Regional-scale effects of eutrophication on ecosystem structure and services of seagrass beds

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Abstract

    Using large-scale field surveys across 12 estuaries in two provinces in Atlantic Canada, we analyzed changes in phytoplankton and benthic macroalgal communities as well as the canopy structure of eelgrass beds and quantified their carbon and nitrogen storage with increasing eutrophication. As eutrophication increased, phytoplankton biomass increased on average 1.8 times and phaeopigments doubled. Among macroalgae, the epiphytic *Ulothrix speciosa* increased 40 times in New Brunswick, and benthic *Ulva lactuca* 670 times in Prince Edward Island covering 61% of the bottom. Eelgrass showed a significant increase in leaf length and declines in shoot density and aboveground and belowground biomass, consistent with increased shading by opportunistic algae. As eelgrass biomass declined, so did the carbon storage capacity of the habitat. Nitrogen storage only declined in belowground eelgrass beds due to increasing tissue nitrogen content above ground with eutrophication. Despite province- and species-specific responses of primary producers to nutrient loading, principal component analysis revealed an overall shift from perennial eelgrass to opportunistic macroalgae and phytoplankton with eutrophication at the regional scale, indicating generalized eutrophication effects on primary producer assemblages.

    Seagrasses are among the most productive habitats in the world (Larkum et al. 2006), and their functions provide essential nutrient cycling (Duarte 2002; Romero et al. 2006), climate regulation (Nellemann et al. 2009; McLeod et al. 2011), sediment stabilization (Orth et al. 2006), and nursery and habitat services (Duarte 2002; Schmidt et al. 2011) that contribute directly and indirectly to human well-being. Despite their ecological importance, anthropogenic activities are increasingly affecting seagrass ecosystems around the world leading to fragmentation, depletion, or localized extinction of the habitat (Lotze et al. 2006; Waycott et al. 2009). Already one third of global seagrass area has disappeared since 1879 (Waycott et al. 2009), while losses in highly affected estuaries and coastal seas have been estimated at 65% of historical abundance (Lotze et al. 2006). With loss rates 14 times greater than those of tropical rainforests (Nellemann et al. 2009), seagrass habitats are among the most threatened ecosystems in the world (Duarte 2002; Waycott et al. 2009).

    In the early 1970s, coastal eutrophication was identified as one of the major contributors to seagrass declines in estuaries (Howarth and Marino 2006), and it continues to be one of the largest contemporary threats to seagrasses on a global scale (Duarte 2002; Orth et al. 2006; Waycott et al. 2009). Anthropogenic nitrogen loading is the main driver of eutrophication in most temperate estuaries and coastal seas (Howarth 1988; Howarth and Marino 2006) and is viewed as one of the most pervasive effects humans have on estuaries around the world (Vitousek et al. 1997). Human activity, particularly agricultural fertilizer use, sewage and wastewater discharges, and burning of fossil fuels, has doubled the reactive nitrogen entering the global nitrogen cycle, with 67% of the nitrogen currently entering coastal waters and a projected 10–20% increase by 2030 (Vitousek et al. 1997; Millennium Ecosystem Assessment 2005). The main effects of nitrogen enrichment of shallow coastal waters on primary producers include a shift in community dominance from perennial seagrasses to phytoplankton and fast-growing opportunistic macroalgae, a change in benthic (Duarte 1995; Short et al. 1995; Borum and Sand-Jensen 1996) and pelagic species composition (Howarth and Marino 2006), and impairment of the system's ability to store and cycle nutrients (McGlathery 2001).

    Under low nutrient conditions, eelgrass beds are associated with abundant and diverse benthic and epiphytic microalgae and macroalgae (Coleman and Burkholder 1994; Jaschinski et al. 2009; Schmidt et al. 2011), where the benthic macroalgae are typically distributed in scattered patches (Hauxwell et al. 2001). Together, epiphytic micro-algae and macroalgae can contribute up to 36% of the biomass and 18–51% of the production in eelgrass beds (Penhale 1977; Borowitzka et al. 2006). Nutrient enrichment, however, shifts the dominance from predominantly red macroalgae and unicellular diatoms to annual green and brown macroalgae and cyanobacteria (Coleman and Burkholder 1994; Hauxwell et al. 2001; Johnson et al. 2005). The loss of the seagrass coupled with a shift in the composition of the algal assemblage changes the quality and production of live and detrital food resources available to consumers, effectively changing the pathway and turnover of carbon and nitrogen through benthic and pelagic food webs, potentially reducing ecosystem stability (McClelland and Valiela 1998; Deegan et al. 2002; Olsen et al. 2011).

    For temperate seagrasses such as eelgrass, light avail-ability is the most important factor controlling growth

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(Dennison and Alberte 1985). Thus, the primary cause of eelgrass loss under increasingly eutrophic conditions is reduced light penetration caused by increased abundance of phytoplankton and total suspended solids (Moore et al. 1996), epiphytic algae on eelgrass blades (Short et al. 1995), drift macroalgal blooms, or any combination of the three (Hauxwell et al. 2001, 2003). Other mechanisms, such as ammonia toxicity, low oxygen concentration, increased sediment sulfides, and anoxia (Pregnall et al. 1984; Goodman et al. 1995) also contribute to reduced growth and production of temperate seagrasses (Hauxwell et al. 2001; Havens et al. 2001), especially when coupled with increased shading (McGlathery 2001).

Our study aimed to examine the effects of eutrophication on the combined and species-specific structure of the phytoplankton and macroalgal assemblage in eelgrass beds as well as eelgrass' canopy structure, nutrient storage, and carbon storage services in Atlantic Canada. Previous studies have assessed eutrophication-induced changes in phytoplankton biomass (Borum and Sand-Jensen 1996), epiphytic macroalgae (Coleman and Burkholder 1995), and benthic macroalgae (Neckles et al. 1993; Hauxwell et al. 2001) in eelgrass beds, as well as eelgrass bed structure (Short et al. 1995), usually in individual locations. However, researchers have paid little attention to the simultaneous changes in all of these primary producer components across regional scales, which is key to our understanding of the general as well as province-specific and variable effects of eutrophication. Also, the species-specific responses of macroalgae have been little studied; this is an important knowledge gap, since they can affect predator–prey interactions (Sunda et al. 2006) and the cycling of carbon and nitrogen through benthic food webs (Olsen et al. 2011), in turn altering fish and invertebrate communities (Deegan et al. 2002). Finally, understanding the effects of nutrient loading on eelgrass habitats and their carbon and nitrogen storage capacity is becoming increasingly urgent because of the importance of coastal vegetated habitats (blue carbon) in mitigating anthropogenic carbon dioxide emissions (McLeod et al. 2011). Therefore, the objectives of our study were to examine (1) phytoplankton biomass and particulate matter, (2) the abundance and composition of benthic and epiphytic macroalgal assemblages, (3) eelgrass canopy structure, and (4) the carbon and nitrogen storage of eelgrass beds across a gradient of eutrophication on a regional scale in Atlantic Canada.

Methods

Study sites—We sampled 12 sites located within larger estuaries in the provinces of New Brunswick (NB) and Prince Edward Island (PEI), Canada, from 27 July to 08 August 2007 (Fig. 1; Table 1). All estuaries contained mostly soft sediment substrate and eelgrass beds as the dominant structuring vegetation. Sites were chosen before data collection based on the range and severity of human uses that would contribute nitrogen to the estuary (Coll et al. 2011) and annual mean nutrient concentrations in PEI rivers (M. van Heuvel unpubl. data) and then grouped into low (L) and high (H) eutrophication after data
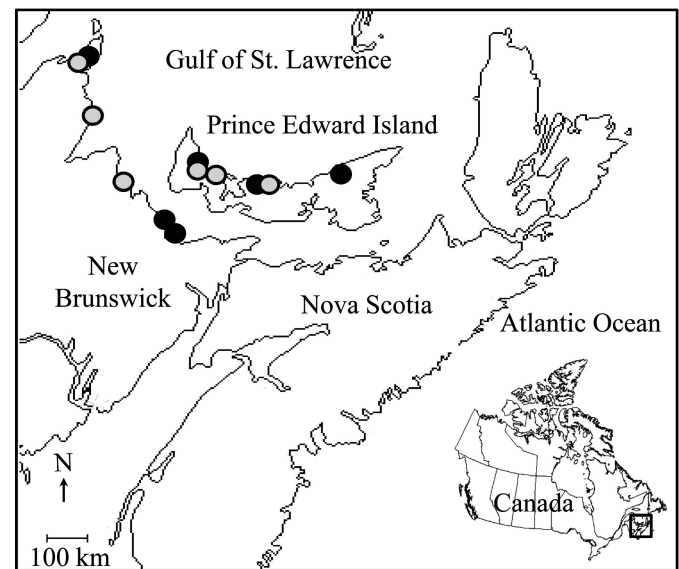

Fig. 1.   Map of study sites experiencing low (gray circle) and high (black circle) levels of eutrophication in the provinces of New Brunswick and Prince Edward Island in Atlantic Canada (*see* Table 1 for more details on each site and Coll et al. 2011 for photos).

collection using percentage tissue nitrogen concentrations in eelgrass blades and roots (*see* "Eelgrass canopy structure and storage services" for collection methods). We used the percentage tissue nitrogen because it is a more integrated measure of nutrient conditions than short-term water column nitrogen concentrations (Duarte 1990; Lee et al. 2004).

Sampling design—At each site, one 50×4 m transect was laid parallel to the shore inside the eelgrass bed ≥ 10 m from the vegetation-bare substrate interface. To assess water column integrated phytoplankton abundance, we manually collected three 1-liter water samples over the transect center using a pipe sampler (weighted tubing 2 m long $\times$ 2.5 cm diameter) at three times of the tide to capture at least one high and low tide at each site. Samples were kept in opaque thermos bottles, filtered on site within an hour of collection, and frozen for storage (*see* below). To assess the eelgrass canopy and benthic and epiphytic (on eelgrass) macroalgae, we sampled 11 quadrats (0.5×0.5 m, with 0.25 m subsections) placed every 5 m along the transect during high tide.

Phytoplankton abundance—To assess the abundance of the phytoplankton community and other particles in the water column, we measured concentrations of chlorophyll a (Chl a) and phaeopigments (μg L^{-1}), total particulate matter concentration (TPM; mg L^{-1}), and its organic (POM; mg L^{-1}) and inorganic (PIM; mg L^{-1}) components. We used a Pall filtration rig with three 200-mL polysulfone filter funnels with a 19.1 mm effective diameter attached to a Welch piston vacuum pump (model 2522) to filter the three water samples per site. For Chl a and phaeopigments, two replicate 70-mL subsamples were filtered through

Table 1. Name (ID), location, and eutrophication level (level) for each study site as well as bottom depth (depth) at high tide, mean water temperature (temp.), and salinity. Also listed is the average of the aboveground and belowground eelgrass (*Zostera marina*) tissue nitrogen content (%N). Temperature and salinity are the mean of three measurements with a standard error range of 0.1–0.8 and 0.1–1.2, respectively.

Site	ID	Location	Level	Depth (m)	Temp. (°C)	Salinity	%N
New Brunswick							
Tabusintac Bay	TB	N47.37 W64.94	low	0.80	25.8	27.5	0.76
Baie St. Simon Sud	SS	N47.73 W64.77	low	1.00	23.7	26.8	0.90
Kouchibouguac Bay	KB	N46.84 W64.94	low	0.75	22.7	27.2	0.91
Baie de Lamèque	LM	N47.79 W64.67	high	1.50	22.7	27.0	1.14
Cocagne Harbour	CG	N46.37 W64.62	high	1.00	27.7	28.8	1.12
Baie de Bouctouche	BT	N46.50 W64.68	high	0.75	27.5	27.7	1.20
Prince Edward Island							
Stanley-Trout River Estuary	ST	N46.47 W63.46	low	1	24.7	27.0	0.72
Freeland River Estuary	FL	N46.66 W63.91	low	0.8	23.2	30.1	0.89
Mill River Estuary	MR	N46.77 W64.08	low	0.8	23	31.3	0.94
Midgell River Estuary	MD	N46.42 W62.63	high	1.5	22.7	24.5	1.13
Southwest River Estuary	SW	N46.48 W63.50	high	0.8	24.4	27.3	1.21
Kildare River Estuary	KD	N46.84 W64.05	high	0.3	27.5	27.0	1.39

0.7-μm Whatman GF/F filters (2.5 cm diameter). All filters were placed in cryovials and stored in liquid nitrogen. Particles from another two replicate subsamples were captured by filtering through precombusted (6 h at 450°C) and weighed 0.7-μm Whatman GF/F filters to estimate TPM, POM, and PIM, but the volume filtered was judged by the appearance of color on the filter and depended on the study site. The volume filtered ranged from 50 to 150 mL but remained constant within a site. The filters were washed twice with 5 mL of 2% ammonium formate to remove salt, placed in a petri dish, and stored on ice.

In the laboratory, Chl *a* concentrations were determined using the Welschmeyer technique (Welschmeyer 1994) by extracting particulate matter retained on the GF/F filters in 90% acetone at -20°C for 24 h and measuring the extract in a Turner Designs 10-005R fluorometer. To convert all Chl *a* to phaeopigment, 5 μL of 10% HCl was added to the extract. Phaeopigment concentration was then estimated by measuring the fluorescence from the acidified extract (Strickland and Parsons 1972). TPM filters were dried (60°C, 24 h), weighed (dry weight), combusted (450°C, 6 h), and weighed again (combusted weight) to estimate POM and PIM.

Benthic and epiphytic macroalgae—To evaluate changes in benthic and epiphytic algal community structure, the same observer estimated the percentage cover of benthic and epiphytic algae in each 0.25-m² quadrat by hovering just above the top of the canopy. Using the 0.25-m subsections as a guide, the percentage cover of each algal species on the bottom and on the eelgrass blades was estimated with respect to the bottom to the nearest 2% whereby the sum of benthic and epiphytic cover could exceed 100%. All algae were identified to the lowest possible taxon in the field and used to calculate species richness (S).

Eelgrass canopy structure and storage services—To examine changes in the canopy structure we used a 0.25 × 0.25 m inset of the sampling quadrat to count shoot density

and measure canopy height of four haphazardly selected shoots from the substrate to the tip of the longest blade to the nearest millimeter. We also examined changes in eelgrass aboveground (blades) and belowground (roots and rhizomes) biomass. We used a sediment core (0.2 m diameter; 0.2 m deep) to collect the aboveground and belowground biomass at three locations (0, 25, and 50 m) along each transect. First, we gently pressed the core in the collection location to leave an outline on the sediment and then cut and removed all of the aboveground tissue from within the outline. Next, we took the entire core sample to estimate belowground biomass. Any aboveground tissue in the core sample itself was added to the weight of all cut blades, which may have led to a slight overestimation of aboveground biomass ($\sim$ 1–2 shoots) in locations of high shoot density.

In the laboratory, all visible epiphytes were scraped from the blades prior to weighing aboveground wet weight to the nearest milligram. All of the blades and roots and rhizomes from 2 to 5 shoots from each core sample were weighed prior to drying (70°C, 48 h) and reweighed to estimate the wet : dry weight ratio. After weighing, the dried aboveground and belowground tissue samples were homogenized separately, and a 50-mg dry weight subsample was analyzed using a Perkin Elmer carbon hydrogen nitrogen 2400 analyzer to estimate tissue carbon (C) and nitrogen (N) content. We used the average aboveground and belowground percentage tissue C and N and biomass per site to estimate the amount of C and N stored (kg m^{-2}) in aboveground and belowground eelgrass beds.

Statistical analysis—For all analyses we used a fully crossed two-factorial design with a fixed province (two levels, NB and PEI) and eutrophication factor (two levels; L and H). Where possible we used parametric statistics and compared the results with the nonparametric technique; however, since the results were the same we only present the nonparametric analyses for consistency across all results. We used univariate permutational analysis of
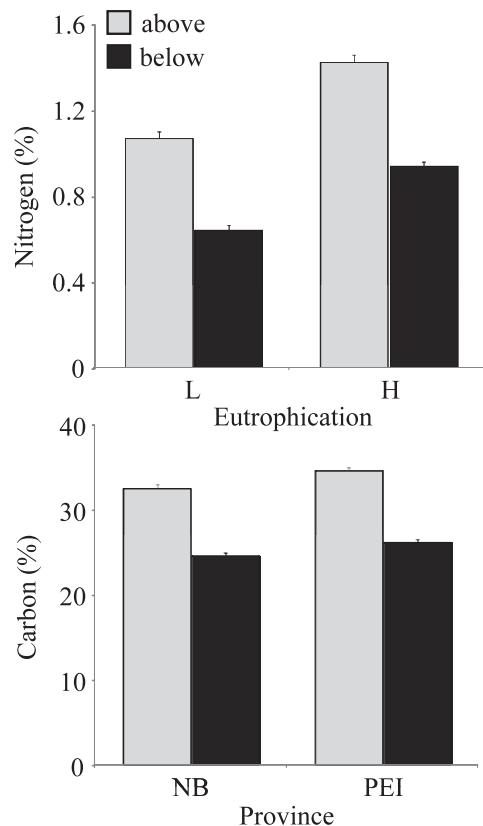
Fig. 2.   Mean aboveground and belowground tissue nitrogen and carbon content (%) of eelgrass (± SE) across low (L) and high (H) eutrophication levels and the provinces of NB and PEI, respectively.

variance (PERMANOVA) on the Euclidean distance matrix of species richness as well as the zero-adjusted Bray–Curtis similarity matrix of the square root trans- formed total abundance of benthic and epiphytic primary producers. Permutational post hoc *t*-tests were used where significant effects were detected (Anderson et al. 2008).

Since some variables were not independent, we used multivariate PERMANOVAs to examine the effects of province and eutrophication on the Euclidean distance matrix of normalized (1) Chl *a* and phaeopigments, (2) PIM and POM, (3) eelgrass shoot density and canopy height, (4) aboveground and belowground biomass, (5) %C and %N tissue content, and (6) overall C and N storage. Where differences were significant we followed up with protected univariate PERMANOVA and post hoc *t*-tests.

Analogous to ANOVA, PERMANOVA also estimates the components of variation for both fixed and random factors as sums of squared fixed effects (divided by the appropriate degrees of freedom) and actual variance components (Anderson et al. 2008). These estimators are directly comparable and useful in determining the relative importance of different terms in the model in explaining the overall variance. Since the estimates are in squared units of either Euclidean distance or Bray–Curtis similarity, the square root ($\sqrt{V}$) is taken to put them back into their original units (Anderson et al. 2008).

Multivariate PERMANOVA was also used to assess the community composition of the benthic and epiphytic algae. We used the zero-adjusted Bray–Curtis similarity matrix of the dispersion weighted algal cover to dampen the fluctua- tions of the metric for near-blank samples in an analogous way to the addition of a constant to the log transformation (Clarke and Gorley 2006). Dispersion weighting divides the cover of each species by their variance to mean ratio calculated from replicate quadrats within a site and then averaged across sites. This only downweights numerically dominant spatially clustered species, decreasing the influence of their erratic cover estimates on the similarity coefficients (Clarke and Gorley 2006). When a significant effect was detected, we used similarity percentages analysis (SIMPER) to identify the species that consistently contributed (dissim- ilarity standard deviation ≥ 1) to the observed dissimilarities (Clarke and Warwick 2001). We used cluster analysis on the zero-adjusted Bray–Curtis similarity matrix of the square root transformed site-averaged benthic and epiphytic cover to identify more general patterns in the community.

Finally, to assess changes in the overall primary producer assemblage, we used principal component anal- ysis (PCA) on the site-averaged concentration of Chl *a*, combined aboveground and belowground eelgrass biomass, and the total cover of benthic and epiphytic macroalgae to evaluate the overall response of the phytoplankton– macrophyte–eelgrass assemblage to eutrophication. We then used regression analysis of the first principal component axis as well as the individual components Chl *a*, benthic and epiphytic algal cover, and eelgrass biomass against percentage tissue nitrogen to examine the effect of eutrophication on a regional scale.

Results

Eutrophication levels—The percentage tissue nitrogen (%N) in eelgrass blades and roots identified two different eutrophication levels in our previously selected sites (Table 1; Fig. 2). The average of the aboveground and belowground %N ranged by 0.22% and 0.27% within the low and high eutrophication levels, respectively, and there was a significant difference in both aboveground and belowground %N between eutrophication levels (Table 2).

Phytoplankton abundance—Chl *a* and phaeopigments, phytoplankton degradation products, increased significantly in the water column with eutrophication (Fig. 3; Table 3), and there was no province effect or interaction between factors. Despite the apparent increase in PIM with eutrophication (Fig. 3), there was no significant effect of eutrophication, province, or their interaction in the multi- variate analysis with POM (Table 3), likely because of the high variability under eutrophied conditions. However, the effect of eutrophication explained the greatest amount of variance in PIM and POM after the residual.

Benthic and epiphytic macroalgae—Overall, 16 species or genera of benthic and epiphytic macroalgae were identified during field surveys: four red (*Ahnfeltia plicata*, *Chondrus crispus*, *Cystoclonium purpureum*, *Rhodochorton purpureum*),

Table 2. Multivariate PERMANOVA results of the effect of province (pr) and eutrophication (etro) on the aboveground and belowground tissue carbon and nitrogen content (%CN) and univariate results for each component of tissue carbon and nitrogen. $\sqrt{V}$ are unbiased estimates of the components of variance for each of the factors in the model. Negative $\sqrt{V}$ values indicate that there is no evidence against the null hypothesis (Anderson et al. 2008). Total degrees of freedom (df) = 30, and res is the residual. Significant effects are in bold.

	Source (df)			
	Pr (1)	Eutro (1)	Pr×Eutro (1)	Res (27)
Multivariate PERMANOVA				
Above and below %CN				
pseudo *F*	**10.4**	0.74	2.49	
p	**0.002**	0.41	0.11	
$\sqrt{V}$	**1.60**	−0.27	0.90	1.99
Univariate PERMANOVA				
% nitrogen above				
pseudo *F*	0.33	**52.8**	0.63	
p	0.58	**0.001**	0.43	
$\sqrt{V}$	−0.003	**0.25**	−0.003	0.13
% nitrogen below				
pseudo *F*	0.29	**67.0**	0.43	
p	0.58	**0.001**	0.53	
$\sqrt{V}$	−0.002	**0.22**	−0.003	0.10
% carbon above				
pseudo *F*	**10.4**	0.74	2.49	
p	**0.004**	0.39	0.12	
$\sqrt{V}$	**1.60**	−0.27	0.90	1.99
% carbon below				
pseudo *F*	**10.5**	0.33	2.51	
p	**0.005**	0.55	0.13	
$\sqrt{V}$	**0.97**	−0.26	0.55	1.20

Fig. 3. Concentrations (mean ± SE) of Chl *a*, phaeopigments, and TPM, POM, and PIM for water samples collected at sites experiencing low (L, white) and high (H, black) levels of eutrophication.

eight green (*Chaetomorpha linum*, *Cladophora rupestris*, *Codium fragile* ssp. *tomentosoides*, *Rhizoclonium* sp., *Spongomorpha* sp., *Ulva intestinalis*, *Ulva lactuca*, *Ulothrix speciosa*) and four brown algal species (*Fucus evanescence*, *Fucus vesiculosus*, *Pilayella littoralis*, *Sphaerotrichia divaricata*). Of these, six were annuals and only *U. speciosa* was an epiphyte on eelgrass.

Total abundance (percentage cover) of the benthic and epiphytic macroalgae was significantly affected by the interaction between province and eutrophication (Table 4); however, eutrophication explained the greatest amount of the variation. Overall, total abundance strongly increased with eutrophication across as well as within provinces (Fig. 4). Species richness significantly increased with eutrophication with no effect of province, nor was there an interaction between factors (Fig. 4; Table 4). Because of overall low species richness, with often only one species per site, we did not further examine diversity patterns.

Cluster analysis of the community composition, based on the average percentage cover (mean of 11 quadrats per site) of each benthic and epiphytic species, revealed that sites with low levels of eutrophication were more similar to each other (73.3%) than to high sites (except one high site
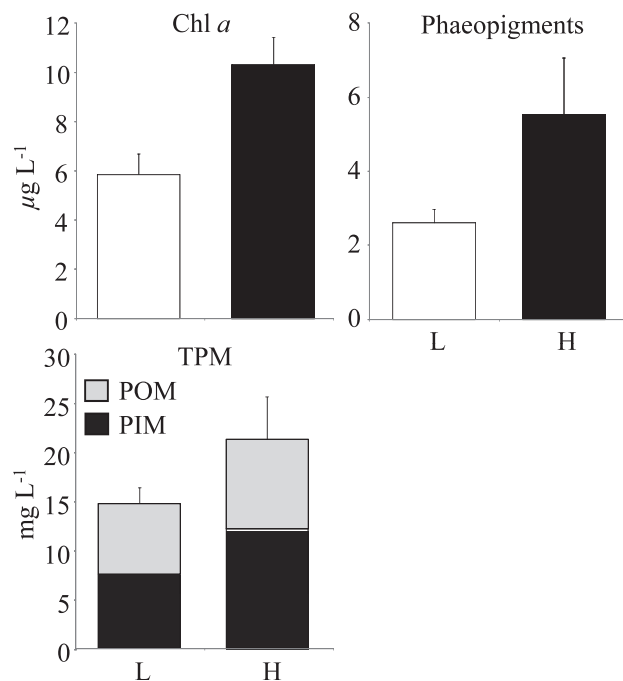
in NB) within the same province (Fig. 4 inset). It also showed that the different responses of the communities to high levels of eutrophication were driving the differences between provinces (Fig. 4 inset). The most important factor in explaining the variance in benthic and epiphytic community composition (multivariate PERMANOVA) was the eutrophication level (Table 3). In NB, community composition significantly differed between low and high levels (post hoc: *p* = 0.001) largely driven by the increase in *U. speciosa* (Fig. 4). In PEI, the significant differences between low and high (*p* = 0.001) were driven by the increase in both *U. lactuca* and *U. speciosa*.

Eelgrass canopy structure—Overall, eelgrass shoot density declined with increasing eutrophication (Fig. 5), but there was a significant province by eutrophication interaction (Table 5). In both provinces, shoot density significantly declined from low to high levels (post hoc: *p* ≤ 0.023; Fig. 5), and the interaction is one of magnitude because shoot density declined more in PEI than NB. Across provinces, the canopy was similarly short under low levels of eutrophication (*p* = 0.69); however, the increase in height from low to high levels was more pronounced in PEI (Fig. 5), resulting in a significant interaction between eutrophication and province (Table 5).

There was a reduction in total eelgrass biomass with increasing eutrophication (Fig. 5). The decline was significant in aboveground biomass in both provinces (Fig. 5; Table 5), but belowground biomass only decreased in PEI (post hoc: *p* = 0.001) leading to a significant interaction between province and eutrophication (Table 5).

Table 3.   Multivariate PERMANOVA results of the effect of province (pr) and eutrophication (eutro) on water column chlorophyll *a* and phaeopigment (Chl *a* and phaeo) and POM and PIM concentrations as well as the univariate results for each component. $\sqrt{V}$ are unbiased estimates of the components of variance for each of the factors in the model. Negative $\sqrt{V}$ values indicate that there is no evidence against the null hypothesis (Anderson et al. 2008). Total degrees of freedom (df) = 35, and res is the residual. Significant effects are in bold.

	Source (df)			
	Pr (1)	Eutro (1)	Pr×Eutro (1)	Res (32)
Multivariate PERMANOVA				
Chl *a* and Phaeo				
pseudo *F*	1.60	**6.60**	0.78	
p	0.18	**0.003**	0.48	
$\sqrt{V}$	0.24	**0.73**	−0.21	1.31
POM and PIM				
pseudo *F*	1.20	1.87	0.24	
p	0.28	0.16	0.80	
$\sqrt{V}$	0.15	0.31	−0.41	1.41
Univariate PERMANOVA				
Chl *a*				
pseudo *F*	2.84	**10.8**	1.77	
p	0.11	**0.002**	0.20	
$\sqrt{V}$	0.27	**0.63**	0.25	0.85
Phaeo				
pseudo *F*	0.65	**3.38**	0.003	
p	0.52	**0.03**	0.92	
$\sqrt{V}$	−0.14	**0.36**	−0.32	0.99

In addition, the belowground biomass under low levels of eutrophication did not differ between provinces ($p = 0.08$) but was significantly lower in PEI under highly eutrophic conditions ($p = 0.001$).

Carbon and nitrogen storage—While the tissue nitrogen content showed a clear increase with eutrophication (*see* "Eutrophication levels" above), the tissue carbon content in aboveground and belowground eelgrass was similar across eutrophication levels but was significantly higher in PEI (Table 2; Fig. 2). The overall carbon and nitrogen storage in aboveground and belowground eelgrass biomass was significantly affected by the province by eutrophication interaction, as were the belowground carbon and nitrogen storage when examined individually (multivariate and protected PERMANOVAs; Table 5). Despite the increase in tissue carbon content (%) across provinces, carbon storage (g m^{-2}) showed a similar pattern to eelgrass biomass whereby total carbon storage declined with eutrophication (Fig. 5). There was no effect of province or eutrophication on aboveground carbon storage (Table 5), nor was there a difference in belowground storage between levels in NB (post hoc: $p = 0.28$) or across provinces under low levels of eutrophication ($p = 0.09$). However, there was significantly more carbon stored in the belowground tissue in NB under high eutrophication levels ($p = 0.001$), and the overall decline in total carbon storage

was driven by the significant decrease in belowground storage in PEI ($p = 0.001$). Total nitrogen storage was similar across eutrophication levels likely because tissue nitrogen increased with eutrophication causing nitrogen storage to differ from the pattern observed in biomass (Fig. 5). There was no change in aboveground nitrogen storage across provinces or eutrophication levels (Table 5), whereas belowground nitrogen storage significantly declined in PEI (post hoc: $p = 0.009$) but not in NB ($p = 0.11$). However, similar to the pattern in belowground biomass, nitrogen storage was significantly lower in PEI under levels of high eutrophication ($p = 0.001$) but was not different between provinces for low eutrophication levels ($p = 0.14$).

Overall community—Principal component analysis of the entire primary producer assemblage showed clear distinctions between the levels of eutrophication (Fig. 6A). There was a negative correlation between overall eelgrass biomass (principal component [PC] 1 eigenvectors: −0.556) and the percentage cover of benthic and epiphytic macroalgae (0.645) and Chl *a* concentration (0.524) in the first principal component separating the low and highly eutrophied conditions. The first principal component explained 70.6% of the variation. The second principal component was divided between the effect of eelgrass biomass (PC2 eigenvector: 0.657) and Chl *a* (0.753) and explained an additional 22.7% of the variation for a cumulative variation of 93.3%. Despite the variability in the response of some of the sites in PC1, there was a significant positive relationship (linear regression, $F_{1,10} = 17.0$, $p = 0.002$) between the PC1 scores and average (blades and roots) tissue nitrogen (Fig. 6B).

We also examined the response of each component of the PCA across the regional scale (Fig. 7). There was a significant positive relationship between average tissue nitrogen and Chl *a* (linear regression, $F_{1,10} = 7.7$, $p = 0.02$; Fig. 7A), and the strongest relationship was with the cover of benthic and epiphytic algae ($F_{1,10} = 21.4$, $p < 0.001$; Fig. 7B). However, the negative relationship between average tissue nitrogen and overall eelgrass biomass was not significant ($p = 0.10$; Fig. 7C).

Discussion

Our large-scale field surveys revealed a general regional shift from perennial eelgrass to opportunistic macroalgae and phytoplankton with increasing eutrophication in Atlantic Canada, and they also revealed some province- and species-specific responses. We further showed that carbon and nitrogen storage of eelgrass habitats declined with increasing eutrophication. Overall, our results demonstrate that eutrophication alters the structure and services of eelgrass beds with potentially wide-ranging consequences on coastal ecosystems and human well-being (Millennium Ecosystem Assessment 2005; Hughes et al. 2008).

Changes in phytoplankton—Owing to their short life-history, phytoplankton respond quickly to enhanced nutrient availability by increasing their biomass and can maintain

Table 4. Multivariate PERMANOVA results of the effect of province (pr) and eutrophication (eutro) on the community composition (community) and univariate results for total cover (%) and richness (S) of benthic and epiphytic macroalgae associated with eelgrass beds. $\sqrt{V}$ are unbiased estimates of the components of variance for each of the factors in the model. Negative $\sqrt{V}$ values indicate that there is no evidence against the null hypothesis (Anderson et al. 2008). Total degrees of freedom (df) = 131, and res is the residual. Significant effects are in bold.

	Source (df)			
	Pr (1)	Eutro (1)	Pr×Eutro (1)	Res (128)
Multivariate PERMANOVA				
Community				
pseudo *F*	**39.1**	**92.2**	**34.6**	
p	**0.001**	**0.001**	**0.001**	
$\sqrt{V}$	**22.4**	**34.6**	**29.7**	29.5
Univariate PERMANOVA				
Cover				
pseudo *F*	**3.82**	**207.9**	**7.25**	
p	**0.019**	**0.001**	**0.003**	
$\sqrt{V}$	**4.69**	**40.1**	**9.86**	22.7
Richness				
pseudo *F*	0.049	**70.6**	1.22	
p	0.84	**0.001**	0.26	
$\sqrt{V}$	−0.09	**0.81**	0.06	0.79

a high biomass under sustained enrichment conditions (Sunda et al. 2006). Like previous studies in seagrass beds (Short et al. 1995; Moore et al. 1996), we documented an increase in phytoplankton biomass (Chl *a*) and phaeopigments, the degradation products of Chl *a*, with increasing nutrient loading. Increase in phytoplankton biomass is a major contributor to particle light attenuation in the water column and has been linked to eelgrass decline (Olesen 1996). Indeed, in our study, eutrophied sites with higher Chl *a* had reduced eelgrass density and biomass and enhanced canopy height, all indicating shading. In addition, increased benthic resuspension because of the decrease in sediment stabilization due to the decline in eelgrass (Christiansen et al. 1981; Olesen 1996) can create a positive feedback loop further promoting eelgrass loss (Duarte 1995).

Changes in benthic and epiphytic macroalgae—To date, much of the work on algae in eelgrass beds has focused on the overall increase in macroalgal abundance with nutrient loading emphasizing problematic species (Hauxwell et al. 2001; McGlathery 2001), while studies at the species level have focused on species presence and absence (Johnson et al. 2005) and benthic and epiphytic microalgae (Neckles et al. 1993; Coleman and Burkholder 1995; Jaschinski et al. 2009). Our study is the first to document species-specific changes in epiphytic and benthic macroalgal abundance in eelgrass beds across different levels of eutrophication on both local and regional scales.

Typically, macroalgal assemblages in temperate seagrass beds with limited nutrient availability are dominated by rhodophytes, whereas those experiencing nutrient loading are dominated mainly by filamentous and foliose chlorophytes (McGlathery 2001; Johnson et al. 2005; Schmidt et al. 2011). In our study, we identified one epiphytic and 15 benthic macroalgal species across all sites, and under high levels of eutrophication the assemblage was dominated by both filamentous and foliose green algal species. Although no work has examined the shifts in benthic macroalgal species assemblages specifically in eelgrass beds, research on ephemeral algae showed changes from predominantly red corticated algae (e.g., *Ceramium* spp., *Polysiphonia* spp.) to foliose (e.g., *Ulva* spp., *Porphyra* spp.) and uniseriate filaments (e.g., *Ulothrix* spp., *Pilayella* spp.) under increasing nutrient enrichment (Karez et al. 2004). Our results showed a clear increase in ephemeral epiphytic and benthic macroalgal cover, but there was a difference in the responding species between provinces. In New Brunswick, the uniseriate filamentous epiphyte *U. speciosa* increased 40-fold in cover, and in Prince Edward Island, the benthic foliose *U. lactuca* increased 670-fold reaching up to 61% cover. Despite using different metrics, our results are consistent with the observed sixfold increase in benthic macroalgal biomass (Valiela et al. 1997), 38-fold increase in macroalgal canopy depth (Hauxwell et al. 2001), and 100-fold increase in epiphyte biomass (Wright et al. 1995) between estuaries with low and high nutrient loading.

The differences in the dominant species between provinces may be driven by a combination of factors, including differences in environmental conditions, differences in climate, differences in propagule supply, and interactions between algal species and consumers, as well as differences in nutrient loading (Lin et al. 1996; Johnson et al. 2005). Short et al. (1995) found that under identical nutrient enrichment conditions, phytoplankton, epiphytes, and macroalgae each independently dominated a mesocosm tank, which was attributed to differences in top-down control. Moreover, foliose macroalgae require higher loading rates than filamentous algae to bloom because they maintain higher individual biomass and have longer turnover times and lower nutrient uptake rates (Duarte 1995; Havens et al. 2001). This may suggest that, despite similar eelgrass tissue nitrogen content, the eutrophied estuaries in Prince Edward Island may have higher nitrogen inputs than New Brunswick. Overall, the changes in macroalgal species composition and abundance will likely have a profound effect on associated animal communities since they provide food and habitat for a variety of species (Deegan et al. 2002; Schmidt et al. 2011).

Changes in eelgrass canopy structure—Eelgrass can show both a physiological and morphological response to nutrient loading and light limitation (Short 1987; Moore et al. 1996; Romero et al. 2006). As nutrient enrichment and light attenuation increase, eelgrass responds by increasing leaf length and decreasing shoot density, biomass, and growth leading to changes in canopy structure and ultimately eelgrass loss (van Lent et al. 1995; Moore et al. 1996; Short and Burdick 1996). Using mesocosm experiments, Short et al. (1995) teased apart the effects of nutrient enrichment and light limitation on eelgrass and found that shoot density and aboveground
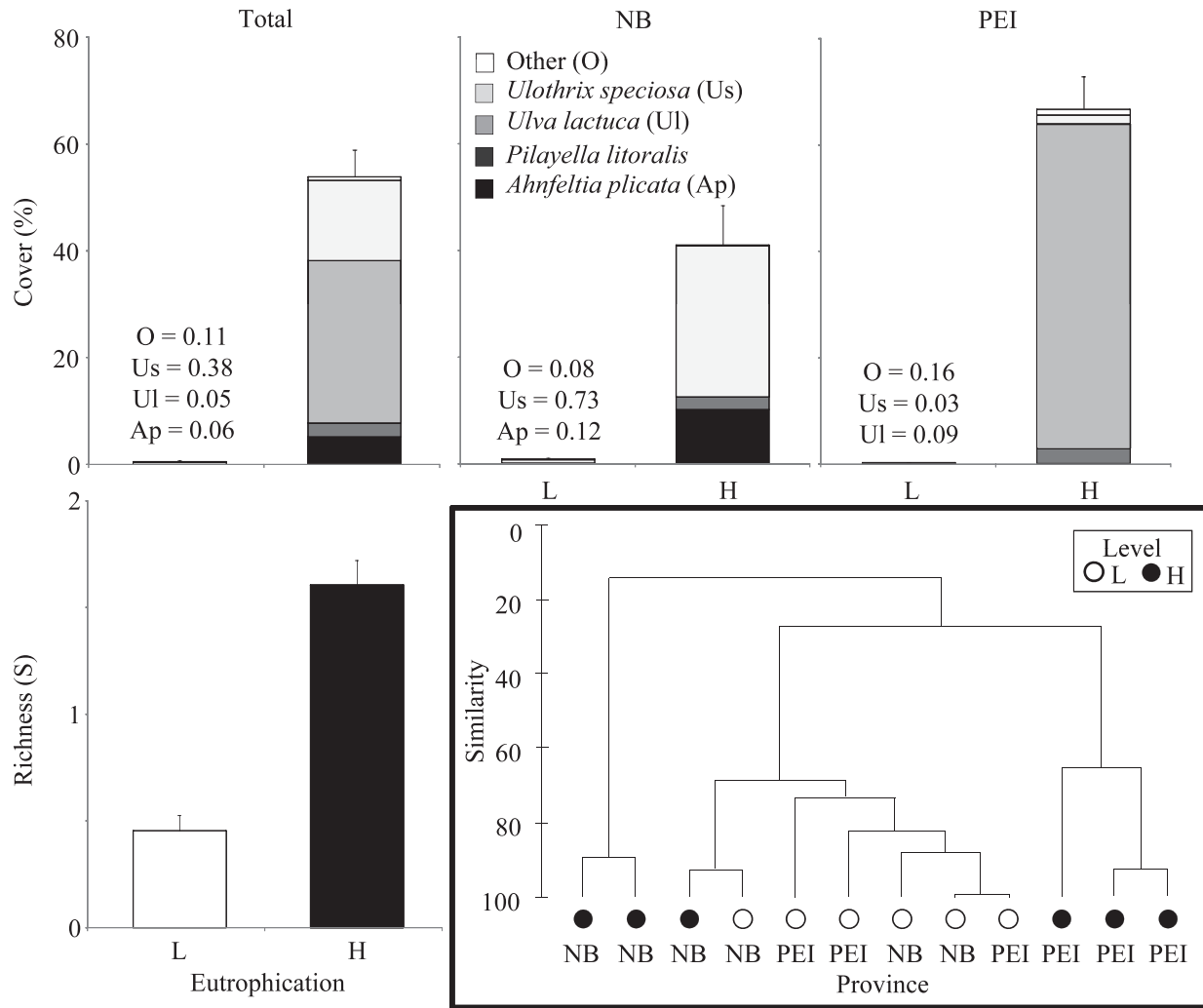
Fig. 4.　Abundance (percentage cover, ± SE) of individual benthic and epiphytic macroalgae, species richness, and inset cluster dendogram on their community composition in eelgrass beds across low (L) and high (H) levels of eutrophication in NB and PEI. The order in the color legend is the order that the macroalgae appear in the bars. Numbers above small bars indicate the percentage of species (abbreviations in legend) with low abundance. White and black bars in the species richness plot represent low and high eutrophication levels, respectively.

biomass both decreased by 50% in all treatments, whereas leaf length only increased by 29% with shade. We found a 45% decline in shoot density, 53% increase in leaf length, and a 27% decline in aboveground and belowground biomass, which is consistent with the effects of shading. Light limitation has been attributed as the main cause of eelgrass decline in increasingly eutrophic estuaries by inhibiting growth and recruitment (Short et al. 1995; Hauxwell et al. 2001, 2003).

The increases in phytoplankton biomass and epiphytic and benthic macroalgal abundance observed in our study have all been documented to increase water column light attenuation (Hauxwell et al. 2001; Brush and Nixon 2002; Carroll et al. 2008). Both water column and epiphyte shading are detrimental for established shoots, whereas shading by macroalgal mats was more detrimental for newly recruiting eelgrass shoots (Hauxwell et al. 2001, 2003). This suggests that the light limitation imposed by the large increases in phytoplankton biomass, epiphyte cover

in New Brunswick, and benthic macroalgal cover in Prince Edward Island contributed to the observed changes in canopy structure of established shoots but may also have a severe effect on the recruitment of new shoots.

Other potential factors that have been implicated in eelgrass decline, whose effects are exacerbated under low light conditions and high temperatures, include alterations in oxygen and ammonium concentrations resulting from the presence of macroalgal canopies (McGlathery et al. 1997; Hauxwell et al. 2001; Havens et al. 2001) and increased sediment sulfides and anoxia (Pregnall et al. 1984; Goodman et al. 1995; Pulido and Borum 2010). Although we did not measure sulfides, sediments from cores collected at each of our study sites smelled increasingly of hydrogen sulfide with eutrophication, especially in Prince Edward Island. In addition, we observed extensive leaf bleaching at two of the highly eutrophied sites in Prince Edward Island, which is indicative of water column anoxia (Pulido and Borum 2010). Once a shoot dies, so does the ramet, and
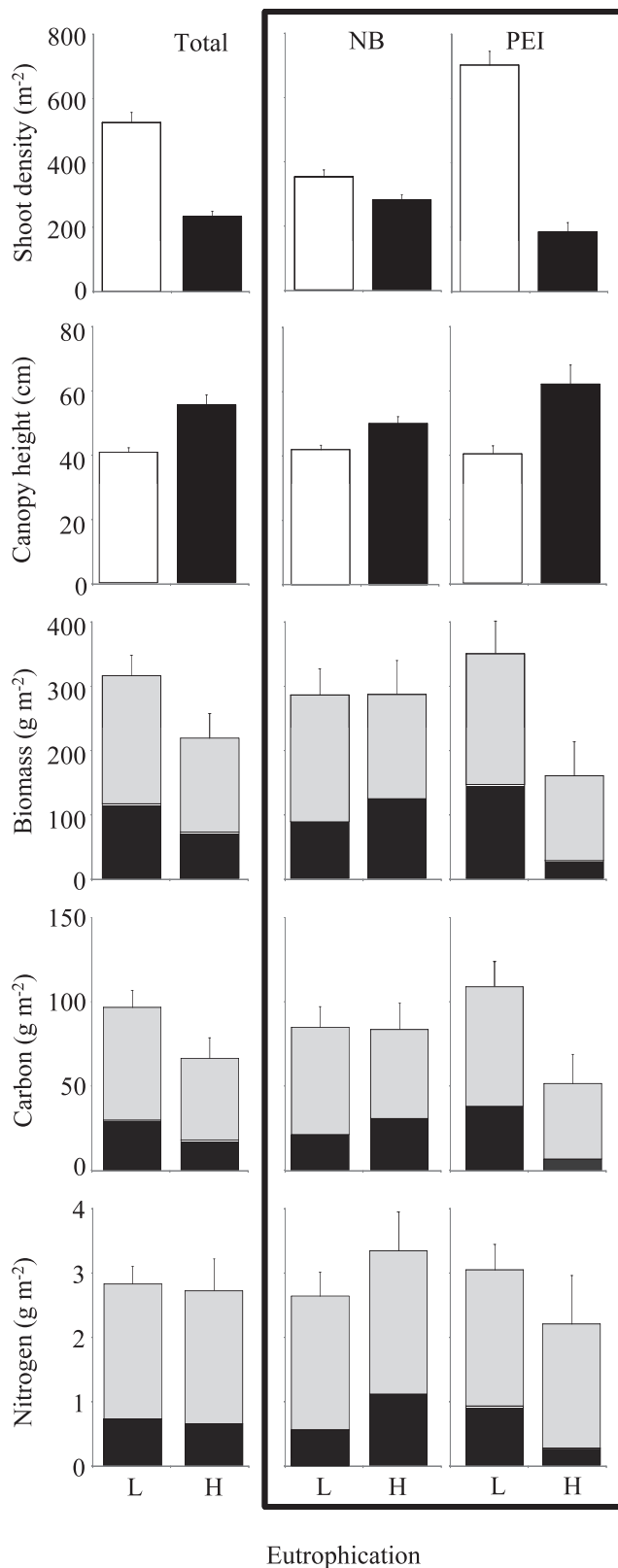
Fig. 5. Canopy structure (shoot density and canopy height, ± SE) of eelgrass beds across low (L, white) and high (H, black) levels of eutrophication in NB and PEI as well as biomass, carbon, and nitrogen storage in the aboveground (light gray) and belowground (dark gray) component of the eelgrass beds.

since eelgrass does not have dormant buds on their rhizomes to form new shoots, it can only recolonize from the seed bank (Greve et al. 2005). Over time with persistent or reoccurring periods of extended anoxia this would lead to a decline in the belowground tissue due to inhospitable growing conditions, to recurring die-offs of the new ramets, and to eventual extirpation as the seed bank is exhausted. This is likely what is happening in Prince Edward Island, since we had an 82% decline in belowground biomass. However, in New Brunswick there was no significant change in belowground biomass, indicating that changes in sediment and water column chemistry were less severe, which was likely due to the differential response of the macroalgal community between provinces: epiphytic macroalgae in New Brunswick compared with extensive benthic algal mats in Prince Edward Island.

Changes in eelgrass carbon and nitrogen storage—Eelgrass tissue nitrogen is known to reflect the nutrient availability of the surrounding waters (Short 1987; Duarte 1990; Lee et al. 2004), and we did document a significant increase in tissue nitrogen across study sites. However, despite this increase in tissue nitrogen, our values were all below the 1.8% benchmark established by Duarte (1990), indicating that eelgrass at our sites was still nitrogen limited. Macroalgae and phytoplankton compete strongly with eelgrass for nutrients (Duarte 1995), which can lead to nutrient limitation even under enriched conditions. Their increased abundance can initially benefit eelgrass by acting as a nutrient "sponge" (Harlin 1978); however, these benefits are quickly outweighed by the adverse effects of biogeochemical changes in the sediment and light limitation (Burkholder et al. 1992).

Coastal rooted macrophytes, like eelgrass, are increasingly being recognized as significant carbon sinks for anthropogenic CO_2, and their global decline seriously threatens the ability of the coastal ocean to sequester carbon (Nellemann et al. 2009; McLeod et al. 2011). As the vegetation changes from eelgrass- to macroalgae- and phytoplankton-dominated, carbon and nitrogen cycling accelerates via increased herbivory and decomposition and long-term storage declines (Duarte 1995; McClelland and Valiela 1998). These changes are reflected in our results where the decline in eelgrass biomass with increasing eutrophication led to an overall reduction of the carbon storage capacity of the habitat. The 82% decrease in belowground carbon storage in Prince Edward Island may have more serious long-term repercussions because it was not offset by the 30% increase in belowground storage in New Brunswick, and belowground storage has the greatest potential for long-term carbon sequestration via burial (Mateo et al. 2006). Although total nitrogen storage did not change with eutrophication because of the increase in tissue nitrogen, there was a significant decrease in belowground nitrogen storage in Prince Edward Island. Therefore, the shift in the dominant primary producer from perennial eelgrass to ephemeral benthic and epiphytic macroalgae and phytoplankton will have profound implications not only for carbon and nitrogen cycling, storage, and sequestration but also for the trophic structure of

Table 5. Multivariate PERMANOVA results of the effect of province (pr) and eutrophication (eutro) on eelgrass characteristics: (1) shoot density (SD) and canopy height (CH), (2) aboveground and belowground biomass (biomass), and (3) aboveground and belowground carbon and nitrogen storage (CN). Univariate PERMANOVA of individual eelgrass characteristics follow. $\sqrt{V}$ are unbiased estimates of the components of variance for each of the factors in the model. Negative $\sqrt{V}$ values indicate that there is no evidence against the null hypothesis (Anderson et al. 2008). Total degrees of freedom (df) = 131, and res is the residual. Significant effects are in bold.

|	Source (df)			
	Pr (1)	Eutro (1)	Pr×Eutro (1)	Res (128)
Multivariate PERMANOVA				
SD and CH				
pseudo F	**8.37**	**46.0**	**21.9**	
p	**0.001**	**0.001**	**0.001**	
$\sqrt{V}$	**0.37**	**0.94**	**0.90**	1.13
Above and below biomass				
pseudo F	2.94	**3.87**	**4.22**	
p	0.058	**0.028**	**0.018**	
$\sqrt{V}$	8.55	**10.4**	**15.6**	25.6
Above and below CN				
pseudo F	0.49	1.29	**4.86**	
p	0.62	0.29	**0.015**	
$\sqrt{V}$	−0.33	0.24	**1.26**	1.90
Univariate PERMANOVA				
SD				
pseudo F	**19.6**	**100.7**	**57.5**	
p	**0.001**	**0.001**	**0.001**	
$\sqrt{V}$	**0.35**	**0.81**	**0.86**	0.65
CH				
pseudo F	2.72	**18.6**	**3.99**	
p	0.09	**0.001**	**0.05**	
$\sqrt{V}$	0.15	**0.48**	**0.28**	0.92
Biomass above				
pseudo F	2.20	**4.15**	2.16	
p	0.11	**0.03**	0.12	
$\sqrt{V}$	6.57	**10.6**	9.13	25.1
Biomass below				
pseudo F	**4.07**	**5.40**	**10.1**	
p	**0.03**	**0.004**	**0.001**	
$\sqrt{V}$	**9.38**	**11.2**	**22.9**	22.4
Carbon above				
pseudo F	0.0005	2.17	0.40	
p	0.98	0.14	0.54	
$\sqrt{V}$	−0.24	0.26	−0.26	1.01
Carbon below				
pseudo F	0.36	3.24	**11.1**	
p	0.54	0.07	**0.002**	
$\sqrt{V}$	−0.17	0.31	**0.93**	0.86
Nitrogen above				
pseudo F	0.07	0.002	0.12	
p	0.77	0.96	0.74	
$\sqrt{V}$	−0.24	−0.25	−0.33	1.04
Nitrogen below				
pseudo F	1.85	0.007	**11.4**	
p	0.19	0.81	**0.001**	
$\sqrt{V}$	0.19	−0.20	**0.96**	0.88
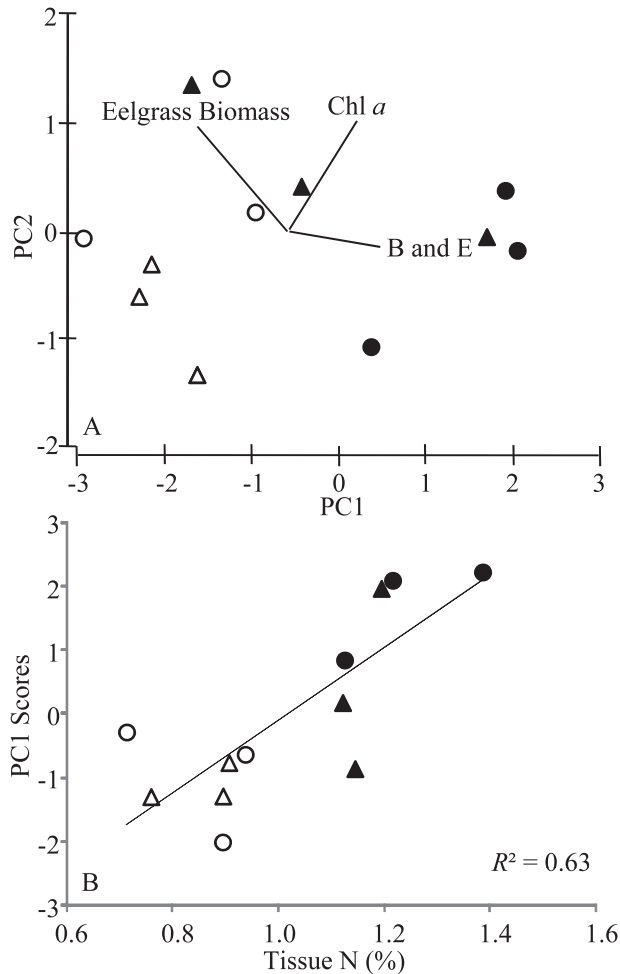
Fig. 6. (A) Principal component analysis using overall aboveground and belowground eelgrass biomass, phytoplankton Chl *a* concentration, and the total percentage cover of benthic and epiphytic macroalgae (B and E) across low (white) and high (black) levels of eutrophication in NB (triangles) and PEI (circles) and (B) linear regression of principal component 1 (PC1) scores against average (blades and roots) eelgrass tissue nitrogen content (%N) across all sites in both provinces.
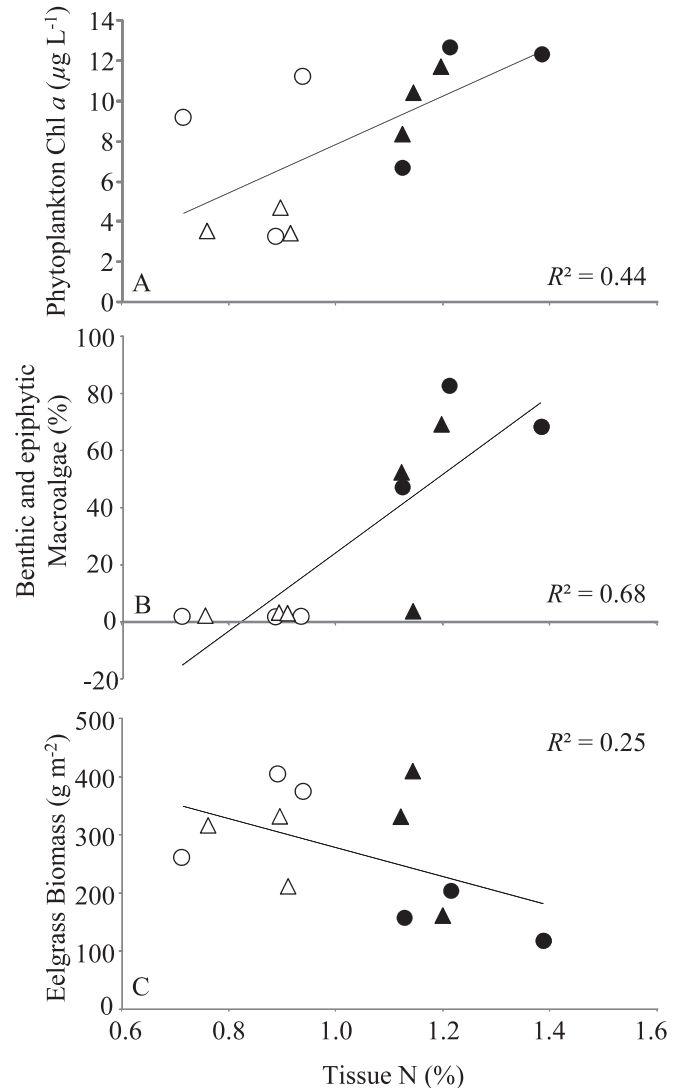

Fig. 7. Regression analysis of (A) Chl *a* concentration, (B) the total percentage cover of benthic and epiphytic macroalgae, and (C) overall eelgrass biomass against average eelgrass tissue nitrogen (%N) content across all sites in NB (triangles) and PEI (circles). Eutrophication levels are indicated with colors (low = open; high = black).

associated food webs in New Brunswick and Prince Edward Island.

Overall community changes and indicators of eutrophication—Our study is the first to integrate changes in different primary producer components with increasing eutrophication into one analysis. Our PCA results clearly distinguished between the two eutrophication levels and showed that all three primary producer components were needed to explain the variation in the response between provinces. Previous studies have reported positive relationships between total water column nitrogen and benthic and epiphytic macroalgal abundance, Chl *a*, and a negative relationship with eelgrass biomass (Borum 1985; Short et al. 1995; Carroll et al. 2008). In our study, we found similar relationships with eelgrass tissue nitrogen, but it was clear that the combination of the three different primary producer components best reflected the overall effect of

eutrophication, highlighting the variable response of individual ecosystems to nutrient loading.

There were two notable omissions in our study on primary producers: benthic and epiphytic microalgae. Benthic microalgal biomass and production frequently equal or exceed the biomass and production of phytoplankton in shallow coastal habitats (Webster et al. 2002), and epiphytic microalgae can, on average, contribute 18% of the productivity of an eelgrass bed (Penhale 1977). Increases in biomass and productivity and a concurrent decrease in diversity, as well as a shift from large diatoms to small diatoms and cyanobacteria with increasing eutrophication, have been documented for both epiphytic and benthic microalgal species (Neckles et al. 1993; Coleman and Burkholder 1994; Hillebrand and Sommer 1997). Both types of microalgae play a significant role in the uptake, cycling, and trophic transfer of both carbon and

nitrogen (Neckles et al. 1993; Sundbäck and Miles 2002; Webster et al. 2002) and, along with their significant contribution to estuarine production, warrant further research into the effects of eutrophication on these two microalgal components, which is essential for a complete picture of the changes in primary producer assemblages in eelgrass ecosystems under increased nutrient loading.

Our large-scale field surveys clearly show that eutrophication of coastal estuaries is profoundly altering the primary producer assemblage, eelgrass canopy structure, and carbon and nitrogen storage capacity of coastal ecosystems at local and regional scales. These results highlight that management responses to eutrophication can be outlined on a regional scale yet may need to be refined on a more local scale where natural variation between estuaries and differences in human activities can modify an ecosystem's response to eutrophication. Without nutrient management, the projected increase in nitrogen loading into coastal waters coupled with warming ocean temperatures and expanding bivalve and finfish aquaculture, as well as other human activities, could push increasingly stressed eelgrass populations in the Canadian Maritimes into a slow decline, ultimately leading to the loss of an essential coastal habitat with potentially severe consequences for associated plant and animal communities as well as human well-being.

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