Journal of Ecology

British Ecological Society

Journal of Ecology 2011, **99**, 445–453 doi: 10.1111/j.1365-2745.2010.01767.x

Seagrass genotypic diversity increases disturbance response via complementarity and dominance

A. Randall Hughes[1]* and John J. Stachowicz[2]

[1]*Coastal and Marine Laboratory, Florida State University, 3618 Highway 98, St. Teresa, FL 32358, USA; and*
[2]*Department of Evolution and Ecology, University of California-Davis, One Shields Avenue, Davis, CA 95616, USA*

Summary

1. Genetic diversity, like species diversity, can enhance resistance or resilience to perturbation. However, we know little about how disturbance intensity affects this relationship or what mechanisms underlie the positive effects of genetic diversity.

2. We experimentally tested the independent and interactive effects of seagrass genotypic diversity (two levels) and disturbance (three levels) on seagrass biomass in a 2-year field experiment.

3. Our results indicate that genotypic diversity enhances seagrass resilience from experimental biomass removal, but only at the highest level of disturbance; in the absence of disturbance, monocultures out-perform polycultures over the short term.

4. Following recovery from the planned experimental disturbance, a natural macroalgal bloom caused a loss of seagrass shoots in our plots. In this case polycultures lost fewer shoots than monocultures (i.e. were more resistant to the disturbance), and this positive effect of genetic diversity persisted until the end of the experiment (1 year in total, including 6 months after all plots had recovered to pre-disturbance densities). At the end of the 2-year experiment, polycultures had higher shoot density and above-ground biomass than monocultures.

5. The positive effects of diversity on shoot density and biomass were caused by both trait-independent complementarity (TIC; due to differential resource use among clones) and positive dominance (due to one genotype achieving high density in both monoculture and polyculture).

6. *Synthesis*. Our results confirm that genetic diversity, like species diversity, can influence disturbance response and does so via similar mechanisms. They also highlight that over longer time frames, these effects are likely to result from a complex mix of dominance and complementarity mechanisms that depend on the traits of the specific taxa involved and the response variables of interest.

Key-words: aquatic plant ecology, biodiversity, community genetics, compensation, disturbance–stability, ecosystem function, eelgrass, genotypic diversity, primary production, resilience, *Zostera marina*

Introduction

The relationship between biodiversity and stability has long interested ecologists, generating a large body of empirical and theoretical research. Although still controversial, the empirical evidence suggests that species diversity most often influences stability by reducing realized disturbance (Hughes *et al.* 2007) via a variety of mechanisms. For example, the 'insurance hypothesis' posits that more diverse communities are more likely to contain taxa capable of withstanding or surviving a given disturbance that can compensate for those that are more susceptible (Tilman 1996; Yachi & Loreau 1999). In this case, these taxa are expected to increase in relative abundance following disturbance, as is the case in the sampling effect in experiments of biodiversity and ecosystem function (Huston 1997; Tilman, Lehman & Thomson 1997). Additionally, diversity may be associated with an increased range of life-history or resource-use traits that enhance the response to disturbance via complementarity or facilitation (Mulder, Uliassi & Doak 2001).

To date, the majority of empirical studies of the diversity–disturbance relationship have focused on diversity at the level of species, yet the mechanisms thought to be responsible for diversity–stability relationships are not specific to any particular taxonomic level (Norberg *et al.* 2001). The operation of these mechanisms depends only on taxa varying in some

*Correspondence author. E-mail: rhughes@bio.fsu.edu

functionally relevant manner; this sort of variation is certainly not restricted to the species level, as studies show that phenotypic variation within species can be as large as that between species (Bangert *et al.* 2005; Shuster *et al.* 2006). In addition, genetic diversity has demonstrated population- and community-level ecological effects similar to those of species diversity (Hughes *et al.* 2008).

Although the number of examples is small, the available data suggest that genetic diversity can also influence population stability by reducing realized disturbance (Schmitt & Antonovics 1986; Boles, Thoendel & Singh 2004; Hughes & Stachowicz 2004; Reusch *et al.* 2005) by increasing resistance (decreased biomass loss) or resilience (recovery to pre-disturbance conditions). In marine systems, two independent studies have shown that genotypic diversity in seagrass (*Zostera marina*) enhances the resistance and resilience of this system to natural disturbances (Hughes & Stachowicz 2004; Reusch *et al.* 2005), and that these positive effects of diversity can be detected in natural populations of *Zostera* (Hughes & Stachowicz 2009). However, these studies involved natural, uncontrolled disturbances, leaving open the question of how seagrass genotypic diversity (and genetic diversity, more generally) influences system response across a range of disturbance levels. Additionally, these studies were relatively short in duration, spanning only a single growing season. In this paper, we examine the effects of seagrass genotypic diversity on seagrass density and biomass in response to experimentally imposed disturbances over the short term (weeks to months) and to larger natural disturbances over the long term ($>$ 1 year). We hypothesized that genotypic diversity would have a positive effect on the response of seagrass plots to experimentally imposed disturbance. However, we expected that these differential responses to disturbance would be temporary, resulting in equivalent performance between monocultures and polycultures at the end of a longer period of time (2 years).

Eelgrass (*Z. marina*, *'Zostera'* henceforth) is a model system for testing the interaction between genotypic diversity and disturbance for a number of reasons. First, *Zostera* reproduces sexually as well as clonally, generating considerable variation in genotypic diversity at small scales in natural populations (Reusch 2001; Hughes & Stachowicz 2009). In addition, genotypic diversity in these populations is important not only for the seagrass itself, but it also influences the abundance and diversity of organisms that rely on it for habitat (Hughes & Stachowicz 2004, Reusch *et al.* 2005). Furthermore, seagrasses are subjected to a wide variety of natural and anthropogenic disturbances, such as grazing (by invertebrates, fishes and birds), storm events, eutrophication, boat grounding and dredging (Short & Wyllie-Echeverria 1996; Orth *et al.* 2006). These varied and common disturbances represent a major threat to seagrasses, which are currently declining throughout their range (Waycott *et al.* 2009). Understanding the role of seagrass genotypic diversity in the response to and recovery from disturbance is increasingly important for the conservation of these valuable habitats.

Materials and methods

STUDY SYSTEM

Bodega Harbor is located *c.* 100 km north of San Francisco Bay, CA, USA. It is a *c.* 2 km^2 marine embayment that is largely flushed on each tidal cycle by a central, dredged shipping channel. *Zostera* is common throughout the low intertidal and subtidal regions of the harbour. This experiment was conducted within an existing seagrass bed on the south-western side of the harbour.

Previous experimental manipulations of genetic diversity in *Zostera* beds (Hughes & Stachowicz 2004; Reusch *et al.* 2005) relied on field-collected clones that were identified using molecular markers, yet this process introduces a number of difficulties. First, the genotyping process is costly and time-consuming. More importantly, however, it is difficult to find adequate numbers of shoots of the same clone to include each clone in both monoculture and polyculture to allow inferences about mechanisms underlying diversity effects. Further, using field-collected shoots from different areas means that genotypic effects may be confounded with local environmental acclimation. To minimize these complications, we propagated eight known genotypes in 'common garden' 300-L outdoor flow-through mesocosms at Bodega Marine Laboratories (see Hughes *et al.* (2009) for details on clonal propagation).

EXPERIMENTAL DISTURBANCE MANIPULATION

We used shoots from the clones propagated in the laboratory to conduct a factorial field experiment in which we manipulated seagrass genotypic diversity (one or six genotypes) and disturbance (0%, 33% or 66% of shoots clipped) and monitored shoot density over time. Shoot density is a non-destructive sampling technique, allowing us to assess how results change over time while avoiding the introduction of additional disturbance that could obscure our disturbance treatments. Shoot density is positively correlated with above-ground biomass in this system (R. Hughes, unpublished data), so it is a reasonable measure of biomass production. We also destructively sampled all plots at the end of the experiment.

The genotypic diversity treatments tested in our experiment fall within the range found in natural *Zostera* populations in Bodega Harbor of 1–15 genotypes m^{-2} with a mean of 4.1 (Hughes & Stachowicz 2009). Because the best way to differentiate between mechanisms of complementarity and selection effects is to compare genotype performance in monoculture and polyculture (Loreau & Hector 2001; Fox 2005), we planted three monocultures (one for each disturbance treatment) of each of the eight genotypes used in the experiment. For the polyculture treatments, we randomly generated eight different combinations of six genotypes and planted one replicate of each per disturbance treatment; by chance, one genotype was present initially in all polyculture treatments. Thus we had replicates of monocultures and polycultures, but no replication of specific compositions within each level of disturbance. We did this because we had limited numbers of shoots available for each genotype, and because in this experiment we were most interested in whether monocultures differed from polycultures rather than comparing the performance of specific genotypes or combinations of genotypes. However, at the end of the experiment, there was no effect of disturbance on any metric of performance, and we removed this factor from the analysis, giving us $N = 3$ per genotype combination in the final analysis. We used six genotypes in our polyculture treatment, because this diversity was well within the range observed in natural populations (i.e. we did not bias our results by using an extremely high diversity) and because this

allowed us to have high diversity plots that varied in genotypic composition.

All treatment plots began with six transplanted shoots, regardless of whether it was a monoculture or polyculture. Limitations on the number of shoots available per genotype necessitated these low initial densities; plots reached densities comparable to surrounding natural seagrass beds in the second year of the experiment (see Results). We also created control plots that received no transplants to estimate natural recruitment of shoots into bare space over the course of the experiment.

In May 2005 we cleared 64.1-m^2 plots in 4 rows of 16 plots (separated by at least 2 m) in an existing seagrass bed in Bodega Harbor. We then buried 16.2 cm high × 41.3 cm wide × 59.7 long containers into each plot and filled them with sieved sediment to exclude all seeds and below-ground vegetation and ensure that there were no differences in the infaunal invertebrate community at the start of the experiment. In preliminary trials, shoot production rates did not differ inside versus outside containers (mean = 1.1 shoots transplant^{-1} month^{-1} inside containers vs. 0.9 shoots transplant^{-1} month^{-1} outside; *t*-test, $P = 0.39$). Treatments were assigned to plots in a randomized complete-block design, with two plots of each disturbance × diversity combination and four control plots per block. Experimental shoots were transplanted into the plots in June 2005, and shoot densities were monitored every 2–4 weeks for the duration of the experiment (June 2007). In November 2005, all plots were surrounded by four 2-m tall pieces of PVC to prevent shoot removal by geese that could confound interpretation of the experiment.

We evaluated the effects of genotypic diversity on the response to disturbance by applying three levels of above-ground biomass removal in January 2006 (8 months post establishment). The removal of above-ground biomass was chosen specifically to simulate grazing by geese, a natural and significant disturbance in Bodega Harbor (Hughes & Stachowicz 2004), although above-ground biomass removal is a common thread among many seagrass disturbances (Short & Wyllie-Echeverria 1996; Orth *et al*. 2006). The specific disturbance levels correspond with variation in goose grazing observed in a previous experiment (Hughes & Stachowicz 2004). Although plots were still increasing in shoot density by vegetative propagation at the time of the experimental disturbance, we wanted to perform the disturbance treatment in an ecologically relevant season (i.e. when goose grazing typically occurs) and before the recruitment of new genotypes by seed (March–April) that could potentially compromise diversity treatments. In early January, we quantified shoot density in each plot; these data were then used to calculate the number of shoots that represented 0%, 33% or 66% of the above-ground biomass. We haphazardly selected the appropriate number of shoots in each plot and removed biomass by manually clipping shoots to the sediment surface. In addition to standardizing the amount of biomass removed within a treatment level, our experimental disturbance allowed us to avoid confounding non-consumptive effects (fertilization, sediment disturbance) that are specific to herbivores, since we wanted to assess whether there is a general effect of diversity on disturbances that causes loss of above-ground biomass.

EXPERIMENTAL DISTURBANCE MANIPULATION: STATISTICAL ANALYSES

We first assessed the effectiveness of our disturbance treatment by conducting a factorial ANOVA on the number of shoots 2 weeks following the experimental disturbance, with block, diversity and disturbance intensity as fixed factors and including all possible interactions. We included pre-disturbance shoot density as a covariate (see Hughes & Stachowicz 2004); in these and subsequent analyses incorporating covariates, our data met the assumption of homogeneity of slopes. To assess the influence of diversity on recovery from disturbance, we performed a repeated-measures ANOVA on all sampling dates during the recovery period (12 weeks), using shoot density 2 weeks following the disturbance as our covariate. There was a significant diversity × disturbance interaction in the repeated measures analysis (Table 1), so we examined the effects of diversity at each sampling date (4, 6, 10 and 12 weeks post disturbance) using planned independent contrasts to compare monoculture and polyculture performance for each disturbance level. Our analysis did not meet the assumptions of sphericity, so we used the Greenhous–Geisser (G–G) adjustment to reduce our degrees of freedom when evaluating time effects. Because there were not significant interactions between diversity and/or disturbance with time, we present data from 6 weeks post disturbance to illustrate the effects of diversity on recovery from disturbance. In these and subsequent analyses, we excluded one plot from our analyses in which *Zostera* transplants failed to establish.

NATURAL DISTURBANCE EVENT

Twelve weeks following our experimental disturbance (April 2006), there were no longer any differences in shoot density due to disturbance, diversity or their interaction (Table 1). Several months later (August 2006), an unexpected loss of shoots occurred across all treatments. This shoot loss was associated with a natural bloom of the green macroalga *Ulva* sp. in our plots and the surrounding seagrass beds of Bodega Harbor. The bloom lasted from May to October, but reached its greatest intensity in late June 2006, peaking at 4 kg wet weight m^{-2}, the greatest biomass recorded during the 4-year period from 2004 to 2008 (Olyarnik 2008). Because of the observed shoot

Table 1. Results of statistical analyses of the interaction between disturbance and genotypic diversity.

	Disturbance (d.f. = 2)		Diversity (d.f. = 1)		Disturbance × Diversity (d.f. = 2)		
Time since disturbance	*F*	*P*	*F*	*P*	*F*	*P*	Covariate
2 weeks	42.19	< 0.0001	0.18	0.67	0.14	0.71	0 weeks
4 weeks	0.56	0.46	0.15	0.70	5.01	0.03	2 weeks
6 weeks	2.97	0.09	0.78	0.38	11.75	0.002	2 weeks
10 weeks	1.44	0.24	1.42	0.24	4.38	0.04	2 weeks
12 weeks	1.09	0.35	0.88	0.36	1.95	0.16	2 weeks
Overall (repeated measures)	1.47	0.25	0.59	0.54	3.64	0.04	

loss following peak *Ulva* abundance and the documented negative effects of high *Ulva* biomass on seagrasses (Hauxwell *et al.* 2001; McGlathery 2001, Olyarnik 2008), we treated this algal bloom as a natural disturbance and continued running the experiment to capture the effect of genotypic diversity on the *Zostera* response to this disturbance.

In June 2007, we measured final shoot density and harvested the seagrass from all plots. We then quantified distance between shoots, and shoot, root and rhizome biomass from haphazardly selected clones (i.e. multiple shoots connected by a single rhizome) from each plot. We also genotyped these sampled clones to assess the performance of the planted genotypes and determine final genotypic composition. Because of the higher probability of not sampling a genotype that was actually present in polyculture than in monoculture (due to the lower initial relative abundance of component genotypes in polyculture), we sampled polycultures with greater intensity: we sampled 48 clones from each polyculture and 24 clones from each monoculture, representing 26–100% of the shoots in each plot (average = 58%). The remaining seagrass biomass was divided into above- and below-ground portions, dried at 60 °C for at least 48 h, and weighed to determine biomass.

NATURAL DISTURBANCE EVENT: STATISTICAL ANALYSES

We ran a factorial ANOVA on shoot density after the *Ulva* bloom dissipated (7 September 2006) with shoot density prior to the decline (26 July 2006) as a covariate, including block, diversity, disturbance intensity and all possible interactions as fixed factors. We also quantified *Ulva* biomass in July 2006 and ran a factorial ANOVA as above to determine if *Ulva* varied by disturbance or diversity treatment. We were unable to reliably sample density in our plots in August 2006 due to a lack of sufficiently low tides during this month.

To evaluate differences between monocultures and polycultures in shoot density, above-ground biomass, below-ground biomass, root : rhizome biomass ratio, and distance between shoots at the end of the experiment, we ran factorial ANOVAS on each response variable individually with block, diversity, disturbance intensity and all possible interactions. Neither the experimental disturbance treatment nor any of its interactions affected any of the response variables during this period ($F \leq 0.78$, $P \geq 0.47$), so we dropped this factor and its interactions from our analyses. Thus, there were three replicates of each monoculture and polyculture combination for this portion of the analysis.

To examine final genotypic composition and relative abundance, we collected tissue samples from each of the haphazardly selected clones in each plot at the end of the experiment. The samples were frozen following collection and later extracted using a modified CTAB protocol, amplified via a polymerase chain reaction, and genotyped at five DNA microsatellite loci specific to *Z. marina* (see Hughes & Stachowicz 2004 for Methods). Shoots differing by zero or one alleles were considered of the same genotype in our analyses (Arnaud-Haond *et al.* 2007). GenClone 2.0 was used to calculate final genotypic diversity.

IDENTIFYING DIVERSITY MECHANISMS

To test whether polyculture performance differed on average from that expected based on performance of the component genotypes in monoculture, we compared observed and expected polyculture values using paired Student's *t*-tests at three occasions in our experiment when there was a significant effect of genotypic diversity: (i) following our experimental disturbance; (ii) following shoot loss during the macroalgal bloom; and (iii) at the end of the 2-year experiment. We calculated expected polyculture values based on the initial genotypic composition of each polyculture, assuming equal relative abundances (i.e. monoculture values were divided by six, the original number of transplants, and then summed for each genotype in a particular polyculture combination). A significant difference between observed and expected values indicates overyielding has occurred.

To further differentiate among diversity mechanisms, we used standard methods (Fox 2005) to partition variation in polyculture shoot density and above-ground biomass at the end of the experiment among three mechanisms: TIC, dominance and trait-dependent complementarity. TIC (equivalent to complementarity *sensu* Loreau & Hector 2001) results when genotypes yield higher biomass in mixture than expected, regardless of absolute monoculture biomass. TDC and dominance (together equivalent to selection *sensu* Loreau & Hector 2001) occur when a particular genotype dominates polycultures, either at the expense of other genotypes (dominance) or in addition to increases in other genotypes (TDC). Because there were no independent or interactive effects of disturbance at this time, we used the three disturbance levels as replicates for each particular genotypic combination. We conducted separate ANOVAS for biomass and density to determine whether the diversity mechanisms differed in their effect size.

Results

EXPERIMENTAL DISTURBANCE MANIPULATION

There was a general increase in shoot density over the 2-year experiment, regardless of disturbance level or genetic diversity (Fig. 1); the final shoot density of 201 ± 14.7 shoots 0.25 m^{-2} (mean ± SE) is similar to that found in undisturbed plots in

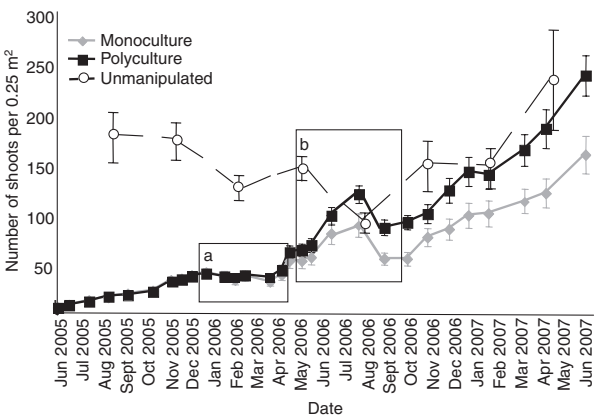

Fig. 1. Seagrass shoot density over the course of the experiment. Grey diamonds represent monocultures; black squares are polycultures. Open circles are from an unmanipulated natural seagrass bed in a nearby experiment (Olyarnik 2008). Error bars represent 1 SE. (a) The experimental disturbance was applied in January 2006. There was a significant interaction between diversity and disturbance during the 12-week period highlighted in the box (see Fig. 2). (b) A natural bloom of the macroalgae *Ulva* began in May 2006 and ended in October 2006, resulting in a loss of shoots across all plots (and in nearby unmanipulated areas). Shoot density in our experimental plots was comparable to the natural shoot density throughout the second year of the experiment.

the surrounding eelgrass bed in a separate study (Olyarnik 2008). Prior to the experimental disturbance there was no effect of diversity treatment on shoot density, with plots of on average c. 40 shoots per container. Our experimental disturbance in January 2006 was effective, resulting in a significant decline in the number of shoots per plot 2 weeks following the disturbance as clipping intensity increased, regardless of diversity (disturbance $F_{2,22} = 42.19$, $P < 0.0001$; Fig. 2a). Not surprisingly, given that we controlled the number of shoots clipped, there was no variation in shoot density due to diversity at this time (diversity × disturbance $F_{2,22} = 0.14$, $P = 0.71$; Fig. 2a). However, there were significant diversity effects on disturbance resilience, measured as shoot re-growth following disturbance (repeated-measures ANOVA: diversity × disturbance $F_{2,22} = 3.64$, $P = 0.04$, see Table 1 for full statistics), and these effects persisted for 10 weeks (time effect: G–G $F_{46,42} = 1.16$, $P = 0.31$).

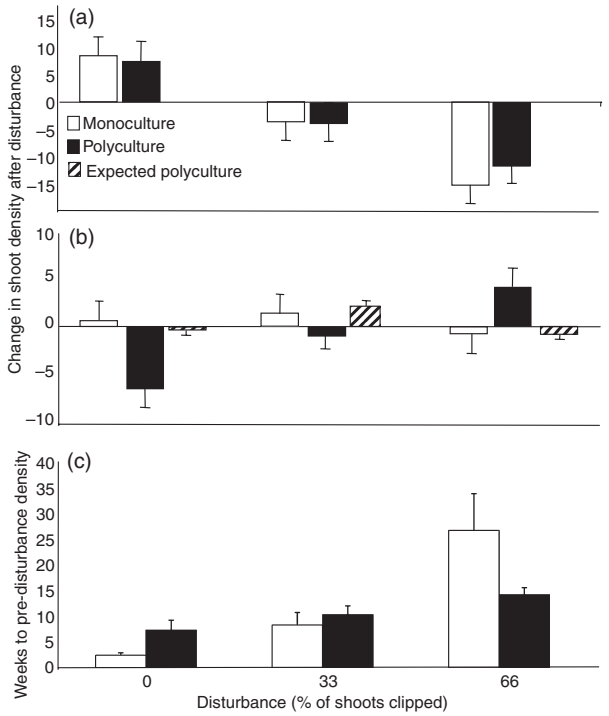

Fig. 2. Clonal diversity and disturbance interact to influence seagrass shoot density. Open bars are monocultures; black bars are polycultures; hatched bars are expected values for polycultures. Error bars represent 1 SE. (a) Seagrass response 2 weeks after an experimental disturbance. Disturbance response is shown as the difference in the number of shoots 2 weeks after the disturbance as compared to immediately prior to the disturbance. As expected, no disturbance plots gained shoots over this period, whereas high disturbance plots lost shoots, regardless of genotypic diversity. (b) Seagrass response 6 weeks after an experimental disturbance. Disturbance response is shown as the difference in the number of shoots at 6 weeks and 2 weeks post-disturbance. Monoculture response did not differ across the disturbance gradient, but polycultures experienced net shoot loss under no disturbance and net shoot gain at the highest level of disturbance. The expected average polyculture performance based on the mean performance of component monocultures differed from observed polyculture performance at both zero and high disturbance. (c) Time to recovery to pre-disturbance shoot density. Differences in shoot re-growth following disturbance (panel b) led to variation in the number of weeks until recovery to pre-disturbance density in the high disturbance treatment: monocultures took almost twice as long to recover as polycultures.

Our experimental disturbance in January 2006 coincided with a seasonal period of low growth (see, e.g. similar period of static shoot density in January 2007; Fig. 1), most likely due to light limitation (Dennison & Alberte 1982). Polycultures and monocultures in the no-disturbance treatment differed in shoot growth during the recovery period (independent contrast, $F_{1,22} = 5.73$, $P = 0.02$): polycultures experienced a slight net decrease in shoots, despite not being clipped. While monocultures exhibited no net change in shoot numbers (Fig. 2b). Shoot mortality and re-growth were equivalent among diversity treatments at intermediate disturbance (independent contrast, $F_{1,22} = 0.54$, $P = 0.47$), and there was a trend for polycultures to gain more shoots than monocultures at the highest level of disturbance (independent contrast, $F_{1,22} = 3.04$, $P = 0.09$).

The observed polyculture change in shoot density was less than expected based on performance of clones in monoculture at zero disturbance (paired *t*-test, $P = 0.06$) but did not differ from expected at intermediate disturbance (paired *t*-test, $P = 0.14$). In contrast, polycultures had greater increases in shoot density than expected at the highest level of disturbance (Fig. 2b, paired *t*-test, $P = 0.01$). The difference in the rate of re-growth between monocultures and polycultures at high disturbance caused the recovery time to pre-disturbance densities to vary by diversity and disturbance (ANOVA: diversity × disturbance $F_{2,22} = 3.23$, $P = 0.05$); polycultures recovered faster than monocultures at high disturbance (independent contrast, $F_{1,22} = 4.94$, $P = 0.04$; Fig. 2c).

NATURAL DISTURBANCE EVENT

Diversity affected shoot density in the presence of the macroalgae *Ulva* sp. Prior to and during the early stages of the bloom (April–July 2006) there was no difference in shoot density between monoculture and polyculture for any disturbance treatments (Fig. 1; diversity $F_{1,22} \leq 1.81$, $P \geq 0.19$). Differences in shoot density were not significant until the end of the bloom in late summer (diversity $F_{1,30} = 4.82$, $P = 0.04$), after a period of shoot loss during a time of year when expansion would normally occur (Fig. 1). All plots lost shoots during this period, yet polycultures lost a smaller percentage of shoots than monocultures (Fig. 3). There was no effect of clipping treatment or diversity on the biomass of *Ulva* during the bloom ($F \leq 1.54$, $P \geq 0.22$), so we attribute the differences in shoot density after the bloom to effects of genotypic diversity on response to the bloom. However, it is also possible that diversity effects in the absence of disturbance simply take time to manifest themselves, and the timing of the bloom coincided with sufficient time elapsing to reach shoot densities characteristic of natural beds (Fig. 1). Regardless, the percentage of shoots remaining in polycultures following the bloom was significantly higher than that expected based on component monocultures (paired *t*-test $P = 0.006$).

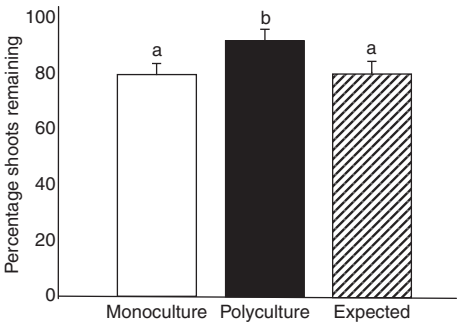

Fig. 3. Seagrass genotypic diversity increases the response to natural disturbance. Open bars are monocultures; black bars are polycultures; hatched bars are expected values for polycultures. Error bars represent 1 SE. The percentage of shoots remaining after a period of shoot loss in summer 2006. Significantly more shoots survived this natural disturbance event in polycultures than in monocultures. In addition, observed polyculture performance exceeded that expected based on component monocultures.

Unlike the transient diversity effect in response to the controlled shoot removal, the positive effect of genotypic diversity following the *Ulva* bloom persisted until the end of the experiment in June 2007, nearly 1 year after the end of the bloom (monoculture mean(SE) shoots 0.25 m^{-2} = 161.9(16.3); polyculture mean(SE) shoots 0.25 m^{-2} = 243.6(16.7); $F_{1,39}$ = 12.31, P = 0.001; Fig. 1). Additionally, there was a corresponding difference in above-ground biomass between polycultures and monocultures ($F_{1,39}$ = 8.00, P = 0.007; Fig. 4a). In contrast, below-ground biomass did not differ between monocultures and polycultures ($F_{1,39}$ = 2.93, P = 0.09; Fig. 4b). Despite the similarity in overall below-ground biomass, the allocation to root or rhizome tissue differed, with polycultures having a greater proportion of root tissue ($F_{1,39}$ = 4.85, P = 0.03; Fig. 4c). Also, the average distance between shoots along a rhizome segment was lower for polycultures than monocultures ($F_{1,39}$ = 6.46, P = 0.01; Fig. 4d), indicating that shoots were packed more closely together in polycultures. This result was largely due to the extremely low shoot spacing of one dominant genotype (mean(SE) distance between shoots = 40.0 ± 2.1 mm) relative to the other genotypes (means ranging from 52.3 to 77.2 mm between shoots). Observed polyculture values were consistently different than expected for all responses except below-ground biomass (paired t-tests $P \leq 0.01$; Fig. 3).

Because we destructively sampled the experimental plots at this time and were able to estimate the relative contributions of different genotypes to final density and biomass, we could assess the degree to which dominance of particular genotypes vs. complementarity among genotypes were responsible for the observed diversity effects. Partitioning the effects of diversity shows that both mechanisms play a role, but their relative importance differs for density and biomass. The positive net diversity effect on density was due to positive TIC, positive dominance and positive TDC (Fig. 5c). Although the strength of these mechanisms did not vary overall ($F_{2,14}$ = 2.42, P = 0.12), examining the different polyculture combinations separately reveals that both dominance and TIC can be quite strong (Fig. 5d). The strong dominance, when it occurred, was driven by the high shoot production of a single genotype, 8 (Fig 5b; labelled 'white' in Hughes *et al.* 2009) that also performed well in monoculture (Fig. 5a). However, genotype 8 was not able to dominate all polyculture combinations, as illustrated by the varying strength of dominance (dominance = 0 in three out of eight polycultures that had this genotype; Fig. 5d). Furthermore, dominance did not play an important role in the diversity effect on above-ground biomass (Fig. 5g); rather, strong and consistently positive TIC created this positive diversity effect (Fig. 5g,h).

The number of genotypes in polycultures remained significantly higher than monocultures at the end of the 2-year experiment (polyculture mean(SE) genotypes = 7.3(0.4), monoculture mean(SE) genotypes = 2.5(0.4); $F_{1,39}$ = 12.32, P = 0.001). Relatively few new clones were identified in our final genetic sampling (mean(SE) new clones per plot = 2.7(0.4); max = 7.0) and, more importantly, these were never

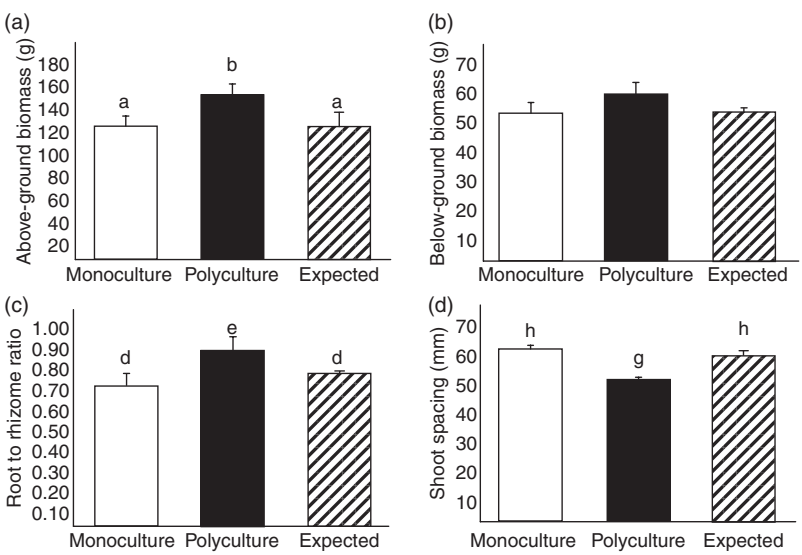

Fig. 4. Positive effects of seagrass genotypic diversity after two years. Open bars are monocultures; black bars are polycultures; hatched bars are expected values for polycultures. Error bars represent 1 SE. (a) Average plot above-ground biomass. (b) Average plot below-ground biomass. (c) Ratio of root biomass to rhizome biomass. (d) Average distance between 1 and 5 shoots on 24 (monoculture) or 48 (polyculture) rhizome segments per plot.

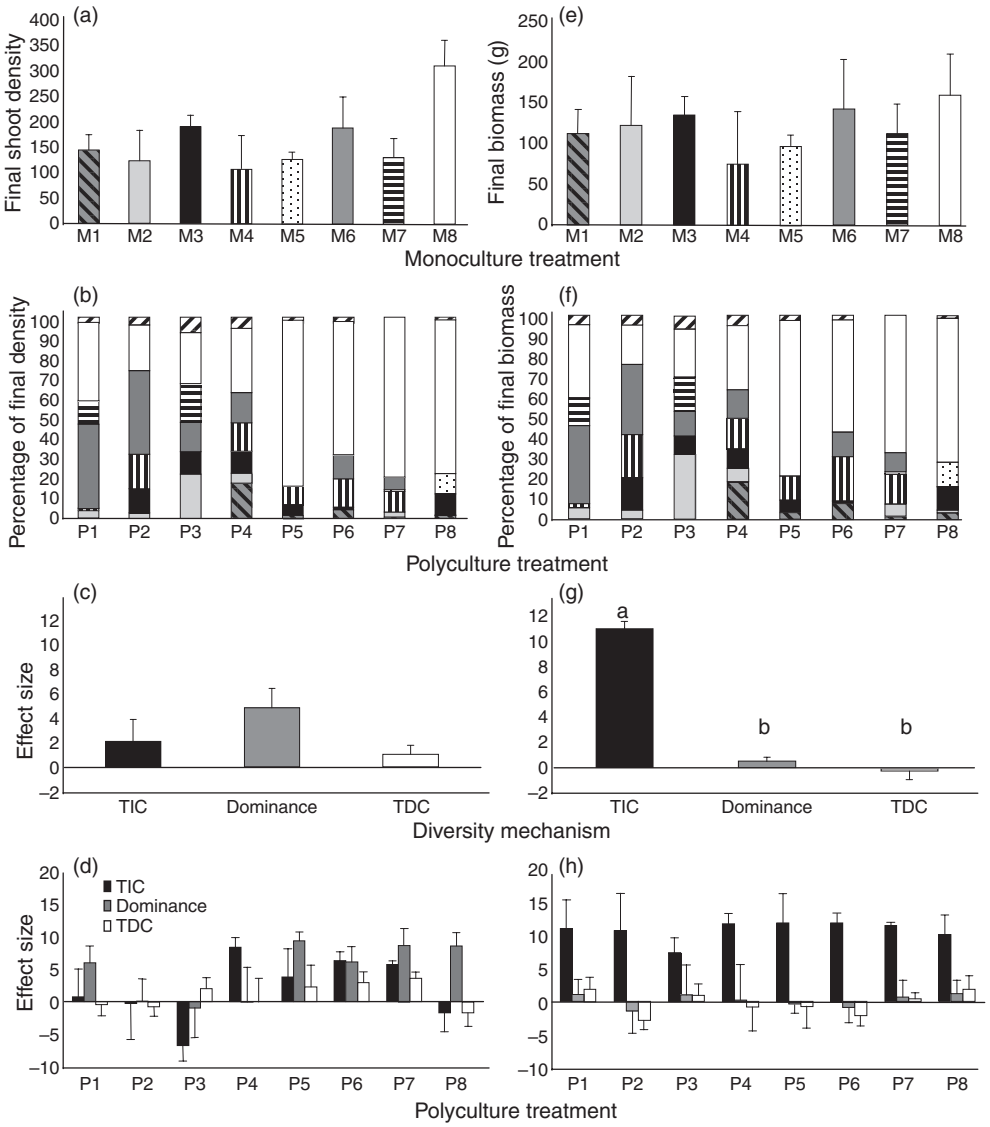

Fig. 5. Genotypic composition of monocultures and polycultures after 2 years. Seagrass (a) shoot density and (e) above-ground biomass in June 2007 in monoculture treatments. Seagrass (b) shoot density and (f) above-ground biomass in June 2007 in polyculture treatments. Shading indicates the relative abundance of component genotypes to final density. Genotypes correspond to the following colours in Hughes *et al.* (2009): 1 (red); 2 (green); 3 (yellow); 4 (orange); 5 (purple); 6 (blue); 7 (grey); 8 (white). (c, g) Overall contribution of TIC (closed bars), dominance (grey bars), and TDC (open bars) to (c) density and (g) above-ground biomass. Letters indicate significant differences ($P \leq 0.05$) according to Tukey's *post hoc* tests. (d, h) Relative importance of TIC, dominance, and TDC in polyculture treatments for (d) density and (h) above-ground biomass.

very abundant (Fig. 5b,f; mean(SE) percentage of total = 6.0(1.6)%). This result is consistent with our observation of very low seedling recruitment into our control plots over the course of the experiment (mean(SE) final shoot density = 16.1(5.3), data not shown). Though genotypic diversity differed from that initially planted at the end of the experiment, we continue to refer to the initial 'monoculture' and 'polyculture' treatments for consistency.

Discussion

Seagrass genotypic diversity enhanced recovery from an experimental disturbance in our study, although these effects were only evident at the highest disturbance intensity. The interaction between diversity and disturbance intensity in this study had not been demonstrated previously, illustrating the importance of controlled, experimental manipulations in order to completely understand the relationship between diversity and disturbance. In particular, the loss of shoots with high diversity in the absence of disturbance is puzzling and hard to explain. The effect was small in magnitude, relatively short-lived (< 12 weeks), and could be a consequence of measurement during a time of year when growth is slow and seasonal declines in density are common (Fig. 1). We did not observe a comparable effect in polycultures during the rest of the experiment or in any other experiment we conducted (e.g. Hughes & Stachowicz 2004). If verified, underyielding by polycultures in the absence of disturbance could have important

conservation and management implications and deserves further attention. The positive diversity effect at high disturbance, combined with the observed increase in disturbance response by polycultures to another high-intensity disturbance (a natural bloom of the macroalgae *Ulva*), corroborates the positive effects of diversity suggested by previous natural (i.e. unmanipulated) disturbances in this system (Hughes & Stachowicz 2004; Reusch *et al.* 2005). In addition, statistical partitioning of the diversity effect indicates that both positive TIC and positive dominance (rather than negative as in Reusch *et al.* 2005) can be important for these effects. However, these statistical methods cannot specify the biological mechanisms underlying diversity effects.

One caveat to the results of our experimental disturbance manipulation is that shoot density was below ambient (albeit not increasing) when we imposed the disturbance, potentially influencing the results. However, the broadly consistent effects of genetic diversity on disturbance response between the experimental disturbance and natural disturbance (which occurred when shoot density was near ambient) – even though they may arise by different mechanisms – increase our confidence that these effects are not dependent on initial shoot density. It is interesting to note that the effects of diversity persisted for much longer, i.e. until the end of the experiment (1 year, Fig. 4), when disturbance occurred at a time when shoot density was near ambient (Fig. 1). There are two potential explanations for this pattern. First, genotypic diversity could have a much stronger and more prolonged impact on shoot density in response to *Ulva* than in response to our experimental disturbance, due either to differences in the intensity or the mechanisms of disturbance. Alternatively, stronger interactions among genotypes once natural densities were reached during year 2 could have resulted in stronger diversity effects, consistent with previous studies suggesting that diversity effects due to complementarity strengthen over time (Cardinale *et al.* 2007, Stachowicz *et al.* 2008). We cannot currently rule out either of these explanations.

Because the experimental clipping process overrode any inherent differences among shoots in their ability to withstand disturbance, the positive impact of diversity in response to our manipulation must have been due to increased shoot production following disturbance. This increase in shoot production in polyculture is consistent with previous studies of seagrass genetic diversity (Reusch *et al.* 2005), as well as manipulations of species diversity (Stachowicz, Bruno & Duffy 2007). In contrast, the positive diversity effect in response to *Ulva* was at least in part due to polycultures losing a smaller percentage of shoots than monocultures (Fig. 3). This observed decrease in the number of shoots came during a seasonal period of shoot gain, so the effects of *Ulva* on *Zostera* were likely greater than indicated by the decline in Fig. 1. In fact, an experimental *Ulva* removal study conducted less than 1 km from our study site at the same time found that ambient *Ulva* biomass of 4.39 kg m^{-2} caused a 50–85% reduction in shoot density in summer 2006 (Olyarnik 2008, Fig. 1). Thus, the *Ulva* biomass of 6.52 kg m^{-2} found in our plots can reasonably be considered a 'high-intensity' disturbance.

When monoculture and polyculture responses differed over the course of the experiment, the observed polyculture response was consistently greater than that expected by the average performance of each of the genotypes in monoculture, indicating that overyielding occurred. At the end of the experiment, all three diversity mechanisms contributed positively to the overall effect on shoot density (Fig. 5c), whereas TIC alone caused diversity effects on above-ground biomass (Fig. 5g). When it occurred, the strong positive dominance effect on shoot density was correlated with a high-relative abundance of a single genotype (genotype 8). This genotype exhibited the highest shoot density in monoculture in this experiment, and also had the highest rate of shoot production in a separate laboratory experiment (Hughes *et al.* 2009). Not only is its shoot production rate high, but its relative allocation to below-ground biomass, and specifically root biomass, is also high (Hughes 2006; Hughes *et al.* 2009); this higher allocation to below-ground reserves may allow it to withstand disturbances and compensate for more susceptible genotypes (i.e. the insurance hypothesis; Tilman 1996; Yachi & Loreau 1999). In contrast to its high density, the total above-ground biomass of genotype 8 is equivalent to that of the other genotypes (Fig. 5e; Hughes *et al.* 2009), perhaps explaining the lack of positive dominance for above-ground biomass in this experiment.

Strong, positive TIC in shoot density is not as easily explained by the traits of individual genotypes, but it does appear to be correlated with the persistence of genotype 4 in polyculture (e.g. polyculture (P) 4, P5, P6, P7; Fig. 5b,c). Genotype 4 differs considerably from the other genotypes in its relative rates of nutrient uptake: it has the highest rate of root ammonium uptake and the lowest rate of leaf nitrate uptake of all of the genotypes used in the experiment (Hughes *et al.* 2009). The association of genotype 4 with positive TIC suggests that variation in resource utilization could drive these density effects. Differences among genotypes in resource use (Hughes *et al.* 2009) could also contribute to complementarity effects on polyculture biomass. However, it is difficult to ascribe complementarity in biomass to the traits of any one genotype because of its consistent strength across replicates of different genotypic composition.

The potential generality of strong, positive dominance and complementarity among genotypes is unclear, as few studies of the ecological consequences of genetic diversity have examined final genotypic composition to differentiate among potential mechanisms of diversity effects (Hughes *et al.* 2008). Even within this single experiment we found that the relative importance of particular mechanisms varied considerably depending on the combination of genotypes in polyculture (also see Hughes, Best & Stachowicz 2010) and the response variable considered. Nonetheless, the apparent contrast between the positive dominance found here and the negative selection effects documented in a similar system (Reusch *et al.* 2005) could be due to the longer experimental duration of our study allowing time for interactions among genotypes to alter relative abundances. Regardless of mechanism, our finding that seagrass genotypic diversity increases the response of this system to controlled and natural disturbance adds to the grow-

ing consensus of the importance of diversity for ecosystem stability (Hughes *et al*. 2007, Stachowicz, Bruno & Duffy 2007). It also highlights that these effects may take time to materialize and are likely to come from a mix of dominance and complementarity effects that depend on the traits of the specific taxa involved.

Acknowledgements

We would like to thank K. Aquilino, S. Attoe, R. Best, N. Bosch, M. Bracken, J. Byrnes, L. Carney, K. Edwards, M. Ferner, A. Fish, K. Hammond, J. Hobbs, D. Kimbro, A. Larson, B. Miner, E. Mullen, E. Mullaney, T. Ng, S. Olyarnik, P. Reynolds, K. Selheim, C. Sorte and B. Steves for help in the field. A. Fish and T. DiMarco provided invaluable assistance in the laboratory. S. Olyarnik provided data for the unmanipulated plots in Fig. 1. R. Best and two anonymous reviewers provided critical comments that improved the manuscript. This work was supported by NSF grant OCE-06-23641 to J.J.S. and A.R.H.

References

Arnaud-Haond, S., Duarte, C.M., Alberto, F. & Serrao, E.A. (2007) Standardizing methods to address clonality in population studies. *Molecular Ecology*, **16**, 5115–5139.

Bangert, R.K., Turek, R.J., Martinsen, G.D., Wimp, G.M., Bailey, J.K. & Whitham, T.G. (2005) Benefits of conservation of plant genetic diversity to arthropod diversity. *Conservation Biology*, **19**, 379–390.

Boles, B.R., Thoendel, M. & Singh, P.K. (2004) Self-generated diversity produces 'insurance effects' in biofilm communities. *Proceedings of the National Academy of Sciences*, **101**, 16630–16635.

Cardinale, B.J., Wright, J.P., Cadotte, M.W., Carroll, I.T., Hector, A., Srivastava, D.S., Loreau, M. & Weis, J.J. (2007) Impacts of plant diversity on biomass production increase through time because of species complementarity. *Proceedings of the National Academy of Sciences*, **104**, 18123–18128.

Dennison, W.C. & Alberte, R.S. (1982) Photosynthetic responses of *Zostera marina* to in situ manipulations of light intensity. *Oecologia*, **55**, 137–144.

Fox, J.W. (2005) Interpreting the 'selection effect' of biodiversity on ecosystem function. *Ecology Letters*, **8**, 846–856.

Hauxwell, J., Cebrian, J., Furlong, C. & Valiela, I. (2001) Macroalgal canopies contribute to eelgrass (*Zostera marina*) decline in temperate estuarine ecosystems. *Ecology*, **82**, 1007–1022.

Hughes, A.R. (2006) *The Ecological Consequences of Genetic Diversity in Eelgrass (Zostera marina)*. University of California, Davis, CA.

Hughes, A.R., Stochowicz, J.J. & Williams, S.L. (2009) Morphological and physiological variation among seagrass (*Zostera marina*) genotypes. *Oecologia*, **159**, 725–733.

Hughes, A.R., Best, R.J. & Stachowicz, J.J. (2010) Genotypic diversity and grazer identity interactively influence seagrass and grazer biomass. *Marine Ecology Progress Series*, **403**, 43–51.

Hughes, A.R., Byrnes, J.E., Kimbro, D.L. & Stachowicz, J.J. (2007) Reciprocal relationships and potential feedbacks between biodiversity and disturbance. *Ecology Letters*, **10**, 849–864.

Hughes, A.R., Inouye, B.D., Johnson, M.T.J., Vellend, M. & Underwood, N. (2008) Ecological consequences of genetic diversity. *Ecology Letters*, **11**, 609–623.

Hughes, A.R. & Stachowicz, J.J. (2004) Genetic diversity enhances the resistance of a seagrass ecosystem to disturbance. *Proceedings of the National Academy of Sciences (USA)*, **101**, 8998–9002.

Hughes, A.R. & Stachowicz, J.J. (2009) Ecological impacts of genotypic diversity in the clonal seagrass *Zostera marina*. *Ecology*, **90**, 1412–1419.

Huston, M.A. (1997) Hidden treatments in ecological experiments: re-evaluating the ecosystem function of biodiversity. *Oecologia*, **110**, 449–460.

Loreau, M. & Hector, A. (2001) Partitioning selection and complementarity in biodiversity experiments. *Nature*, **412**, 72–76.

McGlathery, K.J. (2001) Macroalgal blooms contribute to the decline of seagrass in nutrient-enriched waters. *Journal of Phycology*, **37**, 453–456.

Mulder, C.P.H., Uliassi, D.D. & Doak, D.F. (2001) Physical stress and diversity-productivity relationships: the role of positive interactions. *Proceedings of the National Academy of Sciences*, **98**, 6704–6708.

Norberg, J., Swaney, D.P., Dushoff, J., Lin, J.R. & Levin, S.A. (2001) Phenotypic diversity and ecosystem functioning in changing environments: a theoretical framework. *Proceedings of the National Academy of Sciences USA*, **98**, 11376–11381.

Olyarnik, S.V. 2008. *The Causes and Consequences of Macroalgal Blooms on an Eelgrass (Zostera marina) Community in Bodega Harbor, CA*. University of California, Davis, CA.

Orth, R.J., Carruthers, T.J.B., Dennison, W.C., Duarte, C.M., Fourqurean, J.W., Heck Jr, . K.L., Hughes, A.R., Kendrick, G.A., Kenworthy, W.J., Olyarnik, S.V., Short, F.T., Waycott, M. & Williams, S.L. (2006) A global crisis for seagrass ecosystems. *Bioscience*, **56**, 987–996.

Reusch, T.B.H. (2001) Fitness-consequences of geitonogamous selfing in a clonal marine angiosperm (*Zostera marina*). *Journal of Evolutionary Biology*, **14**, 129–138.

Reusch, T.B.H., Ehlers, A., Haemmerli, A. & Worm, B. (2005) Ecosystem recovery after climatic extremes enhanced by genotypic diversity. *Proceedings of the National Academy of Sciences of the United States of America*, **102**, 2826–2831.

Schmitt, J. & Antonovics, J. (1986) Experimental studies of the evolutionary significance of sexual reproduction. IV. Effect of neighbor relatedness and aphid infestation on seedling performance. *Evolution*, **40**, 830–836.

Short, F.T. & Wyllie-Echeverria, S. (1996) Natural and human-induced disturbance of seagrasses. *Environmental Conservation*, **23**, 17–27.

Shuster, S.M., Lonsdorf, E.V., Wimp, G.M., Bailey, J.K. & Whitham, T.G. (2006) Community heritability measures the evolutionary consequences of indirect genetic effects on community structure. *Evolution*, **60**, 991–1003.

Stachowicz, J.J., Bruno, J.F. & Duffy, J.E. (2007) Understanding the effects of marine biodiversity on communities and ecosystems. *Annual Review of Ecology, Evolution, and Systematics*, **38**, 739–766.

Stachowicz, J.J., Graham, M., Bracken, M.E.S. & Sloboszlai, A.I. (2008) Diversity enhances cover and stability of seaweed assemblages: the role of heterogeneity and time. *Ecology*, **89**, 3008–3019.

Tilman, D. (1996) Biodiversity: population versus ecosystem stability. *Ecology*, **77**, 350–363.

Tilman, D., Lehman, C.L. & Thomson, K.T. (1997) Plant diversity and ecosystem productivity: theoretical considerations. *Proceedings of the National Academy of Sciences*, **94**, 1857–1861.

Waycott, M., Duarte, C.M., Carruthers, T.J.B., Orth, R.J., Dennison, W.C., Olyarnik, S., Calladine, A., Fourqurean, J.W., Heck Jr, K.L. ., Hughes, A.R., Kendrick, G.A., Kenworthy, W.J., Short, F.T. & Williams, S.L. (2009) Accelerating loss of seagrasses across the globe threatens coastal ecosystems. *Proceedings of the National Academy of Sciences USA*, **106**, 12377–12381.

Yachi, S. & Loreau, M. (1999) Biodiversity and ecosystem productivity in a fluctuating environment: the insurance hypothesis. *Proceedings of the National Academy of Sciences USA*, **96**, 1463–1468.

Received 30 June 2010; accepted 29 October 2010
Handling Editor: Rob Brooker