

Plant species diversity and genetic diversity within a dominant species interactively affect plant community biomass

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Summary

1. Both plant species diversity and genetic diversity within a plant species can affect community properties and ecosystem processes. However, the relative contribution of species diversity and genetic diversity to ecosystem functioning is poorly known. Furthermore, ecosystem processes may respond non-additively to interactions between species diversity and genetic diversity. If interactive effects exist, the impact of biodiversity loss may not be predictable from simple assessments of either species diversity or genetic diversity alone.

2. Here, we addressed how plant species diversity and genetic diversity within a dominant species independently and interactively influenced plant community biomass in a Great Lakes sand dune ecosystem. To test the independent effects of diversity, we established two experiments. In one, we manipulated genetic diversity within the dominant dune species, *Ammophila breviligulata*. In the other, we manipulated the number of plant species, excluding *A. breviligulata*. Then, to test for interactive effects, we constructed communities that varied the number of species and levels of genetic diversity within *A. breviligulata*.

3. Although there were no independent effects of either species diversity or genetic diversity within *A. breviligulata* on biomass production in this system, interactive effects of species diversity and genetic diversity significantly influenced overall above-ground biomass production of the plant communities. Specifically, as genetic diversity within the dominant species increased, the relationship between species diversity and community-level biomass shifted from negative to positive. Negative non-additive effects of diversity drove this pattern.

4. Synthesis. These results show, for the first time, that interactions between plant species diversity and genetic diversity within a dominant species can alter biomass production, highlighting the importance of incorporating interactions between levels of biodiversity into our understanding of how biodiversity influences ecosystem function.

Key-words: additive effects, *Ammophila breviligulata*, biodiversity-ecosystem function, dominance, Great Lakes, intersimple sequence repeat, non-additive effects, plant–plant interactions, sand dunes, species richness

Introduction

Native biodiversity is being lost at a rapid rate owing to anthropogenic causes, including habitat destruction, pollution and the spread of non native species (Chapin *et al.* 2000; Butchart *et al.* 2010; Barnosky *et al.* 2011). During the last two decades, this loss has spurred research to elucidate the influence of biodiversity on ecosystem functioning (reviewed by: Loreau, Naeem & Inchausti 2002; Hooper *et al.* 2005). The majority of

these studies have focused on how reductions in the number of plant species influence terrestrial ecosystems (Duffy *et al.* 2007) and show that ecosystem functioning generally declines as species richness is reduced (meta-analyses: Balvanera *et al.* 2006; Cardinale *et al.* 2006, 2011). However, before a species is lost from the community, it will likely suffer losses in genetic diversity owing to shrinking population sizes (Ellstrand & Elam 1993). In addition to having long-term effects on evolutionary potential, the loss of intraspecific diversity can have negative consequences on a shorter time-scale, if variation within species (genetic diversity) plays an analogous role to variation among species (species diversity) in the maintenance

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of ecosystem functioning (Hughes *et al.* 2008). Furthermore, it is possible that genetic diversity and species diversity may interact to influence community structure and ecosystem functioning (Booth & Grime 2003; Vellend & Geber 2005; Vellend 2006), making the consequences of biodiversity loss difficult to predict based on either level of diversity alone.

Although the consequences of intraspecific variation have only recently received attention in the ecological literature, effects of genetic diversity on ecosystem functioning have been clearly documented in some ecosystems (reviewed by Hughes *et al.* 2008). Higher levels of genetic diversity increased the population biomass of goldenrod (*Solidago altissima*) in the field (Crutsinger *et al.* 2006) and of *Arabidopsis thaliana* in the greenhouse (Crawford & Whitney 2010; Kotowska, Cahill & Keddie 2010). Additionally, genetic diversity was positively correlated with population resistance and resilience to disturbance in eelgrass (*Zostera marina*) (Hughes & Stachowicz 2004; Reusch *et al.* 2005). Strong effects of genetic diversity are expected to be particularly prevalent for systems that are characterized by having one or a few dominant species (Whitham *et al.* 2006; Hughes *et al.* 2008).

The mechanisms underlying the positive effects of diversity on ecosystem function can be classified into one of two categories, additive versus non-additive effects (Hughes *et al.* 2008). Additive effects occur when population or community responses can be predicted by summing the responses of component individuals in monoculture. Therefore, populations or communities with greater diversity have an increased probability of including a genotype or species with a large effect on the measured response (lottery model or sampling effect). Non-additive effects, on the other hand, occur when interactions (e.g. facilitation, niche partitioning, competition) among community members cause individuals to perform differently in polyculture relative to monoculture, resulting in a change in the community response relative to additive predictions. Positive non-additive effects can alter ecosystem functioning relative to the additive expectation and have been documented in experimental studies of genetic diversity. For example, diverse populations of *Arabidopsis thaliana* produced 30% more biomass than the additive expectation (Crawford & Whitney 2010), and positive non-additive effects of diversity increased goldenrod (*Solidago altissima*) biomass above what was expected under an additive model (Crutsinger *et al.* 2006). Negative non-additive effects may also occur, for example, if competition between more distant relatives is stronger than competition among close relatives or if negative effects are mediated by a third party (such as herbivores or pathogens) that responds to diversity.

Because of parallels in both the direction and magnitude of ecosystem responses to species diversity and genetic diversity, it has been suggested that the effect of genetic diversity may be as strong, if not stronger, than the effect of species diversity on ecosystem functioning (Crutsinger *et al.* 2006; Hughes *et al.* 2008). However, only one study has compared the magnitudes of effects by independently manipulating both levels of diversity in the same experiment (Cook-Patton *et al.* 2011); here, the magnitude of the effect of genetic diversity

in evening primrose (*Oenothera biennis*) on population-level biomass was comparable with the magnitude of the effect of species diversity on community-level biomass. Furthermore, only one study to date has manipulated a combination of both levels of diversity. In a factorial experiment utilizing eight genotypes of eight plant species representative of limestone grasslands, Fridley & Grime (2010) found that plant genetic diversity did not alter the effect of species diversity on plant biomass. However, neither of these studies have explicitly addressed genetic diversity within a dominant species, which is expected to have large effects on ecosystem functioning. Furthermore, it remains unclear whether the presence versus absence of a dominant species may alter relationships between diversity and ecosystem processes.

Interactive effects between genetic diversity and species diversity are particularly important to investigate, because natural systems are usually composed of several genotypes of many different species. Intraspecific variation is likely to alter interactions among species (Bolnick *et al.* 2003). For example, there is considerable evidence that genetic identity plays a role in competition among plant species (e.g., Turkington & Harper 1979; Lankau & Strauss 2008). Plant genetic identity can also influence the above-ground and below-ground communities associated with plants (e.g. Mooney & Agrawal 2008; Crutsinger, Strauss & Rudgers 2010; Schweitzer *et al.* 2011), as can plant genetic diversity (Crutsinger *et al.* 2006; Johnson, Lajeunesse & Agrawal 2006). These genetically mediated changes in interaction strength and community composition may generate an interactive effect of species diversity and genetic diversity on ecosystem functioning, especially in systems dominated by one or a few plant species, where genetic identity of the dominants can influence their interactions with the rest of the plant community. Therefore, understanding the potential for interactive effects can improve predictions for the consequences of biodiversity loss and better inform restoration efforts.

Here, we addressed how plant species diversity and genetic diversity within a dominant species independently and interactively influenced ecosystem function, utilizing multi-year common garden experiments. Specifically, we addressed the following questions:

- 1 What are the independent effects of plant species diversity and genetic diversity within a dominant species on biomass production?
- 2 How does the presence of the dominant species influence the relationship between species diversity and biomass production?
- 3 Does genetic diversity within the dominant interact with species diversity to change the relationship between species diversity and biomass production?

Materials and methods

STUDY SYSTEM

This experiment was conducted in the dune system surrounding Lake Michigan at Sleeping Bear Dunes National Lakeshore (44° 43.689' N,

86° 07.369' W). Great Lakes sand dunes support plant communities of relatively low species richness (1–5 species m⁻², Crawford, unpubl. data; Cowles 1899), making this an ideal ecosystem for realistic, yet feasible manipulations of species diversity. During primary succession, the dominant plant species, *Ammophila breviligulata* (American beachgrass), is the first colonizer of the dune system, grows primarily via ramets and acts as an ecosystem engineer. Through its ability to tolerate sand burial (Maun & LaPierre 1984), *A. breviligulata* stabilizes sand, which allows the colonization of other plant species less tolerant to sand burial (Olson 1958). *Ammophila breviligulata* is largely self-incompatible (Krajnyk & Maun 1982). It rarely reproduces through seeds owing to low rates of flowering (13% of culms flowering along Lake Michigan, Laing 1958), low seed set (around 30%, Krajnyk & Maun 1982) and poor seedling survival (Laing 1958). However, *A. breviligulata* does readily reproduce asexually, and rhizomes of *A. breviligulata* are likely fragmented and dispersed by wave action (Maun 1984). Natural populations of *A. breviligulata* are typically composed of 1–3 (average of 1.5) genotypes per m² (Fant *et al.* 2008).

PLANT MATERIAL

We collected plant material from Sleeping Bear Dunes National Lakeshore during July 2007. *Ammophila breviligulata* ramets were collected in a roughly 2-m² area from 14 populations and propagated at VansPines Nursery, Holland, Michigan, USA. The populations stretched across *c.* 40 linear km and were separated from each other by at least 1 km to ensure each population was composed of unique genotypes of the largely clonally propagating species. Populations were collected next to the lake where *A. breviligulata* essentially exists as a monoculture along the coast, so each population contained thousands of adult individuals with very few seedlings owing to low recruitment through seed. Populations differed in several above-ground and below-ground traits when grown in the common garden, including: tiller number, size of the area occupied by one plant, maximum height, root thickness and the number of root hairs per mm (see Appendix S1 and Table S2 in Supporting Information). In some systems, collecting populations across such a large spatial scale may raise genetic diversity to a level not expected to occur locally (see Tack, Johnson & Roslin 2012). However, *A. breviligulata* disperses through the water following wave action along the coast (Maun 1984). Therefore, our collection of genetic material is likely to be representative of what could be expected during a colonization episode. Furthermore, there was no apparent correlation between any measured plant trait and geographic distance (Crawford, unpubl. data), suggesting that more distant populations do not display greater functional diversity. Nine other plant species were collected, including four grasses (*Calamovilfa longifolia*, *Elymus canadensis*, *Koeleria pyramidata* and *Schizachyrium scoparium*), four woody species (*Arctostaphylos uva-ursi*, *Prunus pumila*, *Vitis riparia* and *Salix cordata*) and a forb (*Asclepias syriaca*). All species were identified using Voss (1972, 1985, 1996). The woody species were propagated from cuttings collected from three to five mature individuals, and the other species were propagated from seeds collected from a single population, with the exception of *C. longifolia* and *K. pyramidata*, which were collected near the common garden and directly transplanted into the plots. Cuttings and seeds were propagated by Richey Nursery Company, LLC, Spring Lake, MI, USA.

CHARACTERIZING GENETIC DIVERSITY

We examined genetic diversity within and among the populations of *A. breviligulata* using intersimple sequence repeat (ISSR) markers.

These highly variable nuclear markers have previously been used to describe populations of *A. breviligulata* (Fant *et al.* 2008). We used the three primers employed by Fant *et al.* (2008) – (GA)8T, (GA)8C and (CA)8G – to genotype 8 individuals each from our 14 populations. Resulting bands were scored as present/absent and analysed using ANOSIM (PRIMER v6, Clarke & Gorley 2006), which showed that genetic variation among populations was greater than genetic variation within populations (Global $R = 0.80$, $P < 0.01$). Pairwise contrasts revealed that all populations except two (3 and 12) had significantly different banding patterns ($P < 0.05$, Appendix S1 and Table S1). These 2 populations never occurred together in treatments with three populations of *A. breviligulata* and only occurred together in 2 of 28 plots with six populations of *A. breviligulata* (see design, below). Thus, by increasing the number of populations in a community, we increased genetic diversity within *A. breviligulata*. Appendix S1 provides details on the molecular and statistical analyses.

COMMON GARDEN

The common garden was established at a site where the National Park Service demolished homes in 2004 to perform a restoration of the dune habitat. Few plants had colonized the area since demolition (< 0.25 m⁻²); non native species were manually removed, and native species were relocated prior to plot establishment. Mimicking the natural order of colonization, we planted *A. breviligulata* first in mid-October 2007, because land managers reported greater success with fall plantings. In June 2008, we planted the woody species, and we planted the remaining species in July. Plants that died before September 2008 were replanted to maintain the diversity treatments. Plots were watered during the summer of 2008 to promote establishment and were weeded monthly during the growing seasons (May–September) to maintain diversity treatments.

INDEPENDENT DIVERSITY MANIPULATIONS AND MONOCULTURES (INDEPENDENT PLOTS)

To test how species diversity and genetic diversity within a dominant species independently affected biomass production, we established two experiments – one that manipulated species diversity (one, three or six species) and one that manipulated *A. breviligulata* genetic diversity (one, three, or six populations of *A. breviligulata*) (Fig. 1). Species diversity treatments did not include the dominant plant, *A. breviligulata*, in the species pool. Plots with three or six species/populations were established in 1.5 × 1.5 m plots at a density of 24 plants per plot, which is a realistic density for this community. Species and populations were represented by equal numbers of individuals in each plot. These plots were replicated seven times for each treatment. To assess whether non-additive effects occurred in diversity treatments, information on how each species and *A. breviligulata* population performed in monoculture (only one species or one population of *A. breviligulata*) was necessary. Owing to space and labour limitations, these monocultures were planted at the same density as individuals in the diversity plots, but with 12 individuals per plot. Each population monoculture (14 total) and species monoculture (9 total) was replicated three times.

To minimize the potential for quasi-replication – the replication of a specific community in the highest diversity treatment that confounds diversity effects with community composition effects (Huston & McBride 2002) – *A. breviligulata* populations were selected randomly from a pool of 14 and other species were selected randomly from a pool of nine. To avoid increases in the similarity of communities at

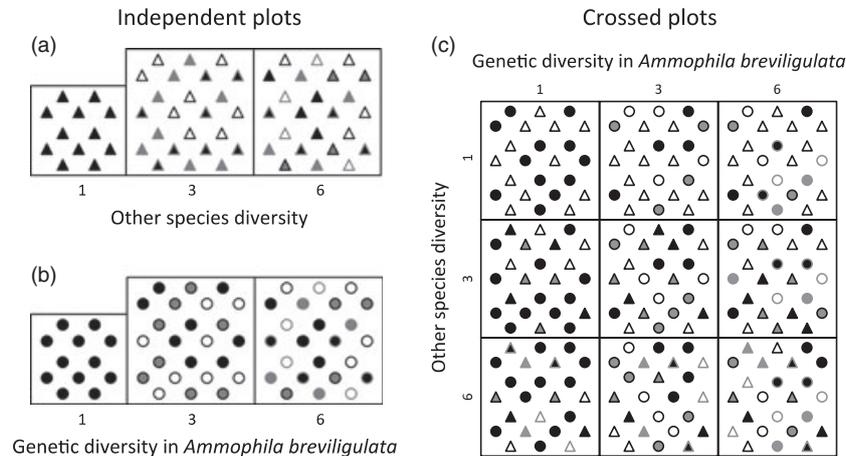


Fig. 1. Diagram of the experimental design for the common garden. Circles with different patterns represent different populations of *Ammophila breviligulata*. Triangles with different patterns represent different plant species. In the independent plots, only one level of diversity, either genetic diversity within *A. breviligulata* or species diversity, was manipulated. In the crossed plots, both species diversity and genetic diversity within *A. breviligulata* were simultaneously manipulated. Crossed plots were composed of $\frac{1}{2}$ *A. breviligulata* and $\frac{1}{2}$ other species. All plots contained 24 plants, except for monocultures, which had 12. Individuals within each plot were randomized and planted equidistant from one another in staggered rows to increase the number of interactions among individuals.

high diversity levels, random combinations were chosen to maximize dissimilarity within treatment combinations. For example, replicates with three species were allowed to have only two of the nine species in common. If a treatment replicate deviated from these stipulations, the replicate was discarded and a new replicate was randomly generated.

MANIPULATION OF SPECIES DIVERSITY \times GENETIC DIVERSITY WITHIN THE DOMINANT SPECIES (CROSSED PLOTS)

To examine how genetic diversity within a dominant species influenced the relationship between species diversity and biomass production, we factorially crossed genetic diversity within *A. breviligulata* (one, three, or six populations) with species diversity (one, three or six species excluding *A. breviligulata*) (Fig. 1). Communities were established in 1.5×1.5 m plots at a density of 24 plants per plot, comprised of 12 individuals of *A. breviligulata* and 12 individuals of the other species. In diverse dune communities where *A. breviligulata* is the dominant grass, it averages 50% of the community composition, making this a realistic community composition for dunes that are past the earliest stages of primary succession. Individuals were randomly assigned to a position in each plot using a staggered grid design to maximize interactions among plants (Fig. 1). Each treatment combination was replicated seven times for a total of 63 plots. Quasi-replication was minimized, and dissimilarity within treatment combinations was maximized as described for the independent plots.

PLANT BIOMASS MEASUREMENTS

After the experiment was fully established for 1 year, we measured above-ground plant biomass in each plot once per month during the growing season (June, July and August 2009). Above-ground plant biomass was estimated non destructively using allometric equations developed for each species from destructive harvests (Appendix S2). To calculate community-level plant biomass, values for individual plant biomass were summed for each plot. The allometric equations method has been commonly used to estimate plant biomass in diver-

sity studies (e.g., Crutsinger *et al.* 2006) and has the advantage of reduced disturbance to plots but assumes that allometry is not affected by plant diversity treatments. Even if traits differ among diversity treatments, it is unlikely that the relationship between traits and biomass changes with treatment. All correlations were significant at $P < 0.0001$ (range $n = 16$ – 28) and explained $\geq 88\%$ of the variation in plant biomass for all species except *A. syriaca* ($r^2 = 0.78$). *Ammophila breviligulata* populations varied significantly in maximum height and tiller number (Appendix S1 and Table S2), which produced variation in estimated above-ground plant biomass. For more detail on the formulation of the allometric equations, see Appendix S2.

STATISTICAL ANALYSES

During the course of the experiment, some mortality occurred. Models incorporating mortality as realized diversity did not differ qualitatively from models using the initially planted diversity, so for ease of interpretation the latter models are presented. In all models, values for biomass were log-transformed prior to analysis to meet assumptions of normality of residuals and homogeneity of variances.

Monoculture performance

Variation in biomass production among populations and species in monoculture may lead to additive effects of diversity – the probability of including a high performing or low performing individual increases as diversity increases. To test whether differences among plant species or *A. breviligulata* populations existed, we analysed data from the monoculture plots using repeated measures mixed models with the fixed factor of either species identity or genetic identity, time and all interactions (Proc MIXED, Kenward-Roger corrected, SAS Institute 2009). Based on Akaike information criterion, we used the heterogeneous Toeplitz structure of model covariances in all models.

Independent diversity effects

Before we tested how species diversity and genetic diversity may interactively influence biomass production, we tested how species

diversity and genetic diversity within a dominant species individually affected biomass production in the independent plots. To compare across treatment levels, the above-ground plant biomass values were doubled for the half-sized monoculture plots. To test how diversity influenced plant biomass, we used repeated measures mixed models as described for the monocultures. The models included either the continuous effect of species diversity or genetic diversity, the fixed effect of time and the diversity \times time interaction.

Dominant species \times species diversity

Next, we tested whether the addition of the dominant species, *A. breviligulata*, altered the relationship between species diversity and biomass production by comparing the slope of the relationship between species diversity and biomass production in the independent plots (which did not contain *A. breviligulata*) to the slope of the relationship between species diversity and biomass production in the crossed plots (which contained $\frac{1}{2}$ *A. breviligulata* and $\frac{1}{2}$ other species) using a repeated measures mixed model, as described previously. The model included the continuous effect of species diversity, the fixed effects of *A. breviligulata* (dominant) presence and time and all possible interactions.

Genetic diversity \times species diversity

Finally, we tested whether genetic diversity in the dominant species interacted with species diversity to alter the relationship between species diversity and biomass production in the crossed plots. The repeated measures mixed model included the continuous effects of species diversity and genetic diversity within *A. breviligulata*, the fixed effect of time and all possible interactions.

Additive versus non-additive effects

When a significant effect of diversity was detected, we tested whether the effect was driven by additive or non-additive diversity effects by conducting Monte Carlo simulations (Crutsinger *et al.* 2006; Johnson, Lajeunesse & Agrawal 2006; Crawford & Whitney 2010). Artificial *in silico* communities matching the composition (species composition and genotypic composition) of each of the experimental diversity plots were constructed by randomly sampling biomass values (with replacement) from individual plants growing in monoculture. We then examined the distribution of community-level plant biomass values for 9999 sets of artificial populations and calculated 95% confidence intervals for each treatment mean. When actual means fell outside these intervals, we inferred non-additive effects of diversity – that is, the effect of diversity on plant biomass was not explained solely by the composition of the community. Monte Carlo simulations were programmed using SAS macro language (SAS Institute 2009) following methods in Crawford & Whitney (2010).

Because monoculture plots were half the size of diversity plots, a greater ratio of plants were on the edge of the plot in monocultures (8 of 12) relative to polycultures (12 of 24) (Fig. 1). Plants on the edges of plots were, on average, slightly larger than plants on the interior of the plot (edge plants: 1.88 ± 0.05 g, interior plants: 1.67 ± 0.05 g); thus, the average biomass per individual is expected to be larger in monoculture than polyculture owing to plot size alone. This did not influence results from analyses of independent diversity effects, as results from analyses using only interior plants did not differ qualitatively from results using all plants. However, this could influence the partitioning of additive and non-additive effects. To con-

rol for this effect, we ran another Monte Carlo simulation where, in addition to matching composition, *in silico* communities matched the placement of individuals by replacing edge plant values in polyculture with edge plant values in monoculture and interior plant values in polyculture with interior plant values in monoculture. Results from these simulations did not differ qualitatively from simulations ignoring edge effects, so only the latter results are presented.

Results

MONOCULTURE PERFORMANCE

In the monoculture plots, *A. breviligulata* was the most productive species, producing, on average, *c.* 50% more biomass than the next most productive species, *K. pyramidata* (Fig. 2). There was no significant effect of *A. breviligulata* genetic identity on plot-level biomass (Table 1), but other work has identified significant variation in plant traits among populations (Crawford, unpubl. data). In species monoculture plots, the identity of the species had a significant effect on plant biomass (Table 1). On average, *K. pyramidata* produced over seven times more biomass than the least productive species, *S. scoparium* (Fig. 2). These differences in species biomass production indicate the potential for an additive effect of species diversity.

INDEPENDENT DIVERSITY EFFECTS

Despite species differences in biomass production, increasing species diversity did not significantly affect community-level plant biomass (Table 1). Genetic diversity within populations of *A. breviligulata* also did not have an independent effect on biomass production (Table 1). The average biomass for communities in which only *A. breviligulata* genetic diversity

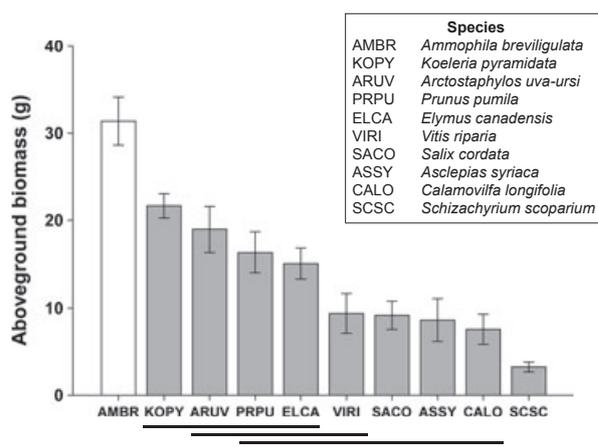


Fig. 2. The average plot-level biomass produced per species in monoculture. *Ammophila breviligulata* produced significantly more biomass than all other species, but different populations of *A. breviligulata* did not produce statistically different amounts of biomass. Bars show means \pm SE. Horizontal lines below the species show the results of a Tukey's *post hoc* analysis of species (excluding *A. breviligulata*) biomass. Species sharing a line do not produce significantly different amounts of biomass.

Table 1. Results from mixed models testing how plant genetic identity (GID), species identity (SID), genetic diversity (GD), and species diversity (SD) influenced above-ground biomass production throughout the field season. ‘Experimental plots’ describes which experiments were compared to test the ‘Model’ which explains the effect(s) being tested. ‘Independent plots’ include plots where genetic diversity or species diversity were manipulated separately. ‘Crossed plots’ includes plots with both levels of diversity manipulated

Community biomass					
Experimental plots	Model	Effects	d.f.	F	P
Monocultures	<i>Ammophila breviligulata</i> genetic identity	GID	13, 28	1.28	0.2806
		Species identity	SID	8, 18	9.79
Independent plots	<i>A. breviligulata</i> genetic diversity	GD	1, 54	0.07	0.7943
		Species diversity	SD	1, 39	1.58
Independent plots v. crossed plots	<i>A. breviligulata</i> presence × SD	<i>A. breviligulata</i>	1, 100	13.51	0.0004
		SD	1, 100	2.27	0.1347
		<i>A. breviligulata</i> × SD	1, 100	1.12	0.2931
Crossed plots	<i>A. breviligulata</i> GD × SD	GD	1, 59	1.98	0.1649
		SD	1, 59	4.28	0.0429
		GD × SD	1, 59	4.51	0.0379

Bold *P*-values were significant at *P* < 0.05.

was manipulated was 61.41 ± 2.54 g, and the average plant biomass for species diversity plots (with no *A. breviligulata*) was 21.47 ± 1.30 g.

DOMINANT SPECIES × SPECIES DIVERSITY

The inclusion of the dominant species did not alter the relationship between species diversity and community biomass (Table 1). Plots with *A. breviligulata* did, on average, produce significantly more biomass than plots without *A. breviligulata* (with: 37.97 ± 1.03 g, without: 21.47 ± 1.30 g), which could be expected given *A. breviligulata*'s greater biomass relative to all other species.

GENETIC DIVERSITY × SPECIES DIVERSITY

Despite the apparent lack of a relationship between species diversity and biomass production, when genetic diversity within the dominant species was accounted for, genetic diversity interacted with species diversity to influence biomass production (Table 1). As genetic diversity increased, the relationship between species diversity and community biomass switched from negative to positive (Fig. 3). When one *A. breviligulata* population was present, community biomass with six other species was *c.* 30% lower than biomass with just one other species. In contrast, when three *A. breviligulata* populations were present, biomass was only about 10% lower for plots of six other species versus plots of one other species. And, when six *A. breviligulata* populations were present, community biomass was nearly 30% greater for plots containing six other species relative to plots with one other species.

ADDITIVE VERSUS NON ADDITIVE EFFECTS

The significant interaction between species diversity and *A. breviligulata* genetic diversity was largely driven by negative non-additive effects, indicating that individuals interacted to reduce community-level plant biomass (Fig. 4). When

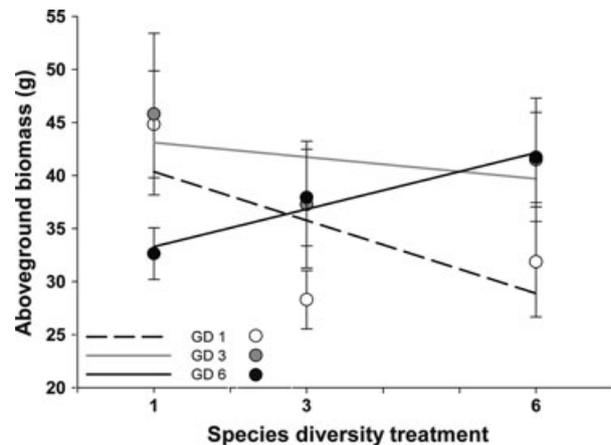


Fig. 3. The average interactive effect of species diversity and genetic diversity within *Ammophila breviligulata* on community-level above-ground biomass in crossed plots.

communities were composed of one population of *A. breviligulata* and more than one other plant species, biomass was *c.* 20% lower than the additive expectation; this effect drove the negative relationships between species diversity and biomass production at low levels of genetic diversity (Fig. 4). When communities were composed of six populations of *A. breviligulata* and only one other species, biomass was also *c.* 20% lower than the additive expectation, and this drove the positive relationship between species diversity and biomass production under high genetic diversity (Fig. 4).

To better understand the underlying causes of negative non-additive effects, we examined the performance of individual species and populations in the treatments (Appendix S3). The experiments were established to test effects of diversity not to track population or species performance in the diversity treatments; each population and each species were not replicated across all levels of the diversity treatments. Therefore, individual plants were used as the unit of replication for these

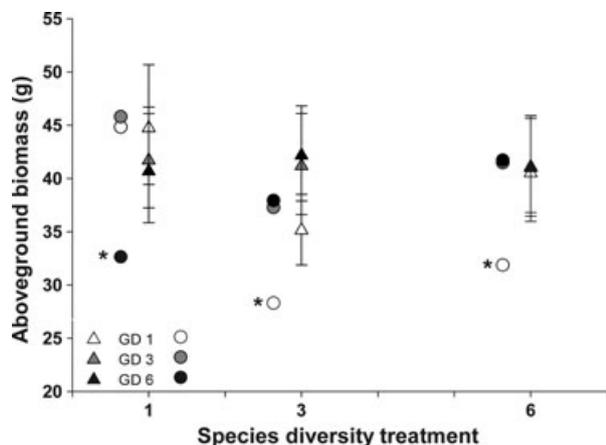


Fig. 4. Additive expectations versus experimental treatment means for the significant interaction between species diversity and genetic diversity on above-ground biomass production. Triangles are the additive expectation based on 9 999 Monte Carlo simulations surrounded by 95% confidence intervals. Actual treatment means are represented by circles and are slightly offset for visual clarity. Asterisks indicate values that differed significantly ($P < 0.05$) from the additive expectation.

statistics rather than plot. While we acknowledge that this approach confounds plot identity with individual performance, it provides a first approximation to the question of which populations or species are driving non-additive responses. Overall, species other than *A. breviligulata* typically performed better in monocultures than in diverse communities across all treatments, suggestive of positive density-dependent intraspecific interactions (Appendix S3 and Table S5 and Fig. S1). In treatment combinations where the diversity effect did not deviate from additive expectations, this negative response to being in a diverse community was counter-balanced by positive effects of diversity on one or more of the *A. breviligulata* populations – thus eliminating a non-additive effect on total biomass (Appendix S3 and Table S5 and Fig. S1). In other words, even though non dominant species performed worse in polyculture than in monoculture, there was no net decline in total community biomass with diversity because of increases in the biomass of the dominant species. However, in treatments where diversity effects were negatively non-additive, the *A. breviligulata* response was also largely negative and was insufficient to counteract the negative response of other species. Therefore, the three cases of negative non-additive effects (Fig. 4) may be explained by a relative decrease in the performance of *A. breviligulata* (Appendix S3 and Fig. S1).

Discussion

Our results show, for the first time, that above-ground plant biomass can be affected by an interaction between species diversity and genetic diversity in a dominant plant species. Despite the fact that the inclusion of a dominant species *per se* did not influence the effect of species diversity on community-level biomass, when the diversity within the dom-

inant species was taken into account, the relationship between species diversity and biomass was altered. Specifically, as genetic diversity within the dominant species increased, the relationship between species diversity and community-level above-ground biomass shifted from negative to positive. This result was influenced by net negative interactions among individual plants, as evidenced by the significant negative non-additive effects of diversity. Our results demonstrate that while, independently, species diversity and genetic diversity within the dominant species may have little effect on plant biomass production, interactions between the two levels of diversity can be key to understanding controls on productivity. This may be particularly important given the growing awareness of the direct interplay between species diversity and genetic diversity in natural systems (Vellend & Geber 2005).

While several studies have examined the relationship between plant biomass and either plant species diversity (e.g. Tilman, Wedin & Knops 1996; Tilman *et al.* 2001) or genetic diversity (e.g. Crutsinger *et al.* 2006), ours is among the first to simultaneously investigate the independent and interactive effects of diversity on above-ground plant biomass. Unlike Cook-Patton *et al.* (2011), who found that the magnitude of biomass increase was the same whether species diversity or genetic diversity was manipulated; we found no significant effect of either species diversity or genetic diversity in our independent diversity manipulations. However, in contrast to the findings of Fridley & Grime (2010), species diversity and genetic diversity interactively influenced biomass production in our experiment. Fridley & Grime (2010) manipulated genetic diversity within all species in their experimental communities, whereas we manipulated genetic diversity only within the dominant plant, *A. breviligulata*. Effects of genetic diversity on ecosystem function are predicted to be particularly important in foundation species, which tend to form monospecific stands where genetic diversity may be more analogous to species diversity (Whitham *et al.* 2006; Hughes *et al.* 2008). In our factorial experiment, *A. breviligulata* was planted as 50% of the total individuals in the plots, but it had produced >80% of the total above-ground biomass after two growing seasons. Therefore, by focusing our manipulation of genetic diversity on *A. breviligulata*, we were probably more likely to detect an interactive effect than had the manipulation targeted non dominant species. Sampling a very small area minimized variation within populations, but the populations were not monoclonal. However, we think this had minimal, if any, effects on our results. The primary potential effect is that it is possible that genetic variation within populations increased the probability that we detected non-additive effects of diversity. Increasing the number of genotypes potentially increased the occurrence of interactions among individuals that significantly influenced biomass even more so than would a manipulation of single genotypes rather than populations.

We found that negative non-additive effects of diversity contributed to the significant interactive effect of species diversity and genetic diversity. Negative non-additive effects

indicate that some individuals performed worse in mixtures than in monocultures. Overall, *A. breviligulata* populations occasionally performed better in mixtures than monocultures, whereas other species tended to perform worse in mixtures than monocultures (Appendix S3 and Fig. S1 and Table S4). However, in the three cases of significantly negative non-additive effects, there was little to no benefit of growing in mixtures for *A. breviligulata* (Appendix S3 and Fig. S1 and Table S4). For communities comprised of one population, the lack of genetic diversity is likely to decrease functional diversity, potentially compromising *A. breviligulata*'s competitive ability against a more diverse plant community (three or six species). This suggests that interactions between *A. breviligulata* and other species are more important for community-level biomass production than interactions among populations of *A. breviligulata* or among other species and that these interactions are mediated by levels of species diversity and genetic diversity within the dominant species. This hypothesis is consistent with the absence of a genetic diversity effect when *A. breviligulata* populations were grown in the absence of interspecific competitors (independent plots). These interactions could be a direct consequence of competition for resources, or an indirect effect mediated through changes in interactions with soil microbes, such as mycorrhizal fungi or soil nematodes (van der Putten, van Dijk & Peters 1993; Bever 2003). In addition, for communities comprised of six populations, the performance of the *A. breviligulata* populations generally increased with higher plant species diversity (Appendix S3 and Fig. S1 and Table S4). In other words, on average, an individual population performed better when competing with diverse populations in the presence of diverse community of plant species than when competing with diverse populations against a single competitor. To our knowledge, such an effect has not been reported by prior studies but may be the consequence of complex competitive hierarchies among individual genotypes and species or may result from intransitive competitive hierarchies (e.g. Lankau & Strauss 2008) that cannot be detected with the current experimental design.

Non additive effects may shift through time or with abiotic conditions. In experiments manipulating species diversity, complementarity, which occurs when species grown in polyculture outperform their monoculture averages, tends to increase through time (Cardinale *et al.* 2007). As plants in the dune system are long lived and productivity is low relative to the other systems where diversity–productivity relationships have been documented (e.g. old-field systems, Tilman, Wedin & Knops 1996; Tilman *et al.* 2001; Crutsinger *et al.* 2006; Johnson, Lajeunesse & Agrawal 2006), we may expect to see non-additive effects shift to become more positive through time. Also, previous work suggests that plant mixtures may show negative non-additive effects under conditions of low fertility, but positive non-additive effects under high fertility conditions (Fridley 2002). Sand dunes are relatively harsh environments with low nutrients and soil moisture, especially recently colonized dunes such as ours. Soil samples from plots prior to planting revealed no detectable nitrogen,

(Crawford, unpubl. data), and nitrogen levels did not reach 1 mg ha^{-1} until 500 years into a dune chronosequence (Lichter 1998). Therefore, the observed negative non-additive responses may be common in the low productivity environment of the dunes, and we may expect to see negative interactions decrease through time as nutrients and soil moisture increase. Conducting similar experiments across a range of productivities could help elucidate the conditions under which negative versus positive non-additive effects of diversity prevail.

While positive non-additive effects of diversity are more often found to contribute to diversity–productivity relationships (e.g. Crutsinger *et al.* 2006; Cardinale *et al.* 2007), negative non-additive effects have been documented. In a disturbed grassland community, three annual plant species produced less biomass when grown in mixture than when grown in monoculture (Polley, Wilsey & Derner 2003). Similarly, negative interactions between algal species caused them to produce less biomass when grown together than when grown separately (Zhang & Zhang 2007). Negative non-additive effects of diversity have also been documented for other ecosystem responses. For example, mass loss during decomposition can be slower for species mixtures than species monocultures (e.g., Nilsson, Wardle & Dahlberg 1999).

Conclusion

Several studies have shown that independently plant species diversity (Tilman, Wedin & Knops 1996; Tilman *et al.* 2001) and genetic diversity (Crutsinger *et al.* 2006; Hughes & Stachowicz 2009; Crawford & Whitney 2010) can influence plant biomass. However, few studies to date have investigated the relative importance of these two levels of diversity (Cook-Patton *et al.* 2011) or their potential for interactive effects (Fridley & Grime 2010). Here, we showed for the first time that plant species diversity and genetic diversity within a dominant species interactively affect above-ground biomass production via non-additive diversity effects. This result has clear implications for conservation and restoration, especially in systems dominated by one or a few species because we show that the effects of species diversity on ecosystem functioning cannot be predicted in the absence of knowledge of the level of genetic diversity within the dominant species. Therefore, it may be imperative that both species diversity and genetic diversity within dominant species be maintained to preserve ecosystem function.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Appendix S1. Detailed methods for genetic analysis of *Ammophila breviligulata* and analysis of trait differences among populations.

Appendix S2. Formulation of allometric equations.

Appendix S3. Further analysis of additive versus non-additive effects of diversity.

Figure S1. Deviation from the additive expectation for each species and each population in the diversity treatments.

Table S1. Results from ANOSIM showing the pair-wise genetic differences between populations of *Ammophila breviligulata*.

Table S2. Results from general linear models testing whether *Ammophila breviligulata* populations significant differed in selected above-ground and below-ground traits.

Table S3. Allometric equations for each plant species.

Table S4. Species codes.

Table S5. Results from general linear models testing if individuals of each species and population performed differently in diversity treatment relative to the monoculture (additive) expectation.

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