Vol. 532: 59–72, 2015
doi: 10.3354/meps11334

MARINE ECOLOGY PROGRESS SERIES
Mar Ecol Prog Ser

Published July 21

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

E. B. Watson[1,4,*], C. Wigand[1], A. J. Oczkowski[1], K. Sundberg[2], D. Vendettuoli[1], S. Jayaraman[1], K. Saliba[1], J. T. Morris[3]

[1]Atlantic Ecology Division, US Environmental Protection Agency, ORD-NHEERL, Narragansett, RI 02882, USA
[2]Baruch Marine Field Laboratory, University of South Carolina, Georgetown, SC 29440, USA
[3]Belle Baruch Institute for Marine & Coastal Sciences, University of South Carolina, Columbia, SC 29208, USA

[4]*Present address:* Department of Biodiversity, Earth & Environmental Science, Academy of Natural Sciences of Drexel University, Philadelphia, PA 19103, USA

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 ^{15}N 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 macroalgae canopies and decaying mats can have detrimental effects on aquatic biota and water quality (Kamer et al. 2001). Episodic macroalgae 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

*Corresponding author: elizabeth.b.watson@gmail.com

through the decomposition of algal biomass (Sfriso & Pavoni 1994). In some shallow coastal lagoons, seasonal macroalgae cycles exert a greater control on water column dissolved nitrogen values than allochthonous inputs (Sfriso 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, macroalage accumulations exceeding our lower dosage level have been reported in the literature (Sfriso 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.

2013). 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-

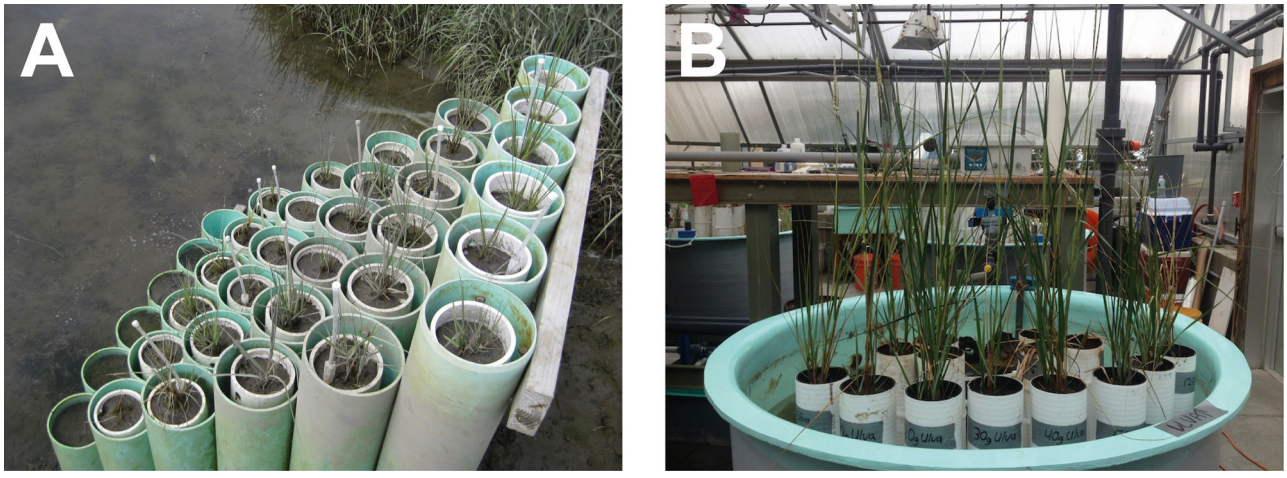

Fig. 1. (A) Marsh organ deployed at Plum Island Sound estuary, Massachusetts, USA; (B) mesocosm tanks in the greenhouse laboratory at the US Environmental Protection Agency (EPA) facility. The Plum Island photo was taken about 5 wk into the experiment, and some of the sods at the lowest elevations had 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)

parative data (see Supplement 1 at www.int-res.com/articles/suppl/m532p059_supp.pdf).

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 micropiezometer 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, $Zn(O_2CCH_3)_2$. 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 H_2SO_4:20 ml porewater) for later nutrient analysis. Porewater samples were analyzed for hydrogen sulfide, orthophosphate and ammonium using spectrophotometeric 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):

$$\text{porewater } [NH_4^+ \text{ or } PO_4^{3-}]\ (\mu\text{mol l}^{-1}) = \frac{\textit{Ulva} \text{ addition } \left(\frac{\text{g}}{\text{pot}}\right) \times [\text{N or P}]\ (\mu\text{mol g}_{\text{dw}}^{-1})}{\text{porewater volume (l)}} \quad (1)$$

where dw represents dry weight. After harvest of aboveground biomass, cut shoots were plugged with silicone and bare soil CO_2 efflux was measured as CO_2 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).

^{15}N enrichment

Living *Ulva* was labeled with a ^{15}N 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-N_2) such that $\delta^{15}N = \frac{R_{\text{sample}} - R_{\text{standard}}}{R_{\text{standard}}} \times 1000$, where R is the ratio of ^{15}N to ^{14}N. Unaltered soils and plants were also analyzed to calculate tracer concentrations.

The enrichment of ^{15}N in mesocosm pots and above- and belowground biomass was calculated using a mass balance approach as the absolute abundance of ^{15}N frequency in terms of atomic percent (atom%) (Currie 2007) (Eq. 2), converted to atom% excess by subtracting the atom% in background samples (atom% $^{15}N_b$) from amended samples (atom% $^{15}N_a$) (Eq. 3). Soil ^{15}N 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 ^{15}N concentration and biomass weights. Detailed calculations can be found in Supplement 2 at www.int-res.com/articles/suppl/m532p059_supp.pdf.

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

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

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 CO_2 flux and belowground biomass. For each pot, associations were measured using mean values for porewater. In 2 cases (porewater PO_4^{3-} and NH_4^+ 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 $\alpha = 0.05$ to $\alpha = 0.005$ (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-

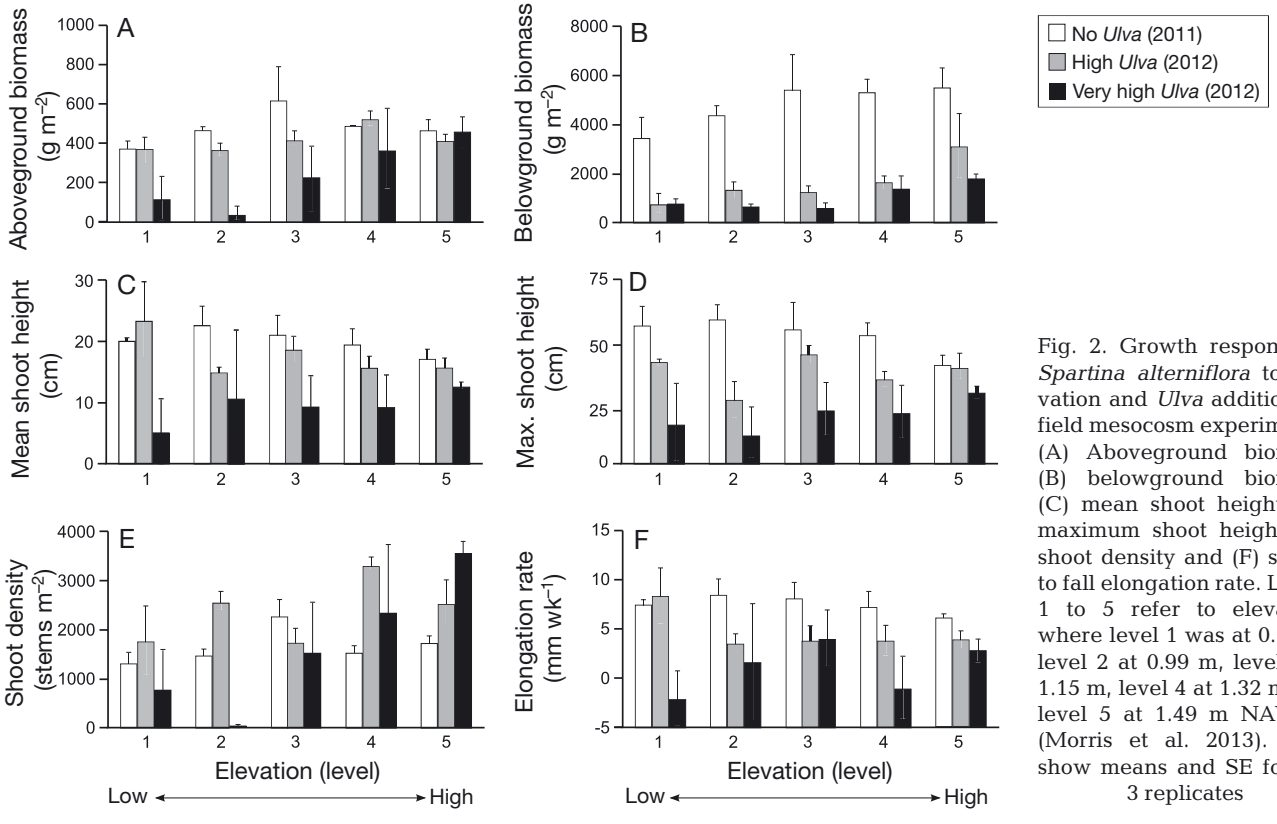

Fig. 2. Growth response of *Spartina alterniflora* to elevation and *Ulva* additions in field mesocosm experiments. (A) Aboveground biomass, (B) belowground biomass, (C) mean shoot height, (D) maximum shoot height, (E) shoot density and (F) spring to fall elongation rate. Levels 1 to 5 refer to elevation, where level 1 was at 0.82 m, level 2 at 0.99 m, level 3 at 1.15 m, level 4 at 1.32 m and level 5 at 1.49 m NAVD88 (Morris et al. 2013). Plots show means and SE for the 3 replicates

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 ($p < 0.05$)

	Ulva addition			Elevation			*Ulva* × elevation	
	F	p	LSD	*F*	p	LSD	*F*	p
Growth and soil response								
Aboveground biomass	4.79	**0.016**	N > HH	3.26	**0.025**	4 > 2,1, 5 > 1	0.89	0.54
Belowground biomass	61.65	**<0.001**	N > H > HH	7.49	**0.0003**	4,5 > 1,2,3	1.09	0.40
Mean shoot height	19.54	**<0.001**	N > H > HH	0.84	0.51		0.81	0.60
Longest shoot height	21.30	**<0.0001**	N > H > HH	0.49	0.75		0.97	0.48
Shoot density	3.51	**0.043**	H > N	4.03	**0.0099**	4,5 > 1,2	2.65	**0.025**
Elongation rate	21.28	**<0.001**	N > H > HH	0.68	0.61		0.99	0.47
Carbon dioxide efflux	14.66	**<0.001**	HH,H > N	4.26	**0.0076**	3,4,5 > 1	0.75	0.65
Tracer								
Soil tracer loss	0.10	0.75		0.18	0.95		0.80	0.54
Shoot tracer recovery	15.54	**0.0008**	H > HH	2.19	0.11		1.19	0.35
Root tracer recovery	13.29	**0.0016**	H > HH	7.86	**0.0006**	4,5 > 1,2,3	2.00	0.13

squared test, a significant association was found between field *Ulva* additions and reduced flowering ($\chi^2 = 56.1$, $p < 0.001$) 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 (NH_4^+) and phosphate (PO_4^{3-}) to

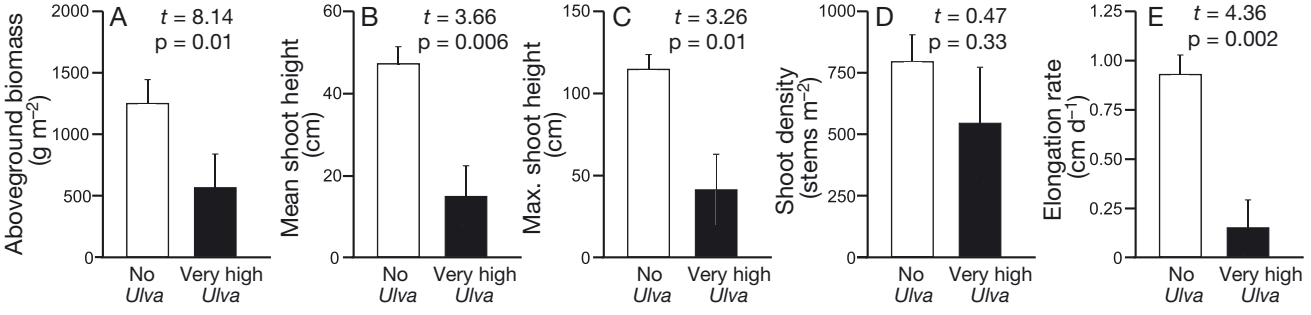

Fig. 3. Growth response of *Spartina alterniflora* to *Ulva* addition treatments in the greenhouse experiment. (A) Aboveground biomass, (B) mean shoot height, (C) maximum shoot height, (D) shoot density and (E) mean shoot elongation rate. Error bars: SE (n = 6 for each treatment)

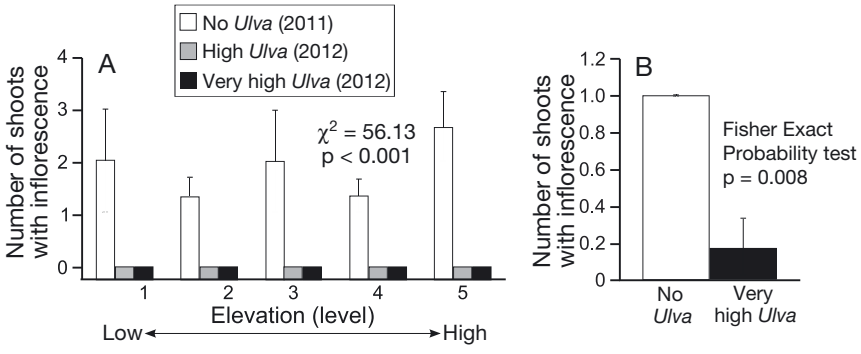

Fig. 4. Flowering response of *Spartina alterniflora* grown in *Ulva*-treated and untreated soils in (A) field and (B) laboratory mesocosms. Error bars: SE

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 28 000 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 CO_2 efflux rates (Fig. 5). Averaging across elevations, increases in porewater salinity, pH, hydrogen sulfide concentrations and soil CO_2 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 CO_2 efflux rates also responded to both elevation and soil treatments: sulfide concentrations were higher at low elevations and in pots receiving *Ulva* additions, while CO_2 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 CO_2 flux rates were further positively interrelated.

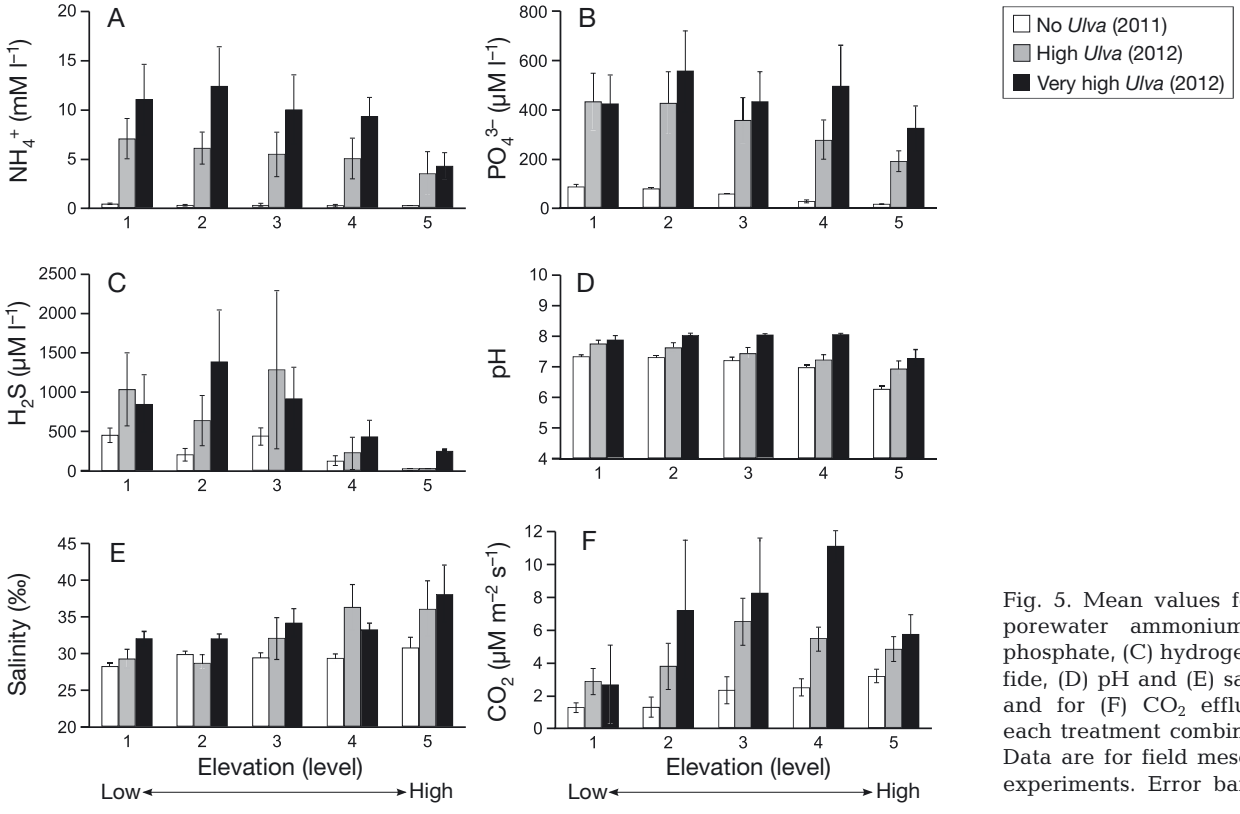

Fig. 5. Mean values for (A) porewater ammonium, (B) phosphate, (C) hydrogen sulfide, (D) pH and (E) salinity, and for (F) CO_2 efflux for each treatment combination. Data are for field mesocosm experiments. Error bars: SE

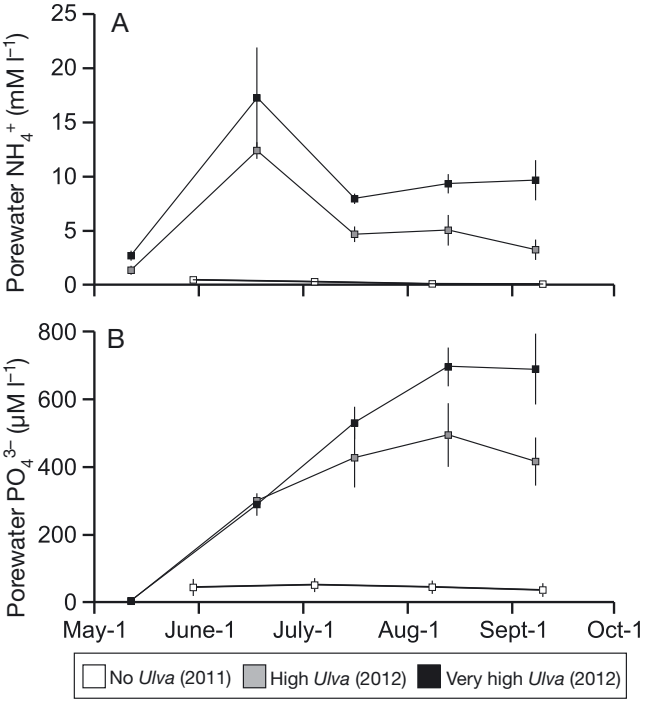

Fig. 6. Variations in (A) ammonium and (B) phosphate over the course of the experiments in field mesocosms. Values were averaged across elevation treatments. Error bars: SE

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 n g^{-1} dry soil. A *t*-test identified significant differences between soils with and lacking *Ulva* additions (t = 8.45; $p < 0.001$).

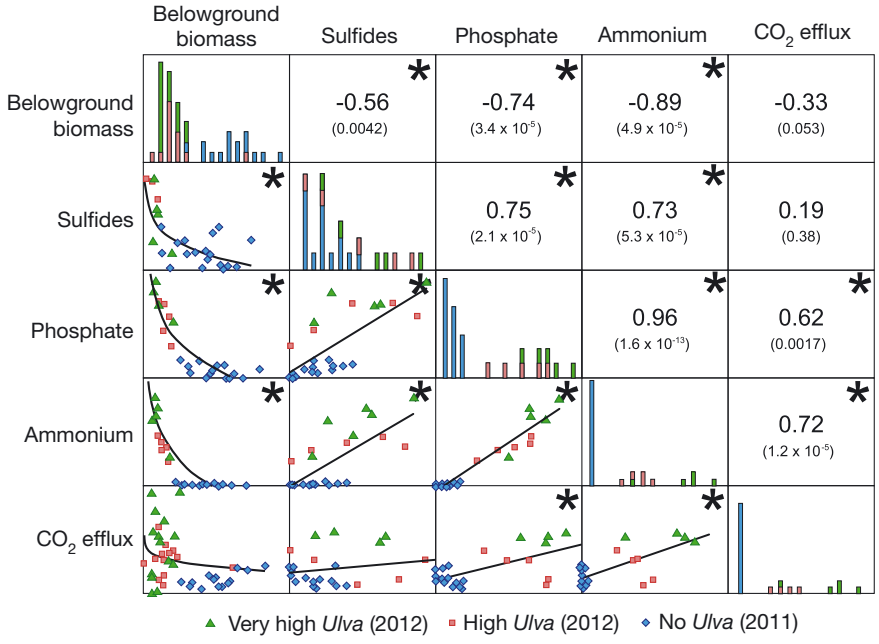

Fig. 7. Correlation matrix showing covariance between belowground biomass, porewater sulfide, ammonium, phosphate and bare soil carbon dioxide flux measured at the end of the growing season for field mesocosm experiments. Histograms show the distribution of measured values across *Ulva* treatments. Numerical values indicate the Pearson correlation coefficient (r) and values in parentheses are p-values for the correlation. The analysis was performed on ln-transformed values for porewater sulfide, phosphate, ammonium and CO_2 efflux (against belowground biomass only). *Correlation coefficients significant at $\alpha = 0.002$

Table 2. Tracer recovery by *Spartina alterniflora* above- and belowground biomass, and tracer loss from soil. Values are mean (and SE) per treatment pot. Elevations are abbreviated from 1 to 5 (1 = lowest, 5 = highest). For belowground biomass, n = 3; for aboveground, sample size varies (0 ≤ n ≤ 3), as some pots had only dead shoots

Elevation	Treatment	Aboveground biomass			Belowground biomass			Mesocosm soils		
		$\delta^{15}N$ (‰)	N (mg)	Tracer recovery (%)	$\delta^{15}N$ (‰)	N (mg)	Tracer recovery (%)	$\delta^{15}N$ (‰)	N (mg)	Tracer loss (%)
1	High *Ulva*	473 (35.6)	68.8 (12.2)	7.05 (2.09)	119 (51.2)	47.5 (23.0)	1.77 (0.99)	48.3 (0.48)	6.42 (0.44)	41.0 (17.1)
1	Very high *Ulva*	69.8 (n = 1)	23.3 (21.8)	1.45 (1.37)	18.9 (6.26)	57.2 (12.1)	0.77 (0.56)	15.9 (1.57)	5.63 (0.42)	38.4 (4.1)
2	High *Ulva*	446 (20.3)	65.5 (11.3)	7.14 (1.05)	174 (33.9)	92.9 (23.4)	3.50 (0.27)	46.8 (1.09)	5.80 (0.34)	36.7 (5.7)
2	Very high *Ulva**				13.5 (2.19)	43.7 (6.36)	0.20 (0.056)	15.1 (0.07)	5.63 (0.28)	52.6 (7.9)
3	High *Ulva*	493 (21.9)	69.7 (10.5)	8.35 (1.16)	164 (49.9)	88.2 (19.4)	3.00 (0.30)	46.3 (1.78)	6.68 (0.82)	31.8 (14.1)
3	Very high *Ulva**	79.3 (2.20)	46.6 (34.8)	3.24 (2.55)	20.4 (4.04)	52.8 (10.8)	0.60 (0.22)	15.8 (0.55)	5.52 (0.16)	47.2 (5.4)
4	High *Ulva*	467 (19.9)	87.0 (6.17)	10.25 (1.32)	205 (41.6)	123 (14.5)	5.86 (0.54)	41.2 (0.54)	6.60 (0.39)	37.6 (10.8)
4	Very high *Ulva**	82.9 (0.35)	88.9 (50.2)	5.89 (3.25)	30.8 (9.14)	113 (37.7)	2.93 (1.63)	14.0 (0.31)	6.69 (0.17)	37.3 (1.5)
5	High *Ulva*	488.4 (24.8)	78.5 (8.78)	6.86 (3.26)	191 (57.8)	261 (93.7)	11.32 (8.09)	43.3 (2.29)	7.49 (0.30)	50.7 (41.2)
5	Very high *Ulva**	83.1 (1.79)	100.0 (9.60)	6.98 (0.91)	43.9 (4.47)	142 (5.30)	4.91 (0.98)	14.3 (1.67)	7.37 (0.16)	28.8 (3.3)

*Some biomass remained for this level, but had been dead for many months, and therefore was excluded as a tracer sink and not sampled

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 macroalage accumulations 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-

anisms include ammonium toxicity, sulfide toxicity and allelopathic compounds.

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 *Arabadiposis 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 amount 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 (Ponnamperuma 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.

Acknowledgements. We acknowledge E. Markham, A. Hanson and A. Pivarnik for assistance with field sampling and laboratory processing, and 3 anonymous reviewers for their helpful comments. G. Thurby, R. Johnson and P. Pelletier provided helpful input on an earlier version of the manuscript, and K. Wasson, A. Woolfolk, R. Eby and R. Kvist Preisler contributed to helpful discussions. The report is tracking number ORD-007487 of the US EPA's Office of Research and Development, National Health and Environmental Effects Research Laboratory, Atlantic Ecology Division. Although the information in this document has been funded by the US EPA, it does not necessarily reflect the views of the agency and no official endorsement should be inferred. The field experiment was partially supported by NSF grant OCE 1238212 to the Marine Biological Laboratory. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

LITERATURE CITED

Aller RC, Aller JY (1998) The effect of biogenic irrigation intensity and solute exchange on diagenetic reaction rates in marine sediments. J Mar Res 56:905–936

Berry J (2011) Marine and freshwater microalgae as a potential source of novel herbicides. In: Kortekamp A (ed) Herbicides and environment. InTech, Rijeka, p 705–734

Bierzychudek A, D'Avanzo C, Valiela I (1993) Effects of macroalgae, night and day, on ammonium profiles in Waquoit Bay. Biol Bull 185:330–331

Boyer KE, Fong P (2005) Macroalgal-mediated transfers of water column nitrogen to intertidal sediments and salt marsh plants. J Exp Mar Biol Ecol 321:59–69

Bradley PM, Morris JT (1990) Influence of oxygen and sulfide concentration on nitrogen uptake kinetics in *Spartina alterniflora*. Ecology 71:282–287

Britto DT, Siddiqi MY, Glass ADM, Kronzucker HJ (2001) Futile transmembrane NH_4^+ cycling: a cellular hypothesis to explain ammonium toxicity in plants. Proc Natl Acad Sci USA 98:4255–4258

Buchsbaum R, Valiela I, Swain T, Dzierzeski M, Allen S (1991) Available and refractory nitrogen in detritus of coastal vascular plants and macroalgae. Mar Ecol Prog Ser 72:131–143

Cantwell MG, Wilson BA, Zhu J, Wallace GT and others (2010) Temporal trends of triclosan contamination in dated sediment cores from four urbanized estuaries: evidence of preservation and accumulation. Chemosphere 78:347–352

Chambers RM, Mozdzer TJ, Ambrose JC (1998) Effects of salinity and sulfide on the distribution of *Phragmites australis* and *Spartina alterniflora* in a tidal saltmarsh. Aquat Bot 62:161–169

Clarke E, Baldwin AH (2002) Responses of wetland plants to ammonia and water level. Ecol Eng 18:257–264

Cline JD (1969) Spectrophotometric determination of hydrogen sulfide in natural waters. Limnol Oceanogr 14: 454–458

Conover WJ, Inman RL (1981) Rank transformation as a bridge between parametric and nonparametric statistics. Am Stat 35:124–129

Currie WS (2007) Modeling the dynamics of stable-isotope ratios for ecosystem biogeochemistry. In: Michener R, Lajtha K (eds) Stable isotopes in ecology and environmental science. Blackwell, Malden, MA, p 450–479

Duarte CM (1995) Submerged aquatic vegetation in relation to different nutrient regimes. Ophelia 41:87–112

Fletcher RL (1996) The occurrence of 'green tides'—a review. In: Schramm W, Nienjuis P (eds) Marine benthic vegetation. Springer, Berlin, p 7–43

Fong P, Donohoe RM, Zedler JB (1993) Competition with macroalgae and benthic cyanobacterial mats limits phytoplankton abundance in experimental microcosms. Mar Ecol Prog Ser 100:97–102

Fong P, Fong JJ, Fong CR (2004) Growth, nutrient storage, and release of dissolved organic nitrogen by *Enteromorpha intestinalis* in response to pulses of nitrogen and phosphorus. Aquat Bot 78:83–95

Fontaine S, Bardoux G, Abbadie L, Mariotti A (2004) Carbon input to soil may decrease soil carbon content. Ecol Lett 7:314–320

Ford RB, Thrush SF, Probert PK (1999) Macrobenthic colonisation of disturbances on an intertial sandflat: the influence of season and buried algae. Mar Ecol Prog Ser 191: 163–174

García-Robledo E, Revsbech NP, Risgaard-Petersen N, Corzo A (2013) Changes in N cycling induced by *Ulva* detritus enrichment of sediments. Aquat Microb Ecol 69: 113–122

Gerard VA (1999) Positive interactions between cordgrass, *Spartina alterniflora*, and the brown alga, *Ascophyllum nodosum* ecad *scorpioides*, in a mid-Atlantic coast salt marsh. J Exp Mar Biol Ecol 239:157–164

Guidone M, Thornber CS (2013) Examination of *Ulva* bloom species richness and relative abundance reveals two cryptically co-occurring bloom species in Narragansett Bay, Rhode Island. Harmful Algae 24:1–9

Hassan SM, Ghareib HR (2009) Bioactivity of *Ulva lactuca* L. acetone extract on germination and growth of lettuce and tomato plants. Afr J Biotechnol 8:3832–3838

Hauxwell J, Cebrián J, Furlong C, Valiela I (2001) Macroalgal canopies contribute to eelgrass (*Zostera marina*) decline in temperate estuarine ecosystems. Ecology 82: 1007–1022

Helsel DR, Hirsch RM (2002) Statistical methods in water resources. US Geological Survey, Reston, VA

Holmer M, Nielsen RM (2007) Effects of filamentous algal mats on sulfide invasion in eelgrass *Zostera marina*. J Exp Mar Biol Ecol 353:245–252

Holmquist JG (1997) Disturbance and gap formation in a marine benthic mosaic: influence of shifting macroalgal patches on seagrass structure and mobile invertebrates. Mar Ecol Prog Ser 158:121–130

Howes BL, Dacey JWH, King GM (1984) Carbon flow through oxygen and sulfate reduction pathways in salt marsh sediments. Limnol Oceanogr 29:1037–1051

Hull SC (1987) Macroalgal mats and species abundance: a field experiment. Estuar Coast Shelf Sci 25:519–532

Johnson DA, Welsh BL (1985) Detrimental effects of *Ulva lactuca* (L.) exudates and low oxygen on estuarine crab larvae. J Exp Mar Biol Ecol 86:73–83

Kamer K, Boyle KA, Fong P (2001) Macroalgal bloom dynamics in a highly eutrophic southern California estuary. Estuaries 24:623–635

Kearney MS, Rogers AS, Townshend JRG, Rizzo E, Stutzer D, Stevenson JC, Sundberg K (2002) Landsat imagery shows

decline of coastal marshes in Chesapeake and Delaware Bays. EOS Trans Am Geophys Union 83:173–178

King GM, Klug MJ, Wiegert RG, Chalmers AG (1982) Relation of soil water movement and sulfide concentration to *Spartina alterniflora* production in a Georgia salt marsh. Science 218:61–63

Kirwan ML, Murray AB, Boyd WS (2008) Temporary vegetation disturbance as an explanation for permanent loss of tidal wetlands. Geophys Res Lett 35:L05403, doi: 10.1029/2007GL032681

Koch MS, Mendelssohn IA (1989) Sulfide as a soil phytotoxin: differential responses in two marsh species. J Ecol 77:565–578

Koch MS, Mendelssohn IA, McKee KL (1990) Mechanism for the hydrogen sulfide-induced growth limitation in wetland macrophytes. Limnol Oceanogr 35:399–408

Koop-Jakobsen K, Giblin AE (2009) Anammox in tidal marsh sediments: the role of salinity, nitrogen loading, and marsh vegetation. Estuar Coast 32:238–245

Lisjak M, Teklic T, Wilson ID, Wood M, Whiteman M, Hancock JT (2011) Hydrogen sulfide effects on stomatal apertures. Plant Signal Behav 6:1444–1446

Lomstein BA, Guldberg LB, Neubauer AA, Hansen J and others (2006) Benthic decomposition of *Ulva lactuca*: a controlled laboratory experiment. Aquat Bot 85:271–281

McLaughlin K, Sutula M, Busse L, Anderson S and others (2014) A regional survey of the extent and magnitude of eutrophication in Mediterranean estuaries of Southern California, USA. Estuaries Coasts 37:259–278

Mendelssohn IA, Morris JT (2000) Eco-physiological controls on the productivity of *Spartina alterniflora* Loisel. In: Weinstein MP, Kreeger DA (eds) Concepts and controversies in tidal marsh ecology. Kluwer Academic, Dordrecht, p 59–80

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

Newton C, Thornber C (2012) Abundance and species composition surveys of macroalgal blooms in Rhode Island salt marshes. Northeast Nat 19:501–516

Newton C, Thornber C (2013) Ecological impacts of macroalgal blooms on salt marsh communities. Estuar Coast 36:365–376

O'Connor MI, Violin CR, Anton A, Ladwig LM, Piehler MF (2011) Salt marsh stabilization affects algal primary producers at the marsh edge. Wetlands Ecol Manag 19: 131–140

Orr M, Zimmer M, Jelinski DE, Mews M (2005) Wrack deposition on different beach types: spatial and temporal variation in the pattern of subsidy. Ecology 86:1496–1507

Pennings SC, Clark SM, Cleland EE, Collins SL and others (2005) Do individual plant species show predictable responses to nitrogen addition across multiple experiments? Oikos 110:547–555

PILTERS (Plum Island Long-Term Ecological Research Site) (2014) Plot-level marsh fertilization experiment. http://ecosystems.mbl.edu/pie/data/lte/LTE-MPDescription.htm

Ponnamperuma FN (1984) Effects of flooding on soils. In: Kozlowski TT (ed) Flooding and plant growth. Academic Press, Orlando, FL, p 9–45

Rossi F (2006) Small-scale burial of macroalgal detritus in marine sediments: effects of *Ulva* spp. on the spatial distribution of macrofauna assemblages. J Exp Mar Biol Ecol 332:84–95

Seitzinger SP, Giblin AE (1996) Estimating denitrification in North Atlantic continental shelf sediments. Biogeochemistry 35:235–260

Sfriso A, Pavoni B (1994) Macroalgae and phytoplankton competition in the central Venice lagoon. Environ Technol 15:1–14

Sfriso A, Marcomini A, Pavoni B (1987) Relationships between macroalgal biomass and nutrient concentrations in a hypertrophic area of the Venice lagoon. Mar Environ Res 22:297–312

Sfriso A, Raccanelli S, Pavoni B, Marcomini A (1991) Sampling strategies for measuring macroalgal biomass in the shallow waters of the Venice lagoon. Environ Technol 12: 263–269

Soulsby PG, Lowthion D, Houston M (1982) Effects of macroalgae mats on the ecology of intertidal mudflats. Mar Pollut Bull 13:162–166

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

Tang YZ, Gobler CJ (2011) The green macroalga, *Ulva lactuca*, inhibits the growth of seven common harmful algal bloom species via allelopathy. Harmful Algae 10:480–488

Tyrrell MC, Dionne M, Eberhardt SA (2012) Salt marsh fucoid algae: overlooked ecosystem engineers of north temperate salt marshes. Estuar Coast 35:754–762

Valdemarsen T, Kristensen E, Holmer M (2010) Sulfur, carbon, and nitrogen cycling in faunated marine sediments impacted by repeated organic enrichment. Mar Ecol Prog Ser 400:37–53

Valiela I, Teal JM (1979) The nitrogen budget of a salt marsh ecosystem. Nature 280:652–656

Valiela I, McClelland J, Hauxwell J, Behr PJ, Hersh D, Foreman K (1997) Macroalgal blooms in shallow estuaries: controls and ecophysiological and ecosystem consequences. Limnol Oceanogr 42:1105–1118

van der Wal D, Pye K (2004) Patterns, rates, and possible causes of salt marsh erosion in the Greater Thames area (UK). Geomorphology 61:373–391

van Hulzen JB, van Soelen J, Herman PMJ, Bouma TJ (2006) The significance of spatial and temporal patterns of algal mat deposition in structuring salt marsh vegetation. J Veg Sci 17:291–298

van Katwijk MM, Vergeer LHT, Schmitz GHW, Roelofs JGM (1997) Ammonium toxicity in eelgrass *Zostera marina.* Mar Ecol Prog Ser 157:159–173

Viaroli P, Bartoli M, Bondavalli C, Christian RR, Giordani G, Naldi M (1996) Macrophyte communities and their impact on benthic fluxes of oxygen, sulphide and nutrients in shallow eutrophic environments. Hydrobiologia 329:105–119

Wang R, Xiao H, Zhang P, Qu L, Cai H, Tang X (2007) Allelopathic effects of *Ulva pertusa*, *Corallina pilulifera* and *Sargassum thunbergii* on the growth of the dinoflagellates *Heterosigma akashiwo* and *Alexandrium tamarense.* J Appl Phycol 19:109–121

Viaroli P, Bartoli M, Azzoni R, Giordani F and others (2005) Nutrient and iron limitation to *Ulva* blooms in a eutrophic coastal lagoon (Sacca di Goro, Italy). Hydrobiologia 550: 57–71

Watson EB, Oczkowski AJ, Wigand C, Hanson AR and others (2014) Nutrient enrichment and precipitation changes do not enhance resiliency of salt marshes to sea level rise in the Northeastern U.S. Clim Change 125:501–509

Editorial responsibility: Erik Kristensen, Odense, Denmark

Submitted: May 21, 2014; Accepted: April 30, 2015
Proofs received from author(s): July 3, 2015