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Genotypic diversity enhances invasive ability of *Spartina alterniflora*

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Abstract

Although genetic diversity is very important for alien species, which have to cope with new environments, little is known about the role that genetic diversity plays in their invasive success. In this study, we set up a manipulation experiment including three levels of genotypic diversity to test whether genotypic diversity can enhance the invasive ability of alien species, in our case the invasive *Spartina alterniflora* in China, and to infer the underlying mechanisms. There was no significant relationship between genotypic diversity and parameters of performance in the first year; however, from the summer of the second year onwards, genotypic diversity enhanced four of the six parameters of performance. After two growing seasons, there were significant positive relationships between genotypic diversity and maximum spread distance, patch size, shoot number per patch, and aboveground biomass. Moreover, abundance of the native dominant species *Scirpus mariqueter* was marginally significantly decreased with genotypic diversity of *S. alterniflora*, suggesting that enhanced invasive ability of *S. alterniflora* may have depressed the growth of the native species. There was no significant difference in most measures of performance among six genotypes, but we observed a transgressive over performance in four measures in multiple-genotype patches. At the end of the experiment, there were significant nonadditive effects of genotypic diversity according to Monte Carlo permutations, in six-genotype, but not three-genotype plots. Our results indicated that both additive and nonadditive effects played roles in the positive relationship between genetic diversity and invasion success, and nonadditive effects were stronger as duration increased.

Keywords: additive effects, ecological genetics, genotypic diversity, invasive species, nonadditive effects, *Spartina alterniflora*

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Introduction

Population genetic theory suggests that intraspecific genetic variation may promote the performance of a species (Hartl & Clark 1997), because high genetic variation increases the probability of advantageous genotypes or that genetic variation may be reshuffled and recombined, generating novel genotypes and providing materials for rapid selection (Lavergne & Molofsky 2007; Prentis *et al.* 2008). Recent experimental manipulations of genetic diversity have shown that genotypic diversity of plants can increase biomass (Hughes & Stachowicz 2004; Crutsinger *et al.* 2006; Kotowska *et al.* 2010; Cook-Patton *et al.* 2011), reduce pathogen levels (Schmid 1994), promote tolerance to or resilience from disturbance (Hughes & Stachowicz 2004, 2009, 2011; Reusch *et al.* 2005), enhance recovery in the face of global warming (Ehlers *et al.* 2008), resist plant invasions (Crutsinger *et al.* 2008b), enhance nutrient uptake (Hughes & Stachowicz 2004) or support more associated species diversity (Johnson *et al.* 2006; Crutsinger *et al.* 2008a; Fridley & Grime 2010). Additive

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mechanisms or selection effects, that is, the higher probability of containing high performance genotypes, are frequently used to explain these effects (Johnson *et al.* 2006). Some studies suggested that nonadditive effects, that is, interactions among different genotypes, can also promote these different aspects of ecosystem functioning (Johnson *et al.* 2006; Crawford *et al.* 2007; Shen *et al.* 2007; Crutsinger *et al.* 2008b). However, the relative importance of these mechanisms is under debate.

Genetic variation is especially important for alien species, which have to cope with novel environments (Sax *et al.* 2007). However, alien species may experience a population bottleneck when they colonize new habitats, generally resulting in decreased genetic variation (Roman & Darling 2007). Some invasive species contain low genetic variation (Dlugosch & Parker 2008), and general advantageous genotypes have been used to explain their invasive success (Le Roux *et al.* 2007). Multiple introductions may rescue invaders from a loss of genetic diversity (Saltonstall 2002; Dlugosch & Parker 2008) and alleviate the negative impacts of low genetic variation. Increasingly, empirical studies have shown that introductions from different sources can increase the genetic variation in the invasive range. For example, reed canary grass (*Phalaris arundinacea* L.) had higher genetic diversity and heritable phenotypic variation in its invasive relative to native ranges. The resulting high evolutionary potential of invasive populations allowed for rapid selection of genotypes with higher vegetative colonization ability and phenotypic plasticity (Lavergne & Molofsky 2007). Although such cases provided evidence that genetic variation may contribute to invasion success, experiments that manipulate the genetic diversity and directly correlate diversity with degree of invasive success using extant introduced populations are needed (Dlugosch & Parker 2008). Furthermore, such manipulation experiments may identify potential mechanisms of invasive success, which provide insights for the management and control of alien plants (Crawford & Whitney 2010).

Smooth cordgrass (*Spartina alterniflora* Loisel.) is a dominant species throughout Atlantic and Caribbean coasts. It was introduced to China in 1979 (Xu & Zhuo 1985), much later than its congener *S. anglica* C.E. Hubbard, a notorious invasive species worldwide, which was introduced to China in 1963. However, *S. alterniflora* is rapidly replacing *S. anglica* in China and increased to >112 000 ha by 2000 (Zhi *et al.* 2007). In <20 years, *S. alterniflora* had become the most influential alien species in coastal China. Although introductions may help the long-distance spread of *S. alterniflora* across coastal China, its strong clonal growth and competitiveness contributed to rapid spreading in native marshes and introduced *S. anglica* communities (Zhang *et al.* 2006; Zhi *et al.* 2007). *S. alterniflora* introduced to China had three source populations: North Carolina, Florida and Georgia of the USA. Individuals from these three sources were mixed in the first introduction site, Luoyuan of Fujian Province (Xu & Zhuo 1985), and gradually introduced to other locations in China. Such a mixture may increase within-population genetic variation. Using microsatellite markers, we found that *S. alterniflora* populations had much higher genetic diversity than *S. anglica* (Shen *et al.*, in preparation), indicating that high genetic variation in *S. alterniflora* might play a critical role in its invasion in China.

In this study, we conducted a manipulation experiment with a series of plots containing plants of single and multiple genotypes. We tracked measures of performance across two growing seasons to answer the following questions: (i) Did genotypic richness promote the clonal invasion success of *S. alterniflora*? (ii) Did genotypic richness enhance the competitive ability of *S. alterniflora* over native species? and (iii) What was the underlying mechanism and did its role change over time?

Materials and methods

The study site

This study was conducted in the intertidal zone of Chongming Dongtan, in the Yangtze Estuary (31°30′42.8″ N, 121°58′44.0″ W). Soil salinity ranged from 2 to 6‰. Mean annual precipitation was 1022 mm. Mean annual temperature was 15.3 °C, with the warmest month in August and the coldest month in January (Wang 2011). The flooding tides are regular and semidiurnal. Based on 31-year records (1978–2008) at the nearest tidal gauge station (Waigaoqiao, approximately 35 km from the study site), local mean sea level is 2.17 m, and the mean tidal range is 2.48 m with the maximum tidal range of 4.43 m (Yuan *et al.* 2011). The natural vegetation was dominated by the annual plant *Scirpus mariqueter* Wang et Tang (Cyperaceae) in the low–mid-parts of intertidal zones and by *Phragmites australis* in the mid–high-parts. In Chongming Dongtan, *Spartina alterniflora* was first found in 1995 and was believed to have arrived there via natural dispersal (Chen *et al.* 2008). By August 2003, it covered an area of 910 ha, about one-third of the vegetated marshes of Chongming Dongtan (Li *et al.* 2006).

Genotyping method

In December 2005, we selected and marked 22 distinct patches of *S. alterniflora*, and each patch was at least

20 m from other patches or scattered individuals of *S. alterniflora*. In each patch, 3–5 leaf samples were taken in different directions and dried by silica gel. DNA was extracted from approximately 50 mg of dried leaves using the modified miniprep cetyltrimethylammonium bromide (CTAB) procedure (Fan *et al.* 2004). Each sample was genotyped at eight microsatellite loci using primers provided by Blum *et al.* (2004). Approximately, 100 ng of DNA was used to seed a 20 μL PCR and amplified following the manufacturer's instruction in a PTC-220 PCR machine (MJ Research Inc., Waltham, MA, USA). Amplification conditions were as follows: an initial 4 min of predenaturation at 94 °C followed by 30 cycles of 30 s denaturation at 94 °C, 45 s annealing at 65 °C and 90 s extension at 72 °C and a final extension step of 72 °C for 2 min. PCR products were resolved by 6% polyacrylamide gel electrophoresis, visualized using silver nitrate staining and manually scored against a sequence ladder of pUC19 DNA/MspI(HpaII) Marker 23 (Fermentas).

Experimental design and data collection

Spartina alterniflora has a strong capacity for clonal growth, and there were usually only a few genotypes in an area of several dozens of square metres. In Jinshan of Shanghai, we took 30 random samples at the scale of about 10 × 20 m, and only four genotypes were found (unpublished data). Therefore, we set a maximum of six genotypes in polyculture plots. We carefully dug out ten patches representing ten different genotypes from 22 patches we had previously marked and then split them into ramets. Six genotypes (see Supplementary materials; Tables S1 and S2, Supporting information) were chosen randomly, and all their connected ramets were transported to a special site convenient to our use. In May 2006, we set up 42 experimental plots (each 0.5 × 0.5 m) in a homogenous marsh dominated by *Sc. mariqueter* with four stakes at the four corners and one stake in the centre of each plot. The minimum distance between plots was 30 m, providing enough space for *S. alterniflora* to spread between plots without interfering with other plots for at least three years. The experimental area was rather flat, and it usually takes <15 min for flood tide to cover the near-shore plots after seaward plots are reached. In each plot, belonging to one of the three levels of genotypic diversity, 24 ramets were planted in June 2006: six plots received a mixture of all six genotypes in equal abundance (six-genotype treatment); 12 plots received a mixture of three genotypes in equal abundance, with six different compositions and two replicates per composition (three-genotype treatment); and 24 plots received single genotypes (single-genotype treatment), with six different genotypes each replicated in four plots. Because of a limited number of ramets of the same genotype, we only set up six replicates of the six-genotype treatment. Such a experimental design is appropriate because the problem of unequal sampling at the different diversity levels is not severe, as long as mean values are compared (Schmid *et al.* 2008).

In multiple-genotype plots, each ramet was marked with a coloured ring circling the stem, with a distinct colour for each genotype. After one month, that is, July 2006, ramet survivals were checked, and in each plot, the number of surviving ramets was reduced to 18 by removing surplus ramets, maintaining equal abundances of different genotypes in multi-genotype plots. Thus, each genotype had six and three ramets in the three- and six-genotype plots, respectively. The 42 plots were randomly arranged with the proviso that plots having same treatment were not adjacent (Fig. S1, Supporting information). Because there was only one species in the native *Sc. mariqueter* community, there was no need to weed before planting *S. alterniflora*.

In August, October and December 2006, and also May, July, September and November 2007, we recorded the shoot number per plot. The height of each shoot and the distance of the furthest shoot from the plot centre were measured to the nearest centimetre. We use 'patch' instead of 'plot' here onwards as individuals may grow out of initial plots (0.5 × 0.5 m), and plots were not of regular form anymore. Size of each patch was estimated by measuring the area embraced by furthest shoots in eight directions. To characterize clonal invasive ability, in addition to the above three characters, we also estimated the aboveground biomass of the planted *S. alterniflora* in each patch. Because the experiment lasted for two growing seasons, we did not use a harvest method, but used a rapid, nondestructive method to estimate aboveground biomass (Thursby *et al.* 2002). The aboveground biomass (y, g) of each shoot was calculated as $y = 0.0013\ H^{1.7863}$ [$R^2 = 0.8736$, $n = 325$, $P < 0.001$; H: shoot height (cm)] established in the study area (Miao *et al.*, unpublished data). Aboveground biomass of each patch was estimated by summing the aboveground biomass of all shoots in the patch. Shoot number per m^2 (i.e. density) and aboveground biomass per m^2 were also estimated.

The experiment was conducted in the native community dominated by *Sc. mariqueter*. Density of *Sc. mariqueter* was 3566.7 ± 276.4 (mean ± SE, $n = 6$) shoots per m^2. To describe the impact of the invasive *S. alterniflora* on the native community, we recorded the shoot number of *Sc. mariqueter* in the 20 × 20 cm area at the centre of each patch in July and September 2007, but not at the end of experiments because this species is annual and most individuals died in November.

Data analyses

Except for maximum spread distance, all response variables were ln-transformed prior to analysis to meet the assumptions of normality and homogeneity of variance. For each census, we examined the difference among six genotypes using one-way analysis of variance (ANOVA) and relationships between genotypic diversity and six response variables, that is, maximum spread distance, patch size, number of shoots, aboveground biomass, density and aboveground biomass per m^2 by linear regression using SPSS v13 (SPSS Inc., Chicago, IL, USA). We also used repeated-measures ANOVA to separate the effects of time and genotypic diversity. Because values of the six parameters did not meet the assumptions of sphericity ($P < 0.001$ in Mauchly's test of sphericity), and estimates of sphericity (ε) of all six parameters were <0.75, Greenhouse–Geisser correction was used to reduce the degrees of freedom associated with the F-value. Repeated-measures ANOVA was carried out using general linear model in SPSS.

To describe the effect of genotypic diversity on performance, we used net effect (LR_{net}) and transgressive over performance (LR_{trans}) for each census using the following equations (Cardinale *et al.* 2007): $LR_{net} = \ln \frac{P_{\bar{p}i}}{P_{\bar{m}i}}$ and $LR_{trans} = \ln \frac{P_{\bar{p}i}}{P_{\max i}}$, where $P_{\bar{p}i}$ is the mean value of the performance (shoot number, biomass, maximum spread distance, or patch size) of six-genotype plots, $P_{\bar{m}i}$ is mean value of the performance of single-genotype plots, and $P_{\max i}$ is mean value of the single-genotype plots with the highest performing genotype.

Monte Carlo simulations were carried out to test whether there were additive (selection) or nonadditive (such as complementarity, facilitation, counteraction) effects in multi-genotype patches in our experiment. Following the general logic of Johnson *et al.* (2006) and Crawford *et al.* (2007), we constructed artificial plots with the same genotypes of each experimental polycultures by re-sampling values from individual genotypes growing in monoculture. The number of bootstrap re-samplings was set to 9999, and 95% confidence intervals were calculated. If the actual means fell inside the intervals, we could infer additive effects of genotypic diversity; otherwise, nonadditive effects were inferred (Crawford & Whitney 2010). Monte Carlo permutations were performed using the R 2.9.0 (R Development Core Team 2010).

To determine whether genotypic diversity of *S. alterniflora* affected abundance of *Sc. mariqueter*, we also analysed the relationship between genotypic diversity and *Sc. mariqueter* abundance using linear regression.

Results

Using eight microsatellite loci, we distinguished 21 genotypes across the 22 patches of *Spartina alterniflora* surveyed; however, samples from the same patch were always of the same genotype. Such a pattern suggested that clonal growth plays a critical role in local spreading after establishment. There was no significant difference in shoot number and aboveground biomass among the six genotypes in single-genotype experimental patches, and the maximum spread distance only showed significant differences among the six genotypes at one sampling time (in September 2007, $P = 0.032$; Fig. S2, Supporting information). Similarly, patch size, density and aboveground biomass per m^2 varied significantly ($P < 0.05$) among genotypes in two to four surveys before November 2007, but were not significantly different at the last survey (Fig. S2, Supporting information).

During the two-year experiment, significant relationships between genotypic diversity and the maximum spread distance of *S. alterniflora* were observed from the summer of the second year onwards (Table 1, July and November of 2007). After two growing seasons, the mean maximum spread distance of six-genotype patches was 27 and 11% larger than that of one- and three-genotype patches, respectively. There was also a significantly positive relationship ($P < 0.05$) between genotypic diversity and patch size in July and November 2007 (Table 1; Fig. 1b). After two growing seasons, the mean area of six-genotype patches was 61% and 32% larger than that of one- and three-genotype patches, respectively.

The number of shoots significantly increased with genotypic diversity in the last three surveys, that is, July, September and November 2007 (Table 1; Fig. 1c). At the end of the experiment, the total number of shoots of six-genotype patches was 74% and 36% greater than that of one- and three-genotype patches, respectively. There was a similar pattern in aboveground biomass (Table 1; Fig. 1d). In November 2007, the aboveground biomass of six-genotype patches was 60% and 36% higher than those of one- and three-genotype patches, respectively. However, genotypic diversity had no significant effects on density or aboveground biomass per area (Table 1). Repeated-measures ANOVA showed that genotypic diversity had no significant impacts on these variables, but they varied significantly across time (Table 2). However, interaction of genotypic diversity and time had significant effects on maximum spread distance and density of *S. alterniflora*.

Linear regression showed that LR_{net} ($F = 15.02$, $P = 0.012$) and LR_{trans} ($F = 12.24$, $P = 0.017$) of

Table 1 Effects of genotypic diversity on maximum spread distance (m), patch size (m^2), number of shoots, aboveground biomass (kg), aboveground biomass per area (kg/m^2) and density (number of shoots/m^2) of *Spartina alterniflora*

	Max. distance		Patch size		Number of shoots		Biomass		Biomass per area		Density	
Time	R^2	*P* (*F*)	R^2	*P* (*F*)	R^2	*P* (*F*)	R^2	*P* (*F*)	R^2	*P* (*F*)	R^2	*P* (*F*)
August 2006	0.01	0.517 (0.43)	0.02	0.421 (0.66)	0.03	0.268 (1.26)	0.02	0.364 (0.84)	<0.01	0.975 (<0.01)	<0.01	0.753 (0.10)
October 2006	0.04	0.232 (1.47)	0.03	0.278 (1.21)	0.04	0.181 (1.85)	0.03	0.271 (1.25)	<0.01	0.701 (0.15)	<0.01	0.899 (0.02)
December 2006	0.03	0.244 (1.40)	0.02	0.323 (1.00)	0.04	0.216 (1.58)	0.03	0.321 (1.01)	<0.01	0.699 (0.15)	<0.01	0.862 (0.03)
May 2007	0.05	0.148 (2.18)	0.05	0.161 (2.04)	0.01	0.546 (0.37)	0.02	0.377 (0.80)	0.07	0.102 (2.81)	0.09	0.060 (3.73)
July 2007	**0.18**	**0.006 (8.43)**	**0.12**	**0.025 (5.43)**	**0.14**	**0.016 (6.39)**	**0.10**	**0.041 (4.47)**	<0.01	0.929 (0.01)	<0.01	0.957 (<0.01)
September 2007	0.08	0.063 (3.66)	0.06	0.129 (2.40)	**0.18**	**0.005 (8.85)**	**0.11**	**0.034 (4.82)**	0.04	0.211 (1.62)	0.07	0.082 (3.19)
November 2007	**0.17**	**0.006 (8.26)**	**0.11**	**0.036 (4.69)**	**0.15**	**0.011 (7.15)**	**0.10**	**0.045 (4.29)**	<0.01	0.815 (0.06)	<0.01	0.840 (0.04)

The effects of genotypic diversity on response variables were analysed by general linear regression. The data, except for maximum spread distance, were ln-transformed to increase the normality of residuals. Bold numerals are significant ($P < 0.05$).

aboveground biomass increased significantly over time. The six-genotype patches yielded more shoots and aboveground biomass than the average of one-genotype patches ($\mathrm{LR}_{\mathrm{net}} > 0$; Fig. 2a) from the beginning of the experiment. However, the six-genotype patches began to yield more shoots and aboveground biomass than the highest performing single-genotype patches ($\mathrm{LR}_{\mathrm{trans}} > 0$) after one year (Fig. 2b). The patch size ($\mathrm{LR}_{\mathrm{trans}} > 0$ in the last date) and the maximum spread distance ($\mathrm{LR}_{\mathrm{trans}} > 0$ in October 2006, July 2007 and November 2007) showed similar trends (Fig 2c, d).

Significant nonadditive effects of genotypic diversity were detected for four of the six response variables. After two growing seasons, six-genotype patches spread faster and had larger patch sizes than predicted from the additive model and produced significantly more shoots and aboveground biomass than expected under additivity (Fig. 3), but not in the first year (data not shown), suggesting that additive effects played a more critical role at the early stages, while nonadditive effects were more important at the later stages. No significant difference was found in density or aboveground biomass per m^2 between observed data and expectations under additivity (Fig. 3). However, all six response variables of three-genotype patches showed no significant difference from expectations under additivity during the experiment (Fig. 3).

Although not significant, there was a negative relationship between genotypic diversity and abundance of *Sc. Mariqueter* (Fig. S3, Supporting information), suggesting that enhanced invasive ability of *S. alterniflora* might have negatively affected the native community. In July 2007, numbers of *Sc. mariqueter* individuals of one- and three-genotype patches had ranges of 0–57 and 0–31, respectively, while there were at most seven *Sc. mariqueter* individuals in six-genotype patches (adjusted $R^2 = 0.051$, $P = 0.106$). In September 2007, no *Sc. mariqueter* was found in six-genotype patches, while single-genotype patches had 0–50 individuals (adjusted $R^2 = 0.068$, $P = 0.093$; Fig. S3, Supporting information).

Discussion

After colonizing a new habitat, an invasive species occupies empty niches or competes with native species, generally increasing in individual number, biomass and occupied area. Therefore, the number of individuals, biomass and area are appropriate indicators of competitive ability, and thus invasive ability, of an alien species or genotype (Lavergne & Molofsky 2007; Vellend *et al.* 2010). Our two-year study revealed a positive relationship between genotypic diversity and patch-level performance of *Spartina alterniflora* (i.e. shoot number and aboveground biomass per patch, patch size and maximum spread distance), indicating that genotypic diversity enhanced invasive ability of *S. alterniflora*. In addition, performance in high genotypic diversity patches led to decreased abundance of the key native species *Sc. mariqueter* in those patches.

Significant positive relationships between genotypic diversity and parameters of invasion success did not always occur in *S. alterniflora* and were only found in surveys ten months after transplanting and onwards, but not in the first growing season, leading to no significant effects of genotypic diversity by repeated-measures ANOVA (Table 2). The repeated-measures

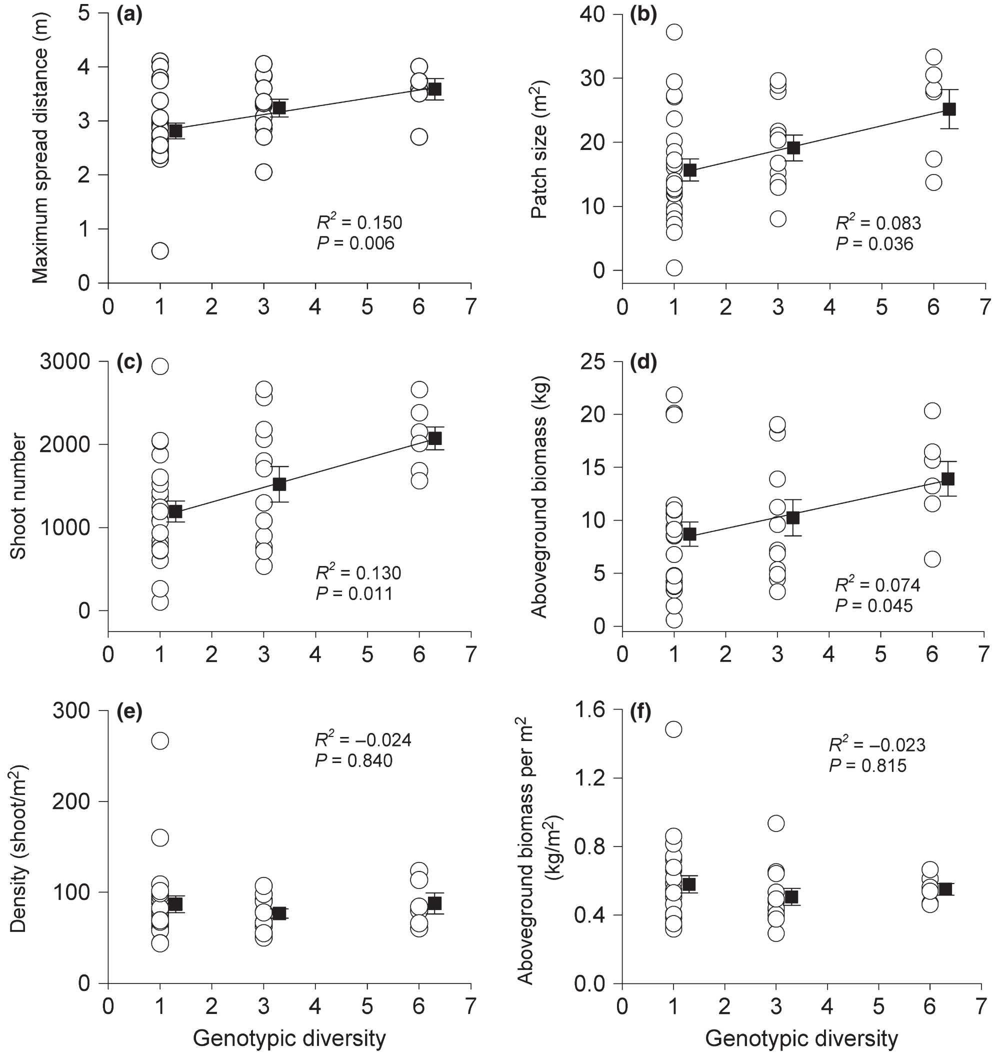

Fig. 1 Relationship between patch-level genotypic diversity and maximum spread distance (a), patch size (b), shoot number (c), aboveground biomass (d), density (e) and aboveground biomass per m^2 (f) of the last census (November 2007). Squares represent mean (±SE) value. Adjusted R^2 and P-value were also shown.

Table 2 Repeated-measures ANOVA of effects of genotypic diversity (GD) and time (T) on maximum spread distance (m), patch size (m^2), number of shoots, aboveground biomass (kg), aboveground biomass per area (kg/m^2), and density (number of shoots/m^2) of *Spartina alterniflora*

	Genotypic diversity				Time				Genotypic diversity × Time			
	d.f.	MS	*F*	*P*	d.f.	MS	*F*	*P*	d.f.	MS	*F*	*P*
Maximum spread distance	2	3.84	2.39	0.106	2.94	72.28	357.97	**<0.001**	5.88	0.63	3.12	**0.008**
Patch size	2	5.98	1.33	0.277	2.42	203.34	456.07	**<0.001**	4.85	0.55	1.23	0.304
Number of shoots	2	5.31	1.84	0.173	2.74	104.93	437.81	**<0.001**	5.49	0.30	1.26	0.286
Aboveground biomass	2	4.68	1.24	0.300	2.47	218.77	844.27	**<0.001**	4.93	0.24	0.91	0.476
Aboveground biomass per area	2	0.19	0.37	0.691	3.24	16.51	74.07	**<0.001**	6.47	0.45	2.01	0.064
Density	2	0.15	0.22	0.802	3.52	12.74	58.49	**<0.001**	7.04	0.46	2.11	**0.046**

The data, except for maximum spread distance, were ln-transformed to increase the normality of residuals. Bold numerals are significant ($P < 0.05$).

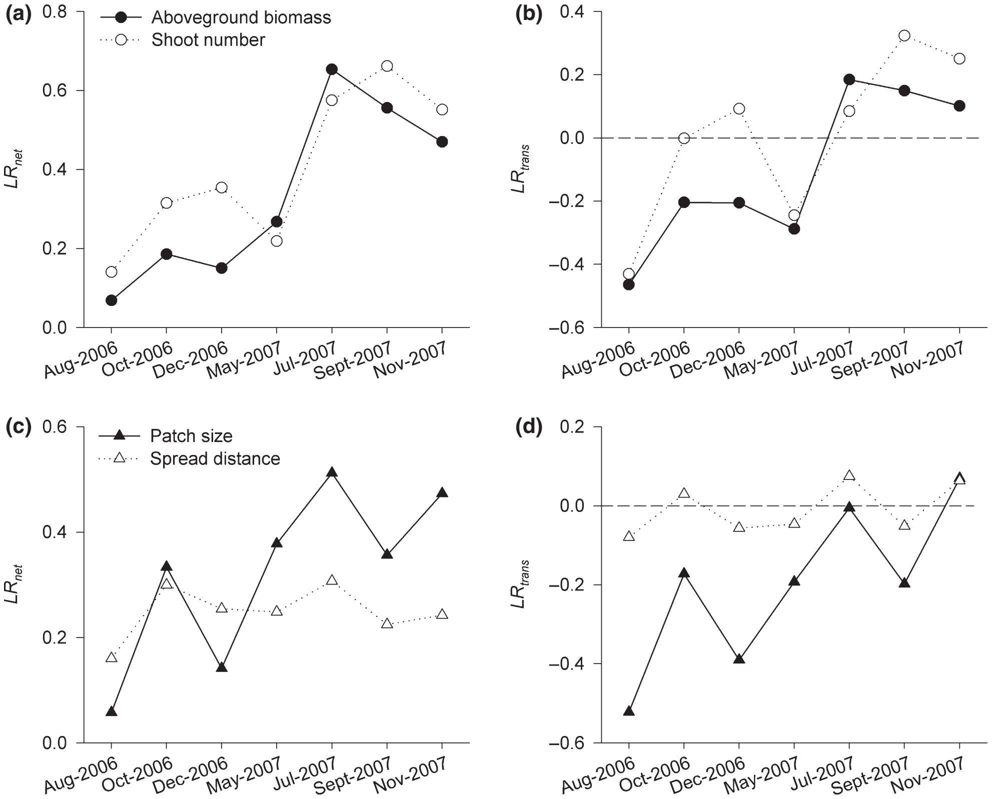

Fig. 2 Net effect (LR_{net}, a) and transgressive over performance (LR_{trans}, b) based on aboveground biomass (filled circles and solid lines) and shoot number (open circles and dotted lines) for six-genotype plots from August 2006 to November 2007. The net effect (LR_{net}) is the ln-transformed ratio of the mean measured variables in six-genotype plots and the mean of the six genotypes when occurring in one-genotype plots. The transgressive over performance (LR_{trans}) is the ln-transformed ratio of the mean measured variables in six-genotype plots and the mean of the genotypes with the best performance (the highest aboveground biomass or the most shoot number) in single-genotype plots.

ANOVA is very conservative in detecting the impact of treatments during multiple observations across time, and significant effects can be observed when the treatment has significant effects in all or most measures. The significant effects of interactions of genotypic diversity and time indicated that impacts of genotypic diversity on maximum spread distance and density changed across time.

Effects of genotypic diversity on parameters of invasive success became significant after ten months, indicating that enhanced invasive ability from genotypic diversity may take some time to develop, possibly for two reasons. Firstly, transplants may require time to acclimatize to the new environment after transplanting. Secondly, effects of genotypic diversity may accumulate and thus become more obvious as time passes. Indeed, a similar relationship was found between genotypic diversity and ecosystem functioning in the seagrass *Zostera marina*. Here, a significantly positive relationship between shoot density and genotypic diversity was recorded in the last three surveys but not in the first three surveys of the experiment (Reusch *et al.* 2005). Using field and mesocosm experiments, Stachowicz *et al.* (2008) also found that complementarity effects of species diversity were stronger in long-term experiments and that short-term experiments lacked sufficient time to allow expression of niche differences. Based on a meta-analysis of biodiversity–ecosystem functioning, Cardinale *et al.* (2007) revealed that strength of over yielding because of complementarity increased with experimental duration; it took approximately 1750 days or approximately 2–5 growing seasons before the most diverse polycultures began to yield more biomass than the highest-yielding monocultures.

In the present study, the similar performances among genotypes in single-genotype patches—in regard to shoot number, aboveground biomass and maximum spread distance—at the end of experiments precluded a strong selection effect on *S. alterniflora* (Table 1; Fig. S2, Supporting information). That is, of the six tested genotypes from Dongtan, none was clearly superior. Similarly, in *Z. marina*, the especially robust genotypes did not explain the observed effects of genotypic diversity (Reusch *et al.* 2005; Hughes & Stachowicz

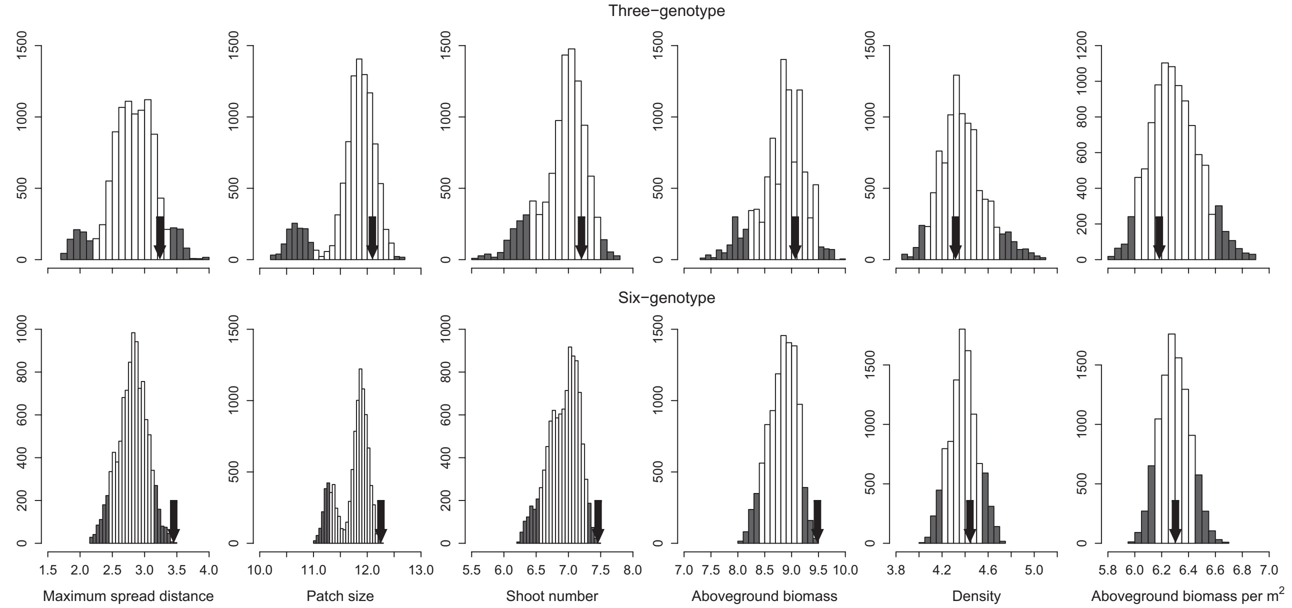

Fig. 3 Nonadditive effects of genotypic diversity on maximum spread distance (m), patch size (cm^2), shoot number, density (number of shoots per m^2), aboveground biomass (g), aboveground biomass per m^2 (g/m^2)of *Spartina alterniflora* of the last census (November 2007). Distribution of trait values for 9999 sets of artificial populations was shown, and the area outside of the 95% confidence intervals was shaded. Arrows indicate the locations of the observed means.

2011). Furthermore, Monte Carlo permutations (Fig. 3) and a transgressive over performance of multi- compared to single-genotype patches (Fig. 2) confirmed that nonadditive effects were probably more important than additive (selection) effects after two growing seasons.

Niche partitioning among genotypes may lead to a positive relationship between genotypic diversity and ecosystem functioning (Loreau & Hector 2001). In the case of niche partitioning, genotypes differ in utilization of resources, such as light or nutrients, and therefore, more genetically diverse plots can utilize resources more efficiently, thus supporting a high shoot number or high biomass per unit area. However, in our case, the absence of significant impacts of genotypic diversity on shoot density or aboveground biomass per unit area and no difference in efficiency of nutrient uptake among plots (X. Y. Wang *et al.*, unpublished data) suggested that niche partitioning might have played a minor role in this study.

The broad umbrella of facilitation among genotypes includes a variety of processes, where the outcome of genotype interactions cannot be predicted a priori because of changes in phenotypes of individual genotypes or in behaviour of other interacting species responding to polycultures vs. monocultures (Hughes *et al.* 2008). For example, the productivity of a plant genotype might be greater in mixtures than in monocultures, or genotypes may protect each other from herbivory or pathogen infestation (Schmid 1994). Differential performance in tolerance and resistance to the same herbivore has been observed among genotypes of *S. alterniflora* in introduced but not in native populations in North America (Garcia-Rossi *et al.* 2003). However, there was very little herbivory in either mono- or polyculture plots during the experiments, as well as in the studied region of the Yangtze Estuary, indicating that associational resistance to herbivory did not play an important role in the facilitation among genotypes. Therefore, genotypes expressing different phenotypes in mixtures and in monocultures probably played a critical role in the positive relationship between genotypic diversity and invasion success. This meant that individual genotypes, in general, performed better when surrounded by other genotypes than by the same genotype. For example, when intra-genotype competition is fierce, productivity of individual genotypes may be greater in mixtures than in monocultures. The dependence of genotype performance on neighbourhood genetic composition was also found in *Anthoxanthum odoratum* (Antonovics & Ellstrand 1984) and *Festuca ovina* (Fridley & Grime 2010).

Although nonadditive effects played a critical role in the significant relationship between genotypic diversity and traits related with invasive ability, selection effects cannot be ruled out and might also play an important role, at least at the early stages. First, there was no significant positive relationship between genotypic diversity and invasive ability during the first year. Second, we found no difference from expectations under additivity in the first year of six-genotype plots (data not shown) and throughout the whole experimental duration of the three-genotype plots using Monte Carlo permutations (Fig. 3). Our results were consistent with those for experimental populations of weedy *Arabidopsis thaliana* (Crawford & Whitney 2010), in which both additive and nonadditive effects contributed to the increased colonization success of increased genetic diversity. The finding that significant nonadditive effects were observed in six-genotype but not in three-genotype patches also suggested that these effects occurred among multiple genotypes, but were absent or weak in low genotypic diversity patches.

To date, most studies have compared the genetic diversity in native and introduced ranges of invasive species. Our manipulation experiment provided direct evidence that genetic diversity could enhance invasive ability of alien species. Identity of genotypes may be important in the earlier stage of introduction or under situations of low genetic diversity. However, nonadditive effects of genetic diversity became stronger as duration increased. These results shed light on the relative roles of additive and nonadditive mechanisms of genetic variation in invasion success.

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X.Y.W.'s research interests include population genetics and ecosystem consequences of invasive plants. D.W.S. is interested in population genetics of invasive plants. J.J. is interested in statistical ecology. N.N.X. is interested in population genetics of wetland plants. S.Y. is interested in population genetics of plants. X.F.Z. is interested in wetland ecology. M.M.S. is interested in plant population genetics. X.Y.C. is interested in population biology and evolution.

Data accessibility

Microsatellite data of the six genotypes are available in Table S1 (Supporting information).

Data deposited in the Dryad repository: doi: 10.5061/dryad.9412sq70.

Supporting information

Additional supporting information may be found in the online version of this article:

Table S1 Microsatellite data and heterozygosity of each genotype of *Spartina alterniflora* used in the present study.

Table S2 Pairwise distance of the six genotypes.

Fig. S1 Spatial arrangement of plots.

Fig. S2 Maximum spread distance, patch size, shoot number, density, aboveground biomass, and aboveground biomass per m^2 of six genotypes.

Fig. S3 Effects of genotypic diversity of the alien *Spartina alterniflora* on abundance of native dominant species *Scirpus mariqueter*.

Fig. S4 Relationship between genotype diversity and variables of performance of *Spartina alterniflora*.