

Plant genotype and nitrogen loading influence seagrass productivity, biochemistry, and plant–herbivore interactions

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Abstract. Genetic variation within and among key species can have significant ecological consequences at the population, community, and ecosystem levels. In order to understand ecological properties of systems based on habitat-forming clonal plants, it is crucial to clarify which traits vary among plant genotypes and how they influence ecological processes, and to assess their relative contribution to ecosystem functioning in comparison to other factors. Here we used a mesocosm experiment to examine the relative influence of genotypic identity and extreme levels of nitrogen loading on traits that affect ecological processes (at the population, community, and ecosystem levels) for *Zostera marina*, a widespread marine angiosperm that forms monospecific meadows throughout coastal areas in the Northern Hemisphere. We found effects of both genotype and nitrogen addition on many plant characteristics (e.g., aboveground and belowground biomass), and these were generally strong and similar in magnitude, whereas interactive effects were rare. Genotypes also strongly differed in susceptibility to herbivorous isopods, with isopod preference among genotypes generally matching their performance in terms of growth and survival. Chemical rather than structural differences among genotypes drove these differences in seagrass palatability. Nitrogen addition uniformly decreased plant palatability but did not greatly alter the relative preferences of herbivores among genotypes, indicating that genotype effects are strong. Our results highlight that differences in key traits among genotypes of habitat-forming species can have important consequences for the communities and ecosystems that depend on them and that such effects are not overwhelmed by known environmental stressors.

Key words: chemical deterrence; clonality; community genetics; detritus; eutrophication; intraspecific variation; isopod; nutritional quality; primary production; *Zostera marina*.

INTRODUCTION

Genetic variation is required for species to adapt to their environment and thus is essential for population persistence as the environment changes. However, genetic variation can also have significant immediate ecological consequences at the population, community, and ecosystem levels (Whitham et al. 2003, Hughes et al. 2008). In foundation species (sensu Dayton 1972), which exert strong influence on ecosystems through their structural and functional attributes, variation among genotypes may be an important source of ecological variation analogous to species diversity in other systems (Whitham et al. 2006). For example, genetic identity and diversity of some tree and herb species can have strong effects on productivity, resistance to invasion and disturbance, decomposition, or nutrient cycling (Driebe and Whitham 2000, Schweitzer et al. 2004, Whitham et al. 2006, Crutsinger et al. 2008b, Vellend et al. 2010). Further, plant genotypic variation can also modify

infestation rate, performance, composition, abundance, and diversity of associated terrestrial and freshwater arthropod communities (e.g., Fritz and Price 1988, Johnson and Agrawal 2005, Wimp et al. 2005, Crutsinger et al. 2006, LeRoy et al. 2006, Tovar-Sánchez and Oyama 2006).

Understanding the biological basis of ecological variation among genotypes may be particularly relevant for long-lived clonal plants, for which one specific genotype can dominate large areas in space (from meters to kilometers) and time (up to thousands of years; e.g., Steinger et al. 1996, Reusch et al. 1999). However, aside from the extensive knowledge acquired for *Populus* hybrid systems (see reviews by Whitham et al. [2006] and Schweitzer et al. [2008]), relatively few studies have demonstrated the biological basis for these effects by characterizing among-genotype variation in the species' traits that influence community and ecosystem processes (e.g., Johnson et al. [2009] for evening primrose *Oenothera biennis*). In addition, our knowledge of the relative contribution of genetic variation vs. other important factors (e.g., species diversity, presence of consumers, environmental conditions) to different ecological processes is still very limited (Hughes et al. 2008).

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For instance, terrestrial plant genotypic variation can sometimes be as important as environmental variation in determining plant and insect abundance and community structure (Johnson and Agrawal 2005, Fridley et al. 2007, Kotowska et al. 2010; but see Johnson et al. 2008, Tack et al. 2010), although genotypic variation may only influence ecological processes in the presence (e.g., Hughes and Stachowicz 2009) or absence (e.g., Maddox and Cappuccino 1986) of environmental stress. Furthermore, understanding the importance of intraspecific genetic variation relative to environmental stressors will also be important for prioritizing conservation and management resources.

Seagrass beds provide a model ecosystem in which to examine the relative importance of genetic vs. environmental variation in influencing ecosystem functioning. These widespread coastal ecosystems are often dominated by a single species of habitat-forming clonal plant that perform critical ecological functions (e.g., provision of trophic resources within and across habitats, nutrient cycling, carbon sequestration; Williams and Heck 2001), and genetic variation among seagrass individuals is known for some key traits (Williams 2001, Hughes et al. 2009). Seagrass ecosystems are also subject to significant natural and human-caused environmental variability (Ralph et al. 2007, Romero et al. 2007). Furthermore, seagrass beds are declining worldwide as a result of human activities (Waycott et al. 2009), and genotypic diversity can increase community resistance to and recovery from disturbance (e.g., Hughes and Stachowicz 2004, Reusch et al. 2005), improving the success of restoration (e.g., Williams 2001).

We conducted a series of experiments to assess the relative importance of genotype identity vs. a known environmental stress (high levels of nitrogen addition; Ralph et al. 2007) in determining seagrass ecological properties and processes at the population (e.g., recruitment, carbon reserves), community (plant–herbivore interactions), and ecosystem (e.g., detritus production) levels. We were particularly interested in the role of nitrogen because changes in its availability can strongly affect plant performance (Ralph et al. 2007, Bobbink et al. 2010) and can modify herbivore–plant interactions through alteration of plant nutritional quality or defensive traits (e.g., Mattson 1980). Furthermore, nitrogen addition and resultant eutrophication is a major threat to coastal plant communities, including seagrass beds (Howarth and Marino 2006, Ralph et al. 2007, Antón et al. 2011).

MATERIALS AND METHODS

Study species

Zostera marina forms extensive monospecific meadows in shallow temperate estuaries worldwide. *Z. marina* beds exhibit high primary productivity, provide habitat and refuge for numerous species, and have a key role in nutrient cycling and sediment stabilization (Williams and Heck 2001). For our experiments, we used four

eelgrass genotypes (distinguished hereafter by naming each a different color) that were collected haphazardly in 2004 from nearby field sites and were propagated in separate outdoor mesocosms at Bodega Marine Laboratory (BML), California, USA (see Hughes and Stachowicz [2004], and Hughes et al. [2009] for details on genotype identification and propagation). In previous work we measured physiological and morphological traits of these genotypes in common gardens and compared them to trait measurements of 20 haphazardly collected genotypes from the field (Hughes et al. 2009). The range of trait variation in the four genotypes used in this study was within the range of the field sample of 20 genotypes, suggesting that the trait variation in these genotypes is representative of what is found in natural beds in Bodega Bay. We used the isopod *Idotea resicata* to assess feeding preferences and performance on seagrass diets, as this is one of the most common seagrass consumers in *Z. marina* beds of the northeastern Pacific (Williams and Ruckelshaus 1993, Hughes et al. 2010).

Experimental procedure

To assess the role of genotype identity and nitrogen loading on seagrass characteristics and herbivore feeding behavior, we conducted a factorial mesocosm experiment with six replicates of each combination of four genotypes and two sediment nitrogen levels. Each replicate mesocosm was 25 cm in diameter and 42 cm deep (~20 L in volume), and was supplied with an independent source of coarse sand-filtered flow-through seawater at a rate of approximately 3.6–4.2 L/min. In each mesocosm, we planted four shoots of a single genotype into sieved, field-collected sediment. To minimize initial differences between genotypes, shoot and rhizome length were standardized to 30 and 2.5 cm, respectively (Hughes et al. 2009).

Nitrogen enrichment was produced by fertilizing the sediment with ammonium sulphate (21 N:0 P:0 K; Lilly Miller, Walnut Creek, California, USA). We opted to enrich the sediment because we were interested in the direct effects of nitrogen addition on seagrass itself; nutrients released into the water would likely have strongly stimulated epiphytic growth, which could have different indirect effects on seagrass growth and herbivory. Thus our experiment would simulate the consequences of an increase in nitrogen availability in the sediment rather than in the water column, as might occur as a result of sewage outfall or aquaculture activities (Holmer et al. 2007, Ralph et al. 2007). Twelve grams of fertilizer were held in a mesh bag inside a capped 50-mL plastic centrifuge tube with holes drilled in the sides (Williams and Ruckelshaus 1993). To impose the treatment uniformly across each mesocosm, each nitrogen-enriched mesocosm contained four equally spaced fertilizer tubes, buried ~4 cm deep in the sediment, which were not removed until the end of the experiment (Lee and Dunton 1999). Sediment and water

column ammonium levels were monitored throughout the experiment using a Lachat 8000 series flow injection autoanalyzer (Lachat Instruments, Loveland, Colorado, USA; Hansen and Koroleff 1999). In the unfertilized mesocosms, ammonium levels ranged between ~38 and 289 $\mu\text{mol/L}$ in pore water and between 2.5 and 9.5 $\mu\text{mol/L}$ in the water column (typical values of field conditions; Dennison et al. 1987, Williams and Ruckelshaus 1993), while in fertilized sediments values were between ~3480 and 8580 $\mu\text{mol/L}$. Ammonium concentrations in the water column of enriched mesocosms were low, ranging between 10.8 and 21.1 $\mu\text{mol/L}$. We chose sediment nutrient enrichment levels that would likely have stressful effects on the plant but that would not cause immediate mortality (see Ralph et al. 2007) to provide a basis for evaluating the strength of variation among genotypes on population, community, and ecosystem processes.

Treatments were randomly assigned to mesocosms, which were located on an outdoor platform under natural light conditions and were reassigned positions every week to minimize potential position effects. We also conducted weekly monitoring of mesocosms to collect detritus and remove any epiphyte growth from seagrass leaves and mesocosm walls. At the end of the experiment (10 weeks) we quantified epiphyte load (grams dry mass) per mesocosm and found no differences between treatments (no genotype effect $F_{3,40} = 1.481$, $P = 0.234$; no nitrogen addition effect $F_{1,40} = 0.399$, $P = 0.531$; no genotype \times nitrogen addition effect $F_{3,40} = 0.464$, $P = 0.709$), thus epiphyte data are not considered further. Two weeks before ending the experiment, leaves were punched with a hypodermic needle just above the meristem to estimate leaf growth.

Quantifying plant traits and production

After 10 weeks, we harvested all shoots from each mesocosm and quantified several metrics of eelgrass performance. Eelgrass physiological and morphological traits respond rapidly (within 2–5 weeks) to environmental conditions (e.g., Dennison and Alberte 1982, Williams and Ruckelshaus 1993, van Katwijk et al. 1997); thus, any initial differences between the measured traits should have been erased. In the laboratory each shoot was separated into rhizomes, roots, leaves, and sheaths, and the number of leaves and inflorescences (on reproductive shoots) were counted. Epiphytes were removed from leaves by gentle scraping, collected in pre-weighed Millipore glass fiber filters (Millipore, Billerica, Massachusetts, USA) and dried at 60°C to a constant mass. Above- and belowground tissue, as well as the detritus collected weekly were dried (at 50°C to constant mass) and weighed separately. We measured recruitment (percentage new shoots), aboveground biomass (grams dry mass), number of leaves per shoot, leaf and rhizome elongation (square centimeters per shoot and centimeters per shoot, respectively), as well as

total belowground biomass (rhizomes and roots) and total detritus produced (grams dry mass).

We analyzed carbon, nitrogen, and total nonstructural carbohydrates in rhizomes from pooled (3–4 shoots) material of each mesocosm. Dry rhizomes were ground to a fine powder. Carbon and nitrogen content were analyzed using a Carlo-Erba elemental analyzer (Carlo-Erba Instruments, Milan, Italy). Carbohydrate reserves were measured using a modified method from Alcoverro et al. (1999). Sugars were solubilized from ground tissue by three sequential extractions in 95% (volume/volume) ethanol at 80°C for 15 min. Starch was extracted from the ethanol-insoluble remaining pellet by dissolving it in 0.1 mol/L NaOH for 24 h at room temperature. Sugar and starch contents of extracts were determined with a spectrophotometer using an anthrone assay with sucrose as standard. Sugar and starch content were combined as total nonstructural carbohydrate content (TNC).

In addition, we measured chemical and structural attributes of eelgrass leaves to determine whether variation in those traits related to herbivore preference detected between treatments. Carbon and nitrogen content were measured from pooled dried and ground leaf material as described previously. Total phenols were analyzed from pooled freeze-dried ground plant material (~4 mg) following a modified Folin-Ciocalteu method (see Bolser et al. 1998). Phenols were extracted with 2 mL of methanol 80% for 24 h, and concentrations were determined with a spectrophotometer using caffeic acid, gallic acid, and ferulic acid as standards (ferulic and caffeic acids are two of the most abundant phenolics in *Zostera marina*; Quackenbush et al. 1986, Vergeer and Develi 1997). The three standards gave similar results and only results using caffeic acid as a standard are reported. We measured plant mechanical properties on fresh material (plant segments of 7 cm in length) using an Instron model 3345 tensiometer (Norwood, Massachusetts, USA), which yields the stress (N/mm^2) required to break the plant tissue when subjected to longitudinal extension.

We analyzed the effect of genotype and nitrogen addition on plant traits using ANOVAs (two fixed crossed factors). For most variables we used mesocosms as replicates ($n = 6$), but for two traits related to herbivore preference (percentage phenols and leaf-breaking strength) we used pooled leaf material ($n = 3$ subsamples per treatment) or the individual leaves from different shoots ($n = 6$), respectively as replicates. Samples of one genotype (blue) from the nitrogen-enriched treatment were not available for phenol analysis. Therefore, variation in phenol content among all genotypes was assessed for unfertilized samples using a one-way ANOVA. A two-way ANOVA (factors genotype and nitrogen addition) was conducted to examine differences among nutrient treatments for the other three genotypes. For all ANOVAs (see also *Herbivore performance*), we checked data for normality and homogeneity of variances and we used Tukey's test

to examine differences among genotypes (Quinn and Keough 2002). When necessary, data transformations were carried out to meet parametric assumptions. In addition, we calculated the relative effect size of the experimental factors (magnitude of effects, ω^2 ; Graham and Edwards 2001) for each significant ANOVA term. Following Graham and Edwards (2001), negative estimates are considered negligible and were set to zero.

Herbivore feeding behavior

To examine the effects of genotype identity and nitrogen addition on herbivore feeding behavior we performed a series of feeding preference assays under controlled laboratory conditions in an indoor flow-through seawater system. For all experiments, isopods were acclimated to laboratory conditions for 24–48 h before starting each assay, during which they were fed freshly collected *Ulva* sp. We placed each isopod in an individual container (10 cm diameter \times 7.5 cm high) with holes for water exchange covered by a 2-mm mesh. Isopods were offered similar amounts of seagrass of multiple genotypes or treatments, each of which was blotted dry of excess water before measuring initial and final wet mass. All seagrass material used for the feeding trials was of similar age and cleaned of epiphytes. Each isopod container was paired with a no-herbivore control to assess changes in eelgrass mass due to factors other than herbivory. Assays were terminated when approximately half of any choice had been consumed. Biomass consumption was estimated as $([H_i \times C_f/C_i] - H_f)$, where H_i and H_f were initial and final wet masses of tissue exposed to herbivores, and C_i and C_f were initial and final masses in controls.

First we conducted four-way choice experiments examining isopod preferences among the four genotypes separately for nitrogen-enriched and unfertilized treatments. In addition, for each genotype, we performed a paired experiment in which we offered a choice between enriched and unfertilized plant material. We detected strong effects of genotype and nitrogen addition, the latter deterring herbivory (see *Results*). To further assess the relative importance of genotype vs. nitrogen addition in driving feeding patterns, we also conducted a two-choice experiment offering a highly preferred genotype subjected to nitrogen addition, which would decrease its palatability, and a low preference but unfertilized genotype.

To test whether structural differences among genotypes or nitrogen addition treatments determined isopod feeding preferences, we repeated preference assays using homogenized tissue that removed structural differences among genotypes, but left chemical and nutritional differences intact. To do this, we freeze-dried plant material and ground it to a fine powder (e.g., Vergés et al. 2007), which we later reconstituted by combining 1.2 g of dried eelgrass with a solution of 1 g of agar in 25 mL of distilled water. Eelgrass was added to the agar solution once it had cooled (but not solidified) to

prevent exposure of plant tissue to extreme heat. The mixture was poured onto a thin plastic 1.5-mm window screen mesh following Hay et al. (1998). The solidified mixture adhered to the mesh and was cut into identical agar rectangles, which were offered to a single isopod in a flow through container ($N = 20$ – 30 per experiment). The amount of each food consumed was quantified by counting the number of “squares” of the window screen that were cleared of food. Control foods not exposed to herbivores never changed in the number of squares covered and were not considered further in the analysis.

For statistical analyses we discarded replicates in which all food was consumed or where isopods failed to feed because they provided no information about feeding preferences. Differences in feeding from the multiple choice experiments with fresh material were analyzed following Prince et al. (2004), using Hotelling’s multivariate (T^2) test on the proportional consumption data corrected by autogenic changes. The multiple choice conducted with agar-based foods was analyzed using Hotelling’s test on the proportional consumption data as modified by Lockwood (1998). We used paired t tests or Wilcoxon signed-ranks paired test (when parametric assumptions were not met) to analyze the two-choice experiments. To assess the overall effect of nitrogen addition on isopod preference we conducted two meta-analyses (one for the fresh material experiment and another one for the agar-based food) where we quantitatively combined the results of the two-choice experiments (see details in Appendix B).

Herbivore performance

Having detected a strong effect of genotype identity on herbivore feeding preferences (see *Results*), we experimentally examined whether these preferences were correlated with isopod growth and survival. Individual isopods were randomly assigned to one of six treatments: an equal mix of all four genotypes (mixed diet), a monotypic diet composed of one of the four genotypes, and a starvation control ($N = 20$ per treatment). Each isopod was held in a 10 cm diameter \times 7.5 cm height mesh (2 mm) cage within a flow-through water table indoors to prevent diatom growth that could provide an alternative food source for isopods. The experiment ran for eight weeks, and we replaced plant material twice per week. We measured isopod length at the beginning (no difference among treatments; $F_{4,80} = 0.207$, $P = 0.934$) and at the end of the experimental period, or when isopods died. Differences in growth (millimeters per day) were analyzed with a one-way ANCOVA (with initial length as the covariate), and difference in survival at the end of the experiment was tested using contingency table analysis.

For this experiment we also measured consumption rates to elucidate whether poor performance was the result of reduced feeding or whether compensatory feeding was occurring. Total eelgrass consumption by isopods fed monotypic vs. mixed diets were compared

using a one-way ANOVA. Relative consumption of the different genotypes in the mixed diet treatment was analyzed using Hotelling's (T^2) test, as before.

RESULTS

Plant traits: production and biomass

Both plant genotype and eutrophication had strong independent effects on plant performance. Nitrogen addition decreased eelgrass performance reducing aboveground and belowground biomass, carbohydrate storage in rhizomes, and leaf growth rate (Appendix A; Fig. 1A, E, H, B), while increasing nitrogen content of rhizomes (Fig. 1G). Independent of nitrogen addition, genotypes differed in aboveground and belowground biomass, number of leaves per shoot, detritus production, recruitment of new shoots, rhizome growth, and rhizome carbon content (Appendix A; Fig. 1A, E, C, D, F). One genotype (red) tended to have a lower frequency of reproductive shoots (~6% of all shoots) than the others (between ~12% and 18%), although differences were not statistically significant (Appendix A). There were virtually no significant interactions between genotype identity and nutrient treatment, and the magnitude of the effect of nitrogen addition and genotype was generally similar (Appendix A). For tests in which both effects could be assessed, genotype effects were significant in 11 out of 17 cases with an average effect size (ω^2) of 0.25, whereas nitrogen addition effects were significant in 10 out of 17 cases with an average effect size of 0.26.

Herbivore feeding behavior

Herbivores exhibited a strong and consistent preference among genotypes, regardless of nitrogen treatment (blue and green more consumed than red and yellow; Fig. 2A, B). When offered a paired choice between nitrogen-enriched or unfertilized eelgrass of the same genotype, isopods tended to prefer unfertilized material (Fig. 3A; Appendix B), despite the fact that leaf material from the nitrogen-enriched treatment had relatively higher nitrogen content, lower C:N ratios, and lower phenols (Appendix A; Fig. 2D, E). Isopod feeding averaged across all paired-choice assays (Fig. 3A) produced a qualitatively similar preference hierarchy among genotypes (Fig. 2A, B), with blue and green genotype being highly consumed (18 ± 2 mg wet mass and 11 ± 2 mg wet mass, of unfertilized eelgrass consumed per isopod in ~48 h, respectively) in comparison to red and yellow (4 ± 2 mg wet mass and 5 ± 1 mg wet mass, respectively).

Both structural and chemical components of eelgrass influenced the feeding choices of isopods, with among-genotype preferences associated with chemical differences (compare Fig. 2A, C) and preferences for unfertilized tissue associated with structural differences (compare Fig. 3A, B). The preference toward unfertilized plant material detected in the two-choice assays (Fig. 3A; Appendix B) was reduced or absent when herbivores

were offered those same tissues ground and reconstituted in agar (i.e., without genotype-specific physical structure; Fig. 3B; Appendix B). Correspondingly, some genotypes from the nitrogen-enriched treatment tended to have increased breaking strength (Appendix A; Fig. 2F), suggesting that nitrogen addition may have increased tissue toughness for some genotypes.

Removing physical structure did not have a strong effect on relative preferences among unfertilized genotypes (Fig. 2A, C), and differences in breaking strength among genotypes did not correlate well with preferences (Fig. 2F). This result suggests that chemical aspects of the genotypes were mostly responsible for feeding preferences. Preferred genotypes (i.e., blue and green) generally had higher nutritional quality (higher percentage N, lower C:N), and lower concentrations of potential deterrents (phenolic compounds) (Appendix A; Fig. 2D, E) that may contribute to these preferences.

When offered a choice between a highly preferred (blue) genotype from the nitrogen-enriched treatment vs. a low preference (yellow) but unfertilized genotype, genotype identity was more important in determining herbivore preference than nitrogen addition, as illustrated by the strong preference for the blue genotype both when offering fresh tissue or agar-based extracts (lower panels in Fig. 3A, B).

Herbivore performance

Growth and survival of isopods was highest on the genotype consistently preferred in choice experiments (blue, Fig. 4A, B). Survivorship was high for isopods feeding on either the blue genotype (most preferred) or the mixed diet, whereas isopods feeding on red genotype (least preferred) had the lowest survivorship ($\chi^2_4 = 12.623$, $P = 0.013$; Fig. 4A). Starved isopods died sooner than those fed any of the genotypes, indicating that isopods could obtain some nutrition from any of the genotypes (Fig. 4A). In addition, isopods fed the blue genotype grew larger than those feeding on red genotype ($F_{4,89} = 2.730$, $P = 0.034$; no significant effect of initial length, $F_{1,89} = 3.080$, $P = 0.083$; Fig. 4B).

Isopods feeding on the blue genotype generally consumed greater amounts of eelgrass than those feeding on other diets (Fig. 4C). In the mixed diet treatment, blue and green were both highly consumed whereas red and yellow were much less consumed (Fig. 4D), as in previous assays (Fig. 2A).

DISCUSSION

We found that eelgrass genotypes varied in traits highly relevant to clonal and population growth as well as to community and ecosystem processes. Moreover, these effects were generally similar in magnitude to, or greater than, those of nitrogen loading, which is broadly appreciated to have strong detrimental effects on seagrasses (see reviews in Moore and Short 2007 and Ralph et al. 2007).

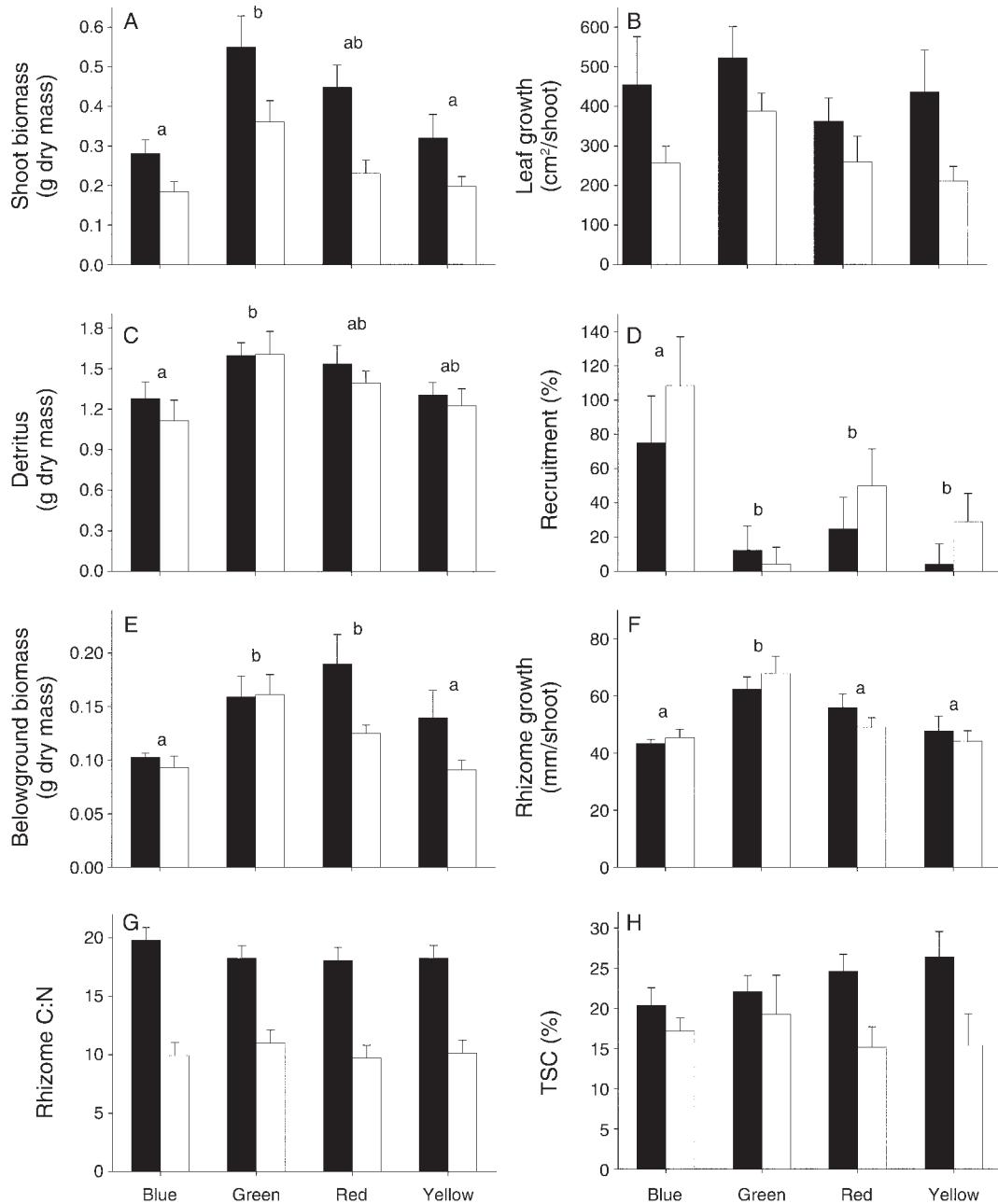


FIG. 1. Above- and belowground plant traits of eelgrass genotypes from unfertilized (black) and nitrogen-enriched (white) mesocosms at the end of the experiment. TSC refers to total nonstructural carbohydrates in rhizomes. Data are means + SE. Significantly different genotypes are labeled with different lowercase letters following Tukey's test ($\alpha = 0.05$). Color names represent the different genotypes (see *Materials and methods*).

Genetic diversity can be critical for eelgrass recovery after disturbance (Williams 2001, Hughes and Stachowicz 2004, 2011, Reusch et al. 2005) and thus ecosystem resilience. Many of the genotypic differences we observed may help explain the resiliency paradigm. For example, in clonal plants, rhizomes and roots provide an essential reservoir of nutrients and carbohydrates to support growth and maintenance of other plant tissues (within and among clonal ramets) during

periods of reduced photosynthesis (Suzuki and Stuefer 1999, Romero et al. 2007). The prevalent differences in belowground biomass among genotypes (Fig. 1E) could thus be particularly critical for storing reserves and maintaining the plant's carbon balance (and thus survival) under stress. In addition, given the importance of asexual spread to recovery and recolonization of seagrasses (Duarte et al. 2007), variation among genotypes in shoot recruitment could strongly influence

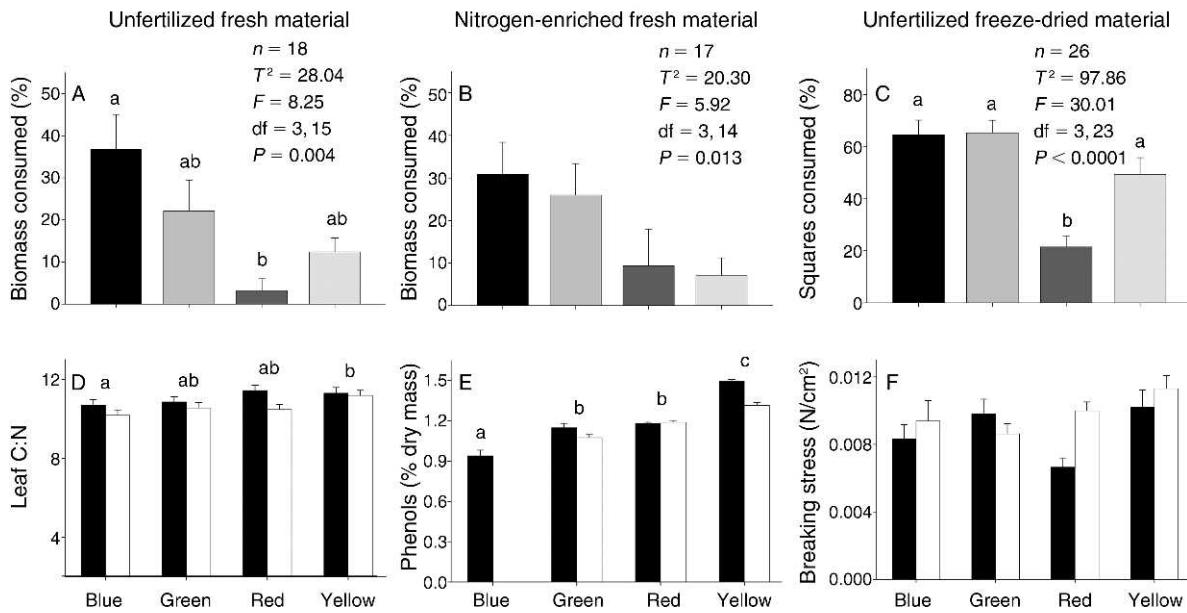


FIG. 2. Results of eelgrass consumption (%) from the multiple choice experiments (top panels) performed with fresh material from the four genotypes (A, unfertilized; B, nitrogen-enriched treatment) and (C) using agar-based foods. Probability, critical F , and T^2 statistics from Hotelling's test are shown, and significant differences among genotypes are marked with lowercase letters. Leaf traits (lower panels) related to feeding preference of the different genotypes from unfertilized (black) and nitrogen-enriched (white) treatments. Significantly different genotypes are labeled with different lowercase letters following Tukey's test ($\alpha = 0.05$). Color names represent the different genotypes (see *Materials and methods*).

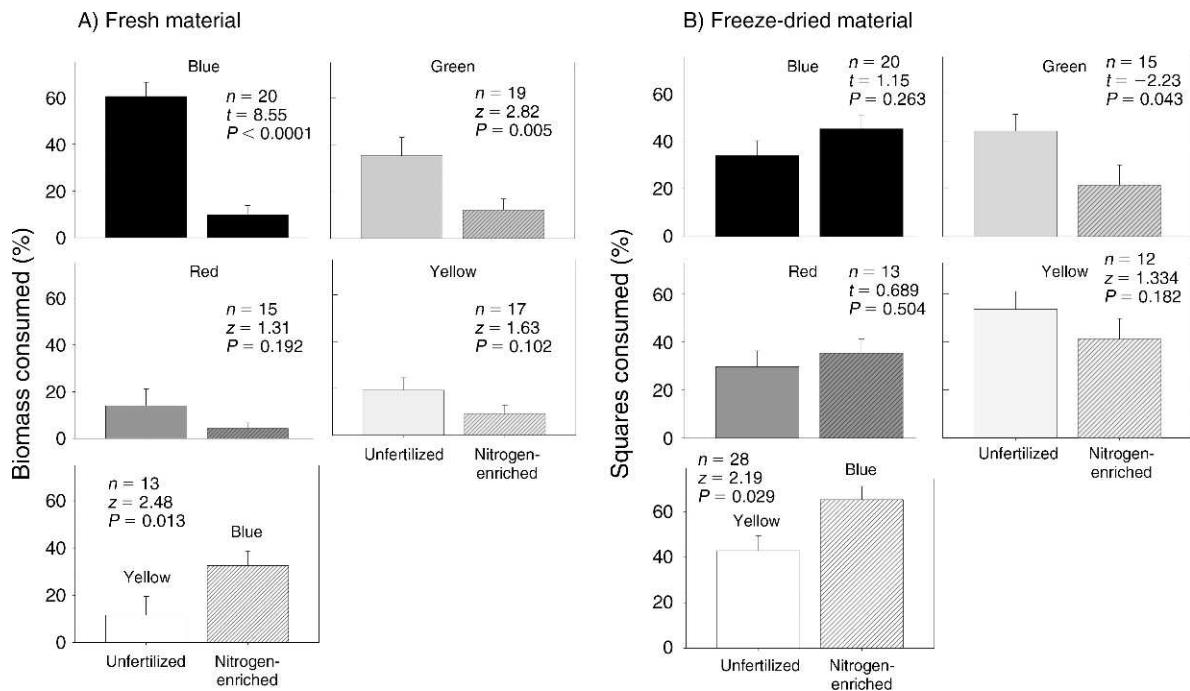


FIG. 3. Results of eelgrass consumption (%) from the two-choice feeding experiments between nitrogen-enriched and unfertilized genotypes using (A) fresh material and (B) agar matrix. Data are means + SE. Probability values and statistics were generated by t tests and Wilcoxon signed-ranks paired tests. The color names represent the different genotypes (see *Materials and Methods*). Panels with only one color name mean that both columns belong to the same genotype.

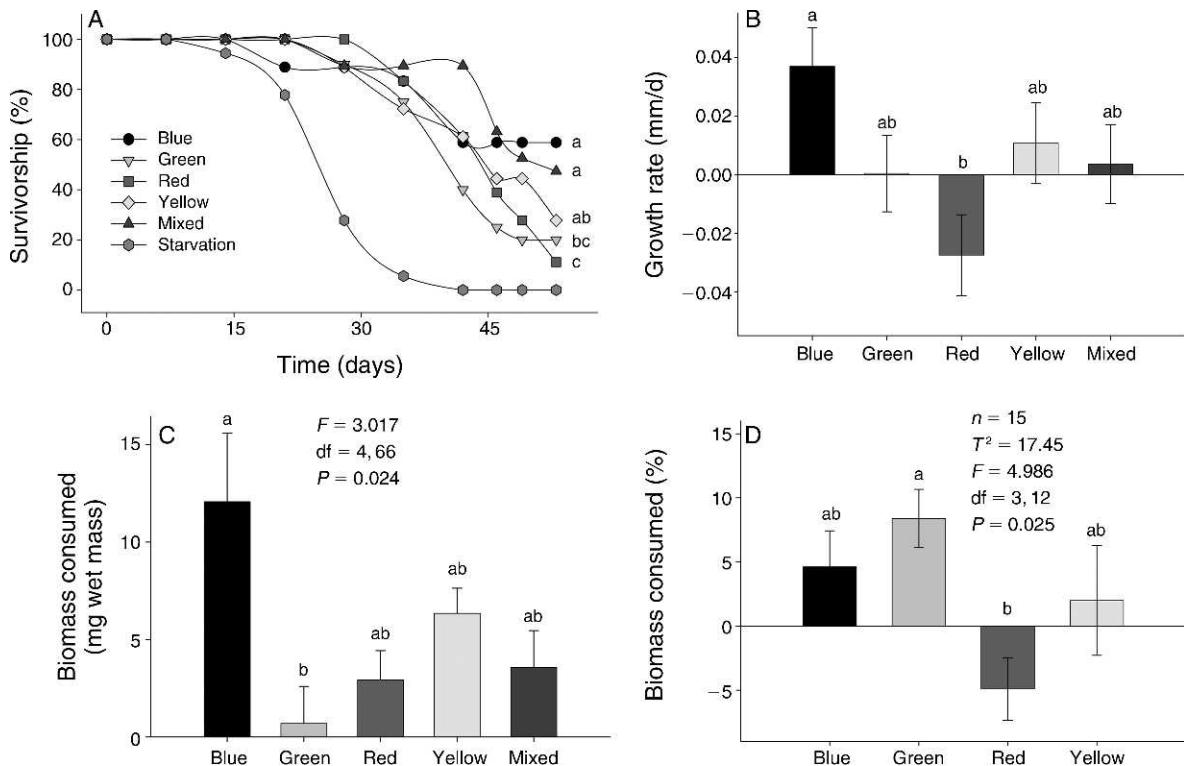


FIG. 4. Performance (A, survivorship; B, growth) and elgrass consumption patterns (C, total biomass; D, relative biomass within the mixed diet) of isopods fed different single-genotype or mixed diets. Significantly different groupings in survival (indicated with lowercase letters) were obtained from χ^2 tests. Results of (C) one-way ANOVA and (D) Hotelling's test are shown for elgrass consumption, with lowercase letters representing significantly different groups. Color names represent the different genotypes (see *Materials and methods*).

recovery rates. Our data also provide some evidence for intraspecific trade-offs; the genotype that produced the largest shoots (green) also produced the fewest, whereas the genotype that produced the largest number of shoots (blue) produced smaller shoots. Also these highly productive genotypes were preferred by herbivores. We examined an insufficient number of genotypes to rigorously test for such trade-offs but, if confirmed, they might form the basis of complementarity observed in other experiments manipulating seagrass genetic diversity (Hughes and Stachowicz 2004, 2011, Reusch et al. 2005, Hughes et al. 2010).

Plant genotype effects can depend on environmental context (with significant genotype \times environment interactions; e.g., Quiring and Butterworth 1994, Stiling and Rossi 1996), and the relative importance of environment vs. genotype may differ among ecological processes (e.g., Johnson and Agrawal 2005, Fridley et al. 2007, Crutsinger et al. 2008a, Johnson et al. 2008). However, we found surprisingly few genotype \times nutrient treatment interactions, despite strong effects of both nitrogen addition and genotype identity on plant and herbivore responses (see also Kotowska et al. 2010). In fact, regardless of negative effects of an environmental stress (eutrophication) on elgrass performance (e.g., reducing shoot biomass and growth as well as carbohy-

drate reserves; Fig. 1; Ralph et al. 2007), ecological differences among genotypes remained important for numerous plant traits affecting population, community, and ecosystem processes. However, because these genotypes differ in their rates of nitrogen incorporation (Hughes et al. 2009), lower (i.e., non-stressful) levels of nitrogen addition may have triggered genotype-specific responses (and thus an interactive effect of environment and genotype).

We focused much of our study on the effects of plant genotype on herbivory because this link had not been previously tested and because of its potential to affect trophic transfer and the nursery and refuge function of seagrass beds (Williams and Heck 2001). Genotypic effects on isopod feeding preferences were generally stronger than the effects of nutrients (Figs. 2A–C and 3) and survival and growth of herbivores was greatest on the preferred genotype (Fig. 4A, B). Thus, we provide evidence that the genotypic composition of an elgrass bed may strongly influence its value to associated animals, providing a potential mechanism for the effects of genotypic composition and diversity on animal abundance and diversity (Hughes and Stachowicz 2004, Reusch et al. 2005).

Our experiments indicate that herbivore preferences among elgrass genotypes are mainly driven by intra-

specific chemical differences. For example, the two most preferred genotypes (blue and green) had higher nutritional quality and lower phenol content than the least preferred genotypes. However, these correlations were not perfect (yellow had highest phenolic content, yet it was consumed more than red), suggesting that isopod preference and performance are likely determined by a combination of these and perhaps other traits. In fact, differential insect herbivory among terrestrial plant genotypes often does not correlate with a single plant trait, but rather with different combinations of plant characteristics (e.g., Fritz and Price 1988, Stiling and Rossi 1996, Larson and Whitham 1997, Karley et al. 2008).

Both low plant nutritional quality and phenolic compounds appear to reduce grazing on seagrasses (see review by Valentine and Duffy 2007, and Buchsbaum et al. 1984, Goecker et al. 2005, Vergés et al. 2007). The phenol content we measured in our genotypes is within the range reported in other studies of *Zostera marina* (0.4–3.5% dry mass; Buchsbaum et al. 1984, 1990, Harrison and Durance 1989) and within the range that modifies fish feeding on other seagrasses (*Thalassia testudinum*, 0.3–1% dry mass; Goecker et al. 2005). In addition, eelgrass genotypes could differ in the relative amounts of different chemical deterrents produced (e.g., Dungey et al. 2000), which could affect herbivore preference and performance (e.g., Poelman et al. 2009). Finally, other aspects of leaf nutritional quality (e.g., sugars, proteins) that we did not measure can affect preference and performance of crustacean grazers (e.g., Pavia and Toth 2000, Hemmi and Jormalainen 2002, Cruz-Rivera and Hay 2003), and may contribute to observed differences in herbivore responses to different eelgrass genotypes.

Eelgrass genotypes also varied in detritus production. Seagrass ecosystems sustain important detritus-based food webs (Fenchel 1977) and are also a major global carbon sink through the accumulation of a refractory detrital pool (Duarte and Cebrian 1996, Mateo et al. 2007). Genotype identity strongly influences riparian and terrestrial litter-based arthropod communities associated with clonal plants (e.g., LeRoy et al. 2006, Barbour et al. 2009) and, ultimately, decomposition rates and nutrient remineralization (e.g., Schweitzer et al. 2004, Crutsinger et al. 2009) by determining the quantity and quality of litter produced. We found strong differences in detritus production among genotypes in *Zostera marina*, with the green genotype producing the most of a high quality (low C:N and low phenol) material, which could have large effects on the ecosystems dependent on eelgrass detritus. Furthermore, considering that some seagrass clones can have significant longevity and extend over large areas (Reusch et al. 1999; S. Arnaud-Haond, *personal communication*), the characteristics of single genotypes could have further large-scale consequences, including for eelgrass restoration (Williams 2001).

Overall, this work highlights that differences in key traits among genotypes of marine habitat-forming species can have important consequences for community and ecosystem processes by affecting crucial ecological properties and interactions with higher trophic levels. Such effects are strong enough that they are not modified or overwhelmed by the addition of a known stressor such as high nitrogen loading, and need to be accounted for to fully understand the functioning of ecosystems based on foundation species and when considering management strategies.

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APPENDIX A

A table showing the results of the two-way ANOVAs examining differences in above- and belowground eelgrass parameters of different eelgrass genotypes submitted to nitrogen addition (*Ecological Archives* E092-152-A1).

APPENDIX B

A table showing the results of the meta-analyses conducted to assess the effects of nitrogen addition on isopod feeding preference in experiments with fresh material and agar-based foods (*Ecological Archives* E092-152-A2).