



# Salt Marsh Aboveground Production in New England Estuaries in Relation to Nitrogen Loading and Environmental Factors

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## Abstract

Aboveground production responses of *Spartina alterniflora* and *S. patens* in estuaries in Massachusetts, USA were assessed in relation to temporal (date) and physical (elevation and distance from creek edge) factors as well as nitrogen loading using stem  $\delta^{15}\text{N}$ , water column dissolved inorganic nitrogen (DIN), and upland nitrogen loading as nitrogen input proxies. All nitrogen input proxies had negative relationships with *S. alterniflora* stem density while stem height and biomass increased or were unaffected. Nitrogen content of *S. alterniflora* increased with stem  $\delta^{15}\text{N}$  but was not related to DIN or upland nitrogen loading proxies. For *S. patens*, stem density, biomass, and height all increased with stem  $\delta^{15}\text{N}$  while nitrogen content decreased. Stem density and biomass also varied with elevation. For *S. alterniflora*, this relationship was parabolic for stem density and declined linearly for biomass. Both stem density and biomass increased linearly for *S. patens*. Across the growing season, *S. alterniflora* stem density decreased, *S. patens* biomass increased, and nitrogen content declined for both *Spartina* species. *S. alterniflora* stem height also decreased with distance from the creek edge. Results show different responses for *Spartina* species to upland and water column nitrogen inputs and provide complementary information to results from controlled fertilization experiments.

**Keywords** Eutrophication ·  $\delta^{15}\text{N}$  · *Spartina* · Stem biomass · Stem density

## Introduction

Anthropogenic impacts to the nitrogen cycle have increased the transfer of nitrogen through rivers to estuaries and coastal waters (Vitousek et al. 1997). This increased anthropogenic nutrient loading in many coastal and estuarine ecosystems (Paerl et al. 2014) including the northeastern United States (Nixon 1995; Bowen and Valiela 2001) poses potential threats to salt marsh vegetation and associated ecosystem services (Turner et al. 2009; Deegan et al. 2012). Salt marsh vegetation could potentially buffer impacts to bordering coastal ecosystems by acting as a nutrient sink (Valiela and Cole 2002) if vegetation responds to increased nutrient supply with increased production. Enhanced primary production

could increase sediment accretion through more effective trapping of suspended particulates and consequently assist marsh platforms in keeping pace with sea level rise (Morris et al. 2002). Vegetation could also be adversely impacted by nitrogen additions (Turner et al. 2009; Deegan et al. 2012) resulting in diminished ecosystem services including nutrient removal. Increased marsh soil microbial respiration in response to nitrogen inputs could lead to marsh subsidence and increased greenhouse gas emissions (Wigand et al. 2009; Martin et al. 2018).

Salt marsh response to increased nutrient loading has primarily been studied through controlled fertilization experiments over the past nearly half century (e.g., Gallagher 1975; Valiela et al. 1975; Darby and Turner 2008a, b; Turner et al. 2009; Morris et al. 2013; Johnson et al. 2016). Individual fertilization treatments have varied in duration from a single year (e.g., McFarlin et al. 2008) to several decades (e.g., Turner et al. 2009; Morris et al. 2013). Studies have also used different application methods (e.g., direct application, additions to tidal water), amounts, and nitrogen forms (e.g., nitrate, ammonium), which may influence observed vegetation responses to eutrophication (Johnson et al. 2016).

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Salt marsh eutrophication responses can also be assessed by opportunistically sampling vegetation from sites with different nitrogen loading levels (e.g., Wigand et al. 2003; Alldred et al. 2017). Fertilization experiments allow the concentration of nitrogen inputs to be controlled through experimental additions, which is advantageous for quantifying salt marsh response to different levels of nutrient input but potentially problematic as a means of understanding marsh response to existing nutrient inputs given challenges in accurately simulating anthropogenic nitrogen inputs. While lacking similar precision surrounding nutrient input levels, a more opportunistic approach can complement controlled studies by using available nitrogen input proxy data to assess relative marsh responses. Examples of potential proxy data include water column nutrients, land use, and nitrogen loading models.

For systems in which wastewater is the dominant nitrogen input, nitrogen stable isotopes ( $^{15}\text{N}/^{14}\text{N}$ ;  $\delta^{15}\text{N}$ ) can act as a proxy for relative eutrophication states due to the demonstrated positive relationship with the percent wastewater contribution to total nitrogen loads (Cole et al. 2004). Nitrogen derived from septic systems becomes increasingly enriched in  $^{15}\text{N}$  as it travels towards the local aquifer due to ammonia volatilization and denitrification processes that preferentially remove  $^{14}\text{N}$  (Aravena et al. 1993). The positive relationship between  $\delta^{15}\text{N}$  and percent wastewater contributions to nitrogen loading has been well documented in estuarine ecosystems in groundwater, particulate organic matter, primary producers, and primary and secondary consumers (McClelland et al. 1997; McClelland and Valiela 1998; Carmichael et al. 2004; Cole et al. 2006; Martinetto et al. 2006; Wigand et al. 2007). This relationship has been documented for both *Spartina alterniflora* and *S. patens* (McClelland et al. 1997; McClelland and Valiela 1998; Martinetto et al. 2006; Wigand et al. 2007; Kinney and Valiela 2018) providing support for the use of these species as sentinels of relative eutrophication states across estuaries for which wastewater is the primary nitrogen source.

In addition to eutrophication and other anthropogenic impacts, salt marsh production can also vary seasonally (Chaisson 2012; Morris et al. 2013) and in relation to abiotic factors (Wigand et al. 2003; Morris et al. 2013; Kulawardhana et al. 2015), which can confound assessments of eutrophication effects (Wigand et al. 2003). To assess these various influences, *S. alterniflora* and *S. patens* stem density, biomass, height, and nitrogen content were compared in relation to nitrogen inputs (water column DIN, upland N-loading, stem  $\delta^{15}\text{N}$  as wastewater proxy), elevation, distance from creek edge, and sampling date from quadrat samples collected in estuaries in Massachusetts, USA.

## Methods

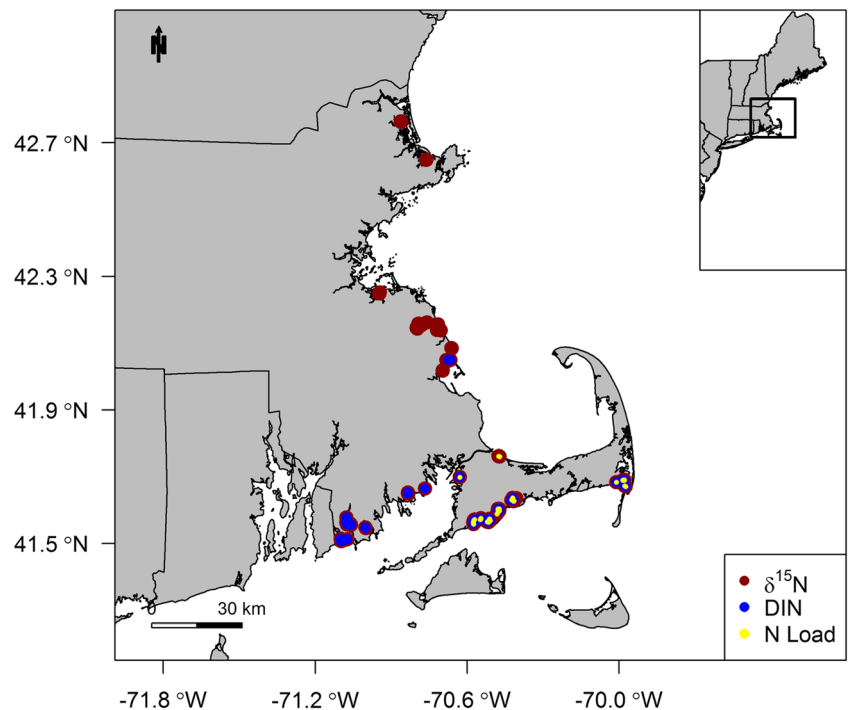
### Field Sampling

Aboveground biomass samples were collected as part of a study of dock shading impacts on salt marsh vegetation (Logan et al. 2018). Samples ( $n=200$ ) were collected from July through September 2014 from sites ( $n=193$ ) along the Massachusetts coastline (Fig. 1) with varying nitrogen loads (e.g., Hamblin Pond: 58 kg/ha/yr., Green Pond: 124 kg/ha/yr., Pocasset River: 302 kg/ha/yr) (Carmichael et al. 2004; Williamson et al. 2017). Estuaries included in this dataset are representative of the range of nitrogen loading conditions reported for southern Massachusetts (Williamson et al. 2017). Most samples ( $n=131$ ) were collected in Massachusetts estuaries on or south of Cape Cod where total nitrogen loading inputs have largely been stable over the past twenty years despite temporal changes in individual nitrogen inputs (e.g., atmospheric deposition, wastewater) (Rheuban et al. 2016; Valiela et al. 2016; Williamson et al. 2017). Eight 1/16 m<sup>2</sup> clip plot samples were collected five meters perpendicular to a dock at each site with individual quadrat locations based on a randomized grid system within an overall square meter area. The sampling area was centered mid-way between the upland and seaward boundaries of the marsh platform. If the site contained both high and low marsh zones, samples were collected from both regions with the two sampling sites centered in the middle of the respective zones. All stems were clipped at the marsh platform surface and stored frozen until later analysis within the following year.

### Laboratory Analyses

Vegetation samples were later thawed, rinsed, and processed for stem density, height, dry biomass, elemental composition, and stable isotope analysis. For each quadrat sample, all live stems were separated by species and counted. Total length measurements were made for the five tallest stems of the dominant *Spartina* species from each quadrat sample ( $\pm 0.1$  mm). Stems were then dried in a drying oven at 70 °C for  $\geq 48$  h and weighed ( $\pm 0.01$  g) separately by species. All dry, live biomass of the dominant *Spartina* species (*S. alterniflora* or *S. patens*) from each site was then homogenized using a blender. The homogenate was precisely measured ( $\pm 0.001$  mg) and various samples between 6 and 7 mg from each station were then packed into a tin capsule. Packed samples were analyzed for percent nitrogen and nitrogen stable isotope ratios ( $\delta^{15}\text{N}$ ;  $^{15}\text{N}/^{14}\text{N}$ ) using an elemental analyzer coupled with an isotope ratio mass spectrometer (EA-IRMS) at the Viking Environmental

**Fig. 1** Map of salt marsh sampling sites. All sites included stem  $\delta^{15}\text{N}$  (‰) data (red) while a subset also included water column DIN ( $\mu\text{M}$ ) (blue) and/or local nitrogen loading (kg/year) (yellow)



Stable Isotope Lab (VESIL) at Salem State University. All nitrogen isotope data are reported in  $\delta$  notation according to the following equation:

$$\delta^{15}\text{N} = \left[ \left( \frac{^{15}\text{N}/^{14}\text{N}_{\text{Sample}}}{^{15}\text{N}/^{14}\text{N}_{\text{Standard}}} \right) - 1 \right] * 1000$$

All isotope samples were calibrated using USGS40 and USGS41 standards and are reported as per mil relative to Air. Reference gas stability on the EA-IRMS system was 0.02‰. For elemental analysis, values were corrected using a sulfanilamide standard, and for each daily run, factors ranged between 0.95 and 1.05. The mean standard deviations of duplicate samples were 0.2% (N,  $n = 41$ ) and 0.1‰ ( $\delta^{15}\text{N}$ ,  $n = 21$ ).

### Environmental and Additional Nitrogen Input Proxy Datasets

Additional environmental data were collected following field sampling. Site elevation ( $\pm 0.1$  m) was estimated using the National Elevation Dataset (NED) (Gesch et al. 2009) with data from the 3D Elevation Program (Carswell Jr 2014) where values are referenced to the North American Vertical Datum of 1988 (NAVD88). Mean tidal range ( $\pm 0.1$  m) was estimated based on available data from the closest tide station gauge obtained from the Massachusetts Ocean Resource Information System (MORIS; [http://maps.massgis.state.ma.us/map\\_ol/moris.php](http://maps.massgis.state.ma.us/map_ol/moris.php)). Distance from the nearest creek edge was based on the shortest straight line distance to the closest marsh edge with direct connectivity to the main water body,

which was estimated ( $\pm 0.1$  m) using the ruler tool in Google Earth v. 6.1. Correlations among co-variates were quantified and evaluated based on Pearson correlation coefficients calculated using the “cor” function in R (R Core Team 2016).

Water column nutrient data were obtained from Buzzards Bay Coalition (Rheuban et al. 2016; Buzzards Bay Coalition 2017) and Center for Coastal Studies (Center for Coastal Studies 2017) datasets. Site average dissolved inorganic nitrogen (DIN) data ( $\mu\text{M}$ ) were used for the 2014 growing season (July–September) from monitoring sites in close proximity to marsh sampling sites ( $n = 110$  samples). The Cape Cod Commission WatershedMVP application 3.1 (The Cape Cod Commission 2017) was used to estimate upland nitrogen loading. For each salt marsh sampling site ( $n = 80$  samples), the total estimated unattenuated nitrogen load (kg/year) was calculated for the bordering upland region. This polygon region extended 25 m laterally along the shoreline bordering each side of the sampling site and 200 m landward into the upland. A 200 m height was included to capture the watershed area most closely associated with nitrogen inputs to bordering waterways (Cole et al. 2006). A 50 m shoreline polygon width was used to account for uncertainty in groundwater flow paths.

### Statistical Analyses

To assess the appropriateness of stem  $\delta^{15}\text{N}$  as a proxy for site nitrogen loading within this dataset, stem  $\delta^{15}\text{N}$  was compared with available measures of both water column and upland nitrogen loading using linear regression. For water column contributions, average site DIN and stem  $\delta^{15}\text{N}$  were regressed

separately for *S. alterniflora* samples collected at high and low elevations since relative DIN contributions to *Spartina* should vary with tidal inundation. An elevation of 0.34 m, the average elevation among sampling sites, was used to delineate high and low elevation classifications.

Generalized additive models (GAMs) were used to examine the relationship between each nitrogen input proxy (DIN, upland N-load, stem  $\delta^{15}\text{N}$ ) and stem aboveground production with the “mgcv” package (Wood 2006) in R. Sites with available DIN data were largely constrained to Cape Cod and southeastern Massachusetts while local N-loading data were only available for Cape Cod estuaries. Sites with stem  $\delta^{15}\text{N}$  data included Cape Cod and southeastern Massachusetts as well as estuaries further north towards the New Hampshire border (Fig. 1). This latter dataset was used to assess both *S. patens* and *S. alterniflora* responses to varying nitrogen inputs and to examine the influence of spatial and environmental variables given its larger size and inclusion of a broader diversity of environmental conditions. Due to small sample sizes, multiple linear regression was used to assess relationships for stem height and nitrogen content with environmental variables for *S. patens*. Diagnostics were performed on these datasets and visually evaluated for normality. The relative importance of each environmental variable was assessed using averaging over orderings with the “lmg” function within the “relaimpo” package (Grömping 2006) in R. Analyses were constrained for the DIN and N-loading datasets to *S. alterniflora* as sample sizes for *S. patens* were limited (DIN: stem density and biomass ( $n = 54$ ), stem height and % N ( $n = 8$ ); upland N: stem density and biomass ( $n = 40$ ), stem height and % N ( $n = 5$ )) and often consisted of only a few stems within an *S. alterniflora*-dominated area.

For each vegetation metric, a set of candidate models was generated with the full model including a nitrogen input proxy (stem  $\delta^{15}\text{N}$ , DIN, or upland N), sampling date, elevation, and distance from creek edge. To account for sampling multiple sites within individual estuaries, all models also included estuary as a random effect. For analyses of stem density, biomass, and height, a Tweedie family of distributions with a log-link was used with  $p$ -values determined for each analysis based on diagnostics using the “gam.check” function within the “mgcv” package. Since nitrogen content data are bound between zero and one, a logit transformation was performed on the *S. alterniflora* nitrogen content data, and GAM analyses were performed on the logit-transformed values using a Gaussian family of errors with an identity link function. DIN was included in GAM models as an interaction with elevation using a scale invariant tensor product based on a hypothesized relationship between tidal inundation and water column nutrient influence.

All possible model combinations were analyzed using the “MuMIn” package (Bartoń 2016) in R with site location (estuary) included in all candidate models as a random effect.

All models were ranked based on Akaike’s information criterion values corrected for small sample sizes ( $AIC_c$ ) using the equation:

$$AIC_c = -2 \text{ Log Likelihood} + 2K + \frac{2k(k+1)}{n-k-1},$$

where  $k$  is the number of parameters and  $n$  is the sample size (Burnham and Anderson 2002, 2004). The first term reflects the deviance and tends to decrease with additional parameters while the second term penalizes model complexity by increasing with additional parameters. By combining these terms,  $AIC_c$  values reflect a balance between improved explanatory power and model complexity among candidate models. The sum of Akaike weights of models and predictor variables was also calculated for models with  $\Delta AIC_c < 7$ , as all models within this  $\Delta AIC_c$  range have some support as best models (Burnham et al. 2011). Akaike weights range from zero to one. Models with higher weights have more support as best models. Variables found in a higher percentage of models will have higher Akaike weight values with variables found in all or none of the considered models having Akaike weight values of one and zero, respectively (Symonds and Moussalli 2011). Percent deviance explained and adjusted  $R^2$  were also calculated to provide absolute measures of model fits and explanatory power as  $AIC_c$  values only reflect how a given model performs relative to other candidate models (Galipaud et al. 2014).

Different subsets of the dataset were used for models relating to each stem characteristic. For stem density and aboveground biomass, *S. alterniflora* analyses were based on all sites for which *S. alterniflora* was present (DIN:  $n = 108$ , upland N:  $n = 79$ , stem  $\delta^{15}\text{N}$ :  $n = 183$ ) and *S. patens* analyses on all sites for which this latter species was present (stem  $\delta^{15}\text{N}$ :  $n = 104$ ). For stem height and nitrogen content analyses, only datasets containing data for each respective species of interest were used. Height [*S. alterniflora* (DIN:  $n = 101$ , upland N:  $n = 79$ , stem  $\delta^{15}\text{N}$ :  $n = 166$ ) and *S. patens* (stem  $\delta^{15}\text{N}$ :  $n = 31$ )] and nitrogen content [*S. alterniflora* (DIN:  $n = 101$ , upland N:  $n = 79$ , stem  $\delta^{15}\text{N}$ :  $n = 168$ ) and *S. patens* (stem  $\delta^{15}\text{N}$ :  $n = 32$ )] models were based on data subsets containing data for each respective species.

Stem  $\delta^{15}\text{N}$  was measured for both species at six sites where samples were collected from both high and low marsh zones, and *S. alterniflora*  $\delta^{15}\text{N}$  was higher with a median difference of +0.9‰. This interspecies difference is consistent with previously reported data that have generally shown a 1–2‰ offset (Deegan and Garritt 1997; Wainright et al. 2000; Wigand et al. 2007) possibly due to partial reliance of *S. patens* on isotopically-light nitrogen derived from nitrogen fixation by fungal symbionts (Wigand et al. 2007). Stem  $\delta^{15}\text{N}$  was increased for *S. patens* ( $n = 32$ ) by 0.9‰ (median difference between species) for direct comparison with *S. alterniflora* ( $n = 168$ ) stem data. *S. alterniflora* and *S. patens* stem  $\delta^{15}\text{N}$

values both increase as a function of percentage of nitrogen contributed by wastewater with similar slopes (Wigand et al. 2007), so following a baseline correction, either species provides a comparable proxy for nitrogen loading dominated by wastewater contributions. Separate analyses were also performed using only the subsets of data with  $\delta^{15}\text{N}$  values derived directly from the species of interest for comparison.

## Results

### Explanatory Variables and Dataset Comparisons

Of the explanatory variables considered for the GAM and linear regression analyses, mean range and elevation were the only strongly correlated variables ( $r = 0.71$ ). Mean range was excluded from further analyses due to this high correlation with elevation.

Analyses based on full datasets using  $\delta^{15}\text{N}$  from both *S. alterniflora* and normalized *S. patens* as well as sub-datasets based only on single species for all analyses showed the same relationships between site  $\delta^{15}\text{N}$  and stem characteristics. Only results for the pooled species analyses are presented as they are larger datasets and better represent the additional physical co-variables.

### Stem $\delta^{15}\text{N}$ , DIN, and Upland N-Loading

*Spartina*  $\delta^{15}\text{N}$  had a significant but weak positive correlation with both water column and upland nitrogen loading proxies. Average site DIN was significantly correlated with *Spartina*  $\delta^{15}\text{N}$  ( $P < 0.01$ ,  $R^2 = 0.08$ ; Fig. 2). When analyzed separately for high ( $>0.34$  m) and low ( $<0.34$  m) elevation sites, the correlation was significant for low ( $P < 0.01$ ,  $R^2 = 0.16$ ) but not high ( $P = 0.48$ ,  $R^2 = 0.01$ ) elevation sites. Bordering upland annual nitrogen loading was significantly correlated with *Spartina*  $\delta^{15}\text{N}$  ( $P < 0.001$ ,  $R^2 = 0.15$ ; Fig. 2). The upland nitrogen correlation was strongly influenced by two outliers with the highest measured *Spartina*  $\delta^{15}\text{N}$  values (12.5 and 15.0 ‰) but only moderate upland nitrogen loading estimates (26.4 and 16.8 kg/yr). The  $R^2$  increased to 0.28 when these outliers were excluded.

The three N-loading proxies showed the same general relationships with *S. alterniflora* stem characteristics for stem density, biomass, and height, but not nitrogen content (Tables S1-S12; Figs. 3, 4, 5 and 6). For the DIN data subset, low elevation sample stem density decreased in relation to water column DIN while stem biomass and height increased (Fig. 3). The DIN and elevation interaction co-variate was part of top models for these three stem metrics, but was absent from all top models ( $\Delta\text{AIC}_c < 7$ ) of nitrogen content. For the upland N-loading subset, stem density decreased while biomass and height both increased in response to this nitrogen

loading proxy although explanatory power was low for all biomass models with the model based only on upland N-load only explaining 5.5% of deviance (Table S6; Fig. 4). Upland N-loading was part of the best models for stem density and biomass but not stem height, which varied mainly with proximity to the creek edge. However, deviance explained was 32% for a GAM based on upland nitrogen loading alone, providing support for its positive association with stem height. For nitrogen content, the upland nitrogen loading co-variate accounted for less than 1% of deviance explained. Stem  $\delta^{15}\text{N}$  followed similar patterns to DIN and upland nitrogen loading for stem density (decreasing), biomass (increasing or unchanged), and height (increasing). Unlike the other nitrogen proxies, stem  $\delta^{15}\text{N}$  was also part of best models for nitrogen content and had a positive linear relationship (Table S12; Fig. 6).

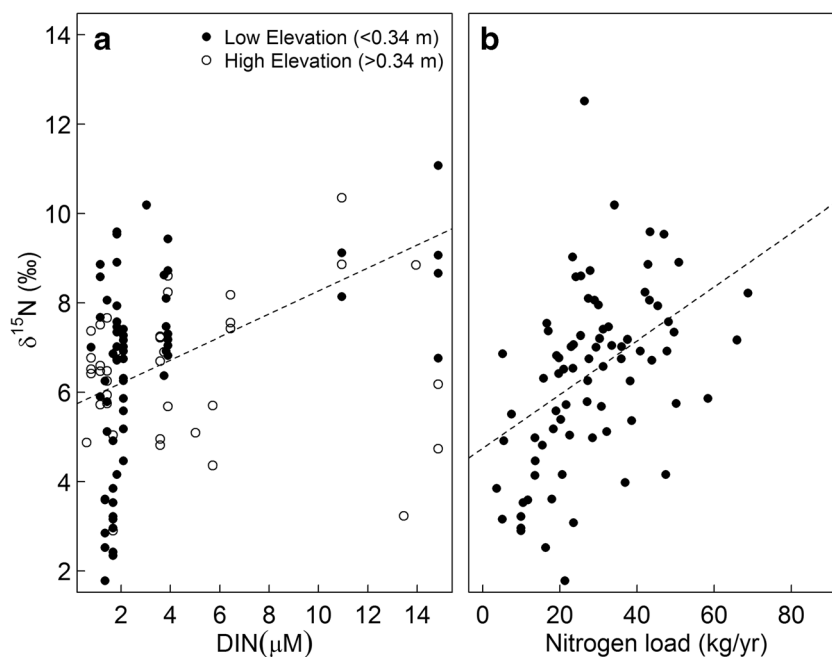
### Full Dataset - *S. alterniflora*

The best model of *S. alterniflora* stem density explained 28.6% of deviance and included stem  $\delta^{15}\text{N}$ , ordinal date, and site elevation (Table S9; Fig. 5). Stem density had a negative linear relationship with stem  $\delta^{15}\text{N}$  and date and a parabolic relationship with site elevation (Fig. 5). Stem  $\delta^{15}\text{N}$  was included in all top models, and  $\delta^{15}\text{N}$ , date, and elevation were all part of the two top models that had combined model weights of almost 0.9. Distance from creek edge was part of three top models, but its inclusion in two of these three instances increased the model  $\text{AIC}_c$  value relative to the equivalent model with distance omitted. A model consisting of distance and  $\delta^{15}\text{N}$  only had a model weight of 0.02 and resulted in an  $\text{AIC}_c$  increase relative to the simpler model, suggesting its limited explanatory power.

The best model for stem biomass included site elevation and explained 20.2% of deviance. Biomass declined linearly with elevation (Table S10; Fig. 5), which had an Akaike weight of 0.71. Fifteen candidate models had  $\Delta\text{AIC}_c$  values  $< 7$ . A model based only on estuarine site had a lower  $\text{AIC}_c$  value than six of these models and had a  $\Delta\text{AIC}_c$  value within three units of the top model. The selected explanatory variables poorly explained observed biomass variability, which instead appeared to mainly differ among estuaries due to factors beyond those considered in this analysis. Consistent with results from the DIN and upland N-loading data subsets, stem  $\delta^{15}\text{N}$  had the second highest Akaike weight with biomass showing an associated moderate linear increase with  $\delta^{15}\text{N}$ .

The best model of *S. alterniflora* stem height explained 46.3% of deviance and included stem  $\delta^{15}\text{N}$  and distance from the creek edge (Table S11; Fig. 6). Both relationships were linear with positive and negative associations for the two respective covariates. Stem  $\delta^{15}\text{N}$  was part of all top models and was the sole covariate in the model with the second lowest

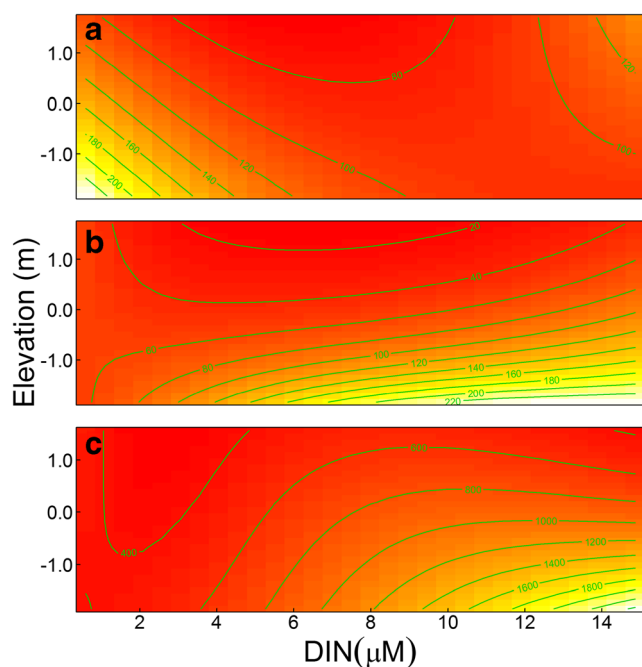
**Fig. 2** *Spartina* stem  $\delta^{15}\text{N}$  (‰) in relation to (a) water column dissolved inorganic nitrogen (DIN) concentrations ( $\mu\text{M}$ ) and (b) upland annual nitrogen load (kg/yr). The dashed line is the best fit linear regression line for (a) high elevation (>0.34 m) samples and (b) all samples



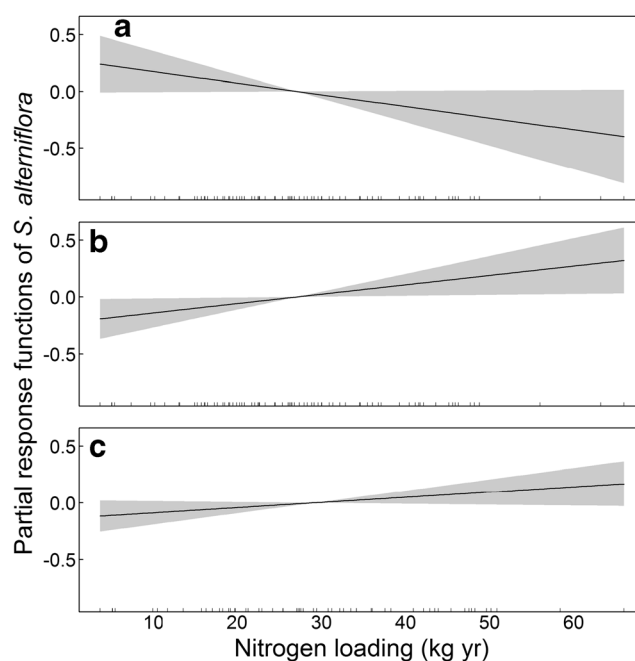
$\text{AIC}_c$  value, which explained 44.8% of deviance. Date and elevation both resulted in increased  $\text{AIC}_c$  values relative to equivalent top models with these co-variables excluded.

Stem nitrogen content was best explained by stem  $\delta^{15}\text{N}$  and sampling date with the top model explaining 33.3% of deviance (Table S12; Fig. 6). Stem  $\delta^{15}\text{N}$  and date were part of

all top models. Nitrogen content had a positive linear relationship with stem  $\delta^{15}\text{N}$ . Nitrogen content decreased sharply during early summer and stabilized during late summer and early fall. Inclusion of elevation and distance from creek edge resulted in increased  $\text{AIC}_c$  values relative to equivalent models based only on date and stem  $\delta^{15}\text{N}$ .

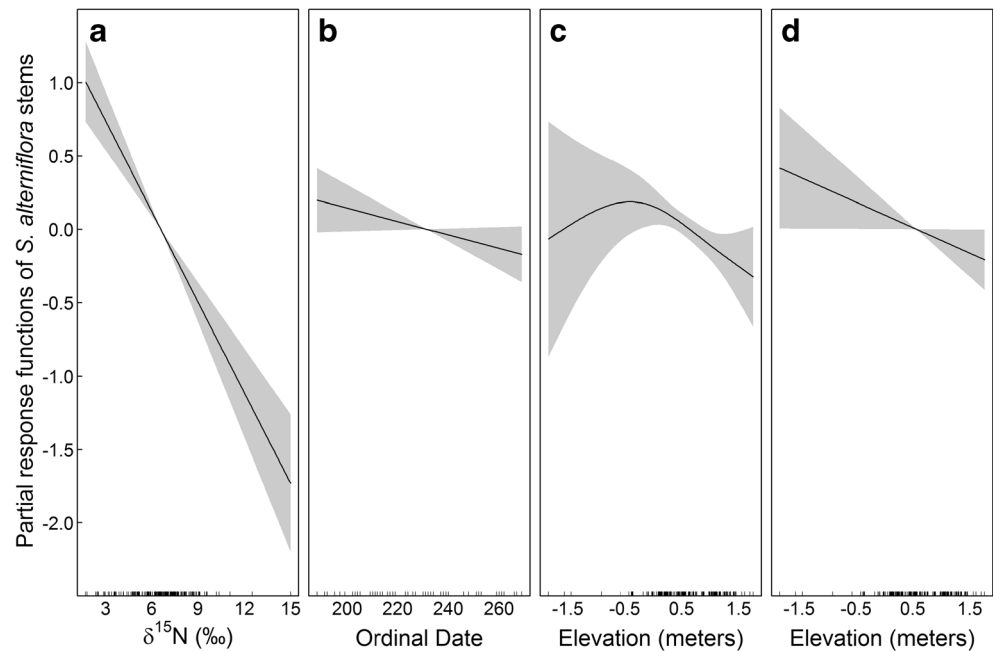


**Fig. 3** Outputs of generalized additive models (GAMs) of *Spartina alterniflora* stem (a) density, (b) biomass, and (c) height in response to the interaction of marsh elevation (m) and water column DIN ( $\mu\text{M}$ ). Areas shaded yellow represent peaks in the respective stem characteristic while darker areas shaded red reflect troughs



**Fig. 4** Partial response plots for *Spartina alterniflora* stem (a) density, (b) biomass, and (c) height in relation to upland annual nitrogen loading estimates (kg/yr). Shaded areas represent two standard errors above and below the smooth estimate, and the tick marks on the x-axis are rug plots showing sampling density

**Fig. 5** Partial response plots for the best candidate model of *Spartina alterniflora* stem (a–c) density and (d) biomass. The best model for stem density included (a) stem  $\delta^{15}\text{N}$  (‰), (b) sampling date, and (c) site elevation (meters) while the best model for biomass included (d) elevation (meters)



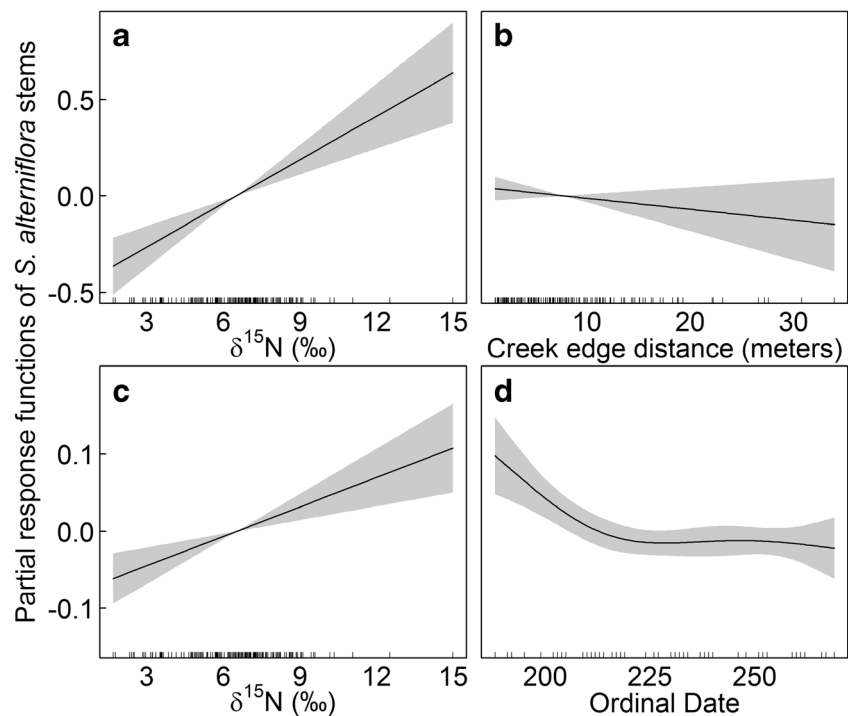
### Full $\delta^{15}\text{N}$ Dataset - *S. patens*

The best model for *S. patens* stem density, which explained 15.6% of deviance, included positive linear associations with both elevation and stem  $\delta^{15}\text{N}$  (Table S13; Fig. 7). Elevation was part of all top models while stem  $\delta^{15}\text{N}$  had an Akaike weight of 0.95. Date and elevation increased model  $\text{AIC}_c$  values when added to models based on elevation and stem  $\delta^{15}\text{N}$  or elevation alone.

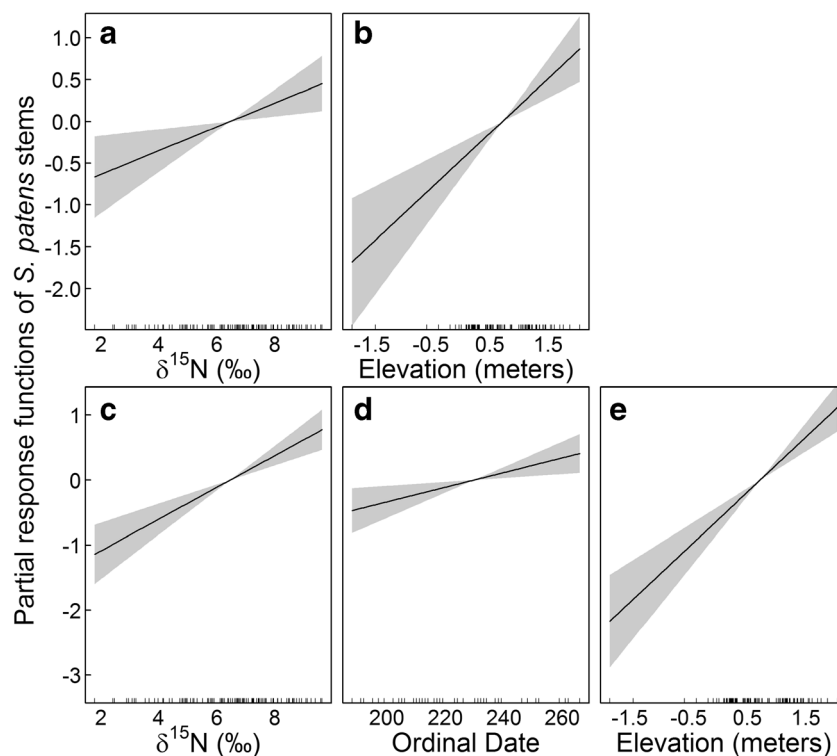
For biomass, the best model accounted for 30.1% of deviance and included positive linear associations with stem  $\delta^{15}\text{N}$ , date, and elevation (Table S14; Fig. 7). Stem  $\delta^{15}\text{N}$  and elevation were included in all top models while date had an Akaike weight of 0.84. Distance from creek edge increased model  $\text{AIC}_c$  values when combined with the other three co-variables.

Stem  $\delta^{15}\text{N}$  was significantly correlated with both stem height and nitrogen content, which was also significantly correlated with sampling date (Table S15;

**Fig. 6** Partial response plots for the best candidate model of *Spartina alterniflora* stem (a–b) height and (c–d) nitrogen content. The best stem height model included (a) stem  $\delta^{15}\text{N}$  (‰) and (b) the distance from the sampling site to the nearest creek edge (meters) while the best model for stem nitrogen content included (c) stem  $\delta^{15}\text{N}$  (‰) and (d) sampling date

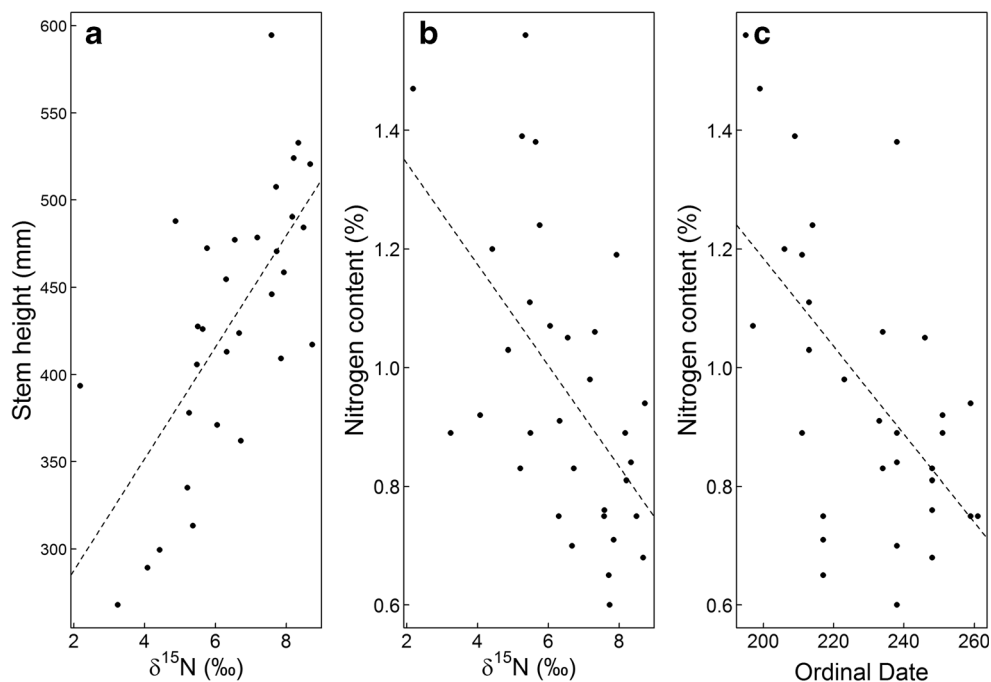


**Fig. 7** Partial response plots for the best candidate model of *Spartina patens* stem (a–b) density and (c–e) biomass. The best stem density model included (a) stem  $\delta^{15}\text{N}$  (‰) and (b) site elevation (meters) while the best model for biomass included (c) stem  $\delta^{15}\text{N}$  (‰), (d) sampling date, and (e) site elevation (meters)



**Fig. 8** *S. patens* stem height and nitrogen content had positive and negative relationships, respectively, with stem  $\delta^{15}\text{N}$  while nitrogen content was negatively related to sampling date (Fig. 8). Stem  $\delta^{15}\text{N}$  had the highest relative importance among explanatory variables for stem height while stem  $\delta^{15}\text{N}$  and ordinal date had similar relative importance values for nitrogen content (Table S15).

**Fig. 8** *Spartina patens* stem (a) height (mm) and (b) nitrogen content (%) in relation to stem  $\delta^{15}\text{N}$  (‰) as well as (c) nitrogen content (%) in relation to sampling date. The dashed line is the best fit linear regression line



## Discussion

The three nitrogen input proxies were related to almost all assessed *Spartina* characteristics and results for aboveground production responses generally agreed with previous controlled fertilization experiments. For *S. alterniflora*, stem density declined while height increased for all three proxies. *S. alterniflora* biomass instead was unaffected or increased indicating a



compensatory increase in average stem mass given the associated decrease in stem number. *S. alterniflora* stem nitrogen content increased with stem  $\delta^{15}\text{N}$  but did not vary as a function of the other two nitrogen input proxies. For *S. patens*, stem density, biomass, and height all increased while nitrogen content decreased with stem  $\delta^{15}\text{N}$ .

Results for *S. alterniflora* agreed with recent findings from a long-term fertilization experiment in the Plum Island estuary in northern Massachusetts (Johnson et al. 2016) while *S. patens* results matched previous plot fertilization studies (Vince et al. 1981; Levine et al. 1998; Wigand et al. 2003; Morris et al. 2013). The Plum Island fertilization experiment resulted in decreased *S. alterniflora* stem density but increased stem mass with the two effects counteracting for no net change in aboveground biomass (Johnson et al. 2016). Stem density also declined with all three nitrogen input proxies across Massachusetts estuaries while aboveground biomass remained constant or increased, consistent with an increase in individual stem mass. Best models for water column DIN and upland N-loading showed a positive relationship with *S. alterniflora* stem biomass, and  $\delta^{15}\text{N}$  was also part of several top models of stem biomass. Previous plot fertilization experiments produced increased aboveground biomass and stem density for *S. alterniflora* (Gallagher 1975; Valiela et al. 1975; Mendelssohn 1979a; Buresh et al. 1980; Vince et al. 1981; Levine et al. 1998; Pennings et al. 2002; Gratton and Denno 2003; Wigand et al. 2003; Bertness et al. 2008; Darby and Turner 2008a, b; McFarlin et al. 2008; Langley et al. 2009; Anisfeld and Hill 2012; Murphy et al. 2012; Morris et al. 2013). Johnson et al. (2016) argued that the responses of aboveground production to their long term fertilization treatment differed from these past studies as a result of differing methods. The Plum Island treatment consisted of tidal delivery of nitrate while past studies used dry application of ammonium to the marsh surface or rooting zone. Stem  $\delta^{15}\text{N}$  was positively related with both *S. patens* aboveground biomass and stem density, which contrasts with the Johnson et al. (2016) findings and may also reflect methodological effects. The Plum Island fertilization experiment was conducted in a system with minimal anthropogenic inputs (Deegan et al. 2007) so nitrogen additions were reliant on tidal transfer from the experimental point source. This experimentally-added nitrogen would contact low marsh zone vegetation regularly, but only briefly interact with high marsh vegetation (Deegan et al. 2007; Johnson et al. 2016). All of the sites sampled across Massachusetts estuaries were associated with private docks, and the homes associated with these structures represent potential upland nitrogen sources (e.g., septic systems) absent from the Plum Island site.

The positive relationship between stem nitrogen content and stem  $\delta^{15}\text{N}$  observed across Massachusetts estuaries for *S. alterniflora* was consistent with increases following fertilization in previous studies (Vince et al. 1981; Stiling et al. 1991; Gratton and Denno 2003; Deegan et al. 2007; McFarlin et al. 2008; Murphy et al. 2012) while the observed negative relationship

for *S. patens* differed (Vince et al. 1981; Deegan et al. 2007; Johnson et al. 2016). The increase in stem nitrogen content with stem  $\delta^{15}\text{N}$  is consistent with vegetation incorporating the additional nitrogen made available by wastewater inputs. For *S. patens*, previous fertilization experiments demonstrated increased stem nitrogen content (Vince et al. 1981) or no effect (Deegan et al. 2007; Johnson et al. 2016), in agreement with observed increases (Vince et al. 1981) or lack of fertilization effects (Deegan et al. 2007; Johnson et al. 2016) on aboveground biomass. The negative relationship between stem nitrogen content and stem  $\delta^{15}\text{N}$  in Massachusetts estuaries could reflect more limited nitrogen availability in the high marsh zone with available anthropogenic nitrogen used for growth. Nitrogen content was the only stem characteristic with different responses among the three nitrogen input proxies as neither DIN or upland N-loading were related to *S. alterniflora* values.

The seasonal decline in stem nitrogen content for both *Spartina* species mirrored previous observations for *S. patens* (Deegan et al. 2007), *S. alterniflora* (Mendelssohn 1979b; Morris 1982; Ornes and Kaplan 1989; Morris 1991; Deegan et al. 2007), and *S. maritima* (Cartaxana and Catarino 1997). These declines across the growing season are likely due to the dilution of stem nitrogen with increased growth and accumulation of aboveground biomass (Morris 1982; Cartaxana and Catarino 1997).

The increasing trend in stem height in relation to the three nitrogen input proxies is similar to past results showing increased aboveground production with fertilization, but only partly matched the more tempered response measured at the Plum Island fertilization site (Johnson et al. 2016). Increases in *S. alterniflora* height have been reported consistently for fertilization experiments performed along the U.S. East Coast (Buresh et al. 1980; Vince et al. 1981; Levine et al. 1998; Pennings et al. 2002; McFarlin et al. 2008; Anisfeld and Hill 2012; Murphy et al. 2012) including tall form *S. alterniflora* at the Plum Island site. Height responses for *S. patens* varied with experimental approach with direct fertilization inducing a positive height response (Levine et al. 1998) and tidal delivery producing no detectable changes (Johnson et al. 2016). Like stem density and biomass, these differing aboveground production responses could be explained by the mode of application; direct treatment would provide greater access to excess nitrogen than tidal delivery for the high marsh zone.

Relationships between *Spartina* aboveground production and elevation follow physical and biological factors that vary across marsh zones. The observed parabolic relationship between *S. alterniflora* stem density and elevation supports modeled results of *S. alterniflora* distribution (Morris 2007) in which the lower elevation limits production due to abiotic stress while the upper bound could be limited by additional physical stressors like drought or hypersalinity (Morris et al. 2013) as well as competitive exclusion (Bertness and Ellison 1987; Bertness 1991a). Stem height declined with distance

from creek edges for *S. alterniflora*, similar to observed patterns elsewhere in the northeast U.S. (Wigand et al. 2014) and the transitioning from tall form *S. alterniflora* near creek edges towards short form *S. alterniflora* higher along the marsh platform. Tall form *S. alterniflora* has lower stem densities than short form (Valiela et al. 1978), which would further contribute to observed increases in stem density from lowest to mid-elevation sites. Higher elevation samples were often taken in areas with co-occurring *S. patens*, the competitive dominant of *S. alterniflora* (Bertness and Ellison 1987; Bertness 1991a). *S. alterniflora* biomass instead decreased linearly with elevation, similar to experimental results (Morris et al. 2013). The decline in biomass for higher elevations is consistent with the reduced stem density and likely due to the same abiotic and biological stressors. The lack of a parabolic relationship for biomass may be due to the influence of tall form *S. alterniflora* at the lowest elevations since this growth form would have lower stem density but possibly higher biomass than short form *S. alterniflora* found at higher elevations. *S. patens* stem density and biomass were both positively related to elevation. Samples were collected from the mid-point of each respective marsh zone, so samples collected from the high marsh zone would include the upper bounds of *S. alterniflora* distribution where stem density and biomass are reduced due to abiotic stress and competitive exclusion. For *S. patens*, samples would include the lower distributional bounds where more frequent tidal inundation and associated hypoxia would limit growth. The upper limit of distribution where *S. patens* would be displaced by competitive dominants like *Juncus gerardii* occupying the terrestrial border (Bertness 1991b) was not sampled, so only the positive relationship with elevation was evident.

Stem density declined across the growing season for *S. alterniflora* while *S. patens* biomass increased. Seasonal declines in *S. alterniflora* stem density could be due to self-thinning (Chaisson 2012) or tidal action (Cranford et al. 1989) while *S. patens* biomass increases follow consistent production rates throughout the growing season in the northwest Atlantic (Connor and Chmura 2000). *S. alterniflora* biomass in northern salt marshes is less variable from July to September (Connor and Chmura 2000; Morris et al. 2013), so sampling date was not part of the best models for biomass for this species.

## Conclusions, Caveats, and Next Steps

Model results demonstrate some enhancement of aboveground production for *S. patens* in response to increased wastewater inputs estimated through stem  $\delta^{15}\text{N}$  and mixed responses for *S. alterniflora* based on all three nitrogen input proxies, which collectively could result in both positive and negative eutrophication effects. Increased aboveground production for *S. patens* (stem density, biomass, and height) suggest that the high marsh zone can partly buffer nitrogen

loading effects by incorporating excess available nitrogen into live biomass. This response translates to increased aboveground productivity and possible enhancement of certain ecosystem services (e.g., detrital energy source, benthic invertebrate habitat). Increased primary production could also facilitate sediment accretion by enhancing trapping of suspended sediment (Morris et al. 2002), although this mechanism may be of less importance to marsh stability in systems with low sediment supplies (Alldred et al. 2017). Declining trends in *S. alterniflora* stem density could result in reduced sediment accretion and erosion buffering capabilities (Gleason et al. 1979) although increases in stem biomass could actually promote particle deposition due to reduced turbulent kinetic energy over the marsh surface (Mudd et al. 2010). Compensatory increases in stem height and biomass avoid any net losses of aboveground production so contributions to detrital food web per unit area may not be diminished across nitrogen loading conditions.

This analysis only assessed aboveground production, and negative impacts of eutrophication to salt marsh habitat have mainly been observed in belowground characteristics (Valiela et al. 1976; Buresh et al. 1980; Darby and Turner 2008a, b; Langley et al. 2009; Turner et al. 2009; Deegan et al. 2012; Alldred et al. 2017). Belowground biomass functions in erosion resistance and maintenance of marsh elevation. Eutrophication-induced reductions in belowground production can inhibit these functions and compound effects of sea level rise (Deegan et al. 2012). While aboveground production in Massachusetts estuaries showed positive or neutral relationships with nitrogen loading, negative impacts to belowground production could reduce detrital production through net loss of salt marsh acreage as a result of erosion and creek bank collapse (Deegan et al. 2012). Salt marshes in Long Island, New York with varying land uses and nitrogen inputs had 60 to 70% declines in belowground biomass in systems with higher nitrogen inputs despite an observed positive albeit weak relationship between aboveground production and available DIN (Alldred et al. 2017).

Each of the nitrogen input proxies used in this analysis is likely only a coarse approximation of actual nitrogen available to the accompanying salt marsh samples. Best models using each proxy had relatively low percent deviance explained values, and this limited explanatory power is probably related to the coarseness of these proxies. For nitrogen loading estimates, the upland area polygon may not have accurately reflected the actual sources impacting *Spartina* in a given sample site. The amount of nitrogen reaching the salt marsh from sampled areas would also be influenced by biological and abiotic factors not considered in this analysis (e.g., buffering from upland vegetation). Water column DIN concentrations are only point estimates and inadequate sampling frequency can result in inaccurate representations of nitrogen inputs at a seasonal timescale (Costa et al. 1992). DIN includes the most biologically active forms of nitrogen, so

DIN concentrations can be low during the growing season due to rapid uptake by phytoplankton and may not reflect system nitrogen loads (Costa et al. 1992). Indeed, water column nitrogen showed only weak positive correlations with nitrogen loading rates for many of the systems included in this study (Williamson et al. 2017). As salt marsh vegetation occupies the interface between uplands and estuarine water bodies, nitrogen from both of these sources can contribute to salt marsh production and so water column nutrients or upland nitrogen sources alone only reflect part of the nitrogen budget. The weak relationship between stem  $\delta^{15}\text{N}$  and DIN in this dataset is likely at least partly a reflection of this latter caveat as the relationship improved for samples from low elevation sites where more regular tidal inundation would increase the influence of water column nutrients on salt marsh production. Cole et al. (2004) observed a similarly weak ( $R^2 = 0.05$ ) positive correlation between water column DIN and aquatic plant  $\delta^{15}\text{N}$  and attributed the weak correlation to plant usage of both porewater and water column nitrogen. Macrophytes that rely entirely on water column nutrients showed a stronger, significant correlation between  $\delta^{15}\text{N}$  and DIN (Cole et al. 2004).

The relatively weak relationship between stem  $\delta^{15}\text{N}$  and both DIN and upland N also suggests limitations to the  $\delta^{15}\text{N}$  nitrogen input proxy approach, whose efficacy is reliant upon wastewater acting as the dominant nitrogen source across study sites. Inorganic fertilizer runoff from lawns or agricultural fields would actually impart lower stem  $\delta^{15}\text{N}$  values and thus confound the presumed positive  $\delta^{15}\text{N}$ -nitrogen loading relationship as such fertilizers have low  $\delta^{15}\text{N}$  due to their atmospheric nitrogen origin (Kendall et al. 2007). Sections of the Westport River watershed, for example, have a high percentage of agricultural activity in addition to residential development (Howes et al. 2013). The analysis of *Spartina*  $\delta^{15}\text{N}$  and upland nitrogen loading relationships included several outliers (low stem  $\delta^{15}\text{N}$ , high nitrogen loading rates) from sites located near a golf course, a likely source of  $^{15}\text{N}$ -depleted nitrogen (Flipse Jr. and Bonner 1985). Various natural process within estuaries (e.g., nitrification and denitrification) can also influence baseline  $\delta^{15}\text{N}$  values (Cifuentes et al. 1989; Horrihan et al. 1990) and would further weaken the relationship between nitrogen loading and *Spartina*  $\delta^{15}\text{N}$ . Nutrient recycling is another important nitrogen source in salt marshes (Valiela and Teal 1979; Hopkinson and Schubauer 1984; White and Howes 1994). In cases where anthropogenic nitrogen inputs are low, internal recycling and other processes would influence stem  $\delta^{15}\text{N}$  and weaken its relationship with total nitrogen inputs. The high degree of variability in stem  $\delta^{15}\text{N}$  among sites with low water column DIN concentrations (Fig. 2) is consistent with this decoupling of the  $\delta^{15}\text{N}$ -nitrogen loading relationship with low loads. Similarly, the relationship between nitrogen loading and stem  $\delta^{15}\text{N}$  is asymptotic, so  $\delta^{15}\text{N}$  is also less suitable as a nitrogen input proxy at highest nitrogen loading levels (Kinney and Valiela 2018). Such caveats limit the general

applicability of this  $\delta^{15}\text{N}$ -proxy approach to systems where wastewater is the principle anthropogenic nitrogen source and overall nitrogen loading is moderate.

Many biological and environmental variables that can potentially influence vegetation aboveground production were not considered in this analysis. Salinity can have an interactive effect with nutrient loading on *Spartina* biomass and nitrogen uptake (Medeiros et al. 2013; MacTavish and Cohen 2017). Competition can also influence *Spartina* aboveground production with interactive effects of both salinity and nutrient levels (Legault II et al. 2018). Failure to account for such additional drivers of aboveground production likely contributed to relatively low percent deviance explained among GAMs.

Despite these caveats, this nitrogen input proxy approach provides information on salt marsh aboveground production responses to coastal eutrophication that complements results of controlled fertilization experiments. By making use of existing nitrogen loading conditions, this study avoids potential methodological biases associated with artificial nutrient addition simulations in controlled experimental studies. Sampling sites subjected to anthropogenic inputs over many decades also allows chronic eutrophication effects to be assessed that may not be captured in controlled fertilization experiments (Allred et al. 2017). When applied to a large sample size, this approach also avoids possible biases of site-specific effects and provides a cost-effective means of assessing eutrophication effects across a range of nitrogen loading conditions and marsh habitat types. Generalized additive models provide a robust, flexible framework for evaluating marsh production responses across such nitrogen loading gradients.

Researchers employing this approach in future studies should select sites with appropriate nitrogen loading characteristics and include additional biological and environmental data. For example, a  $\delta^{15}\text{N}$  tracer approach should be limited to sites that vary in terms of percent wastewater inputs but are otherwise similar. Field sites for use with more conventional input proxies (e.g., water column nutrients, land use patterns, nitrogen loading models) should be selected based on a priori identification of availability of such data in close proximity to marsh sampling locations. Finally, future studies employing this approach should assess both above and belowground production metrics and consider additional biological and environmental variables (e.g., salinity, competitive interactions).

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