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Are large macroalgal blooms necessarily bad? nutrient impacts on seagrass in upwelling-influenced estuaries

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Abstract. Knowledge of nutrient pathways and their resulting ecological interactions can alleviate numerous environmental problems associated with nutrient increases in both natural and managed systems. Although not unique, coastal systems are particularly prone to complex ecological interactions resulting from nutrient inputs from both the land and sea. Nutrient inputs to coastal systems often spur ulvoid macroalgal blooms, with negative consequences for seagrasses, primarily through shading, as well as through changes in local biogeochemistry. We conducted complementary field and mesocosm experiments in an upwelling-influenced estuary, where marine-derived nutrients dominate, to understand the direct and indirect effects of nutrients on the macroalgal–eelgrass (*Zostera marina* L.) interaction. In the field experiment, we found weak evidence that nutrients and/or macroalgal treatments had a negative effect on eelgrass. However, in the mesocosm experiment, we found that a combination of nutrient and macroalgal treatments led to strongly negative eelgrass responses, primarily via indirect effects associated with macroalgal additions. Together, increased total light attenuation and decreased sediment oxygen levels were associated with larger effects on eelgrass than shading alone, which was evaluated using mimic algae treatments that did not alter sediment redox potential. Nutrient addition in the mesocosms directly affected seagrass density, biomass, and morphology, but not as strongly as macroalgae. We hypothesize that the contrary results from these parallel experiments are a consequence of differences in the hydrodynamics between field and mesocosm settings. We suggest that the high rates of water movement and tidal submersion of our intertidal field experiments alleviated the light reduction and negative biogeochemical changes in the sediment associated with macroalgal canopies, as well as the nutrient effects observed in the mesocosm experiments. Furthermore, adaptation of ulvoids and eelgrass to high, but variable, background nutrient concentrations in upwelling-influenced estuaries may partly explain the venue-specific results reported here. In order to manage critical seagrass habitats, nutrient criteria and macroalgal indicators must consider variability in marine-based nutrient delivery and local physical conditions among estuaries.

Key words: *eelgrass; estuary; eutrophication; macroalgae; nutrients; oceanic upwelling; Oregon, USA; species interactions;* Ulva *spp.;* Zostera marina.

Introduction

Anthropogenic nutrient inputs to the environment have had broad repercussions across ecosystems, including terrestrial, freshwater, and marine systems (Smith et al. 1999, Galloway et al. 2003, Shibata et al. 2014). These nutrients can be incorporated directly into communities via their uptake by producers. However, they can also operate through indirect means, including mediating competition between primary producers or altering food webs in a diversity of systems (e.g., Micheli 1999, Carpenter et al. 2001, Havens et al. 2001, Bowman et al. 2008, Bobbink et al. 2010, Deegan et al. 2012). Knowledge of key nutrient pathways and their resulting interactions can be used to mitigate and alleviate the numerous environmental problems associated with elevated nutrient inputs. However, such interactions can be complicated, nuanced, and context specific, supporting management recommendations derived from comparative and local studies (Shibata et al. 2014). Although not unique, coastal systems are particularly prone to complex ecological interactions resulting from nutrient inputs from land and sea, and the strong physical forcing at play at the nearshore margin.

Land-based nutrient inputs to estuarine systems can have multiple negative effects including hypoxia, die-offs of fish and invertebrate species, and harmful phytoplankton and macroalgal blooms (Bricker et al. 2008). These effects are a consequence of eutrophication, which can be broadly defined as "... the accelerated production of organic matter (sensu Nixon 1995, 2009), particularly algae, in a water body. It is usually caused by an increase in the amount of nutrients being

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discharged to the water body" (Bricker et al. 1999). Seagrasses have been particularly impacted by eutrophication both through the direct effects of nutrients and their indirect effects through macroalgal blooms (see Hemminga and Duarte 2000, Touchette and Burkholder 2000, Romero et al. 2006, Burkholder et al. 2007 for reviews). However, nutrient and macroalgal effects, and their influence on seagrasses, can be context specific and dependent on local and regional circumstances (Duarte 1995, Hessing-Lewis et al. 2011). Thus, an improved understanding of their relative roles, and mechanisms through which they operate, is necessary for eutrophication management across diverse coastal systems.

Studies have shown that nutrients can have both positive and negative direct effects on seagrasses. Most commonly, nutrient enrichment has positive effects on seagrasses, especially in nutrient-limited systems (Udy and Dennison 1997, Burkholder et al. 2007). However, some research also indicates that high nutrients can have negative effects on seagrass growth when elevated nitrogen uptake causes carbon limitation, resulting in structural weakness and ensuing direct lethal effects (Burkholder et al. 1992, 1994, Van Katwijk et al. 1997, Brun et al. 2002, Invers et al. 2004, Touchette and Burkholder 2007).

There are also indirect negative effects of nutrients on seagrasses through large algal blooms, including phytoplankton, epiphytes, and macroalgae (i.e., Duarte 1995, Short et al. 1995, Moore and Wetzel 2000, Havens et al. 2001, Nixon et al. 2001, Cardoso et al. 2004, Burkholder et al. 2007). In many temperate systems, macroalgae are often nutrient limited, and can respond quickly to increased nutrient concentrations (Duarte 1995, Valiela et al. 1997). Of primary concern are blooms of fast-growing macroalgae, such as the ulvoids (*Ulva* spp.), which have often been found to negatively affect seagrasses by forming canopies and shading, as well as changing the local biogeochemical environment (McGlathery 2001). Macroalgal blooms can be thick (e.g., 0.25 to >2 m in height in the water column; Sfriso et al. 1992, Hauxwell et al. 2001), of high biomass (e.g., 50–310 g dry mass/m^2; Pregnall and Rudy 1985, McGlathery 2001), and cause such low light levels that negative physiological consequences are common (e.g., Hauxwell et al. 2001). In addition, low oxygen concentrations caused by increased macroalgal respiration (during non-photosynthetic periods) and degradation of organic matter can increase the sulfide concentrations in the sediment and ammonium in the water column, which can also negatively affect seagrasses (Pregnall et al. 1984, Goodman et al. 1995, Krause-Jensen et al. 1996, van der Heide et al. 2008). Moreover, the structure of macroalgae itself can result in decreased water movement, which reduces advection of oxygen and nutrients within the water column, and alters nutrient cycling systemwide (McGlathery et al. 2007). Direct mechanical effects of macroalgae on seagrasses and their epiphytes can also occur as it drifts or accumulates in large aggregations (Irlandi et al. 2004).

Determining the direction, magnitude, and threshold of the direct and indirect effects of nutrients on the macroalgae–seagrass interaction is important to the management of this critical nearshore habitat, which provides important ecosystem services (Antón et al. 2011, Barbier et al. 2011). Mounting evidence suggests that seagrass responses to nutrient inputs are likely to show substantial variation depending on the source of nutrients and the physical environment of the estuary (Martinetto et al. 2011, Thomsen et al. 2012). For example, in contrast to what is known about the negative effects of eutrophication on the east coast of North America, some estuaries adjacent to upwelling zones, such as the California Current upwelling system along the west coast of North America, have shown different patterns, and may not be nutrient limited. In many of these systems, marine-derived nutrients seasonally dominate estuaries, as a result of nearshore oceanographic processes rather than terrestrial nutrient loading. Here, blooms of ulvoid macroalgae can be as large as those associated with seagrass declines elsewhere, but few negative effects on the dominant seagrass species, *Zostera marina* L. have been detected (Thom 1990, Kentula and DeWitt 2003, Brown et al. 2007, Jorgensen et al. 2010, Hessing-Lewis et al. 2011, Hessing-Lewis and Hacker 2013, but see Nelson and Lee 2001, Olyarnik and Stachowicz 2012). Further, in these systems, the effects of high nutrient conditions (driven by upwelling events) on eelgrass growth are unclear. In a multiscale, five-year study in Pacific estuaries along the Oregon and Washington, USA coast, Hessing-Lewis and Hacker (2013) documented that at a local scale (Coos Bay, Oregon), *Zostera marina* L. production was positively associated with increases in nitrogen as a result of upwelling. At a regional spatial scale, however, production evidently responded negatively. These scale-dependent trends and correlative studies from upwelling-influenced estuaries suggest alternative hypotheses regarding nutrient effects and the dominant pathways through which they operate. To illuminate the dominant interactions, yield comparative insight with other estuaries, and guide appropriate management of nutrient inputs to coastal systems, we tested these hypotheses experimentally.

We conducted two experiments that manipulated both nutrients and macroalgae in a factorial design: one in the field, the other in controlled tank mesocosms. The field research took place at the mouth of Coos Bay, where there are commonly large ulvoid macroalgal blooms interacting with the eelgrass, *Zostera marina*. The mesocosm research was conducted in outside mesocosm tanks at the Hatfield Marine Science Center in Newport, Oregon. Our goal was to explore four alternative scenarios that could govern eelgrass: (1) direct nutrient effects alone, (2) macroalgae effects alone, (3) the

interactive and additive effects of nutrients and macroalgae together, or (4) no effects of either nutrients or macroalgae. In both the field and laboratory, we (1) evaluated the potential direct effects of nutrients alone by augmenting the ambient nutrient concentrations in the local water column, (2) assessed the effects of macroalgae by manipulating the algal canopy, and (3) examined the potential for interactive effects by crossing macroalgae with nutrient manipulations. For both macrophytes, we focused on the response of common bioindicator metrics (biomass, density, morphology) over the duration of the experiments. In addition to determining the direction and magnitude of the primary nutrient pathways affecting eelgrass, we aimed to parse out key mechanisms responsible for interactions, specifically light limitation, hydrodynamics, and biogeochemical mechanisms. To isolate the effects of light from those of nutrient feedbacks resulting from macroalgal decomposition and re-mineralization, we employed mimic algae, which decreased the light but had no biogeochemical effects. The use of both experimental venues also allowed us to consider the role of hydrodynamics in upwelling-influenced estuaries, and to compare our findings to other estuaries with decreased tidal currents and increased nutrient retention.

Materials and Methods

Experiment location and design

The field experiment was conducted at Fossil Point (43°22′3″ N, 124°18′11″ W), near (~3.5 km) the mouth of Coos Bay, Oregon, along its primary shipping channel. The site was exposed to high water movement caused by tidal flux, waves entering the estuary from the exposed coast, and wakes from boat traffic. Tides in Coos Bay are mixed semidiurnal, with a mean tidal range of 2.3 m at the mouth, generating substantial tidal currents, with average flows of over 1 m/s throughout the estuary (Rumrill 2006). As a component of a corollary study (Hessing-Lewis et al. 2011), background dynamics of eelgrass and macroalgae biomass were also quantified at this site from June 2007 to April 2009 (Appendix A).

From June to September 2009, we manipulated macroalgal cover by adding or removing ulvoid macroalgae from large plots of eelgrass (2.4 m^2) located along the main channel at elevations of −0.1 to +0.1 m mean lower low water (MLLW). Plots were delineated with garden fencing (50 cm high, 5-cm mesh) anchored at the corners with PVC stakes and reinforced along the perimeter with bamboo rods. Our manipulations focused on the most dominant ulvoid macroalgae, *Ulva linza* L. and *Ulva lactuca* L. Macroalgal treatments also included addition of mimic macroalgae (described in *Macroalgal treatments*) and control plots open to ambient macroalgal conditions. Macroalgal treatments (ambient macroalgae [control], macroalgae added [addition], macroalgae removed [removal], and mimic macroalgae added [mimic]) were crossed with nutrient addition treatments (ambient, +nutrients) in a fully crossed design. Three replicate blocks were separated by 30–50 m. Within each replicate block, the eight treatments were randomly assigned to plots separated by 3–5 m, for a total of 24 plots. This spacing was sufficient to allow for independence of nutrient treatments in this highly diffusive environment.

The mesocosm experiment was conducted at Oregon State University's Hatfield Marine Science Center, Newport, Oregon, in 18 flow-through cylindrical tanks (80 cm tall, 90 cm diameter, 0.64 m^2 area) in an open area exposed to direct sunlight. Bay water was pumped from the adjacent Yaquina Bay at high tide and filtered (50 μm) before circulating through the tanks. Water entered all tanks from spigots near the top of the tanks, and exited via 65-cm tubular drains located at the center of each tank. Flow rates of ~5 L/min were controlled so that all tanks had turnover rates of ~17 times per day. Water levels in the mescocosm tanks were kept at a constant height, and water movement was low compared to the field experiment. Yaquina Bay is located 150 km north of Coos Bay and its nutrient conditions are also highly upwelling-influenced during summer months (Brown and Ozretich 2009). Macroalgal blooms in the marine zone of Yaquina Bay are similar in magnitude to those found in Coos Bay (Hessing-Lewis and Hacker 2013).

Collections of macroalgae and eelgrass for the mesocosm experiment were made in early summer 2009 in Yaquina Bay, at Idaho Point (44°37′1″ N, 124°1′43″ W, 4.5 km from the estuary mouth). Once collected, eelgrass shoots (including 5 cm of rhizome) were transplanted into plastic buckets (23 cm tall) containing sediment (10–15 cm deep) collected from the same site (infauna, primarily polychaetes, were not removed from the substrate). Seven shoots were transplanted into each bucket, and seven buckets were placed at the bottom of each mesocosm tank. Shoots were allowed to acclimate to tank conditions for one month from May to June 2009. During this time, dead or unhealthy shoots were replaced, so that initial shoot density and condition was similar across all tanks. Then, six treatments, comparable to field treatments (i.e., three macroalgal treatments; removal, addition, mimic, crossed with two nutrient treatments; ambient, +nutrients) were randomly applied in three replicate tanks ($N = 18$ total). Tank surfaces were scrubbed and eelgrass blades were cleaned manually every week in order to reduce epiphytic fouling (primarily diatoms). This allowed for manual mixing of the water column and macroalgae manipulations, beyond that caused by water flowing into the tank from the spigot. Fauna, such as juvenile crabs, snails, and amphipods were removed manually when possible.

Macroalgal treatments

To quantify macroalgae in our experiments, we used volume as a surrogate measurement for biomass in the

field (Robbins and Boese 2002). Based on a two-year data set relating macroalgal volume (mL/0.25 m^2) to biomass (g/0.25 m^2; all mass shown as dry mass) in Coos Bay ($N = 199$, $R^2 = 0.82$, $P < 0.001$), our measurements of volume were converted to dry mass as follows: log(macroalgal biomass) = 1.08 × log(macroalgal volume) − 3.49; see Hessing-Lewis and Hacker (2013). The same volume-to-biomass conversion equation was used in the field and mesocosm experiments.

In the field experiment, addition consisted of pulsed addition of macroalgae every month during low tide (160 000 mL [12.7 kg] in June, August, and September, and 140 000 mL [11.0 kg] in July), or five times the ambient macroalgal volume in non-addition treatments, which varied by month. Macroalgae was collected by hand in areas adjacent (~100 m) to the field experiment, where it had accumulated as drift or was loosely attached to the sediment. Biomass was measured volumetrically before application at low tide. At flood tide, macroalgal entrainment was actively encouraged by manually sinking floating macroalgae so that it remained negatively buoyant, and reduced drift outside of the plots. Macroalgae was manually removed from both removal and mimic treatments every month during low tide, and left untouched in control plots. Our response variable was macroalgal volume in permanently marked central quadrats (0.25 m^2) in each plot before monthly macroalgal additions.

In the mesocosm experiment, we initially added 8000 mL (0.5 kg) of macroalgae in July. Based on volume per substrate area, this was comparable to field plot additions of 60 000 mL (4.4 kg), which is about three times lower than field addition treatments. Smaller volumes were used in mesocosms compared to field plots because field estimates indicated that about two-thirds of the macroalgae were lost between monthly additions, and we wanted to maintain similar volumes of macroalgae in both experiments. At monthly intervals (August and September) we re-measured macroalgal volume per tank, and added fresh macroalgae to maintain addition treatment quantities at 6000 mL (0.4 kg) in August and 8000 mL (0.5 kg) in September. The mesocosm removal served as the control and approximated field removal conditions.

We used silicon-impregnated rip-stop nylon to mimic algae for both mesocosm and field mimic treatments. Sheets of green nylon (approximating the color of ulvoids) were cut into rectangles (40 cm wide × 75 cm long). Two sheets were overlaid so that their lengths were perpendicular, and secured around a central bundle of rocks using a cable tie to create a "unit." The rocks served as anchors for the positively buoyant mimic fronds. Mimics were added to both mesocosm tanks and field plots at equivalent densities, in order to ensure substrate coverage and imitate the physical structure of macroalgal canopies found within the addition treatments. To achieve this, 30 mimic units were added to each mimic tank, and 40 mimic units were added to each plot in the field experiment. In both experiments, mimic and addition macroalgae were intercalated between eelgrass shoots to minimize physical damage. In the mesocosm, macroalgae were also intercalated between shoots, and placed within and between buckets to ensure full coverage within the tank. In the field, mimic units were arranged throughout the plot, but more densely in the central area, where eelgrass dynamics were closely monitored. The "biomass" of nylon for the mimic canopies was approximately one-third that of the addition canopies, but their light reduction capabilities were higher. One sheet of nylon attenuated the equivalent amount of light as three average sheets of ulvoid macroalgae. Average light reduction was ~16%, or a difference of ~2240 between photosynthetic photon flux density (PPFD; μmol photon·m^{-2}·s^{-1}) measurements of incident light and those taken with the sensor completely obscured below the nylon. Organisms, such as worms, amphipods, anemones, and snails also recruited to the mimics in the field, and sediment accreted in their crevices, in a similar fashion to that observed for large sheets of ulvoids observed in the field. While the mimic units did fray, they did not decompose as macroalgae does.

Nutrient treatments

In the tanks, we used nutrient diffuser tubes containing Osmocote (Scotts, Marysville, Ohio, USA) slow-release fertilizer (mol/L N:P:K ratio of 19:6:12) to enrich the water in the mesocosms. Osmocote was suspended in thin mesh within small diffuser tubes (20 cm long, with 12 holes) suspended 20 cm above the tank bottom. Two 150-g tubes (300 g total per tank) were initially deployed in July 2009 (for 39 d), with a redeployment of fresh fertilizer in August (for 56 d; Table 1). This dosage (469 g/m^2) is comparable to that used in other nutrient addition experiments in subtidal seagrass beds (i.e., 500 g/m^2 at N:P of 19:1 [Antón et al. 2011), 1250 g/m^2 at N:P of 16:3 [Heck et al. 2006]). Because diffusion rates were likely much higher in the field experiments, we added about three times the nutrients (g Osmocote/m^2) in an effort to augment the nutrient concentrations in the field experiments. To disperse nutrients (per 2.4-m^2 plot), five nutrient diffuser tubes were arrayed in a horseshoe configuration around the central quadrat of each plot, allowing access for repeated measurements. Each tube (5.08 cm diameter) was 60 cm long; 30 cm above- and 30 cm belowground. Within each tube, 500 g of Osmocote (2500 g total per 2.4-m^2 plot, or 1042 g/m^2) was suspended in mesh bags from the top of the tubes, and nutrients dispersed into the water column via 20 2.4 cm diameter holes drilled into the aboveground segment of each tube. Control tubes of the same dimensions, with no holes or nutrients, were placed in the ambient treatments in order to control for the physical structure of the tubes. Nutrient treatments were first applied in July 2009, and after 45 d, the fertilizer was gathered and weighed to determine the

TABLE 1. Physicochemical differences between field and mesocosm experiments in Coos Bay and Yaquina Bay, Oregon, USA.

Parameter, by experiment and treatment	Parameter values
Osmocot dissolution rate	
Field	
7 Jul–20 Aug	38.2 ± 0.88 g/d
21 Aug–5 Oct	41.3 ± 0.72 g/d
Mesocosm	
16 Jul–24 Aug	3.0 ± 0.07 g/d
24 Aug–19 Oct	1.7 ± 0.14 g/d
Chalk block dissolution rate	
Field (2 Jun–20 Jun)	4.49 ± 0.19 g/d
Mesocosm (2 Jun–21 Jun)	0.41 ± 0.03 g/d
Summer month nitrogen (DIN) concentration (NO_3^- + NO_2^- + NH_4^+)	
Field, Jul (n = 3 per treatment)	
Ambient, 5 cm	0.17 ± 0.00 mg/L (12.18 ± 0.06 μmol/L)
+Nutrient, 5 cm	0.78 ± 0.23 mg/L (55.38 ± 16.26 μmol/L)
Field, Aug (n = 9 per treatment)	
Ambient, 0 cm	0.10 ± 0.01 mg/L (7.45 ± 0.42 μmol/L)
Ambient, 5 cm	0.10 ± 0.00 mg/L (7.08 ± 0.22 μmol/L)
Ambient, 20 cm	0.10 ± 0.01 mg/L (7.03 ± 0.98 μmol/L)
+Nutrient, 0 cm	10.37 ± 4.66 mg/L (740.14 ± 332.54 μmol/L)
+Nutrient, 5 cm	0.12 ± 0.01 mg/L (8.65 ± 0.94 μmol/L)
+Nutrient, 20 cm	0.11 ± 0.02 mg/L (7.87 ± 1.30 μmol/L)
Coos Bay data: May–Oct 2009	
South Slough monitoring program, high tide values	0.20 ± 0.03 mg/L (14.05 ± 1.87 μmol/L); range = 0.04–0.34 mg/L (3.21–24.34 μmol/L)
Mesocosm, Jul and Aug (n = 9 per treatment)	
Ambient, 5 cm	0.30 ± 0.01 mg/L (21.70 ± 0.77 μmol/L)
+Nutrient, 5 cm	0.42 ± 0.01 mg/L (29.93 ± 0.95 μmol/L)
Yaquina Bay data: May–Oct 2002 and 2003 (Brown and Ozretich 2009)	
NO_3^- + NO_2^-†	11.3 ± 8.8 μmol/L; range: 0.0–31.5 μmol/L
NH_4^+ + SD	3.6 ± 1.7 μmol/L; range: 0.4–9.0 μmol/L
Summer month phosphate (PO_4^{3-}) concentration	
Field, Jul means (n = 3 per treatment)	
Ambient, 5 cm	0.01 ± 0.00 mg/L (0.19 ± 0.00 μmol/L)
+Nutrient, 5 cm	0.01 ± 0.00 mg/L (0.32 ± 0.04 μmol/L)
Field, Aug means (n = 9 per treatment)	
Ambient, 0 cm	0.01 ± 0.00 mg/L (0.22 ± 0.02 μmol/L)
Ambient, 5 cm	0.01 ± 0.00 mg/L (0.21 ± 0.02 μmol/L)
Ambient, 20 cm	0.01 ± 0.00 mg/L (0.20 ± 0.02 μmol/L)
+Nutrient, 0 cm	0.10 ± 0.04 mg/L (3.25 ± 1.37 μmol/L)
+Nutrient, 5 cm	0.01 ± 0.01 mg/L (0.45 ± 0.03 μmol/L)
+Nutrient, 20 cm	0.01 ± 0.00 mg/L (0.42 ± 0.04 μmol/L)
Coos Bay data: May–Oct 2009	
South Slough monitoring program, high tide values	0.04 mg/L ± 0.00 (1.38 ± 0.12 μmol/L); range: 0.02–0.06 mg/L (0.56–2.01 μmol/L)
Mesocosm, Jul and Aug (n = 9 per treatment)	
Ambient, 5 cm	0.007 ± 0.00 mg/L (0.24 ± 0.01 μmol/L)
+Nutrient, 5 cm	0.008 ± 0.00 mg/L (0.27 ± 0.01 μmol/L)
Yaquina Bay data: May–Oct values 2002 and 2003 (Brown and Ozretich 2009)	1.4 ± 0.8 μmol/L; range: 0.0–2.9 μmol/L
Temperature	
Field	13.53° ± 0.02°C; range: 8.4–19.0°C
Mesocosm	12.23° ± 0.06°C; range: 11.95–12.73°C
Salinity	
Field	32.93 ± 0.01 ppt; range: 29.6–35.8 ppt
Mesocosm	33.21 ± 0.06 ppt; range: 33.15–33.37 ppt
Dissolved oxygen	
Field	8.47 ± 0.02 mg/L; range: 4.5–14.3 mg/L
Mesocosm	9.83 ± 0.10 mg/L; range: 8.80–10.72 mg/L
pH	
Field	8.25 ± 0.00; range: 7.5–8.8
Mesocosm	8.07 ± 0.02; range: 7.97–8.28

TABLE 1. Continued.

Notes: Mean values and standard errors are reported across all treatments and replicates, except where noted otherwise. Osmocote (Scotts, Marysville, Ohio, USA) and chalk block dissolution rates are measured as a change in dry mass. Mean monthly summer (May–October 2009) nutrient concentrations for the field were based on nutrient grab samples collected at high tide at the South Slough National Estuarine Research Reserve (SS NERR; Oregon, USA) systemwide monitoring protocol (SWMP) Boathouse site (SWMP protocol available from NOAA [2015]). The Boathouse site is located ~3 km from the Fossil Point (43°22′3″ N, 124°18′11″ W; near the mouth of Coos Bay) experiment site. Data are converted from μmol/L to mg/L as follows: DIN (NO_3^- + NO_2^- + NH_4^+) (μmol/L)/71.39 = DIN mg/L, and PO_4^{3-} (μmol/L)/32.9 = PO_4^{3-} mg/L. Field measurements of temperature, salinity, DO, and pH were collected using YSI datasondes (Yellow Springs Instruments Model 6600; Yellow Springs, Ohio) located in the water column at the Charleston Bridge site (located ~3.5 km from the Fossil Point experiment site). Data were collected continuously at 15-min intervals, and June–September means are reported. All flagged data not meeting SWMP quality check protocol were removed from this summary. Mesocosm measurement of temperature, salinity, dissolved oxygen (DO), and pH were from spot measurements in tanks.

† Error measure is ±SE.

amount dissolved. Fresh fertilizer (1500 g per plot) was added for another 45 d to replace the amount dissolved (Table 1).

We collected water samples to quantify the level of nutrient enrichment by the Osmocote additions. Samples were collected though Tygon tubing (Saint-Gobain, La Défense, France) attached to a 50-mL plastic syringe. All water samples were kept on ice in a cooler prior to filtration (Whatman DF/F filters; GE Life Sciences, Little Chalfont, UK) in the lab. All samples were frozen before analysis of dissolved inorganic nitrogen (DIN) and phosphate (PO_4^{3-}) by the University of Washington Marine Chemistry Lab (Seattle, Washington). In the field, we compared samples gathered as the tide flooded control plots with and without nutrient addition (+nutrient vs. ambient). Three samples were collected from one replicate block at a distance of 5 cm from the nutrient diffuser tubes in July following nutrient manipulations. In August, following the second application of nutrient treatments, we compared samples at three distances from the nutrient diffuser tubes (0, 5, 20 cm) on three dates from one replicate to quantify diffusion rate (Table 1). In the mesocosms, three water samples were also collected from each of three replicate control/ambient and control/+nutrient treatments. Tank samples were collected at 5 cm from the diffuser tubes in both July and August (Table 1).

Eelgrass responses

We measured shoot density (shoots/area), shoot length (first rootlet node to tip), and sheath length (a proxy for growth; Gaeckle et al. 2006) monthly in both experiments (June–October 2009). In the field, eelgrass measurements were focused on the central permanently marked quadrat (0.25/m^2) of the plot. Five density measurements (shoots/0.0625 m^2) were also taken haphazardly throughout the quadrat. Because initial densities, shoot lengths, and sheath lengths differed among plots, our response variable was percentage of change, i.e., ([final density − initial density]/initial density) × 100% for both field and tank experiments. Length of eelgrass shoots was haphazardly measured within a radius of 0.5 m around the nutrient diffuser tubes. Sheath length was measured in a subset of five shoots within the central quadrat, and 15 shoots haphazardly located throughout the plot. In the mesocosm experiment, change in density and morphometrics of all shoots per bucket were recorded and averaged by tank (buckets were nested within tanks). For shoot length and sheath length, we analyzed percentage of change between all individual measurement dates.

At the end of the experiment, total biomass (dry mass) was determined by collecting all shoots from the central quadrats of the plots and in the entire bucket, washing and scraping all epiphytes from eelgrass in the lab, and drying at 60°C for 24 h. In the mesocosms, we also measured (1) clippings obtained from trimming eelgrass to the top of the water line (trimmed biomass), and (2) material that had sloughed from the shoots (sloughed biomass), both of which were collected on a weekly basis to prevent self-shading.

Physical parameters

Light measurements of PPFD were measured with a LI-193 Spherical Quantum Sensor (LI-COR, Lincoln, Nebraska, USA). In the field, we measured light levels on five days from 18 to 22 August and three days from 17 to 19 September as the tide ebbed or flooded within the plots (deployments ranged from 3 h 35 min to 1 h 20 min). Sampling events spanned the timeframe just before or after the field sites were exposed and accessible by the tides, and included a range of tidal heights and times of day (all daylight hours). Sensors were vertically oriented and attached to a stake placed in the substrate within the plot at 20 cm above the substrate. On each event, we recorded light at 5-min intervals for 30 s in three plots simultaneously, using a LI-1400 three-channel datalogger (LI-COR). Light was measured in all replicate blocks, and in ambient nutrient treatments in all macroalgal treatments. In the mesocosms, light was measured at 3-min intervals over 30 s at two depths (5 cm and 30 cm from surface) on 14 occasions from July through October. Percentage of surface irradiance in the mesocosms was calculated as mean PPFD readings at 30 cm depth/mean PPFD readings at 5 cm below the water line (surface), while total attenuation was the difference between surface

and depth readings (measured in μmol photon·m^{-2}·s^{-1}). Light measurements were recorded at daylight hours at different times on different dates to capture among-treatment differences across a range of ambient light conditions, and incorporate the effect of tank shading at different hours of the day.

We measured redox potential (field, $N = 4$; mesocosm, $N = 5$; see Appendix B for measurement dates) at the sediment surface (within the top 5 cm) as a measure of sediment oxidation and biogeochemical activity in both the field plots and the mesocosm buckets using an Orion Star probe (Thermo Electron, Waltham, Massachusetts, USA) fitted with a platinum electrode. We measured temperature, salinity, dissolved oxygen, and pH on 13 dates (see Appendix B) at a depth of 30 cm in the water column of the mesocosm tanks using a HI 9828 multiparameter water quality portable meter (Hanna Instruments, Woonsocket, Rhode Island, USA) equipped with a pH/ORP sensor.

We used chalk blocks (die-keen dental chalk; Heraeus Kulzer, South Bend, Indiana, USA) to obtain an integrative and relative measure of water movement, wave action, and sediment scouring in field and tank environments (i.e., Porter et al. 2000, Dudas et al. 2009). In June 2009, pre-weighed blocks were screwed into short PVC stakes, which were then placed at the same height with reference to the sediment in both field plots and mesocosm buckets. Dissolution rates were estimated as dry mass loss per day throughout deployments (18 d in field, 19 d in mesocosms).

Statistical analyses

To assess effects of macroalgal and nutrient treatments on eelgrass, and other measured physical parameters, we used a model selection approach based on the Akaike information criterion (AIC). Mixed-effects models were formulated to determine which model best explained the observed data for each response metric (Bolker et al. 2009) using the nlme package in R (Pinheiro et al. 2009, R Development Core Team 2009). We first determined the random effects structure for the full model using restricted maximum likelihood estimation (REML), then we determined the best-fit model for different candidate models differing in fixed effects using maximum likelihood (ML) estimation (Zuur et al. 2009). Model structure for the random effects was based on the hierarchical design of the experiment and considered both random intercept and slope models based on AIC minimization. Random effects were individual buckets nested within each replicate tank for the mesocosm experiment or replicate blocks for the field experiment. For field light measurements and water column metrics, measurement date (time) was considered a random effect. Model fit was examined visually to inspect for normality, minimize variance heterogeneity, and reduce nonindependence in the residuals. Alternative distributions were also considered for analysis of non-Gaussian response metrics, including Poisson/negative binomial distributions (for overdispersed count and density data) and binomial distributions (for proportional data). Correlation and variance structures were evaluated to improve model fit, in accord with the assumption of independence (i.e., through time) and equal variance (i.e., between factors; see Appendix B for details on model structure for each response variable). Nutrient and macroalgal treatments, and their interactions, were considered fixed effects in all the models.

To test hypotheses regarding factors governing eelgrass metrics, we compared the following candidate models for each response metric: (1) the effect of nutrients alone (N), (2) the effect of macroalgae alone (M), (3) the additive effects of nutrients and macroalgae (N + M), (4) the interactive effects of nutrients and macroalgae (N × M), and (5) a null model (null) with no macroalgae or nutrient effects (estimated intercept only). For models considering repeated measurements through time, candidate models included interactions of all factors with time (T; N × T, M × T, N + M + T, N × M × T), as well as a model considering the effect of time alone (T) independent of M and N treatments (Appendix B). This model selection approach allowed us to examine individual, additive, and interactive effects of nutrients and macroalgae, and to illuminate their potential indirect and direct effects on eelgrass. To determine the most parsimonious model(s) explaining the data, we used simple difference calculations (delta AIC_c [Akaike's information criterion corrected for sample size]; the difference between a model AIC_c and the lowest observed AIC_c value) and Akaike weights (the model likelihood normalized by the sum of all model likelihoods). AIC_c scores for all models were computed using the AICcmodavg package in R (Mazzerole 2013) and are reported in Appendix C. The best-fit models (Table 2) have delta $AIC_c < 2$, indicating substantial support for these models, and Akaike weights closer to 1.0, indicating greater confidence in the model (Burnham and Anderson 2002).

To determine differences between treatment groups, we computed effect sizes (averaged over top models) and their 95% confidence intervals (all effects different from 0 reported in Appendix D). Differences were calculated across individual measurement time steps, as well as across the full experiment, independent of time. This can be seen as an information-theoretic alternative to multiple comparisons (e.g., Burnham et al. 2011). For all response metrics, we used model-estimated parameters from the best-fit models (first and second top models presented, where appropriate) to compute model predictions for the factorial experiment (Bolker 2015). These predictions are presented graphically with the observed data (Figs. 1–4; Appendix E). Finally, we used ANOVA results from simple linear models implemented in R to verify the treatment effects related to nutrient enrichment, including Osmocote dissolution rates and water nutrient concentrations.

TABLE 2. Top models resulting from AIC-based model selection for response metrics in field and mesocosm experiments.

Response metric and best model	Delta AIC_c	AIC_c weight
A) Field experiment		
Macroalgal volume (mL)		
N + M	0	0.96
Eelgrass shoot density (% change) per central quadrat (0.25 m^2)		
N	0	0.96
Null	0.48	0.48
Eelgrass shoot density (% change) per haphazard quadrat sample (0.0625 m^2)		
Null	0	0.74
Final eelgrass biomass (g)		
Null	0	0.97
Eelgrass shoot length (% change)		
Null	0	0.35
N	0.59	0.26
Time	0.71	0.25
Eelgrass sheath length (% change)		
Time	0	0.84
Redox potential (mV)		
Time	0	0.53
N × T	0.6	0.39
PPFD (μmol photon·m^{-2}·s^{-1})		
Null	0	0.99
B) Mesocosm experiment		
Macroalgal volume (mL)		
Time	0	1
Eelgrass mean shoot density (% change/rep)		
N + M	0	0.6
M	1.51	0.28
Final eelgrass biomass (g/shoot)		
N × M	0	0.98
Eelgrass shoot length (% change/bucket)		
M × T	0	0.64
M + N + T	1.49	0.3
Eelgrass sheath length (% change/bucket)		
M + N + T	0	0.63
M × T	1.25	0.34
Trimmed eelgrass biomass (g)		
N × M × T	0	0.99
Sloughed eelgrass biomass (g)		
M + T	0	0.87
Redox potential (mV)		
N × M × T	0	0.93
Total light attenuation (μmol photon·m^{-2}·s^{-1})		
M × T	0	0.57
N + M + T	0.62	0.42
Temperature (°C)		
M	0	0.4
N + M	0.58	0.3
Null	1.94	0.15
Salinity (ppt)		
Null	0	0.63
Dissolved oxygen (mg/L)		
M	0	0.72
pH		
M	0	0.72
N + M	1.88	0.88

Notes: All response metrics are shown per replicate. All lengths were measured in centimeters, all biomass in grams dry mass. Eelgrass density refers to change in mean shoots per replicate. In each model, macroalgae and nutrients were fixed effects in a linear mixed-effects model (see Appendix B for model structures). Models considered were N (nutrient effect alone), M (macroalgae effect alone), N + M (macroalgae additive with nutrients), N × M (macroalgae interactive with nutrients), and null (no nutrient, macroalgae, or temporal effect). Interactions with time (T), as well as time alone, were evaluated for all models with a temporal component. PPFD refers to photosynthetic photon flux density, and rep to replicate. The best models were selected based on the strength of the model given the data, based on AIC minimization (delta AIC_c values >2 AIC) and weight of evidence (highest AIC_c weights). See Appendix C for AIC_c tables for all models.

RESULTS

Success of nutrient and macroalgal treatments

Slow-release fertilizer (Osmocote) and chalk block dissolution rates were ~20 and ~10 times greater, respectively, in the field than in the mesocosm experiments (Table 1). Dissolution rates among macroalgal treatments (i.e., addition, removal, mimic, control), however, were similar in both mesocosm and field experiments (field, $F_{3,18} = 0.61$, $P = 0.62$; mesocosm, $F_{2,13} = 0.79$, $P = 0.47$).

In both experiments, slow-release fertilizer increased the nutrient concentrations (Table 1). In the field, in July, both DIN and PO_4^{3-} concentrations were higher in the +nutrient than in the ambient treatments (Table 1; DIN, $F_{1,4} = 7.06$, $P = 0.06$; PO_4^{3-}, $F_{1,4} = 11.33$, $P = 0.03$). In August, when we examined dilution potential, both DIN and PO_4^{3-} decreased with distance from the dispensers (nutrient × distance, $F_{2,12} = 4.84$, $P = 0.04$ and $F_{2,12} = 4.19$, $P = 0.04$, respectively). In the field experiment, nutrient concentration dropped off sharply with distance from the dispensers, with the highest nutrient concentrations occurring adjacent to the tubes (0 cm distance) for both nutrients. For DIN and PO_4^{3-}, nutrient enrichment was limited to within 5 cm of the tubes. In the mesocosms, mean nutrient concentrations were also higher in the +nutrient treatments (Table 1; DIN, $F_{1,17} = 7.06$, $P < 0.001$; PO_4^{3-}, $F_{1,17} = 11.33$, $P < 0.001$). Based on the high ambient nutrient concentrations found in the tanks (Table 1), and their fast turnover rates, we calculated high nutrient loading values of approximately 2160 mg DIN/d (154 000 μmol/L DIN/d) for ambient treatments, and 3000 mg DIN/d (215 884 μmol/L DIN/d) for +nutrient treatments. Given the greater water flow in the field, rough

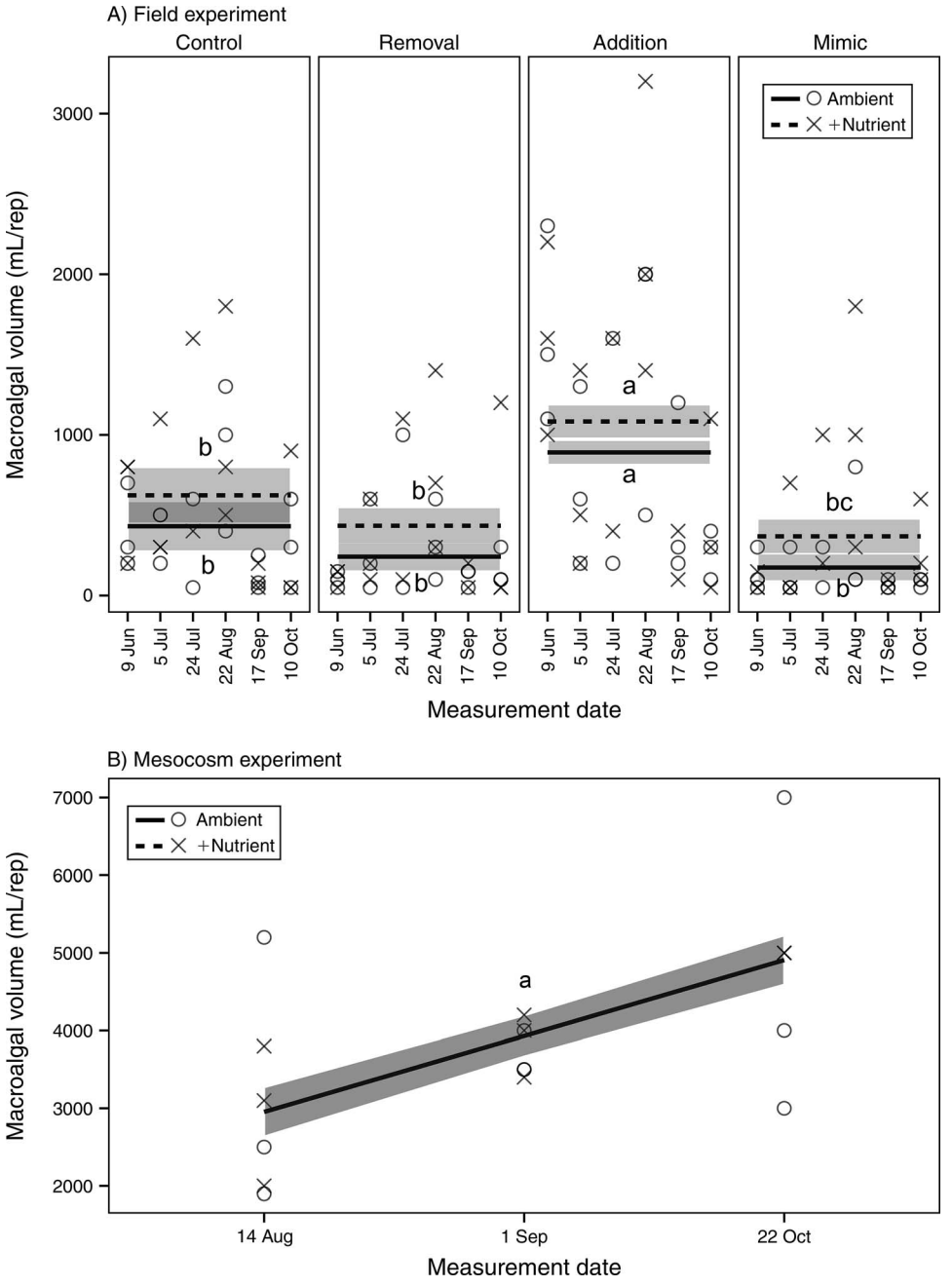

FIG. 1. Final macroalgal volume (observed and predicted) from the field and mesocosm experiments grouped by nutrient (ambient, +nutrient) and macroalgal (removal, addition, control, mimic) treatments. Observed changes are points (ambient [open circles], +nutrient [x's]). Observational data presented are averaged to the level of the random effect determined from model selection. Predicted changes are lines based on the best-fit (AIC-based) models to the data (ambient [solid line], +nutrient [dashed line]), with gray shading indicating (±) SE (see Appendix B for full model specifications). Lowercase letters group treatments that show strong evidence for differences (see Appendix D for effect sizes). (A) Field experiment macroalgal volume (mL) per central quadrat (0.25 m^2) macroalgal treatment replicate (rep). (B) Mesocosm experiment macroalgal volume (mL) per addition tank replicate.

estimates of nutrient loading to those plots were much higher (about three orders of magnitude).

In the field experiment, nutrient and macroalgal effects on macroalgal volume were additive (Table 2A, Fig. 1A). Macroalgal biomass values tended to be higher for +nutrient treatments, but values across nutrient treatments overlapped, and no large differences were detected between them (Fig. 1A; Appendix D). Macroalgal volume was greatest for addition treatments (1083.60 mL/0.25 m^2 [ambient] to 890.78 mL/0.25 m^2

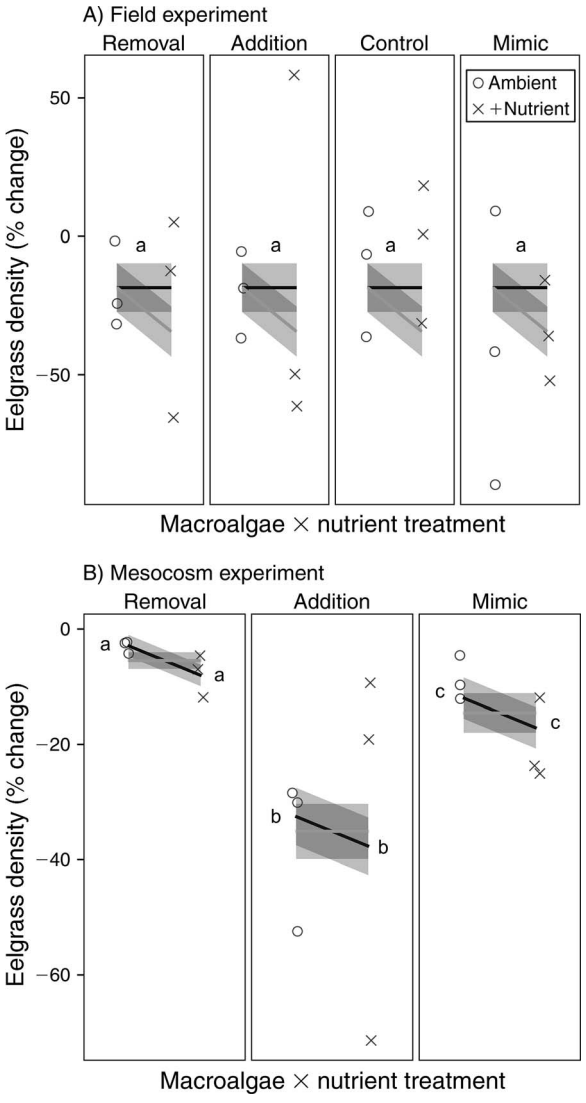

FIG. 2. Change in eelgrass density (observed and predicted) in the field and mesocosm experiments, grouped by nutrient (ambient, +nutrient) and macroalgal (removal, addition, control, mimic) treatments (see Fig. 1 caption for symbol definitions). For models with evidence supporting more than one model, best-supported model is shown by a black line, secondary model with strong support is shown by a gray line (Table 2, Appendix C). Lowercase letters group treatments that show strong evidence for differences (see Appendix D for effect sizes). (A) Field experiment change in eelgrass density (number of shoots) per central quadrat (0.25 m^2) replicate from the initial date (June) to end date (September). (B) Mesocosm experiment change in eelgrass density (number of shoots per bucket) per replicate tank from the initial date (July) to end date (September).

[+nutrient]), which differed from both control and removal treatments (Fig. 1A; Appendix D). Macroalgal volumes in mimic treatments were the lowest, about three (+nutrient) to five (ambient) times lower than addition values, reflected in the largest effect sizes among treatment factors (Appendix D). Differences associated with macroalgal manipulations were also detected between mimic and control (ambient only) treatments (Fig. 1A; Appendix D).

Although mesocosms likely presented a more controlled environment for examination of nutrient effects, the hypothesis that nutrients directly affected macroalgal volume was not supported (Table 2B; Appendix D). Rather, macroalgal volume was variable across treatments and replicates, and overall, increased between consecutive measurement dates (Fig. 1B).

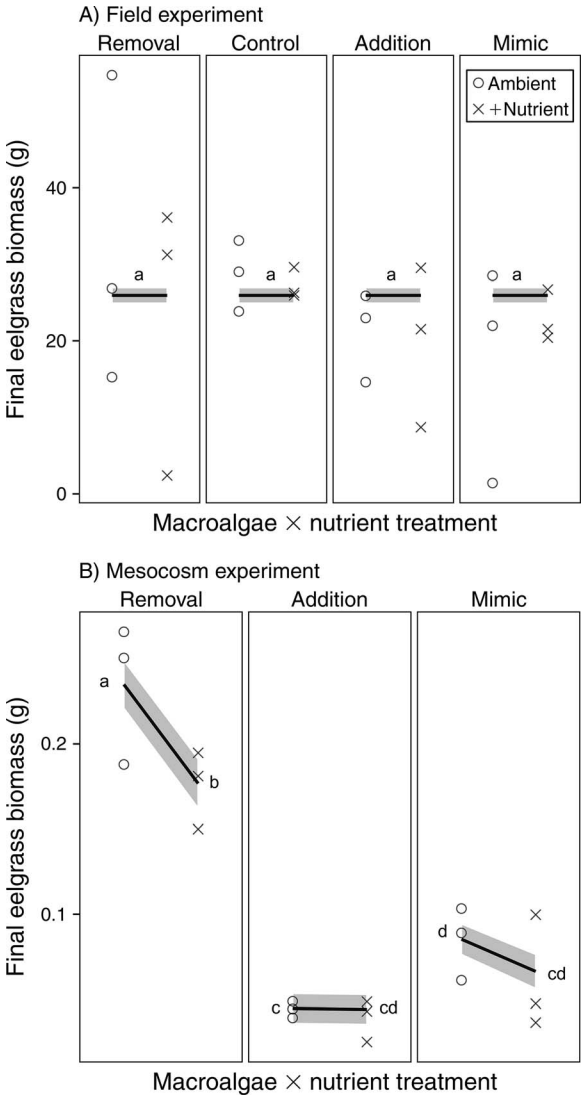

FIG. 3. Final eelgrass biomass (observed and predicted) from the field and mesocosm experiments, grouped by nutrient (ambient, +nutrient) and macroalgal (removal, addition, control, mimic) treatments (see Fig. 1 caption for symbol definitions). Lowercase letters group treatments that show strong evidence for differences (see Appendix D for effect sizes). (A) Field experiment final eelgrass biomass (g dry mass) per central quadrat (0.25 m^2) replicate. (B) Mesocosm experiment final eelgrass biomass per shoot (g dry mass) per replicate tank.

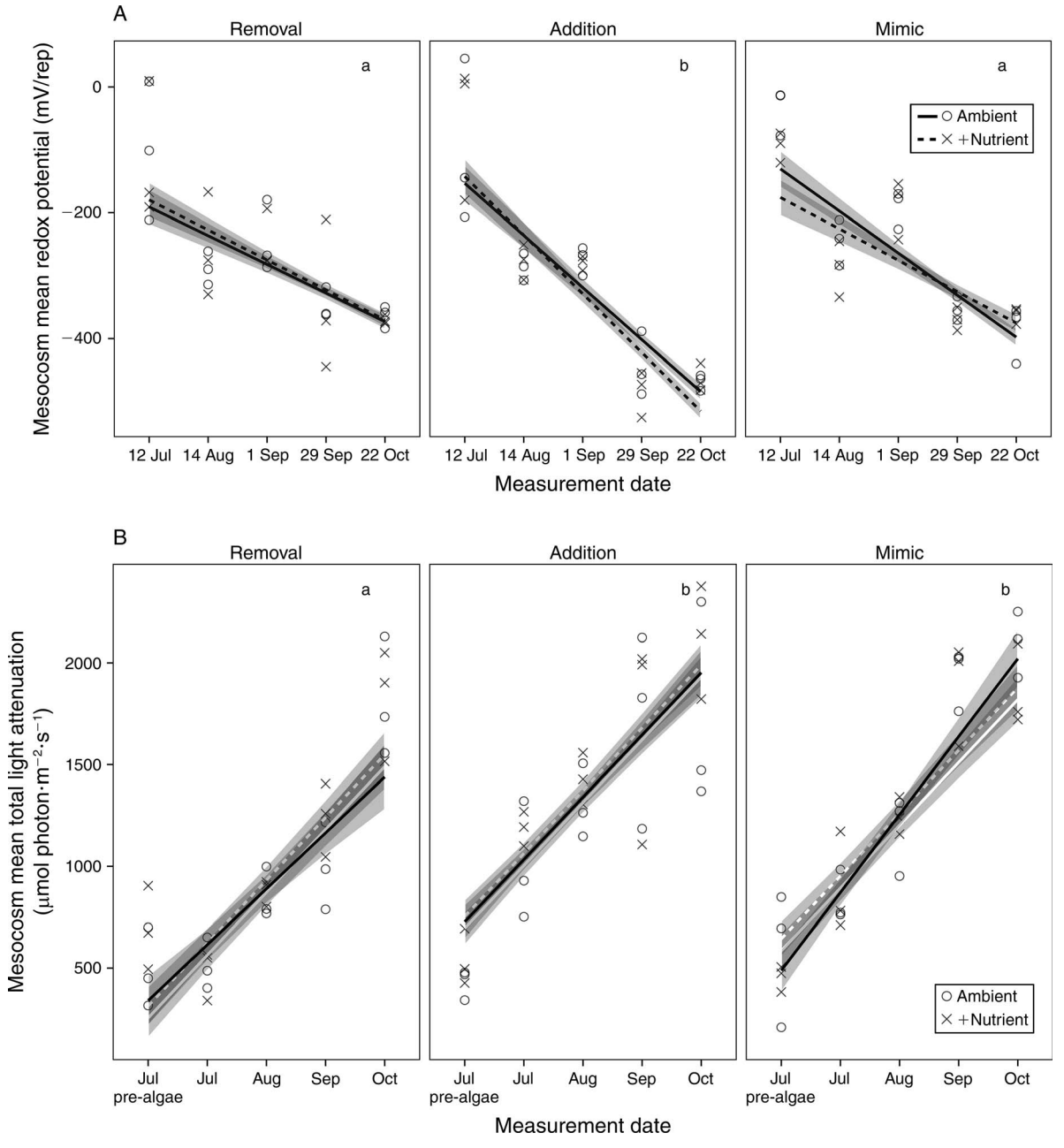

FIG. 4. Light and sediment oxygen levels in the mesocosm experiment (observed and predicted) by measurement dates spanning the experiment duration. Data are grouped by nutrient (ambient, +nutrient) and macroalgal (removal, addition, control, mimic) treatments (see Fig. 1 caption for symbol definitions). For models with evidence supporting more than one model, best-supported model is shown by a black line, secondary model with strong support is shown by a dashed white line (Table 2, Appendix C). Lowercase letters group treatments that show strong evidence for differences (see Appendix D for effect sizes). (A) Redox potential value (mV) per replicate tank per measurement date. (B) Mean total light attenuation per replicate tank per date (monthly averaged data). Total light attenuation calculated as mean PPFD (photosynthetic photon flux density; μmol photon·m^{-2}·s^{-1}) at 5 cm below the water line (surface) minus mean PPFD at 30 cm depth per tank. Pre-algae refers to July measurements before macroalgal treatment, all other dates are post-macroalgal treatment.

Eelgrass responses to treatments

In the field experiment, eelgrass density declined through time (Fig. 2A; change in eelgrass density $<$ 0% for all treatments). Both nutrient treatments and no treatments (null model) explained the observed declines (Table 2A), but differences between nutrient treatments were minimal (Fig. 2A; Appendix D). Except for change in shoot length, no other eelgrass metric (haphazard quadrat density, biomass [Fig. 3A], and sheath length) responded to any treatment (Table 2A; top models: null

and time). Shoot length was affected, to some degree, by nutrient additions (Table 2A; top models: null, nutrient, and time), but no differences between nutrient treatments were detected (Appendices D and E). The lack of eelgrass responses to nutrient and macroalgal treatments in the field experiment may be due to high variation among replicate blocks (Figs. 2A and 3A).

Contrary to the field, all eelgrass metrics, except for sloughed biomass, responded to a combination of macroalgal and nutrient manipulations in the mesocosms (Table 2B; interactive and additive models dominate). We found declines in density (percentage of change), biomass (final and trimmed), and length (shoot and sheath) associated with both treatments, through time (Appendices D and E). Macroalgal treatments alone (density declines) and with time (sloughed biomass) also explained the declining trends (Table 2B; Appendix E). Across most metrics, the effects of macroalgal treatments were much greater than those of nutrient treatments (Appendix D). For example, decreases in eelgrass density were associated with both treatments (Table 2B; top model: N + M) but differences between macroalgal treatments were responsible for the largest negative percentage of change (Fig. 2B; addition > mimic > removal). Differences between nutrient treatments were weak (ambient vs. +nutrient; Appendix D). For final eelgrass biomass (Table 2B; top model: N × M), differences between nutrient treatments were found for all but the addition treatment (Fig. 3B; Appendix D). Similar patterns in the dominance of macroalgal treatment effects were also observed for change in eelgrass shoot/sheath lengths and trimmed/sloughed biomass (Appendix D). Differences between addition and other treatments were large (Appendix D), resulting in the largest declines (Appendix E: Trimmed eelgrass biomass, eelgrass shoot length) and lowest values (Appendix E: Sloughed eelgrass biomass, eelgrass sheath length).

Physical responses to treatments

As with the eelgrass responses in the field experiment, redox potential measurements and light levels (PPFD) were minimally affected by the treatments (Table 2A; top models: time and null; Appendix E). Redox potential also varied with nutrient additions through time (Table 2A), but no strong effects were detected among treatments (Appendix D).

In the mesocosms, redox potential varied with both treatments and time (Fig. 4A, Table 2B; top model: N × M × T). Total light attenuation varied with macroalgae, nutrients, and time (Fig. 4B, Table 2B; top models: M × T, N + M + T). Again, as with eelgrass responses, macroalgal treatments had greater effects than nutrient treatments for both redox potential and PPFD (Fig. 4; Appendix D; no difference between nutrient treatments). For redox potential, the steepest declines were associated with the addition treatment, and macroalgal effects (addition vs. removal/mimic) increased through time from initial differences of ~37–55 mV to final differences of ~86–145 mV (Fig. 4A; Appendix D).

For total light attenuation, the greatest increase was associated with both addition and mimic treatments, which differed from the removal treatments (Fig. 4B). Again, the largest differences through time were found between addition and removal (475.95 μmol photon·m^{-2}·s^{-1}) followed by mimic and removal (309.86 μmol photon·m^{-2}·s^{-1}). Light conditions were sampled more sporadically in the field (e.g., on different dates and recording periods, both ebb and flood tides), and no differences were detected between treatments (control, removal, addition, and mimic averages across all dates and measurement periods: 164, 236, 208, and 161 μmol photon·m^{-2}·s^{-1}, respectively; Appendix D)

In the mesocosm experiment, minor differences in water column physical characteristics were detected (temperature, dissolved oxygen [DO], light, salinity, and pH; Table 2B, see Table 1 for average values across all treatments). For example, across the full experiment, temperature differences in macroalgal treatments were <0.5°C (Appendix E; removal [12.4°C], addition [12.4°C], mimic [12.2°C]). Similarly, differences across treatments in DO were small (Appendix D: ~1.2 mg/L), with predicted values ranging from 10.14 mg/L (removal) to 10.19 mg/L (addition) and 9.98 mg/L (mimic; Appendix E). Comparably small differences also occurred across treatments for pH (Appendices D and E; removal [8.07], addition [8.10], and mimic [7.97]).

Discussion

In this upwelling-influenced estuary, the hypothesis that nutrient and/or macroalgal biomass manipulations negatively affected eelgrass under field conditions was not supported (Figs. 2A and 3A, Table 2A; Appendix E). This suggests that in the field, the threshold response for eelgrass to elevated nutrients and high volumes of macroalgae is higher than the values generated by our manipulations. This lack of negative effects is consistent with our previous research in these estuaries. Macroalgal blooms were neither associated with temporal declines in eelgrass among estuaries in the Pacific Northwest (Hessing-Lewis and Hacker 2013) nor within the marine zone of a single, high-nutrient estuary (Coos Bay; Hessing-Lewis et al. 2011). Observational data collected at the same site and over the same time period as the field experiment shows positive, not negative, correlations between eelgrass and macroalgae (Appendix A). Thus, eelgrass in Pacific Northwest upwelling-influenced estuaries responds differently to nutrient inputs and macroalgal blooms than what has been observed under conditions of land-based eutrophication (see also Kaldy 2009). However, the mesocosm results did show negative effects of macroalgae and, to a limited extent, nutrients. Here, macroalgae and nutrients, and the biogeochemical changes they created, were important predictors of eelgrass declines, biomass, and morphological change (Figs. 2B and 3B, Table 2B; Appendix E).

Potential mechanisms for negative interactions in the mesocosm experiment

As with other research on the mechanistic causes of seagrass declines (Short and Wyllie-Echeverria 1996, McGlathery 2001), changes in sediment conditions and water column light associated with macroalgal additions explained the observed declines in eelgrass in the mesocosm experiment (Fig. 4; Appendix D). We found decreased sediment redox potential (a proxy for oxygen) in all the treatments, but it was especially acute in the addition treatment (Fig. 4A). This decline is likely driven by macroalgal decomposition, although other explanations are possible. For example, oxygen transport to the sediments via eelgrass shoots is likely reduced as a result of photosynthetic reduction from canopy shading. Interestingly, DO in the water column was slightly lower in mimic treatments compared to the addition and removal treatments (Appendix D), suggesting that the living macroalgal canopy had little effect on water column DO. While seagrasses can often tolerate low sediment oxygen conditions (Terrados et al. 1999), anoxia can lead to the production of toxic sulfide compounds, which interfere with nitrogen metabolism (Pregnall et al. 1984). This process can have negative effects, especially in concert with other stressors (e.g., light reduction and its effects on photosynthetic oxygen production; Goodman et al. 1995, Koch and Erskine 2001, Borum et al. 2005). In the field experiment, sediment redox potential values were within the range of values observed in mesocosms, although variability between measurement dates was high (Table 2A; Appendix E). Hence, it is unlikely that changes in sediment oxygen conditions in the mesocosms were solely responsible for the observed negative effects on eelgrass.

Decreased light produced by the macroalgal additions likely played a large role in the decline of eelgrass in the tank environment (Fig. 4B, Table 2B). Temporal declines in light conditions occurred in all treatments, but shading effects were greatest for addition and mimic treatments (Appendix D), where surface irradiance was substantially reduced (removal, addition, mimic; 49%, 30%, 31% mean μmol photon·m^{-2}·s^{-1} per replicate [rep], respectively). The latter two values are <34%, which was the limit for negative effects on eelgrass observed by Ochieng et al. (2010). The associated declines in eelgrass (biomass, density, shoot/sheath length) have been well documented (e.g., Orth and Moore 1983, Hauxwell et al. 2003, 2006). However, in the Pacific Northwest, eelgrass productivity is maximized at irradiance values of 350–550 μmol photon·m^{-2}·s^{-1} (Thom et al. 2008). Eelgrass is within or above this range in the tank environment (removal, addition, mimic; mean light values 30 cm below the water surface: 908, 523, 556 μmol photon·m^{-2}·s^{-1}·rep^{-1}, respectively; mean total light attenuation: 977, 1314, 1297 μmol photon·m^{-2}·s^{-1}·rep^{-1}, respectively). Thus, we conclude that elevated shading by real and mimic macroalgae in the mesocosm experiment is a contributing cause of eelgrass declines, but additional biogeochemical changes in oxygen likely contributed to the macroalgal treatment effects we observed (Figs. 2B and 3B).

Direct negative effects of nutrients on eelgrass in the mesocosm experiment

Although macroalgae had a much larger effect than nutrients on eelgrass (Figs. 2 and 3; Appendices D and E), nutrients did have weak negative effects, most clearly when macroalgae were absent (i.e., Fig. 3B). Nutrients could have had direct physiological effects, and/or indirect biogeochemical effects, that had knock-on effects for eelgrass. Physiological effects are strongly dependent on specific plant features and local conditions (Romero et al. 2006). In some cases, nutrient enrichment can result in elevated nutrient uptake in eelgrass leaves, and if sustained, can lead to negative structural consequences for seagrasses via internal carbon limitation (Burkholder et al. 1992, Touchette and Burkholder 2000). Direct toxic effects of ammonium may also cause high mortality in eelgrass (Van Katwijk et al. 1997), but this effect has not been well studied across a diversity of systems (Kaldy 2009). A recent study suggests that eelgrass adapted to strong ocean upwelling is unaffected by nitrogen toxicity observed elsewhere due, in part, to accompanying low water temperatures (Kaldy 2014).

While direct nutrient effects might play a role in the mesocosm eelgrass declines, other mechanisms influencing nutrient acquisition could also be important. One possibility, and an important caveat to our manipulations, is the effect of adding nutrients to an already high-nutrient ambient environment combined with the lack of strong mixing in the tanks. This might have led to a carbon- (or other nutrient-) depleted boundary layer around the eelgrass (Koch 1994). While persistent boundary layer effects are unlikely, given the water flow and cleaning treatments in the tanks, they could have led to short periods of carbon limitation, decreased photosynthetic rates, and reduced final stem density and biomass relative to the ambient treatment. Moreover, carbon or other limiting nutrients might explain the weak role of nutrients in the field experiment (Table 2A; percentage of change in density and shoot length), as well as the larger spatial trends we have observed between estuaries (Hessing-Lewis and Hacker 2013). Regionally, we found that lower eelgrass biomass could not be explained by macroalgal blooms alone, but was also negatively correlated with marine nutrient input (based on a proxy measurement, upwelling strength). Strictly controlled experiments, in which the ambient nutrient conditions are modified and leaf tissue constituents (i.e., percentage of nitrogen, $\delta^{15}N$) analyzed, would allow determination of whether augmented nutrients are being directly incorporated into eelgrass tissue or if some indirect mechanism is at work.

Direct effects of nutrients on macroalgae

Our experiments tested the potential for additional nutrient inputs to affect not only eelgrass, but also augment the high seasonal abundance of ulvoid macroalgae (Pregnall and Rudy 1985, Kentula and DeWitt 2003, Hessing-Lewis and Hacker 2013). Nutrient addition alone did not affect macroalgal volume in the mesocosms, and in the field, the addition of nutrients (Table 2A; top model: N + M) was not associated with large differences between nutrient treatments (Fig. 1A; Appendix D). This indicates that either (1) diffusion rates were too high to stimulate a macroalgal response, or (2) macroalgae was not nutrient limited, but rather, uptake rates were saturated or near-saturated in these estuaries. Because background nutrient levels are high in upwelling-influenced systems (Table 1; e.g., Coos Bay mean summer DIN = 14.05 μmol/L and Yaquina Bay mean summer $NO_3^- + NO_2^- = 11.3$ μmol/L, mean $NH_4^+ = 3.6$ μmol/L), macroalgae may already have been near nutrient saturation. In fact, although we documented increases in nutrient concentrations in the tanks (mean = 29.93 μmol/L [0.42 mg/L]), the very high ambient concentrations (Table 1; mesocosm mean = 21.70 μmol/L [0.30 mg/L]; data from Brown and Ozretich 2009) and loading rates, could have overwhelmed the macroalgae and resulted in no appreciable response to experimental enrichment.

In a global comparison of ulvoid macroalgae nutrient limitation, Teichberg et al. (2010) found that macroalgal growth rates increased linearly with annual DIN concentrations up to 100 μmol/L, but addition of NO_3^- primarily increased growth rates in estuaries where ambient DIN was low (~<10 μmol/L). In upwelling-influenced estuaries, however, summer nitrogen concentrations associated with nutrient-rich ocean waters can be as high as 31.5 μmol/L (Brown and Ozretich 2009), which may explain the lack of macroalgal response in our experiments. Alternatively, while DIN may not be limiting under higher nutrient concentrations, PO_4^{3-} might be (Wheeler and Bjornsater 1992, Teichberg et al. 2010). Overall, nutrient limitation and saturation are not well studied in Pacific Northwest estuaries (Kaldy 2009, but see Williams and Ruckelshaus 1993), and will require further investigation.

Potential for hydrodynamics to mitigate negative effects

As well as physiological adaptations to high background nutrient concentrations, physical hydrodynamic conditions may mitigate the negative effects we observed in the mesocosms (Table 2B, Figs. 2B and 3B) by mediating light, biogeochemical processes, and local nutrient conditions. In the field, high water exchange across the experimental plots, and the estuary as a whole, likely contributed to the lack of treatment effects (Table 2A). The flushing time for the entire Coos Bay estuary is relatively fast (6–8 tidal cycles or 3 days), due in part to large tidal velocities (peaks of ±1.1 m/s, mean of 0.4 m/s; Roegner and Shanks 2001, Rumrill 2006). High currents and water motion caused by tides and waves are reflected in the high dissolution rates of chalk blocks and Osmocote observed in the field (Table 1). Water turnover rates in the mesocosm experiment (17 times/day), although high, were much lower than in situ conditions, and the tank environments were not subject to high currents or semidiurnal tides.

Differences in current flow rates and tidal activity affected not only the structure of the water column itself, but also the formation of the macroalgal canopy in the two experiments. In the field, the augmented addition treatments, as well as the other macroalgal treatments, underwent daily changes in canopy shape, with the ebb and flood of the tides occurring twice daily, with an average amplitude of 2.3 m (Rumrill 2006). At the field site, we observed aggregations of floating macroalgae above the sediment surface during the flooding tide. These aggregations were 5–30 cm thick, with eelgrass blades penetrating through the ulvoid canopy and upward into the water column. During periods of emersion, some leaves were exposed and lay flat above mats of accumulated macroalgae. Eelgrass blades in the tank environment, by contrast, had no such opportunity to be released from macroalgal shading. In the tanks, canopy heights were deeper (30–50 cm) and eelgrass blades could not penetrate through the canopy as easily.

Physical differences between experiments may also have contributed to the weak direct nutrient effects in the field (Table 2A: density and length). In the mesocosms, nutrient additions led to increases in nitrogen and phosphorus concentrations in the water column (Table 1). However, differences with ambient conditions were small and within the range of values found in Yaquina Bay and Coos Bay waters during the summer season (Table 1; Brown and Ozretich 2009). In the field, we found that diffusion rates of nutrients were very high, and were only different from ambient nutrient concentrations closely adjacent to the diffuser tubes (Table 1). Therefore, while we added large amounts of fertilizer, with the potential to create very high nutrient concentrations (i.e., maximum DIN values of 740 μmol/L adjacent to diffuser tubes), water movement likely diffused nutrients throughout the larger estuarine system, and diluted the direct effects of elevated nutrient concentrations on eelgrass (Table 2). Advection plays a major role in this system (Table 1), and the augmented nutrients in the field may not have been concentrated enough to affect uptake by macrophytes, except at high tide and at close proximity to the nutrient diffuser tubes. In fact, as Worm et al. (2000) documented in their comparison of nutrient enrichment studies, it may be hard to differentiate nutrient effects from the inability to manipulate nutrient concentrations. However, across a range of physical conditions and background nutrient concentrations, they found that coated slow-release fertilizer successfully augmented nutrient concentrations. Similarly, nutrient concentrations increased locally in the manipulated plots, but our sampling frequency may not have captured the temporal

variability of these additions. Thus, more frequent nutrient sampling and replenishment seems necessary in highly advective systems such as ours.

In sum, the contrasting hydrodynamics between experimental venues not only affected flushing rates, but also resulted in the observed differences in macroalgal canopy structure and localized dilution of nutrient additions. We hypothesize that the local hydrodynamic environment in the field experiment was a key factor in alleviating the negative effects of macroalgal shading, sediment hypoxia, and the potential direct and indirect nutrient effects found in the mesocosms.

Implications for cross-system eutrophication management

Even though nutrient enrichment may have dramatic repercussions in some systems (Teichberg et al. 2010, Howarth et al. 2011), macroalgal blooms and nutrient concentrations, by themselves, may not necessarily signal eutrophication (Bricker et al. 2003). Our work suggests that two key factors control the negative interactions documented for seagrasses and macroalgae: (1) local hydrodynamics, and (2) estuarine nutrient history. Eutrophication management in the United States uses regional assessment models (i.e., Assessment of Estuarine Trophic Status [ASSETS] and National Estuarine Eutrophication Assessments [NEEA]) that include aspects of these two factors. Flushing and dilution potential are used as measures of an estuary's susceptibility to nutrient inputs, based on the premise that short residence times and increased flushing rates decrease the intensity of blooms or alleviate them altogether (Valiela et al. 1997). Furthermore, the threshold nutrient concentration at which eutrophication status is evaluated is based on anthropogenic nutrient loading relative to background conditions (Bricker et al. 2003). However, these regional assessments lack the knowledge necessary to accurately interpret the local ecological ramifications of macroalgal blooms on seagrass distribution (primary and secondary indicators in NEEA; Bricker et al. 1999). Understanding these local effects may be especially important for estuaries on the Pacific coast of the US, which have the highest number of "data deficient" estuaries reported in national assessments (Bricker et al. 1999).

Management of specific estuaries should focus on monitoring metrics that assess the mechanistic causes of eelgrass decline and apply across a large range of environmental conditions. For instance, we advocate monitoring macroalgal canopy heights relative to the mean plant height of eelgrass beds, and light and sediment conditions resulting from canopy conditions. This recommendation stems from our results, and findings from other Northeastern Pacific studies, that show differences in the location and physical structure of macrophyte production. Nelson and Lee (2001) report negative effects of macroalgae on seagrass in subtidal systems with persistent canopies, and we have found negative effects in shallower riverine sections of estuaries where shorter plants and reduced sediment conditions (associated with ulvoid macroalgae) co-occur (Hessing-Lewis et al. 2011). In California, USA, the effects of macroalgal blooms on sediment conditions (Sutula et al. 2014) and macrobenthic fauna (Green et al. 2014) have recently been assessed to understand the threshold effects of macroalgal blooms there. Again, the macroalgal blooms documented to have negative effects in California estuaries were much lower than peak ambient biomass observed in Yaquina Bay (Appendix A; 400–500 g/m^2), and suggest that mechanistic benchmarks (e.g., sediment oxygen conditions or canopy height) might provide better integrated measures of the effects of macroalgal blooms than biomass alone.

Management responses for upwelling-influenced estuaries must also consider future scenarios, especially climate-related increases in nutrient inputs in conjunction with other oceanographic changes associated with upwelling, such as hypoxia and ocean acidification (Scavia et al. 2002, Chan et al. 2008, Bakun et al. 2010, Iles et al. 2012, Shibata et al. 2014). Furthermore, watershed nutrient delivery in this region will likely increase because of projected increases in coastal development and population growth (Bricker et al. 1999). Together, these inputs may result in whole-scale, substantive changes from background nutrient concentrations, with the potential to push macrophytes beyond their current capacities to uptake nutrients and withstand nutrient effects. Evaluating the strength of evidence for the interactive effects of nutrients and other key climate-related parameters (e.g., temperature, pH, and DO) on macroalgae–seagrass interactions will require a mix of experimental approaches, and long-term monitoring data. Coupled, this approach is necessary to guide current and future management of these critical estuarine habitats.

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Supplemental Material

Ecological Archives

Appendices A–E are available online: http://dx.doi.org/10.1890/14-0548.1.sm

Data Availability

Data associated with this paper have been deposited in Dryad: http://dx.doi.org/10.5061/dryad.j9m92