The relative importance of trait vs. genetic differentiation for the outcome of interactions among plant genotypes

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Abstract. Functional trait differences and genetic distance are increasingly used as metrics to predict the outcome of species interactions and the maintenance of diversity. We apply these ideas to intraspecific diversity for the seagrass Zostera marina (eelgrass), by explicitly testing the influence of trait distance and genetic relatedness on the outcome of pairwise interactions among eelgrass genotypes. Increasing trait distance (but not relatedness) between eelgrass genotypes decreased the likelihood that both would persist over a year-long field experiment, contrary to our expectations based on niche partitioning. In plots in which one genotype excluded another, the biomass and growth of the remaining genotype increased with the trait distance and genetic relatedness of the initial pair, presumably due to a legacy of past interactions. Together these results suggest that sustained competition among functionally similar genotypes did not produce a clear winner, but rapid exclusion occurred among genotypes with distinct trait combinations. Borrowing from coexistence theory, we argue that fitness differences between genotypes with distinct traits overwhelmed any stabilizing effects of niche differentiation. Previously observed effects of eelgrass genetic diversity on performance may rely on nonadditive interactions among multiple genotypes or sufficient environmental heterogeneity to increase stabilizing forces and/or interactions.

Key words: biodiversity; coexistence; ecosystem function; functional traits; genetic diversity; genetic relatedness; intraspecific interactions; trait diversity; Zostera marina.

INTRODUCTION

For decades, ecologists have been interested in how functional traits influence interacting species to determine the outcome of competition (e.g., Hardin 1960, MacArthur and Levins 1967) and ecosystem structure and functioning (e.g., Hector et al. 1999, Tilman 1999, Heemsbergen et al. 2004, Wójcik and Mittelbach 2007). More recently there has been interest in how the same principles apply to trait variation among individuals within species (Hughes et al. 2008, Bolnick et al. 2011). Although these studies show clear evidence that trait differences affect the outcome of interactions both among and within species, choosing which traits matter to the outcome of interactions a priori is often challenging and context dependent (Naeem and Wright 2003). Furthermore, within-species trait-based approaches may be entirely infeasible where continuous traits are involved, except where genotypic diversity is low, because of the effort needed to measure traits on a large number of genotypes.

Alternatively, genetic relatedness could be used as a proxy for trait differentiation, integrated across a number of traits. A growing body of work takes this approach for interspecific comparisons, based on the idea that phylogenies can reflect integrated phenotypic differences among taxa (Felsenstein 1985, Harvey and Pagel 1991). Reanalysis of species richness manipulations finds that phylogenetic diversity of an assemblage (e.g., total branch length in a phylogeny) is often a better predictor of assemblage productivity than the number of species or functional groups (Cadotte et al. 2008, 2009, Flynn et al. 2011). More generally, the outcome of interspecific interactions can be predicted by the phylogenetic distinctiveness of the interacting species, with more closely related species competing more intensely, leading to lower group productivity and/or greater probability of competitive exclusion (Maherali and Klironomos 2007, Burns and Strauss 2011, Violland et al. 2011). These patterns are hypothesized to result from a relationship between evolutionary divergence and divergence in traits relevant to the outcome of competition, although this link is not always clearly demonstrated (but see Violland et al. 2011). While not all traits relevant to the outcome of interactions are evolutionarily conserved (see, e.g., Best

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and Stachowicz 2013, Best et al. 2013), phylogenetic distance sometimes provides an integrated measure of many traits that is more strongly correlated with ecological functioning than species richness, per se. The same approach may prove useful within species, where the genetic relatedness of individuals may be correlated with overall phenotypic differences. This, in turn, could make estimates of relatedness a stronger predictor of the performance of interacting genotypes than measures of genotypic richness. For example, reanalysis of genotypic richness manipulations found that relatedness was a significantly better predictor of total biomass accumulation than genotypic richness, and this was probably due to a strong correlation between relatedness and trait diversity (Stachowicz et al. 2013). However, these experiments used assemblages with a limited range of relatedness, and trait distance and genetic relatedness were positively (not negatively) correlated in this limited sample of genotypes and mixtures. Genetic relatedness need not be tightly correlated with trait diversity, especially where there is strong selection on ecologically relevant traits. In these cases, phenotypic differentiation often exceeds what might be predicted by genetic distance, and for many traits there may be little correlation between the two (Reed and Frankham 2001, McKay and Latta 2002).

Genetic relatedness could also have direct effects on assemblage or individual performance through inbreeding or outbreeding depression (e.g., Charlesworth and Charlesworth 1987, Ralls et al. 1988, Crnokrak and Roff 1999, Keller and Waller 2002) or kin recognition (Dudley and File 2007). However, whether such effects are strong enough to affect the outcome of interactions or assemblage biomass accumulation is not yet clear.

In this paper, we explicitly test the effects of trait differences and genetic relatedness on the interactions between eelgrass (*Zostera marina*) genotypes. Eelgrass forms extensive monocultures in bays and estuaries throughout the northern hemisphere, where it is often the only vascular plant, and provides critical habitat for fishes and invertebrates, while buffering shorelines from erosion and playing a key role in nutrient cycling (Williams and Heck 2001). Eelgrass reproduces sexually as well as vegetatively, and genotypic diversity varies at scales of meters (1–15 genotypes/m² in Northern California; Hughes and Stachowicz 2009). Genotypes can grow highly intertwined, with as many as four unique genotypes occupying a 10 × 10 cm area, so multiple genotypes potentially interact at a fine scale. Eelgrass genotypes differ in traits such as individual growth, nutrient uptake, susceptibility to herbivores, and detrital production (Ehlers et al. 2008, Hughes et al. 2009, Tomas et al. 2011), and these differences in phenotypes lead to overyielding and complementarity in field experiments (Hughes and Stachowicz 2004, 2011, Reusch et al. 2005).

We build on this previous work to ask how pairwise trait distance and relatedness affect both the outcome of intraspecific interactions (the likelihood of competitive exclusion) and the productivity of the assemblage (plot-level biomass and leaf growth rate). In addition, we examine the relationship between relatedness and trait distance to see if relatedness can be used as a proxy for trait distance. Based on the theory underpinning positive biodiversity–ecosystem function relationships, we hypothesize that relatedness and trait distance are negatively correlated, and that greater trait differentiation between genotypes increases resource partitioning, such that pairs with a greater trait distance are more likely to coexist and achieve higher overall biomass. Alternatively, large trait differences among genotypes could enhance the probability of competitive exclusion if these trait differences lead to large fitness differences between genotypes within the local environment. This idea that the breadth of available niche space and fitness (trait) differences among species or genotypes interact to affect competition and performance is the basis of contemporary theories of coexistence among species (Chesson 2000, Hubbell 2001, Adler et al. 2007, Mayfield and Levine 2010, Turnbull et al. 2012). Here we apply these ideas to the maintenance of intraspecific diversity and the relationship between intraspecific diversity and assemblage productivity.

**Materials and Methods**

We used six unique eelgrass genotypes in our field experiment that were known to vary in their genetic relatedness to one another and in their phenotypic traits (Appendix S3). Genotypes were propagated in outdoor tanks at the Bodega Marine Lab, Bodega, California, USA, to produce enough shoots to allow deployment of five replicates of all possible pairwise combinations of the six genotypes. We delineated genotypes and estimated relatedness using 11 microsatellite loci selected from a pool of >30 loci designed specifically for *Zostera marina* (Appendix S2; see Reusch et al. 1999, Reusch 2000, Oetjen and Reusch 2007a, Oetjen et al. 2010). We determined the relatedness of all genotype pairs using a regression-based measure of the number of shared alleles calibrated by the frequency of those alleles in the population using the program STORM (Frasier 2008). We estimated population allele frequencies using 260 genotypes collected at five sites and three tidal heights throughout Bodega Harbor in May 2012.

**Trait distance measurements**

We previously measured numerous traits for these six genotypes (Hughes et al. 2009), including traits related to biomass (aboveground, belowground, and ratio of above to belowground), growth rate (new...
shoots produced, and leaf and rhizome growth rate, morphology (max root length), nutrient uptake rate (nitrate uptake of the shoots and ammonium uptake of the roots), and photosynthetic rate (light saturated and light limited). From these trait data, we calculated trait distance between all possible genotype pairs using standard methods (e.g., Petchey and Gaston 2002). We used the trait data for all of the traits that varied significantly among genotypes (all traits except for aboveground biomass and photosynthetic rate, 24 traits in total) to create a trait matrix where each trait’s values were standardized to have a mean = 0 and variance = 1. We then used the R “dist” function, R version 3.0.3 (R Core Team 2014) to produce a Euclidean distance matrix of the multivariate trait distance between all genotype pairs. We also used a principal components analysis to account for correlations among traits and used principal component scores for PC1 and PC2 to calculate trait distances between all pairs of genotypes in two-dimensional trait space. We performed the PCA using scaled and centered (mean = 0 and variance = 1) trait values and used a correlation matrix to calculate principal components. The analysis was performed in R 3.0.3 using the prcomp function (R Core Team 2014), which performs the PCA using singular value decomposition of the data matrix.

Field experiment

In July 2011, we planted five replicates of each of the 15 possible pairwise combinations of the six genotypes and their monocultures in the field for a total of 105 plots. We cleared five 85 cm × 13 m rows of all eelgrass shoots and rhizomes in an existing intertidal eelgrass bed in Bodega Harbor, California, USA. Each row was spaced by 1 m from adjacent parallel rows and roughly followed a depth contour and was thus considered a block for analysis. Each block had 21 plots with one replicate of the 15 pairs and the six monocultures. We inserted plastic containers (19.5 cm long × 14.5 cm wide × 12.5 cm high) lined with 2 mm diameter mesh into each plot and filled them with field-collected sediment. Each container was spaced 33 cm apart within a row (block) and 33 cm from the edge of the row (block). The level of the sediment in the container was even with that of the surrounding sediment and the edge of the container protruded about 3 cm above the sediment to prevent plants from growing outside of the containers. We randomly assigned each of the 21 genotype combinations (15 pairs and six monocultures) to a container within each block.

We planted two shoots of each genotype for each relatedness pair into the containers, and four shoots of a given genotype in monoculture plots. Prior to planting, we removed all epibionts from each shoot and standardized them to 30 cm of shoot and 2.5 cm of rhizome. We arranged all four shoots in a square pattern, evenly spaced, with rhizomes oriented such that elongation would be in the direction of other shoots in the container. We recorded shoot density monthly and measured leaf growth on 10 randomly selected shoots at the end of the experiment using the hole-punch method (Williams and Ruckelshaus 1993).

After 1 yr of growth, we harvested all of the eelgrass from each container. Each physically connected section of eelgrass was placed into individually labeled ziploc bags and transported a short distance (~2.5 km) back to the marine lab, where they were stored in a refrigerator until processing. For each segment of ramet, we took a tissue sample for genetic analysis. We measured growth as leaf area (length that the hole-punch mark traveled from the base of the sheath × shoot width). Next, we divided the biomass of each ramet into shoots, roots, rhizome, and reproductive shoots. These biomass samples were dried at 60°C for at least 48 h before weighing. We also counted the number of shoots and measured various aboveground and belowground morphological parameters on each shoot, but all of these correlated strongly with total dry biomass measures so we do not present them.

Re-genotyping

The tissue samples collected for genotyping were processed in the same manner as the original genetic analysis. Each sample was identified as one the original six genotypes or a new unique genotype. A new genotype established in three of the 105 plots and these were excluded from analysis because the timing of colonization and traits of these new genotypes were unknown.

Statistical analysis

All analyses were done using R 3.0.3 (R Core Team 2014). The relationship between relatedness and trait distance was analyzed using a Mantel test (vegan package) to account for nonindependence of the data caused by the presence of the same genotype in multiple pairwise combinations.

Using logistic regression, we tested the effects of relatedness and trait distance of original pairs on the likelihood that each genotype would be lost (excluded) from the plot. To do this, we assessed the survival of each genotype individually for all plots, such that each plot had two entries, one for each genotype, and the response variable was binomial survivorship. We included genotypic identity in the model to test if survival varied among genotypes. We excluded from this analysis monocultures and plots where both genotypes failed to establish.

We used generalized linear mixed models to assess the effects of relatedness and trait distance on
plot-wide eelgrass performance measured as above- and belowground biomass and leaf growth rate. The analyses were performed using the MCMCglmm package in R, which uses Markov chain Monte Carlo techniques, and included exclusion (exclusion vs. coexistence), relatedness, and trait distance as fixed effects and block as a random effect in the model. We specified a Gaussian distribution for the response variables and used priors that correspond to an inverse-Gamma distribution with shape and scale parameters equal to 0.001. We tested for differences in performance among monocultures of the six genotypes using the ANOVA function in the CAR package in R, including a block effect.

**RESULTS**

**Trait distance vs. relatedness**

Our principal components analysis showed that many of the traits that we measured were correlated and that the six genotypes covered a broad range of trait space (Fig. 1A). The first two principal components accounted for 42% and 25% of the variation, respectively. Higher order PCs contributed minimally to explaining variation in traits and were not considered further. PC1 was negatively associated with ammonium uptake rate and new shoot production and positively associated with the ratio of above- to belowground biomass. PC2 was negatively associated with the biomass and growth of the terminal shoot and positively associated with nitrate uptake rate. The six genotypes fall in all quadrants of the trait PCA (Fig. 1A), with some genotype pairs having more similar traits than others. Genotypes differ across both axes and different traits contribute to the multivariate trait distance between different genotype pairs (Fig. 1A).

However, across all 15 possible combinations, pairwise multivariate trait distance was uncorrelated with pairwise genetic relatedness (Mantel statistic $r = 0.19$, $P = 0.263$; Fig. 1B; Appendix S1). Although this means that relatedness cannot be used as a proxy for trait distance among these six genotypes, it does allow us to assess the relative influence of relatedness and trait distance on coexistence and performance. There were also no strong correlations between relatedness and trait distance for any individual traits and only a few that were significant close to $P = 0.05$. Correcting for multiple comparisons (24 traits in all) using the Bonferroni correction resulted in none of these being significant at $P < 0.05$. There was also no correlation between relatedness and the two-dimensional trait distance measured as distance between genotypes across the two primary principal components (Mantel statistic $r = 0.33$, $P = 0.129$). Our two trait distance measures

![Fig. 1. (A) Principal components biplot depicting how genotypes 1–6 of the eelgrass *Zostera marina* differ in their traits across the two most significant principal components axes. Arrows represent the loadings for the 24 different trait measurements (top and right axes) and numbers depict the PC scores for the six genotypes (bottom and right axes). For simplicity, instead of labeling all traits, six of the major trait groupings are listed in the area where their arrows occur. (B) The relationship between relatedness and trait distance of all possible pairwise combinations of the six genotypes. We determined the relatedness of all genotype pairs using a regression-based measure of the number of shared alleles calibrated by the frequency of those alleles in the population using the program STORM (Frasier 2008). We estimated population allele frequencies using 260 genotypes collected in May 2012 at five sites and three tidal heights throughout Bodega Harbor, California, USA. From the trait data, we calculated trait distance between all possible genotype pairs using standard methods (e.g., Petchey and Gaston 2002).](image-url)
(summed Euclidean distance among all traits vs. distance on PCA axes) were highly correlated ($r = 0.92$, $P < 0.0001$).

**Effect of trait distance and relatedness on coexistence of genotypes**

Eelgrass failed to permanently establish in 28 of 105 plots. The percentage of plots failing to establish was the same for monocultures and pairs (28% and 26%, respectively), and trait distance, relatedness, genotypic identity, or block did not affect the likelihood of both genotypes being lost. Most (23/28) of the plots with complete mortality failed to produce any new shoots before all shoots died in the plot, suggesting that complete mortality as a result of failure of transplants to establish was probably due to dislodgement by currents, burrowing organisms, or floating algal mats rather than due to any interactions among shoots. Thus we excluded these plots from the remainder of the analyses.

Among plots that contained eelgrass at the end of the experiment, exclusion of one genotype occurred in 58% of the plots originally planted with two genotypes, hereafter referred to as “exclusion plots” (compared to “coexistence plots,” which are those in which both genotypes persisted). The best-fitting model predicting genotype survival included both trait distance from the competitor and identity of the focal genotype, and this model was a better fit than models including either predictor alone (Table 1; $P = 0.026$). As pairwise trait difference increased, the likelihood of one of the two genotypes being excluded also increased (Fig. 2B). Genotypes 5 and 6 were more frequently excluded (excluded from 50% and 44%, respectively, of plots where they were initially planted), whereas genotype 1 was only excluded in 13% of the plots in which it was initially planted (Fig. 2A, Table 1). Trait distance, rather than particular trait values, influenced the likelihood of exclusion. The two genotypes most likely to be excluded (genotypes 5 and 6) occur in opposite quadrants of trait space (Fig. 1A), with genotype 5 having particularly high nitrate uptake rate and low terminal shoot mass/growth rate and vice versa for genotype 6. Similarly, the two genotypes least likely to be excluded (genotypes 1 and 2) also occurred in opposite quadrants of trait space (Fig. 1A), differing in ammonium uptake and the ratio of above:belowground biomass.

**Effects of trait distance and relatedness on plot-level biomass and leaf growth**

The effect of genetic relatedness and trait distance on total biomass achieved in a plot depended on whether or not both genotypes remained at the end of the experiment (Table 2: interaction between whether exclusion occurred and the effect of trait distance or relatedness). Thus, we analyzed the data from exclusion plots and coexistence plots separately. If both genotypes remained, there was no effect of trait distance or relatedness on biomass or leaf growth rate (Fig. 3A, C, Table 2), but if only one genotype remained, increasing trait distance and relatedness of the planted pair increased the biomass (stronger relationship with belowground biomass vs. aboveground, and stronger with trait distance vs. relatedness) and growth rate (stronger relationship with relatedness) of the surviving genotype (Fig. 3B, D, Table 2).

Biomass and leaf growth did not differ among the six genotypes in monoculture (Fig. 4; aboveground biomass $P = 0.58$, belowground biomass $P = 0.95$, growth $P = 0.70$), but did increase with decreasing tidal elevation (block effect $P < 0.001$). Because there

<table>
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<th>Variable</th>
<th>Model 1 Coeff.</th>
<th>Model 1 SE</th>
<th>Model 1 Wald Z</th>
<th>Model 1 P</th>
<th>Model 2 Coeff.</th>
<th>Model 2 SE</th>
<th>Model 2 Wald Z</th>
<th>Model 2 P</th>
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<td>--</td>
<td>--</td>
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<td>1.08</td>
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<tr>
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<td>1.03</td>
<td>-2.59</td>
<td>0.009</td>
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*Notes.* Table shows the coefficients, their standard error, the Wald Z statistic, and associated P values. For model 1, AIC = 127.48; for model 2, AIC = 129.11. In both models Genotypes 2–6 are compared to genotype 1 for survivorship (genotype 1 had the highest survivorship). Genotypes 4, 5, and 6 are all significantly less likely to survive (more likely to be excluded). We also tested the overall effect of genotype (all genotypes together) in the models using the wald.test function in the aod package of R.3.0.3. The chi square statistics and degrees of freedoms for the overall effect of genotype are $\chi^2 = 11.6$, df = 5 and $\chi^2 = 11.8$, df = 5 for models 1 and 2, respectively.
was no difference among monocultures in performance, the expected performance (biomass or leaf growth rate) of all pairs of genotypes was the same (equal to the mean of all monocultures). The mean monoculture belowground biomass is plotted on each panel of Fig. 3 as a dashed line to facilitate comparisons between monocultures and mixtures. Coexistence plots, on average, performed better than plots originally planted as monocultures, although not significantly so (Fig. 3A). In exclusion plots, the performance relative to monocultures depended on trait distance. Plots planted with low trait distance pairs performed consistently worse than monocultures, whereas those with high trait distance tended to have higher biomass than monocultures (Fig. 3B).

The pattern of increasing performance with trait distance or relatedness of original pair in exclusion plots is not explained by the expected performance of the genotypes that dominate and remain at the end of the experiment. There is no correlation between plot belowground biomass in exclusion plots and the expected belowground biomass in that plot, based on which genotype remains and its belowground biomass in monocultures ($P = 0.96, R^2 < 0.001$). Moreover, the pattern of increasing belowground biomass with trait distance of the original pair remains even if we exclude plots in which genotype 1 dominated ($P = 0.01, R^2 = 0.28$), which includes many of the highest trait distance plots. Finally, in the low trait distance exclusion plots, which are the ones that deviated most from the expected performance based on monocultures, there were several different genotypes that remained at the end of the experiment and they each performed worse in the low trait distance pairs than in monocultures, or when they excluded their partner in higher trait distance pairs (e.g., genotypes 2 and 3 in Fig. 3B, D). Thus, the pattern of increasing performance with trait distance in exclusion plots is not driven solely by the identity or traits of the persistent genotype, but rather by some legacy of past interactions with the other genotype in the original pair and how similar their traits were.

**DISCUSSION**

Genetic relatedness was not correlated with our multivariate trait index, nor was it strongly correlated with the values for any individual trait, and is not a good proxy for the overall trait differentiation we observed among these genotypes. Correspondingly, trait distance had a stronger and more consistent effect on the outcome of pairwise interactions (exclusion, biomass, and weak effect on leaf growth) than did relatedness (leaf growth, and weak effect on biomass). However, the positive effects of relatedness and trait differentiation on biomass and growth were only manifest when one of the two planted genotypes had been excluded. This suggests that the effects of relatedness we saw probably represent unmeasured trait differentiation rather than an effect of kin recognition. We will draw on theory of coexistence among species to interpret our results and suggest the potential mechanisms underlying the effects of trait distance on competitive exclusion and biomass accumulation among genotypes of eelgrass.

The likelihood of exclusion increased with trait distance in our experiment. This is contrary to the prediction that trait differentiation should promote coexistence by reducing overlap in resource use among competitors. However this prediction hinges on stabilizing forces, such as resource partitioning, being sufficiently large to offset any fitness differences between genotypes caused by the trait differences and allow for frequency-dependent population dynamics (Adler et al. 2007). If stabilizing forces are weak at the scale of these interactions, then trait differences can increase the likelihood of exclusion, because one genotype will have higher fitness in that particular environment. Conversely, genotypes with very similar traits may coexist longer in this situation because fitness differences are small and neither genotype is clearly the superior competitor (cf. Hubbell’s [2001] neutral theory of biodiversity).
<table>
<thead>
<tr>
<th>Model</th>
<th>Belowground biomass</th>
<th>Aboveground biomass</th>
<th>Leaf growth rate</th>
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<tr>
<td></td>
<td>Estimate</td>
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<tr>
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<tr>
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<tr>
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<tr>
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<td>Relatedness</td>
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<td>−0.23</td>
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**Notes.** Predictor variables evaluated include continuous measures of trait distance and relatedness, and exclusion, a binary variable donating if the plot resulted in coexistence or exclusion. Block was included as a random effect. Models are presented with the posterior mean of the estimates (Estimate), 95% credible intervals (equivalent to 95% confidence intervals), and pMCMC values (testing if the parameter is significantly different from zero, analogous to $P$-values at $=0.05$).
Our data are consistent with the idea that, in this experiment, stabilizing forces were weak and the outcome of competition had more to do with fitness differences caused by differential matching of traits to the environment. The significant effect of genotypic identity on exclusion rate also supports this hypothesis because certain genotypes consistently dominated and excluded their paired genotype, suggesting that they were often superior competitors under the conditions in our experiment (Table 1). For example, of the 16 plots containing genotype 1, only five ended with coexistence, and when exclusion occurred, genotype 1 was more likely to be the survivor (9/11 replicates). All of the pairs containing genotype 1 were above the mean trait distance of all pairs, and so the dominance of this genotype contributed to the relationship between trait distance and exclusion rate. However, genotypic identity and trait distance both contribute independently to the probability of exclusion (Table 1), so the pattern is not driven by genotype 1 alone. Furthermore, the two genotypes most likely to exclude others (1 and 2) have very different traits and are at opposite ends of the PC1 trait axis (Fig. 1A). Thus, although some genotypes consistently dominate, the traits that allow them to do so differ among genotypes.

In contrast, among pairs with less trait differentiation, no genotype consistently dominated and, in many cases, the winner of a given pairwise combination varied among replicates. Out of the seven mid and low trait distance combinations in which multiple

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FIG. 3. Effects of trait distance (A, B) and relatedness (C, D) on belowground biomass in both coexistence (A, C) and exclusion plots (B, D). Graphs show the residuals of belowground biomass by block to better visualize the effects of trait distance while taking into account block effects. Results from exclusion plots are coded by the genotype remaining at the end of the experiment; the key shows which symbol corresponds to each genotype. The dashed line represents the average value for monocultures; the solid lines are regressions; $r = 0.53$ in (B) and $r = 0.37$ in (D). Similar patterns were observed with leaf growth rate (not shown).

FIG. 4. Residuals of mean belowground biomass, originally measured in g (after accounting for the block effect) by genotype in monoculture. Error bars are 95% confidence intervals. A similar pattern was observed for aboveground biomass and for growth rate.
replicates resulted in exclusion, only three had the same genotype win in all replicates. This is consistent with low trait distance pairs having small fitness differences, and the outcome of intergenotypic competition being stochastic when fitness differences are small.

Many of the same mechanisms underlying the effects of trait distance on the coexistence of genotypes can also affect their aggregate performance. Surprisingly, we found that trait distance affected the total biomass accumulated only in plots in which one genotype had been excluded. If pairs with high trait distance have one genotype that is clearly a superior competitor and more likely to exclude its pair, it may do so more quickly than in pairs with low trait distance where exclusion occurs. Thus, the pace of competitive exclusion may influence final plot-scale biomass. In pairs with high trait distance, if one genotype excludes the other quickly, then it functions as a monoculture and should achieve approximately the same biomass as monoculture plots. This is, in fact, what we found with the high trait distance exclusion plots (Fig. 3B, D).

In pairs with low trait distance, the genotypes may be closer to competitive equals and it may take longer for one genotype to be excluded. If intergenotypic competition is stronger than intragenotypic competition, possibly due to response to self vs. non-self recognition (Dudley and File 2007), then the remaining genotype in low trait distance pairs may have a lower biomass compared to monocultures because it will have experienced intergenotypic competition for a longer period. Again this is the pattern we see in our data (Fig. 3B, D) and could explain the relationship between trait distance and biomass in the exclusion plots. However, we have no data on the time to exclusion in our plots with which to evaluate this mechanism.

Previous experiments with these genotypes have found strong complementarity when many genotypes coexist in larger plots (e.g., Hughes and Stachowicz 2011), and this effect was stronger in plots with greater trait distance (Stachowicz et al. 2013). Although this initially seems at odds with our finding that trait distance did not affect biomass accumulation in coexisting pairs, previous demonstrations of complementarity occurred in plots with six or more genotypes (see also Hughes and Stachowicz 2004, Reusch et al. 2005). This suggests that many genotypes, particular combinations of genotypes, and/or a more heterogeneous environment are required for sufficiently broad trait variation to lead to biomass overyielding.

Contrary to the intuitive and commonly held assumption that relatedness and trait differences should be correlated, our results found no support for the use of relatedness as a proxy for trait distance, even though we considered a large number of traits relevant to the performance of eelgrass. We acknowledge that this conclusion is tentative, as it is drawn from only six genotypes and 11 markers. However, the lack of correlation is perhaps not too surprising, given that the traits we measured were selected because they relate to plant fitness and thus are likely under selection, and the markers we used to estimate relatedness are neutral or possibly linked to unmeasured traits. For our work, four of the 11 microsatellite markers that we used to estimate relatedness are anonymous markers and putatively neutral. The other seven markers come from an EST library and are gene-linked, with putative gene function based on BLASTX hit searches showing results related to seed maturation, cytochrome c (electron transport chain in mitochondria), apoposy (embryo development in flowering plants), and bHLH like protein (transcription factor) (Oetjen and Reusch 2007a, b, Oetjen et al. 2010). It is unlikely that any of these would directly affect any of the traits we measured. Thus, it is also possible that our estimates of relatedness might be correlated with some other unmeasured traits. Indeed, the direct effect of relatedness on biomass accumulation and leaf growth rate that we found was only in the exclusion plots (Table 2), making it difficult to imagine how kin recognition might underlie these effects. The anticipated availability of genomic scale data for Zostera (Wissler et al. 2009) might improve the prospects of genetic data being used as a proxy for functional variation, but for now, this remains a prospect rather than a reality.

There is a growing interest in incorporating intraspecific variation, genetic diversity, and evolutionary processes such as selection and drift into our understanding of community ecology (Neuhauser et al. 2003, Vellend 2010, Bolnick et al. 2011). One manifestation of this interest is the emerging evidence that intraspecific genetic diversity is an important driver of the maintenance of species diversity (Booth and Grime 2003, Lankau and Strauss 2008, Fridley and Grime 2010) and can influence community structure and ecosystem functioning (e.g., Whitham et al. 2006, Hughes et al. 2008). However, the assumption that trait differences among individuals necessarily lead to trade-offs in space or time that promote diversity and higher aggregate performance may not be universal. Our results remind us that trait variation among genotypes can also reduce diversity when fitness differences under particular conditions are sufficiently large to result in competitive superiority of one genotype. Thus some caution is needed in the application of two common assumptions (1) that the genetic relatedness of individuals predicts overall similarity in traits and (2) that increasing trait differentiation consistently leads to coexistence and increased performance. However, scaling up our results from pairwise experiments to assemblages of interacting genotypes on the scales that occur in nature remains a significant challenge, but one with the potential reward of aiding in the conservation and restoration of the genotypic diversity of key foundation species such as eelgrass.

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LITERATURE CITED


