**Ulva additions alter soil biogeochemistry and negatively impact Spartina alterniflora growth**

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ABSTRACT: Decaying mats of Ulva can be washed into salt marshes by the tides as large wrack deposits, especially in eutrophic estuaries, where they can negatively impact marsh vegetation. Using field and laboratory experiments, we examined the effects of decomposing Ulva on Spartina alterniflora growth, soil biogeochemistry and nitrogen dynamics. High levels of Ulva exposure resulted in reductions in above- and belowground biomass, while lower levels of Ulva exposure resulted in reductions in only belowground biomass. Porewater ammonium in soil that contained decomposing Ulva quickly attained potentially toxic levels. In addition, amending soil with Ulva led to elevated porewater concentrations of sulfide and trithiane, an organosulfur compound and potential biocide. Use of a ¹⁵N tracer documented plant uptake of Ulva-derived nitrogen, but higher nitrogen availability did not stimulate growth. Our findings support the hypothesis that decaying Ulva mats may create hotspots of adverse physiochemical conditions in salt marshes. However, because our Ulva additions were higher than typically found in coastal marshes, additional field and laboratory studies are needed to establish more firmly whether similarly adverse responses are observed under natural conditions.

KEY WORDS: Salt marsh · Eutrophic estuary · Salt marsh loss · Allelochemicals · Allelopathy

INTRODUCTION

Nuisance blooms of opportunistic macroalgae of the genera Ulva, Cladophora, Chaetomorpha and Gracilaria are found worldwide (Duarte 1995, Valiela et al. 1997, Kamer et al. 2001) and are often associated with cultural eutrophication (Fletcher 1996). While macroalgae are a natural part of estuarine ecosystems, prodigious macroalgal canopies and decaying mats can have detrimental effects on aquatic biota and water quality (Kamer et al. 2001). Episodic macroalgal blooms can damage seagrass beds and impact phytoplankton populations through reduction of light penetration (Fong et al. 1993, Valiela et al. 1997) and depletion of water column oxygen (Sfriso et al. 1987). Decaying algal mats are associated with anoxia (Hull 1987), low redox potentials (Sfriso & Pavoni 1994) and sulfide and ammonium accumulation (Bierzychudek et al. 1993, Holmer & Nielsen 2007), which have detrimental effects on seagrass and infauna (van Katwijk et al. 1997, Hauxwell et al. 2001). Some species of Ulva have also been noted to have growth-inhibiting effects on phytoplankton (Wang et al. 2007, Tang & Gobler 2011) and shellfish (Johnson & Welsh 1985) through allelochemical exudates.

Macroalgae also play a key role in nutrient dynamics in eutrophic coastal ecosystems. They can assimilate pulsed nutrient inputs from an estuary (Fong et al. 2004) and are a strong indicator of elevated nutrient loads (McLaughlin et al. 2014). Macroalgae may mediate and recycle nutrient releases to the estuary...
through the decomposition of algal biomass (Srisko & Pavoni 1994). In some shallow coastal lagoons, seasonal macroalgae cycles exert a greater control on water column dissolved nitrogen values than allochthonous inputs (Srisko et al. 1987).

In addition to ecological effects on benthic and pelagic zones in estuaries, macroalgae wrack may also impact the upper intertidal zone, including beaches, dunes and salt marshes via wave run-up and tidal action (Boyer & Fong 2005). Decaying algal mats have beneficial effects on sand beaches and dunes, where they provide refugia for mobile invertebrate fauna (Holmquist 1997), serve as a food source (Soulsby et al. 1982) and release nutrients (Orr et al. 2005). Macroalgae accumulations are known to have both facilitative and inhibitory effects on salt marsh plant growth. Salt marshes are nitrogen-limited (Valiela & Teal 1979) and often experience a positive growth response to fertilization (Pennings et al. 2005, Morris et al. 2013). Previous field and laboratory studies have shown that macroalgae presence can enhance marsh plant growth (Gerard 1999, Boyer & Fong 2005, Newton & Thornber 2013). However, mats of algal wrack can be quite dense and pervasive in salt marshes, and can become entangled with and break the culms of Spartina alterniflora, the dominant species of the Atlantic salt marsh ecosystem (Newton & Thornber 2012). In addition, some field studies using Ulva spp. have found neutral or negative impacts of macroalgae accumulations on marsh plant growth (Hulzen et al. 2006, O’Connor et al. 2011, Newton & Thornber 2013). Temporary vegetation loss associated with disturbance (Kirwan et al. 2008), such as that which occurs with Ulva deposition, may exacerbate ongoing marsh vegetation loss seen in large portions of North America and Europe (Kearney et al. 2002, van der Wal & Pye 2004).

In this study, we examined the effects of Ulva soil additions prepared to simulate large macroalgae blooms associated with extremely eutrophic conditions on S. alterniflora growth, soil biogeochemistry and nitrogen dynamics. Our hypothesis was that decaying Ulva mats may create hotspots of adverse physiochemical conditions in salt marshes, similar to those reported for some benthic and tidal flat habitats (Johnson & Welsh 1985, Holmer & Nielsen 2007). As decomposition and biogeochemical interactions are redox-dependent (Bradley & Morris 1990), we additionally identified interactions between Ulva decay and elevation. We used these results to draw inferences regarding soil interactions and potential ecosystem effects of algae-mediated nutrient transfer in salt marshes.

**MATERIALS AND METHODS**

A series of field and greenhouse experiments was conducted, growing Spartina alterniflora in Ulva-treated, and untreated control soils. Field experiments were carried out at the Plum Island Sound estuary (PIE), Massachusetts, USA, which is among the largest of the salt-marsh-dominated estuaries in New England (Koop-Jakobsen & Giblin 2009). The greenhouse experiment was conducted at the US Environmental Protection Agency (EPA) laboratory in Narragansett, Rhode Island.

**Field mesocosm experiments**

The combined effects of Ulva additions and inundation on the growth of S. alterniflora were examined using field experiments. During the 2011 growing season, S. alterniflora was grown in experimental mesocosms in soils lacking Ulva additions, but varying in inundation regime. During the 2012 growing season, S. alterniflora was grown under similarly varying inundation levels, but in soils with 2 levels of Ulva additions. One treatment was calculated to roughly correspond to Ulva mat density found in highly eutrophic estuaries (e.g. 1 kg m⁻² dry wt Ulva; Viaroli et al. 2005), converted to a dosage of ~5 g l⁻¹, presuming the Ulva mat impacted benthic soil to a depth of 20 cm. A higher treatment level (16 to 18 g l⁻¹) was also formulated. While this treatment level is probably artificially higher than found under field conditions, macroalgae accumulations exceeding our lower dosage level have been reported in the literature (Srisko et al. 1991). We also initially expected the Ulva to decompose slowly, and to use this higher dosage to simulate impacts of Ulva across a growing season, where separate or episodic blooms may deposit.

Early in the growing season in 2012, 30 short-form S. alterniflora sods were collected from the marsh platform in the PIE. Short-form S. alterniflora was used in the experiment as previous research at the site had suggested that it responded strongly to changes in inundation (Morris et al. 2013). Sods were collected using a steel soil-coring device, measuring 5 cm in diameter by 15 cm in length, and care was taken to collect undamaged sods. Sods were planted in round white PVC plant pots, which were attached to a framework commonly referred to as a ‘marsh organ’—a field mesocosm design consisting of replicate rows of plants at different elevations relative to the tidal frame, and therefore subjected to different inundation treatments (see details in Morris et al.
The marsh organ was situated in a draining pond on the marsh platform. The 30 pots in total were arranged in 5 rows of 6 pots each (Fig. 1). The relative elevation difference between rows was 16 to 17 cm, and the absolute height of the pots ranged from 0.82 to 1.49 m relative to the North American Vertical Datum of 1988 (NAVD88), and from 47 cm below to 17 cm above mean high water. Pots measured 10 cm in width and 40 cm in length. The length was chosen to provide constant space for root and rhizome growth, and pots were separated from native marsh sediments at their bases by a nylon screen to allow for the exchange of water through the soil column. The growth experiment ran from 10 May 2012 to 10 September 2012.

Soil for mesocosm pots was composed by mixing native estuarine sediment collected from a PIE tidal creek (which did not contain Ulva) with additions of dried, coarsely ground Ulva, labeled with $^{15}$N tracer. While Ulva soil additions are regularly used in studies to produce homogenous mixtures (e.g. Rossi 2006, García-Robledo et al. 2013), and burial occurs in the field (Ford et al. 1999), Ulva accumulations in salt marshes are more common as mats entangled with marsh macrophyte stems (Newton & Thornber 2012). By using soil additions rather than covering plants with Ulva wrack, we were able to examine whether impacts of marsh Ulva accumulations extend beyond the physical damage of shoot breakage and photosynthetic reduction associated with blanketing.

Living Ulva was collected from the intertidal zone in Warwick, Rhode Island (Apponaug Cove; 41.69° N, 71.45° W) and cultured in seawater for several days under full sunlight conditions in a greenhouse laboratory tank (salinity 28 to 32 psu) in the presence of $K^{15}$NO$_3$ (99.7% $^{15}$N, Cambridge Isotope Laboratories). Specimens were a mixture of Ulva compressa and Ulva rigida (Guidone & Thornber 2013). Ulva was then dried at 65°C for at least 48 h, ground with a mortar and pestle and manually homogenized. Two levels of Ulva treatments were tested: ‘very high’ treatment pots (3 at each elevation, n = 15) received 60 g of dried Ulva (18.5 g l$^{-1}$), while ‘high’ treatment pots (also 3 per row, n = 15) received 15 g of dried Ulva (4.6 g l$^{-1}$). Treatments were randomized along rows.

As we did not have a control for this experiment we used a near-identical experiment, run in 2011 at the same site, where Ulva additions were not employed. The growth conditions were nearly identical (i.e. same plant source, collection method, location, pots, elevations and soil homogenization method), but differed slightly in timing (29 April to 12 September 2011 vs. 10 May to 10 September 2012). Interannual differences in temperature, precipitation and inundation contributed additional variability. To help address this concern, a comparison of peak biomass and porewater characteristics monitored in adjacent S. alterniflora plots was performed by comparing 95% confidence intervals for mean peak biomass and monthly porewater data (PILTERS 2014). As no significant differences were found between growth and porewater data from 2011 and 2012, this analysis supports the use of the 2011 experiment as com-

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Fig. 1. (A) Marsh organ deployed at Plum Island Sound estuary, Massachusetts, USA; (B) mesocosm tanks in the greenhouse laboratory at the US Environmental Protection Agency (EPA) facility. The Plum Island photo was taken about 5 wk into the experiment, and some of the sods at the lowest elevations had died or were experiencing chlorosis (yellowing). The very lowest level was unused by this experiment. The EPA photo was taken at the time of harvest (mid-September).
S. alterniflora height and density were monitored at the beginning and end of the experiment, and standing aboveground biomass was harvested at the end of the growing season, washed to remove salts and sediment, and dried to constant weight. Belowground biomass was assessed at the end of the growing season by weighing the root and rhizome dry mass retained by a 500 μm sieve after wet-sieving. The number of shoots supporting inflorescences was monitored monthly.

Porewater was collected from each pot from a depth of 20 cm using a permanently installed micro-piezometer outfitted with a 0.5 μm nylon mesh frit. To clear the piezometer and collect fresh porewater, the maximum volume of water possibly contained by the piezometer (5 ml) was discarded, and a fresh sample was pulled using a syringe. For 2011, porewater samples were obtained from each pot monthly. For 2012, porewater samples were collected monthly at 1 pot per elevation and Ulva addition treatment. A first sample for hydrogen sulfide analysis (2 ml) was obtained and preserved (1:1) within 1 min with a 0.22% solution of zinc acetate, Zn(O2CCH3)2. A larger sample was then obtained, filtered (0.2 μM) in the field and analyzed in the laboratory for salinity using a refractometer, and for pH using litmus paper (pHydrion Vivid, 6-8, Micro Essential Laboratory). The sample was then preserved with sulfuric acid (120 μl 6 M H2SO4:20 ml porewater) for later nutrient analysis. Porewater samples were analyzed for hydrogen sulfide, orthophosphate and ammonium using spectrophotometric methods (Cline 1969, Strickland & Parsons 1972). We also estimated concentrations of ammonium and phosphate that would be liberated by decomposing Ulva in field mesocosm porewater assuming mineralization of Ulva nitrogen and phosphorus to porewater ammonium and phosphate, using Eq. (1):

$$\text{porewater } [\text{NH}_4^+ \text{ or } \text{PO}_4^{3-}] (\mu\text{mol l}^{-1}) = \frac{\text{Ulva addition } (\frac{g}{\text{pot}}) \times [\text{N or P}] (\mu\text{mol g}^{-1})}{\text{porewater volume (l)}}$$  (1)

where dw represents dry weight. After harvest of aboveground biomass, cut shoots were plugged with silicone and bare soil CO2 efflux was measured as CO2 concentrations at 1 s intervals for periods of 3 min with a Licor LI-8100 gas analyzer system and dome. Emission rates were calculated using regression of concentration against time to generate fluxes (slopes).

15N enrichment

Living Ulva was labeled with a 15N tracer, prior to introduction into field mesocosms, to establish the fate of nitrogen liberated by Ulva decomposition. Ulva-treated soil and plant samples, collected at the beginning and end of the experiment, were analyzed for nitrogen composition and stable isotope ratios using a Vario Micro Cube elemental analyzer coupled to an Isoprime stable isotope ratio mass spectrometer. Isotopic composition is expressed in per mille notation relative to the reference standard (air-N2) such that $\delta^{15}N = \frac{R_{\text{sample}} - R_{\text{standard}}}{R_{\text{standard}}} \times 1000$,

where R is the ratio of 15N to 14N. Unaltered soils and plants were also analyzed to calculate tracer concentrations.

The enrichment of 15N in mesocosm pots and above- and belowground biomass was calculated using a mass balance approach as the absolute abundance of 15N frequency in terms of atomic percent (atom%) (Currie 2007) (Eq. 2), converted to atom% excess by subtracting the atom% in background samples (atom% 15Nb) from amended samples (atom% 15Na) (Eq. 3). Soil 15N tracer loss for soil over the duration of the experiment was calculated as the difference between the weight of tracer added and the weight of the tracer recovered, divided by the weight of the tracer added. Tracer uptake in above- and belowground biomass pools was computed as the product of the excess 15N concentration and biomass weights. Detailed calculations can be found in Supplement 2 at www.int-res.com/articles/suppl/m532p059_supp.pdf.

$$\text{atom}%^{15}N = \frac{[^{15}N]/[^{14}N + ^{15}N] \times 100}{1}$$  (2)

$$\text{atom}% \text{ excess }^{15}N = \text{atom}%^{15}N_a - \text{atom}%^{15}N_b$$  (3)

Greenhouse experiment

To verify that negative impacts of Ulva additions were repeatable, a follow-up greenhouse experiment was performed in which S. alterniflora was grown under one inundation regime, but similarly grown in soils treated with, or lacking, Ulva. In the follow-up, experimental details were altered (soil and plant collection site, plant ecotype, Ulva source) to increase confidence in the robustness of our results.
Ulva was collected from the subtidal zone in Narragansett Bay (Conimicut Point; 41.72° N, 71.36° W), dried, hand ground with a mortar and pestle, and mixed with soil. Soil was collected from salt marshes from several Narragansett Bay locations and homogenized using a cement mixer. Ulva was added to soil at weights of 60 g per 10 cm × 35 cm pot (16.2 g L⁻¹), with 6 pots receiving Ulva additions and 6 serving as untreated controls. Small sods of tall-form S. alterniflora were collected from Bristol, RI (Colt State Park; 41.68° N, 71.30° W), using the same small steel coring device. Semi-diurnal tides were simulated in mesocosms using a standpipe and pumps (Watson et al. 2014) and pots were alternated weekly between high and low shelves to simulate spring and neap tides (Fig. 1). The experiment was run for 93 d: 9 June 2013 to 10 September 2013.

S. alterniflora height and density were monitored at the beginning and end of the experiment, and standing aboveground biomass was harvested at the end of the growing season, washed to remove salts and sediment, and dried to constant weight. The number of shoots with inflorescences was monitored biweekly.

Many of the variables measured in the field experiment were omitted from the follow-up greenhouse study, as our purpose was not to replicate the previous experiment, but to ensure that our principal result (negative effects of Ulva on salt marsh plant growth) was repeatable. The follow-up experiment also altered many experimental details (tall-form S. alterniflora was used, plant and soil collection locations were 150+ km distant), to test the universality of our results.

In addition, a chemical analysis was performed on a small subset of treated and untreated soils from the greenhouse experiment and dried Ulva to investigate the presence of potentially toxic cyclic sulfur compounds. Dithiolane and trithiane have been associated with allelopathic effects on phytoplankton (Tang & Gobler 2011). We analyzed our soils and dried Ulva for 1,3-dithiolane and 1,3,5-trithiane content. For chemical analysis, freeze-dried samples were extracted by the accelerated solvent extraction method using methylene chloride (Cantwell et al. 2010). Extract volume was reduced and solvent exchanged to hexane, with a final volume adjusted to 1 ml. Extracts were analyzed by gas chromatography–mass spectrometry (Agilent 5975C series gas chromatograph equipped with a mass selective detector) using a 30 m × 0.30 mm inner diameter J&W DB 5-ms capillary column.

Statistical analysis

For the field mesocosm, 2-way ANOVA was used to evaluate effects and interactions of Ulva additions and elevation on plant growth response and related variables. Data from the 2011 experiment were also included, and rank-transformations were employed, as raw and log-transformed data did not meet assumptions for a strictly parametric test (Conover & Inman 1981). Fisher’s least significant difference (LSD) post-hoc test was used to identify significant differences between treatment groups. Where a significant interaction was identified for 2-way ANOVAs, a simple main effects follow-up analysis was performed. For the greenhouse experiment, a series of t-tests was used to compare plant growth response variables in pots treated with Ulva and untreated control pots. Flowering data were analyzed using a non-parametric nominal approach using contingency tables. The association between lack of flowering and Ulva additions was tested for significance using Pearson’s chi-squared test in the case of the field experiments and Fisher’s exact probability test (an analog to the Pearson’s test for small sample sizes) for the greenhouse experiment. To facilitate comparisons with other studies, above- and belowground biomass values were analyzed as g m⁻² and shoots m⁻², and shoot elongation rates were calculated as the change in average shoot height, per experimental unit, for the full growth period (spring to fall).

For the mesocosm experiment, a multiple comparison test for porewater was not performed, as only one value per treatment combination per elevation period was available, and results varied through time. For the greenhouse experiment, a t-test was performed on concentrations of 1,3,5-trithiane in Ulva-treated and untreated soils to compare plant growth and soil organosulfur content with and without Ulva additions. To explore covariation between soil parameters and biomass from field mesocosms, a correlation matrix was produced. The variables used were concentration of porewater ammonium, phosphate and sulfide, and bare soil CO₂ flux and belowground biomass. For each pot, associations were measured using mean values for porewater. In 2 cases (porewater PO₄³⁻ and NH₄⁺ against belowground biomass) data were log transformed to meet the assumption of homoscedasticity of residual error values. Owing to the high level of covariation present in biogeochemical variables, we used a Bonferroni correction to adjust the significance level from α = 0.05 to α = 0.005 (0.05/10 comparisons) to identify significant correlations.
Multiple comparison tests were conducted in SAS v.9 and correlation tests were completed using Stat Plus Excel extension for Mac. The Pearson’s chi-squared and Fisher’s exact probability tests were computed manually. Statistical analyses were performed following Helsel & Hirsch (2002).

RESULTS

Plant growth response

*Spartina alterniflora* growth was altered by *Ulva* soil additions in field mesocosms (Fig. 2, Table 1). Aboveground biomass was unchanged by the high *Ulva* treatment, but was significantly reduced by the very high *Ulva* treatment. Biomass also responded to elevation, with a strong trend of lower biomass where the very high *Ulva* treatment was combined with low elevation (Fig. 2). End-of-season belowground biomass, mean shoot height, elongation rate and longest shoot height were also significantly reduced by *Ulva* additions, with the greatest growth achieved without *Ulva* additions and the lowest growth achieved with the very high *Ulva* treatment (Fig. 2, Table 1). For shoot density there was a significant interaction between *Ulva* addition and elevation, and hence a simple main effects model was performed. This model found *Ulva* additions had a significant effect on shoot density for 3 of 5 elevation treatments. At level 2, *Ulva* additions significantly reduced shoot density, while at levels 4 and 5, *Ulva* additions were associated with enhanced shoot density. However, the simple main effects model found few significant differences among elevation treatments (1, 2 < 5 for *Ulva* additions; Fig. 2).

The greenhouse experiment was able to reproduce the negative impacts of *Ulva* soil additions on *S. alterniflora* growth seen in field experiments. Aboveground biomass, mean shoot height, maximum shoot height and elongation rate were significantly reduced for *S. alterniflora* grown in *Ulva*-treated soils in greenhouse mesocosms (Fig. 3; belowground biomass was not measured).

Flowering was also affected by *Ulva* additions. For field mesocosms, 1 to 5 inflorescences were found per untreated pot and flowering occurred across elevation treatments at the end of the growing season; however, flowering was completely absent for plants grown in *Ulva*-treated soil (Fig. 4A). For laboratory mesocosms, plants grown in *Ulva*-treated soil were 5 times less likely to flower than those grown without *Ulva* additions (Fig. 4B). Using a Pearson’s chi-
squared test, a significant association was found between field Ulva additions and reduced flowering ($\chi^2 = 56.13, p < 0.001$) in field mesocosms. For the greenhouse experiment, a Fisher’s exact probability test also indicated a significant association between reduced flowering and Ulva additions ($p = 0.008$).

Soil response
High porewater nutrient concentrations were found in Ulva-treated soils in the field mesocosms (Fig. 5). Both levels of Ulva additions increased porewater ammonium ($\text{NH}_4^+$) and phosphate ($\text{PO}_4^{3-}$) to

Table 1. Summary of 2-way ANOVA for effects of Ulva addition and elevation on growth, soil and tracer recovery. To report significant differences between treatments for the Fisher’s LSD post-hoc tests, Ulva treatments are abbreviated: N: no Ulva, 2011; H: high Ulva, 2012; HH: very high Ulva, 2012. Elevations are abbreviated from 1 to 5 (1 = lowest, 5 = highest). Bold: significant effects ($p < 0.05$)

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Fig. 3. Growth response of Spartina alterniflora to Ulva addition treatments in the greenhouse experiment. (A) Aboveground biomass, (B) mean shoot height, (C) maximum shoot height, (D) shoot density and (E) mean shoot elongation rate. Error bars: SE ($n = 6$ for each treatment)

Fig. 4. Flowering response of Spartina alterniflora grown in Ulva-treated and untreated soils in (A) field and (B) laboratory mesocosms. Error bars: SE
concentrations that were 40 to 70 times and 7 to 10 times greater, respectively, than found in untreated mesocosms from 2011 (Fig. 5A,B). To put these values in perspective, the ammonium concentrations expected from the immediate decomposition of the added Ulva would have been 7 and 28 mM (i.e. 7000 to 28000 μM) in the high and very high Ulva treatments, respectively, which was consistent with what was measured in porewater. The theoretical release of phosphate from the decomposed Ulva in the high and very high treatments was 200 and 800 μM, exactly what was measured. Values for porewater ammonium and phosphate (for ambient and high Ulva) also varied with elevation, with higher concentrations in lower-elevation pots, for all Ulva treatments.

Ulva soil treatments were usually associated with elevated hydrogen sulfide concentrations, pH, salinity and bare soil CO₂ efflux rates (Fig. 5). Averaging across elevations, increases in porewater salinity, pH, hydrogen sulfide concentrations and soil CO₂ effluxes in Ulva treatments were 8–15, 5–12, 110–1000 and 68–330%, respectively. Examining salinity across elevations revealed a trend towards higher soil salinity in pots where Ulva additions were combined with high elevation. Conversely, porewater pH declined with increasing elevation, independent of Ulva treatment (Fig. 5). Porewater hydrogen sulfide concentrations and CO₂ efflux rates also responded to both elevation and soil treatments: sulfide concentrations were higher at low elevations and in pots receiving Ulva additions, while CO₂ efflux rates were higher at high elevations and in pots receiving Ulva additions.

Porewater nutrient concentrations changed over time (Fig. 6). Within 3 d of preparing Ulva-treated soil, porewater ammonium concentrations were 4 to 10 times greater than spring measures conducted on untreated soil for the previous year (comparison of 13 May 2012 with 31 May 2011). And within 1 mo, concentrations spiked to 50 to 68 times greater than untreated soil (i.e. 10 to 20 mM); after this time, concentrations declined (Fig. 6A). Porewater phosphate concentrations were low immediately after soil additions, but increased steadily for the first 3 mo of the experiment, after which they plateaued (Fig. 6B).

Significant negative relationships were found between belowground biomass and porewater ammonium, phosphate and sulfide concentrations (Fig. 7). Porewater sulfide, nutrients and CO₂ flux rates were further positively interrelated.

**Fig. 5.** Mean values for (A) porewater ammonium, (B) phosphate, (C) hydrogen sulfide, (D) pH and (E) salinity, and for (F) CO₂ efflux for each treatment combination. Data are for field mesocosm experiments. Error bars: SE
Nutrient uptake

Between 29 and 53% of the $^{15}$N tracer, added as Ulva, was lost from soils over the 5-mo field experiment (Table 2). Tracer recovery from soil was apparent in above- and belowground biomass at the time of harvest but no trends were found regarding treatment or elevation (Table 1). Recovery for shoots ranged from 0 to 10.3%, with high-dosage Ulva mesocosms having greater shoot tracer recovery rates than the very high Ulva treatment (Table 2). Recovery of tracer from belowground biomass was similar to that for aboveground biomass, with values ranging from 0.2 to 11.3%. Similar to aboveground measures, greater tracer recovery occurred in mesocosm pots with lower Ulva exposure (Table 2).

Organosulfur compounds

Recovery of chemicals from Ottawa sand, used as a carrier and spiked to ~40 ng g$^{-1}$, ranged from 87 to 109%, indicating that both 1,3-dithiolane and 1,3,5-trithiane were extractable. Neither 1,3-dithiolane nor 1,3,5-trithiane was detected in the dried Ulva samples, but the latter was found in both Ulva-treated and untreated soils. Soils treated with high levels of Ulva, collected at the end of the experiment, had concentrations of 1,3,5-trithiane ranging from 126 to 198 ng g$^{-1}$ dry soil, while untreated soil had concentrations ranging from 12.3 to 15.3 ng g$^{-1}$ dry soil. A $t$-test identified significant differences between soils with and lacking Ulva additions ($t = 8.45$; $p < 0.001$).
DISCUSSION

Growth reductions

This study has identified negative impacts of Ulva soil additions on a salt marsh macrophyte independent of shading or photoinhibition effects, including reductions in above- and belowground biomass, shoot height and shoot elongation rate. In addition, plant growth variables also responded to elevation, with higher growth achieved under less inundated conditions (high elevation). These results may help explain previous studies where algae additions enhanced growth in non-tidal experiments (Boyer & Fong 2005, Newton & Thornber 2013), but negative impacts were noted in field experiments (van Hulzen et al. 2006, Newton & Thornber 2013), or where macroalgae removals increased growth (Tyrrell et al. 2012). This discrepancy may be explained by the effect of soil oxidation. When plants are grown under non-tidal conditions, and in our experiment at high elevations, soils are more oxidized, and levels of soil hydrogen sulfide and ammonium do not accumulate to levels likely to impact plant growth. In contrast, where plants are grown with Ulva additions under tidal conditions, and in our experiment at high levels of inundation, anoxic conditions occur and levels of ammonium and sulfides are able to accumulate to potentially stressful levels. These results suggest that Spartina alterniflora growing at low tidal elevations, or under poorly drained conditions, is at higher risk of suffering adverse effects of Ulva than S. alterniflora growing at higher tidal elevations. This differential impact of macroalgal accumulation on marshes as a function of tidal height is also supported by field studies. Two areas in the USA known for rapid marsh loss (Jamaica Bay, New York, and Elkhorn Slough, California) are locations where rapid tidal flooding increases (due to hydrological alterations) co-occur with high nutrient loads and intermittent massive (up to 10 kg m\(^{-2}\)) Ulva accumulations on marshes. These observations, combined with experimental evidence, suggest that high nutrient loads, manifesting as massive macroalgae blooms, may exacerbate coastal marsh loss.

Possible mechanisms for reduction of S. alterniflora growth

We propose, and review the evidence for, potential mechanisms to account for the negative impacts of Ulva additions on S. alterniflora growth. These mech-
Vascular plants experience ammonium toxicity when supplied with high concentrations of NH$_4^+$, or high NH$_4^+$/NO$_3^-$ ratios (Britto et al. 2001). This syndrome is characterized by chlorosis (yellowing) of leaves, depressed growth, reductions in root:shoot and fine:coarse root ratios, and plant mortality (Britto et al. 2001). While ammonium toxicity is a known ecosystem stressor for aquatic vegetation (van Katwijk et al. 1997), toxicity levels are known to co-vary with soil pH and range across several orders of magnitude (15 μM to 15 mM), making it difficult to estimate toxic concentrations for salt marsh taxa. Studies of plants grown in treatment wetlands subjected to various levels of ammonium showed reductions in growth at concentrations of 5 to 10 mM (Clarke & Baldwin 2002). This is similar to the range of peak concentrations seen in our field mesocosm experiment. As S. alterniflora expressed symptoms of ammonium toxicity, such as growth reductions, depressed root:shoot ratios and chlorosis (Fig. 1), we hypothesize that at least some of the negative effects of Ulva additions noted in our study were due to elevated porewater ammonium concentrations. These concentrations greatly exceed the range of 10 to 100 μM porewater NH$_4^+$ typically observed in the field (PILTERS 2014).

Hydrogen sulfide is also a potent phytotoxin and was found at elevated levels in our Ulva-treated soils. In waterlogged soils, hydrogen sulfide is produced metabolically by prokaryotes that oxidize organic compounds and use sulfate as a terminal electron receptor (Valdemarsen et al. 2010). In salt marshes, hydrogen sulfide has been associated with decreased above- and belowground productivity and nutrient uptake (King et al. 1982, Koch & Mendelssohn 1989). Porewater sulfide concentrations in excess of 1000 μM are detrimental to growth for S. alterniflora (Koch et al. 1990, Mendelssohn & Morris 2000). Porewater values ≥1000 μM were found in our 3 lower-elevation field mesocosm treatments, and represented values that were substantially higher than those found in untreated soils. However, elevated sulfide levels are unlikely to be the sole cause of reduced growth for 2 reasons. First, belowground growth was reduced for the 2 highest elevations and, for these pots, sulfide concentrations were <500 μM. Second, porewater sulfide values of 1000 μM are relatively common for Atlantic salt marshes (e.g. Chambers et al. 1998), and therefore probably do not explain why some of the mesocosm pots contained few live shoots at the end of the growing season.

Our experiment also identified an association between Ulva soil additions and potentially elevated concentrations of organosulfur compounds. These compounds were not found in samples of dried Ulva, but were found in Ulva-treated soils. Hence, it is possible that, in addition to porewater hydrogen sulfide, other sulfur-containing compounds related to Ulva decomposition may play a role in the negative impacts found in this study. Ulva is often considered allelopathic (Wang et al. 2007), and tomato and lettuce appear to be sensitive to the allelopathic compounds of Ulva lactuca (Hassan & Ghareib 2009). Algicidal compounds have been noted to have inhibitory effects on growth of the model organism Arabidiposis thaliana (Berry 2011). Although evidence for allelopathy in our experiments is indirect, we suspect that allelochemicals may play some role in the negative impacts on S. alterniflora growth found in our study.

In conclusion, we propose that Ulva soil additions reduced growth of S. alterniflora by 3 potential mechanisms: elevated porewater ammonium and sulfide concentrations, and the presence of organosulfur compounds. We feel confident that elevated hydrogen sulfide concentration was not the sole stressor for plant growth, but without further experimentation we are uncertain of the specific roles of ammonium toxicity, organosulfur compounds, other unmeasured stressors (e.g. anoxia), or potential synergistic effects.

**Implications**

**Flowering**

The inhibition of flowering by Ulva additions has not been reported by previous field manipulations (Newton & Thornber 2013), although a similar result was found in an unpublished field study conducted at Elkhorn Slough, California (K. Wasson pers. comm.) involving Ulva spp. and the marsh succulent Sarcocornia pacifica. Although floral development in marsh macrophytes has received little attention relative to crop plants, the association between Ulva additions and low rates of floral development found in this study may be related to the elevated levels of ammonium or hydrogen sulfide found in porewater in Ulva-treated soils. Hydrogen sulfide, in particular, has recently been identified as a signaling molecule in plants (Lisjak et al. 2011), and the elevated concentrations found in the Ulva-treated pots may have interfered with floral development. Reductions in...
sexual reproduction linked to biogeochemical impacts of ecosystem processes may reduce the capacity of S. alterniflora populations to respond to impacts of global change.

Biogeochemistry

The results of our experiments provide new information on potential biogeochemical impacts of Ulva in salt marshes. First, we noted that a strong pulse of ammonium was released immediately upon preparing the soil additions. After only 2 d, ammonium concentrations were elevated over background concentrations, and within 1 mo, porewater ammonium concentrations (10 to 20 mM) were similar to those expected if all of the Ulva-N had been mineralized to ammonium (7 to 28 mM).

These results are in accord with previous in situ and mesocosm experiments that have documented the very rapid decomposition of Ulva mats (Buchsbaum et al. 1991). Additionally, Ulva-N is principally mineralized as ammonium, even under oxidized conditions (Lomstein et al. 2006). Taken in sum, these results suggest that decaying Ulva mats deposited in salt marshes provide a rapid pulse of NH$_4^+$-N that, depending on the N concentration of Ulva and thickness of the mat, may elevate NH$_4^+$ concentrations to levels that may not be assimilated by salt marsh plants. This interpretation is also supported by our use of a $^{15}$N tracer. Plants that received high dosages of Ulva had higher porewater ammonium, but reduced uptake of Ulva-derived N.

Most biogeochemical responses to Ulva additions were not dose-dependent. Porewater ammonium, sulfide and phosphate concentrations and carbon dioxide flux did not scale equally with the weight of Ulva added. Mean concentrations of ammonium and phosphate in porewater were only 75 and 33% higher, respectively, in the very high Ulva treatment than in the high treatment, even though they contained 4 times the weight of Ulva. Similarly, sulfide concentrations were only 19% greater in the very high Ulva treatments and carbon dioxide flux rates were only 47% higher. We interpret this non-linearity as a result of bacterially mediated processes. Transformations may have been limited by bacterial activity, via a saturation effect (Aller & Aller 1998), or alternatively, may have been promoted by priming. Priming effects have been observed when labile organic inputs to soil stimulate microbial mineralization of pre-existing organic matter (Fontaine et al. 2004). Given that impacts of Ulva additions did not scale with the weight of Ulva added, our results suggest that even much lower levels of Ulva exposure may have similar effects on soil biogeochemistry.

The results reported here also suggest that the biogeochemical response to Ulva additions varies with inundation regime. At low tidal elevations, sulfide and ammonium accumulated to high levels. In contrast, soils higher in the intertidal zone showed reduced values of these compounds. More aerated conditions can promote the oxidation of sulfides to sulfates (Howes et al. 1984) and facilitate coupled nitrification–denitrification (Seitzinger & Giblin 1996). In addition, more aerated conditions could also promote gaseous diffusion or volatilization (Ponnampерuma 1984). Overall, growth impacts appear to be elevation- or redox-dependent, with submerging or poorly drained marshes therefore more vulnerable to the biogeochemical impacts of macroalage accumulations.

Comparison with field conditions

How do these soil addition experiments compare with field conditions? While burial of Ulva does occur (Ford et al. 1999), and dried Ulva has been used in studies to produce soil homogenates (e.g. Rossi 2006, García-Robledo et al. 2013), Ulva is more common in salt marshes as wrack entangled in stems of marsh macrophytes. Additionally, the weights of the soil treatments tested, particularly the higher dosages, were higher than typically occur in the field. Assuming that Ulva mats impact benthic soil to a depth of 20 cm, our high dosage (ca. 5 g l$^{-1}$) roughly corresponds to a field dosage of 1 kg m$^{-2}$ dry wt, or 10 kg m$^{-2}$ wet wt of Ulva; values that are realistic (e.g. Viaroli et al. 2005), but only for highly eutrophic estuaries. Ulva accumulations exceeding 10 kg m$^{-2}$ wet wt have been reported (Sfriso et al. 1991), but are undoubtedly rare. Additionally, our initial attempt to simulate episodic deposition of Ulva using higher soil additions was unsuccessful because of rapid decomposition: we had assumed that the Ulva would decompose slowly over the growing season, which turned out not to be the case. Lastly, some of the ammonium or other potential phytotoxins released from Ulva mats accumulating in salt marshes may be washed away by the tides, limiting effects on marsh vegetation. In summary, many elements of our experiment represent a worst-case scenario: maximum Ulva mass in a dry and labile form, and burial to prevent tidal flushing. Our results are therefore mainly applicable to only eutrophic estuaries where large rafts of Ulva deposit in marshes,
and additional field and laboratory studies are needed to establish whether similarly adverse responses are observed under less dramatic conditions.

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LITERATURE CITED


Watson et al.: Negative impacts of Ulva
decline of coastal marshes in Chesapeake and Delaware Bays. EOS Trans Am Geophys Union 83:173–178


Morris JT, Sundberg K, Hopkinson CS (2013) Salt marsh primary production and its responses to relative sea level and nutrients in estuaries at Plum Island, Massachusetts, and North Inlet, South Carolina, USA. Oceanography 26:78–84


Strickland JDH, Parsons TR (1972) A practical handbook of seawater analysis. Fisheries Research Board of Canada, Ottawa


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