

## DIVERSITY ENHANCES COVER AND STABILITY OF SEAWEED ASSEMBLAGES: THE ROLE OF HETEROGENEITY AND TIME

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**Abstract.** Generalizations regarding the mechanisms underlying the effects of plant diversity on ecosystem processes, and whether the patterns transcend study systems remain elusive. Many terrestrial plant diversity manipulations have found that plant biomass increases with diversity, but most marine studies find little or no effect of seaweed diversity on producer biomass or production. However, differences in experimental approach (field vs. mesocosm) and duration (years vs. weeks) between published terrestrial and marine experiments confound the interpretation of these differences in response to changing diversity. We conducted a three-year field manipulation of seaweed diversity on intertidal rocky reefs in central California, USA, to examine the effect of diversity on seaweed cover. We found that diversity increased standing algal cover and decreased the availability of free space relative to monocultures, but this effect took nine months to materialize. Furthermore, diverse assemblages did not consistently exceed the best performing monocultures until 18 months after the experiment was initiated, suggesting that the effect of diversity strengthens over time. Overall, diversity's effect was consistently stronger than that of individual species and not attributable to the influence of any particular species (sampling effect) because (1) polycultures eventually achieved higher cover than even the best performing monoculture and (2) monocultures rarely differed much, precluding a strong sampling effect. Instead, mechanisms such as facilitation and differential use of microhabitats in a heterogeneous environment likely caused the higher cover in polycultures. Our findings contrast with short-term experiments with other seaweeds but are similar to longer-term experiments with terrestrial plants, suggesting that experimental design and approach, rather than inherent differences between marine and terrestrial ecosystems, underlie contrasting responses among systems. We argue that experiments conducted in the field, and for a greater length of time, allow for the manifestation of a greater number of potential mechanisms ofoveryielding in diverse communities, increasing the likelihood of observing a strong diversity effect.

*Key words:* algae; compensation; diversity; field experiment; heterogeneity; intertidal; seaweed; stability.

### INTRODUCTION

The relationship between species diversity and ecosystem properties such as stability, productivity, and resistance to perturbation has long been a topic of theoretical interest to ecologists (Elton 1958, May 1974). In recent years, however, accelerating loss of biodiversity due to human activities has renewed interest in what the actual functional consequences of biodiversity in ecosystems might be. Additionally, the recognition that natural clines in diversity across environmental or biogeographic gradients could produce parallel gradients in productivity or stability has led to a broadening of the scope of this research beyond applied conservation concerns.

An influential series of field experiments, conducted primarily in terrestrial grasslands, demonstrated that the

identity and number of plant species in a system could affect the production and accumulation of biomass (Tilman 1999, Loreau et al. 2002, Hooper et al. 2005). However there is still considerable debate over the mechanisms most responsible for these effects and their importance relative to other known drivers of ecosystem functioning. Complementary roles played by different species are often cited as the mechanism underlying diversity–productivity relationships, however a recent meta-analysis argues that the available data often do not generally support this claim (Cardinale et al. 2006). Instead, Cardinale et al. (2006) argue that current experimental results are most consistent with greater function in diverse communities being driven by sampling effects, the greater likelihood of diverse communities containing species that have dominant effects on ecosystem function (Huston 1997). However, Cardinale and colleagues also point out that the limited temporal and spatial scale of many experiments may reduce the likelihood of complementary properties of

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species being expressed. While the absolute scale of an experiment itself may not be important, increasing plot size should increase heterogeneity in resources or environmental conditions that would promote the expression of species differences and lead to diversity effects via resource use complementarity (Cardinale et al. 2004). Indeed, meta-analysis suggests that the strength of overyielding due to complementarity increases with experimental duration (Cardinale et al. 2007 and references therein), although few experiments are conducted for sufficiently long periods of time to provide an adequate test of this hypothesis.

A controversy also exists over whether the nature and magnitude of the effect of diversity, are consistent across ecosystems. Averaged across all experimental manipulations, there is no difference in the effect of diversity in terrestrial and aquatic systems (Cardinale et al. 2006). However, in contrast to the consistent effect of terrestrial plant diversity (Cardinale et al. 2006), only roughly half of seaweed diversity manipulations found evidence for an effect of richness on biomass or production, and these effects were generally very weak (e.g., Bruno et al. 2005, 2006; see review in Stachowicz et al. 2007). It is possible that this marine-terrestrial difference is evidence that ecosystem production or biomass may be influenced more by herbivores or predators than by plant diversity in marine systems (Duffy 2002).

Alternatively, there are several methodological differences between terrestrial plant and marine algal experiments that might contribute to these contrasting findings. Terrestrial experiments are typically established by sowing seeds in desired combinations into plots of bare ground (e.g., Hector et al. 1999, Tilman 1999) and occasionally by removal of species from established plots of natural vegetation (e.g., Lyons and Schwartz 2001). The response is measured in terms of biomass production over the course of one to several growing seasons. In contrast, seaweed diversity manipulations often involve transplanting adults onto an artificial substrate and tracking their growth or photosynthetic rate in mixtures and monocultures (e.g., Bruno et al. 2005, 2006; but see Allison 2004). This is in part due to the relative ease of transplanting seaweeds (which lack roots) and the rapid growth rate of individuals, and in part due to the difficulty of culturing them from spores due to their complex life histories which often involve multiple, morphologically or ecologically distinct, phases, making "seeding" very difficult. Regardless, as a consequence of this difference, seaweed diversity experiments typically measure growth rates of the individuals they transplant, rather than population-level growth, which includes clonal spread and sexual recruitment. This approach could restrict the opportunities for species to respond to interspecific interactions and environmental heterogeneity, potentially limiting the expression of niche differences among species.

In particular, seasonal variation can be an important part of the mechanism underlying diversity effects in other systems (Stachowicz et al. 2002, Fargione and Tilman 2005), and this variation might be missed in studies of short duration. Furthermore, substratum heterogeneity, like heterogeneity of soils for plants in terrestrial systems, can influence seaweed species composition and performance (e.g., Lubchenco 1983, Airoldi 2000). Assembling seaweed communities on artificial substrates that purposefully lack such heterogeneity may reduce the ability for niche differences to be expressed. We know of only one study examining the community consequences of seaweed diversity (functional group richness) on unaltered natural substrate (Allison 2004). This study did find significant effects of richness on the resistance and resilience of the seaweed assemblage to disturbance (extreme heat stress), although the lack of replication of all groups in monoculture precluded a complete assessment of the relative strength of diversity and identity and the mechanisms underlying any diversity effects.

In this study, we used a field-based, removal approach to manipulate seaweed species richness on natural rocky substrate over the course of three years. On mid-high intertidal portions of rocky reefs in central and northern California, four common perennial taxa comprise >85% of seaweed cover and are typically organized in a patchwork mosaic that appears to be maintained by a combination of disturbance, competition, and herbivory (Sousa 1984, Foster et al. 1988, 1991; but see Foster et al. 2003). These four species represent a diversity of morphologies, life history strategies, functional groups, and evolutionary lineages: the turf forming red alga *Endocladia muricata*, the foliose red alga *Mastocarpus papillatus*, the canopy forming brown alga *Pelvetiopsis limitata*, and the turf forming green alga *Cladophora columbiana*. In some locations with a smooth, steep gradient of tidal exposure, species sort out into horizontal bands. However, waves, sea spray, and a heterogeneous rock substrate blur or eliminate these patterns over much of these species' ranges (Foster 1990, Foster et al. 2003). The relatively low species diversity of this system, and the fact that monocultures or near monocultures do exist in some locations, allows us to assess the consequences of changing of diversity at levels that occur across natural environmental gradients. Understanding the influence of species diversity and composition on seaweed cover is important for understanding overall community structure because seaweed cover (1) regulates the space available for colonization by sessile invertebrates, microalgae, and other macroalgae; (2) is an important determinant of the structural complexity of the habitat and microenvironmental conditions; and (3) represents the primary food resource for the local food web (Foster et al. 1988, 1991).

Here we report the effect of algal diversity on the standing amount of, and variability in, seaweed cover over time, as measures of ecosystem productivity and

stability, respectively. We then compare the strength of the effect of diversity with that of species composition to facilitate comparisons with previous studies. Finally, we use data on the total cover and seasonal phenology of each species in monoculture and polyculture to evaluate potential mechanisms underlying the effects of seaweed richness. We compare our findings both with other long-term field based studies of terrestrial plant communities and previous shorter-term studies using seaweeds.

## METHODS

### *Study system*

The study was conducted at Bodega Marine Reserve, California (38°19'12" N, 123°4'24" W). We selected the mid-high intertidal zone mainly for logistical reasons: the long duration of exposure of this zone to air was sufficient to allow enough time for the extensive plot establishment and maintenance activities required of the experiment. Most of the seaweed cover (>85%) is composed of the four perennial seaweeds mentioned previously; the remainder of the cover is mostly ephemeral species, especially *Ulva* spp. and *Porphyra* spp. Although these ephemeral species can be locally or seasonally abundant, the maintenance of monocultures of either of these species would have been impossible due to strong seasonality in their abundances. Nevertheless, these species may contribute significantly to the short-term production of these communities on an episodic basis, so we quantified the abundances of these (and all other) seaweeds in our plots. Abundant sessile invertebrates in this area included the California mussel (*Mytilus californianus*) and several barnacle species (e.g., *Chthamalus dalli*, *Balanus glandula*). There was also a diverse mobile invertebrate epifauna including snails, limpets, chitons, crabs, amphipods and polychaetes that live amongst the seaweed. Animal response to algal diversity and composition will be reported separately (J. Stachowicz and A. Chaudoin, unpublished data).

### *Plot establishment and censusing*

In March 2004, we established 72 1.5-m circular plots in the mid-high intertidal zone. These plots are large relative to the size of the dominant seaweeds allowing hundreds of individuals per plot. We located plots on relatively level rock surfaces (avoiding areas with vertical slopes or large tidepools), though all rock surfaces did contain natural roughness and heterogeneity. In May 2004, and again in July 2004, we estimated percent cover of seaweeds and sessile invertebrates to establish baselines for each plot. We assessed percent cover using a modified version of a random point quadrat to sample a circular plot. The perimeter of a circular hoop of 1.5 m diameter was divided into 17 equally sized sections. A random point was selected within each of these sections to serve as the terminus for a line radiating from a ring in the center of the circle. The circle was then divided into six concentric rings of

equal area, and we chose one randomly selected point along each radial line within each concentric ring to produce a total of 102 randomly selected points that were distributed throughout the 1.5 m diameter sampling area to ensure equal sampling intensity of the entire plot. To estimate percent cover in each plot, we centered the hoop on the plot, and at each of the predetermined sampling points, we recorded all species that were present in both the canopy and understory (thus percent cover could be >100%). After the July 2004 census, plots were sorted into 12 blocks of six plots each. Where possible, assignment of plots to blocks was conducted using natural geographic features. For example, if six plots were near each other on a single reef, they were assigned to a single block. However, a few larger reefs contained up to 18 plots. In those cases, we used hierarchical agglomerative cluster analysis (Ward's method, Euclidean distance measure) to assign plots to blocks based on similarities in the cover of target seaweed species, mussels (*Mytilus californianus*), and unoccupied space. Plots were then randomly assigned within each block to one of six treatments: a *Pelvetiopsis* monoculture, a *Mastocarpus* monoculture, an *Endocladia* monoculture, a *Cladophora* monoculture, a four-species polyculture consisting of only the four target seaweed species, and an unmanipulated control.

Treatments were initiated in August and early September 2004 by removing all non-target seaweeds from each plot. For monocultures this meant removing all seaweeds except one target species, whereas in the polycultures all seaweeds except the four target species were removed. We quantified seaweed wet and dry tissue mass (60°C to constant mass) removed from each plot to establish the relationship between percent cover and biomass. Because the seaweed biomass removed from the polyculture and some monoculture plots (e.g., *Pelvetiopsis*) was often less than that removed from other plots, we removed additional biomass of target species from these plots to ensure that there were no differences between treatments with respect to biomass loss or disturbance associated with seaweed removal. In polycultures, this meant removing an amount of all four seaweed species to achieve a similar biomass removal to monocultures; extra biomass removal in polycultures was distributed among the four species in proportion to their abundance in the plot. As a result, after the application of treatments through the initial weeding, neither biomass removed nor post-weeding percent cover differed among treatments (ANOVA for cover,  $F_{4,55} = 0.571$ ,  $P = 0.68$ ; for biomass,  $F_{4,55} = 1.71$ ,  $P = 0.16$ ). Because of seaweed regrowth from holdfasts and recruitment of new individuals, we continued to weed plots throughout the experiment to maintain the treatments. If amounts removed differed among treatments, we also continued to remove additional biomass of target seaweed species to equalize the effects of disturbance due to biomass removal and avoid confounding diversity and composition treatments with

disturbance. By summer 2006, weeding consisted mostly of removing ephemeral seaweeds from plots, and few adjustments to equalize biomass removed across treatments were required.

The experiment thus began as a replacement-type experimental design in which abundance of each species was lower in polyculture than monoculture to allow all plots to begin with equivalent cover. We recognize that this type of design can conflate increasing diversity with reductions in intraspecific density, posing problems for the interpretation of results (e.g., Benedetti-Cecchi 2004). Manipulating abundance factorially with diversity to control for this was not possible because maintaining abundance at prescribed levels would have compromised the interpretation of responses in algal cover, which was our primary response variable. However, because external recruitment and vegetative spread occurred for all species, abundances of target species in polyculture could increase throughout the experiment eliminating initial differences in intraspecific densities in monocultures vs. polycultures. Indeed, for much of the experiment after year 1, cover of individual species in polyculture differed little from that in monoculture (see *Results*), suggesting little change in the strength of intraspecific interactions within increasing diversity.

We sampled plots quarterly (January, April, July, and October) from October 2004 to July 2007 for algal cover using a 1 m diameter hoop that was otherwise identical in design to the one described already. This left a 25-cm radial buffer around the edge of each plot to minimize edge effects. Hoops were rotated for each sampling period to provide a new set of random points relative to permanent plot markers. Because of the strong linear correlation between biomass and percent cover (removed biomass (in kilograms) =  $0.0513 \times$  surveyed percent cover;  $R^2 = 0.69$ ), we continued measuring percent cover only to avoid destructive sampling that might influence the effectiveness or outcome of the treatments. At two different time periods during the summer of 2007, we also estimated desiccation rates in each plot by measuring mass loss of  $2 \times 2 \times 1$  cm agar cubes (batch recipe: 14 g agar + 1 L water) with an initial mass of 4 g, either on bare rock, on turf algae, or under foliose algae during the low tide. Four cubes were placed in each plot, two on bare rock and two on or under seaweed. In monocultures, the two "seaweed" cubes were placed either on (turf species) or under (foliose species) the target seaweed; in polycultures, one cube was placed under each of the foliose species (*Mastocarpus* and *Pelvetiopsis*). We recorded the weather (foggy vs. sunny) and measured change in mass of each cube over the duration of one low tide.

#### *Statistical analyses*

We used repeated-measures analysis of variance (RM ANOVA) to assess the effect of time, block, and weeding treatment on total seaweed cover. Our intent in

replicating treatments through blocking was solely to account for spatial heterogeneity in background environmental conditions. We assume the lack of a block  $\times$  treatment interaction and we use the mean-square error as our error term for testing our treatment effects. We qualitatively evaluated this assumption by examining the ordering of treatments within each block using cell mean plots (Quinn and Keough 2002). Because the assumption of sphericity was never met, we reduced our degrees of freedom using the Greenhouse-Geisser adjustment (Quinn and Keough 2002). Where there was a significant time  $\times$  treatment interaction, we ran individual ANOVAs for each time period, and adjusted significance levels using a sequential Bonferroni procedure (e.g., Quinn and Keough 2002). In these individual ANOVAs, we partitioned the treatment sums of squares into an *a priori* contrast between the polyculture and the monoculture treatments (richness effect,  $df = 1$ ; see also Bruno et al. 2005). The remaining treatment sums of squares is then due to variation among monocultures, and thus consists of a test of variation in cover due to species identity ( $df = 3$ ). Significance of richness and identity effects was assessed using the error MS from each individual ANOVA as the denominator in the  $F$  test. To compare the relative effect size of richness and identity we calculated omega-squared ( $\omega^2$ ) for each (Graham and Edwards 2001). We also tested whether (1) the polyculture treatment differed from the unmanipulated control and (2) the polyculture differed from the best performing monoculture with Tukey's *hsd* or Ryan's  $Q$  post hoc tests (Quinn and Keough 2002). We tested whether seaweed species diversity and identity exhibited greater variation in seaweed cover by comparing the coefficients of variation (CV) of the cover of the four manipulated species over time in polycultures and monocultures. We used data from April 2005 to April 2007 for these analyses to avoid including the large variation in cover immediately after the initial weeding and establishment of treatments.

#### RESULTS

Polycultures achieved higher total cover, and had less bare rock, than any of the monocultures, though significant diversity effects did not materialize until nine months after initial application of treatments (Fig. 1). Repeated-measures ANOVA indicated a significant time  $\times$  treatment interaction ( $F_{55, 594} = 3.24$ ,  $P < 0.0001$ ) as well as main effects of time ( $F_{11, 44} = 14.7$ ,  $P < 0.0001$ ), block ( $F_{11, 54} = 2.48$ ,  $P < 0.0001$ ), and treatment ( $F_{5, 54} = 17.0$ ,  $P < 0.0001$ ) on total seaweed cover (Fig. 1A;  $df$  presented are uncorrected; Greenhouse-Geisser adjustment results in  $\epsilon = 0.65$ , all  $P$  values still  $< 0.0001$  with correction). We found similar effects of treatment and time on bare space (Fig. 1B; time  $\times$  treatment interaction,  $F_{55, 594} = 1.69$ ,  $P = 0.002$ ) as well as main effects of time ( $F_{11, 44} = 5.23$ ,  $P < 0.0001$ ), block ( $F_{11, 54} = 3.69$ ,  $P = 0.0006$ ), and treatment ( $F_{5, 54} = 8.01$ ,  $P < 0.0001$ ;  $\epsilon = 0.49$ , all  $P$  values still  $\leq 0.02$  with correction).

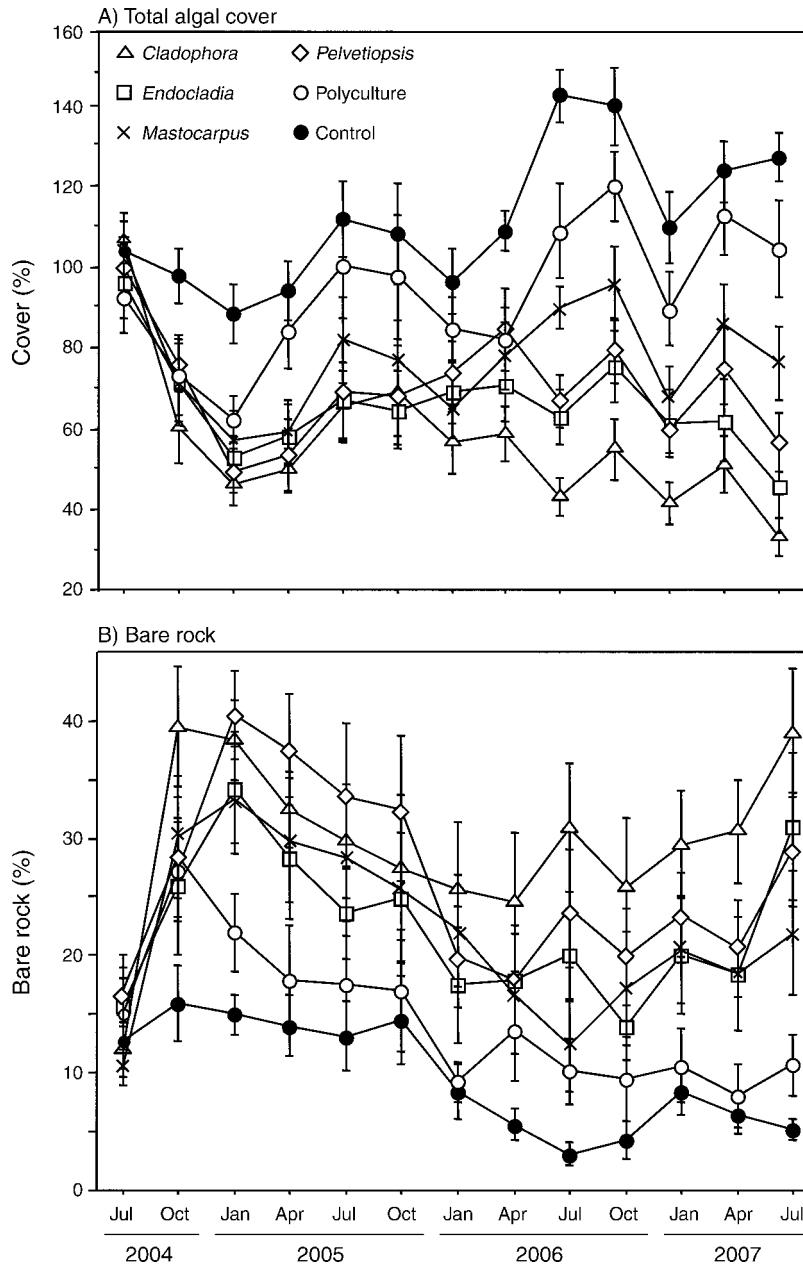


FIG. 1. Time series comparing (A) total seaweed cover and (B) bare rock in polycultures, controls, and monocultures. Points and error bars indicate means  $\pm$  SE. Note that (1) the difference between polyculture and monocultures widens over time and (2) the identity of the best performing monoculture also changes over time. Control designates plots that were not manipulated.

One replicate of a polyculture treatment was obliterated by seal haul-out early in the experiment, and is omitted from the analysis.

To better visualize the effects of diversity relative to species composition, we plotted total seaweed cover as a function of species richness and composition for the late summer/fall of each year, when biomass was at its seasonal maximum (Fig. 2). These figures illustrate that the strength of the diversity effect increases over the duration of the experiment from no effect in 2004, just

months after establishment, to a maximum in 2006 and 2007.

To examine how the effect of algal diversity varied over the course of the experiment in more detail, we conducted individual ANOVAs on each sampling date. Partitioning the effect of treatment at each sampling date into the variation among monocultures (species identity) and between monoculture and polyculture (diversity) confirmed that the effect of diversity was strong and consistent relative to that of species identity,

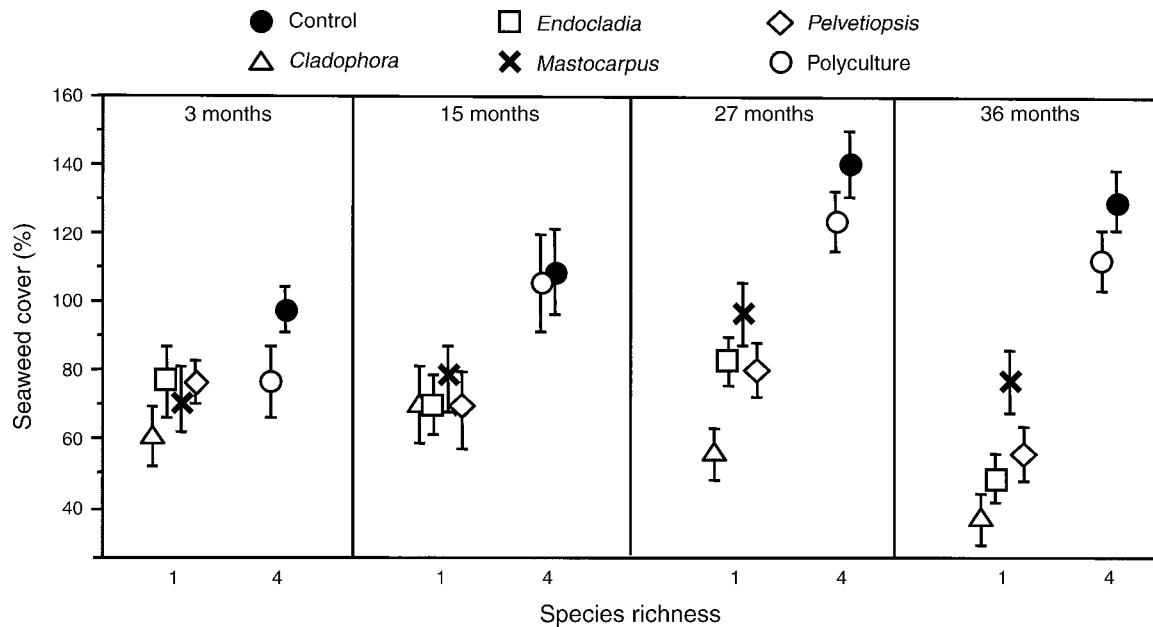


FIG. 2. Seaweed cover (mean  $\pm$  SE) as a function of algal species richness and composition (species in key) after 3, 15, 27, and 36 months.

which was weaker and often not significant (Fig. 3). We also calculated  $\omega^2$  for the block effect and found that for most sampling dates after April 2005 the effect of diversity was at least as strong as, and often stronger than, the block effect, especially toward the end of the experiment (April 2005, July 2006–July 2007, Fig. 3). Given that blocks reflected natural variation in cover among plots due to unmeasured factors such as wave exposure, bare space, and cover of mussels, the magnitude of the diversity effect relative to the block effect indicates the high importance of diversity in explaining variation in this system.

We used post hoc tests to further evaluate differences among treatments within each sampling date. Comparing each monoculture to the polyculture provided a crude test of the sampling effect. Beginning in April 2005, the polyculture diverged significantly from the monocultures, and was no longer different from the control ( $P > 0.05$ , Ryan's  $Q$ ). From July of 2005 to January of 2006, seaweed cover in the polyculture exceeded all but one of the monocultures, though the identity of the best performing monoculture differed among sampling dates (Table 1). Between April 2005 and the end of the experiment, total seaweed cover did not differ between polyculture and control (LS means contrast test,  $P > 0.10$ ), except in April and July of 2006 ( $P < 0.01$ ). In April of 2006, there was an unexplained loss of cover in a few of the polyculture plots, but by July, the polyculture returned to high cover levels, where it remained greater than any of the monocultures through the end of the experiment (Figs. 1 and 2, Table 1). Applying a sequential Bonferroni adjustment to control for having performed separate tests on each

sampling date did not change any of our conclusions; whenever diversity significantly influenced cover it did so with  $P \leq 0.001$ , and even the most extreme  $P$  value for diversity effects would have been tested at  $0.05/12 = 0.004$ . On two dates, block effects that were significant with unadjusted  $P$  values, were no longer significant after adjustment.

Agar blocks placed in the plots on a sunny day in September 2007 to measure desiccation rates lost less mass in polycultures than all other treatments (Fig. 4). Both position (in algae < bare rock,  $F_{1,264} = 55.7$ ,  $P < 0.0001$ ) and the treatment ( $F_{5,264} = 5.39$ ,  $P < 0.0001$ , polycultures < each monoculture) had a strong effect on water loss. There was no interaction between the two factors ( $F_{5,264} = 1.92$ ,  $P = 0.10$ ), although differences among treatments in water loss from agar blocks placed on or under algae (open bars, Fig. 4) were less than those on bare rock (solid bars). There was also a block effect ( $F_{11,264} = 5.63$ ,  $P < 0.0001$ ), but no strong interactions between block and other variables were apparent. Additional trials performed on foggy days showed no effect of treatment on desiccation under these conditions, due to low rates of mass loss (mean  $\pm$  SE across all treatments on foggy days =  $0.016 \pm 0.009$  g/h vs.  $0.121 \pm 0.007$  g/h for sunny days).

Weeding to remove perennial competitors only slightly increased the cover of the target seaweed species in the monocultures (Fig. 5). Using repeated-measures ANOVA for each species, we compared the cover of particular species in that species' monoculture with its cover in polyculture. All four species showed a strong effect of time ( $P < 0.0001$  in all cases) and no effect of treatment (mono vs. polyculture vs. control;  $P > 0.10$ ).

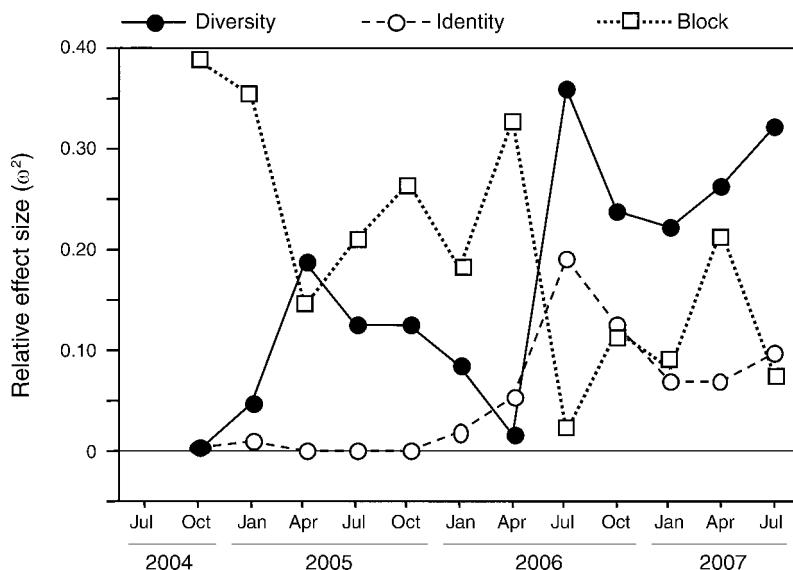


FIG. 3. Relative effect size ( $\omega^2$ ) of seaweed species richness and identity on total seaweed cover from orthogonal partition of “treatment” effects in ANOVA conducted on each date. Block effect size is presented for comparison.

There was an interaction between time and treatment for *Mastocarpus* and *Pelvetiopsis* ( $P = 0.009$  and  $0.0001$ , respectively, after Greenhouse-Geisser adjustment,  $\epsilon = 0.44\text{--}0.52$ ), due to a seasonal increase in the cover of these species in monoculture relative to the polyculture (Fig. 5C, D). This was confirmed by Tukey post hoc tests run after individual ANOVA on dates where treatment effects were significant at  $P < 0.05$  prior to correction sequential Bonferroni correction ( $P < 0.05$ , monoculture  $>$  polyculture during January–April 2006 for *Pelvetiopsis*; April–October 2006 for *Mastocarpus*). Individual ANOVAs for each sampling date rarely showed significant treatment effects once the sequential Bonferroni correction was applied, but such corrections may be overly conservative. There was no time  $\times$  treatment interaction for *Endocladia* ( $P = 0.63$ ), though there was a trend for greater *Endocladia* cover in monoculture in summer 2006, and only a weak interaction for *Cladophora* ( $P = 0.065$ ;  $P = 0.14$  after G-G adjustment). On balance, these data suggest that individual species are only minimally able to compensate for the loss of other species, and when compensation occurs, it is seasonally restricted.

Among the treatments, the coefficient of variation in cover (CV) was lowest in the polycultures, intermediate in *Mastocarpus* and *Pelvetiopsis*, and greatest in *Cladophora* and *Endocladia* (Fig. 6). Partitioning the effects of treatment between richness and identity, we found strong effects of richness ( $F_{1,42} = 11.6$ ,  $P = 0.001$ ) and only marginally significant effects of identity ( $F_{3,42} = 3.05$ ,  $0.05 < P < 0.10$ ), and no effect of block ( $F_{11,42} = 0.91$ ,  $P = 0.54$ ). This likely reflects clear seasonal trends in perennial cover in monocultures of *Pelvetiopsis*, *Endocladia*, and *Mastocarpus* that were absent in the polyculture (Fig. 5). For example, *Mastocarpus* consistently reached a seasonal maximum in July in both 2005 and 2006 (Fig. 5D), whereas *Pelvetiopsis* had a less well-defined summer maximum, but a clear winter minimum (Fig. 5C). The high CV of *Cladophora* was probably due to the low mean cover of this species rather than its variability (Figs. 5 and 6). However, identical results were obtained for other metrics of variability (Gaston and McCardle 1994) including standard deviation of log-transformed cover and the average deviation among successive time periods.

TABLE 1. Results of post hoc Ryan’s  $Q$  tests of individual ANOVA on each sampling date.

Species	2004			2005			2006				2007		
	Oct	Jan	Apr	Jul	Oct	Jan	Apr	Jul	Oct	Jan	Apr	Jul	
<i>Cladophora</i>	A	A	<b>B</b>	B	B	B	A	<b>C</b>	<b>C</b>	<b>B</b>	<b>C</b>	<b>C</b>	
<i>Endocladia</i>	A	A	<b>B</b>	B	B	AB	A	<b>BC</b>	<b>BC</b>	<b>B</b>	<b>BC</b>	<b>BC</b>	
<i>Mastocarpus</i>	A	A	<b>B</b>	B	B	AB	A	<b>BC</b>	<b>BC</b>	<b>B</b>	<b>BC</b>	<b>B</b>	
<i>Pelvetiopsis</i>	A	A	<b>B</b>	AB	AB	B	A	<b>B</b>	<b>B</b>	<b>B</b>	<b>B</b>	<b>BC</b>	
Polyculture	A	A	A	A	A	A	A	A	A	A	A	A	

Notes: Different letters indicate significant differences in means among treatments ( $P < 0.05$ ). Boldface type indicates dates when the polyculture cover was greater than the best monoculture.

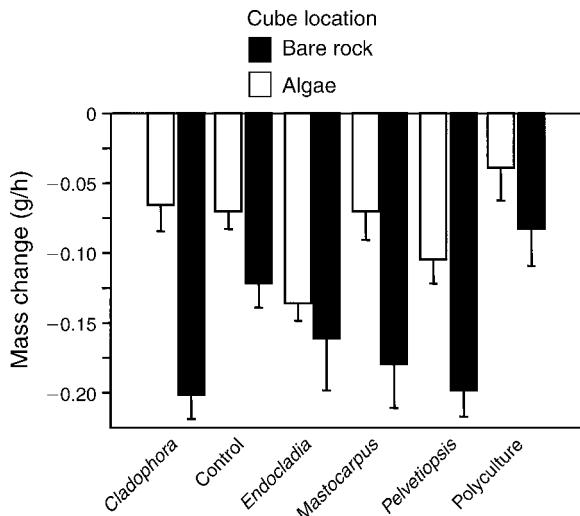


FIG. 4. Effect of algal species richness and identity on desiccation on a sunny day, measured as mass loss of agar cubes placed in each plot either on bare rock or on algae. ANOVA finds significant effects of both treatment (polyculture > all monocultures, with controls as intermediate) and position (bare rock > on or under algae), but no interaction between the two factors. Data presented are means and SE.

DISCUSSION

Previous studies have demonstrated strong effects of grazers and abiotic conditions on seaweed cover in the rocky intertidal (see review in Menge and Branch 2001). Our field experiments showed that even in the context of natural levels of grazing and variation in environmental conditions, diversity increased standing seaweed cover and decreased the availability of free space to a level virtually indistinguishable from unmanipulated control plots. This is consistent with the idea that diversity increases recovery after disturbance. The magnitude of the diversity effect also increased as the experiment progressed (Fig. 3), a pattern also found in some terrestrial experiments (Tilman et al. 2002, Cardinale et al. 2007) and models (Cardinale et al. 2004). The effect of diversity was consistently stronger than that of species identity (Fig. 3) and not attributable to the strong effect of any particular species (sampling effect) because polycultures achieved higher cover than even the best-performing monoculture (Figs. 1 and 2, Table 1). Instead, the data suggest that mechanisms such as niche partitioning or facilitation underlie the higher cover in polyculture. Regardless of the specific mechanism, seaweed cover in this habitat has important, cascading effects on the structure and composition of the rest of the intertidal community through effects on (1) availability of primary space for colonization by

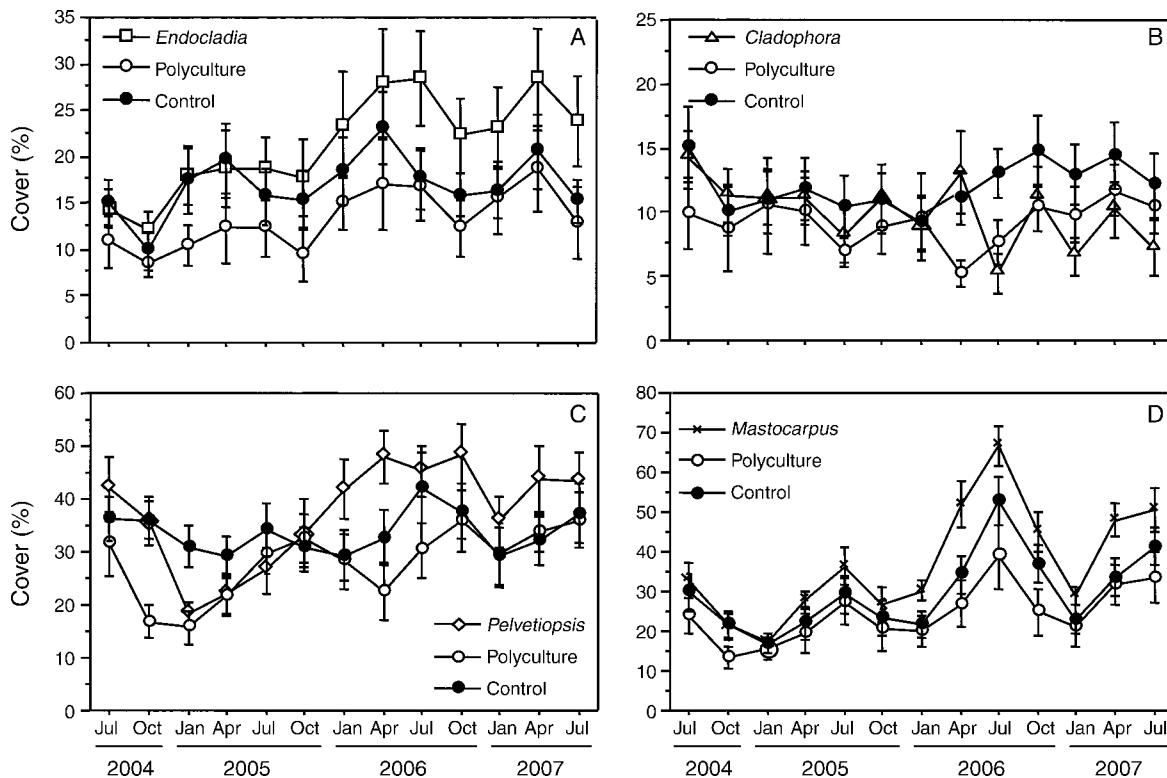


FIG. 5. Cover (mean ± SE) of each target seaweed in polyculture and in monoculture over the course of the experiment to illustrate the degree to which individual species compensate for the removal of other species.

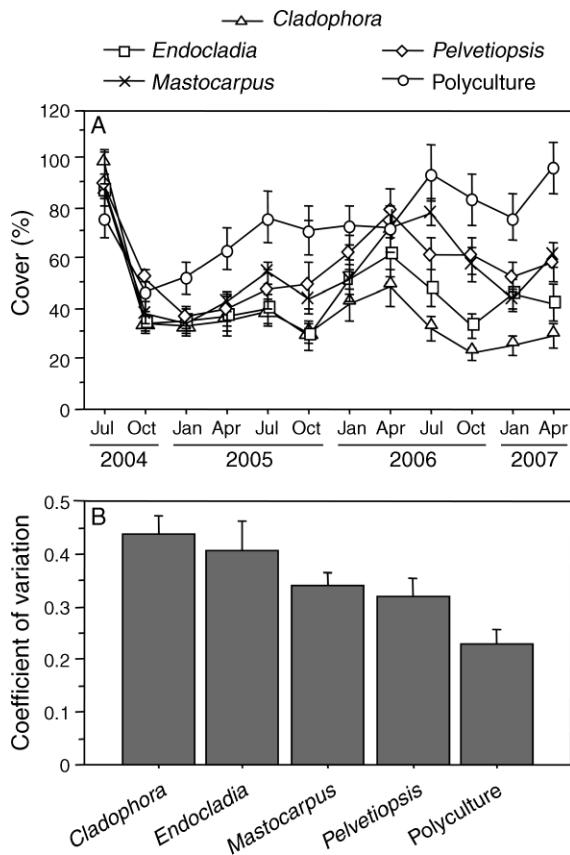


FIG. 6. (A) Time series of the cover (mean  $\pm$  SE) of perennial seaweeds (the four target species: *Pelvetiopsis*, *Mastocarpus*, *Endocladia*, and *Cladophora*) as a function of treatment. (B) coefficient of variation (CV) over time in perennial seaweed cover (mean  $\pm$  SE) as a function of treatment. CV data are calculated from April 2005 to April 2007 to avoid effects of initial biomass loss during treatment application.

other macroalgal taxa as well as sessile invertebrates, (2) microclimatic conditions and structural complexity of the habitat, and (3) the availability of food resources for the local food web (e.g., Dean and Connell 1983, Foster et al. 1988, 1991, 2003, Bertness et al. 1999). Because our plots started out with equal cover, and cover and biomass were closely correlated, the effect of diversity likely applies to production as well as biomass. Thus we suspect that the effects of diversity on community organization extend far beyond effects on seaweed cover.

Our results differ from those of most published experiments assessing the effect of seaweed diversity on productivity or biomass in that most find weak effects of richness and stronger effects of species identity (see review in Stachowicz et al. 2007). A recent meta-analysis across terrestrial and aquatic habitats (Cardinale et al. 2006) detected more consistent richness effects that the authors argued were largely explained by the strong effects of particular species present in polyculture

(sampling effects). The meta-analysis concluded that relatively few studies provided solid evidence for consistent diversity effects not attributable to sampling effects. In contrast, our data provide clear evidence of such effects, and below we discuss several mechanisms that could underlie these effects and why these may not have been detected in other seaweed diversity manipulations. We avoided using the sampling vs. complementarity partition (e.g., Loreau and Hector 2001) because of difficulties in calculating both (1) expected yield, due to the long duration of the experiment and the large (unmeasured) amount of external recruitment, and (2) relative yield due to negative production values for some time periods in monoculture. Nonetheless, our data showed only weak differences in cover among monocultures, precluding a strong sampling effect.

#### Temporal complementarity

Differences in seasonality among the species in our experiment could contribute to greater total cover (and lower variability) in polycultures relative to monocultures. In the second year of the experiment (2005–2006), the difference between the polyculture and the best monoculture, though sizable, was often not statistically significant (Table 1). However, the identity of the best performing monoculture varied among seasons (in year 2, summer = *Pelvetiopsis*; winter = *Mastocarpus*; Table 1). Differences among species in seasonality can produce a form of temporal complementarity, which produces greater cover in polyculture than any one monoculture when integrated across the entire year (see also Stachowicz et al. 2002, Fargione and Tilman 2005). Seasonality in seaweed abundance or reproduction is common, yet temporal complementarity would only be manifest when experiments are conducted over a sufficiently long period of time or across a sufficiently heterogeneous environment to allow species differences to be expressed. Indeed, a combined analysis of individual studies that each found weak or no effects of diversity on total biomass or production at a single location or point in time showed that diverse mixtures exhibit less variability in production across environmental gradients than monocultures (Kertesz 2006).

#### Spatial heterogeneity and niche partitioning

Seaweed cover in polycultures was greater than all monocultures from July 2006 through the remainder of the experiment. Temporal complementarity cannot explain why polycultures consistently outperform the best monoculture on a given sampling date, and thus other mechanisms such as spatial niche partitioning, complementary resource use, or facilitation warrant consideration. Some data suggest that each of the four species preferentially occupy somewhat different microhabitats, partially defined by microtopographical heterogeneity. For example, there is correlative evidence that *Cladophora* is restricted to moister microclimates than the other species (Doty 1946, Bracken et al. 2007),

and in our plots *Cladophora* was mostly restricted to cracks and small depressions that help retain moisture at low tide. Correspondingly, *Cladophora* cover in monoculture was never different from controls (Fig. 5), despite the presence of considerable bare space (Fig. 1B), indicating that other seaweeds did not limit *Cladophora* abundance in our plots.

Each of the other three species did increase in cover in the monocultures relative to the controls, although this expansion was often short-lived and seasonal, generally with a summer maximum and winter minimum. Seasonal patterns in seaweed cover in monoculture could reflect expansion into suboptimal habitats during favorable periods (summer) and contraction during less favorable periods (winter). For example, *Pelvetiopsis* appears restricted to microtopographical high spots, and often develops rotting scars or lesions when excessively submerged (J. Stachowicz, *personal observation*; C. Hays, *personal communication*), possibly due to infection by pathogens (see Rugg and Norton 1987 for *Pelvetia canaliculata*). Additionally, both *Pelvetiopsis* and *Mastocarpus* experience seasonal contractions in cover that could be due to removal of large individuals by winter storms (Carrington 1990, Wolcott 2007) or extreme temperatures during winter midday low tides (e.g., Sousa 1979). *Endocladia* was the only species that achieved consistent expansion relative to the control and polyculture, but still 20–25% of the rock surface was bare, indicating that plenty of space went uncolonized by macroscopic organisms, even after three years. The data suggest that most species are only marginally capable of expanding into suboptimal microhabitats, meaning that diversity could promote greater and less variable seaweed cover by ensuring consistent occupation of distinct microhabitats. The consistently low availability of bare space in both polycultures and controls supports this idea (Fig. 1B) and is consistent with other experiments showing that species diversity reduces resource availability (e.g., Stachowicz et al. 2002, Fargione et al. 2003).

Microhabitat complementarity does not preclude the operation of other mechanisms. For example, mortality due to desiccation on sunny days could be mitigated by reduced water loss in the polycultures relative to monocultures (Fig. 4), which might lead to greater rates of survival of seaweed early-life history stages and thus more rapid recruitment or regeneration. Complementarity in nutrient utilization is also a possible mechanism for greater resource use and productivity in polycultures, as previous studies find differences among species in affinity for different forms of nitrogen (Bracken and Stachowicz 2006). Because our plots spend significant time each day in air and water (>50% in air on average; exact durations vary with lunar phase), complementarity could also result because some species (e.g., *Mastocarpus* [Bell 1993]) have greater rates of photosynthesis in water than air, whereas others (e.g., *Endocladia* [Johnson et al. 1974]) show opposite patterns.

#### *How general are our findings?*

The strong effect of diversity in our experiments contrasts with findings of other experiments with seaweeds in marine systems (review in Stachowicz et al. 2007). Exploring the differences between our experiment and other studies may shed light on the mechanisms by which diversity does and does not affect seaweed cover. Most previous experiments on the effects of seaweed diversity on production or biomass have been far shorter than our experiment (weeks to a few months). Thus, most measure growth rates of transplanted individuals, rather than population level growth, which includes clonal spread and sexual recruitment. Some of the mechanisms we suggest above could apply equally to individual or population responses (complementarity of photosynthesis in air vs. water; complementary nutrient preferences), but others (microhabitat preferences, temporal complementarity, reduced desiccation leading to greater survival of recruits) are more likely to operate in experiments such as ours that follow populations over time and allow external recruitment (see also Allison 2004). Further testing is needed, but the contrast between our results and those of most previous seaweed diversity manipulations suggests that diversity may have a stronger effect on population-level response variables that are expressed over the longer-term under heterogeneous field conditions.

Had we run our experiment for less than nine months, we would have found no difference among treatments in total seaweed cover (Fig. 1). Further, “transgressive overyielding” in which mixtures outperform all monocultures did not occur until 18 months. The time required in our study system to detect these effects could be a consequence of the colder water and exposure to air, which resulted in slower growth rates relative to previous experiments performed subtidally and in warmer climates. However, we suspect that the effect of diversity in our experiments is more a consequence of (1) the spatial and temporal heterogeneity that occurred within individual plots and (2) that we allowed sufficient time for natural recruitment and clonal spread to occur across this heterogeneous landscape. We suspect that had we performed this experiment on smooth artificial substrates in the field, at a location with smooth rock composition (e.g., sandstone), or for a very short period of time we would have reached similar conclusions to previous studies. That substrate heterogeneity limits species’ ability to form high-cover monocultures is consistent with our own observations and those of others who report greater predominance of monocultures that form distinct tidal bands at sites that lack small-scale heterogeneity in the rock surfaces (see, e.g., Doty 1946, Foster 1990). Additional investigation is needed to test this idea, but mathematical modeling efforts (Cardinale et al. 2004) and meta-analysis of previous experiments (Cardinale et al. 2007) are consistent with the ideas that (1) the effect of diversity should increase with increasing experimental duration

and (2) that increasing spatial scale should increase the strength of diversity due to the inclusion of a greater diversity of patch types, suggesting that this could be a general phenomenon.

The role of heterogeneity in determining the diversity–function relationship remains to be conclusively and explicitly demonstrated, but if confirmed, this could help resolve the conditions and scales over which diversity plays an important role in determining ecosystem functioning, ultimately leading to increased predictive ability. Indeed explicit recognition of the role of environmental heterogeneity has helped reconcile the effect of scale on the relationship between species diversity and invasibility (Davies et al. 2005). Focusing on the role of heterogeneity in space and time may help reconnect diversity–function studies with the study of species coexistence and niche theory from which they developed.

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