POPULATION ECOLOGY - ORIGINAL PAPER

# Morphological and physiological variation among seagrass (*Zostera marina*) genotypes

**A. Randall Hughes · John J. Stachowicz · Susan L. Williams**

**Abstract** Intraspecific variation in habitat-forming species can have important ecological consequences at the population, community, and ecosystem level. However, the contribution of genetic variation among individuals to these effects is seldom documented. We quantified morphological and physiological variation among genotypes of a marine foundation species, the seagrass *Zostera marina*. We grew replicate shoots of eight genetically distinct *Zostera* individuals collected from Bodega Bay, California, in a common garden environment and then quantified shoot production and morphology, nutrient uptake, and key photosynthetic parameters. We found that genotypes differed in shoot production, biomass, and both root and shoot nutrient uptake rates, even when corrected for genotype-specific biomass differences. In addition, the rank order of uptake ability differed for ammonium and nitrate, indicating that genotypes may exhibit resource partitioning of different forms of nutrients. Our results suggest that both niche complementarity among genotypes and the sampling/selection effect could contribute to previously observed positive effects of seagrass clonal diversity on resource utilization and biomass production. Further, they highlight that genotypic variation in key traits of habitat-forming species could have measurable effects on community structure and function.

**Keywords** Biodiversity · Coexistence · Community genetics · Genetic diversity · Niche complementarity

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## Introduction

Intraspecific variation in factors such as morphology, behavior, and resource use can have important population, community, and ecosystem-level effects. For instance, plants commonly exhibit inter-individual variation in secondary chemistry or palatability, with strong effects on plant community dynamics, herbivore fitness, and nutrient mineralization (Lankau and Strauss 2007; Schweitzer et al. 2004; Taylor et al. 2003). Despite the growing recognition of the importance of such variation to ecological processes (Bolnick et al. 2003; Clark et al. 2004), and a long history in evolutionary biology of partitioning phenotypic variation into component parts (Wright 1920), ecologists have relatively little understanding of the exact source of observed phenotypic variation within species. In particular, the contribution of differences among genotypes to variation in ecologically relevant population traits is seldom understood aside from a few well-studied systems (e.g., *Populus* hybrid complexes; Schweitzer et al. 2004; Whitham et al. 2006).

Variation among genotypes may be particularly important in foundation species (sensu Dayton 1972), which by their presence alter abiotic conditions and/or create habitat for other species. Because these species perform many of the same ecosystem processes associated with multiple species in other systems, variation among genotypes may

increase community stability or reliability over time (Norberg et al. 2001; Tilman et al. 1997a). In fact, functional differentiation among genotypes is a prerequisite for ecological effects of genetic diversity (Hughes et al. 2008). Selection effects and niche partitioning are two mechanisms that account for non-additive diversity effects, and both rely on functional differences among genotypes (or species) in productivity or resource use, respectively (Cardinale et al. 2007; Hughes et al. 2008; Huston 1997; Tilman 1999). Despite growing evidence for the ecological importance of genetic diversity (Hughes et al. 2008), there is far less information regarding the nature of the functional variation among individuals that causes these effects.

We quantified the degree of morphological and physiological variation among genotypes of the seagrass *Zostera marina*. *Zostera* is a key marine foundation species, contributing to enhanced primary production, secondary production, and nutrient cycling in soft-sediment systems throughout the Northern Hemisphere (Moore and Short 2006). Despite documented variation in morphology, photosynthetic parameters, and nutrient uptake within and across seagrass beds (Dennison and Alberte 1982; Romero et al. 2006), the role of differences among genotypes in this variability is not known. Because *Zostera* plays such a major role in the structuring of the ecosystem, understanding the degree of genotypic variation is important to more than just the population biology of the species. Several previous studies have shown that increased genetic or genotypic diversity at the plot scale can lead to higher shoot densities, lower nutrient levels, and increased abundance or diversity of epifaunal invertebrates (Hughes and Stachowicz 2004; Reusch et al. 2005; Williams 2001). In addition, *Zostera*'s ability to reproduce clonally allows single genotypes to dominate some areas (Reusch et al. 1999a); thus any variation among genotypes in their effect on the ecosystem and associated processes such as primary or secondary production could be maintained for long periods of time and influence large areas in space. Finally, *Zostera* is the target of extensive restoration in some areas (Orth et al. 2006), and ecological variation among genotypes could play a previously unrecognized role in the success of these efforts.

## Materials and methods

### Experimental design

We conducted a common garden experiment to quantify variation within and between *Zostera* genotypes in morphology, shoot and biomass production, photosynthetic rate, and nutrient uptake rate. Genotypes were identified using five DNA microsatellite loci designed specifically for this species (Reusch et al. 1999b; Reusch et al. 2000). Because of the difficulty of finding adequate numbers of shoots of the same genotype in the field, we propagated eight known genotypes in adjacent 300-l outdoor flow-through mesocosms at Bodega Marine Laboratory (BML) under ambient light and temperature conditions. Before planting the seagrass, we added 8 l of sieved field-collected sediment to each mesocosm. Each mesocosm was then initiated with a single terminal shoot collected from one of eight different locations at 0.5 m mean lower low water tidal height in Bodega Harbor, California, in July 2004. The genetic distinctiveness of each of the original shoots was verified. Periodically during the propagation period we added equivalent numbers of *Phyllaplysia taylori* to each tank; these opisthobranch molluscs graze on epiphytic algae growing on *Zostera* but do not consume the seagrass itself (A. R. Hughes, unpublished data). No other organisms were intentionally added to the mesocosms, although some species (e.g., limpets, abalone, anemones) did colonize the tanks via the flow-through seawater system. Flow was maintained at a rate of approximately 60–70 ml s$^{-1}$. Mesocosms were monitored weekly for the presence of flowering shoots or seagrass grazers; any present were removed.

In June 2006 we harvested ten terminal shoots (hereafter, 'clones') from each genotype for use in this experiment (see Thayer et al. 1984; Dennison et al. 1987 for diagrams of *Zostera* morphology). Seagrass photosynthetic capacity, pigment ratios, growth rates, and morphology acclimate rapidly to new environmental conditions when transplanted into different habitats, common gardens, and cultures (e.g., Dennison and Alberte 1985; Dennison and Alberte 1986; McMillan 1978). The 2-year acclimation period in mesocosms thus should have minimized environmental carryout from the habitat. To further minimize potential variation due to growing conditions, we propagated the ten terminal shoots of each genotype in individual pots in a common garden environment in the lab for 10 weeks. We did the same with 20 field-collected terminal shoots later confirmed to be from genetically distinct individuals. At least one new shoot per 'parent' was produced during this period. Shoot and rhizome lengths were standardized to 30 and 2.5 cm, respectively, to minimize initial size differences. The clones were planted in sieved sediment in individual 15-cm-diameter pots and randomly assigned to locations in a single flow-through raceway at BML. The raceway was located outdoors under natural light conditions. Clones were randomly reassigned to different positions in the raceway every 2 weeks to minimize position effects.

After 10 weeks in the common garden, we assessed the performance of each clone with respect to the following metrics: (1) morphology and production (rhizome length,

maximum root length, number of vegetative and reproductive shoots produced, shoot biomass, root biomass, and rhizome biomass); (2) nutrient uptake (shoot nitrate uptake, root/rhizome ammonium uptake); and (3) photosynthetic rate. Shoots that died ($n = 3$) or produced only flowering shoots ($n = 7$) were excluded from the analyses. To analyze variation among genotypes, we first conducted separate multivariate ANOVA (MANOVA) on the morphology/production and nutrient uptake data, with *Zostera* genotype as a random factor and a blocking factor for date when the clones were processed. When the MANOVA indicated significant differences among genotypes, we then conducted ANOVA for each response variable. There were no significant block × genotype interactions, so we omitted this term from our model. When there was a significant effect of genotype, we calculated broad sense heritability ($H^2$) as the ratio of the genetic to total component of variance, an appropriate measure for clones (Lynch and Walsh 1998). Data from the field-collected shoots were not included in our statistical analyses, but the mean and range of these shoots are presented on the figures to allow an assessment of whether the performance of any of the propagated genotypes was extreme relative to a larger sample of genotypes. Response variables were log transformed as necessary to meet assumptions of normality and homogeneity of variance. We present untransformed data in the figures. All analyses were conducted using JMP 5.0 (SAS Institute, Cary, NC). The specific methods and statistics for each of these categories are described in greater detail below.

Morphology and production

We quantified the total number of shoots and total rhizome length at the end of the common garden experiment as measures of production and spatial spread, respectively. We also measured maximum root length to examine the potential for variation in rooting depth. Finally, we quantified the biomass of shoot, root, and rhizome tissue that was dried at 60°C for at least 48 h; these measurements were conducted separately for the original terminal shoots and any new tissue produced during the common garden experiment.

Nutrient uptake

Following the 10-week common garden period, we separated the original terminal shoot from any new shoots. We clipped a 1.5-cm section of the 3rd rank leaf from the original terminal shoot for measures of photosynthetic rate (see below), and then used these original terminal shoots in nutrient uptake experiments. To examine the role of genotypic variation in nutrient uptake, we quantified biomass-specific leaf nitrate uptake and root/rhizome ammonium uptake using individual two-compartment chambers as in

Terrados and Williams (1997). Although all tissues are capable of utilizing multiple forms of nitrogen, we chose to test leaf nitrate and root/rhizome ammonium because these forms of nitrogen are most available in the water column and sediments, respectively. Roots/rhizomes were compartmentalized from leaf shoots by inserting the terminal shoot through a slit in a water-tight stopper separating a 500-ml transparent cylinder for the leaves from an opaque 40-ml chamber for the roots/rhizome. A submersible pump delivered turbulent water flow at 15.1 ml s$^{-1}$ to the leaf chamber to prevent mass transfer limitation of uptake. The root compartment was unstirred and left aerobic because *Zostera* root and rhizomes release oxygen during active photosynthesis (Caffrey and Kemp 1990). The chambers were placed in a flow-through seawater table in a cold room at BML to maintain a constant ambient temperature (approximately 15°C). Photosynthesis-saturating light (860 μmol photons m$^{-2}$ s$^{-1}$) was provided by four quartz halite lamps placed over the water table. Due to a limited number of nutrient chambers, we ran the experiments in batches of 14–15 clones at a time over a period of 3 weeks.

Nitrogen-free artificial seawater spiked to known initial concentrations (see below) was used in both the shoot and root/rhizome compartments. Prior to turning on the pumps and lights, we collected an initial 5-ml water sample from both the shoot and root compartments. We then collected a 5-ml sample from each leaf chamber every 45 min for 4 h. A final 5-ml water sample was taken from the root chambers at the end of the experiment. Shoot compartment (nitrate) samples were frozen until analysis using a Lachat 8000 series flow injection auto analyzer. Root compartment (ammonium) samples were placed in acid-washed test tubes, kept in the dark, and analyzed spectrophotometrically (Koroleff 1976).

We conducted two separate uptake experiments (on consecutive days) for each clone to examine the effect of nutrient limitation on nitrate uptake. In one experiment, we spiked the artificial seawater in the root/rhizome compartments to 100 μM using a 0.1 M ammonium chloride solution (Orion), which approximates average sediment porewater ammonium concentrations in Bodega Harbor (A. R. Hughes, unpublished data). In the second experiment, no ammonium was added to the root chamber, resulting in initial ammonium concentrations of approximately 7 μM. In all experiments, we used a 0.1 M sodium nitrate solution (Orion) to spike seawater for the shoot compartment to an initial nitrate concentration of approximately 15 μM, a representative concentration for the study area (Smith et al. 1989).

Change in nitrogen concentration was standardized by leaf biomass (nitrate) and root and rhizome biomass (ammonium); results did not differ when root biomass only was used for ammonium uptake (data not shown). We used nonlinear

regression in order to estimate the biomass-specific first order rate constant ($k$) for change in concentration over time. Given that biomass can vary among genotypes (see "Results"), we also calculated an estimate of total nutrient uptake for the experimental period [biomass-specific uptake rate (mol $g^{-1}$ dry) $\times$ biomass (g dry)]. To determine whether shoot nitrate uptake varied in response to the presence or absence of belowground ammonium, we conducted a factorial ANOVA with genotype and ammonium treatment (presence or absence) as fixed factors.

### Photosynthetic rate

We generated photosynthesis (as oxygen evolution) versus irradiance ($P$ vs. $I$) curves for each clone. As described above, after 10 weeks in the common garden environment we clipped a 1.5-cm section 25 cm above the leaf sheath on the 3rd rank leaf of the original terminal shoot to be used in laboratory experiments of oxygen evolution. These shoot clippings were held in flow-through seawater for a minimum of 24 h; this time period is sufficient to allow the tissue wounds from clipping to heal (S. L. Williams, unpublished data). We placed this tissue in a 2.3-ml cuvette (Rank Brothers model) held at constant temperature (12°C) and measured the change in oxygen concentration over a 3-min period at each of six irradiances: 75, 250, 500, 1,000, 1,500, and 2,000 μmol photons $m^{-2}$ $s^{-1}$. The relationship between oxygen concentration and time was linear at each irradiance. We then dried and weighed the seagrass tissue after the experiment to standardize productivity by biomass. These data were fit to a nonlinear model using Talling's equation (Talling 1973) to estimate light-saturated photosynthesis ($P_{max}$) and the light-limited slope ($\alpha$).

### Effects of intraclonal competition

To assess the correspondence between physiological and growth measurements of single shoots and more natural situations in which multiple shoots are present initially, we compared variation among genotypes from this experiment with independent laboratory experiments using the same eight genotypes. The experimental design of these two additional experiments was similar to that described above, with the exception that two physically separated shoots (i.e., clones) of each genotype were planted initially rather than a single shoot (i.e., intraclonal competitors were present from the start of the experiment). In each of the two separate laboratory experiments, two clones of each genotype were planted in sieved sediment in individual 15-cm-diameter pots and randomly assigned to locations in six mesocosms at BML. Shoot and rhizome lengths were standardized to 30 and 2.5 cm, respectively, to minimize initial size differences. At the end of 12 weeks, we quantified total

shoot production, aboveground biomass, belowground biomass, root to rhizome biomass ratio, rhizome length, and maximum root length.

We conducted a factorial ANOVA with genotype and experiment type (single vs. pair) as fixed factors to assess whether genotypic differences varied in the presence or absence of intraclonal competition. The presence of a significant genotype × experiment interaction would indicate that the effect of genotype is not consistent across the separate experiments.

### Trade-offs in performance

We were interested a priori in two potential performance trade-offs among genotypes: allocation to vegetative versus reproductive shoot allocation, and aboveground nitrate uptake rate versus belowground ammonium uptake rate. In addition, we calculated the pair-wise correlations between the following response variables (grouped by genotype) to examine whether other trade-offs were evident in our data and whether these varied by genotype: vegetative shoot production, rhizome length, maximum root length, aboveground biomass, root biomass, rhizome biomass, the ratio of root to rhizome biomass, $P_{max}$, $\alpha$, and biomass-specific ammonium and nitrate uptake rates.

### Prediction of clonal identity

To examine whether genotypes can be predicted based solely on their similarity in ecological performance, we performed a linear discriminant analysis (DA) using the same response variables listed above as our predictors or input variables. We first input these observations along with the known genotypic identity; we then used the resultant discriminant functions to predict the genotypic identity of the same set of observations. The effectiveness of the DA is based on the percentage of observations that are assigned to the correct class (i.e., genotypic identity).

## Results

### Morphology and production

*Zostera* genotypes varied in biomass production and a variety of ecologically relevant morphological measures (Fig. 1). Broad-sense heritability values for these and other response ranged from 18.7 to 36.5%. In addition to differences in shoot production (genotype $P < 0.001$, $H^2 = 29.4$; Fig. 1a) and belowground biomass (genotype $P < 0.001$, $H^2 = 25.9$; Fig. 1c) production, genotypes varied in the allocation of biomass to different tissues (e.g., the ratio of root to rhizome biomass, genotype $P = 0.001$, $H^2 = 30.4$,
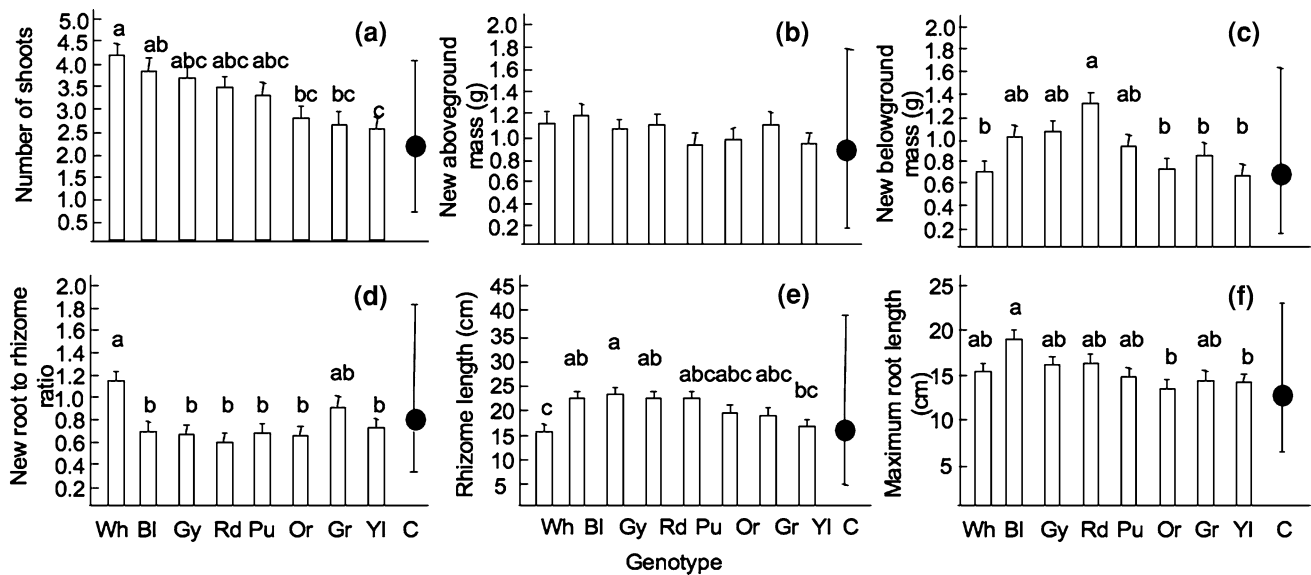
**Fig. 1** Production and biomass variation among eight *Zostera* genotypes following a 10-week common garden experiment. **a** Shoot production. **b** Aboveground biomass produced during experiment. **c** Belowground biomass produced during experiment. **d** Ratio of root to rhizome biomass produced during experiment. **e** Rhizome length. **f** Maximum root length. Genotypes were coded by tag color [white (*Wh*), blue (*Bl*), gray (*Gy*), red (*Rd*), purple (*Pu*), orange (*Or*), green (*Gr*), yellow (*Yl*)]. Control (*C*) shoots were collected from the field at the start of the experiment. Means plus SE for genotypes ($n = 7–10$); means and range for field-collected shoots (C, $n = 15$). *Different letters* indicate significant differences ($P < 0.05$) based on Tukey's post-hoc tests

Fig. 1d; the ratio of above to belowground biomass, genotype $P < 0.001$, $H^2 = 24.2$). Spatial spread (rhizome length; genotype $P = 0.001$, $H^2 = 23.3$; Fig. 1e) and rooting depth (maximum root length; genotype $P = 0.01$, $H^2 = 18.7$; Fig. 1f) also differed significantly. Interestingly, no one genotype maximized (or minimized) all response variables, or even those that would appear to be highly correlated (e.g., number of shoots and shoot mass; Fig. 1). Instead, the rank order of genotypes varied considerably from one response to the next (Pearson's $r$; $P > 0.05$). Only the rank order for belowground biomass, root to rhizome biomass, and rhizome length were significantly correlated (Pearson's $r$ $P < 0.01$ for each of these pairwise correlations).

Nutrient uptake

The eight *Zostera* genotypes examined here displayed significant variation in belowground biomass-specific ammonium uptake rate ($k$; genotype $P = 0.02$, $H^2 = 28.9$; Fig. 2d). As with newly produced tissue, these genotypes also differed in total (newly produced plus tissue originally planted) belowground biomass (genotype $P < 0.001$, $H^2 = 25.8$; Fig. 2e). As a result, estimated total ammonium uptake varied by genotype (genotype $P < 0.001$, $H^2 = 32.4$; Fig. 2f), yet the rank order of estimated ammonium uptake (Fig. 2f) was different from the rank order of biomass-specific uptake (Fig. 2d).

Biomass-specific shoot uptake of nitrate also varied by genotype, both in the absence (genotype $P < 0.001$, $H^2 = 18.9$; Fig. 2a) and presence (genotype $P < 0.001$, $H^2 = 23.3$) of belowground ammonium. Shoot nitrate uptake was higher overall when ammonium was absent from the roots/rhizomes (nutrient treatment $P < 0.001$), and there was no genotype × ammonium treatment interaction ($P = 0.99$). Because total aboveground biomass did not vary across genotypes (genotype $P > 0.05$; Fig. 2b), the rank order of estimated total nitrate uptake (Fig. 2c) was identical to the biomass-specific uptake rate (Fig. 2a).

Photosynthetic rate

Neither $P_{max}$ nor $\alpha$ differed among *Zostera* genotypes (genotype $P > 0.50$).

Effects of intraclonal competition

Genotypic effects on seagrass biomass and morphology were largely consistent in the presence and absence of clonemates (Table 1). However, two genotypes (blue and white) had significantly higher aboveground biomass in the presence of clonemates, leading to an interaction between genotype and experiment type. Maximum root length also exhibited a significant genotype × experiment interaction: most genotypes had longer roots on average in the presence of clonemates, although some (gray, purple and white) were not statistically longer and one genotype (blue) even showed the opposite pattern.
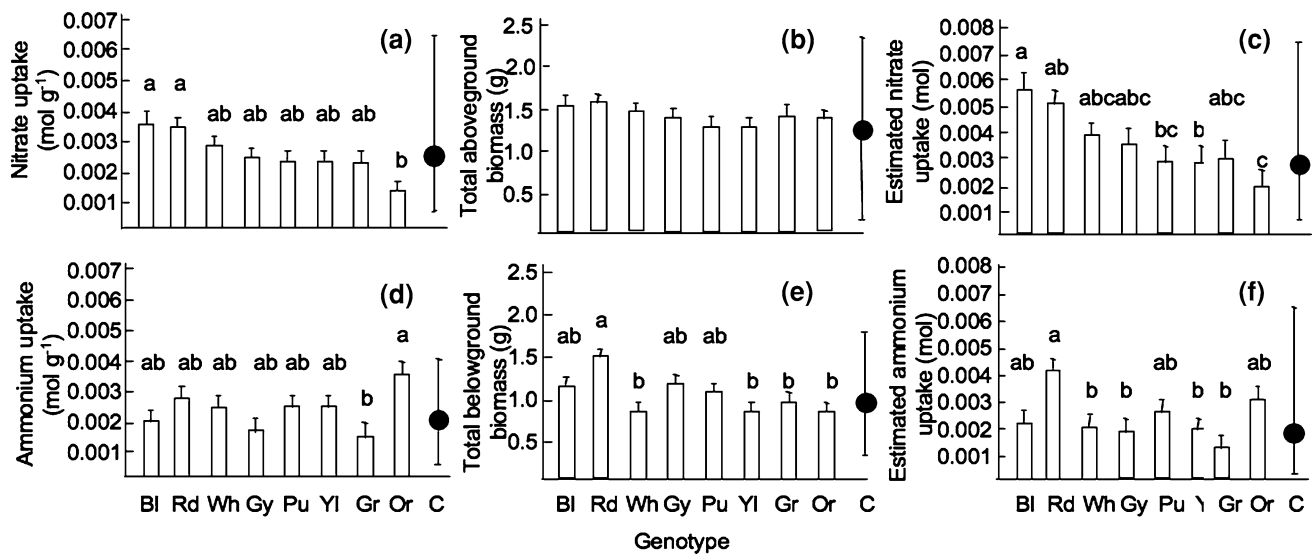
**Fig. 2** Nutrient uptake variation among eight *Zostera* genotypes following a 10-week common garden experiment. **a** Biomass-specific shoot nitrate uptake. **b** Total aboveground biomass. **c** Estimated total nitrate uptake. **d** Biomass-specific root to rhizome ammonium uptake. **e** Total belowground biomass. **e** Estimated total ammonium uptake. Genotypes were coded by tag color. C shoots were collected from the field at the start of the experiment. Means plus SE for genotypes ($n = 7$–10); means and range for field-collected shoots (C, $n = 15$). *Different letters* indicate significant differences ($P < 0.05$) based on Tukey's post-hoc tests. For abbreviations, see Fig. 1

**Table 1** Results of comparison of experiment type (with or without intraclonal competition)

| Response | $R^2$ | Genotype ($df = 7$) | | Experiment type ($df = 1$) | | Genotype × experiment ($df = 7$) | |
|---|---|---|---|---|---|---|---|
| | | $F$ | $P$ | $F$ | $P$ | $F$ | $P$ |
| Shoot production | 0.4 | 6.24 | **<0.0001** | 26.49 | **<0.0001** | 0.89 | 0.52 |
| Aboveground biomass | 0.24 | 2.50 | 0.02 | 1.87 | 0.17 | 3.20 | **0.004** |
| Belowground biomass | 0.34 | 6.56 | **<0.0001** | 6.37 | 0.01 | 1.15 | 0.34 |
| Root to rhizome ratio | 0.36 | 8.23 | **<0.0001** | 1.87 | 0.17 | 0.87 | 0.53 |
| Maximum root length | 0.45 | 1.86 | 0.08 | 51.42 | **<0.0001** | 2.65 | **0.01** |
| Rhizome length | 0.24 | 3.49 | **0.002** | 0.01 | 0.94 | 1.54 | 0.16 |

Bold indicates significant effect

### Trade-offs in performance

Although reproductive shoot production did not vary by genotype (genotype $P = 0.13$), there was a significant trade-off between vegetative and reproductive shoot allocation overall ($R^2 = 0.41$, $P < 0.0001$). In contrast, there was no strong correlation between genotypic nitrate uptake and ammonium uptake ($R^2 = 0.13$, $P = 0.38$; Fig. 3), although the genotype with the lowest biomass-specific nitrate uptake rate did have the highest biomass-specific ammonium uptake rate.

Few correlations among response variables for clones within genotype were significant, regardless of the particular genotype being considered (39 out of 288 possible correlations, or 13.5%), most likely due to the low number of clones of each genotype. Rhizome length/belowground biomass and $P_{max}/\alpha$ were consistently positively correlated across genotypes (75 and 62.5% of possible correlations,
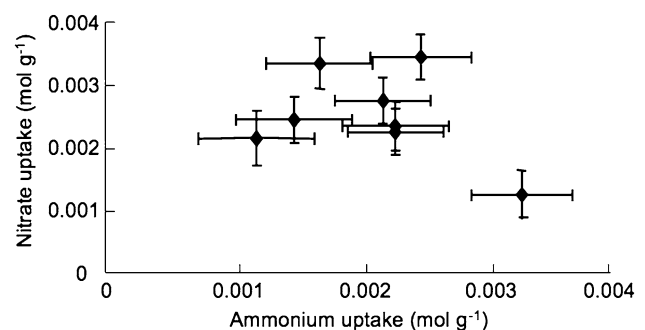


**Fig. 3** Potential trade-offs in *Zostera* performance (means ± SE). Biomass-specific nitrate uptake versus biomass-specific ammonium uptake by genotype

respectively). Of the other significant correlations, a greater percentage was positive (71.4%) than negative (28.6%). In addition, the nature of the correlations varied by genotype,
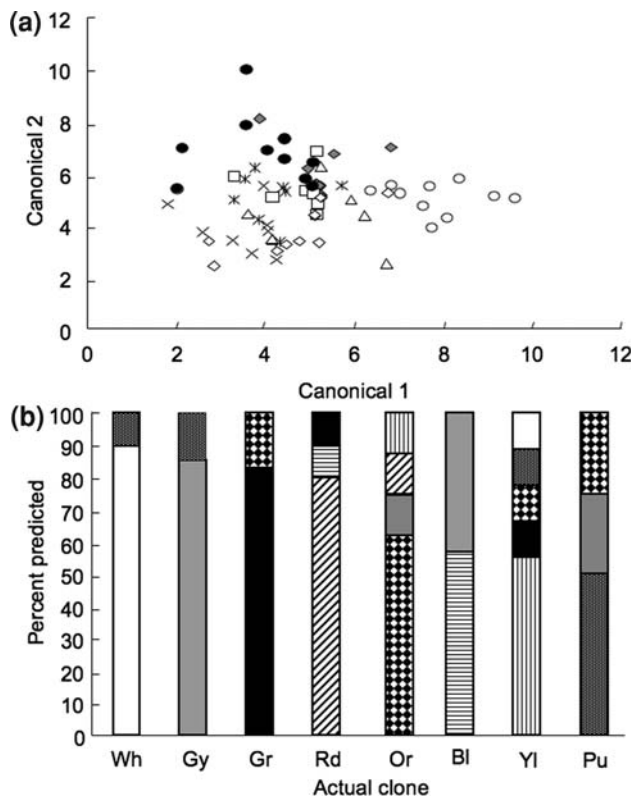
**Fig. 4** Results of discriminant analysis. **a** Genotypic means along primary discriminant axes. Genotypes are distinguished by shapes: Wh (*open circles*), Gy (*open square*), Gr (*open triangle*), Rd (*solid circle*), Or (*crosses*), Bl (*shaded diamond*), Yl (*open diamond*), Pu (*star*). **b** Predicted versus actual *Zostera* clonal identity. Genotypes are distinguished by shading of the bars: Wh (*open*), Gy (*light gray*), Gr (*black*), Re (*right diagonal*), Or (*squares*), Bl (*horizontal stripe*), Yl (*vertical stripe*), Pu (*dark gray*). For abbreviations, see Fig. 1

with some exhibiting a higher percentage of significant correlations among response variables (range = 2.8–22.2% across genotypes).

Prediction of clonal identity

The DA classified 71% of the clones into the correct genotype (compared to a null hypothesis of 12.5% if clones were assigned randomly). However, the probability that a clone was misclassified varied by genotype (Fig. 4b): clones were reliably assigned to four of the genotypes with 80% or greater accuracy, while predictions for the remaining four genotypes were considerably less accurate, presumably because these genotypes displayed greater variability among clones in the measured performance traits (Fig. 4a).

**Discussion**

We found significant variation among *Zostera* genotypes for a wide variety of morphological and physiological responses; only aboveground biomass, flowering shoot production, and photosynthetic parameters did not vary consistently by genotype. In addition, there was no single genotype that maximized all performance measures simultaneously, providing evidence for functional differentiation among genotypes. These results imply that variation in niche space may contribute to genotypic coexistence in a manner analogous to differences in resource use among species (Tilman 1982). If so, it is possible that the ecological differences that we and others (Proffitt et al. 2003; Schweitzer et al. 2004; Johnson and Agrawal 2005) have identified among genotypes may contribute to the maintenance of genetic diversity in natural populations (e.g., high levels of seagrass genotypic diversity in some areas; Hughes and Stachowicz, in press). However, it is important to note that our results represent upper estimates of genetic variance, as they cannot rule out environmental carry-over or maternal effects.

We focused our analyses on eight *Zostera* genotypes that we propagated in the laboratory to ensure adequate replication of each genotype and provide a comparison for additional experiments. Despite that fact that these genotypes were initially selected haphazardly from the field with no a priori indication of morphological differences, they display significant variation that is relevant to community- and ecosystem-level processes. More importantly, the eight genotypes examined in this experiment appear to be representative of the variation present in natural settings, given that their average responses fell within the range exhibited by a broader array of field-collected genotypes (Figs. 1, 2). Given reductions in *Zostera* genetic variation (Williams 2001; Williams and Davis 1996), the conservation implications of these results and previous work in seagrass systems (e.g., Williams 2001; Hughes and Stachowicz 2004; Reusch et al. 2005) are clear: seagrass restoration efforts should include a diversity of genotypes (by increasing the spacing between transplants from a single bed, and/or by collecting transplants from multiple beds if inbreeding/outbreeding effects are known to be minimal; Ruckelshaus 1995) to enhance functional diversity and increase the likelihood of long-term persistence in the face of changing conditions.

In addition to significant variation among genotypes, we found that nitrate uptake rate was consistently higher when ammonium was abundant belowground than when ammonium was negligible. Leaf-root interactions governing nitrogen uptake in *Zostera* are complex (Iizumi and Hattori 1982; Short and McRoy 1984; Thursby and Harlin 1982) and a comprehensive kinetic understanding is lacking, but we are unaware of any other study that has shown a stimulation of nitrate uptake in the presence of belowground ammonium. Our goal in this study was to assess the potential for genotypic variation in nutrient uptake, but an

interesting future direction will be to further examine the relative importance of and interactions between nitrate and ammonium uptake above- and belowground.

Our findings support conclusions from genetic diversity manipulations that multiple mechanisms likely contribute to the observed effects of genotypic diversity on ecosystem functioning (Hughes et al. 2004; Reusch et al. 2005; Johnson et al. 2006; reviewed in Hughes et al. 2008). For example, significant variation among genotypes in shoot production could lead to more diverse plots being more productive on average via a sampling effect (Huston 1997), given that a mixture is statistically more likely to contain a highly productive clone. In addition, if these more productive clones also have a competitive advantage, then they may increase in relative abundance (the selection effect; Tilman et al. 1997b) and further contribute to positive diversity effects. Of course the opposite can also occur: productive genotypes in monoculture can perform poorly in mixture, contributing to a negative selection effect (Reusch et al. 2005). Our results are also consistent with the finding that complementarity can contribute to positive effects of genotypic diversity (Reusch et al. 2005; Crutsinger et al. 2006) and indicate that niche partitioning is a likely mechanism, at least in seagrass systems. *Zostera* genotypes varied in their rate of uptake of both nitrate and ammonium, and different genotypes maximized these two responses; thus, total nitrogen uptake might be greater in more diverse beds. Furthermore, maximum rooting depth varied by genotype, suggesting that our laboratory uptake experiments (which effectively equalized rooting depth across clones) may have underestimated partitioning of belowground resource uptake.

Evolutionary biologists have long recognized the importance of genetically based variation within species—indeed every example of the operation of natural selection requires that genotypes differ in fitness-related traits. Despite this recognition by evolutionary biologists and the obvious implications for conservation and restoration, ecologists have been slow to appreciate the potential importance of genetic variation among individuals in ecological experiments. As we demonstrate here, it can explain a substantial portion of the observed variation ($R^2 \leq 0.48$) in many responses, including the ecosystem-level functions of primary production and nutrient acquisition. Furthermore, it is clear that habitat-forming plant species play an important role in organizing a wide range of ecosystems from forests, to marshes to kelp beds (Bruno et al. 2003; Ellison et al. 2005; Halpern et al. 2007). We now have several studies that demonstrate that key traits of these foundation species vary among plant genotypes (reviewed by Whitham et al. 2006). Thus, genetic variation in foundation species may often have measurable effects on community composition, structure, and function and should not be ignored.

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