Spartina alterniflora $\delta^{15}$N as an indicator of estuarine nitrogen load and sources in Cape Cod estuaries

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Highlights: (limited to 85 characters per highlight, 5 highlights)

- We compared $\delta^{15}$N values in above-ground cordgrass to nitrogen loads in 7 estuaries.
- We sampled 3 months over 2 growing seasons to look for seasonality differences.
- We found that samples should be collected during the peak growing season.
- $S. alterniflora$ $\delta^{15}$N is a highly sensitive indicator of nitrogen load.
- $S. alterniflora$ $\delta^{15}$N is more sensitive at incipient levels of eutrophication.
ABSTRACT

$\delta^{15}$N values of coastal biota have been used as indicators of land-derived N-loads and sources to estuarine systems and should respond predictably to differences in nitrogen and be sensitive to changes in nitrogen, preferably at the low end of eutrophication. We evaluated *Spartina alterniflora* as an indicator species of N-loads and sources of $\delta^{15}$N throughout the growing season, and compared the average $\delta^{15}$N to estuarine nitrogen loads and sources for several estuaries receiving different watershed N-loads. $\delta^{15}$N of *S. alterniflora* differed among estuaries, and these differences were maintained even as $\delta^{15}$N declined during the end of the growing season. $\delta^{15}$N values increased with increasing nitrogen loads to the subestuaries and with increasing percent wastewater-derived nitrogen load. The response of $\delta^{15}$N of *S. alterniflora* to increased N loads was greater at low N-loads, and decreased as N-loads increased, suggesting that *S. alterniflora* is a good indicator of incipient nitrogen load.

*Keywords: waste water; seasonality; salt marsh; New England*
INTRODUCTION

Nitrogen inputs from watersheds to coastal aquatic ecosystems depend in some measure on the land covers present on the watershed (Koppelman 1978, Lee and Olson 1985). The relative importance of forest, agricultural, and residential land uses makes for important contrasts in the nitrogen loads received by coastal environments from atmospheric deposition, fertilizer use, and wastewater disposal (Valiela et al. 1997, 2000; Latimer and Charpentier 2010). Studies of these contrasting sources and land uses show that stable isotopic measurements on water (York et al. 2007), sediment (Struck et al. 2000, Castro et al. 2007, Church et al. 2006), and producers and consumers in the coastal food webs (Martinetto et al. 2006, Corbisier et al. 2006, Baeta et al. 2008, Fox et al. 2009, Fry 2009) may reveal the relative importance of the different sources of land-derived nitrogen (Valiela et al. 2000). Biota reflect δ^{15}N values associated with land-derived nitrogen, specifically wastewater nitrogen, and could be indicators of land-derived nitrogen loading and sources to estuaries (McClelland et al. 1997, Fry et al. 2000, Cole et al. 2005, Pruell et al. 2006, Wigand et al. 2007, and others). For a particular species to be a useful indicator of anthropogenic nitrogen, there should be a reliable response to differences in nitrogen supply and sensitivity to changes in nitrogen sources.

Salt marsh plants, such as Spartina alterniflora (salt marsh cordgrass), are present at the boundary between land and sea along a broad geographic span on the coasts of North America, positioning S. alterniflora to take up nitrogen from both estuarine and groundwater sources and making them a potential indicator of land-derived nitrogen entering estuaries. S. alterniflora is a common and widely distributed indicator species, and the δ^{15}N could not only monitor the degree of land-sea coupling by nitrogen transport, but may also reflect changing land covers on coastal
watersheds. For example, the greater the contribution of wastewater from a watershed, the heavier was the $\delta^{15}N$ of cordgrass (and other producers) growing on the coast of Cape Cod, Massachusetts (McClelland et al. 1997, Cole et al. 2005) and elsewhere (Cole et al. 2005, Wigand et al. 2001, 2007, Bannon and Roman 2008). Similarly, the greater the proportion of residential land use or population density, the heavier the $\delta^{15}N$ signatures in cordgrass present in receiving waters (Wigand et al. 2001, Bannon and Roman 2008, Bruland and MacKenzie 2010). These studies have linked $\delta^{15}N$ of cordgrass to coastal land use, which suggests that this species could become a useful monitoring tool, particularly because residential development and wastewater contribution to estuarine nitrogen loads are increasing, especially in the northeastern United States (Valiela et al. 1992, Bowen and Valiela 2001a, Valiela and Bowen 2002, Filoso et al. 2004, Bricker et al. 2007).

Cordgrass is sensitive to supply of external nitrogen. Experimental studies (Valiela and Teal 1974; Valiela et al. 1973, 1975; Nixon and Oviatt 1973; Chalmers 1979; Gallagher 1975; Sullivan and Daiber 1974; Levine et al. 1998; Caffrey et al. 2007; Broome et al. 1975; Deegan et al. 2012) in salt marsh fertilization have shown that stem height, biomass, and production in $S. alterniflora$ increased with higher nitrogen inputs and, similarly, $\delta^{15}N$ of cordgrass also responds to wastewater sources. The fertilization experiments were done at the m$^2$ spatial scale, which raises the question whether these results can be meaningfully scaled-up to a larger or even regional spatial scale, as needed in studies of watershed-estuary nutrient-mediated linkages.

To use $\delta^{15}N$ as useful proxies that detect land use and source differences, we need information as to the variation that might be expected in $\delta^{15}N$ of $S. alterniflora$ and how best to
sample to ensure reliable results. There might be seasonal and inter-annual differences in δ\(^{15}\)N that need to be considered.

Variability in δ\(^{15}\)N in *S. alterniflora* would affect its utility as an indicator of nitrogen loads. There might be seasonal changes in nitrogen uptake, translocation, flowering and senescence that might alter δ\(^{15}\)N in grasses. Studies in Rhode Island and Massachusetts have found modest monthly variability and inter-annual variability, but no consistent pattern of δ\(^{15}\)N in *S. alterniflora* leaves over a growing season (Pruell et al. 2006, Drake et al. 2008). Changes in δ\(^{15}\)N of *S. alterniflora* leaves during senescence and higher below-ground δ\(^{15}\)N values in root and rhizomes (Currin et al. 1995, Drake et al. 2008, White and Howes 1994b) suggests selective remineralization of isotopically heavier nitrogen toward the end of the growing season, as found in rice (Wada and Hattori 1991). Longer term decadal-scale trends might also be detectable if land covers change enough to result in different land-derived inputs. Potentially, multi-year changes in the δ\(^{15}\)N of *S. alterniflora* growing in affected receiving estuaries could reflect longer term changes in nitrogen loading. These long term changes may be a useful way to track incipient development of land-derived nitrogen enrichment.

In this study we first assess growth and δ\(^{15}\)N of *S. alterniflora* stands in estuaries that receive different nitrogen inputs from their watersheds, to discern whether the responses of *S. alterniflora* δ\(^{15}\)N to different watershed-scale nitrogen supply rates were similar to responses to fertilization at m\(^2\) scales. We then examine whether there were differences in δ\(^{15}\)N of *S. alterniflora* associated with season, land-derived nitrogen loads, and with contribution to nitrogen loads by wastewater. Finally, we test whether changes in land use on the contributing watersheds over a period of 15 years can be identified in changes in δ\(^{15}\)N in *S. alterniflora*. 
METHODS

Site Description

The Waquoit Bay system is located on the south shore of Cape Cod at 41.5626° N, 70.5220° W. It is made up of seven subestuaries whose watersheds receive a wide range of N loads [Childs River, Eel River, Quashnet River, Hamblin Pond, Jehu Pond, Sage Lot Pond and Timms Pond (Figure 1, Table 1)] as a result of different land uses and the use of septic systems for wastewater disposal. The seven subestuaries are connected through Waquoit Bay, but receive most of their water supply from groundwater and several independent streams and rivers. 

Spartina alterniflora is present along the banks of all seven subestuaries.

Growth response of S. alterniflora to different external nitrogen loadings

To examine the effect of different nitrogen loads on growth of S. alterniflora, we measured stem heights of ten tallest shoots growing in 1 m² areas within each of the Waquoit Bay in September of 2008. Locations were chosen away from the creek bank, on the salt marsh platform, approximately 2–3m from the bank. The S. alterniflora growth form that we measured was intermediate between the tallest creek bank plants and the shortest plants found closest to the high marsh border associated with Spartina patens. The land-derived nitrogen loads to these subestuaries within the Waquoit Bay estuarine system differ (Table 1). Height can be taken as a proxy for over-all response to nitrogen loads, based on reports of highly significant relationships of 10 tallest measurements to aboveground biomass in quadrats containing S. alterniflora and other marsh grasses (aboveground biomass in g m⁻²=9.477 height in cm⁻38.722, R=0.862, P<0.001) and to external nitrogen supply (Vince et al. 1981).
To ascertain whether responses to watershed-scale nitrogen loads by *S. alterniflora* were similar to responses obtained by fertilization of plots at the m² level, we compared ten tallest data from the Waquoit estuarine system to similar data from long-term enrichment experiments done in Great Sippewissett salt marsh (Table 2). These comparisons aimed to show if data obtained at m² scale could be reasonably scaled up to larger regional spatial contexts, and if increased nitrogen loads via fertilizer have similar effects on *S. alterniflora* growth as increased nitrogen loads to the watershed.

*Growth response of S. alterniflora to different external nitrogen loadings*

The Waquoit Bay system’s seven subestuaries tidal range across the system is relatively small (40-60 cm) and there is approximately 10-20 cm vertical relief across the salt mash gradient between the mean low tide and mean high tide marks, similar to the tidal range found at Great Sippewisset Marsh (Rietsma et al. 2011). All of the subestuaries have *S. alterniflora* marsh along their length with marsh size depending on the degree of development and encroachment on the water’s edge. To test whether δ¹⁵N of *S. alterniflora* changed seasonally or among years, depended on land-derived nitrogen load, or was related to the contribution to nitrogen loads made by wastewater disposal, we randomly collected five shoots of *S. alterniflora* in each of five approximately 1 m² locations around the shore of each of the Waquoit Bay subestuaries. The plants collected for δ¹⁵N analysis were located close to the creek-bank, as all sites consistently had salt marsh habitats fringing the waterway. Since we aimed to develop a practical protocol for use of *S. alterniflora* in a monitoring program, we used sampling that could be replicated in a variety of settings.
To define seasonal and inter-annual changes, the procedure was repeated every other month during the growing season for two years (20 and 21 June, 10 and 19 August, and 3 and 4 October 2005, 22 and 31 May, 11 and 12 July, and 25 and 27 September 2006). Samples were collected, dead leaves removed, and live leaves were rinsed with deionized water, dried at 60°C and ground into powder using a Retsch Mixer Mill. The five shoots from each sampling location were combined into one sample and homogenized. \( \delta^{15}N \) of samples were measured at the University of California Davis Stable Isotope Laboratory.

Decadal changes in nitrogen loads and in \( \delta^{15}N \) of S. alterniflora

To examine sensitivity of \( \delta^{15}N \) in S. alterniflora to decadal changes in land use on watersheds, and the consequent changes in land-derived nitrogen loads, we compared previously published nitrogen loads (Valiela et al. 2000) that were based on 1990 land use data, and S. alterniflora \( \delta^{15}N \) collected 1995-1996 (McClelland et al. 1997) to updated nitrogen loads and \( \delta^{15}N \) data we obtained in the 2000s. The decadal-scale comparison was equivalent to a real-time experiment in regional nitrogen loads and their effect on \( \delta^{15}N \) of the indicator species.

To calculate the 2000-era land-derived nitrogen loads we used watershed delineations of the basins draining into each of the Waquoit Bay and other Cape Cod estuaries (Valiela et al. 1992, Kroeger et al. 2006a). We also obtained land cover data and aerial photographs from 1990 and 2005 in GIS format from the Planning Offices of the towns of Falmouth and Mashpee. These delineation and land use files were needed as inputs necessary to run calculations of nitrogen loads using the Nitrogen Loading Model (NLM) (Table 3, available on http://nload.mbl.edu).

NLM uses land use inputs within a delineated watershed and calculates the fates of nitrogen from wastewater, fertilizers, and atmospheric deposition unto the watershed, and keeps track of the
fate of nitrogen from these sources as the nitrogen traverses soils, vadose zones, and travels in aquifers on its way to receiving estuaries. We used NLM to estimate nitrogen loads for 1990 and 2005 (Table 4).

RESULTS AND DISCUSSION

Response of S. alterniflora growth to land derived nitrogen loads

The standing crop of the S. alterniflora canopy, estimated from the height of the shoots, was significantly larger in estuaries with higher nitrogen loads (Figure 2). The slopes of stem height vs. nitrogen load in the Waquoit Bay subestuaries and the Great Sippewissett Marsh experimental plots were not significantly different.

Response of stable isotopic measurements in S. alterniflora

Seasonal variation in $\delta^{15}$N

Seasonal variation in $\delta^{15}$N in shoots of S. alterniflora was minimal (Figure 3), as also reported by Pruell et al. (2006) in Narragansett Bay, Rhode Island salt marshes and Drake et al. (2008) in the Plum Island, Massachusetts salt marsh. The only exception detected in the Waquoit Bay data was during October, when $\delta^{15}$N values were significantly lower according to each paired student $t$-tests within each estuary (Figure 3). To facilitate comparisons of our values to those from other studies, we did not include the October results in the comparisons below. The September 2006 samples were not found to be significantly different and were included in the comparisons below.

$-\text{N load effects on $\delta^{15}$N}$
\[ \delta^{15}\text{N} \] of S. alterniflora in Waquoit Bay and other Cape Cod estuaries increased sharply as loads began to increase and then tapered-off at higher loads (Figure 4). This result is similar to what was reported for macroalgal \( \delta^{15}\text{N} \) (Valiela et al. 2000, Cole et al. 2005, Wigand et al. 2007).

Wastewater as percent of nitrogen load

Across multiple estuarine systems and with rigorous seasonal sampling protocols, \( \delta^{15}\text{N} \) of S. alterniflora in Cape Cod estuaries increased significantly as wastewater increased as percent of the total nitrogen load (Figure 5), a result supported by those of previous studies (McClelland et al. 1997, Cole et al. 2005, Wigand et al. 2003). This relationship is most likely related to heavier signatures found in wastewater-derived nitrogen flowing through groundwater into receiving estuaries (McClelland and Valiela 1997, Valiela et al. 2000).

Effects of long-term land-cover changes on \( \delta^{15}\text{N} \) in S. alterniflora

There have been substantial changes in land use on the Waquoit Bay sub-watersheds between 1990 and 2005, and the changes were calculated using GIS information available from MassGIS and Towns of Falmouth and Mashpee, Massachusetts (Tables 3 and 4). The major change has been rapid progression of urbanization at the expense of natural land covers. For example, the percent residential land use in Childs River has gone from 27 to 39, and the natural forest cover has decreased by 16%.

To obtain a measure of the changes in nitrogen loads that followed the changes in land cover between 1990 and 2005 (Figure 6 top), we used NLM to estimate the loads for 2005, and compared these estimates to those published for 1990 (Valiela et al. 1997, Valiela et al. 2000). We found substantial increases in nitrogen loads during these 15 years between the 2005 NLM results and those published in 1990 (Valiela et al. 1997, Valiela et al. 2000; Figure 6, top). For
example, in Childs River, the most developed of the watersheds, there was a 41% increase in
nitrogen load, by about 200 kg N ha\(^{-1}\) yr\(^{-1}\), over the 15-year time interval, Hamblin Pond saw an
increase of 66% in total nitrogen load. The Waquoit Bay watershed as a whole saw an increase in
nitrogen load of 28% through the period. On those same watersheds, the increases in residential
land use were 12%, 11%, and 5%, respectively: even a small percent increase in residential land
use translated to a relatively large increase in total nitrogen load.

The \textit{S. alterniflora} \(\delta^{15}\text{N}\) values for 2006 in Sage Lot Pond, Quashnet River and Childs
River subestuaries were significantly heavier than those reported for 1994 \((t=0.004, \text{Figure 6},
\text{bottom})\), but the increases did not differ among the three estuaries we studied (Figure 6, bottom).

Since the slope of stem height vs. nitrogen load in the Waquoit Bay subestuaries did not
differ statistically from the Great Sippewissett experimental plots, these regional-scale findings
were similar to results of m\(^2\)-scale experimental studies (Valiela and Teal 1974, Valiela et al.
1975). Given the similar elevation and hydroperiod of the two sites, we conclude that increased
nitrogen supply led to similar increases in above ground biomass and production in both spatial
scales, since we have shown that biomass can be significantly predicted from height data in these
nitrogen supports increased growth of salt marsh grasses is of applied importance. Increased
stem height is evidence of a physiological response by the plants to an increase in available
nitrogen, a response that appears to be linear in nature and suggests a direct relationship between
\textit{S. alterniflora} above ground growth and land-derived nitrogen load (Figure 2). We should note
that the stimulation of growth takes place even at rather high nitrogen loads, judging from
experimental additions in the Great Sippewissett plots (Figure 2) and elsewhere (Nixon and
Oviatt 1973, Broome et al. 1975, Chalmers 1979). The similarity between the experiments conducted at different scales indicates that the effects of increased nitrogen on *S. alterniflora* are measurable at both m² and watershed scale, and that the effect of nitrogen load from a watershed on a natural marsh are not dissimilar from the effect of nitrogen loads on an experimentally fertilized marsh. The linkage between *S. alterniflora* growth and nitrogen load can be utilized by managers to identify salt marshes with higher land-derived nitrogen loads. Source of watershed-derived nitrogen, specifically wastewater, was also evident in the δ¹⁵N values of *S. alterniflora*. There was some insignificant variation over the growing season, as supported by Pruell et al. (2006) in Narragansett Bay, Rhode Island salt marshes and Drake et al. (2008) in the Plum Island, Massachusetts salt marsh. However, only the October values were significantly different from the rest of the growing season, most likely as a result of senescence (Figure 3). The October data were collected after flowering had taken place, and plants began senescing. Most studies of δ¹⁵N in *S. alterniflora* collected samples during the summer growing season (Cole et al. 2005, Wigand et al 2007), before physiological changes associated with senescence altered the δ¹⁵N of above ground tissues (White and Howes 1994b, Currin et al. 1995, Wada and Hattori 1991). For this reason, we recommend that future collection of *S. alterniflora* shoots for δ¹⁵N analysis take place during the summer, preferably during the same month, for comparisons between estuaries. In this study, we have averaged the δ¹⁵N values between May and September to represent the full range of values, but have omitted the October values as they are likely affected by senescence. In spite of variation, the growing season δ¹⁵N values appear to retain the pattern of heavier δ¹⁵N in *S. alterniflora* from subestuaries with higher nitrogen loads, reflecting nitrogen sources. Wastewater nitrogen is delivered to estuarine plants and macroalgae via groundwater
and estuarine water. Groundwater source $\delta^{15}N$ averaged 9.5‰ in Childs River groundwater and 5.8‰ in Quashnet River groundwater (McClelland and Valiela 1998) and Childs River estuarine water $\delta^{15}N$ ranged between 13‰ and -9‰ (York et al. 2007). The $\delta^{15}N$ we reported for *S. alterniflora* in the same subwatersheds (Figures 3, 4), was lighter than the groundwater values reported by McClelland and Valiela (1998) and heavier than the estuarine water reported by York et al. (2007), suggesting that the plants are either fractionating $\delta^{15}N$ that they are taking up from groundwater, or taking up a mixture of nitrogen from groundwater and estuarine water sources. Either way, the $\delta^{15}N$ of *S. alterniflora* reflects the $\delta^{15}N$ coming from the watershed.

In some Cape Cod estuaries, wastewater-derived nitrogen made up as much as 75% of total nitrogen loads entering estuaries (Table 4). Other major sources (fertilizers, atmospheric deposition) add smaller amounts (Table 4). The $\delta^{15}N$ of *S. alterniflora* in receiving estuaries therefore take up nitrogen with isotopic signatures that portray the major sources, which in our case come from wastewater disposal within the watershed. Percent wastewater contribution to total nitrogen load facilitates the comparison of $\delta^{15}N$ of *S. alterniflora* across estuaries with a wide range of nitrogen loads by constraining the independent variable to values between 0 and 100%. This constraint is responsible for the linear relationship between percent wastewater and $\delta^{15}N$ of *S. alterniflora* (Figure 5).

The shape of the relationship between $\delta^{15}N$ and total land derived nitrogen load (Figure 4) illustrates that $\delta^{15}N$ of marsh cordgrass changes more rapidly in response to small changes at low nitrogen loads than at higher nitrogen loads. This indicates that *S. alterniflora* has a higher sensitivity as an indicator of nitrogen loading at lower nitrogen loads. The greater sensitivity of $\delta^{15}N$ in *S. alterniflora* to changes in nitrogen load at the lowest end of the range results in an
asymptotic shape to the relationship between \( S. \ alterniflora \ \delta^{15}N \) and N load (Figure 4). This result is similar to what was reported for macroalgal \( \delta^{15}N \) (Valiela et al. 2000, Cole et al. 2005, Wigand et al. 2007). The overall shape of Figure 4 shows that in the Waquoit Bay system, beyond some nitrogen load, therefore, there are no detectable further increases in \( \delta^{15}N \). The mechanism underlying the asymptote remains to be identified, but is most likely caused by the interaction between external, groundwater and estuarine-derived sources and internal sediment microbial processes. As an indicator, this sensitivity at lower nitrogen loads makes \( S. \ alterniflora \) more valuable to managers and scientists wishing to identify systems at the early stages of eutrophication, when management efforts might be able to control or even reduce the impacts of nitrogen loading on fragile coastal systems.

We have so far discussed information collected from different estuaries, whose watersheds furnish different nitrogen loads, some relatively low, some higher; this has been described as a space-for-time substitution approach (Kolasa and Pickett 1991), and has been used successfully in a variety of contexts, including soil development on the island of Hawaii (Crewes et al. 1995, Vitousek and Farrington 1997). The Cape Cod watershed-estuary systems offer the possibility of a real-time course test of the implications of the space-for-time conclusions. In our real-time comparisons of 1990 and 2005 nitrogen loads we found that nitrogen loads in Childs River, Quashnet River and Sage Lot Pond all increased, largely due to an increase in wastewater nitrogen. The \( \delta^{15}N \) of \( S. \ alterniflora \) collected in 1994 and 2006 also increased. The increase in Sage Lot Pond by 6 kg N ha\(^{-1}\) yr\(^{-1}\) between 1990 and 2005, resulted in a somewhat larger increase in \( \delta^{15}N \) in \( S. \ alterniflora \) than the 206 kg N ha\(^{-1}\) yr\(^{-1}\) increase over the same 15 years in Childs River. This is not surprising, given the apparent higher sensitivity to small changes at low
nitrogen loads than at higher nitrogen loads. The relatively small changes in response to nitrogen load increases in the highest nitrogen load estuary suggest that *S. alterniflora* might be best implemented as an indicator species in lower-loaded estuaries. Although limited to only three subestuaries, the results of our real-time study in these three estuaries agree with the results of the space-for-time substitution approach used in the other Cape Cod estuaries.

CONCLUSIONS

- The standing crop of the *Spartina alterniflora* canopy was significantly larger in estuaries with higher nitrogen loads in both a meter-scale experiment and a watershed-scale comparison.
- δ¹⁵N values of above-ground *S. alterniflora* reflect the land-derived nitrogen loads and percent wastewater contribution to total nitrogen loads to the salt marsh.
- *S. alterniflora* used as an indicator of land-derived nitrogen loading is best collected during the growing season, before senescence and preferably within the same month for cross-site comparisons.
- *S. alterniflora* is a more sensitive indicator of nitrogen loads at lower total nitrogen loads, making it an ideal indicator species for managers wishing to identify early incidences of eutrophication.
- Real time comparisons of δ¹⁵N values of above-ground *S. alterniflora* over 15 years reflect changes in land-derived nitrogen loads, as well as the higher sensitivity of *S. alterniflora* δ¹⁵N as an indicator of incipient eutrophication.
ACKNOWLEDGMENTS

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Table 1. Nitrogen loads of sub-watersheds to the estuary within the Waquoit Bay estuary system modeled from 2005 land use data (Kinney unpublished data) and 1990 land use data (Valiela et al. 2000) and nitrogen loads to other Cape Cod estuaries.

<table>
<thead>
<tr>
<th>Watershed</th>
<th>Area of estuary* (ha)</th>
<th>1990 Modeled N load</th>
<th>2005 Modeled N load</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>kg N yr(^{-1})</td>
<td>kg N ha(^{-1}) yr(^{-1})</td>
</tr>
<tr>
<td>Timms Pond</td>
<td>14</td>
<td>92</td>
<td>7</td>
</tr>
<tr>
<td>Sage Lot Pond</td>
<td>70</td>
<td>596</td>
<td>9</td>
</tr>
<tr>
<td>Hamblin Pond</td>
<td>59</td>
<td>1662</td>
<td>28</td>
</tr>
<tr>
<td>Jehu Pond</td>
<td>90</td>
<td>2648</td>
<td>29</td>
</tr>
<tr>
<td>Eel Pond</td>
<td>48</td>
<td>2965</td>
<td>61</td>
</tr>
<tr>
<td>Quashnet River</td>
<td>28</td>
<td>9622</td>
<td>341</td>
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<tr>
<td>Childs River</td>
<td>14</td>
<td>5886</td>
<td>436</td>
</tr>
<tr>
<td>Little Pond(^a)</td>
<td>28</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mashpee River(^b)</td>
<td>10</td>
<td>2531</td>
<td>250</td>
</tr>
<tr>
<td>Green Pond(^b)</td>
<td>19</td>
<td>2596</td>
<td>126</td>
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<tr>
<td>Great Pond(^b)</td>
<td>39</td>
<td>4868</td>
<td>137</td>
</tr>
</tbody>
</table>

* Includes area of salt marsh
\(^a\)Valiela unpublished data, \(^b\)Cole et al. 2005
Table 2. Nitrogen loads applied to experimentally fertilized 10-m radius Great Sippewissett Marsh plots.

<table>
<thead>
<tr>
<th>Plot treatment</th>
<th>Fertilizer applied g N m^{-2} wk^{-1}</th>
<th>N load* kg N ha^{-1} yr^{-1}</th>
</tr>
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<tbody>
<tr>
<td>Control</td>
<td>0</td>
<td>12</td>
</tr>
<tr>
<td>Low</td>
<td>0.85</td>
<td>182</td>
</tr>
<tr>
<td>High</td>
<td>2.52</td>
<td>532</td>
</tr>
<tr>
<td>Extra-high</td>
<td>7.56</td>
<td>1572</td>
</tr>
</tbody>
</table>

* Includes atmospheric deposition on the marsh surface as in Bowen and Valiela (2001b).
Table 3. NLM, nitrogen loading model, inputs for the Waquoit Bay estuarine system based on 1990 (Valiela et al. 1997, 2000) and 2005 (this study) land use.

<table>
<thead>
<tr>
<th></th>
<th>Childs River</th>
<th>Eel River</th>
<th>Quashnet River</th>
<th>Hamblin Pond</th>
<th>Jehu Pond</th>
<th>Sage Lot Pond</th>
<th>Timms Pond</th>
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<th>Whole Bay</th>
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2005

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<th>Timms Pond</th>
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Table 4. NLM, nitrogen loading model, results (kg N yr\(^{-1}\) and %) for the Waquoit Bay estuarine system for 1990 and 2005 land use.

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**Total N to estuary by source**

| Atmospheric deposition** kg N yr\(^{-1}\) | 1266             | 1393             | 530            | 533             | 3569                 | 3596                 | 359             | 359             | 617             | 620             | 238               | 241                | 92              | 92              |
| **Wastewater** kg N yr\(^{-1}\) | 3535             | 5564             | 2031           | 2774           | 1947                 | 3272                 | 1084            | 2039            | 1436            | 1780            | 34                | 172                | 0               | 0               |
| **Fertilizer** kg N yr\(^{-1}\) | 733              | 965              | 322            | 567            | 2274                 | 2480                 | 219             | 357             | 595             | 643             | 324               | 341                | 0               | 0               |
| **intercepting pond input** kg N yr\(^{-1}\) | 353              | 388              | 82             | 90             | 1833                 | 2053                 | 0               | 0               | 0              | 0              | 0                 | 0                  | 0               | 0               |
| **Total N** kg N yr\(^{-1}\) | 5886             | 8310             | 2965           | 3964           | 9622                 | 11401                | 1662            | 2756            | 2648            | 3042            | 596               | 754                | 92              | 92              |
| % atmospheric N:        | 22                | 17                | 18             | 13             | 37                   | 32                   | 22              | 13              | 23             | 20             | 40                | 32                 | 100             | 100             |
| % septic N:             | 60                | 67                | 69             | 70             | 20                   | 29                   | 65              | 74              | 54             | 58             | 6                 | 23                 | 0               | 0               |
| % fertilizer N:         | 12                | 12                | 11             | 14             | 24                   | 22                   | 13              | 13              | 22             | 21             | 54                | 45                 | 0               | 0               |
| % change Total N        | 41                | 34                | 18             | 18             | 0                    | 0                    | 0               | 0               | 0              | 0              | 0                 | 0                  | 0               | 0               |
| % change in atm deposition | 10                | 1                 | 1              | 0              | 0                    | 0                    | 0               | 0               | 0              | 0              | 0                 | 0                  | 0               | 0               |
| % change in waste water | 57                | 37                | 68             | 68             | 88                   | 88                   | 24              | 407             | 0              | 0              | 0                 | 0                  | 0               | 0               |
| % change in fertilizer  | 32                | 76                | 9              | 63             | 8                    | 5                    | 0               | 0               | 0              | 0              | 0                 | 0                  | 0               | 0               |
Figure 1. Map of Cape Cod estuaries included in this study.

Figure 2. Stem heights of *Spartina alterniflora* compared to N loads of Waquoit Bay subestuaries (black circles, ± SE, $y = 0.05x + 47.23$, $R^2 = 0.74$, $F = 4.55$, $P = 0.01$) and those in Great Sippewissett Marsh experimental plots (grey circles, ± SE, $y = 0.08x + 44.12$, $R^2 = 0.94$, $F = 93.56$, $P < 0.0001$). Slopes are not significantly different ($t = 1.43$, $\alpha > 0.05$).

Figure 3. $\delta^{15}$N of *S. alterniflora* vs. sampling month, ± SE. Samples were collected during the 2005 and 2006 growing seasons. (2-way ANOVA results for all months except October: $F = 0.98$, n.s.).

Figure 4. $\delta^{15}$N of *S. alterniflora* vs. N load in Waquoit Bay subestuaries (black circles, ± SE) and other Cape Cod estuaries (white circles). Regression statistics for all estuaries: ($y = 0.87 \ln (x) + 1.86$, $F_{reg} = 17.09$, $P = 0.001$).

Figure 5. $\delta^{15}$N of *S. alterniflora* vs. percent contribution of wastewater to total N load in Waquoit Bay subestuaries (black circles, ± SE) and other Cape Cod estuaries (white circles) ($y = 0.04x + 3.64$, $F_{reg} = 10.74$, $P = 0.008$).

Figure 6. (Top) 1990 versus 2005 modeled N loads for 3 Waquoit Bay subestuaries: Sage Lot Pond, Quashnet River, and Childs River as compared to the 1:1 line (dashed). The slope of the regression ($y = 0.72x + 12.98$, $R^2 = 0.98$, $F = 51.75$, $P = 0.08$) is significantly different from the 1:1 line ($t = 5.88$, $\alpha < 0.03$). (Bottom) $\delta^{15}$N values for *S. alterniflora* collected from each of the 3 subestuaries of Waquoit Bay in 1994 and 2006 compared to the 1:1 line (dashed). The slope of the regression ($y = 1.04x - 0.63$, $R^2 = 0.99$, $F = 39417.39$, $P = 0.003$) is significantly different from the 1:1 line ($t = 7.25$, $\alpha < 0.01$).
Figure 1
Figure 2
Figure 3

S. alterniflora $\delta^{15}$N (%)

Sampling month

CR
ER
QR
HP
JP
SLP
TP

Apr-05
Jun-05
Aug-05
Oct-05
Dec-05
Feb-06
Apr-06
Jun-06
Aug-06
Sep-06
Figure 4

N load (kg N ha\(^{-1}\) yr\(^{-1}\))

$S. alterniflora \delta^{15}N$ (‰)
Figure 5

Waste water contribution to N load of S. alterniflora δ15N (‰)

S. alterniflora δ15N (%)

% Waste water contribution to N load
Figure 6

1990 Modeled N load (kgN ha\(^{-1}\) yr\(^{-1}\))

2005 Modeled N load (kgN ha\(^{-1}\) yr\(^{-1}\))

2006 S. alterniflora $\delta^{15}$N (‰)

1994 S. alterniflora $\delta^{15}$N (‰)