

Herbivores, tidal elevation, and species richness simultaneously mediate nitrate uptake by seaweed assemblages

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Abstract. In order for research into the consequences of biodiversity changes to be more applicable to real-world ecosystems, experiments must be conducted in the field, where a variety of factors other than diversity can affect the rates of key biogeochemical and physiological processes. Here, we experimentally evaluate the effects of two factors known to affect the diversity and composition of intertidal seaweed assemblages—tidal elevation and herbivory—on nitrate uptake by those assemblages. Based on surveys of community composition at the end of a 1.5-year press experiment, we found that both tide height and herbivores affected seaweed community structure. Not surprisingly, seaweed species richness was greater at lower tidal elevations. Herbivores did not affect richness, but they altered the types of species that were present; seaweed species characterized by higher rates of nitrate uptake were more abundant in herbivore-removal plots. Both tide height and herbivores affected nitrate uptake by seaweed assemblages. Individual seaweed species, as well as entire seaweed assemblages, living higher on the shore had greater rates of biomass-specific nitrate uptake, particularly at high ambient nitrate concentrations. Grazed seaweed assemblages exhibited reduced nitrate uptake, but only at low nitrate concentrations. We evaluated the effect of seaweed richness on nitrate uptake, both alone and after accounting for effects of tidal elevation and herbivores. When only richness was considered, we found no effect on uptake. However, when simultaneous effects of richness, tide height, and herbivores on uptake were evaluated, we found that all three had relatively large and comparable effects on nitrate uptake coefficients and that there was a negative relationship between seaweed richness and nitrate uptake. Particularly because effects of richness on uptake were not apparent unless the effects of tide height and herbivory were also considered, these results highlight the importance of considering the effects of environmental context when evaluating the consequences of biodiversity change in more realistic systems.

Key words: consumers; context-dependency; diversity; nitrate uptake; seaweeds; tide height.

INTRODUCTION

A variety of factors, including physical stress, herbivores, and nutrient availability, determine the structure of producer assemblages (Menge and Sutherland 1987, Proulx and Mazumder 1998, Hillebrand et al. 2007). Historically, the many factors influencing community structure were evaluated independently (Agrawal et al. 2007), but more recent experiments have incorporated multiple factors, quantifying, for example, the interactive effects of physical disturbance, nutrients, and/or herbivores on producer communities (Nielsen 2001, Moon and Stiling 2004). We now appreciate that the diversity, composition, and biomass of producer assemblages are simultaneously influenced by a variety

of factors, including consumers (Hillebrand et al. 2007), nutrient availability (Hillebrand et al. 2007), and disturbance (Menge and Sutherland 1987). Here, we consider how the composition of a producer assemblage is altered by physical stress and consumers and evaluate how those consumer- and stress-mediated changes in community structure affect the ability of that assemblage to take up limiting nutrients, highlighting important feedbacks between multiple factors affecting producer communities.

Given ecologists' recent focus on the effects of biodiversity changes on biogeochemical processes such as nutrient uptake (Tilman 1999, Cardinale et al. 2006), surprisingly little attention has been simultaneously paid to consumers, physical stress, and nutrient availability, all of which have the potential to alter the rates of resource use by plant communities. For example, herbivores can both reduce nutrient availability to primary producers by consuming tissues or structures that are necessary for nutrient uptake (Brown 1994, Bracken and Stachowicz 2007) and increase availability by enhancing nutrient recycling (Williams and Carpen-

Manuscript received 9 July 2010; revised 15 November 2010; accepted 2 December 2010. Corresponding editor: P. T. Raimondi.

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ter 1988, McNaughton et al. 1997). Furthermore, herbivores, stress, and nutrients do not operate in isolation. Physical stress and nutrient availability can interact to determine bottom-up effects on plant quality and herbivory (Moon and Stiling 2004), and nutrient additions increase tissue nutrient levels in seaweeds, leading to more effective top-down control by grazers (Hauxwell et al. 1998). As ecologists make biodiversity manipulations more realistic by running experiments in the field (Stachowicz et al. 2008), it is necessary to take into account factors such as stress and consumers that also have the potential to affect biogeochemical processes, especially because nutrient availability (Fridley 2002, Boyer et al. 2009), consumers (Wojdak 2005, Bruno et al. 2008), and physical stress (Bulling et al. 2010) can alter the relationship between biodiversity and processes such as nutrient availability and primary production. We therefore also examine whether factors such as physical stress and consumers, by virtue of their effects on nutrient acquisition, can mask the effect of producer diversity on resource use.

We simultaneously evaluated the effects of consumers, physical stress, and seaweed biodiversity on intertidal seaweeds' acquisition of nitrogen, an essential growth-limiting nutrient (Howarth and Marino 2006). We conducted a 1.5-year press experiment where we manipulated herbivory at two tide heights (representing different intensities of physical stress) in a fully crossed experimental design and monitored the effects of our experimental treatments on algal community structure. At the conclusion of the experiment, we measured the nitrate uptake rate of the algal assemblage found in each plot to evaluate the effects of tide height and herbivory on nitrate uptake. Finally, we quantified the effect of seaweed species richness on uptake, both alone and after accounting for tide height and herbivory, to evaluate the relative importance of diversity and environmental context as mediators of nitrate uptake by seaweed assemblages.

MATERIALS AND METHODS

Field experiment and observations

Our field experiment began in early March 2007 and was maintained until the end of September 2008. Plots were established on wave-protected rocky intertidal reefs in the Bodega Marine Reserve, California, USA (38.32° N, 123.07° W). We cleared 20 circular plots, 0.5 m in diameter, of all algae and sessile invertebrates, but we were careful not to disturb the herbivores in the plots. Ten "high" plots were located at the upper edge of the *Pelvetiopsis limitata* (Setchell) N. L. Gardner zone (2.2 ± 0.5 m above mean lower-low water [MLLW]), and 10 "low" plots were at the lower edge of the *Pelvetiopsis* zone (1.7 ± 0.4 m above MLLW). The center-to-center vertical distance between the plots was only 0.5 m, but that translated to a substantial difference in the time that plots spent submerged, based on daytime temperatures (i.e., warmer when exposed, colder when submerged) in

the plots recorded every 5 minutes for 7 months using TidbiT dataloggers (Onset Computer Corporation, Pocasset, Massachusetts, USA). Low plots were submerged for an average of 4.2 h each day, whereas high plots were submerged for only 0.5 h each day.

All plots were surrounded by a 3 cm wide circular border of Z-Spar Splash Zone Compound (Carboline Co., St. Louis, Missouri, USA) painted with copper antifouling hull paint (Trinidad VOC, KOP-COAT, Inc., Rockaway, New Jersey, USA), which limited incursions by limpets and chitons (Cubit 1984, Aquilino et al. 2009). We chose to surround all plots with copper paint because it ensured that any toxic effects of the paint (e.g., on seaweed growth) would impact all plots identically (Johnson 1992). Limpets will still reluctantly cross a copper-paint border, and copper paint does not deter snails, so these borders simply assisted us in our efforts to limit herbivore access to herbivore-removal plots. It was therefore necessary to manually remove herbivores from herbivore-removal plots in order to maintain low abundances. We removed all herbivores from 10 randomly selected plots, five in each tide-height zone, every two weeks; herbivores were not manipulated in the other 10 plots. These removals resulted in an 89% reduction in herbivore abundances in removal plots relative to controls, based on low-tide plot censuses conducted every 3 months. On average, herbivore-removal plots contained 34.2 ± 15.0 (mean ± standard error) herbivores per m² at the end of the experiment, whereas control plots contained 305.4 ± 106.5 herbivores per m² (generalized linear model [log link, Poisson distribution], $\chi^2 = 1193.5$, $P < 0.001$; O'Hara and Kotze 2010), including snails, limpets, chitons, amphipods, isopods, and an occasional crab. Herbivores were more abundant lower on the shore ($\chi^2 = 108.5$, $P < 0.001$), which was largely driven by greater abundances of turban snails (*Tegula funebralis* A. Adams; $\chi^2 = 43.6$, $P < 0.001$) and limpets ($\chi^2 = 59.3$, $P < 0.001$) in low plots; littorine snail densities were greater in plots higher on the shore ($\chi^2 = 25.6$, $P < 0.001$). Littorines were the most common herbivores, followed by limpets (*Lottia* spp.) and *Tegula*. Abundances of littorines ($\chi^2 = 697.8$, $P < 0.001$), limpets ($\chi^2 = 75.1$, $P < 0.001$), and *Tegula* ($\chi^2 = 5.0$, $P = 0.025$) were all reduced in herbivore-removal treatments, whereas the rarer species (amphipods, isopods, chitons, and crabs) were not affected.

At the conclusion of the experiment, we censused all of the plots to determine abundances of invertebrates, cleared all macroalgal material, separated it by species, and dried it to constant mass at 60°C. We used these data on dry seaweed biomass (g/m²), seaweed richness (the number of species), and seaweed evenness (Pielou's *J*, the relative abundance of species) to evaluate effects of tide height and herbivory on seaweed biomass and diversity. Samples of representative species (*Pelvetiopsis*, *Mastocarpus papillatus* [C. Agardh] Kützing, and *Blidingia minima* [Nägeli ex Kützing] Kylin) were cleaned of all epiphytes, rinsed in de-ionized water,

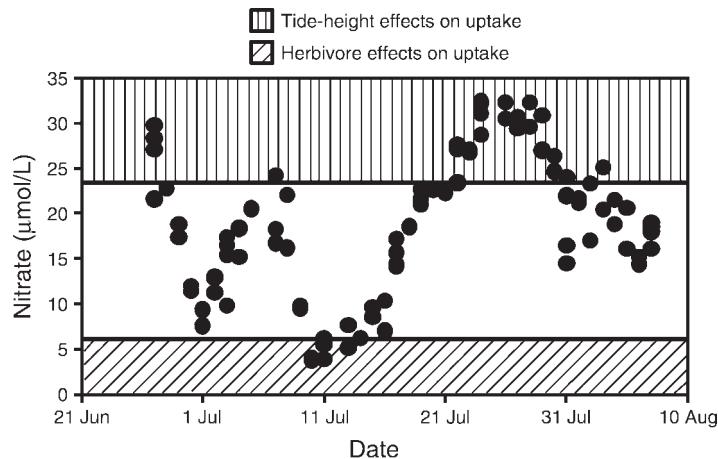


FIG. 1. Variation in water-column nitrate concentrations adjacent to our experimental plots. Data are nitrate concentrations ($\mu\text{mol/L}$) measured in water samples collected daily during the summer upwelling season of 2008. Tidal elevation (area in vertical stripes) affects nitrate uptake at concentrations $>23.0 \mu\text{mol/L}$, whereas herbivores (area in diagonal stripes) reduce nitrate uptake at concentrations $<6.5 \mu\text{mol/L}$.

dried at 60°C , ground to a fine powder, and analyzed to determine the percentage of nitrogen in the algal tissue (Carlo-Erba Flash EA 1112; CE Elantech, Lakewood, New Jersey, USA). We analyzed the percentage of N in tissue of three additional species, *Corallina vancoveriensis* Yendo, *Endocladia muricata* (Endlicher) J. Agardh, and *Ulva californica* Wille, during the summer of 2007. To quantify the variation in nitrate available to seaweeds in our experimental plots, we collected daily low-tide water samples in the Bodega Marine Reserve from 27 June 2008 through 7 August 2008, which was during the summer upwelling season when nitrate concentrations are high (mean \pm standard error: $19.2 \pm 0.7 \mu\text{mol/L}$) but variable, ranging from 3.8 to $32.4 \mu\text{mol/L}$ (Fig. 1). Nitrate concentrations in those samples were determined using a QuickChem FIA 8500 autoanalyzer (Lachat Instruments, Loveland, Colorado, USA).

Nitrate uptake by seaweed assemblages

To quantify the effects of tide height and herbivores on nitrate uptake by seaweed assemblages, we used data from our field experiment to create 20 unique laboratory assemblages that replicated the relative abundances of upright algal species found in each of the 20 plots in the field (Appendix A). All seaweeds from our field plots were dried to determine final biomass, so collections for uptake experiments were made in the vicinity of our plots but do not reflect the same history of herbivory, as seaweeds used in uptake experiments were exposed to ambient herbivore levels. Thus, our experiments evaluated the effects of herbivore-mediated changes in seaweed species composition on uptake, but did not account for potential effects of herbivores on uptake rates of individual seaweeds within a species (e.g., Bracken and Stachowicz 2007). We measured uptake in 16 2-L cylindrical chambers made from optically pure acrylic (see Plate. 1). The chambers were contained in a

chilled, re-circulating water jacket, also constructed of optically pure acrylic, which maintained chamber temperatures at $12.08 \pm 0.04^{\circ}\text{C}$. Each chamber was plumbed with a submersible pump (model LC-2-CPMD; March Manufacturing, Glenview, Illinois, USA), which generated mean water velocities of $18.1 \pm 3.1 \text{ cm/s}$ (Pollak-Reibenwein and Joeppen 2007) measured using an acoustic velocimeter (Vectrino, Nortek AS, Vangkroken, Norway). Mean turbulence intensities were $16.9\% \pm 1.5\%$ of mean velocity, and maximum velocities reached 30.0 cm/s within individual chambers. Water flow in the chambers was therefore similar to flow conditions we have measured at our field sites (M. Bracken and S. Williams, *unpublished data*). High-velocity ($>10 \text{ cm/s}$), turbulent flow conditions were necessary, as both nitrate uptake and photosynthesis rates of seaweeds are reduced at lower current speeds due to the formation of diffusion boundary layers (Hurd et al. 1996). At higher, more realistic current velocities, the relationship between nitrate concentration and uptake typically saturates at high concentrations and is usually fit using the Michaelis-Menten equation (Lobban and Harrison 1994).

The water jacket was surrounded by four 129-cm 54W T5 HO light fixtures (Model 1123; Current, Inc., Vista, California, USA) equipped with four SlimPac 10 000K daylight and four 460-nm actinic fluorescent bulbs. In addition, two similar 61-cm fixtures (Model 1120) were placed at each end of the water jacket. These fixtures provided photosynthetically active radiation ($1873 \pm 40 \mu\text{mol photons}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) within the chambers which was similar to that experienced by seaweeds in the field and sufficient to saturate photosynthesis (S. Williams and M. Bracken, *unpublished data*).

We measured biomass-specific uptake rates for each assemblage ($n = 20$, Appendix A) and for all component species present in any of the assemblages ($n = 18$ species,

TABLE 1. Nitrate uptake parameters of intertidal seaweeds at high and low tide heights, based on Michaelis-Menten fits to the relationship between initial NO_3^- concentration (K_s) and biomass-specific uptake (V_{\max}).

Species†	V_{\max} ($\mu\text{mol}\cdot\text{h}^{-1}\cdot\text{g}^{-1}$)		K_s ($\mu\text{mol/L}$)		V_{\max}/K_s	
	High	Low	High	Low	High	Low
Chlorophyta						
<i>Blidingia minima</i> ‡	65.9	14.7	114.8	25.6	0.57	0.58
<i>Cladophora columbiana</i> ‡		8.3		3.7		2.27
<i>Ulva californica</i> ‡	158.9	53.1	214.9	61.6	0.74	0.86
Rhodophyta						
<i>Callithamnion pikeanum</i> ‡		1.8		5.3		0.34
<i>Ceramium pacificum</i> ‡		10.9		18.7		0.58
<i>Corallina vancouveriensis</i>		0.2		10.6		0.02
<i>Cumagloia andersonii</i> ‡		23.4		34.5		0.67
<i>Endocladia muricata</i> ‡	7.6	4.3	23.3	9.4	0.33	0.45
<i>Gelidium coulteri</i>		1.1		35.1		0.03
<i>Mastocarpus papillatus</i> ‡	24.8	10.5	67.2	40.7	0.37	0.26
<i>Mazzaella flaccida</i>		10.8		49.0		0.22
<i>Microcladia borealis</i> ‡		5.9		9.6		0.62
<i>Odonthalia floccosa</i> ‡		11.8		24.7		0.48
<i>Plocamium violaceum</i> ‡		1.5		1.8		0.81
<i>Polysiphonia hendryi</i> ‡		2.5		4.4		0.56
<i>Porphyra perforata</i> ‡	275.2	10.4	574.0	13.6	0.48	0.76
Heterokontophyta						
<i>Pelvetiopsis limitata</i> ‡	13.1	4.5	55.4	14.4	0.24	0.32
<i>Silvetia compressa</i>		0.7		2.8		0.24

Note: Empty cells indicate species that were absent at that height.

† See <http://www.algaebase.org> for species authorities.

‡ High-uptake species ($V_{\max}/K_s > 0.25$). Note that *Pelvetiopsis* had $V_{\max}/K_s > 0.25$ only in low plots.

Table 1). Uptake measurements for each assemblage and species were conducted using individuals collected from the same tide height as the field plot. This meant that uptake rates of both “high” and “low” individuals were evaluated for several species. Each chamber contained 12 g (wet mass) of seaweeds, with biomass partitioned among component species to match their relative abundances in the field (Appendix A). Seaweeds were maintained in ambient flowing seawater overnight after collection, which ensured full hydration before uptake was evaluated. Because of the small size of many of the algal thalli, it was not practical to affix them to the substratum in the chambers, so thalli were allowed to move freely in the water column during uptake trials. Uptake experiments were conducted in artificial seawater (33 psu) prepared using Instant Ocean (Aquarium Systems, Mentor, Ohio, USA), spiked with a NaNO_3 stock solution. Initial nitrate concentrations in the chambers were approximately 2, 15, 30, and 50 $\mu\text{mol/L}$, with additional higher concentrations occasionally run for assemblages or species when uptake did not saturate at lower concentrations. For each species and assemblage, we ran two replicates at each initial nitrate concentration. After allowing 10 minutes for equilibration, we collected 6 mL of water from each chamber every 15 minutes for 2 hours and analyzed nitrate concentrations using a QuickChem FIA 8500 autoanalyzer (Lachat Instruments, Loveland, Colorado, USA).

Algae were dried to constant mass at 60°C to obtain the biomass of each species or assemblage.

We then calculated the biomass-specific uptake rate of every assemblage and species ($\mu\text{mol}\cdot\text{h}^{-1}\cdot\text{g}^{-1}$) as a function of the initial nitrate concentration ($\mu\text{mol/L}$). We used nonlinear curve-fitting techniques (PROC NLIN in SAS v. 9.2; SAS Institute 2008) to determine the parameters of the Michaelis-Menten relationship for each species and assemblage:

$$V = (V_{\max} \times S) / (K_s + S) \quad (1)$$

where V ($\mu\text{mol}\cdot\text{h}^{-1}\cdot\text{g}^{-1}$) was the uptake rate, V_{\max} ($\mu\text{mol}\cdot\text{h}^{-1}\cdot\text{g}^{-1}$) was a parameter that defined the maximum uptake rate of the species or assemblage, S ($\mu\text{mol/L}$) was the initial nitrate concentration, and K_s ($\mu\text{mol/L}$; substrate constant) was a parameter that defined the initial concentration at $V_{\max}/2$. Michaelis-Menten fits were verified by comparing them to linear fits using the corrected Akaike information criterion (AIC_c ; Burnham and Anderson 2002).

We derived a third parameter, the nitrate uptake coefficient V_{\max}/K_s (Bracken and Stachowicz 2007), which represents the slope over the initial non-saturating range of nitrate concentrations in the relationship between nitrate concentration and uptake. Greater V_{\max}/K_s values are therefore indicative of more effective uptake at low nitrate concentrations. We used this coefficient to classify seaweeds as high-uptake ($V_{\max}/K_s > 0.25$) or low-uptake ($V_{\max}/K_s < 0.25$) species (Table

1). This classification allowed us to determine whether herbivores were selectively removing species characterized by high nitrate uptake.

We used the parameters from the Michaelis-Menten relationship for each assemblage to predict uptake by that assemblage across the range of nitrate concentrations (from 3.8 to 32.3 $\mu\text{mol/L}$) we observed during the summer of 2008 on the northern California coast (Fig. 1). We substituted nitrate concentrations (S) at 0.5 $\mu\text{mol/L}$ intervals from 3.5 to 32.5 $\mu\text{mol/L}$ (i.e., 3.5, 4.0, 4.5, ..., 32.5 $\mu\text{mol/L}$) into the Michaelis-Menten equation for each of the 20 assemblages to calculate the uptake rate (V) across the range of nitrate concentrations we observed adjacent to our plots. Then, for each interval along the gradient, we used two-way ANOVAs evaluating nitrate uptake as a function of height, herbivores, and height \times herbivores to quantify the effects of herbivore removals and tide height on uptake. This allowed us to determine the range of concentrations over which tide height vs. herbivores affected uptake (Appendix B).

Seaweed diversity and nitrate uptake

We evaluated potential mechanisms underlying the observed negative relationship between seaweed species richness and the nitrate uptake coefficient (see *Results*) by calculating two metrics of the effects of diversity on uptake. We calculated D_{mean} as an indicator of non-transgressive overyielding:

$$D_{\text{mean}} = (P - M_{\text{mean}})/M_{\text{mean}} \quad (2)$$

where P was the uptake coefficient of a given polyculture and M_{mean} was the predicted uptake coefficient based on a weighted average of the component species' uptake coefficients when measured in monoculture (Power and Cardinale 2009). Similarly, we calculated D_{max} as an indicator of transgressive overyielding:

$$D_{\text{max}} = (P - M_{\text{max}})/M_{\text{max}} \quad (3)$$

where M_{max} was the uptake coefficient of the best-performing component species when measured in monoculture (Loreau 1998a). Note that $\ln(D_{\text{mean}} + 1) = \text{LR}_{\bar{m}}$, and $\ln(D_{\text{max}} + 1) = \text{LR}_{\bar{m}}$, as defined by Cardinale et al. (2006). Because D_{mean} declined with increasing species richness, we also calculated D_{mean} values for each level of richness (2, 3, ..., 13 species) by using the parameter estimates from a general linear model relating D_{mean} to richness, tide height, and herbivory (Table 2) and holding height, herbivory, and height \times herbivory values constant. We then compared each of these to a null hypothesis of $D_{\text{mean}} = 0$ using one-sample t tests with 15 degrees of freedom.

Statistical analyses

We analyzed most of our data using two-way analyses of variance (PROC GLM in SAS v. 9.2; SAS Institute 2008), with plots (or the assemblages from those plots) as experimental units. These experiments were designed

TABLE 2. General linear models describing the effects of species richness, herbivory, and tide height on nitrate uptake coefficients (V_{max}/K_s).

Source	df	F	P	ω^2
a) Richness alone				
Richness	1,18	0.20	0.659	-0.043
b) Richness, height, and herbivory				
Richness	1,15	6.34	0.024	0.178
Height	1,15	6.34	0.024	0.178
Herbivory	1,15	9.80	0.007	0.293
Height \times herbivory	1,15	0.14	0.715	-0.028

Note: Analyses are based on Type III sums of squares, so each term is corrected for all other terms in the model.

to evaluate the effects of tide height and herbivory, and our general linear models (GLMs) evaluated response variables, including species richness, evenness (Pielou's J), biomass (g/m^2), and parameters of the Michaelis-Menten relationship (V_{max} , K_s , and V_{max}/K_s), as a function of height, herbivory, and height \times herbivory. We also used GLMs to evaluate the relationship between seaweed species richness and nitrate uptake coefficients, both alone (i.e., uptake as a function of richness) and after accounting for tide height and herbivory (i.e., uptake as a function of richness, tide height, herbivory, and height \times herbivory). To compare the relative importance of richness, tide height, and herbivory as factors explaining nitrate uptake, we partitioned the variance explained by each factor, calculating the semipartial ω^2 statistic as an estimate of relative effect size (Graham and Edwards 2001). We used similar GLMs to evaluate how metrics of non-transgressive (D_{mean}) and transgressive (D_{max}) overyielding and the proportional biomass of high-uptake species changed with the species richness, both alone and after accounting for tide height and herbivory. All data were analyzed after verifying normal distributions and homogeneity of variances and transformed ($\ln[y + n]$, where $0 \leq n \leq 1$) as necessary to meet GLM assumptions. We used Type III sums of squares, so effects of each term were evaluated after accounting for all other terms. Tables showing details of all GLMs are available in Appendix C.

RESULTS

Field experiment

The number of seaweed species was greater in plots that were lower on the shore ($F_{1,16} = 14.72$, $P = 0.0015$; Fig. 2a). However, herbivores had no overall effect on seaweed richness ($F_{1,16} = 1.82$, $P = 0.1964$), and their effects did not differ with tide height ("height \times herbivory" interaction, $F_{1,16} = 0.27$, $P = 0.6112$). Where herbivores were present, seaweed evenness was greater in plots at lower tidal elevations (height \times herbivory interaction, $F_{1,16} = 5.02$, $P = 0.0396$; Fig. 2b).

Total seaweed biomass ($[\text{g dry tissue}]/\text{m}^2$) was greater in plots that were lower on the shore ($F_{1,16} = 33.36$, $P <$

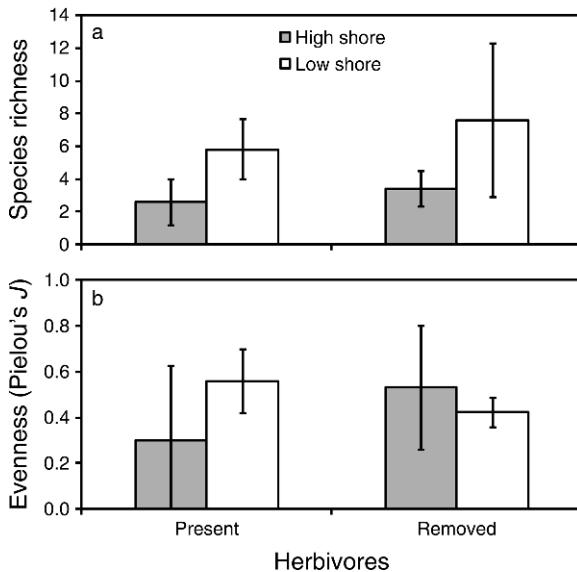


FIG. 2. Effects of tide height and herbivory on seaweed diversity. (a) Plots lower in the intertidal zone had higher seaweed richness, but there was no effect of herbivores on richness. (b) Evenness was higher lower on the shore when herbivores were present. Values are means \pm 95% confidence intervals.

0.0001), and herbivores reduced total seaweed biomass ($F_{1,16} = 4.56$, $P = 0.0485$; Fig. 3a). These effects of height and herbivores on total biomass were entirely driven by effects on the biomass of species with relatively high nitrate uptake rates (those with $V_{\max}/K_s > 0.25$; Table 1). Specifically, high-uptake biomass was greater at lower tidal elevations ($F_{1,16} = 54.51$, $P < 0.0001$) and in plots where herbivores were removed ($F_{1,16} = 4.56$, $P = 0.0485$; Fig. 3b), but neither tidal elevation ($F_{1,16} = 3.70$, $P = 0.0725$) nor herbivores ($F_{1,16} = 0.55$, $P = 0.4684$) affected the biomass of low uptake species (those with $V_{\max}/K_s < 0.25$; Fig. 3c). High variance in low-uptake biomass higher on the shore (Fig. 3c) was due to the presence or absence of *Pelvetiopsis*, which was patchily distributed at the upper edge of its tide-height range. To explore a potential mechanism for disproportionate effects of herbivores on high-uptake species, we evaluated the relationship between the uptake coefficients (V_{\max}/K_s , Table 1) and tissue nitrogen levels (percent N, %N) of several species at different tidal elevations and found that species with higher uptake coefficients were characterized by greater amounts of tissue nitrogen ($F_{1,7} = 22.35$, $P = 0.0021$, $R^2 = 0.76$). Tissue %N was unrelated to either V_{\max} or K_s ($P > 0.08$).

Nitrate uptake by seaweed species and assemblages

Because our measurements of nitrate uptake were carried out under realistic flow conditions, we found that per-gram nitrate uptake ($\mu\text{mol}\cdot\text{h}^{-1}\cdot\text{g}^{-1}$) was a saturating function of initial nitrate concentration ($\mu\text{mol/L}$; Fig. 4). Based on comparisons with linear fits

using the corrected Akaike information criterion (AIC_c), there was an 80% overall probability that the Michaelis-Menten equation was the best model, and it could never be excluded from the confidence set of candidate models (i.e., Akaike weights were never $< 10\%$ of those for a linear fit to the data). In contrast, linear fits were excluded from the confidence set of candidate models for assemblages from 10 of the 20 plots. However, whereas Michaelis-Menten fits were more appropriate overall, the proportion of cases in which linear fits were included in the possible set of candidate models was much higher for plots higher on the shore. For four assemblages, three of which were from high plots, there was a $> 50\%$ probability that a linear fit was the best model. Linear uptake kinetics are consistent with a higher demand for dissolved nitrogen, as indicated by the very high V_{\max} and K_s values we observed in assemblages from high plots (Fig. 5a, b).

We evaluated the effects of tide height and herbivores on parameters of the Michaelis-Menten equation: V_{\max} , K_s , and V_{\max}/K_s . Both average V_{\max} values ($\mu\text{mol}\cdot\text{h}^{-1}\cdot\text{g}^{-1}$; $F_{1,16} = 5.49$, $P = 0.0324$) and average K_s

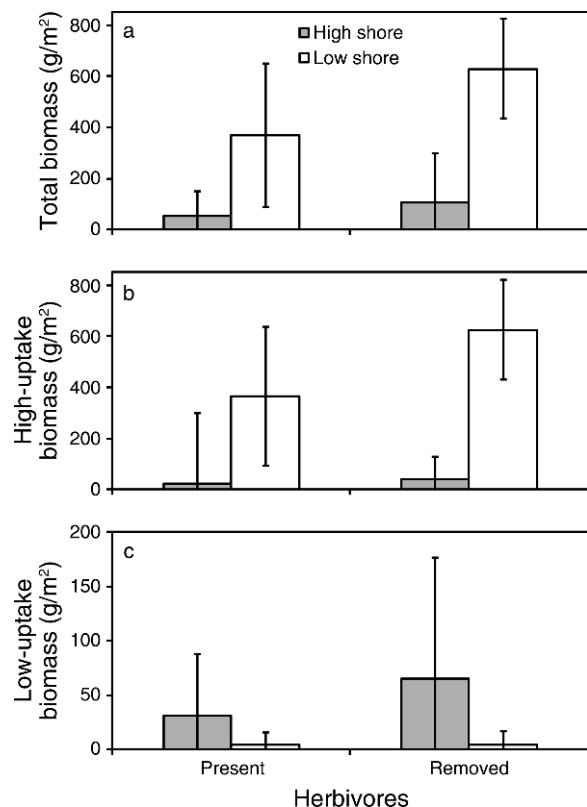


FIG. 3. Effects of tide height and herbivores on seaweed biomass. (a) Plots lower in the intertidal zone and subjected to experimental herbivore removals contained greater total dry seaweed biomass. (b) We found similar patterns with respect to tide height and herbivore effects on the biomass of high-uptake species. (c) In contrast, neither tide height nor herbivores affected biomass of low-uptake species. Values are means \pm 95% confidence intervals.

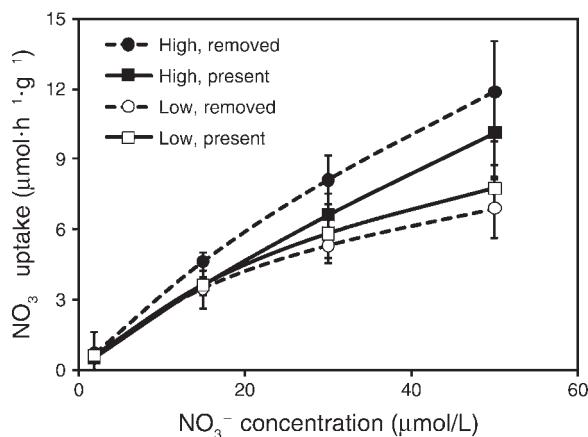


FIG. 4. Effects of herbivory (herbivores present or removed) and tide height (high or low) on nitrate uptake rates of seaweed assemblages. Data are relationships between initial nitrate concentration ($\mu\text{mol/L}$) and biomass-specific rates of nitrate uptake ($\mu\text{mol}\cdot\text{h}^{-1}\cdot\text{g}^{-1}$). Values are means \pm standard errors.

values ($\mu\text{mol/L}$; $F_{1,16} = 8.22$, $P = 0.0112$) were greater for assemblages higher on the shore, but neither V_{max} ($F_{1,16} = 0.84$, $P = 0.3722$; Fig. 5a) nor K_s ($F_{1,16} = 2.31$, $P = 0.1484$; Fig. 5b) differed between grazed and ungrazed assemblages. Greater V_{max} and K_s values are associated with an assemblage's ability to more effectively take up nitrate at relatively high nitrate concentrations, and we found that when nitrate concentrations were $\geq 23.0 \mu\text{mol/L}$ (i.e., high-nitrogen conditions associated with coastal upwelling), average per-gram uptake rates were greater for assemblages from higher tidal elevations ($F_{1,16} > 4.50$, $P < 0.05$; Fig. 1). When nitrate concentrations exceeded $23 \mu\text{mol/L}$, seaweed assemblages living higher on the shore were characterized by uptake rates 26–36% higher than those living lower on the shore (Appendix B).

Average uptake coefficients (V_{max}/K_s) were not affected by tidal elevation ($F_{1,16} = 0.86$, $P = 0.3663$) but were reduced in grazed assemblages ($F_{1,16} = 4.52$, $P = 0.0495$; Fig. 5c). When nitrate concentrations were $< 6.5 \mu\text{mol/L}$ (i.e., low-nitrogen conditions associated with downwelling), average uptake rates were greater in assemblages from herbivore-removal plots ($F_{1,16} > 4.50$, $P < 0.05$; Fig. 1). At low nitrate concentrations, from 3.5 to $6.5 \mu\text{mol/L}$, removal of herbivores resulted in a 17–20% increase in nitrate uptake (Appendix B).

Effects of seaweed species richness on uptake parameters

Richness did not affect V_{max} or K_s , either alone (i.e., without taking into account tide height and herbivory; $F_{1,18} \leq 2.50$, $P \geq 0.1311$) or after accounting for tide height and herbivory ($P \geq 0.52$), suggesting that uptake was unrelated to richness at high nitrate concentrations. We also found no relationship between richness alone and the uptake coefficient V_{max}/K_s ($F_{1,18} = 0.17$, $P = 0.6825$; Table 2a; Fig. 6a). However, after accounting for

the effects of tide height and herbivores, we found a negative correlation between seaweed richness and the uptake coefficient ($P = 0.0236$; Table 2b; Fig. 6b). Richness influenced uptake at low nitrate concentrations, but only when considered together with other factors that potentially affect uptake. We evaluated V_{max}/K_s as a function of richness, herbivores, tide height, and herbivores \times height to assess effect sizes (semipartial ω^2 statistics) associated with each factor and found that the magnitudes of richness ($\omega^2 = 0.18$), height ($\omega^2 = 0.18$), and herbivore ($\omega^2 = 0.29$) effects were all relatively high and reasonably comparable (Table 2b).

To evaluate potential mechanisms underlying this decline in uptake with increasing richness, we examined the relationship between richness and two metrics of diversity effects— D_{mean} , a measure of non-transgressive overyielding, and D_{max} , a measure of transgressive overyielding (Loreau 1998a)—after accounting for height, herbivores, and height \times herbivores. To examine

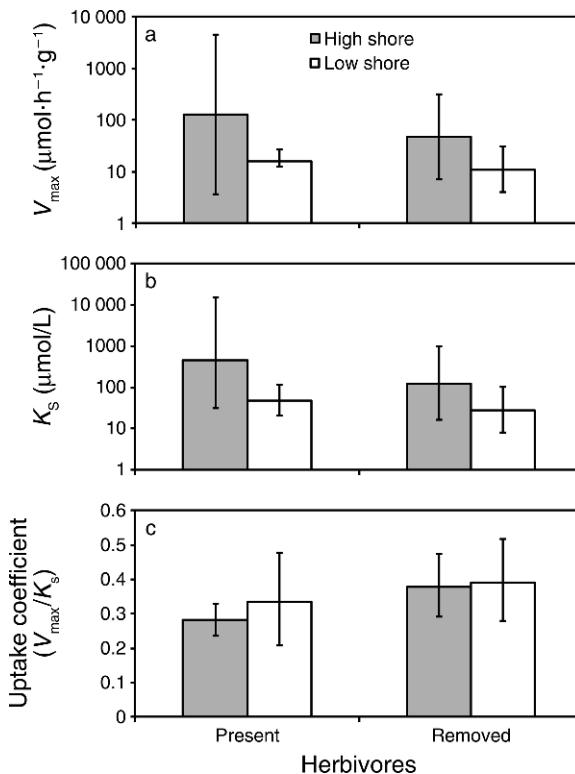


FIG. 5. Effects of herbivory and tide height on nitrate uptake parameters of seaweed assemblages. Data are parameters of Michaelis-Menten fits to relationships between initial nitrate concentration ($\mu\text{mol/L}$) and per-gram nitrate uptake ($\mu\text{mol}\cdot\text{h}^{-1}\cdot\text{g}^{-1}$). (a) Values for V_{max} were greater at higher tidal elevations, but herbivores had no effect on V_{max} . (b) Values for K_s were also greater for assemblages higher on the shore, and herbivores did not affect K_s . (c) In contrast, the nitrate uptake coefficient (V_{max}/K_s) was reduced by herbivores, but unaffected by tidal elevation. Values are back-transformed means \pm 95% confidence intervals of data that were analyzed after log-transformation.

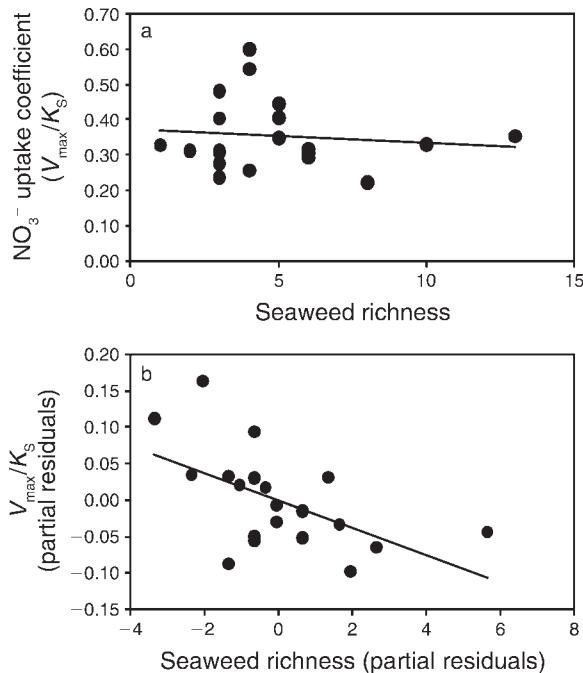


FIG. 6. Relationships between species richness and nitrate uptake in seaweed assemblages. (a) When the N uptake coefficient (V_{\max}/K_s) was evaluated as a function of richness alone, there was no relationship between richness and uptake. However, when richness effects on uptake were evaluated after accounting for effects of herbivory and tide height on uptake, richness declined with uptake. (b) To illustrate this, the residual uptake (i.e., after accounting for herbivory, tide height, and herbivory \times tide height) is plotted as a function of residual richness.

whether the decline in uptake was due to changes in the functional identity of species, we also examined the relationship between richness and the proportion of high-uptake species in an assemblage. After accounting for effects of herbivores and tide height, we found that neither D_{\max} ($F_{1,15} = 1.45$, $P = 0.2474$) nor the proportion of high-uptake species ($F_{1,15} = 1.14$, $P = 0.3031$) changed as richness increased. However, we found that richness ($F_{1,15} = 4.74$, $P = 0.0458$), herbivores ($F_{1,15} = 7.34$, $P = 0.0161$), and tide height ($F_{1,15} = 6.88$, $P = 0.0192$) all influenced D_{mean} . D_{mean} declined with increasing richness and, after accounting for the relationship between D_{mean} and richness, was greater in grazed assemblages and in plots lower on the shore. As richness increased, D_{mean} values were initially positive—for assemblages containing two species, $D_{\text{mean}} = 0.765$ ($t = 2.34$, $df = 15$, $P = 0.0337$)—but became indistinguishable from the null hypothesis of $D_{\text{mean}} = 0$ as richness increased.

DISCUSSION

By simultaneously considering the effects of tide height, herbivores, and biodiversity, we have shown that the uptake of nitrate by seaweed assemblages is context-dependent and that the effects of diversity on

nitrate uptake may only become apparent when other factors that affect uptake (e.g., tide height, herbivores) are accounted for. Our results highlight the fact that nitrate uptake can change over distances on the scale of meters and time-scales on the order of days. These changes, associated with tidal elevation, herbivory, and nitrate availability (Figs. 4 and 5), can occur at both the individual level and the level of the entire producer assemblage within a location. For example, for all six seaweed species present in both high and low plots, maximum nitrate uptake rates (V_{\max}) and half-saturation constants (K_s) were higher for seaweeds collected higher on the shore (Table 1).

These effects scaled up to the community level; V_{\max} and K_s values were higher for seaweed assemblages from high-shore plots (Fig. 5a, b). High and low plots were separated by an average (center-to-center) vertical distance of 0.5 m, making the differences in maximum uptake rates we observed even more striking (Fig. 5a). These differences were at least partly due to the fact that nitrate uptake rates of some high-zone assemblages did not saturate as nitrate concentrations increased (i.e., linear fits were somewhat better than Michaelis-Menten fits to the data), despite field-realistic water flows in our experimental chambers. Not only the parameters of the relationship between nitrate concentration and nitrate uptake, but also the shape of the relationship, appear to change with tidal elevation (Fig. 4). These differences are consistent with local acclimation or adaptation by seaweeds living higher on the shore to compensate for an 88% reduction in time submerged (Phillips and Hurd 2004, Hays 2007); seaweeds in our high plots actually live above the predicted mean higher-high water mark (1.7 m above MLLW) and therefore rely primarily on splashing by waves at high tide for nitrogen. However, the degree of compensation is not great. Based on measurements of submersion time (see *Methods*) and biomass-specific uptake (Fig. 4), we calculate that during upwelling (i.e., when $[\text{NO}_3^-] > 23 \mu\text{mol/L}$ and tide height significantly affects uptake), average daily nitrate uptake ($\mu\text{mol}\cdot\text{g}^{-1}\cdot\text{d}^{-1}$) is still 84% lower in high-shore assemblages because the difference in time submerged is so large.

Herbivore effects on uptake were also important (Table 2), though only at low nitrate concentrations. Herbivore-mediated changes in the seaweed community resulted in lower nitrate uptake coefficients (V_{\max}/K_s) but did not influence either the maximum uptake rates (V_{\max}) or the half-saturation constants (K_s) of algal assemblages. Thus, whereas tidal elevation affected uptake at high ambient nitrate concentrations (i.e., V_{\max} and K_s ; Figs. 1, 5a, b), herbivore effects were evident only at low nitrate concentrations (i.e., V_{\max}/K_s ; Figs. 1, 5c). The removal of herbivores was associated with disproportionate increases in the biomass (g/m^2) of high-uptake species (those with $V_{\max}/K_s > 0.25$; Fig. 3), suggesting that herbivores may be selectively removing species characterized by high uptake rates. Our data



PLATE 1. Experimental chambers used to measure nitrate uptake by seaweeds: (left) chambers arrayed in the chilled water jacket; (right) close-up view of a seaweed assemblage. Photo credits: E. Jones.

relating uptake coefficients to tissue nitrogen levels—species characterized by higher V_{\max}/K_s had higher tissue %N (see also Pedersen and Borum 1996)—provides a possible ecological-stoichiometric mechanism for this selectivity, as herbivores have been shown to preferentially consume high-nitrogen tissues or species (Kimmerer and Potter 1987), including marine macroalgae (Hauxwell et al. 1998, Bracken and Stachowicz 2007). The importance of uptake at low nitrate concentrations may seem counterintuitive in a nitrate-replete upwelling ecosystem. However, even during the spring and summer upwelling season, nitrate can be depleted to $< 6.5 \mu\text{mol/L}$ for five or more days during downwelling conditions (Fig. 1, Wilkerson et al. 2006), and the strong correlation we observed between V_{\max}/K_s and tissue %N suggests that uptake at low concentrations may influence N storage by these seaweeds.

We used two metrics, D_{mean} and D_{max} , to assess mechanisms underlying the negative relationship we observed between seaweed richness and nitrate uptake coefficients (V_{\max}/K_s , Fig. 6b). The decline in D_{mean} with increasing richness indicates that interspecific interactions shifted from complementary (i.e., non-transgressive overyielding) at low richness toward competitive (i.e., underyielding) at high richness. Importantly, the relationship between seaweed diversity and nitrate uptake only became apparent when we considered the richness and relative abundance patterns we found in the field. Our related experiments suggest that there is no

relationship between richness and uptake of either nitrate or ammonium for random assemblages of species, highlighting the need to consider realistic patterns of diversity change in order to understand the consequences of changing biodiversity in natural ecosystems (Bracken et al. 2008; M. Bracken and S. Williams, *unpublished data*).

Those same experiments also suggest that the relationship between seaweed species richness and nitrogen uptake may depend on the form of nitrogen being used and quantified. Bracken et al. (2008) found a positive relationship between richness and ammonium uptake, which contrasts with the negative relationship between richness and nitrate uptake we show here. We also found a negative relationship between richness and nitrate uptake in our related experimental manipulations of seaweed diversity (M. Bracken and S. Williams, *unpublished data*). Ammonium is regenerated locally by consumers in the intertidal community, whereas nitrate is an external subsidy to the system via coastal upwelling in the adjacent nearshore ocean (Bracken and Nielsen 2004, Aquilino et al. 2009). Terrestrial studies linking nutrient uptake and diversity have generally found positive relationships (Tilman 1999, Cardinale et al. 2006). The contrast between those terrestrial results and the negative relationship we found between seaweed richness and nitrate uptake may reflect the importance of local feedbacks between primary producers, consumers, and nutrient availability in many terrestrial systems

(Loreau 1998b) that may not occur in this case because a large fraction of the available nutrients (i.e., upwelled nitrate) comes from outside the local system.

Here, we manipulated herbivory across two tidal elevations to determine the effects of two known drivers of changes in intertidal community structure and found that both factors influenced nitrate uptake. Because of the masking effects of tide height and herbivory on nitrate uptake, we found no relationship between seaweed richness and uptake when richness was considered alone (Table 2a, Fig. 6a). However, when we evaluated richness together with tide height and herbivory, it became apparent that all three factors had relatively strong and comparable effects on uptake when ambient nitrate concentrations were low (Table 2b, Fig. 6b).

In order to understand the effects of biodiversity changes on communities and ecosystems, it is necessary to move beyond artificially assembled communities in laboratory, mesocosm, or greenhouse settings and evaluate relationships that more closely approximate those found in realistic, less-controlled field conditions (Bracken et al. 2008, Stachowicz et al. 2008). Not only do these experiments more effectively capture natural variability that is difficult or impossible to recreate in the lab (Stachowicz et al. 2008), but they also take into account, either explicitly or implicitly, other factors that potentially influence key physiological parameters such as nitrate uptake. However, in evaluating the results of more realistic experiments, a failure to account for these other factors may result in a failure to see a diversity effect where one is actually present.

ACKNOWLEDGMENTS

We thank A. Carranza, M. Cockrell, T. Hazen, A. Ramsower, R. Rockwood, C. Sorte, A. Szoboszlai, H. Weiskel, and R. Zerebecki for research assistance. J. Byrnes and two anonymous reviewers provided comments that greatly improved the manuscript. This research was supported by the National Science Foundation (grants OCE-0549944 to S. L. Williams and M. E. S. Bracken and DBI-0753226 to S. L. Williams and E. Sanford for the Bodega Marine Laboratory REU Program) and the Three Seas Program at Northeastern University. This is contribution number 276 of the Northeastern University Marine Science Center and a contribution of the Bodega Marine Laboratory, UC-Davis.

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APPENDIX A

A table showing richness and biomass (g wet tissue mass) of species in each experimental assemblage (*Ecological Archives* E092-087-A1).

APPENDIX B

A table showing how the effects of herbivores and tide height on nitrate uptake change across the range of observed nitrate concentrations (*Ecological Archives* E092-087-A2).

APPENDIX C

Tables showing general linear models of herbivore and tide height effects on community structure and nitrate uptake of seaweed assemblages (*Ecological Archives* E092-087-A3).