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Grazer diversity interacts with biogenic habitat heterogeneity to accelerate intertidal algal succession

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Abstract. Environmental heterogeneity contributes to coexistence by allowing species with different traits to persist when different species perform best at different times or places. This interaction between niche differences and environmental variability may also help explain relationships between biodiversity and ecosystem functioning, but few data are available to rigorously evaluate this hypothesis. We assessed how a biologically relevant aspect of environmental heterogeneity interacts with species diversity to determine ecosystem processes in a natural rocky intertidal community. We used field removals to factorially manipulate biogenic habitat heterogeneity (barnacles, bare rock, and plots that were 50/50 mixes of the two habitat types) and gastropod grazer species richness and then tracked algal community succession and recovery over the course of 1 yr. We found that herbivore diversity, substrate heterogeneity, and their interaction played unique roles in the peak abundance and timing of occurrence of different algal functional groups. Early successional microalgae were most heavily grazed in diverse herbivore assemblages and those with barnacles present, which was likely due to complementary feeding strategies among all three grazers. In contrast, late successional macroalgae were strongly influenced by the presence of a habitat generalist limpet. In this herbivore's absence, heterogeneous habitats (i.e., mixtures of bare rock and barnacles) experienced the greatest algal accumulation, which was partly a result of complementary habitat use by the remaining herbivores. The complex way habitat identity and heterogeneity altered grazer-algal interactions in our study suggests species' differences and environmental heterogeneity both separately and interactively contribute to the relationship between biodiversity and ecosystem functions.

Key words: *biodiversity-ecosystem functioning; complementarity; environmental heterogeneity; niche differentiation; plant-animal interactions; rocky shore; seaweeds; succession.*

INTRODUCTION

Environmental heterogeneity, or variability in environmental conditions over space and time, is widely viewed as a contributor to the maintenance of species diversity (Chesson 2000, Adler et al. 2013). If species are sufficiently distinct in their response to environmental conditions, environmental heterogeneity can provide a stabilizing mechanism that promotes diversity by allowing species to exploit different spatial or temporal niches (Pacala and Tilman 1994). The same heterogeneity that promotes diversity also likely underlies the effect of biodiversity on ecosystem functioning. Environmental heterogeneity should lead to different species contributing maximally to ecosystem functioning in different microenvironments and thus can enhance resource-use complementarity at the scales at which the heterogeneity occurs. Sampling effects commonly found in biodiversity-ecosystem function (hereafter, BEF) experiments (Wardle 1999) may be reduced relative to complementarity under

heterogeneous conditions, because the likelihood that one species will outperform, and potentially competitively exclude, all others decreases (Cardinale et al. 2004). Furthermore, maintenance of functioning in heterogeneous environments may require a greater number of species simply because more environments are sampled in which different species perform best (Stachowicz et al. 2008b). Environmental conditions in natural systems vary across space and time, often at multiple scales. Thus, a clear picture of how diversity affects ecosystem processes in natural ecosystems requires explicitly incorporating environmental heterogeneity in BEF research (Duffy 2009, Hillebrand and Matthiessen 2009).

Theory predicts that in order for complementarity to generate BEF relationships, community members must differ in the traits they express (Tilman et al. 1997, Yachi and Loreau 1999, Cardinale et al. 2000, 2004, Norberg et al. 2001). Thus, diversity—function relationships should be stronger in environments that lead to differential trait expression among species (e.g., Stachowicz et al. 2008a). Environmental heterogeneity in space or time should facilitate expression of trait variation, but relatively few experiments have directly tested how heterogeneity influences BEF relationships. No clear answer

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has yet emerged, despite many experiments demonstrating that heterogeneity can promote species coexistence (e.g., Huffaker 1958, Sommer 1984, Vivian-Smith 1997, Harpole and Tilman 2007). Laboratory experiments manipulating resource supply or habitat characteristics over space have found all possible outcomes: weaker diversity effects in heterogeneous environments (Tiunov and Scheu 2005), no effect of heterogeneity on ecosystem function (Weis et al. 2008), and enhanced complementarity (Wacker et al. 2008, Griffin et al. 2009, Cardinale 2011). Observational studies complement the finding of theory and lab experiments that heterogeneity in time and space increases complementary resource use by diverse assemblages (Tylianakis et al. 2008). However, field experiments testing the influence of environmental heterogeneity on ecosystem functioning are uncommon. A key challenge for interpreting such experiments is the degree of matching of the scale at which heterogeneity is manipulated with the scale at which heterogeneity actually occurs and trait differences are expressed in nature. In this paper, we experimentally investigate the role of small-scale habitat heterogeneity (generated by patchiness in the abundance of habitat-modifying barnacles) and grazer species richness and composition on algal recovery from disturbance in a natural rocky shore community.

On rocky intertidal shores, empirical evidence suggests that the effect of gastropod herbivores on algal abundance depends on local habitat characteristics. On the west coast of the United States, for example, littorine snails tend to be more abundant and exert stronger control on algae when barnacles are present because their small size allows them to graze interstitially among barnacle tests (Farrell 1991, Geller 1991, but see Harley 2006). In contrast, limpets tend to better control algae in the absence of barnacles because their larger size inhibits them from reaching between barnacle tests to graze (Geller 1991), thus barnacles can potentially inhibit or facilitate of algae depending on herbivore composition (Farrell 1991, Mrowicki et al. 2014) and barnacle density or patchiness. This potential complementarity in herbivore effects on substrates with different barnacle densities could result in the lowest cover of algae in areas with heterogeneity in barnacle cover where both types of herbivores are present. Alternatively, habitat heterogeneity could lead to strong effects of generalist species, capable of having an impact in many types of habitats. Although grazing gastropods, often particular species, can be important determinants of algal community structure and function (e.g., O'Connor and Crowe 2005, Griffin et al. 2010, Mrowicki et al. 2015), their importance can vary with environmental conditions in ways that seem unpredictable (Mrowicki et al. 2014), but may be related to habitat composition and heterogeneity. In laboratory studies, enhanced substrate heterogeneity in the laboratory (mixtures of topographically complex and flat substrates) leads to a strong effect of rocky shore gastropod species richness on algal biomass, whereas single species

have dominant influences on homogenous substrates (Griffin et al. 2009). Effects of substrate topography on rocky shore communities have been investigated at several scales (e.g., Harlin and Lindbergh 1977, Lubchenco 1983, Menge et al. 1985, Johnson 1994), yet field studies typically compare only treatments that vary in their average roughness or texture and do not explicitly consider within-patch variation in microtopography (but see Loke and Todd 2015). Such variation is likely a common feature of many environments as a result of non-uniform distribution of biogenic habitat-forming organisms (Bulleri et al. 2015).

In this study, we manipulated herbivore diversity and biogenic substrate heterogeneity by removing species from natural, "intact" assemblages, and we examined how algal community development responded to these manipulations over 13 months. We predicted that the snails would forage more efficiently on barnacle covered substrate due to easier access to small crevices between barnacle tests, whereas the limpets would forage better on bare rock (Jernakoff 1983, Farrell 1991, Geller 1991). We anticipated the strongest effects of herbivore diversity under heterogeneous conditions due to greater potential for niche complementarity between limpets and snails.

MATERIALS AND METHODS

We used field removal experiments to alter the richness and composition of gastropod grazers (*Lottia digitalis*, *Lottia scabra*, and littorine snails) and the cover of barnacles on a southwest-facing vertical rock (granodiorite) wall in the high intertidal zone on the rocky coast of Bodega Marine Reserve, California (38°19'7.3" N, 123°4'26.7" W). *Lottia digitalis* is often associated with barnacles or macroalgae, whereas *Lottia scabra* uses bare rock where it establishes home sites (Haven 1971). Barnacles seem to inhibit both the movement of limpets as well as their ability to feed effectively on rugose surfaces, whereas littorine snails are well-known to use barnacles as habitat, especially dead tests (Geller 1991). We used two species of limpets because they were both abundant and because we predicted they might interact with barnacle substrate in distinctive ways due to differences in movement: *Lottia digitalis* move frequently and lack home scars, whereas *L. scabra* move around to forage, but always return to the same home sites when the tide falls (Jesse 1968). Thus, *L. digitalis* may sample more habitats more frequently, and therefore be more adept at grazing in a heterogeneous environment than *L. scabra*, whereas *L. scabra* may graze a smaller area more intensely and consistently. We could not reliably distinguish between the two species of littorine snails we used (*Littorina plena* and *L. scutulata*) in the field, making field manipulation of each separately impractical. However, these sibling species exhibit very similar morphologies and life histories and consume similar algal prey (Mastro et al. 1982, Chow 1987), and from laboratory observations we found no evidence for differential habitat use.

In mid-July 2010, the rock wall supported a community of three co-occurring herbivore taxa: *Lottia digitalis* (average density \pm SE = $208 \pm 17 \text{ m}^{-2}$), *L. scabra* ($162 \pm 13 \text{ m}^{-2}$), and littorine snails (*Littorina plena* and *L. scutulata*, $1970 \pm 160 \text{ m}^{-2}$), as well as high cover of barnacles (*Balanus glandula* and *Chthamalus dalli*, $73 \pm 1\%$) at 1–2 m above mean lower-low water. Other grazers and habitat-forming sessile invertebrates were rare or absent, occurring at densities $<4 \text{ m}^{-2}$.

In late July 2010, we established 60 20×20 cm plots initially containing $\geq 70\%$ barnacle cover, ≥ 4 individuals each of *Lottia digitalis* and *L. scabra*, and ≥ 40 littorine snails. We applied a 3-cm wide border of marine epoxy (Z-spar A-788 Splash-Zone compound) around each plot, and we covered the epoxy plot border with Tree Tanglefoot (Tanglefoot Company, Grand Rapids, Michigan, USA) to discourage grazer movement in or out of the plots (Aquilino and Stachowicz 2012). We removed all visible algae and created three habitat heterogeneity treatments: two low heterogeneity treatments with (1) no barnacles, in which we scraped away all barnacles, or (2) full barnacles, in which we left all barnacles intact, and one high heterogeneity treatment in which we removed all barnacles from one half of the plot and left the other intact. Thus, low heterogeneity plots either had low or high biogenic substrate topography (i.e., flat vs. bumpy), but were relatively uniform in that topography, whereas high heterogeneity plots contained roughly equal amounts of flat and bumpy substrate. The relatively hard nature of the rock at our site means that scraping barnacles did not lead to observable changes in surface characteristics relative to rock surfaces naturally lacking barnacles.

We crossed these heterogeneity treatments with four herbivore treatments: *Lottia digitalis* alone, *L. scabra* alone, littorine snails alone, and all three species together. Pilot experiments in which we attempted to add herbivores in desired combinations resulted in mortality of most individuals, especially limpets. Therefore, we achieved desired treatments by removing non-target species from intact assemblages (a deletion experiment, e.g., O'Connor and Crowe 2005); target species density was allowed to vary naturally within a plot. We reapplied Tanglefoot every 2–4 weeks, at which time we also counted all visible invertebrates and removed unwanted individuals that had entered the plots and removed barnacle recruits that settled in cleared areas using a pin. To assess the effects of treatments, we visually estimated percent cover of algae (Diether et al. 1993) every three to 6 weeks, depending on tide and wave conditions.

In January and February 2011, likely due to large waves and possible rock scour, we lost nearly all limpets in seven plots, so these plots were removed from all analyses. Our final analysis contained three *L. scabra*/no barnacle plots, four *L. scabra*/full barnacle plots, three *L. digitalis*/full barnacle plots, four *L. digitalis*/no barnacle plots, four multi-species/no barnacle plots, and five plots each of all other treatments.

Littorine snails colonized removal plots more rapidly than limpets (see *Results*, Appendix S1: Fig. S1). We conducted an additional study in August/September 2013 to estimate treatment effectiveness over timescales shorter than our sampling of the experiment by establishing 12 experimental plots with the same barnacle treatments used in the main experiment ($n = 4$ for each treatment). We counted all littorine snails in these plots and then removed them. We then counted the number of littorines present in the plots after 1 d, and approximately weekly thereafter for six weeks, at which point we repeated littorine removal and assessed colonization again.

Statistical analysis

We tested effects of treatments on algal percent cover, as well as trends over time, by fitting a series of generalized additive mixed-effects models (GAMMs) predicting cover of three major algal groups: microalgae, ephemeral macroalgae, and perennial macroalgae that appear sequentially in the course of algal succession in this system (Aquilino and Stachowicz 2012). To help explore the importance of herbivore identity, richness, and abundance, we also fitted GAMMs describing the effects of treatments on herbivore density separately. Trends over time were smoothed using thin plate regression splines, fitted separately for herbivore treatments, along with fixed effects of herbivore treatment, substrate treatment, and their interaction. Because plots were sampled multiple times during the experiment, we modeled plot as random intercepts. Neither algal percent cover nor herbivore density data can fall below zero, so GAMMs used a Poisson error distribution and log link function, which generally produced sensible predictions (see *Results*). To test specific questions about relationships among treatments, we used a series of orthogonal a priori contrasts that compare each level of a factor to the mean of the subsequent treatments (Helmert contrasts; Venables and Ripley 2002). We arranged levels of herbivore and substrate treatments in these models to test for differences between means of: (1) three vs. one herbivore species (richness effect); (2) littorine snails vs. limpets (group effect); (3) *Lottia digitalis* vs. *L. scabra* (limpet species effect); (4) heterogeneous vs. homogeneous substrates (heterogeneity effect); and (5) full vs. no barnacles (substrate type effect). We also included the overall interaction of herbivore \times substrate and the specific contrasts that tests for species richness \times heterogeneity and species richness \times substrate type effects.

We separately explored how substrate and grazer composition influenced algal community development by comparing the distribution of algal cover on each side of the plots with the heterogeneous substrate treatment (in which barnacles were removed from only one half of the plot). Trends were modeled as described earlier with fixed effects of herbivore treatment, side of the plot, and

their interaction. Non-independence of data points was accounted for using random intercepts for plot.

In deletion experiments such as ours, herbivore density will initially vary among species composition treatments. In order to test for effects of herbivore treatments due to compositional differences rather than differences in herbivore density, we explored data graphically and fit a series of generalized linear mixed effects models with Poisson error distributions and log link functions to model algal responses as functions of different combinations of limpet and littorine snail densities, herbivore treatments, and their interactions, which we compared using Akaike Information Criterion. Herbivore densities were centered and scaled to median of zero and standard deviation of one after natural log transformation of littorine density. We used random intercepts for survey dates and plots to account for repeated measurements and changes in microalgal cover over time.

Herbivore habitat utilization on either side of heterogeneous plots was analyzed as a binomial response of presences (number on barnacle side) and absences (number on barnacle-free side). This presence/absence data was modeled as a function of herbivore identity and herbivore treatment using a generalized linear mixed-effects model with a binomial error distribution and logit link function. In this model, herbivore treatments consisted of responses of each herbivore in three-species polycultures (e.g., littorines in the no removal treatment) and monocultures from which only the focal herbivore was not removed (e.g., littorines in the littorine-only treatment). Non-independence of repeated measurements was accounted for by allowing the response to vary randomly across time (random slopes) in different plots (random intercepts).

All statistical routines were performed in R 3.2.0 (R Core 2015). We used the function “*gam*” in the “*mgcv*” package (Wood 2006), which also uses package “*nlme*” (Pinheiro et al. 2015), to perform generalized additive mixed modeling of algal and herbivore time series. For models of plot side usage by herbivores and algal responses to herbivore density, we used the function “*glmer*” in the “*lme4*” package (Bates et al. 2014) and “*lmerTest*” to calculate *P* values (Kuznetsova et al. 2015).

RESULTS

Effects of herbivore diversity and substrate heterogeneity on algal abundance

Herbivore composition and substrate heterogeneity interactively influenced algal species composition and rates of algal accumulation (Fig. 1, Table 1), though the relative importance of treatments and their interactions varied among different algal groups. Microalgae (composed largely of benthic diatoms) generally increased rapidly and then declined over time, as expected during algal succession and as a result of seasonal variation (Appendix S2: Fig. S1). However, within this seasonal

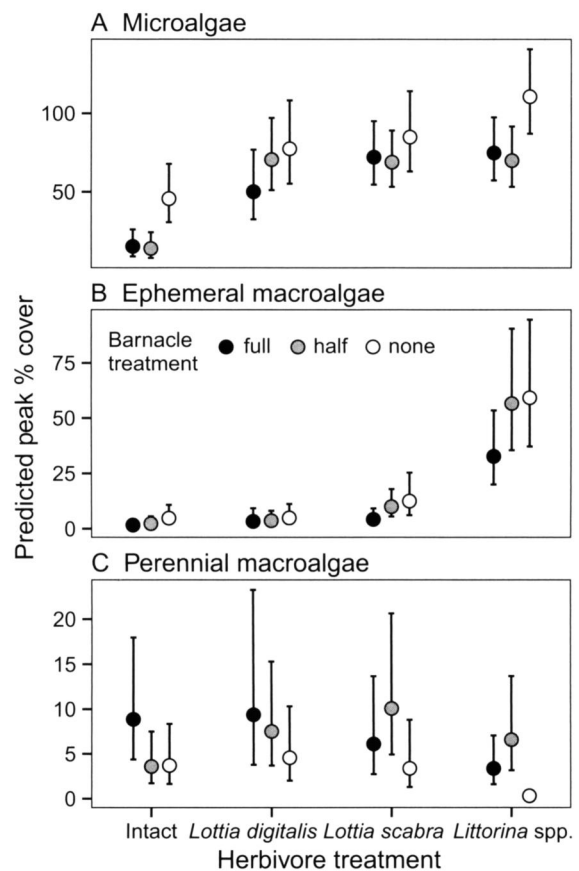


FIG. 1. Peak percent cover (mean \pm 95% confidence interval) of (A) microalgae, (B) ephemeral macroalgae, and (C) perennial macroalgae predicted by generalized additive mixed models (log link) using herbivore treatments and barnacle cover treatments as fixed factors. Herbivore treatments included intact herbivore assemblages (all three herbivores) and three assemblages from which two non-target herbivores were regularly removed. Barnacle cover treatments as follows: full = no barnacle removal (black circles), half = barnacles removed from one side of plot (gray circles), none = all barnacles removed from plot (white circles). Analyses of microalgal and macroalgal percent cover used the full time series, which are presented in Appendix S2. Note the different y-axis scales in each panel.

pattern, there were large effects of herbivore composition and more subtle effects of barnacle cover on the timing of the decline and magnitude of the peak abundances (Fig. 1, Table 1). Microalgal cover was suppressed to lowest levels when all herbivores were present (richness effect, $P < 0.001$), only exceeding 50% cover in the absence of barnacles (richness \times type, $P = 0.02$). In all other herbivore treatments microalgal cover exceeded 50% at peak cover, regardless of barnacle treatment, and reached as high as 99% cover (Appendix S3: Plate S1). Microalgae established more slowly when both limpet species were removed than in plots with either or both limpet species, but these littorine-only plots also supported high cover of microalgae for longer than other herbivore treatments (Appendix S2: Fig. S1). Thus, succession proceeded more slowly when only littorines were

TABLE 1. Results of generalized additive mixed models describing the effects of herbivore removal and barnacle removal on percent cover of microalgae, ephemeral macroalgae, and perennial macroalgae.

Response Source	df	Microalgae		Ephemeral macroalgae		Perennial macroalgae	
		F	P	F	P	F	P
Herbivore treatment	3	28.94	<0.001	19.35	<0.001	5.09	0.002
Richness (3 species—1 species)	1	30.42	<0.001	12.10	<0.001	2.36	0.125
Group (Littorines—Limpets)	1	16.30	<0.001	32.83	<0.001	10.83	0.001
Limpets (<i>Lottia digitalis</i> — <i>L. scabra</i>)	1	8.18	0.004	8.83	0.003	2.16	0.143
Substrate treatment	2	14.55	<0.001	4.41	0.013	8.94	<0.001
Heterogeneity (half barnacles—others)	1	5.02	0.026	0.13	0.715	5.93	0.015
Type (full barnacles—no barnacles)	1	20.86	<0.001	8.82	0.003	13.62	<0.001
Herbivore × substrate	6	2.32	0.033	0.39	0.886	2.58	0.018
Richness × heterogeneity	1	3.03	0.083	0.43	0.515	6.76	0.010
Richness × type	1	5.47	0.020	0.30	0.743	5.28	0.022

Notes: Model terms were tested using Wald tests of significance, conditional on smoothing parameter estimates (not shown). Terms below treatment effects and the interaction are a priori Helmert contrasts to test specific hypotheses outlined in the *Methods*. Numerator degrees of freedom were the same in all three ANOVAs, and residual degrees of freedom were 439.9, 445, and 434.7, respectively. Plot was used as a random intercept to account for repeated measurements.

present. Barnacle-free plots supported slightly greater microalgal cover than plots with barnacles regardless of herbivore treatment (Fig. 1A; substrate type effect, $P < 0.001$).

Macroalgae established after several months, as expected, but establishment varied among herbivore treatments (Fig. 1B, Table 1; Appendix S2: Fig. S2). Early-successional macroalgae included species in the genera *Ulva* and *Pyropia* (formerly *Porphyra*). While *Ulva* appeared in many plots at three months, especially in littorine and *Lottia scabra* treatments, it was largely replaced by *Pyropia* after nine months. *Pyropia* cover increased dramatically at the 1 year mark in plots with littorines only (group effect, $P < 0.001$), while either limpet species alone (and in combination) completely suppressed cover of early successional macroalgae throughout the experiment.

Late successional, perennial macroalgae consisted almost entirely of *Mastocarpus papillatus*, and cover was determined by an interactive effect of the herbivore and substrate treatments (Fig. 1C, Table 1; Appendix S2: Fig. S3). Perennial cover increased with herbivore richness, but this effect depended on the heterogeneity and type of substrate present (Fig. 1C, Table 1; richness × heterogeneity, $P = 0.01$; richness × type, $P = 0.022$). Plots with all three herbivores had greatest perennial increased cover in plots with full barnacle cover, but similar high cover was observed in *Lottia digitalis* only plots with full barnacle cover (Fig. 1C). However, when *Lottia digitalis* was removed (i.e., *Lottia scabra* and littorine snail treatments) perennials established fastest in the heterogeneous substrate treatment. In the *Lottia scabra* treatment, this trend is possibly due to greater use of barnacle-free areas by *L. scabra* (see *Results* below), as well as lower mobility of *L. scabra* relative to *L. digitalis*. Although perennial algal cover was relatively low, this was also

case in all plots prior to manipulation, which was likely the result of high initial grazer density; so in at least some treatments, perennial algae recovered to pre-experiment levels. Thus, the rate of algal community recovery was determined by an interactive effect of herbivore richness and substrate heterogeneity.

Analysis of macroalgal distribution between barnacle and barnacle-free sides of the heterogeneous habitat treatment revealed two notable results (Table 2; Appendix S2: Fig. S4). First, ephemeral macroalgae only accumulated in treatments or parts of treatments where herbivory was reduced the most: when both species of limpet were removed and on the side without barnacles, where there were fewer littorines (Fig. 2B; herbivore × side, $P < 0.001$). Second, perennials only established on the side with barnacles in plots without *Lottia digitalis* (Fig. 2C; herbivore × side, $P < 0.001$). The littorine treatment provides some evidence for competitive suppression of perennials by ephemerals; on the side with barnacles, where littorine density was high, ephemeral cover was lower and correspondingly perennial cover was greater. Conversely, on the barnacle-free half, there were few littorines, greater ephemeral cover, and little perennial cover (Fig. 2).

Treatment effects on herbivore density

Herbivore removal treatments were generally effective, although all species fluctuated in abundance over the course of the experiment in unmanipulated treatments (Appendix S4: Fig. S1), as a result of natural seasonal recruitment and mortality patterns (Best et al. 2014). Limpet removals maintained near-zero densities of removed species throughout the experiment. This resulted in total limpet densities (*Lottia digitalis* + *L. scabra*) in intact herbivore assemblages being initially higher than

TABLE 2. Results of generalized additive mixed models describing the effects of herbivore removal and side of plot on percent cover of microalgae, ephemeral macroalgae, and perennial macroalgae within the half barnacle treatment.

Response Source	df	Microalgae		Ephemeral macroalgae		Perennial macroalgae	
		F	P	F	P	F	P
Herbivore treatment	3	7.94	<0.001	7.48	<0.001	0.51	0.675
Richness (3 species—1 species)	1	12.51	<0.001	6.17	0.014	0.02	0.875
Group (Littorines—Limpets)	1	0.02	0.883	9.12	0.003	0.12	0.729
Limpets (<i>Lottia digitalis</i> — <i>L. scabra</i>)	1	4.94	0.027	7.40	0.007	1.37	0.243
Plot side (bare—barnacles)	1	7.24	<0.001	0.04	0.851	90.74	<0.001
Herbivore × side	3	0.42	0.740	6.27	<0.001	20.37	<0.001
Richness × side	1	0.63	0.083	1.42	0.235	4.94	0.027

Notes: Model terms were tested using Wald tests of significance, conditional on smoothing parameter estimates (not shown). Terms below herbivore treatment and the interaction with plot side are a priori Helmert contrasts to test specific hypotheses outlined in the *Methods*. Numerator degrees of freedom were the same in all three ANOVAs, and residual degrees of freedom were 293.9, 303.7, and 304.1, respectively. Plot was used as a random intercept to account for repeated measurements.

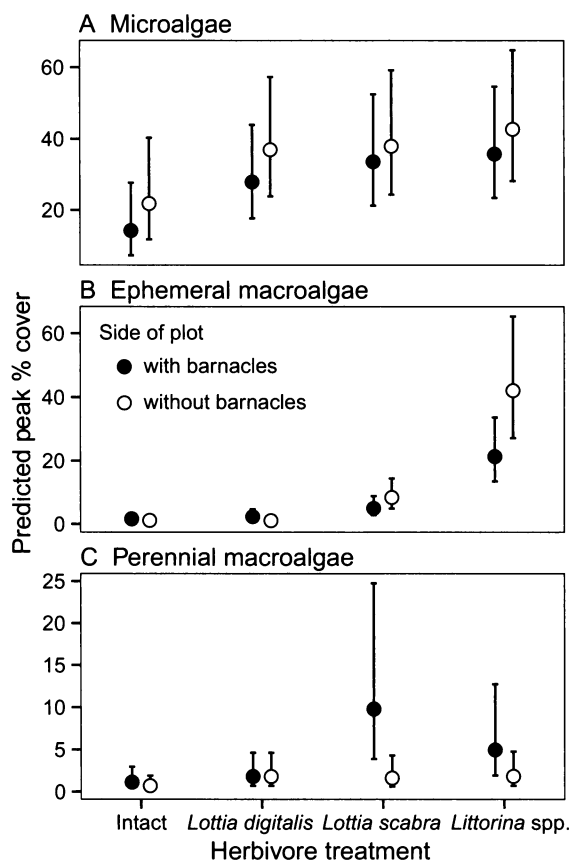


FIG. 2. Peak percent cover (mean ± 95% confidence interval) of microalgae (A), ephemeral macroalgae (B) and perennial macroalgae (C) in the half barnacle treatment predicted by generalized additive mixed models. Here, barnacles were removed from one half of the plot and macroalgal cover is shown on both sides with (black circles) and without (white circles) barnacles. Herbivore treatments included intact herbivore assemblages (All three herbivores) and three assemblages from which two non-target herbivores were regularly removed. Analyses of algal cover used the full time series, which are presented in Appendix S2. Note the different y-axis scales in each panel.

in assemblages in which either species of limpet was removed, but this difference declined to zero by the end of the experiment (Appendix S4: Table S1, Fig. S1). This suggests that the remaining species compensated numerically for the removal of its congener.

Littorine snail removal treatments reduced littorine density, but less persistently than for limpets. Treatments were maintained by snail removal every 2–4 weeks, and our independent experiments examining littorine recolonization found that it took four weeks for littorines to recover to pre-removal density in areas with full barnacle cover (Appendix S1: Fig. S1). Littorines also increased in abundance over time across all treatments, and there appeared to be only modest differences among grazer treatments in littorine densities during the second half of the experiment during a period of high littorine abundance (Appendix S4: Fig. S1).

Effects of herbivore density on algal abundance

Herbivore density did not affect macroalgal responses differently in different treatments. However, limpet and littorine densities did affect microalgae differently among treatments (Fig. 3). To isolate the effect of grazer richness versus abundance, we ran models with a restricted dataset that (1) excluded the treatment where both limpet species were removed (i.e., littorine only treatment), and (2) excluded the final two sampling dates (microalgal cover was zero in most plots at that point in the experiment). Because herbivore densities were centered in these models, our parameter estimates for herbivore treatments effects compare microalgal cover at the median number of limpets and littorines found across all treatments with limpets (10 limpets and 35 littorines per plot).

The best supported model included interactions between herbivore densities and herbivore treatment (Appendix S4: Table S2). In intact herbivore assemblages with both limpet species density of limpets and littorines

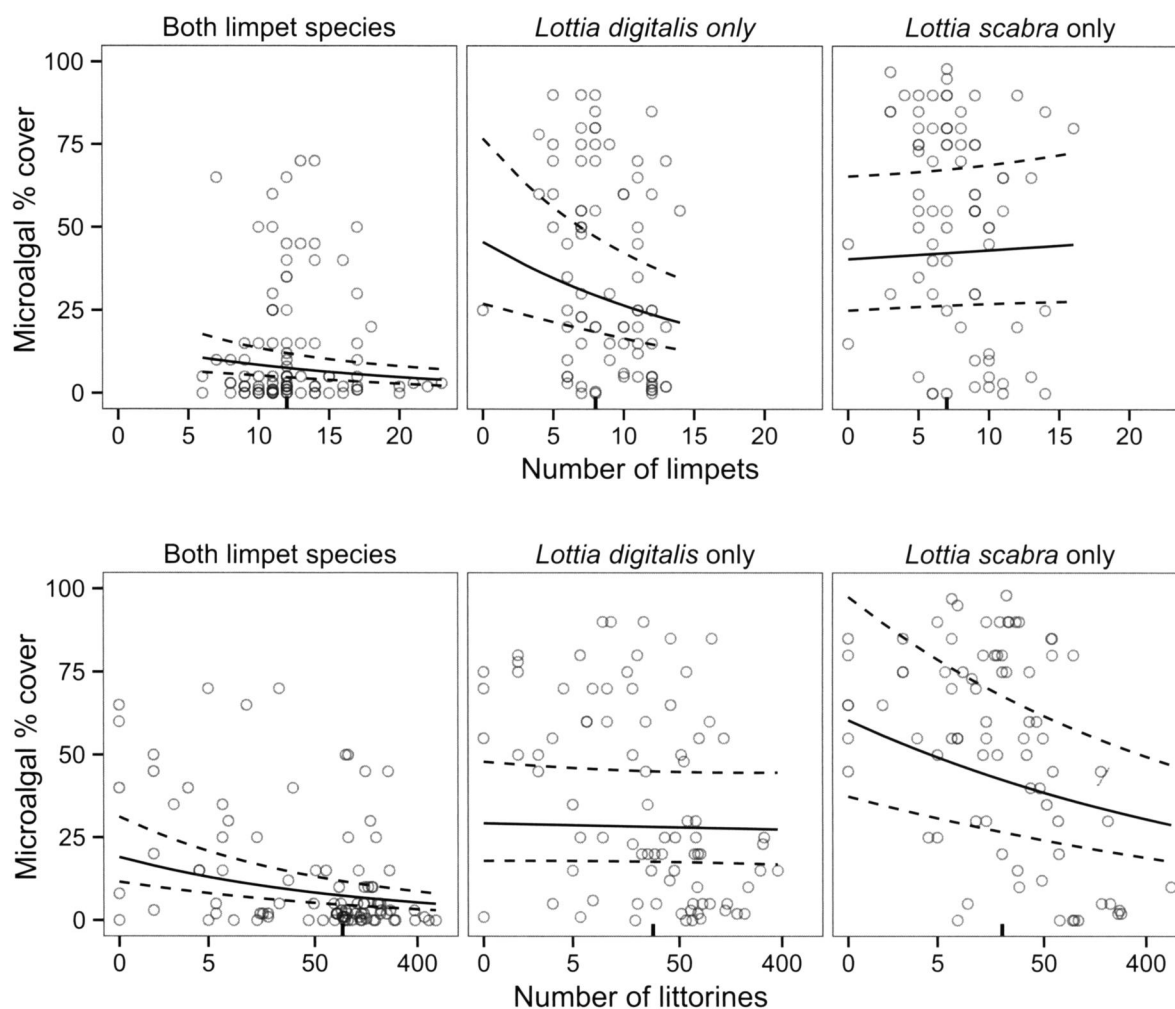


FIG. 3. Effects of limpet and littorine density on microalgal percent cover in treatments with at least one limpet species. Microalgal cover was predicted as a function of limpet density, littorine density, herbivore treatment, and their interactions using a generalized linear mixed model (log link) with random intercepts for sampling dates and plots. Because densities of both limpets and littorines were included in the model, we show effects of density of each herbivore group at the median density of the other group in a particular herbivore treatment, indicated by the black bar above the x -axis in each panel. Dashed lines show 95% confidence interval around the slope. Note the log scale for predictions across littorine densities.

had a modest negative effect on microalgae, and these effects weakened in treatments with a single limpet species (Fig. 3; Appendix S4: Table S3). We also found an interaction between limpet and littorine densities, such that the effect of one herbivore's density on microalgae weakened (became more positive) as the other herbivore increased in density. These effects of density, however, did not fully explain differences in microalgal cover across treatments, and we still detected an effect of grazer diversity on microalgae independent of herbivore density (Fig. 3). At the median densities of limpets and littorines across all treatments, microalgal cover in plots with both limpet species was roughly one-third of that in plots with one limpet species ($z = -6.77$, $P < 0.001$; Appendix S4: Table S3), which suggested that microalgal grazing by the different herbivores is complementary in some way.

Microhabitat use by grazers

Within heterogeneous plots, the abundances of herbivores in each half of the plot varied among species, illustrating differential habitat use (Fig. 4; Appendix S4: Table S4). Littorines were found more often on the side of the plot with barnacles ($P < 0.001$), regardless of whether limpets were removed or not. Further, littorine densities in plots with no barnacles were on average 27% of the density found in plots containing barnacles (Appendix S4: Fig. S1, Table S1). *Lottia digitalis* showed no difference in substrate use (49% on side with barnacles, $P = 0.9$), while *Lottia scabra* was more often found on the side without barnacles ($P < 0.001$). *Lottia scabra* tended to be found on the side without barnacles more often in intact herbivore assemblages

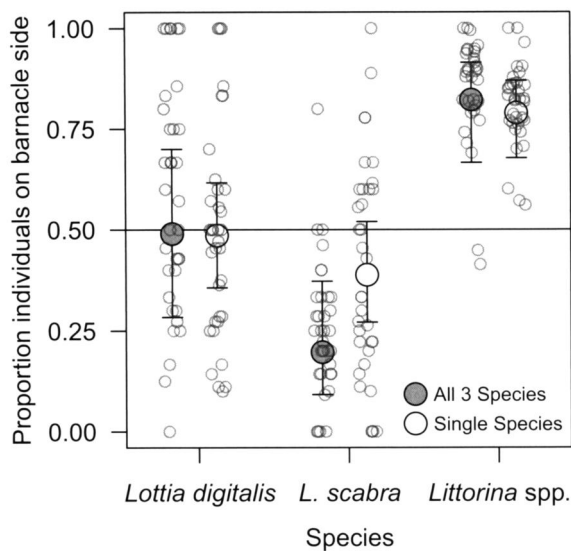


FIG. 4. Herbivore locations in the heterogeneous substrate treatment throughout the experiment. Data, shown in open circles, are proportion of each herbivore species on the side of plot with barnacles, along with model predictions and 95% confidence intervals for each species' location either within intact herbivore assemblages (filled grey circles) or in treatments where the other two herbivores were removed (filled white circles). Herbivore densities on each side of the plot were modeled using a single generalized linear mixed model (see text and Appendix S4), but we display the data here as proportions. The horizontal line represents an equal proportion of individuals on each side of the plot, or equal use of habitat types. Data points are jittered to reduce data overlap.

than in treatments where other species were removed (20% vs. 40% on side with barnacles, $P = 0.03$), suggesting that the presence of competitors may influence this species' habitat use.

DISCUSSION

Previous studies suggest that species' niche differences might only be expected to influence ecosystem processes when environmental heterogeneity creates opportunity for these differences to be expressed (Stachowicz et al. 2008b, Weis et al. 2008). The simple intertidal community we investigated features three herbivore taxa whose habitat-use traits relate well to variation in the spatial features of their rocky landscape, as was evident in their differential use of barnacle-covered and bare rock substrates (Fig. 4). We found evidence for greater herbivore impact on algae when all species of herbivore were present, driven both by strong effects of particular species (Fig. 1B, C) and complementary effects of several species (Figs. 1A, C, and 3). The effect of habitat on grazer-algal interactions was more complex than we initially expected; some, but not all, of these diversity effects were influenced by habitat heterogeneity. We explain these results in more detail herein, emphasizing the role of habitat type and heterogeneity in mediating the complex

interactions between herbivore diversity and the abundance and composition of the primary producer community.

In early successional communities of ephemeral microalgae, the three herbivore species mixture reduced algal cover to the lowest level, regardless of habitat type. While some of this effect was due to different limpet and littorine densities across herbivore treatments, in both treatments in which one limpet species was removed the remaining limpet species compensated numerically (Appendix S4: Fig. S1). Given equivalent total limpet density, neither limpet species alone reduced microalgal cover to levels as low as both species in combination, even after accounting for an influence of littorine density (Fig. 3), suggesting that numerical compensation did not lead to full compensation in grazing function. This might be attributable to complementary feeding strategies of the two limpets. The greater mobility and larger size of *Lottia digitalis* likely translates to a greater spatial extent of grazing but lower intensity, whereas grazing by *Lottia scabra* is intense but spatially restricted due to its territoriality. Limpet effects on microalgae were largely independent of habitat type, but this was not the case for littorines. When barnacles were removed the density of littorine snails was reduced and microalgae covered an additional 30% of plot surfaces, even when both limpet species were present (Fig. 1A). Thus, habitat composition enhanced the extent to which all three species combined to maintain low microalgal cover. Together these results suggest that all three herbivore species contribute uniquely to controlling microalgal abundance, with an influence of habitat type, but little influence of habitat heterogeneity per se.

In contrast, later successional macroalgae were influenced most strongly by a single species, the habitat generalist, *Lottia digitalis*. Specifically, these algae were restricted to the habitat (barnacles; Farrell 1991) that offered the maximum possible refuges from this herbivore when it was present. However, when *L. digitalis* was removed (*L. scabra* and littorine monocultures), the abundance of perennial macroalgae was greatest in the heterogeneous substrate treatment (Fig. 1C). Within these heterogeneous plots, total macroalgal abundance (perennials + ephemerals) was greatest on the barnacle side for *L. scabra* and greatest on the bare side for littorines (Fig. 2B, C), which inversely corresponded to the distribution of these species among habitat types (Fig. 4). This suggests that heterogeneity may mitigate effects of grazer loss on algal recovery by allowing the expression of niche differences among remaining community members (*L. scabra* and littorines) that are weaker interactors. Our finding that species differed in how their grazing responded to habitat heterogeneity emphasizes that the implications of diversity loss for ecosystem functioning depend on whether species lost are habitat specialists or generalists. Complementarity may emerge in heterogeneous environments only after generalists or keystone species are lost from communities. In kelp forests, for

example, complementary effects of invertebrate predators may only become important for suppressing herbivores in the absence of large vertebrate predators such as sea otters (Byrnes et al. 2006).

Importantly, our results also suggest that the effects of species loss depend critically on environmental characteristics (Allison 2004, Duffy 2009, Griffin et al. 2009, O'Connor et al. 2015, but see Mrowicki et al. 2015). If we had conducted our experiment only on bare rock, for instance, we would have concluded that the two limpet species had redundant effects on perennial algal recovery and only weak complementary effects on microalgae. Yet in barnacle-covered and heterogeneous plots stronger evidence of complementarity emerges. While the complexity of interactions we observed makes it difficult to generalize, this difference may contribute toward explaining why different experiments reach such different conclusions about the mechanisms underlying diversity effects (see, e.g., Stachowicz et al. 2008a, b, Mrowicki et al. 2014, 2015).

The match of organism body size, mobility, and behavior to the scale of substrate heterogeneity should be an important determinant of how consumers influence their resources. We manipulated heterogeneity on a scale that is commonly found in the field. Patches with and without barnacles occur adjacent to one another at scales of 10s of centimeters in our system, well within the crawling range of intertidal gastropods (J. Stachowicz, *unpublished data*). Thus, the heterogeneity in our experiments likely modifies herbivore impacts in natural systems. In terrestrial systems, variation in topography on comparable spatial scales creates sufficient environmental heterogeneity to increase the benefit of growing diverse stands of crops (reviewed by Brooker et al. 2015). More generally, spatial environmental heterogeneity can increase diversity by providing space in which each species has the advantage over others (Chesson 2000, Adler et al. 2013); this would likely lead to greater total production in diverse stands with a heterogeneous environment, although field tests of this at the proper scale are few. Just as niche differentiation alone does not guarantee high local diversity (Chesson 2000), trait diversity in the absence of resource diversity or environmental heterogeneity need not lead to enhanced functioning.

Biologically relevant heterogeneity can also occur at different scales. At sub-centimeter scales, empty barnacle tests or gaps between individuals provide shelter for littorine snails, increasing their grazing impact. At the scale of our plots, the treatment of barnacles, bare rock, or a 50/50 mix determined the scale at which some species were complementary. Diversity itself influenced habitat complementarity in heterogeneous plots; the presence of *Lottia digitalis* enhanced habitat partitioning of *Lottia scabra* and littorine snails (Fig. 4; Appendix S4: Table S4). Other aspects of complementarity appeared independent of substrate heterogeneity and instead were the result of different foraging strategies among species that may be consistent across habitats. At even larger spatial

and temporal scales, seasonal variation and rock topography have been implicated as underlying the complementarity among seaweed species in their effects on biomass accumulation (Stachowicz et al. 2008b).

Experimental tests such as ours that focus on environmental heterogeneity in one or a few niche dimensions (e.g., habitat) have demonstrated that heterogeneity affects the diversity-ecosystem function relationship (Griffin et al. 2009, Cardinale 2011). Yet the effects of even the simple form of heterogeneity considered here were complex. If niche separation and coexistence often (if not always) occur simultaneously along multiple niche axes, it follows that environmental heterogeneity relevant to ecosystem functioning should also be multidimensional and as challenging to characterize as the niche itself. However, it is clear that effects of diversity on ecosystem processes depend not just on trait variation, but also on the existence and scale of variation in environmental conditions. Ecologists are beginning to investigate relationships between biodiversity and multiple ecological functions at larger scales (e.g., Pasari et al. 2013, Angelini et al. 2015), and we predict that empirical studies that explicitly incorporate biologically relevant forms and scales of environmental heterogeneity will provide the most useful advances in our understanding of the feedbacks between biodiversity and environment (Loreau et al. 2003, Duffy 2009).

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