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Genotypic diversity in a non-native ecosystem engineer has variable impacts on productivity

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ABSTRACT: Ecosystem engineers alter local community structure and ecosystem functioning, and these impacts can be magnified when engineer populations are genetically diverse. Introduced populations typically have lower genetic diversity, which in theory constrains the positive effect of genetic diversity on ecosystem functioning. We tested for genetic diversity–function relationships in the introduced red alga *Gracilaria vermiculophylla*. Within high-salinity mudflats of Atlantic estuaries of North America, dozens of *G. vermiculophylla* thalli are glued onto individual worm tubes by the polychaete *Diopatra cuprea* (Onuphidae). A field survey in one estuary showed that these algal patches represent clonal fragments of between 6 and 18 multi-locus microsatellite genotypes. Within-patch genotypic diversity correlated with *G. vermiculophylla* biomass and epibacterial density, but had little to no effect on invertebrate or epifloral abundances, nor invertebrate diversity. To experimentally confirm these variable effects, we outplanted monoclonal and polyclonal (8 genotypes) patches into the mid and low intertidal for 28 days in spring and summer. We detected no impacts of genotypic diversity on primary productivity in spring nor in the summer at the low intertidal, but net primary productivity increased 300 % in polyclonal relative to monoclonal patches in the mid intertidal during the summer. Genotypic diversity had little to no effect on any metric of secondary productivity or diversity. We conclude that modest increases in genotypic diversity can increase productivity of an introduced ecosystem engineer, but these impacts vary temporally and spatially for reasons that remain unclear. We also conclude that positive effects of genotypic diversity on primary productivity may not necessarily translate into secondary productivity.

KEY WORDS: Intraspecific variation · Introduced species · Ecosystem engineer · Community processes · Ecosystem functioning

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INTRODUCTION

Ecosystem engineers are species that alter community structure and ecosystem processes (Parker et al. 1999) by creating novel biogenic substrate (Crooks 2002, Levine et al. 2003, Byers et al. 2006, Hastings et al. 2007). Recent studies indicate that the genetic diversity of plant or animal engineers can magnify these effects, and in particular tend to increase primary and secondary productivity and resistance to disturbance (Hughes & Stachowicz 2004, 2009, 2011, Reusch et al. 2005, Crutsinger et al. 2006, 2008, Hughes et al. 2008, Kotowska et al. 2010, Hanley et al. 2016). One weakness of this growing literature is that studies have been largely restricted to vascular plants and invertebrates and neglect other taxa. Seaweeds, for example, commonly structure nearshore communities (Witman & Dayton 2001), and diverse assemblages of seaweed species have greater efficiency in nutrient use (Bracken & Stachowicz 2006)

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and sunlight (Tait et al. 2014), and greater recovery from disturbance (Aquilino & Stachowicz 2012). However, to our knowledge, no tests of genetic diversity–function relationships within seaweed species have been published.

The introduction of ecosystem engineer species to non-native habitats can yield local ecological impacts that are both negative and positive. For example, invasive freshwater plants form dense canopies that outcompete native plants, but facilitate macroinvertebrate communities by providing refuge (Schmitz et al. 1993, 1997). Similarly, some non-native macroalgae outcompete local seagrasses and seaweeds (e.g. Thomsen et al. 2013), but also house abundant epifaunal assemblages who use them as refuges from abiotic and biotic stresses (Byers et al. 2012, Gribben et al. 2013, Wright et al. 2014, Bishop & Byers 2015). However, predicting these impacts is complicated by consideration of within-species genetic diversity. This is because introduced populations tend to have relatively lower levels of genetic diversity (as measured by neutrally evolving markers) relative to native populations (Wares et al. 2005, Dlugosch & Parker 2008), which potentially constrains the positive effects of genetic diversity on ecosystem functioning. Alternatively, it may be that despite such genetic bottlenecks, enough genetic variation in phenotypic traits exists in non-native populations (Warwick et al. 1987, Kaufman & Smouse 2001, Maron et al. 2004) to generate diversity–function relationships. Previous work has focused on how genetic diversity and identity of introduced populations influence invasibility (Vellend et al. 2010, Wang et al. 2012, Matesanz & Sultan 2013), but tests of the effects of genetic diversity on other ecological processes (i.e. secondary production, community composition, etc.) are relatively uncommon.

Gracilaria vermiculophylla ([Ohmi 1958] Papenfuss 1967) is a non-native red alga from the northwestern Pacific that has been introduced into most temperate estuaries of the northern hemisphere (Kim et al. 2010). Within Atlantic estuaries of the southeastern United States, *G. vermiculophylla* is glued by the decorator polychaete *Diopatra cuprea* (Onuphidae) to its tube cap, creating small-scale patches (100–200 cm^2) with high *G. vermiculophylla* biomass (see inset image in our Fig. 1; Byers et al. 2012). Under some conditions, the interaction appears to be mutually beneficial: *D. cuprea* actively decorates its tube cap with *G. vermiculophylla* and gains increased access to epifaunal amphipods, and *G. vermiculophylla* remains anchored to the substrate and thereby minimizes its loss to either the elevated marsh or the low-light, deep subtidal of the estuary (Kollars et al. 2016). Other macroalgal species are historically rare on these mudflats because of highly turbid waters and a lack of substrate for attachment (Byers & Grabowski 2014). *G. vermiculophylla* invasion success results in local ecosystem engineering because it facilitates more than 10 epifaunal phyla by adding biotic structure to a relatively homogenous mudflat (Nyberg et al. 2009, Byers et al. 2012, Hernández Cordero & Seitz 2014). In addition, *G. vermiculophylla* contains a unique bacterial community relative to co-occurring algal species, increases sediment bacteria, and alters sediment bacterial communities (Lachnit et al. 2011, Gonzalez et al. 2014, C. E. Gerstenmaier & E. E. Sotka unpubl. data).

Because the novel interaction between *D. cuprea* and *G. vermiculophylla* contributes to local dominance of *G. vermiculophylla* with its strong effects on associated biotic communities, we predicted that polycultures of *G. vermiculophylla* (multiple genotypes) could have higher primary production and alter abundance and structure of epibiotic communities relative to monocultures (single genotype). To examine this hypothesis, we first conducted a field survey to assess the relative importance of genetic diversity and tidal height on *G. vermiculophylla* standing biomass, epiphyte, epifaunal, and bacterial abundance on the algal surface, and invertebrate diversity. Our sampling unit was *G. vermiculophylla* patches on *D. cuprea* tubes (hereinafter *G. vermiculophylla* patches), with a spatial scale of approximately 100–200 cm^2 each. We then performed a manipulative field experiment at 2 time points (spring and summer) in which we outplanted *G. vermiculophylla* patches with either 1 or 8 genotypes and measured *G. vermiculophylla* growth and the epibiotic community after 4 wk.

Fig. 1. Wappoo Creek Mudflat with an inset photograph of a *Diopatra cuprea* tube cap and associated *Gracilaria vermiculophylla* (image: Erik Sotka; inset image: Edna Diaz Negron)

MATERIALS AND METHODS

Field survey

All work occurred at the Fort Johnson mudflat in Charleston Harbor, South Carolina (32° 45′ 4.70″ N, 79° 54′ 5.14″ W). To determine natural genotypic diversity and associated epifaunal abundance and diversity, and epifloral and bacterial abundance, we collected 20 *Diopatra cuprea* tube caps separated by at least 1 m and all of their associated *Gracilaria vermiculophylla* pieces at 2 tidal heights (0.09 m mean lower low water [MLLW] and −0.09 m MLLW) in May 2014. We collected samples while submerged by at least 15 cm of water in order to retain mobile epifauna. In the lab, all epifauna and epiphytes were removed through multiple freshwater rinses and manual removal and preserved in 70% ethanol. We collected bacteria by swabbing both sides of every thallus with a single sterile 6 inch (~15 cm) cotton swab, and placed the swab in 10 ml of 10% formalin. We inspected all thallus fragments of *G. vermiculophylla* anchored to the *D. cuprea* tube cap for sex and ploidy (gametophyte, sporophyte, or vegetative; Bellorin et al. 2004), and placed a 1–5 cm piece of each fragment into a 1.5 ml microcentrifuge tube filled two-thirds with silica powder (Actíva) for genetic analyses. We weighed the remainder of the individual, and placed it in a pre-weighed aluminum foil packet at 70°C for 72 h, or until there was no change in mass.

We visually identified epibiota to the lowest taxonomic level. We measured the wet mass of each epiphytic taxon, recorded the abundance of epifaunal taxa and calculated the Shannon diversity index in R:vegan (version 2.15.1, Oksanen et al. 2013).

We determined bacterial abundance through epifluorescent microscopy. We vortexed bacterial samples for 30 s, transferred 1 ml of sample to a sterile 1.5 ml microcentrifuge tube, filtered and stained 300 µl of each sample with SYBR Gold (Life Technologies) for 4–6 min, placed the filter on the slide, and counted bacteria at 100× magnification. At least 10 randomly chosen fields of view, or enough to yield 100 bacterial cells, were counted on each filter. We ran each sample in triplicate, and calculated bacterial density (N) as $N = N_{cell} \times C_1 \times C_2 \times vol^{-1}$, where $C_1 = 4.79 \times 10^4$ or the number of fields per slide as determined by the area of the field of view divided by the area of the filter, $C_2 = 1.16$ and is a correction factor for samples fixed in formalin, and vol = volume of water filtered (or 300 µl). This equation was calculated for every field, and density was averaged across all fields viewed in the slide. Bacterial counts linearly increased with *G. vermiculophylla* biomass across 3 independent samples ($p = 0.014$, $R^2 = 0.897$; $p = 0.008$, $R^2 = 0.927$; $p = 0.002$, $R^2 = 0.976$) (Supplement 1, Fig. S1-1, at www.int-res.com/articles/suppl/m556p079_supp.pdf), suggesting that we did not reach the saturation point of the cotton swab.

Genetic analysis

We extracted total genomic DNA from the dried *G. vermiculophylla* thallus using the NucleoSpin® 96 plant kit (Macherey-Nagel) according to the manufacturer's instructions, except for the cell lysis buffer step in which samples were left at room temperature for 1 h rather than heating to 65°C for 30 min. Samples were eluted in 100 µl elution buffer. We chose 7 microsatellite loci (Supplement 2) (Kollars et al. 2015, Krueger-Hadfield et al. 2016) that were polymorphic in our focal population.

We performed simplex PCR using a Bio-Rad T100™ Thermal Cycler: 10 µl final volume, 100 nM of labeled forward primer, 150 nM of unlabeled forward primer, 250 nM of unlabeled reverse primer, 1× reaction buffer, 1.5 nM $MgCl_2$, 250 µM dNTP, 0.5 U *Taq*, 2 µl of DNA. The PCR program included: 2 min at 95°C, followed by 30 cycles of 30 s at 95 °C, 30 s at 55°C; and finally, 5 min at 72°C. One µl of each PCR product was added to 10 µl of loading buffer containing 0.35 µl of size standard (GeneScan 500 LIZ, Applied Biosystems) plus 9.7 µl of Hi-Di Formamide (Applied Biosystems). The loading mix was run on a 3130xl ABI fragment analyzer. Genotypes were scored manually using GeneMapper ver. 4 (Applied Biosystems) by 2 independent scorers. Due to problems with automated binning (see Matschiner & Salzburger 2009), raw allele sizes were binned using TANDEM (Matschiner & Salzburger 2009). We determined multilocus genotypes (MLGs) using GenAlEx ver. 6.5 (Peakall & Smouse 2012) and Genclone ver. 2.0 (Arnaud-Haond & Belkhir 2007). For each MLG, we used Genclone to estimate P_{sex}, which is the probability for a given MLG to be observed in N samples as a consequence of different sexual reproductive events. If P_{sex} was >0.05, the MLGs were considered as distinct genets. If P_{sex} was <0.05, the MLGs were considered as ramets of the same genet.

Field experiment

To assess whether genotypic diversity of a *G. vermiculophylla* patch influenced patch growth rate or

abundance and community structure of associated epibionts, we outplanted replicate monoclonal (8 thalli from 1 individual) and polyclonal (8 thalli of 8 individuals) at 0.09 m MLLW (mid) and −0.09 m MLLW (low) during the spring (27 April to 25 May 2014) and summer (10 July to 8 August 2014). The seaweed biomass (12 g wet mass) and number of genotypes in the polyculture (6–8 genotypes) were typical for patches surveyed on this mudflat (see 'Results').

Prior to each of the spring and summer trials, we haphazardly collected 100 *D. cuprea* tube caps and associated *G. vermiculophylla* from at least 1 m apart over the mudflat between 0.09 and −0.09 m MLLW (23–25 April and 9 July 2014). For transfer to the lab, we placed *G. vermiculophylla* and the associated *D. cuprea* tube cap in re-sealable plastic bags. In the lab, we removed the largest thallus from the *D. cuprea* tube cap and removed all epiphytes and epifauna. A small fragment of each thallus was preserved in silica for microsatellite genotyping. We patted dry each thallus 3 times before obtaining wet mass, and kept any thallus >30 g wet mess until we obtained 30 thalli for use in the field experiments. For the April-May experiment, the 30 thalli were ultimately determined to represent 22 different genotypes, with 16 unique and 5 genotypes repeated between 2 and 5 times, while in the July-August experiment, the 30 thalli represented 21 genotypes, with 18 unique genotypes and 3 additional genotypes were each repeated 4 times.

We determined sex and ploidy in the same manner as the field survey. We divided each field-collected individual into equal biomass pieces (1–1.5 ± 0.05 g) and assigned them randomly to mono- and polyclonal treatments at both tidal heights. A given MLG was used between 2 and 43 different times in our experiment (average of 11 per tidal height) because MLG biomass varied upon collection (Supplement 3). Each experimental unit (or patch) was constructed with three 10–15 cm long 3-strand ropes, which held 8 individuals. Two of the ropes contained 3 individuals, each separated by 2 turns of the rope (approximately 2 cm), while the third rope contained 2 individuals (Fig. 2). We marked each rope with 1 of 3 colors to differentiate the thalli. For polyclonal patches, we randomly assigned the location of each genotype on and among the ropes. We recorded the initial weight for each individual (between 1 and 1.5 g ± 0.05) in relation to both the color of the rope and the location on the rope. We zip-tied the 3 ropes together and attached them to a 30 cm long piece of PVC by a zip-tie through a hole drilled approximately 3 cm from the top of the PVC pipe.

Fig. 2. Ropes before deployment in field experiments. Letters indicate location of each individual on the rope: T = top, M = middle, B = bottom

We transplanted paired sets of monoclonal and polyclonal patches approximately 0.5 m apart and separated from the next pair by at least 1 m, and had transects at both tidal heights (0.09 and −0.09 m MLLW) (Supplement 3). We buried the PVC posts until only the ropes were visible and all *G. vermiculophylla* individuals were above the mud line. We attached a HOBO® Pendant Temperature/Light data logger (Onset) to a 30 cm long PVC post and placed it in the middle of the transect at both tidal heights in order to monitor light levels and temperature at the sediment surface. After 4 wk, we removed the samples by placing a re-sealable plastic bag over the *G. vermiculophylla* ropes and posts while under approximately 15 cm of water. We snipped the zip-tie connecting the ropes to the pole, sealed the bag, and brought the re-sealable bags with samples to the lab.

We split the experimental breakdown over 2 d, with samples being stored at 4°C until processing. Once in the lab, we processed the samples for the same response variables as in the field survey. We determined relative growth rate (hereinafter RGR) of a patch following the method of Hoffmann & Poorter (2002). This method compares the natural log of the sample's end wet mass to the natural log of its starting wet mass in relation to how long the sample was in the field.

Statistical analysis

Because of time and resources, not all thalli could be genotyped at all 7 microsatellite loci; consequently, the biomass of amplified thalli per tube cap

in the field survey was between 38 and 95% of the total biomass. In the following field survey analyses, we included the 14 *G. vermiculophylla* patches in which the amplified biomass was 90% or more of the total biomass.

For the field survey, we tested the covariate effect of genotypic diversity and total biomass, and the fixed, categorical effect of tidal height on each response variable (epifaunal abundance, epiphyte biomass, bacterial abundance, and invertebrate diversity) in R (2.15.1; R Core Team 2012) using ANCOVAs permuted 1000 times (Anderson 2001) and the backwards elimination of non-significant interaction terms. Permutation tests were used because non-normal residuals and heteroscedasticity remained after data transformation. To examine the effects of the fixed effect of tidal height and the covariate of genotypic diversity on biomass, we ran a 2-way permuted ANOVA, after an outlier test indicated that 1 data point fell outside the 95% confidence ellipse.

For the field experiment, we evaluated the influence of the full model including the fixed factors of genotypic diversity treatment and tidal height, and the covariate final biomass, and all interactions in a series of 2-way ANCOVAs permuted 1000 times on each response variable: epiphyte biomass, epifaunal abundance, bacterial abundance, and invertebrate diversity. We examined the effects of tidal height, genotypic diversity treatment, and their interaction on RGR using a permuted ANOVA. While we set up polyclonal patches with 8 individuals, the final number of unique genotypes was sometimes lower than 8 because of loss during the experiment or the presence of identical MLGs. As a result, the number of final genotypes in the May polycultures ranged from 1 to 8, while in July it ranged from 4 to 8. We excluded from analysis any polyculture that had 5 or fewer distinct MLGs, as we assumed that low-diversity treatments would have genotypes that are less able to effectively interact. We ran 100 permuted ANCOVAs and ANOVAs (each replicate was run 1000 times) in which we randomly removed all but 1 of each replicated genotype for the analysis. Analyses that included the full or permuted dataset did not differ in biological interpretation, and thus we report only the permuted dataset results.

We also tested the effect of genotypic diversity on the RGR of individual, unique genotypes. For this analysis, we calculated the RGR of each genotype by taking the sum of the initial and final weights within a patch and substituting them into the Hoffmann & Poorter (2002) equation. These RGR values were then averaged across mono- versus polyclonal treatments, and tested using a paired *t*-test with genotype as a blocked independent replicate (See Supplement 4, Fig. S4-1). Because genotypes differed in the frequency with which they were deployed within the polycultures (Supplement 3), we used linear regressions to assess whether genotype frequency correlated with (1) mean genotype RGR in the experiment or (2) the difference in RGR for each genotype between polyculture and monoculture. Because none of these regressions were significant (Supplement 4, Figs. S4-2 & S4-3), we concluded that our results for growth performance were not biased by differential use of genotypes.

To test for additive versus non-additive effects when we saw differential growth between monocultures and polycultures, we coded resampling methods to create our expected growth for polycultures (see Johnson et al. 2006 for analogous example). For each polyculture replicate, we randomly sampled one of each genotype in monoculture and estimated the RGR for the simulated patch. We compared the mean RGR from 1000 simulated polycultures to the mean observed polyculture RGR using a 1-sample *t*-test. We used additive partitioning to determine the relative contribution of dominance, trait-dependent complementarity (TDC), and trait-independent complementarity (hereinafter complementarity) effects in differences among polycultures (Loreau & Hector 2001, Fox 2005). Complementarity results when genotypes are more productive in polyculture than monoculture, regardless of how well they performed in monoculture. Dominance results when better-performing genotypes in monoculture dominate polycultures at the expense of worse-performing genotypes in monoculture. Trait-dependent complementarity is similar to dominance but does not occur at the expense of other genotypes. Trait-dependent complementarity and dominance effect make up the selection effect of Loreau & Hector (2001). We used a 1-sample *t*-test to determine if these effects were significantly different than zero.

We employed PRIMER 6 (Primer-E) to look for any differences in epibiotic community between tidal heights and mono- versus polycultures. The data matrix consisted of 34 species in the May field experiment and 30 species in the July field experiment. We 4th-root-transformed all data prior to analysis to remove any biases between rare and abundant species. We used the semimetric Bray-Curtis distance to calculate distances between each pair of observations. We tested any observed differences between heights or mono- versus polyculture using ANOSIM and analyzed community similarity using SIMPER.

RESULTS

Field survey

Across 386 *Gracilaria vermiculophylla* individuals from 20 patches, we found 116 different MLGs. The number of genotypes within a single patch (i.e. attached to a single *Diopatra cuprea* tube cap) was 10.9 ± 0.3 (mean ± SE; range: 6–18; Fig. 3). Larger *G. vermiculophylla* patches had significantly more genotypes (Fig. 3, Table 1A; $R^2 = 0.478$, p = 0.011).

The abundance of epifauna on *G. vermiculophylla* patches correlated positively with patch dry mass (p = 0.004) and negatively with tidal height (p = 0.048; Table 1A; Supplement 5, Fig. S5-1). There was a significant interaction effect of *G. vermiculophylla* dry mass and tidal height on bacterial abundance, where bacteria increased with patch size in the mid intertidal and decreased with patch size in the low intertidal (p = 0.001; Supplement 5, Fig. S5-2). Bacterial abundance also positively correlated with genotypic diversity (p = 0.023; Supplement 5, Fig. S5-3). There were no effects of patch size, genotypic diversity, nor tidal height on epiphyte wet mass or invertebrate diversity.

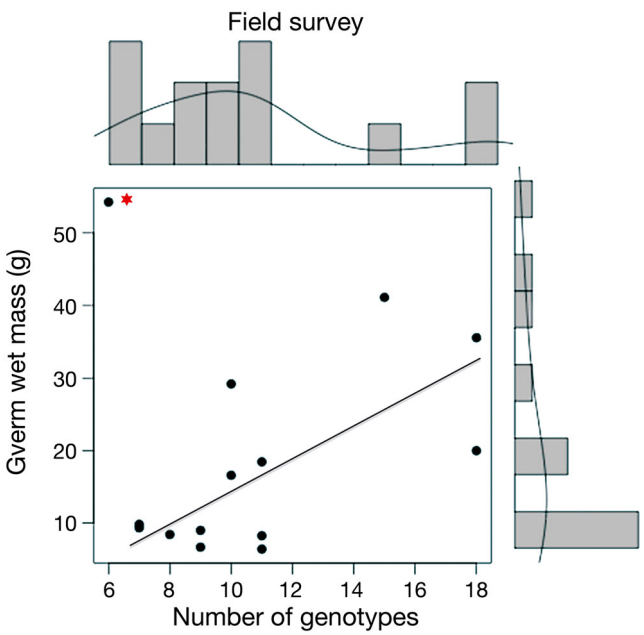

Fig. 3. Scatterplot and histograms of total biomass versus number of genotypes within *Gracilaria vermiculophylla* ('Gverm') patches in the May field survey. Red asterisk indicates an individual outlier that falls outside the 95% confidence ellipse (not shown). Black line indicates a best-fit regression with the outlier removed. Top axis shows histogram of the number of genotypes in a patch. Right axis shows histogram of *G. vermiculophylla* patch weight

Field experiments

During the May field experiment, 95% of all patches lost biomass during the experiment, an indication either of severe environmental stress, senescence following a peak in reproductive output of diploid thalli (S. A. Krueger-Hadfield pers. obs.), or both. The mean patch growth rate of monocultures and polycultures in the low intertidal was −0.317 and −0.334, respectively, while in the mid intertidal growth rates were −0.0634 and −0.062, respectively. Patch growth rate (as measured by RGR per day) was greater in the low intertidal but did not vary between genotypic diversity treatments (Table 1B, Fig. 4, left panel). In the July experiment, relatively few patches lost biomass. Polyclonal patches grew faster than monoclonal patches, low intertidal patches grew faster than mid intertidal patches, but genotypic diversity and tidal height did not interact significantly (p = 0.141; Table 1C, Fig. 4, right panel). The positive effect of genotypic diversity on primary productivity was primarily driven by mid intertidal replicates, whose polyclonal patches had over 300% higher growth rate than monoclonal patches, on average. This patch-level increase in RGR was not reflected at the level of individual genotypes, which grew at statistically indistinguishable rates within polyclonal and monoclonal patches (Supplement 4, Fig. S4-1).

We further explored the significant and positive diversity effect on primary productivity in the summer mid intertidal. The growth rates of polyclonal *G. vermiculophylla* patches in the summer mid intertidal was significantly higher than expected from the growth rate of monocultures (Fig. 5A: $t = 209.9$, p < 0.001). The total biological effect, as determined by additive partitioning, for the summer mid intertidal was statistically different from 0 at 1.847 (p < 0.001). The relative contributions of dominance, TDC, and complementarity to the total biological effect were statistically different from 0 (p < 0.001) at −0.076, −1.21, and 3.133, respectively.

We detected some effects of tidal height and patch biomass on associated epibiota. In May, epifaunal abundance and invertebrate diversity positively related to *G. vermiculophylla* dry mass (Table 1B; Supplement 6, Figs. S6-1 & S6-2). Epiphyte biomass is affected by an interaction between tidal height and patch genotypic diversity; specifically, epiphyte biomass increases in the mid intertidal in polycultures only (data not shown). In July, epifaunal abundance correlated positively with *G. vermiculophylla* dry mass and negatively correlated with tidal height, and this dry mass effect was stronger in the lower inter-

Table 1. ANCOVA of the (A) May field survey (n = 6–8 per tidal height), (B) May field experiment (n = 13–30 per tidal height), and (C) July field experiments (n = 28–30 per tidal height). All ANCOVAs were permuted 1000 times. For the field experiments, we ran 100 permuted ANCOVAs where we randomly removed all but 1 of each replicated genotype (mean p-values are reported as p*). We have only reported p* as there was no difference in significance between p* and the full dataset permutation p. Bold: significant at p < 0.05; –: indicates an interaction term that was removed because it was not significant; *G. vermiculophylla*: *Gracilaria vermiculophylla*; na: not applicable; MvP: monoculture vs. polyculture

(A)	Epiphyte wet mass			Epifaunal counts			Bacterial density			Invertebrate diversity			*G. vermiculophylla* wet mass		
	df	*F*	p	df	*F*	p	df	*F*	p	df	*F*	p	df	*F*	p
May field survey															
Dry mass (D)	1	0.00	0.99	1	15.59	**0.004**	1	3.51	0.097	1	0.04	0.834	na	na	na
Genetic diversity (GD)	1	0.85	0.386	1	0.20	0.673	1	7.75	**0.023**	1	0.02	0.882	1	11.4	**0.011**
Tidal height (TH)	1	1.41	0.276	1	5.47	**0.048**	1	3.83	0.088	1	0.24	0.635	1	0.47	0.533
D × GD	–	–	–	–	–	–	–	–	–	–	–	–	na	na	na
D × TH	–	–	–	–	–	–	1	16.5	**0.001**	–	–	–	na	na	na
GD × TH	–	–	–	–	–	–	–	–	–	–	–	–	na	na	na
D × GD × TH	–	–	–	–	–	–	–	–	–	–	–	–	na	na	na
Error	9			9			8			9			10		

(B)	Epiphyte wet mass			Epifaunal counts			Bacterial density			Invertebrate diversity			*G. vermiculophylla* growth		
	df	*F*	p*	df	*F*	p*	df	*F*	p*	df	*F*	p*	df	*F*	p*
May field experiment															
Dry mass (D)	1	3.07	0.075	1	6.63	**0.014**	1	0.01	0.937	1	5.07	**0.022**	na	na	na
Tidal height (TH)	1	0.01	0.944	1	2.77	0.092	1	0.86	0.361	1	0.88	0.324	1	13.1	**0.001**
MvP	1	1.48	0.244	1	0.80	0.376	1	0.81	0.377	1	1.26	0.267	1	0.0	0.973
D × TH	–	–	–	–	–	–	–	–	–	–	–	–	na	na	na
D × MvP	–	–	–	–	–	–	–	–	–	1	3.91	**0.046**	na	na	na
TH × MvP	1	4.52	**0.031**	–	–	–	–	–	–	–	–	–	na	na	na
D × TH × MvP	–	–	–	–	–	–	–	–	–	–	–	–	na	na	na
Error	81			82			82			81			83		

(C)	Epiphyte wet mass			Epifaunal counts			Bacterial density			Invertebrate diversity			*G. vermiculophylla* growth		
	df	*F*	p*	df	*F*	p*	df	*F*	p*	df	*F*	p*	df	*F*	p*
July field experiment															
Dry mass (D)	1	3.32	0.073	1	57.41	**<0.001**	1	5.33	**0.016**	1	5.45	**0.024**	na	na	na
Tidal height (TH)	1	3.21	0.055	1	13.08	**0.001**	1	20.23	**<0.001**	1	0.14	0.687	1	66.2	**<0.001**
MvP	1	0.17	0.744	1	0.01	0.905	1	0.21	0.615	1	1.53	0.231	1	4.3	**0.049**
D × TH	–	–	–	1	4.88	**0.023**	–	–	–	–	–	–	na	na	na
D × MvP	–	–	–	–	–	–	–	–	–	–	–	–	na	na	na
TH × MvP	–	–	–	–	–	–	–	–	–	–	–	–	1	2.2	0.141
D × TH × MvP	–	–	–	–	–	–	–	–	–	–	–	–	na	na	na
Error	112			111			112			112			112		

tidal (Table 1C; Supplement 7, Fig. S7-1). Bacterial abundance also correlated with dry mass (Table 1C; Supplement 7, Fig. S7-2). Invertebrate diversity also correlated positively with *G. vermiculophylla* dry mass (Table 1C; Supplement 7, Fig. S7-3). Genotypic diversity did not alter any of these metrics of the epibiotic community in May or July (Table 1B,C and Supplement 8).

The multivariate analysis of the epifaunal communities revealed a significant shift between tidal heights (May: R = 0.113, p = 0.001; July: R = 0.354, p = 0.001; Supplement 9, Fig. S9-1), but no difference between mono- and polyclonal patches (R = –0.017, p = 0.916; R = –0.01, p = 0.765; Supplement 9, Fig. S9-1) at either tidal height. In May, Caprellid sp. amphipods, *Atilla* sp. polychaetes, the amphipod *Gammarus mucronatus* (Say), the polychaete *Alitta succinea* (Leuckart), and the polychaete *Dipolydora socialis* (Schmarda) caused the shift in tidal height communities. In July, the polychaete *D. socialis*

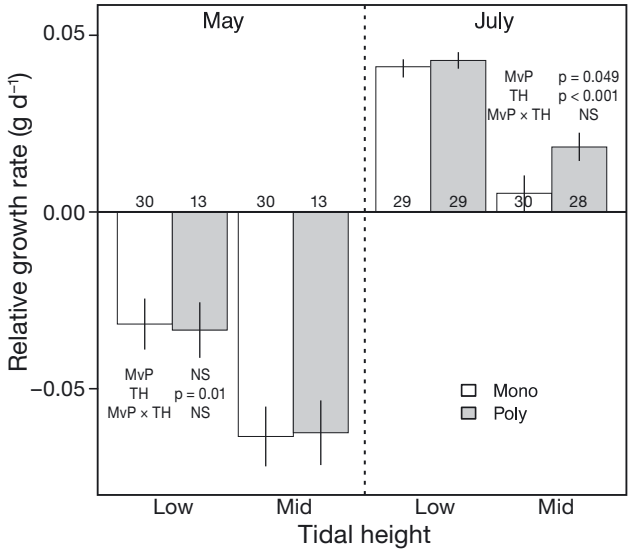

Fig. 4. Mean (± SE) relative growth rate of *Gracilaria vermiculophylla* for the May (left panel) and July (right panel) field experiments. Results of the permuted ANOVAs are presented for each experiment. Mono = monoculture, Poly = polyculture, MvP = monoculture vs. polyculture, TH = tidal height, NS = not statistically significant at α = 0.05. Numbers indicate sample size

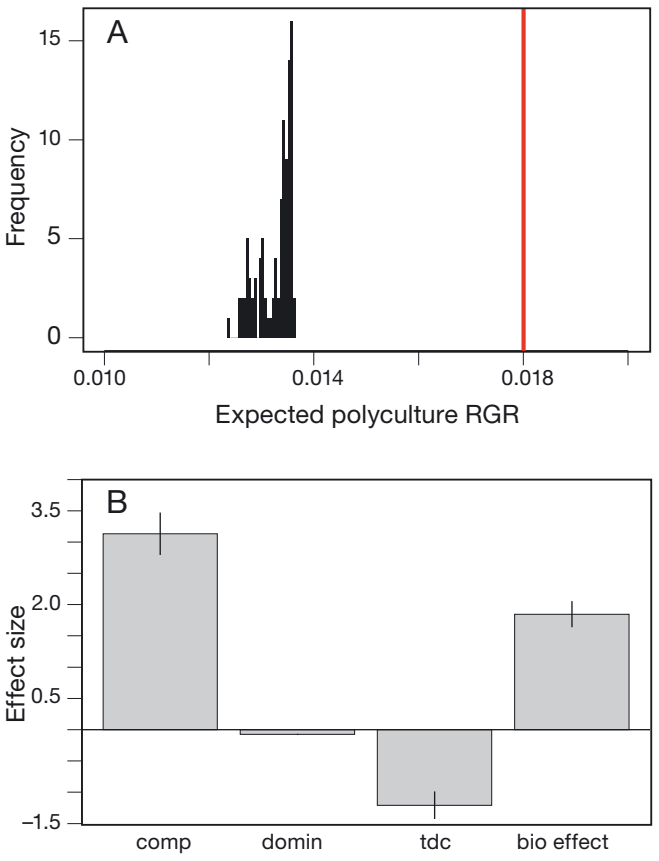

Fig. 5. (A) Histogram of the expected relative growth rates (RGRs) of the polyculture determined by randomly recreating polyculture patches. Red line indicates the observed average mean RGR of the polyculture patches. (B) Effect size of positive complementarity effects (comp: mean = 3.133, t = 9.162, $p < 0.001$), negative selection effects, or dominance effects (domin: mean = −0.076, t = −8.123, $p < 0.001$), trait-dependent complementarity (TDC: mean = −1.21, t = −5.484, $p < 0.001$), and the overall biodiversity effect (bio effect: mean = 1.847, t = 8.7492, $p < 0.001$) during the July mid intertidal trial. Error bars represent SE

(Schmarda), the amphipod *G. mucronatus*, and the hermit crab *Pagurus longicarpus* (Holthius) (Supplement 9, Table S9-1) were responsible for the shift in tidal height communities.

DISCUSSION

Overall, we detected variable impacts of genotypic diversity on primary productivity of *Gracilaria vermiculophylla* patches and virtually no impact on secondary production of macroalgal epibiota. To our knowledge, this represents the first test within any macroalga of whether within-species genotypic diversity has impacts on ecosystem functioning. We address our interpretation for these patterns in turn.

We detected no impacts of genotypic diversity on primary productivity in spring nor in the summer at the low intertidal, but net primary productivity increased 300% in polyclonal relative to monoclonal patches in the mid intertidal during the summer. The lack of genetic diversity effects contradicts most previous studies on plant ecosystem engineers, which tend to report positive results of genotypic diversity on standing biomass and primary production (Hughes et al. 2008). However, Hughes & Stachowicz (2009) did detect spatial and temporal differences in the effect of *Zostera marina* genetic diversity on shoot density, and in particular, a positive effect only in the winter when the eelgrass was under stress. This leads us to suggest that in *G. vermiculophylla*, genetic diversity increases productivity only in a moderately stressful environment (as determined by tidal exposure and light intensity; Supplement 10, Figs. S10-1 & S10-2), likely by buffering individuals from the stress. By extension, monoclonal or polyclonal patches do not grow in highly stressful conditions (spring), and polyclonal patches do not benefit from increased genetic diversity when conditions are near optimal and growth of all patches is high (summer, low intertidal).

The non-additive benefit of diversity within polyclonal *G. vermiculophylla* patches in the summer mid intertidal appears to reflect positive complementarity of genotypes (Fox 2005; our Fig. 5B) and negative selection effects (i.e. dominance effect and trait-dependent complementarity; Fox 2005), which imply that genotypes that did not generally perform well in monoculture performed well in polyculture. These same results have emerged in other marine populations (Reusch et al. 2005, Hughes & Stachowicz 2011, Aguirre & Marshall 2012b). We note that per-genotype growth rates did not differ between mono- and polyculture treatments (Supplement 4, Fig. S4-1). It appears that particular genotype combinations engender higher patch-level growth rates, but this complementarity benefit is not uniformly strong across all genotypes and their combinations.

The positive complementarity effect could be explained by a number of ecological mechanisms including increases in growth rates, declines in rate of loss to either biotic (e.g. herbivory, fouling) or abiotic forces, or some mix of these forces. Algal species can differ profoundly in nutrient usage, and assemblages of complementary species allow for overall greater production rates than species in monoculture or assemblages of competitors (Cardinale et al. 2002, Bracken & Stachowicz 2006). While a similar partitioning of resources may explain within-species complementarity in *G. vermiculophylla*, we did not test that possibility. It is unlikely that higher growth rates of polycultures reflect lower herbivory or fouling pressure. Polycultures and monocultures were statistically indistinguishable in the numbers of foulers (epiphytes or bacteria) and mobile epifauna, including putatively herbivorous taxa (*Ampithoe valida*, *Sesarma reticulatum*, *Synidotea laevidorsalis*). One caveat is that we did not measure herbivory by omnivorous fishes and invertebrates that occasionally consume *G. vermiculophylla* in South Carolina (L. Haram unpubl. data). These macro-consumers may prefer monoculture *G. vermiculophylla* patches.

In contrast, sampling effect played very little role in explaining the diversity effect. A sampling effect generates polycultures with greater growth rates when polycultures more frequently include genotypes that are highly productive or benefit most strongly from polyculture patches (see Huston 1997, Tilman et al. 1997, Hughes et al. 2008). Moreover, *G. vermiculophylla* genotypes more frequently used in polycultures did not have greater performance in polycultures than monocultures (Supplement 4, Fig. S4-2) nor have greater growth rates overall (Supplement 4, Fig. S4-3). The lack of effects of genotypic diversity on epibiota associates was somewhat surprising, given that multiple studies have also shown positive secondary effects on other ecosystem engineers (Hughes & Stachowicz 2004, Reusch et al. 2005, Crutsinger et al. 2006). In the case of *G. vermiculophylla*, the field surveys and experiments revealed weak to no effects of diversity on epiphyte, bacterial or epifaunal abundance, or on invertebrate diversity. This was consistent when analyses occurred on either a per-biomass (analyses not shown) or per-area basis (Table 1). We also did not see effects of genotypic diversity on epifaunal species assemblages (Supplement 9), as was seen in other systems (e.g. Johnson et al. 2006).

The lack of a significant impact of genotypic diversity on associated epibiota might be related to the ecology of the organisms inhabiting *G. vermiculophylla*. Most organisms on *G. vermiculophylla* rarely consume *G. vermiculophylla*, as we found low numbers of herbivorous mesograzers (0.1 ± 0.02 ind. patch^{-1}). Rather, most epibiota use *G vermiculophylla* as habitat for protection from consumers or abiotic stress, or as substrate for prey on mudflats that are devoid of much biological or physical structure (Johnston & Lipcius 2012, Wright et al. 2014, Bishop & Byers 2015, Kollars et al. 2016). Mesograzers often make host choices based on food quality of tissues, which varies profoundly among conspecific plants (for review, see Sotka et al. in press). In contrast, epifaunal non-herbivores likely make host choices based on traits that have little to do with tissue quality (e.g. Sotka et al. 1999) and more to do with morphological complexity (e.g. Hacker & Steneck 1990). Thus, it is possible that monoclonal and polyclonal patches of similar size and complexity are functionally equivalent habitats to the non-herbivorous taxa that dominate these algal patches.

Our results and their interpretation are tempered by 2 limitations of our experimental design. First, our field survey was gathered from a relatively small area (~100 m^2) of the mudflat, which may mean that our initial range of trait variation was small (sensu Fridley & Grime 2010). If we had used genotypes from a wider swath of invaded habitat, we might have seen a diversity effect at a broader spatial scale and on higher trophic levels. If we had used this broader range of traits, however, then the results may be less relevant to the small spatial scales at which the algae and associated organisms interact in nature. Second, we used field-collected genotypes, and thus all responses in the field survey and experiments were influenced not only by an individual's genetic constitution but also by its environmental history. To minimize environmental history, we would need to rear individuals in the same environment prior to the start of the experiment.

CONCLUSIONS

Overall, genotypic diversity had variable effects on primary productivity and little to no effect on any metric of secondary productivity or diversity. We propose that under moderately stressful conditions, genotypic diversity can have positive effects on net primary production in the non-native species *Gracilaria vermiculophylla*. This suggests that under the appropriate environmental conditions, genetic diversity of introduced species can increase productivity, and this in turn may give rise to more population- and patch-level diversity by increasing biomass and greater frequencies of sexual reproduction and somatic mutations (see also Aguirre & Marshall 2012a,b, Wang et al. 2012). The positive impact of modest genetic diversity could thus increase the spread and persistence of non-native populations, exacerbate impacts on the community and ecosystem, and make removal efforts more difficult.

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