modeled as increasing quadratically with the mean. Sample sizes differed among response variables: changes in grazer and eelgrass biomass, epiphyte loading, macroalgal biomass, and grazer and shoot mortalities all $n = 14$; shoot and rhizome responses all $n = 310$ – 317 (multiple observations per mesocosm); grazing trial responses all $n = 34$ – 37 (multiple observations per trial). For the shoot and rhizome responses, in which there were multiple observations per mesocosm, we accounted for possible pseudoreplication by including a random intercept for each mesocosm. To assess the sensitivity of all our models to any individual mesocosm, we refit our models jackknifing out each mesocosm: we removed each mesocosm in succession, fit the same models, and plotted the median predictions.

Regression models with continuous predictors (i.e., encompassing the linear regressions, GLMs, and linear mixed effects models used here) are a powerful tool to analyze mesocosm experimental data, especially compared to ANOVA models with categorical predictors (Cottingham et al., 2005). Regression models with continuous predictors (here the linear and quadratic effects of pH) estimate fewer parameters than the same models fit as ANOVAs with the predictor turned into a categorical variable; this increases statistical power (Cottingham et al., 2005). Furthermore, the estimated regression coefficients on the continuous predictors allow for inference about the shape of the relationship. The coefficient associated with pH describes the linear changes in a response with changes in pH, and the coefficient associated with pH² describes a possible quadratic shape. Response variables that are log-transformed change exponentially with pH and pH² when back transformed. Our base model lets the relationship shift up or down with the addition of nutrients and the interaction model also allows the slope of pH and a response variable to vary with the addition of nutrients, effectively tilting the response-pH relationship (Figure 1c–f).

We fit our models in a Bayesian framework with the package *rstanarm* 2.13.1 (Stan Development Team, 2016a) for the statistical software *R* 3.3.2 (R Development Core Team, 2015). The package *rstanarm* implements regression models in *STAN* (Stan Development Team, 2016b), which are fit with Hamiltonian Markov chain Monte Carlo sampling. Fitting the models in a Bayesian framework let us weakly constrain the magnitude of coefficients based on prior knowledge, thereby reducing the chance of model overfitting, and let us calculate the probability of a coefficient being in specific ranges (e.g. the probability of a coefficient being >0). We used weakly informative priors: Normal (0, 2) on slope coefficients, Normal (0, 20) on intercepts, half-Student-*t* (3, 0, 5) on the residual standard deviation for linear regression models and standard deviation of any random intercepts (i.e., degrees of freedom of 3, mean of 0, and a scale of 5), and half-Student-*t* (3, 0, 20) on the dispersion parameter in the negative binomial GLMs (with small values of the dispersion parameter representing over dispersion compared to the Poisson). For continuous predictors that were fit on the natural scale (not log transformed), we adjusted the response units so that the coefficients would be on a similar scale and therefore these priors would be reasonable (e.g. using units of 10 g instead of g; units are indicated in the scatterplots). We fit the models with 2,000 iterations across four chains, discarding the first 1,000 iterations of each chain as warm-up. We ensured the chains had converged by inspecting the chains visually, checking that \hat{R} values (the potential scale reduction factor) for all parameters were <1.05 , and checking that effective sample sizes for all parameters were >200 (Gelman et al., 2013).

3 | RESULTS

The pH values in the mesocosms reflected conditions in local eelgrass habitats (Fig. S2), as well as pH values beyond current and projected conditions (Feely et al., 2010), which allowed us to investigate functional relationships with carbonate chemistry. Nutrient treatments reflected highly nutrient-loaded estuaries (Burkholder et al., 2007). The mean pH readings (on the total scale, pH_T) in our mesocosms were within the targeted ranges (Table 1 and Fig. S3). The pH in the experimental mesocosms tracked the prescribed treatment conditions, but was slightly elevated in some mesocosms due to sampling midday when pH is elevated by photosynthesis. Our nutrient enriched treatments had elevated NO_3 (independent samples *t* test: $t = 6.76$, $df = 12$, $p < .00005$) and PO_4 ($t = 6.93$, $df = 12$, $p < .00005$; Table S2). Nutrient treatments were 35% greater for NO_3 (ambient mean = $6.37 \mu\text{M}$, enriched mean = $9.88 \mu\text{M}$) and 38% greater for PO_4 (ambient mean = $0.51 \mu\text{M}$, enriched mean = $0.82 \mu\text{M}$), respectively. Overall, nutrient concentrations were low compared to local, nutrient enriched estuaries, which can exceed $200 \mu\text{M}$ NO_3 (Hughes et al., 2016). However, the nutrients were sampled weekly just prior to replenishing, and when combined with high rates in nutrient uptake from the primary producers, especially with elevated temperatures (Bulthuis, 1987; Fong, Fong, & Fong,

2004), could have underestimated the available nutrients. Afternoon temperatures averaged 20.3°C and peaked at 22.3°C during the experiment, which is similar to the 5-year trend where the eelgrass and associated assemblages were collected (Fig. S2). Parameters such as salinity and TA did not vary on an ecologically relevant scale among treatments (Table 1 and Fig. S3).

3.1 | Community interaction experiment

Our results after the 24-day mesocosm experiment support the hypothesis (Figure 1) that grazers would respond quadratically and algae would increase exponentially with increased acidification (Figure 2). Despite these results, eelgrass displayed weak linear or exponential increases with reductions in pH and displayed high resilience in the face of global stressors (Figure 2). We also detected strong effects of nutrient enrichment on some response variables (change in seahare biomass, algal epiphytes consumed, and shoot mortality) across all the interactions we investigated (Figure 2), yet we did not detect any notable interaction between pH and nutrients in our response variables (Fig. S4). Furthermore, our modeling results were relatively robust to a jackknife procedure that removed data from individual mesocosms, suggesting the results are not heavily influenced by individual mesocosms with high leverage (Fig. S5).

As hypothesized, the responses of grazers to ocean acidification were quadratic. Change in biomass of both seahares (*P. taylori*) and isopods (*I. rescacata*), showed a negative (dome-shaped) quadratic relationship with pH with probabilities of 0.97 and 1.00, respectively (Figure 3a,b, see Figure 2 and Table S2 for coefficients and probability densities for each response variable), and both peaked in the pH 7.45 treatment. Although the shape of the functional relationship between the change in mean biomass and pH were similar across both species, the absolute changes in biomass were quite different. The seahare biomass increased across all treatments (mean net change in biomass = $+2.1 \text{ g FW} \pm 0.45 \text{ SE}$) while isopod biomass decreased across all treatments (mean net change in biomass = $-0.6 \text{ g FW} \pm 0.07 \text{ SE}$). Seahare mortality was low and reflected the positive (U-shaped) quadratic relationship (probability = .97) between biomass and pH, with the lowest mortality at 7.45 pH (Fig. S6a). In contrast, isopod mortality was high across all treatments (mean = $4.1 \pm 0.48 \text{ SE}$ per mesocosm), but unlikely to be related to pH (Fig. S6b). Elevated nutrient conditions were associated with a 1.8 (0.3–3.2 95% CI) g FW decrease in mean seahare biomass (Figure 3a, probability = .99), and we only detected slightly increased isopod mortality (probability = .92) with elevated nutrients (Figure 2 and Fig. S6b).

Algal biomass displayed exponential increases with ocean acidification, but did not respond to nutrient enrichment treatments (Figures 2 and 3c,d, Table S2). The probability of exponential increases in epiphyte loading and macroalgal production with decreasing pH was 0.95 and 0.99 respectively (Figure 3c,d). However, there was moderate probability (0.82) of a negative quadratic effect for epiphyte loading, due to a flattening of the relationship between epiphyte loading and pH at low pH values. There was a 51% modeled increase in epiphyte loading with a pH decrease from 7.86 to 7.45,

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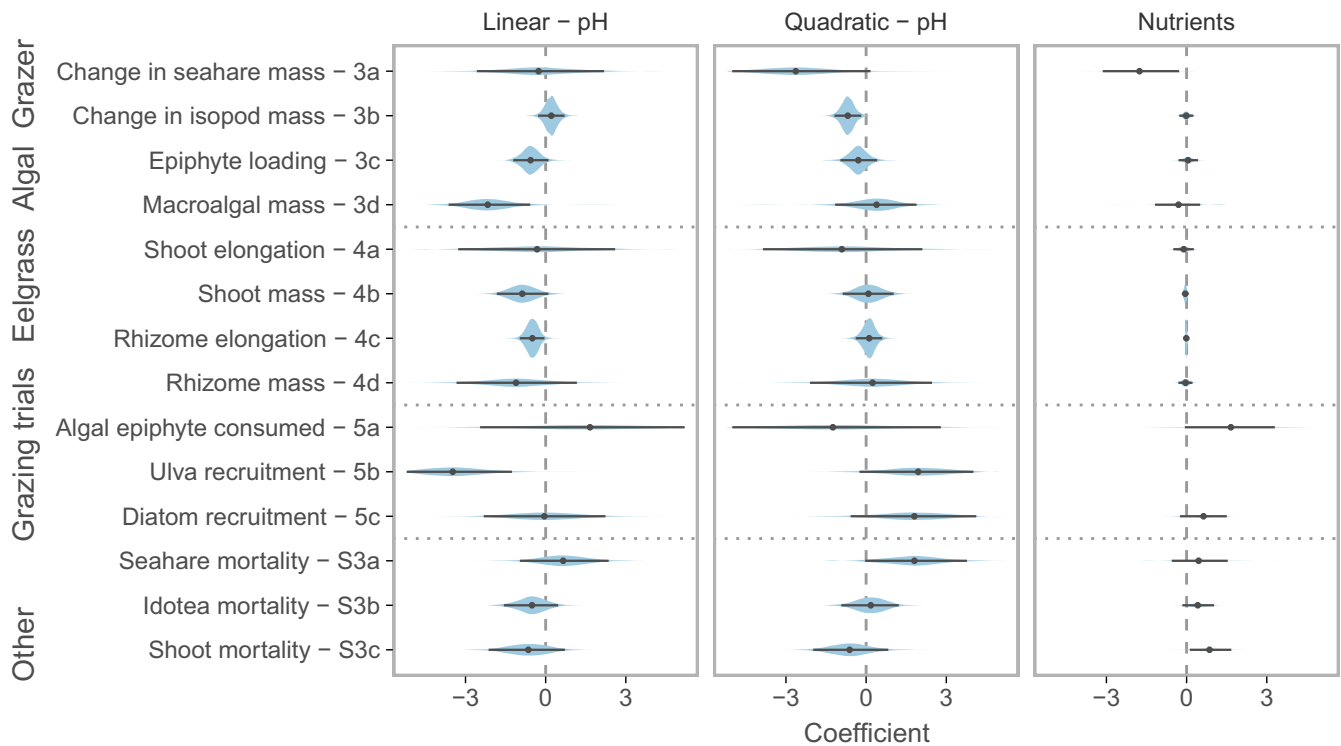


FIGURE 2 Coefficient estimates for all response variables. Shown are posterior densities (violin-shaped polygons), median estimates (points), and 95% credible intervals (line segments) for orthogonal linear and quadratic effects of pH and the effect of adding nutrients on each response variable (the corresponding figure number is noted for each response). Note that some models are fit in log space or with a log-link and some are fit on a natural untransformed scale (see Methods and Tables S1 and S2). See Figures 3–5, Fig. S6 for units but note that the magnitude of the linear and quadratic effects reflects the orthogonal polynomial transformation and is not interpretable in the original units (Chambers & Hastie, 1992)

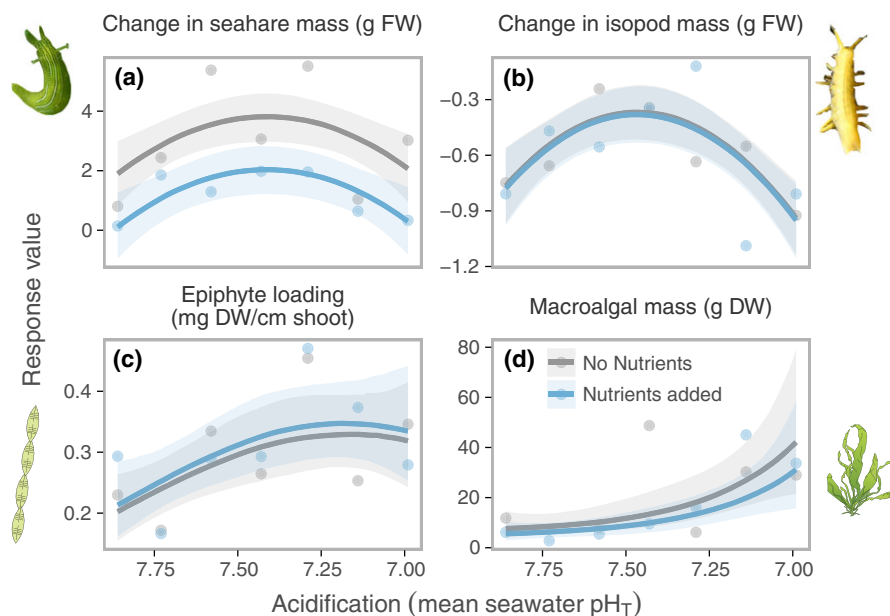


FIGURE 3 Grazer and algal responses to global stressors. (a) The change in seahare (*Phyllaplysia taylori*) biomass ($n = 14$), (b) change in isopod (*Idotea resicata*) biomass ($n = 14$), (c) algal epiphyte loading ($n = 14$), and (d) macroalgal (*Ulva intestinalis*) biomass ($n = 14$) over the 24-day mesocosm experiment (Tables S1 and S2). Lines represent median posterior estimates, shaded areas represent 80% credible intervals, and points represent the data

while there was little change in the modeled response of epiphytes between pH 7.45 and 7.0 (Figure 3c). Macroalgal biomass (all *Ulva intestinalis*) increased exponentially with acidification, with an approximately sixfold increase across the pH treatment range (Figure 3d).

Despite increased macroalgal and epiphyte growth in acidic conditions, which can increase competition for light and space, eelgrass persisted in all treatments. Unlike the quadratic responses in grazers and exponential changes in macroalgae, eelgrass responses to ocean acidification were linear or undetectable (Figures 2 and 4a–d; Table S2). Over the span of the experiment, eelgrass in mesocosms experienced a net decline in biomass (mean = -34.1 ± 6.7 SE g FW), which was likely caused by minor senescence related to the warm seawater temperatures in the region at the end of the growing season. The log of shoot mass and rhizome elongation had positive linear responses to acidification (probabilities = .96, .99, respectively; Figure 4b,c), whereas we found little evidence that shoot elongation or rhizome mass varied with pH. Furthermore, we found little evidence that eelgrass response variables were affected by nutrient levels (Figures 2 and 4). Combined, these results indicate resilience to ocean acidification and nutrient loading. Shoot mortality was the only eelgrass response variable that was primarily explained by nutrients (probability = .99) with 2.4-fold (1.1–5.1 95% CI) higher shoot mortality in nutrient enriched treatments (Fig. S6c). We note that we did not observe direct grazing on eelgrass by seahares or isopods during the course of the 24-day experiment.

3.2 | Algal recruitment and grazing experiment

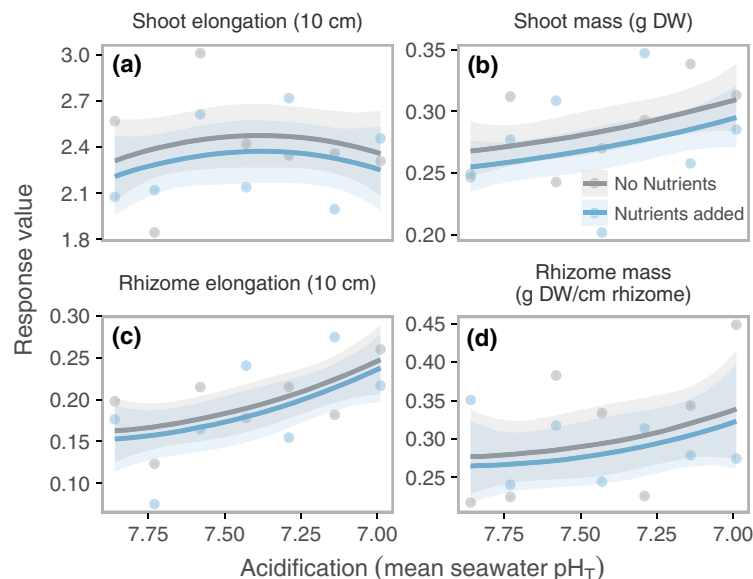
Our grazing trial results indicated that algal epiphytes were strongly affected by nutrients with varying responses to ocean acidification (Figure 5; Table S2). Over the relatively short timeframe of the grazing trial (6 hr), we did not detect a strong relationship between seahare grazing rates and pH. Instead, grazing rates were higher in

nutrient enriched conditions (probability = .97), but with considerable uncertainty in the magnitude of the effect (Figure 5a). When broken into the primary algal types (*Ulva* and diatoms), we found different treatment effects on recruitment. Nutrients had a large effect on *Ulva*—without nutrient enrichment there was no *Ulva* recruitment (Figure 5b). In nutrient enriched conditions, *Ulva* recruitment showed an exponential increase with decreasing pH (probability = 1.0), which was similar to the response of *Ulva* biomass in the 24-days mesocosm experiment (Figure 3d). Diatom percent cover, however, showed a positive (U-shaped) quadratic relationship with pH with a probability of 0.93 (although somewhat sensitive to removing one mesocosm; Fig. S5), and diatom recruitment was 1.9-fold (0.8–4.5 95% CI) higher when nutrients were added (probability = .92; Figure 5c).

4 | DISCUSSION

Resilience models are often used to describe how ecosystems will respond to increased disturbance or stress (Holling, 1973; Scheffer et al., 2001). These models are often founded on a firm understanding of species interactions that allow predictions of how a system might respond to stressors, and can therefore be especially important for developing management tools for ecosystems threatened by global environmental change (Bernhardt & Leslie, 2013). As species change their behavior, acclimate, or adapt to compensate for the changes in their environment, however, it is unclear how nonlinear relationships between organisms and their environment will propagate through ecosystems, as well as whether the interactions governing ecosystem dynamics will be reorganized. In our model assemblage with well-defined species interactions (Hughes et al., 2004, 2013; Williams & Ruckelshaus, 1993), we found resilience in habitat-forming seagrass despite nonlinear relationships between ocean acidification and grazers, which are known to influence seagrass ecosystem state under current conditions (Burkholder et al.,

FIGURE 4 Eelgrass responses to global stressors. (a) Eelgrass (*Zostera marina*) mean shoot mass ($n = 317$), (b) mean shoot mass ($n = 316$), and (c) rhizome elongation ($n = 311$), (d) mean rhizome mass ($n = 310$) over the 24-day mesocosm experiment (Tables S1 and S2). Lines represent median posterior estimates and shaded areas represent 80% credible intervals. In these panels, points represent mean data per mesocosm for visualization. The underlying model included all data and a random intercept for mesocosm



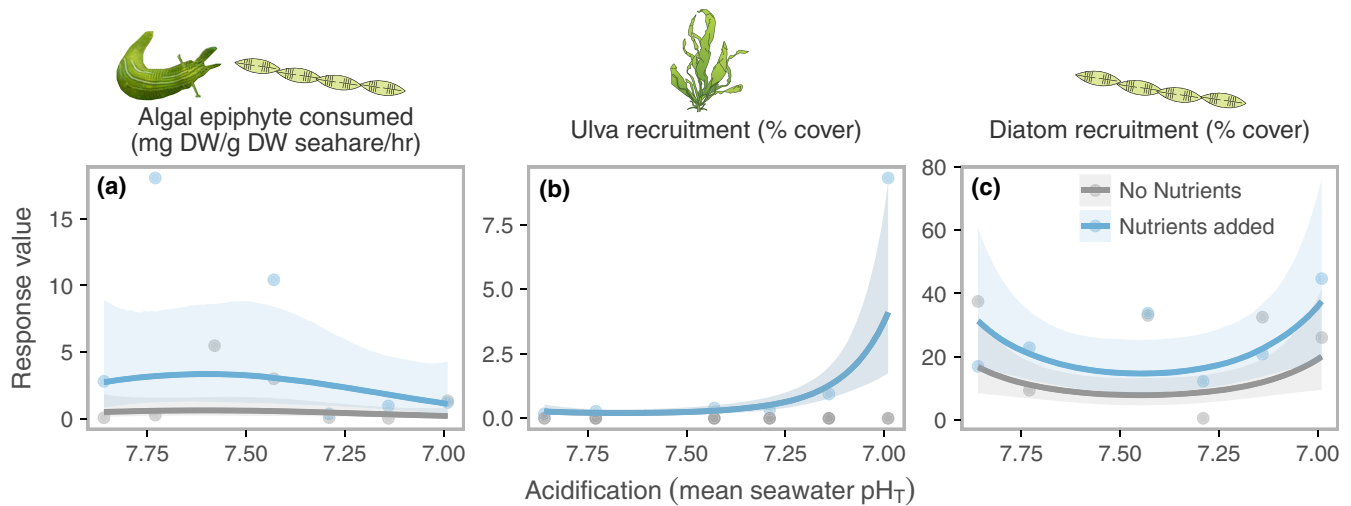


FIGURE 5 Grazing and algal recruitment responses to global stressors. (a) Mean algal epiphyte consumption by seahares ($n = 36$), (b) mean % cover of *Ulva* recruitment ($n = 34$), and (c) and mean % cover of diatom recruitment ($n = 34$) used in 6 hr grazing trials (Tables S1 and S2). Recruitment occurred over 5 days in each treatment mesocosm prior to the grazing trial. Lines represent median posterior estimates, shaded areas represent 80% credible intervals, and points represent the data

2007; Duffy et al., 2015; Hughes et al., 2013; Orth & Van Montfrans, 1984; Valiela et al., 1997; Williams & Ruckelshaus, 1993). These results suggest that important species interactions could be reshuffled under different global change regimes, creating a situation where nonlinear dynamics at lower levels of organization do not translate to ecological regime shifts.

In our experiment, seagrass was able to adequately compensate for the increasing levels of competition with macroalgae, despite failure of grazers to compensate for increases in algal biomass caused by acidification and nutrient loading. As predicted, grazer biomass (and associated algal consumption) was eventually reduced when conditions became increasingly more acidic. This drop in herbivore biomass coincided with an increase in macroalgal biomass (and a similar trend in epiphyte loading) under acidic conditions. Seagrass, however, was able to grow more under acidic conditions with elevated algal biomass. This resilience could be due to direct effects of acidification on seagrass physiology that compensate for any decreasing light levels associated with algae (Zimmerman et al., 2015)—causing a shift in the strength of the species interaction. These results are consistent with previous research that demonstrated that seagrass can persist even with elevated macroalgal (Hessing-Lewis, Hacker, Menge, & Rumrill, 2011; Hughes et al., 2016; Thomsen et al., 2012) and epiphyte loading (Ruesink, 2016) when it is not limited by light.

The effect of pH on short-term grazing rates did not align with the modeled effects of pH on herbivore biomass in our experiment (Figure 5a–c), suggesting that the mechanisms underlying the change in herbivore biomass may have been related to changes in energetic demand or food assimilation with pH, rather than changes in consumption. More in-depth analyses of herbivore metabolism, consumption, and energy assimilation could provide important insight into potential breakdowns in herbivory with acidification. Our results also indicate that lower pH conditions could amplify the effects of

nutrients on *Ulva* recruitment. Previous research has indicated that some grazers may prefer diatoms over *Ulva* recruits (Kitting, 1984), which could be a mechanism underlying the lower grazing rates in low pH. If grazers have an affinity for diatoms vs. *Ulva* recruits, an increase in *Ulva* recruitment could indirectly deter grazing in low pH. Since ephemeral macroalgae can thrive under acidic and nutrient enriched conditions (Fong et al., 2004; Harley et al., 2012; Koch et al., 2013; Young & Gobler, 2016), further research is needed to determine the mechanisms underlying changes in grazing and grazer biomass with acidification and nutrient loading. While grazer biomass declined at a specific pH value, it is unclear how changes in fitness, population dynamics, and density of grazers over longer time frames could affect the relationship between carbonate chemistry, nutrient concentrations and grazing pressure in the future.

Overall, acidification produced more effects on eelgrass community responses than nutrients (Figure 2; Table S2). This, combined with the lack of linear interactions between pH and nutrients, indicates that ocean acidification could be a stronger driver of eelgrass community dynamics than nutrient enrichment. This finding is consistent with those predicted in other studies simulating ocean acidification with other stressors. For example, in Chesapeake Bay, it has been predicted that seagrass will fare well under more acidic conditions that stimulate seagrass productivity, even with high temperature and algal epiphyte stressors (Zimmerman et al., 2015). However, we note that eelgrass in our mesocosm experiment also suffered from higher mortality under nutrient conditions (Fig. S6c). Previous research has linked nutrient (nitrate) enrichment with increased eelgrass mortality under high temperatures due to nutrient imbalances that are unrelated to light availability (Burkholder, Mason, & Glasgow, 1992). Combined, these results indicate that certain stressors can act independently on factors driving resilience in seagrasses.

Managers and policy makers are challenged with developing strategies to predict ecological thresholds and to improve resilience

in the face of global environmental change, such as ocean acidification and continued local threats. Our results suggest that the ecological processes governing ecosystems may shift in future environments, which could limit our ability to scale-up the effects of environmental change on any individual species or interaction to larger ecosystem dynamics based on our current understanding of the processes governing community structure. Continued focus on understanding how the strength of species interactions will change in future conditions may lend insight into potential ecological thresholds or resilience mechanisms with continued acidification (Zimmerman et al., 2015).

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DATA ACCESSIBILITY

All data and code for this study are available at <https://github.com/bbhughes/seagrass-resilience>, and are archived at <http://doi.org/10.5281/zenodo.842853>.

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REFERENCES

- Alsterberg, C., Eklöf, J. S., Gamfeldt, L., Havenhand, J. N., & Sundbäck, K. (2013). Consumers mediate the effects of experimental ocean acidification and warming on primary producers. *Proceedings of the National Academy of Sciences of the United States of America*, 110, 8603–8608.
- Andersen, T., Carstensen, J., Hernández-García, E., & Duarte, C. M. (2009). Ecological thresholds and regime shifts: Approaches to identification. *Trends in Ecology and Evolution*, 24, 49–57.
- Bernhardt, J. R., & Leslie, H. M. (2013). Resilience to climate change in coastal marine ecosystems. *Annual Review of Marine Science*, 5, 371–392.
- Best, R. J., & Stachowicz, J. J. (2012). Trophic cascades in seagrass meadows depend on mesograzers: Variation in feeding rates, predation susceptibility, and abundance. *Marine Ecology Progress Series*, 456, 29–42.
- Bruno, J. F., Stachowicz, J. J., & Bertness, M. D. (2003). Inclusion of facilitation into ecological theory. *Trends in Ecology & Evolution*, 18, 119–125.
- Bulthuis, D. A. (1987). Effects of temperature on photosynthesis and growth of seagrasses. *Aquatic Botany*, 27, 27–40.
- Burkholder, D. A., Heithaus, M. R., Fourqurean, J. W., Wirsing, A., & Dill, L. M. (2013). Patterns of top-down control in a seagrass ecosystem: Could a roving apex predator induce a behaviour-mediated trophic cascade? *Journal of Animal Ecology*, 82, 1192–1202.
- Burkholder, J., Mason, K., & Glasgow, H. B. Jr (1992). Water-column nitrate enrichment promotes decline of eelgrass *Zostera marina*: Evidence from seasonal mesocosm experiments. *Marine Ecology Progress Series*, 81, 163–178.
- Burkholder, J. M., Tomasko, D. A., & Touchette, B. W. (2007). Seagrasses and eutrophication. *Journal of Experimental Marine Biology and Ecology*, 350, 46–72.
- Campbell, J. E., & Fourqurean, J. W. (2014). Ocean acidification outweighs nutrient effects in structuring seagrass epiphyte communities (ed Nilsson C). *Journal of Ecology*, 102, 730–737.
- Chambers, J. M., & Hastie, T. J. (1992). *Statistical models in S*. Pacific Grove, CA: Wadsworth & Brooks/Cole.
- Connell, S. D., & Ghedini, G. (2015). Resisting regime-shifts: The stabilising effect of compensatory processes. *Trends in Ecology and Evolution*, 30, 513–515.
- Cornwall, C. E., Hepburn, C. D., Pritchard, D., Currie, K. I., McGraw, C. M., Hunter, K. A., & Hurd, C. L. (2012). Carbon-use strategies in macroalgae: Differential responses to lowered pH and implications for ocean acidification. *Journal of Phycology*, 48, 137–144.
- Cottingham, K. L., Lennon, J. T., Brown, B. L. (2005). Knowing when to draw the line: Designing more informative ecological experiments. *Frontiers in Ecology and the Environment*, 3, 145–152.
- Crain, C. M., Kroeker, K., & Halpern, B. S. (2008). Interactive and cumulative effects of multiple human stressors in marine systems. *Ecology Letters*, 11, 1304–1315.
- Doak, D. F., & Morris, W. F. (2010). Demographic compensation and tipping points in climate-induced range shifts. *Nature*, 467, 959–962.
- Duffy, J. E., Macdonald, K. S., Rhode, J. M., & Parker, J. D. (2001). Grazer diversity, functional redundancy, and productivity in seagrass beds: An experimental test. *Ecology*, 82, 2417–2434.
- Duffy, J. E., Reynolds, P. L., Boström, C., Coyer, J. A., Cusson, M., Donadi, S., ... Stachowicz, J. J. (2015). Biodiversity mediates top-down control in eelgrass ecosystems: A global comparative-experimental approach. *Ecology Letters*, 18, 696–705.
- Feely, R. A., Alin, S. R., Newton, J., Sabine, C. L., Warner, M., Devol, A., ... Maloy, C. (2010). The combined effects of ocean acidification, mixing, and respiration on pH and carbonate saturation in an urbanized estuary. *Estuarine, Coastal and Shelf Science*, 88, 442–449.
- Fong, P., Fong, J. J., & Fong, C. R. (2004). Growth, nutrient storage, and release of dissolved organic nitrogen by *Enteromorpha intestinalis* in response to pulses of nitrogen and phosphorus. *Aquatic Botany*, 78, 83–95.
- Gelman, A., Carlin, J. B., Stern, H. S., Dunson, D. B., Veharti, A., & Rubin, D. B. (2013). *Bayesian data analysis*, 3rd ed. London: Chapman and Hall.
- Gonzalez, A., & Loreau, M. (2009). The causes and consequences of compensatory dynamics in ecological communities. *Annual Review of Ecology, Evolution, and Systematics*, 40, 393–414.
- Harley, C. D. G., Anderson, K. M., Demes, K. W., Jorve, J. P., Kordas, R. L., Coyle, T. A., & Graham, M. H. (2012). Effects of climate change on global seaweed communities. *Journal of Phycology*, 48, 1064–1078.
- Hessing-Lewis, M. L., Hacker, S. D., Menge, B. A., & Rumrill, S. S. (2011). Context-dependent eelgrass-macroalgae interactions along an estuarine gradient in the Pacific Northwest, USA. *Estuaries and Coasts*, 34, 1169–1181.
- Hilbe, J. M. (2011). Negative binomial regression. *Public Administration Review*, 70, 1–6.
- Holling, C. (1973). Resilience and stability of ecological systems. *Annual Review of Ecology, Evolution, and Systematics*, 4, 1–23.
- Huey, B., & Stevenson, R. D. (1979). Integrating thermal physiology and ecology of ectotherms: A discussion of approaches. *American Zoologist*, 19, 357–366.

- Hughes, A. R., Bando, K. J., Rodriguez, L. F., Williams, S. L., & Davis, U. C. (2004). Relative effects of grazers and nutrients on seagrasses: A meta-analysis approach. *Marine Ecology Progress Series*, 282, 87–99.
- Hughes, B. B., Eby, R., Van Dyke, E., Tinker, M. T., Marks, C. I., Johnson, K. S., & Wasson, K. (2013). Recovery of a top predator mediates negative eutrophic effects on seagrass. *Proceedings of the National Academy of Sciences of the United States of America*, 110, 15313–15318.
- Hughes, B. B., Hammerstrom, K. K., Grant, N. E., Hoshijima, U., Eby, R., & Wasson, K. (2016). Trophic cascades on the edge: Fostering seagrass resilience via a novel pathway. *Oecologia*, 182, 231–241.
- Kitting, C. L. (1984). Selectivity by dense populations of small invertebrates foraging among seagrass blade surfaces. *Estuaries*, 7, 276–288.
- Koch, M., Bowes, G., Ross, C., & Zhang, X. H. (2013). Climate change and ocean acidification effects on seagrasses and marine macroalgae. *Global Change Biology*, 19, 103–132.
- Kroeker, K. J., Kordas, R. L., Crim, R., Hendriks, I. E., Ramajo, L., Singh, G. S., ... Gattuso, J. P. (2013). Impacts of ocean acidification on marine organisms: Quantifying sensitivities and interaction with warming. *Global Change Biology*, 19, 1884–1896.
- Martin, S., Rodolfo-Metalpa, R., Ransome, E., Rowley, S., Buia, M. C., Gattuso, J. P., & Hall-Spencer, J. (2008). Effects of naturally acidified seawater on seagrass calcareous epibionts. *Biology Letters*, 4, 689–692.
- Martinez-Crego, B., Arteaga, P., Tomas, F., & Santos, R. (2016). The role of seagrass traits in mediating *Zostera noltei* vulnerability to mesograzers. *PLoS ONE*, 11, 1–19.
- Melzner, F., Stange, P., Trübenbach, K., Thomsen, J., Casties, I., Panknin, U., ... Gutowska, M. A. (2011). Food supply and seawater pCO₂ impact calcification and internal shell dissolution in the blue mussel *Mytilus edulis*. *PLoS ONE*, 6, e24223.
- Morris, R. H., Abbott, D. P., & Haderlie, E. C. (1980). *Intertidal invertebrates of California*. Stanford: Stanford University Press.
- Nogueira, P., Gambi, M. C., Vizzini, S., Califano, G., Tavares, A. M., Santos, R., & Martinez-Crego, B. (2016). Altered epiphyte community and sea urchin diet in *Posidonia oceanica* meadows in the vicinity of volcanic CO₂ vents. *Marine Environmental Research*, 127, 102–111.
- Orth, R. J., & Van Montfrans, J. (1984). Epiphyte-seagrass relationships with an emphasis on the role of micrograzing: A review. *Aquatic Botany*, 18, 43–69.
- Prado, P., Romero, J., & Alcoverro, T. (2010). Nutrient status, plant availability and seasonal forcing mediate fish herbivory in temperate seagrass beds. *Marine Ecology Progress Series*, 409, 229–239.
- R Development Core Team (2015). *R: A language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing. Retrieved from <http://www.R-project.org/>
- Reich, P. B., Tilman, D., Isbell, F., Mueller, K., Hobbie, S. E., Flynn, D. F. B., & Eisenhauer, N. (2012). Impacts of biodiversity loss escalate through time as redundancy fades. *Science*, 336, 589–592.
- Reynolds, L. K., Carr, L. A., & Boyer, K. E. (2012). A non-native amphipod consumes eelgrass inflorescences in San Francisco Bay. *Marine Ecology Progress Series*, 451, 107–118.
- Ruesink, J. L. (2016). Epiphyte load and seagrass performance are decoupled in an estuary with low eutrophication risk. *Journal of Experimental Marine Biology and Ecology*, 481, 1–8.
- Scheffer, M., & Carpenter, S. R. (2003). Catastrophic regime shifts in ecosystems: Linking theory to observation. *Trends in Ecology & Evolution*, 18, 648–656.
- Scheffer, M., Carpenter, S., Foley, J. A., Folke, C., & Walker, B. (2001). Catastrophic shifts in ecosystems. *Nature*, 413, 591–596.
- Stan Development Team (2016a). rstanarm: Bayesian applied regression modeling via Stan. R package version 2.13.1.
- Stan Development Team (2016b). Stan Modeling Language Users Guide and Reference Manual. Version 2.
- Thomsen, M. S., Wernberg, T., Engelen, A. H., Tuya, F., Vanderklift, M. A., Holmer, M., ... Silliman, B. R. (2012). A meta-analysis of seaweed impacts on seagrasses: Generalities and knowledge gaps. *PLoS ONE*, 7, e28595.
- Valiela, I., McClelland, J., Hauxwell, J., Behr, P. J., Hersh, D., & Foreman, K. (1997). Macroalgal blooms in shallow estuaries: Controls and eco-physiological and ecosystem consequences. *Limnology and Oceanography*, 42, 1105–1118.
- Williams, S. L. (2001). Reduced genetic diversity in eelgrass transplantations affects both population growth and individual fitness. *Ecological Applications*, 11, 1472–1488.
- Williams, S. L., & Ruckelshaus, M. H. (1993). Effects of nitrogen availability and herbivory on eelgrass (*Zostera marina*) and epiphytes. *Ecology*, 74, 904–918.
- Young, C. S., & Gobler, C. J. (2016). Ocean acidification accelerates the growth of two bloom-forming macroalgae. *PLoS ONE*, 11, e0155152.
- Zhu, K., Chiariello, N. R., Tobeck, T., Fukami, T., & Field, C. B. (2016). Nonlinear, interacting responses to climate limit grassland production under global change. *Proceedings of the National Academy of Sciences of the United States of America*, 113, 10589–10594.
- Zimmerman, R. C., Hill, V. J., & Gallegos, C. L. (2015). Predicting effects of ocean warming, acidification, and water quality on Chesapeake region eelgrass. *Limnology and Oceanography*, 60, 1781–1804.

SUPPORTING INFORMATION

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