Spartina alterniflora δ^{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 δ^{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* δ^{15}N is a highly sensitive indicator of nitrogen load.
- *S. alterniflora* δ^{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 $\delta^{15}N$ 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 $\delta^{15}N$ 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 δ^{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 δ^{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 δ^{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, δ^{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 δ^{15}N as useful proxies that detect land use and source differences, we need information as to the variation that might be expected in δ^{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. alternifora* 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 m^2 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 m^2 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 m^2 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 δ^{15}N 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 m^2 locations around the shore of each of the Waquoit Bay subestuaries. The plants collected for δ^{15}N 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. δ^{15}N of samples were measured at the University of California Davis Stable Isotope Laboratory.

Decadal changes in nitrogen loads and in δ^{15}N of S. alterniflora

To examine sensitivity of δ^{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* δ^{15}N collected 1995-1996 (McClelland et al. 1997) to updated nitrogen loads and δ^{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 δ^{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 δ^{15}N

Seasonal variation in δ^{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 δ^{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.

–N load effects on δ^{15}N

δ^{15}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 δ^{15}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, δ^{15}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 ground water into receiving estuaries (McClelland and Valiela 1997, Valiela et al. 2000).

Effects of long-term land-cover changes on δ^{15}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 *S. alterniflora* δ^{15}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, Figure 6, 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 marsh grasses (Valiela et al 1975, Vince et al. 1981). Finding that external watershed-derived 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 *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 m^2 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 δ^{15}N 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 δ^{15}N 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 δ^{15}N 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 δ^{15}N analysis take place during the summer, preferably during the same month, for comparisons between estuaries. In this study, we have averaged the δ^{15}N 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 δ^{15}N values appear to retain the pattern of heavier δ^{15}N 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 δ^{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 δ^{15}N ranged between 13‰ and -9‰ (York et al. 2007). The δ^{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 δ^{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 δ^{15}N of *S. alterniflora* reflects the δ^{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 δ^{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 δ^{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 δ^{15}N of *S. alterniflora* (Figure 5).

The shape of the relationship between δ^{15}N and total land derived nitrogen load (Figure 4) illustrates that δ^{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. atlerniflora* has a higher sensitivity as an indicator of nitrogen loading at lower nitrogen loads. The greater sensitivity of δ^{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* δ^{15}N and N load (Figure 4). This result is similar to what was reported for macroalgal δ^{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 δ^{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 δ^{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 δ^{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.
- $\delta^{15}N$ 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 $\delta^{15}N$ 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* $\delta^{15}N$ as an indicator of incipient eutrophication.

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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.

Watershed	Area of estuary* (ha)	1990 Modeled N load		2005 Modeled N load	
		kg N yr^{-1}	kg N ha^{-1} yr^{-1}	kg N yr^{-1}	kg N ha^{-1} yr^{-1}
Timms Pond	14	92	7	92	7
Sage Lot Pond	70	596	9	754	11
Hamblin Pond	59	1662	28	2756	47
Jehu Pond	90	2648	29	3042	34
Eel Pond	48	2965	61	3964	82
Quashnet River	28	9622	341	11401	404
Childs River	14	5886	436	8310	616
Little Pond[a]	28			7590	268
Mashpee River[b]	10	2531	250		
Green Pond[b]	19	2596	126		
Great Pond[b]	39	4868	137		

* 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.

	Fertilizer applied	N load*
Plot treatment	g N m^{-2} wk^{-1}	kg N ha^{-1} yr^{-1}
Control	0	12
Low	0.85	182
High	2.52	532
Extra-high	7.56	1572

* 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.

			Childs River	**Eel River**	**Quashnet River**	**Hamblin Pond**	**Jehu Pond**	**Sage Lot Pond**	**Timms Pond**	**Head of the Bay**	**Whole Bay**
1990	drainage area	ha	875	356	2084	260	427	134	69	99	4235
	# houses		1232	718	740	340	519	14	0	99	3662
	Houses w/i 2 yr of groundwater travel	#	446	236	123	212	144	0	0	48	1209
	area of wetlands	ha	0	0	140	0	0	0	0	0	140
	cranberry	ha	5	0	22.45	9	3	0	0	0	39.45
	other agriculture	ha	14	5	59.49	0	0	0	0	1	79.49
	area of golf courses	ha	0	0	18.66	0	15	12	0	0	45.66
	Other turf area	ha	83	13	211.72	1	2	9	0	4	323.72
	other impervious (commercial, industry)	ha	14	10	159.73	1	0	1	0	3	188.73
	Area of non-intercepting ponds	ha	0	0	0	0	5	15	0	7	27
	area of intercepting ponds	ha	9	2	29	0	0	0	0	0	40
2005	drainage area	ha	875	356	2084	260	427	134	69	99	4235
	# houses		2001	982	1273	712	588	71	0	133	5760
	Houses w/i 2 yr of groundwater travel	#	579	320	148	255	289	0	0	83	1674
	area of wetlands	ha	0	0	140	0	0	0	0	0	140
	cranberry	ha	5	0	22.45	11	3	0	0	0	41.45
	other agriculture	ha	14	5	59.49	0	0	0	0	1	79.49
	area of golf courses	ha	0	0	18.66	0	16	12	0	0	46.66
	Other turf area	ha	83	13	211.72	1	2	9	0	4	323.72
	other impervious (commercial, industry)	ha	63	10	171	1	1	2	0	3	251
	Area of non-intercepting ponds	ha	0	0	0	0	5	15	0	7	27
	area of intercepting ponds	ha	9	2	29	0	0	0	0	0	40

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

		Childs River		**Eel River**		**Quashnet River**		**Hamblin Pond**		**Jehu Pond**		**Sage Lot Pond**		**Timms Pond**	
		1990	2005	1990	2005	1990	2005	1990	2005	1990	2005	1990	2005	1990	2005
N to watershed															
Atmospheric N:		13125	13125	5340	5340	31260	31260	3900	3900	6405	6405	2010	2010	1035	1035
Septic N:		10622	17252	6190	8467	6380	10976	2931	6139	4475	5070	121	612	0	0
Fertilizer N:		4424	5910	2067	3638	13340	14469	909	1684	3652	3956	2079	2189	0	0
N from intercepting ponds	kg N yr^{-1}	542	597	125	138	2820	3158	0	0	0	0	0	0	0	0
Total N		28714	36885	13723	17583	53800	59863	7740	11722	14531	15431	4210	4811	1035	1035
% atmospheric N:		46	36	39	30	58	52	50	33	44	42	48	42	100	100
% septic N:		37	47	45	48	12	18	38	52	31	33	3	13	0	0
% fertilizer N:		15	16	15	21	25	24	12	14	25	26	49	46	0	0
% change Total N			28		28		11		51		6		14		0
% natural vegetation		73	61	75	68	64	61	84	73	83	81	69	65	100	100
% loss of natural vegetation			16		9		4		13		2		5		0
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		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		66		15		27		0
% change in atm deposition			10		1		1		0		1		1		0
% change in waste water			57		37		68		88		24		407		0
% change in fertilizer			32		76		9		63		8		5		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

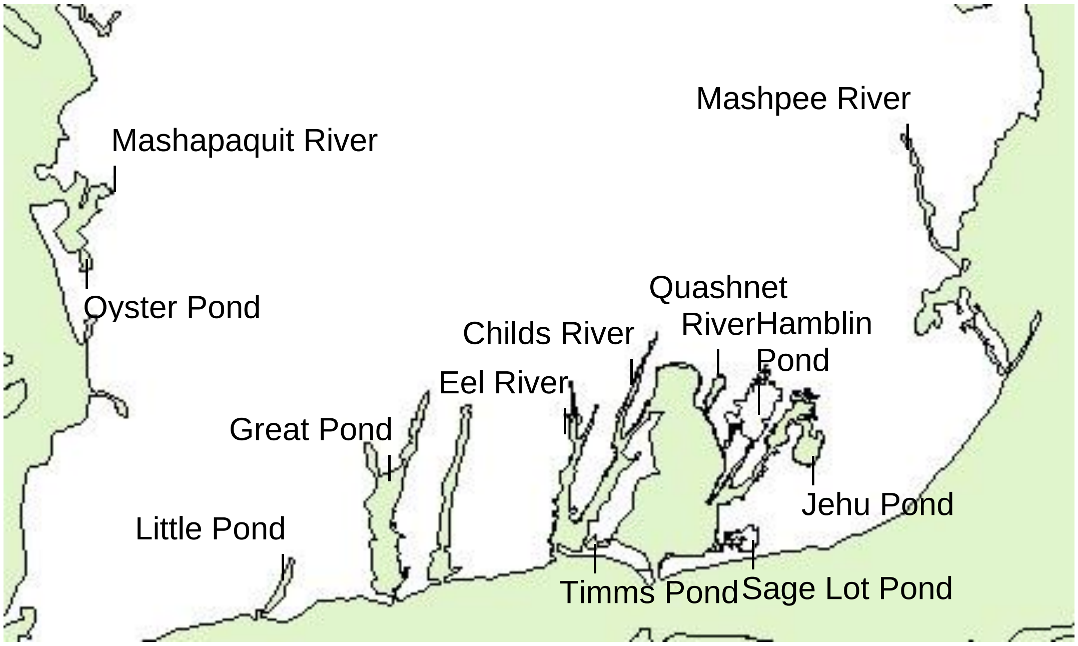
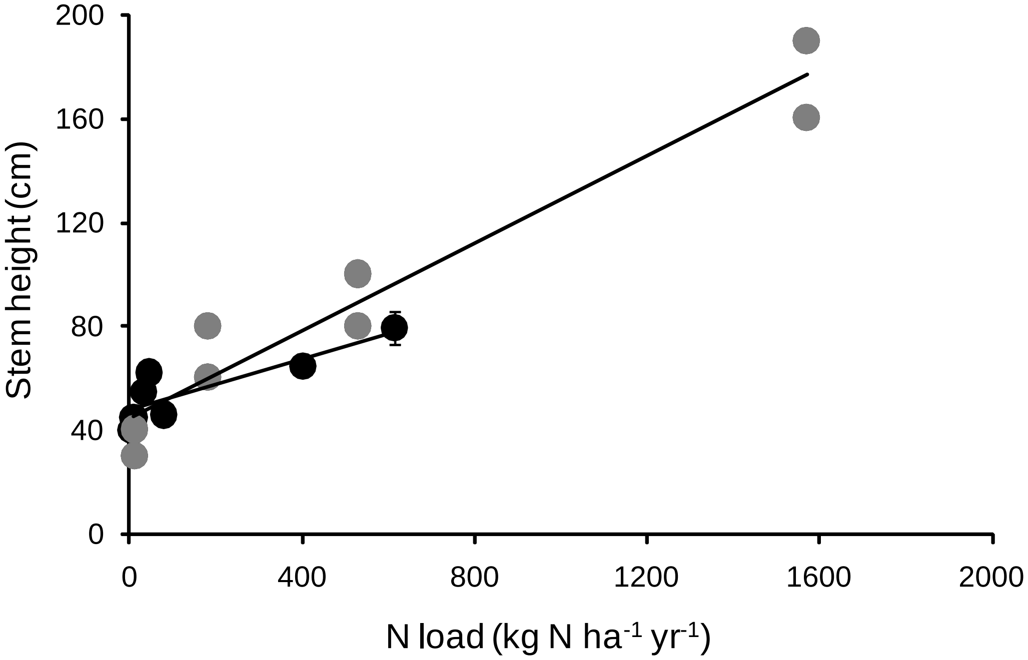
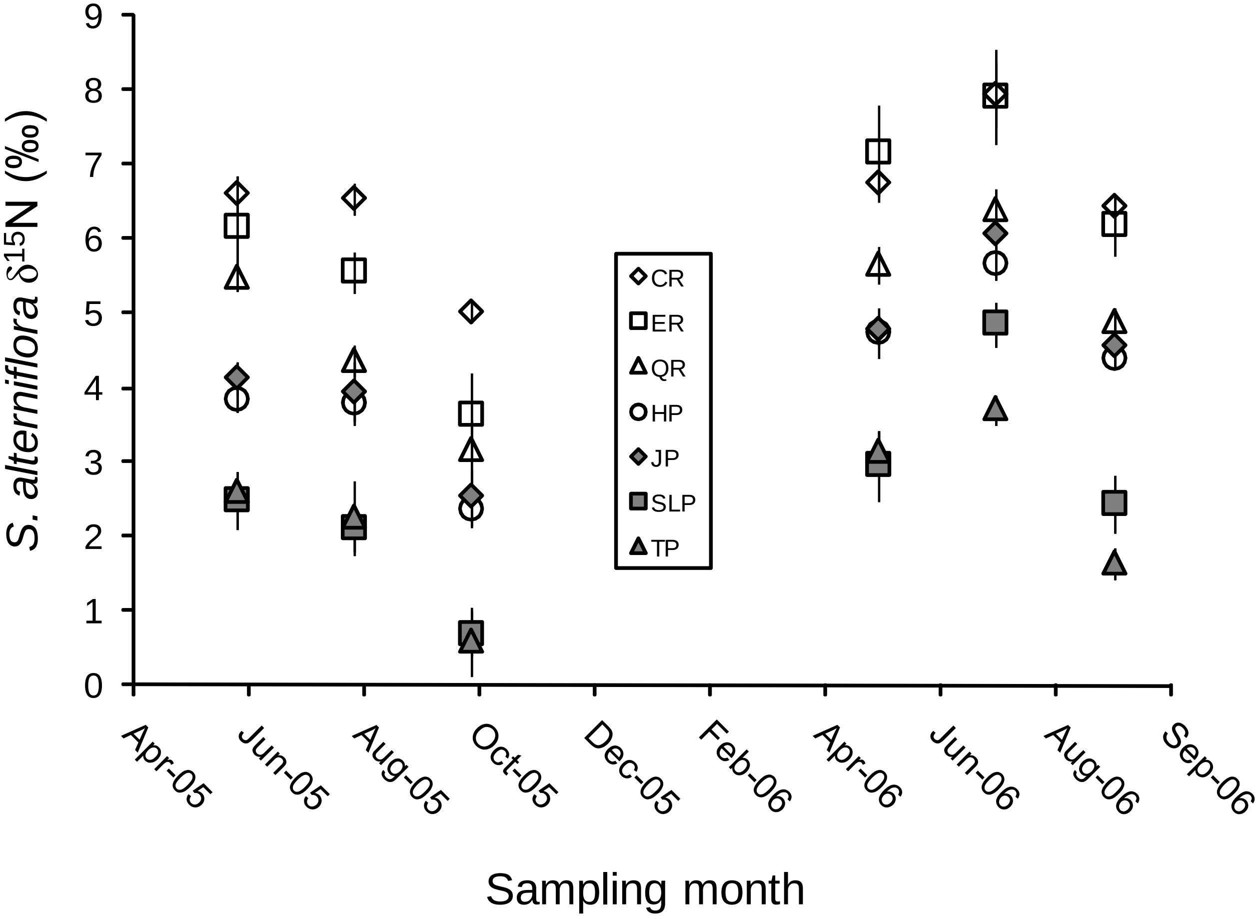

Mashpee River
Mashapaquit River
Oyster Pond
Quashnet River
Hamblin Pond
Childs River
Eel River
Great Pond
Jehu Pond
Little Pond
Timms Pond
Sage Lot Pond

Figure 2



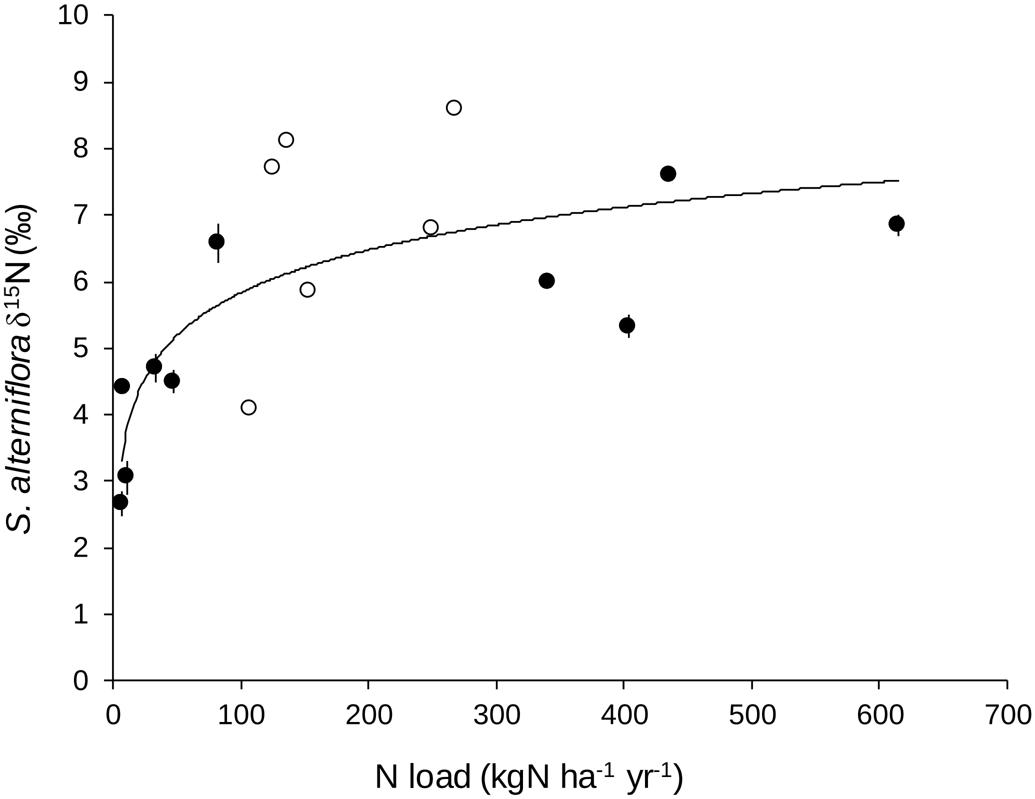
200
160
120
80
40
0
Stem height (cm)
0
400
800
1200
1600
2000
N load (kg N ha^{-1} yr^{-1})

Figure 3

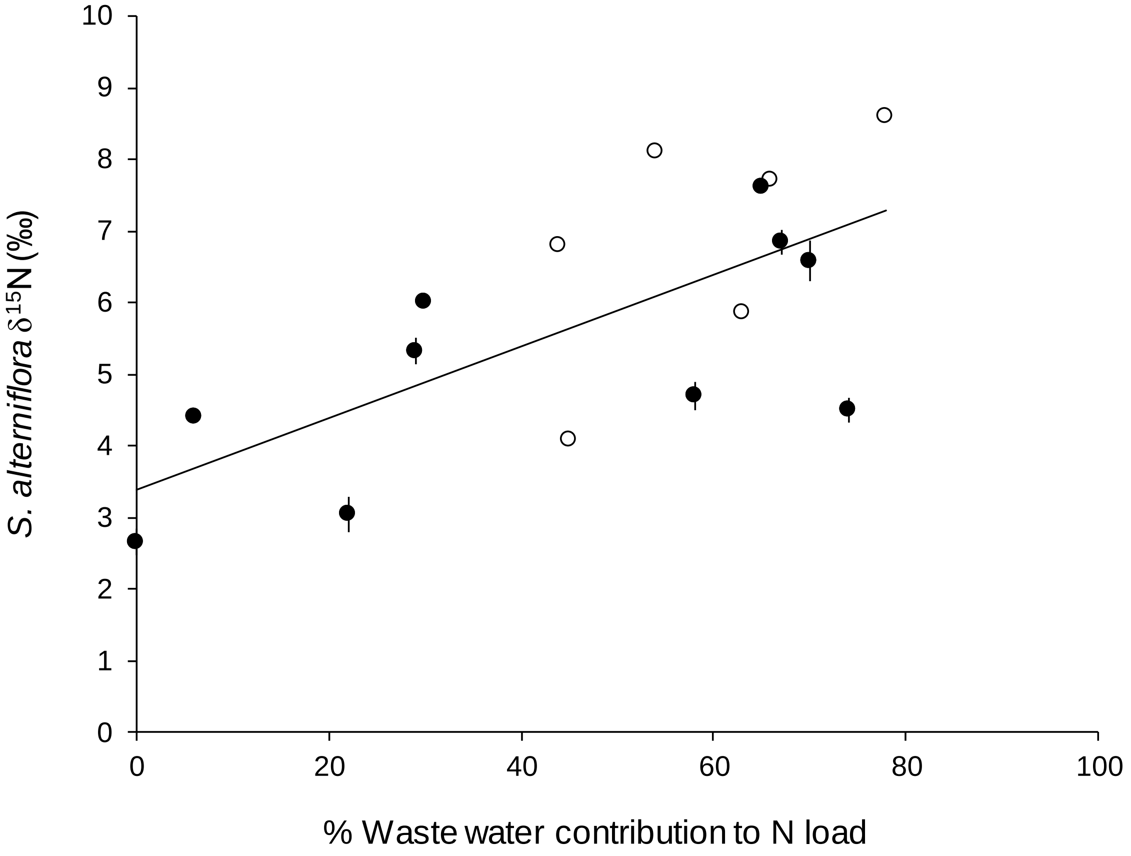


9
8
7
6
5
4
3
2
1
0
S. alterniflora δ15N (‰)
Apr-05
Jun-05
Aug-05
Oct-05
Dec-05
Feb-06
Apr-06
Jun-06
Aug-06
Sep-06
Sampling month
CR
ER
QR
HP
JP
SLP
TP

Figure 4

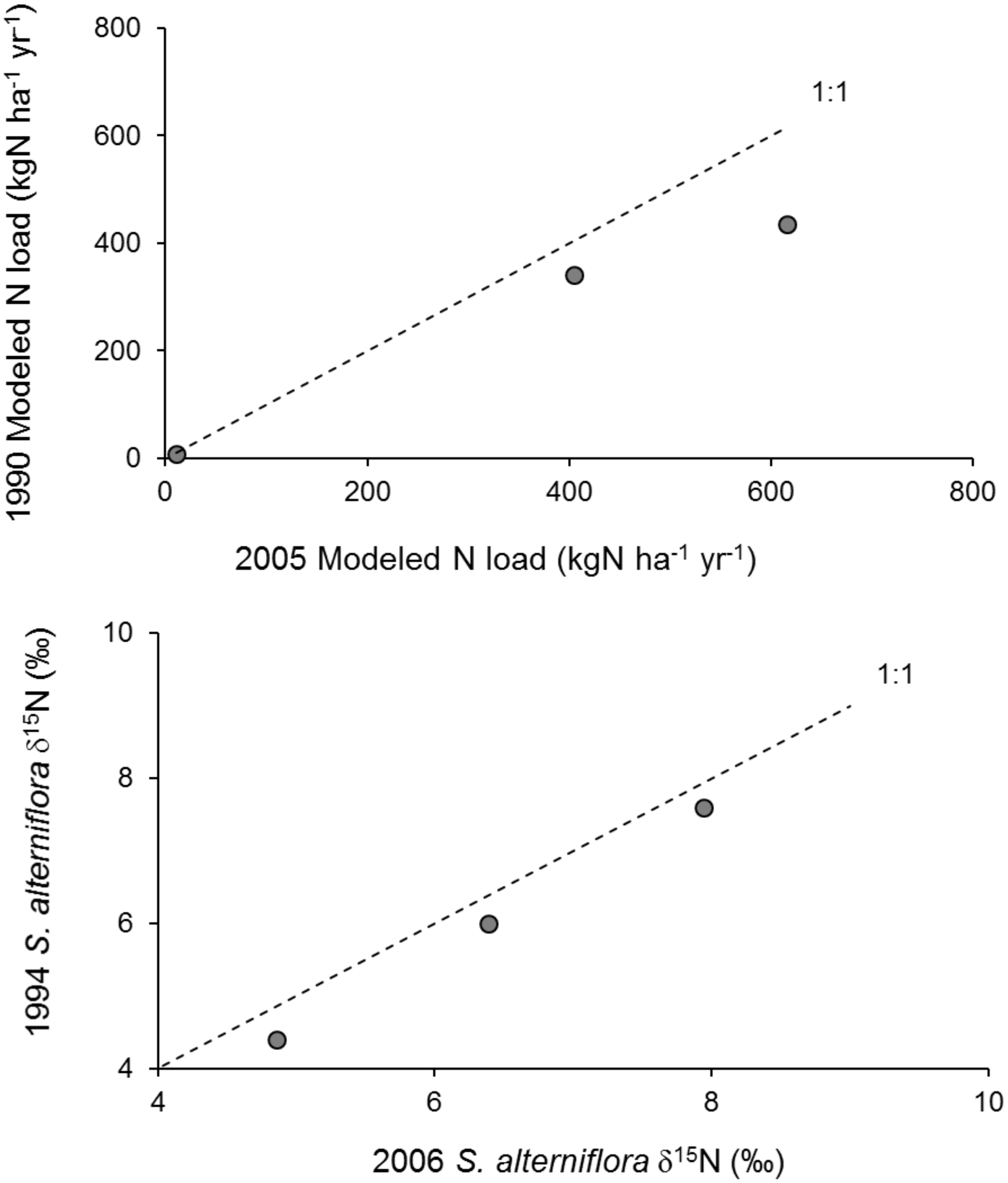


10
9
8
7
6
5
4
3
2
1
0
0
100
200
300
400
500
600
700
S. alterniflora δ15N (‰)
N load (kgN ha-1 yr-1)



$S.$ *alterniflora* δ^{15}N (‰)
10
9
8
7
6
5
4
3
2
1
0
0
20
40
60
80
100
% Waste water contribution to N load

Figure 6


1990 Modeled N load (kgN ha^{-1} yr^{-1})
800
600
400
200
0
1:1
0
200
400
600
800
2005 Modeled N load (kgN ha^{-1} yr^{-1})
1994 *S. alterniflora* δ^{15}N (‰)
10
8
6
4
1:1
4
6
8
10
2006 *S. alterniflora* δ^{15}N (‰)