

Experimental Nutrient Enrichment of a New England Salt Marsh: Plant
productivity and community composition responses.

A thesis presented

By

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Abstract

Potential impacts of anthropogenic nutrient loading were tested experimentally in tidal salt marshes of the Plum Island Sound estuary in northern Massachusetts. The objective was to understand the impacts of moderate N and P enrichment (target of $70\mu\text{M NO}_3^-$ and $5\mu\text{M PO}_4^{3-}$) on a number of ecosystem functions. Changes in plant production, as well as, shifts in vegetation patterns, are one set of predicted responses to nutrient enrichment. The study focused on two pairs of tidal creeksheds (Sweeney/West and Clubhead/Nelson); each pairing included an experimental (nutrient enriched) and a reference (ambient nutrient levels) treatment. Sweeney in the first pairing was fertilized for 2 years (2004-2005) and Clubhead in the second pairing was fertilized for 1 year (2005). Changes in community composition and species distribution were not detected among creeks after 2 years of enrichment. However, small but significant responses in aboveground growth (stem height and g cm^{-1} shoot height) in *Spartina alterniflora* and *Spartina patens* were recorded in 2004 and 2005. These limited responses to nutrient loading argue that these marshes have the capacity to absorb low level eutrophication without major impacts to plant community structure and ecological function, at least in the short term. The ability to monitor long term vegetation responses to chronic nutrient loading is important and this study provides important baseline data. Detailed vegetation maps outlining community borders walked in the field using GPS were created for Sweeney and West creeks in 2004. A second baseline data set includes species frequency and cover along 50 X 1 m strip transects, six distributed along each creek. Comparisons between transect data and vegetation maps indicate transects are reasonable samples for the creekshed. In addition, transect data shows similar plant community structure within creeks and within creek pairings, as well as, significant differences in mean elevation (MLLW) among creek pairs and among species. Significantly greater mean elevation of short *S. alterniflora* in 2005 compared to *S. patens* suggests the importance of surface topography, rather than absolute elevation, to marsh species distribution.

Introduction

Human activities are stressing coastal environments on local, regional, and global scales. Eutrophication of estuaries and near-shore waters, global warming, accelerating sea level rise, and the spread of introduced species are among the most important and well documented factors altering the structure and functions of coastal systems (NRC 1994, IPCC 2001). In the face of these and other anthropogenic stressors, the sustainability of coastal ecosystems is the cause of great concern among ecologists and environmentalists today (Emeis et al. 2001).

Coastal regions are host to the world's most heavily populated areas. Worldwide, approximately 400 million people live within 20 km of a coastline (Smith et al. 2000) and 75 % of the human population lives in a coastal area (Von Bodungen and Turner 2001). Coastal salt marshes and estuaries are among the most productive systems in the world and arguably provide more resources to humans on a per area basis than any other (Emeis et al. 2001). Along with their direct economic values, salt marsh systems also have an enormous range of ecological functions. These systems produce and export large amounts of organic material to coastal waters (Teal and Howes 2000), as well as provide habitat for many invertebrates, fish, and birds (Boesch and Turner 1984, NRC 1995). For this reason, the potential impacts of direct coastal salt marsh ecosystem loss and functional degradation are of particular concern.

Despite their ecological functions and economic values, salt marshes are disappearing at an unprecedented rate. Filling and dredging of the marshes have resulted in direct losses, while tidal restrictions have aided in marsh degradation, allowing the introduction and

establishment of brackish species (Fell et al 2000). Along the U.S. Atlantic coastline, the total area of tidal marsh has decreased by 20% over the last century (Teal and Howes 2000 purple book). On the same time scale, coastal development alone is responsible for a 30% (2000 ha) reduction of salt marsh land in Connecticut and a 20% reduction in coastal wetlands (Fell et al. 2000)

In addition to human activity, accelerating sea level rise is also contributing to the disappearance and degradation of salt marsh land. Globally, mean sea level has been rising 1-2.5 mm per year for the last 150 years (Gornitz et al. 2001). However in southern New England (CT) sea level rise has accelerated from 1mm/yr in the mid-19th century to 2.5-3.0 mm/yr today (Donnelly et al. 2004). Long-term survival of salt marshes requires that vertical growth or accretion track sea level rise. Marsh accretion is driven by the interacting effects of sedimentation, belowground production, and decomposition (Bricker-Urso et al. 1989, Turner 2004, Cahoon 1995). Recorded trends over recent decades, suggest that marsh accretion in many areas is not keeping pace. Since 1900, the 50 % reduction of salt marsh islands found in Jamaica Bay, NY is being attributed to sea level rise (Gornitz et al. 2001). Sea level rise is also a major factor in tremendous marsh loss on the Gulf Coast in Louisiana where the greatest reduction in North American coastal wetlands is occurring. Here marshland is disappearing at an alarming rate of 100 km² per year (Penland and Ramsey 1990).

The sustainability of salt marsh estuarine systems is further complicated by the global increase in anthropogenic nitrogen loading. Cultural eutrophication remains a large issue for coastal scientists today. Industrial fixation of nitrogen, including the use of fertilizers and burning of fossil fuels has, over the past century, more than doubled the annual amount of

useable nitrogen globally (Vitousek et al. 1997). Nitrogen flux to coastal tributaries in New England is predicted to be 5-20 times higher than pre-industrial periods with an average riverine N flux greater than $1,000 \text{ kg N km}^{-2} \text{ yr}^{-1}$ (Jaworski et al. 1997, Howarth et al. 1996). This is evident by the steady increase in nutrient loading per hectare of marshland along the U.S. Atlantic coastline (Teal and Howes 2000 purple book).

It is well documented that nutrient loading causes accelerated eutrophication in estuaries (Cloern 2001). Among the recorded impacts on estuarine water and benthos, are increased primary production from phytoplankton and macroalgae, as well as greater organic matter in the water column leading to altered nutrient cycles (i.e. anoxia) and degraded water quality (Nixon 1995, Cloern 2001). The effects of these altered ecosystem components has been a reduction in higher trophic level production and diversity. Effects of high N loads on eelgrass communities are particularly well documented at Waquoit Bay on Cape Cod, Massachusetts. Research suggests that the reduction in light resulting from increases in macroalgae was responsible for the notable losses of eelgrass beds and a cascade of other ecosystem function losses in nutrient-enriched locations (Hauxwell et al. 2006, Havens et al. 2001).

Despite considerable focus on nutrient enrichment in estuarine waters, little is known about its effect on salt marsh communities. Of particular concern are potential changes in marsh accretion to track sea level rise. Physical stressors on the environment such as nutrient enrichment can alter the ability of the marsh surface to keep up with relative sea level rise. Accretion is a balance between sedimentation and belowground plant production relative to decomposition and the erosion of organic peat and inorganic sediments (Bricker-

Urso et al. 1989). Changes to any of these factors could alter the balance sustaining marsh accretion and disrupt the system's ability to track sea level rise.

Nutrient enrichment could result in either an increase or decrease in marsh accretion. Increased microbial activity through the direct assimilation of nitrogen from the water column (Howarth and Hobbie 1982) could increase rates of peat decomposition, thereby causing marsh subsidence. On the other hand, past research has shown N to be the limiting nutrient in marsh plant production (Valiela 1984). With the experimental addition of fertilizer, plot studies have shown increases in above ground plant production, specifically *Spartina alterniflora* (Valiela and Teal 1974, Gallagher 1975, Mendelsohn 1979). Increases in total plant production allows for trapping of more sediments and potential greater peat formation and marsh accretion rates. However, shifts in resource allocation toward greater aboveground production and less belowground production could also occur (Bertness and Pennings 2000), resulting in slower peat build-up and slower rates of accretion.

Nutrient enrichment can also affect species composition and plant community structure by altering the dynamics of species competition (Levine et al. 1998). Although there is some variation, typical northeast U.S. Atlantic coast salt marshes exhibit similar plant community structure. *Spartina alterniflora* (cordgrass) dominates the low marsh (intertidal creek banks) which is flooded daily with tidal waters and *Spartina patens* (saltmeadow hay) dominates the less frequently flooded high marsh. The short form *S. alterniflora* also occurs on the high marsh in poorly drained areas along with *Juncus gerardii* in the drier areas and *Distichlis spicata* in disturbed and changing sites (Niering and Warren 1980). Past studies in New England (Bertness and Ellison 1987) have suggested that competition is the factor driving these zonation patterns. With the addition of fertilizer however, plot studies have

shown significant changes in species distribution by reversing competitive abilities (Levine et al. 1998, Bertness and Pennings 2000). Although *S. patens* typically out competes *S. alterniflora* forcing it almost exclusively to the low marsh, in fertilized plots *S. alterniflora* was more abundant than *S. patens*. Similarly, fertilization resulted in *S. patens* out competing *J. gerardii* and *D. spicata* out competing both *S. patens* and *J. gerardii* (Levine et al. 1998). These changes in species composition can also lead to decreases in marsh accretion as the species that allocate fewer resources to below ground production are predicted to become more abundant (Brewer et al. 1998, Bertness and Pennings 2000).

Although, numerous studies have been done on the effects of nutrient enrichment on marsh angiosperms (Valiela and Teal 1974, Gallagher 1975, Mendelssohn 1979, Levine et al. 1998), all of the production and vegetation experiments have been small plot studies (1-10m²). While valuable, these spatially small studies can raise questions of generality (Diamond 1986 purple book) as interactive effects, occurring at larger spatial scales cannot be taken into account. Extrapolating small plot finds to ecosystem level processes, thus, should be done with caution. For example, previous salt marsh studies typically used 1m² plots that at low tide were sprinkled with an inorganic dry fertilizer at amounts of 20 to > 1000 g N m² y⁻¹. In these studies very high amounts of fertilizer were needed (100 – 500 g N m² y⁻¹) to elicit a response in above ground production of *Spartina* (Valiela and Teal 1974, Gallagher 1975, Mendelssohn 1979). However, *Spartina* have shown limited uptake of NO₃⁻ from the water column (Wright et al. 1996), indicating that most NO₃⁻ uptake occurs below ground. Anthropogenic nutrient loading usually occurs through the water column, so the direct application of fertilizer to the ground does not simulate real world situations.

Large-scale ecosystem studies are rare in coastal research as they are often expensive, labor-intensive and difficult to control compared to small-scaled plot studies. Understanding the potential impacts of coastal eutrophication on the marsh ecosystem requires large-scale studies that more closely simulate real world situations and involve multiple interacting processes. The TIDE (Trophic cascades and interacting control processes in a detritus-based aquatic ecosystem) project is one such study. TIDE is a 4-year whole ecosystem experiment studying the interactive effects of nutrient loading and top predator species removal in 4 tidal creeksheds, (each ca 6 ha) located in the salt marshes of the Plum Island Sound Estuary in northern Massachusetts.

The salt marshes that dominate the Plum Island Sound Estuary have been ditched and mowed extensively over the last century, but the waters of the Plum Island Sound estuary have relatively low concentrations of NO_3^- (5 μM) and PO_4^{3-} (1 μM) and the marshes thus, remain largely unaffected by nutrient enrichment. Plant zonation patterns are typical of New England salt marshes featuring a mosaic of relatively pure stands. Tall *S. alterniflora* occupies the low marsh and *S. patens* dominates the high marsh along with short form *S. alterniflora* in poorly drained sites. This paper reports on just one component of the TIDE project, the response of the salt marsh plant community to chronic low-level nutrient loading. Focusing on vegetation, this paper will examine 3 of those components in response to increased nutrients: 1) aboveground and belowground production, 2) plant community composition, and 3) marsh accretion.

Salt marsh nutrient enrichment at a large scale has not been previously attempted. Nutrient loading levels in the TIDE project are approximately 30 g N m²yr⁻¹, an order of magnitude less than most in previous small plot studies. Depending on elevation and

flooding frequency, different community types will only see a percentage of that loading amount, therefore any responses are expected to be small. If loading at these levels does influence plant production, it is predicted to occur in the tall and short *S. alterniflora* as these communities will be exposed to longer periods of flooding. Tidal water percolates through the intertidal peat in tall *S. alterniflora* habitats as the intertidal zone is flooded daily for hours at a time. Short *S. alterniflora* is also exposed to nutrient enriched water for periods of time in poorly drained areas on the high marsh. Responses are not expected in *S. patens* as these areas are not flooded frequently and are typically well drained.

Using plot experiments, changes were observed in community composition after only 2 years of dry fertilization with loadings equivalent to the TIDE project (Levine et al. 1998). Despite similar loading levels, the chances of a response are still unknown due to differing methods of nutrient application. If species do respond, the most probable shift would be an increase of high marsh short *S. alterniflora* and *D. spicata* and less *S. patens*. Findings from Levine *et al.* (1998) suggest these community types will out compete *S. patens* and *J. gerardii* in nutrient enriched locations.

Study Site

Plum Island Sound Estuary is a large (60 km²) salt marsh dominated estuary in northern Massachusetts. Located between Cape Ann and the New Hampshire border the estuary has a tide range of 2-4 m and major tributaries include the Parker and Rowley Rivers.

Four specific research sites (Fig. 1) within this system were chosen according to similar creek and creekshed size, as well as similar vegetation (Deegan 2006). West, Sweeney, Clubhead, and Nelson are located at the upper regions of small tidal creeks that

end with each creek dividing into two relatively equal-sized branches. Each branch is 400-500 m in length and each study site, the creekshed for both branches, is ca. 6 ha. The creeks were paired (West-Sweeney and Nelson-Clubhead) based on plant community structure and position in the estuary. The West and Sweeney pair is further up the estuary and their sites have slightly lower salinity levels compared to Nelson and Clubhead, that are closer to the Sound.

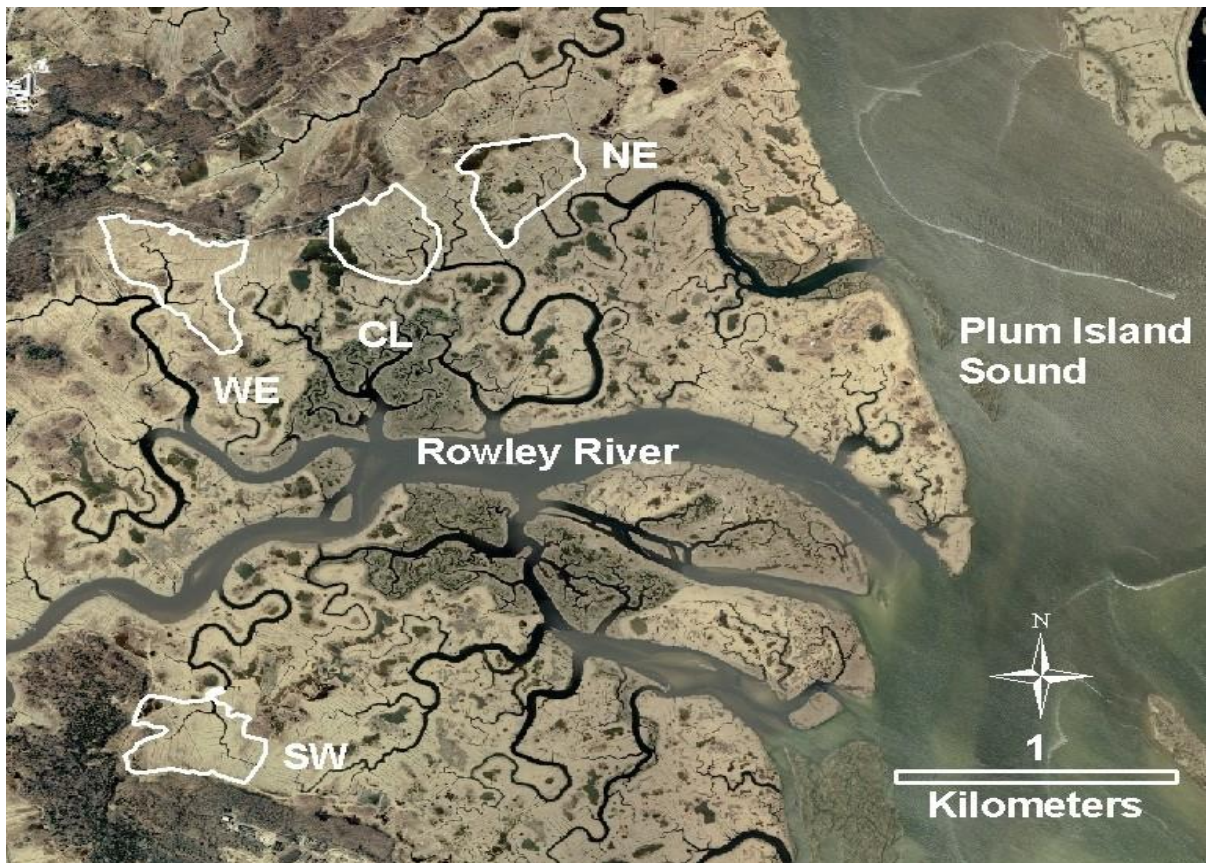


Fig. 1. 0.5 m orthophotograph (MassGIS 2002) of the salt marsh study site in the Plum Island Sound estuary. The small outlined areas represent the creeksheds that are the focus of the study (SW=Sweeney Creek, WE=West Creek, CL=Clubhead Creek, NE=Nelson Creek).

Within each creek pairing, one was used as a reference and the other was designated the experimental creek. Reference creeks (West and Nelson) were flooded with ambient creek water only. The experimental creeks (Sweeney and Clubhead) were enriched to 16X

the ambient N and P levels. Nutrients were added to Sweeney and Clubhead creeks at least 100 m below the creek branches, so both branches of each creek were flooded with nutrient enriched water. A second manipulation, block-netting used to reduce a predator fish species (mummichog), was applied to one branch at every creek; the other branch remained open.

Nutrient enrichment levels for this experiment were chosen based on other estuarine systems determined to be eutrophic (Cloern 2001, Oviatt et al. 1986). In eutrophic systems increased NO_3^- is usually accompanied by an increase in PO_4^{3-} (Cloern 2001). Therefore NO_3^- and PO_4^{3-} were added together to the creek water in the ratio of 15:1. Nutrients were pumped into creek water daily for the duration of each flooding high tide throughout the growing season (May to October) and the pumped turned off when the tide began to ebb. Mixing was uniform throughout the water column both horizontally and vertically at the confluence, thus both branches received the same nutrient enrichment. The rate of addition for the nutrient solution changed as water flux changed to maintain a target concentration of $70 \mu\text{M NO}_3^-$ and $4 \mu\text{M PO}_4^{3-}$.

The sampling schemes were focused on 6 transects (3 per branch) that ran approximately normal to the creek. Each transect started in the center of the creek and extended a ca. 50 m up onto the high marsh. The transects were spaced ca. 100-150 m apart and were labeled according to their distance from the confluence: Transect 1 was closest and transect 3 was the furthest upstream. Each transect included the major plant zones of tall *S. alterniflora*, *S. patens*, and short *S. alterniflora*. Transects provided a consistent and uniformed reference for inter-annual sampling for each creek.

Sampling was done from June to October during the summers of 2003, 2004, and 2005. Nutrients were not added to any of the creeks in 2003. The first year was used to

establish protocols and to collect baseline information for the 4 creeks. In 2004, nutrients were added to Sweeney and baseline data collection was continued for the Nelson and Clubhead pair. In 2005 nutrients were added to Sweeney for the second year and Clubhead was enriched for the first time. So for this experiment, there is replication in both time (Sweeney enriched for 2 years) and space (Sweeney and Clubhead enriched).

Methods

Aboveground Plant Production

Aboveground production samples were collected for tall *S. alterniflora* (SAT), *S. patens* (SP), and short *S. alterniflora* (SAS). 25 individual shoots of each community type, located haphazardly within 4 m of each transect were cut at the marsh surface and refrigerated within 2 hours. Each shoot was washed individually to remove sediments, measured for length, dried at 80° C for 12-48 hours and weighed. Length and dry weight were used to calculate weight per unit length.

Each year (2003-2005), samples were collected from late May to early September. In 2004 samples were collected 7 times throughout the summer for Sweeney (enriched) and West (reference). Nelson and Clubhead were sampled only 4 times. In 2005, all 4 creeks were sampled every other week for a total of 6 times for each creek. Shoots within a creek were collected on the same day and shoots for creek pairs were collected within the same week.

Stem density, shoots per unit area, was measured in the late summer in 2004 and 2005 for each of the three major species (SAT, SP, SAS). For each community type, four haphazardly located points were sampled off of each transect line. Because of differences in

densities among community type, sampling unit areas were different for each; SAT 0.25 m² quadrat, SP 0.008 m² circle, and SAS 0.0625 m² quadrat.

Belowground Production

Belowground production was sampled using cores. Cores of sediment, 7.5 cm by 30 cm were taken from the ground and replaced by nylon screen bags (~2 mm mesh) of the same size containing potting soil (2004) and sand (2005). Cores were deployed in early June and removed in September. Upon removal, the cores were sieved (6 mm mesh); roots and rhizomes retained were dried (80° C/24 hrs) and weighed. Growth was expressed as g dry weight per day.

Belowground production was sampled in 2004 and 2005. In 2004, cores were taken in each of the three community types (SAT, SP, SAS) at each creek; one community per transect on each creek branch (16/creek). In SAT zones, 4 cores were deployed and 2 each in SP and SAS zones. 8 cores/branch and 16 cores/creek were sampled for an N=64. In 2005, only the SAT zones were sampled. Ten cores (20/creek) were placed haphazardly along the low marsh of each branch for an N=80.

Plant Nitrogen Content

Nitrogen and carbon content for the three community types (SAT, SAS, SP) collected from June, July, and August in 2004 were determined using PerkinElmer 2400 Series II CHNS/O analyzer (PerkinElmer Life and Analytical Sciences Inc., Wellesley, Massachusetts). Three samples from each community type per creek were analyzed.

Community Composition

Plant community composition was sampled using visual estimates of percent cover of all angiosperm species in contiguous 1m² plots along each transect, extending from the lower limit of SAT ca 45 m onto the high marsh, for a total of 50 plots/transect. Mean percent cover and frequency (% plots in which a species was recorded) were determined for each species and community type found along each transect. Data was collected at all 4 creeks during the month of July in 2003, 2004, and 2005. Cover and frequency of community composition were compared among creeks and between creeks using SIMPER and ANOSIM (Primer).

Trimble (4800 receivers and TSC1 controller) global positioning system (GPS) was used to create vegetation maps of Sweeney and West creeksheds in 2004. Borders of monocultures and mixed stands were walked in the field and recorded. Using Geographic Information Software (GIS), this data was then overlaid onto 0.5 m orthophotographs (MassGIS 2002; Massachusetts Mainland State Plane Coordinate System) of each creek using Arcview 9.0. Once into GIS, the points were digitized creating polygons. The total area for each monoculture or mixed stand (polygon) was then calculated.

Elevation was measured using a Trimble Total Station surveyor (1 cm vertical resolution). Elevation was recorded at every meter along each transect, starting at the beginning of the low marsh in the tidal creek and running 50 m up onto the high marsh. Mean transect elevations were recorded during the months of July and August for each creek in 2005. Elevation datum was mean lower low water established using “tidesticks” as described by Smith and Warren (in press). Tidal corrections were made from Ipswich and

NOAA tide recordings. Two permanent benchmarks were established at each creek using tidesticks. Measurements were taken at each benchmark 5 times throughout the summer during spring tides.

Results

Nutrient Enrichment

Approximately 20,000 kg of NO_3^- and 1,000 kg of PO_4^{3-} was added to Sweeney in 2004 and again to Sweeney and Clubhead in 2005. In 2004, this addition successfully increased the ambient nutrient levels (2.5 μM of NO_3^- and 1 μM of PO_4^{3-}) in Sweeney by 10X and in 2005 increased levels in both creeks by 20X. The goal of 70 μM of NO_3^- and 5 μM of PO_4^{3-} was not reached in the first year of the treatment. Average concentrations for the 2004 season were 30 μM of NO_3^- and 2.6 μM of PO_4^{3-} . The second year of the treatment resulted in nutrient levels close to the target in both experimental creeks; 2005 concentration of incoming tidal water for Sweeney averaged 66 μM of NO_3^- and for Clubhead 78 μM of NO_3^- . NO_3^- concentrations in the ebbing water of both creeks was reduced ca. 10 μM , demonstrating NO_3^- uptake by the creek (Deegan 2006).

Aboveground Production

In July of the first treatment year (2004) growth curves for SAT, SP, and SAS from Sweeney began to diverge from those in West. By August, mean shoot height for the three was significantly greater in Sweeney compared to West (t-Test $P < 0.05$) (Fig.2, A). Results differed slightly in 2005: SAT mean height in Sweeney was greater over June and July, but in mid-August there were no differences and by late August West was taller. At the end of

the season Sweeney SP was again significantly taller and there were no differences in SAS (Fig. 2, B). SAT mean weight per linear cm of shoot (g cm^{-1}) was significantly greater in Sweeney both treatment years. Sweeney SAS g cm^{-1} was also greater in both years, but only significantly so in 2004. SP g cm^{-1} showed no differences between the fertilized and reference creeks in either treatment year (Fig. 2, C & D).

In 2004, a non-treatment year for Nelson and Clubhead, SAT, SAS, SP mean shoot heights were taller in Nelson, SAS significantly so (Fig.2, A). Nelson g cm^{-1} was also significantly greater in 2004 for SAT and SAS (Fig.2, C). In 2005, Nelson mean SAT height was taller throughout most of the season, but the difference was not significant. SP in Nelson ended the season significantly taller and there were no differences in SAS in 2005 (Fig. 2, B). Nelson SAT and SP g cm^{-1} was significantly greater than Clubhead and again there were no differences in SAS (Fig 2, D).

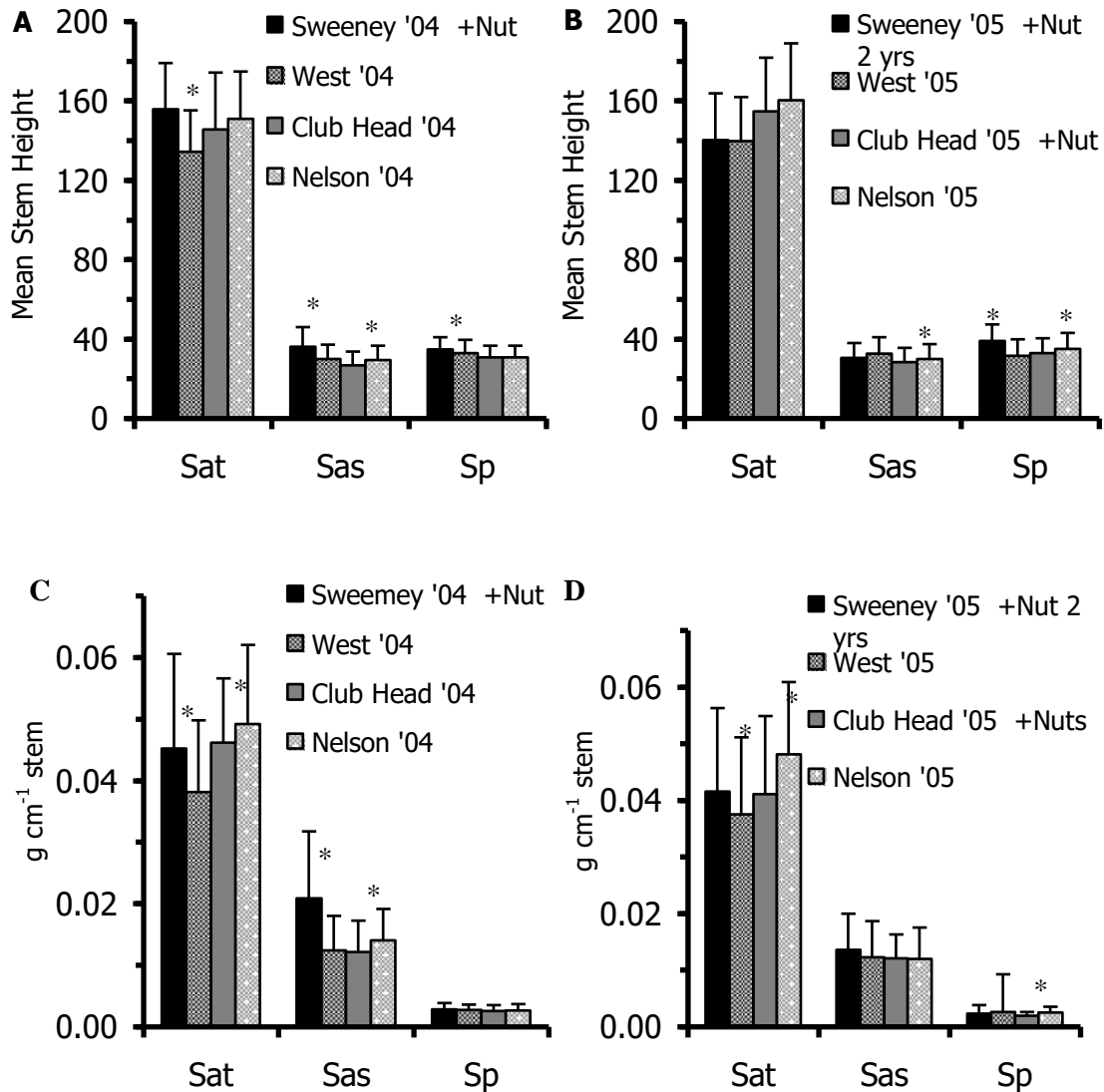


Fig. 2 Mid August mean stem heights (cm) and mean g cm⁻¹ shoot (\pm SD) for tall *S. alterniflora* (SAT), short *S. alterniflora* (SAS), and *S. patens* (SP) in all four creeks collected in 2004 and 2005 A) Stem height in 2004, B) g cm⁻¹ in 2004, C) Stem height in 2005, D) g cm⁻¹ in 2005. Paired creek means (Sweeney vs. West and Clubhead vs. Nelson) were compared by t-test; significant differences ($P < 0.05$) are marked *)

Stem density results in 2004 and 2005 showed no clear pattern for any of the community types between creeks (Table 1). After the second treatment year in 2005, West SAT was significantly greater than the other three creeks and there were no differences in

SAS. SP stem densities from Sweeney and West in 2005 were greater than those from Clubhead and Nelson, with only West being significantly different (Tukey's $P < 0.05$).

Table 1. 2005 Stem density (mean stems/unit sampling area) homogenous groups by Tukey's ($\alpha = 0.05$).

Creeks	Tall <i>S. alterniflora</i>			Short <i>S. alterniflora</i>		<i>S. patens</i>		
	N	1	2	N	1	N	1	2
Clubhead	24	43.3		24	83.5	24	40.2	
Nelson	24	49.2		24	83.5	24	44.6	
Sweeney	24	51.5		24	84.9	24	50.1	50.1
West	24		65.8	21	95.8	24		55.5
Sig. P		0.239	1.000		1.000		.067	.543

Belowground Production

In-growth core root and rhizome dry weight, as a measure of belowground production resulted in no clear pattern between the creeks in either 2004 for all three community types, or 2005 for SAT. In 2004 there were no significant differences among creeks in any of the communities. After the second treatment year SAT means fell into two homogenous sub-groups (Tukeys $\alpha = 0.05$) (Fig.3).

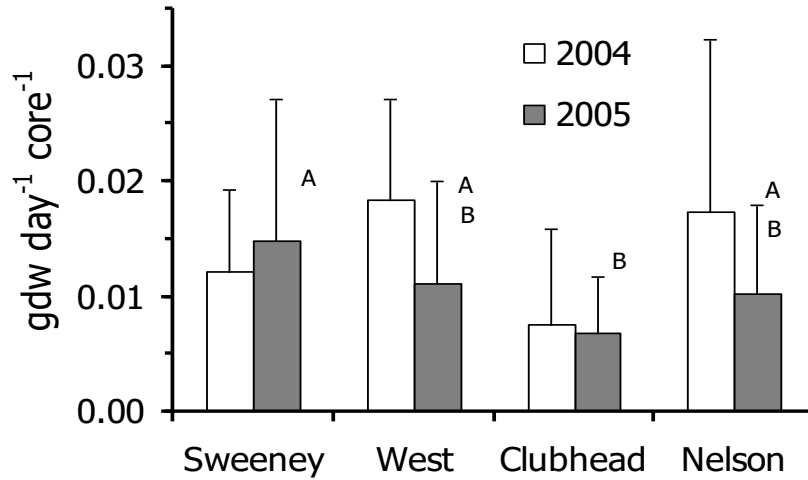


Fig. 3. Mean below ground production of SAT in 2004 and 2004 \pm SD. There were no significant differences among creeks in 2004 (ANOVA, $P = 0.152$) and two homogenous sub-groups, A and B, in 2005 (Tukey's $\alpha = 0.05$).

Nitrogen Content

Aboveground nitrogen content measured from samples in August of 2004 was significantly greater in SAS (t-test, $P=0.016$) and SAT ($P=0.036$) in Sweeney compared to West (Fig. 4). There was no difference in SP between the reference and fertilized creeks. Nitrogen content in Clubhead compared to Nelson was very similar for all 3 community types, with no significant difference. Belowground N content did not differ significantly between the creeks. SAT was slightly greater in Sweeney, but SAS was greater in West.

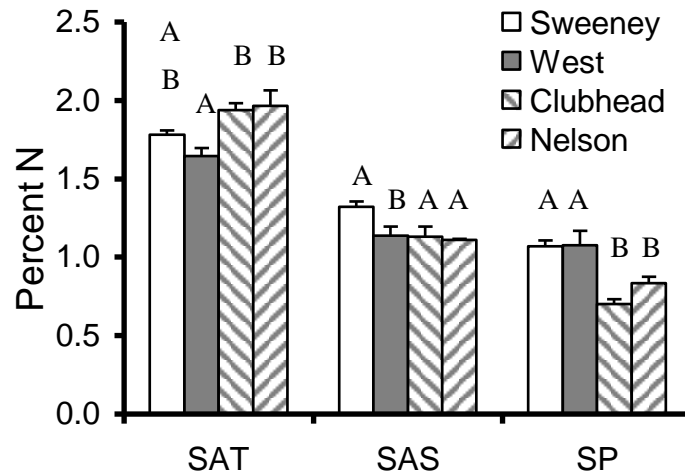


Fig. 4 Nitrogen content (\pm SE) in aboveground samples collected in August at each creek for SAT, SAS, and SP. Means with the same letter are not significantly different (Tukey's $P < 0.05$).

Vegetation

Species cover along transects, along with GPS mapping (Fig. 5 & 6) confirm plant species distribution patterns characteristic of New England salt marshes (Miller and Egler 1950, Niering and Warren 1980, Bertness and Ellison 1987). In all four creeks, low marsh was an SAT monoculture and SP provided the greatest cover on the high marsh, with SAS second, followed by *D. spicata* (DS). Pooling all 24 transects, mean frequencies were SP ca. 80%, SAS ca. 50%, DS ca. 40%.. *J. gerardi* (JG), *Triglochin maritima* (TM) and *Atriplex patula* (AP) had the lowest frequencies of occurrence and contributed little to total cover along transects or in the maps.

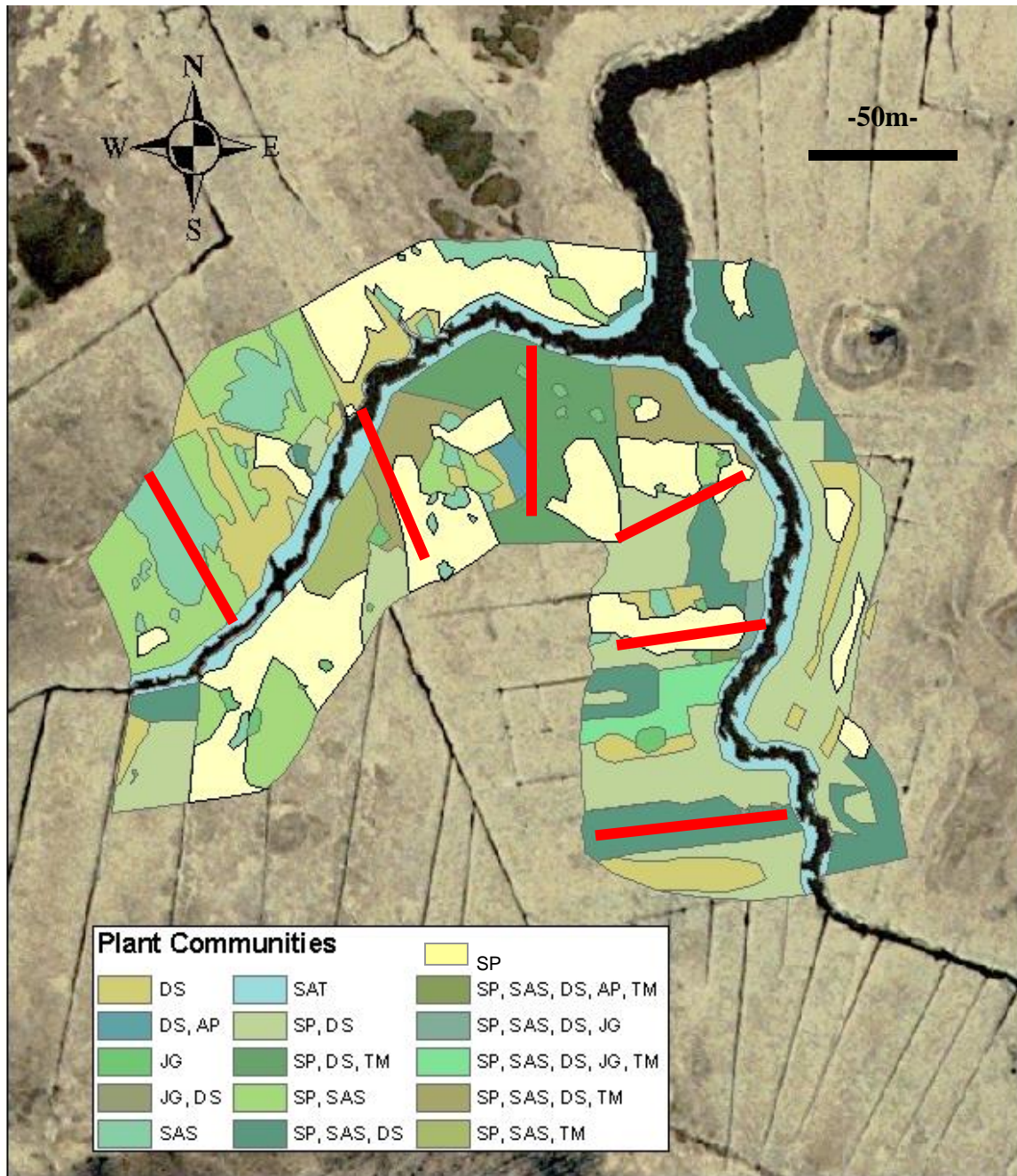


Fig. 5. Vegetation map of Sweeney (2004) labeling major community types of monocultures and mixed stands. Borders were walked in the field using GPS and were overlaid onto 0.5m orthophoto (MassGIS 2002) in GIS (Arcview 9.0). *S. patens* (SP), tall *S. alterniflora* (SAT), short *S. alterniflora* (SAS), *D. spicata* (DS), *J. gerardii* (JG), *A. patula* (AP), *T. maritima* (TM). Red lines mark the locations of sampling transects.

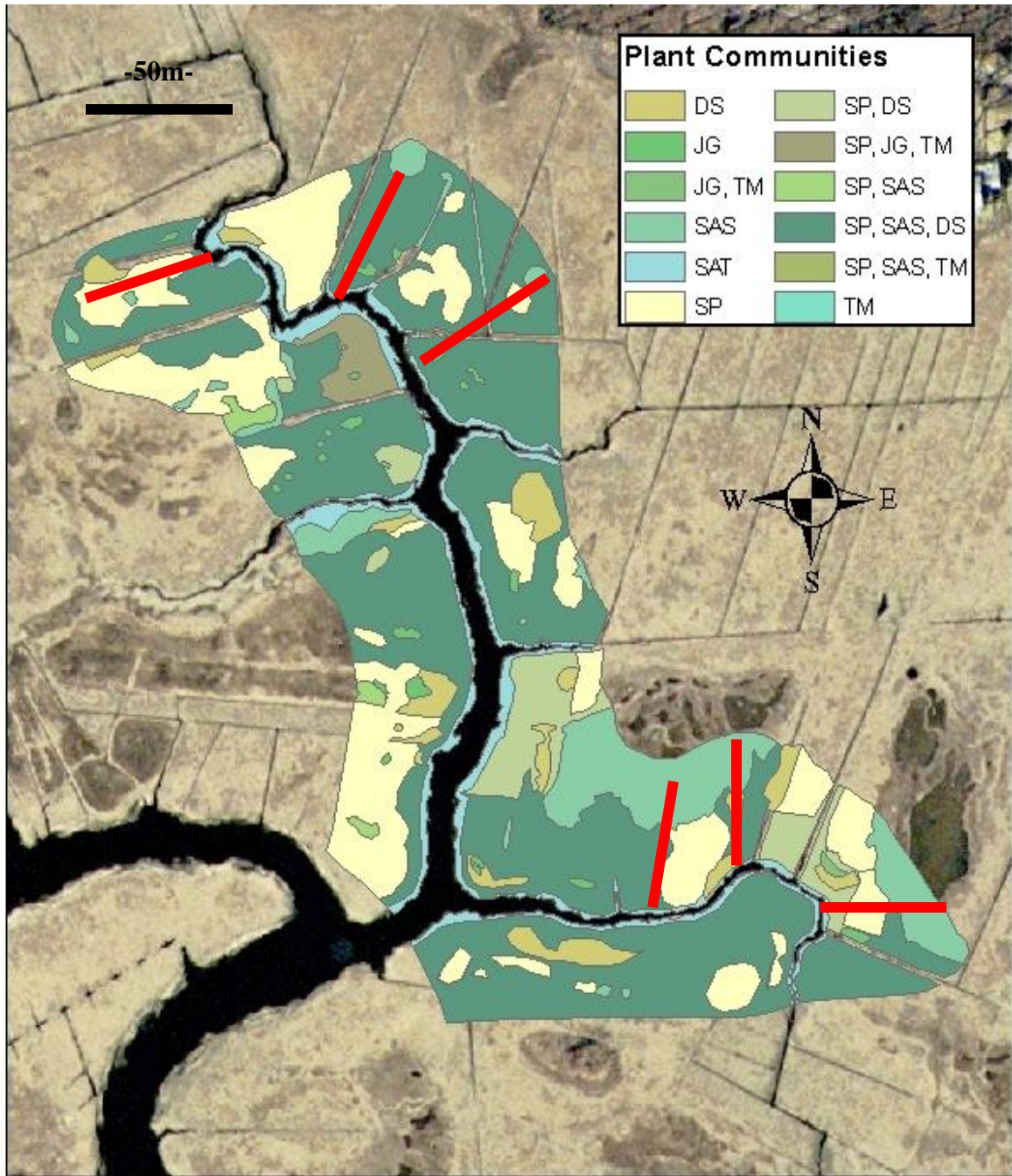


Fig. 6 Vegetation map of West (2004) labeling major community types of monocultures and mixed stands. Borders were walked in the field using GPS and were overlaid onto 0.5m orthophoto (MassGIS 2002) in GIS (Arcview 9.0). *S. patens* (SP), tall *S. alterniflora* (SAT), short *S. alterniflora* (SAS), *D. spicata* (DS), *J. gerardii* (JG), *A. patula* (AP), *T. maritima* (TM). Red lines mark the location of sampling transects.

Comparing transect communities using species cover with SIMPER found high similarity within creeks (Table 2), with Nelson the most consistent and Sweeney the least.

Table 2. Percent similarity in community structure among transects within each creek from SIMPER analysis using mean species cover in quadrats.

Creek	% Similarity		
	No Transformation	Sq. Root Transformed	4th Root Transformed
Sweeney	74.1	79.1	82.9
West	78.9	81.1	84.6
Clubhead	67.9	77.8	84.8
Nelson	73.6	83.9	89.7

With the same quadrat cover data ANOSIM found no significant difference between Sweeney and West (P=0.18) nor between Clubhead and Nelson (P=0.16). Sweeney and West were more similar to each other than to either of Clubhead or Nelson (Table 3).

Table 3. ANOSIM comparisons of plant community structure among creeks based on mean species cover in quadrats along transects. P < 0.05 *: communities are not similar.

Creek Comparison	Not Transformed		SqRt Transformed		4th Rt Transformed	
	R ²	P	R ²	P	R ²	P
Sweeney-West	0.113	0.130	0.094	0.182	0.054	0.297
Sweeney-Clubhead	0.282	*0.044	0.242	*0.050	0.187	0.054
Sweeney-Nelson	0.170	0.121	0.241	0.056	0.037	*0.019
West-Clubhead	0.183	0.071	0.143	0.090	0.222	*0.045
West-Nelson	-0.059	0.688	0.052	0.301	0.339	*0.006
Clubhead-Nelson	0.042	0.235	0.079	0.157	0.091	0.136

Bold indicates the designated creek pairings.

Mean cover from transect data of SAT in the low marsh and SP on the high marsh did not differ greatly among the four creeks (Fig. 7). Clubhead and Nelson both had more SAS than Sweeney and West. DS abundances were variable between years and creeks, but

differences were minor. Using mean cover in quadrats as well as mean frequency of occurrence along transects within each creekshed, ANOSIM found no differences between years for any of the creeks (Table 4).

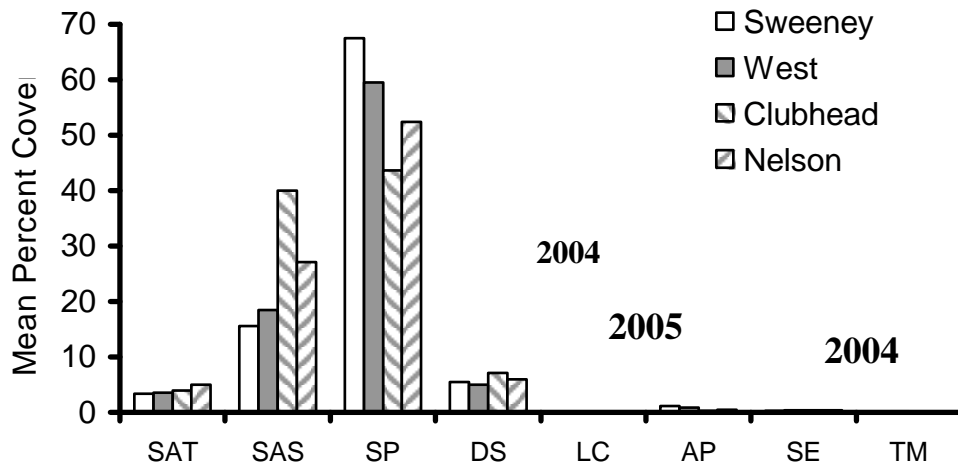


Fig. 7. Mean cover for all species present along transects in 2004 and 2005. Tall *S. alterniflora* (SAT), short *S. alterniflora* (SAS), *S. patens* (SP), *D. spicata* (DS), *Limmonium carolinum* (LC), *A. patula* (AP), *Salicornia europaea* (SE), *T. maritima* (TM).

Table 4. ANOSIM comparison of vegetation within creeks between years 2004-2005.

	Mean Cover		Mean Frequency	
	R ²	P	R ²	P
Sweeney	-0.054	0.680	-0.014	0.939
West	-0.133	0.890	-0.039	0.545
Clubhead	-0.020	0.465	-0.137	0.933
Nelson	-0.128	0.896	0.107	0.812

ANOVA of 2005 mean transect elevations relative to mean lower low water shows distinct differences between the creeks (high marsh only) (Fig 8). West had the lowest mean elevation (2.90 m) followed by Sweeney (2.95 m) and both were significantly different (Tukey's $P < 0.05$) from Nelson (3.01 m) and from Clubhead (3.02 m).

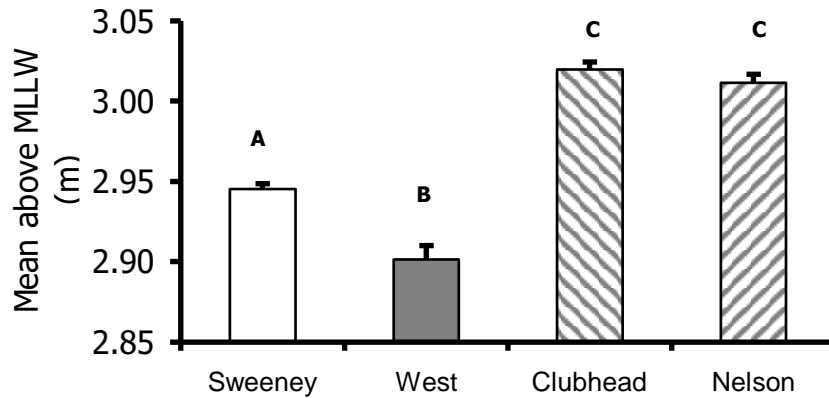


Fig. 8 Mean elevation ($SE\pm$) relative to LLW along transects for each creek in 2005. Means with the same letter are not significantly different (Tukey's; $P<0.05$).

Hypsographic curves for SAT, SAS, SP, and DS were created from the combined cover and elevation points along transects at each creek (Fig. 9). SAT occurs predominantly at elevations between 1.8 m and 2.6 m, and not at all below 1.5 m. SAS, SP, and DS all display tight curves with peak cover ca. 2.9 m - 3.1 m before rapidly dropping off (Fig. 9, A & B). At 3.1 m SAS mean cover was greatest at ca.70%, while SP was ca. 40%. Highest DS cover was ca. 45% at 3.0 m

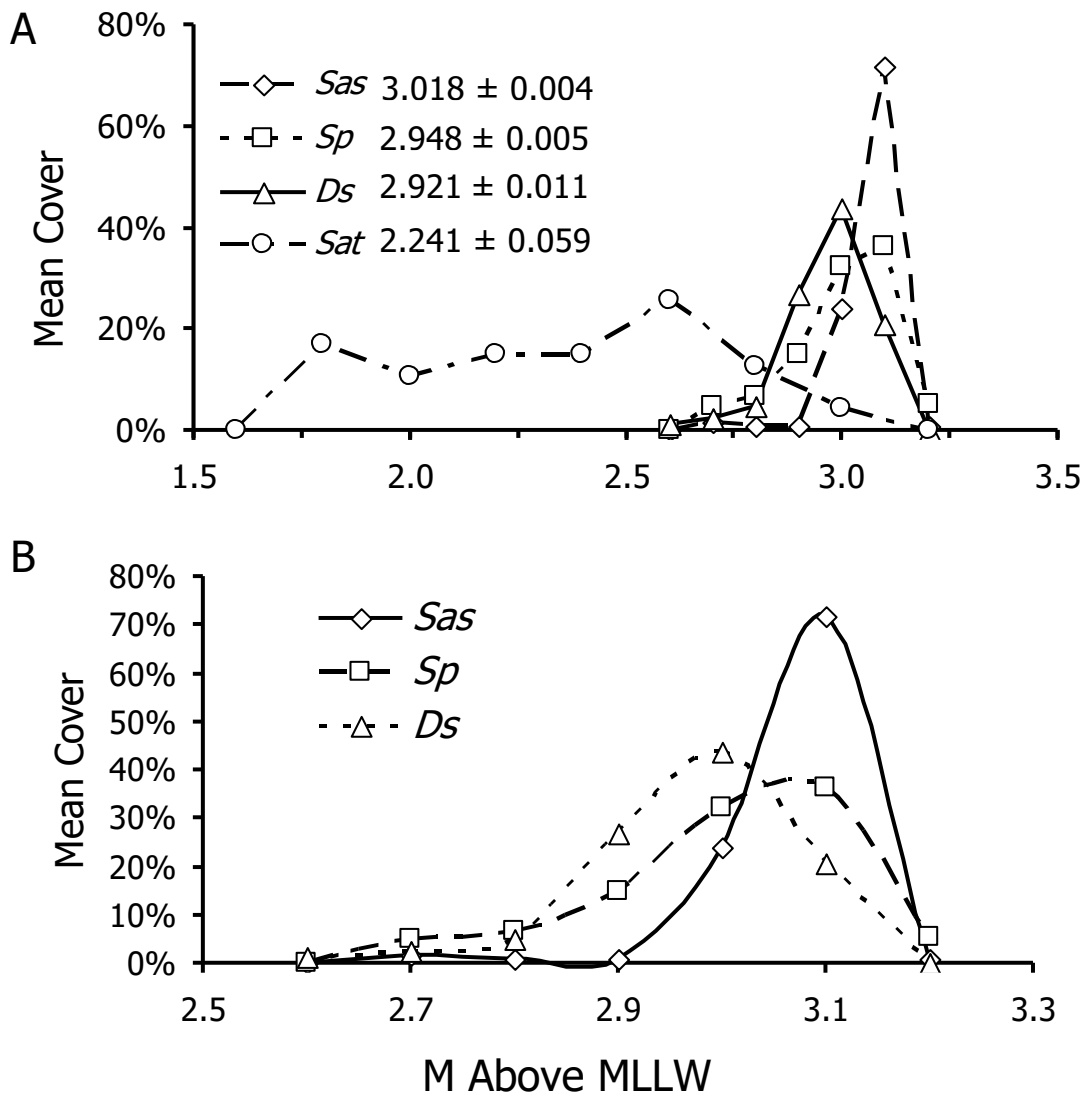


Fig. 9 Hypsographic curves of combined elevation points and mean cover along transects in all 4 creeks. A) SAS, SP, DS, and SAT curves present with mean elevations (MLLW \pm SE) B) SAT curve not present; note change in scale

Transect vegetation and elevation data from each creek, was used to compare mean elevation differences between plant communities (Table 5). Surveying (Trimble Total Station) quadrats at all 4 creeks with SP or SAS cover >75% found significant differences (Tukey's $P < 0.05$) in mean elevation among all of the communities. SAS recorded the highest mean elevation (3.02 m) and was significantly different from SP (2.95 m). SP, SAS,

and SAT were the only species to occur in quadrats with cover >75%. Mean elevation of quadrats with DS cover >40% were significantly different from SAT and SAS, but not SP (Table 5).

Table 5. 2005 Mean species elevation (MLLW) homogenous groups between creeks by Tukey's ($\alpha=0.05$). Cover in quadrats SP >75%, SAS >75%, DS>40%

Creeks	N	SP				N	SAS		N	DS		
		1	2	3	4		1	2		1	2	3
West	175	2.89				39	2.98		19	2.85		
Sweeney	211		2.93			38	2.99		17	2.90	2.90	
Nelson	141			2.99		96		3.03	22		2.93	2.93
Clubhead	118				3.03	65		3.05	24			2.99
Sig. P		1.00	1.00	1.00	1.00		0.91	0.33		0.28	0.65	0.10

Significant differences were also recorded comparing individual species elevations across creeks (Table 6). Differences in mean elevations of SP among all 4 creeks were significant (Tukey's $P<0.05$) and SAS in West and Sweeney was significantly different from Clubhead and Nelson. Differences in DS mean elevations were significant in both West and Sweeney compared to Clubhead, as well as, West from Nelson. Consistent with individual mean creek elevations (Fig. 8), all three community types recorded highest mean elevations at Clubhead and lowest at West (Table 5).

Table 6. Homogenous groups of mean species elevation by Tukey's ($\alpha=0.05$).

Species	N	Mean Elevation (MLLW)		
		1	2	3
SAT	47	2.24		
DS	82		2.92	
SP	645		2.95	
SAS	238			3.02
Sig. P		1.000	0.543	1.000

Discussion

Nutrient Loading

Dividing the total amount of N added over a season by creekshed area gives an average annual loading to both Sweeney and Clubhead creeksheds of ca. 30 g N/m²/y. Within the creeksheds, however, different habitats (community types) saw varying levels of nutrient loading depending on flooding and frequency duration. According to Deegan *et al.* (2006), loading levels within the semi-diurnally flooded *S. alterniflora* intertidal zone were greater at ca. 60 g N/m²/y while loading to the *S. patens* dominated high marsh was just ca. 7-15 g N/m²/y. The consistent decrease in NO₃⁻ concentration in the water column from flooding to ebbing tides indicates uptake of NO₃⁻ by the marsh creek and communities. Deegan *et al.* (2006) calculate a retention of 30-50% of the total added N in the fertilized sites. This result suggests, that on average the real annual uptake was ~10 g N/m²/y for the system, ~20 g N/m²/y in tall *S. alterniflora*, and ~1-5 g N/m²/y in the high marsh.

Aboveground Production

Although the loading levels in this investigation are an order of magnitude or greater less than previous studies (Valiela and Teal 1974, Gallagher 1975, Mendelsohn 1979), results demonstrate a small, but significant response by *S. alterniflora* to nutrient enrichment. After the first treatment year in 2004, height and g cm^{-1} in SAT and SAS at the fertilized creek (Sweeney,) were significantly greater than the reference creek (West) (Fig. 2, A & C). Response in the second treatment year (2005) was less clear-cut; there was no difference in height, but g cm^{-1} was again greater in both *S. alterniflora* forms and the difference was significant in SAT (Fig. 2, B & D). Because the *S. patens* habitat is better drained and less frequently flooded than tall and short *S. alterniflora*, a response was not expected in this community. Results from 2005, however, also suggest a possible response from *S. patens* in the fertilized creek (Fig. 2, A & C). After no differences were seen between creeks in 2004, stems from Sweeney in 2005 were significantly taller and denser (g cm^{-1}) than West.

Results from the lower estuary creek pair, Clubhead and Nelson differ from Sweeney and West. Aboveground production in 2004, a non-treatment year for this creek pair, shows Nelson producing taller stems throughout the season than Clubhead (Fig. 2, A). Figure 2, C shows significant differences between Nelson and Clubhead in g cm^{-1} for SAT and SAS in Nelson. In 2005, despite a year of fertilization, stem height in the three community types remained greater in the reference creek (Nelson); differences were significant in SP and SAS (Fig. 2, B). SAT and SP g cm^{-1} in Nelson also remained greater in 2005, while no differences were seen in SAS (Fig. 2, D).

The results from Sweeney and West do indicate a significant response to nutrient enrichment in all three community types. However, the results from Clubhead and Nelson,

along with their differing position in the estuary and higher salinity levels compared to Sweeney and West, argue that perhaps gradients in physical factors, such as salinity, contribute to different spatial responses within a marsh system and with the levels used they can overwhelm the effects of nutrient loading.

Stems per unit area did not show any differences between the reference and treatment creeks. This was not unexpected though, as similar results have been shown in past fertilization studies (Valiela 1983). This indicates that the aboveground response to nutrient enrichment is strictly in reference to increases in shoot height and weight per linear cm.

Concentrations of the predator fish species, *Fundulus heteroclitus*, were dramatically decreased (target 80%) in one branch of each creek (Deegan et al. 2006). Comparing creek branches in 2004 and 2005 in both fertilized and unfertilized creeks found no consistent differences in aboveground production related to *F. heteroclitus* removal. The similarity between creek branches indicates that predator species removal is not a factor in aboveground plant production.

Belowground Production

There is no indication from the results that belowground production in any community type responded to nutrient enrichment in 2004 (Fig. 3). Explanations for this could include the low sampling number (16 per creek) and large spatial scale availability in belowground production. The 2005 sampling, focused solely on the SAT intertidal habitat with a much greater sampling size (20 per creek). As in 2004, no was no difference in 2005 between the fertilized and unfertilized creeks. Studies on a newly created *Spartina foliosa* dominated marsh (California) are consistent with these results. It was found that in

fertilization experiments, <2% of the added N was incorporated into root and rhizome production and that there is only a small trend toward increased belowground production (Boyer et al. 2000).

Nitrogen Content

Nitrogen content from the plant samples in 2004 of Sweeney and West supports the suggestion of a plant response to nutrient loading. SAT and SAS aboveground nitrogen content was significantly greater in the fertilized creek (Fig. 4), but no difference was recorded in SP. These results are similar to aboveground production results where a response in 2004 was recorded in SAT and SAS, but not SP (Fig. 2, A & C). Differences in N content between Clubhead and Nelson in 2004 were not significant (Fig. 4). Increased N content has been recorded in past studies with similar plant responses to fertilization (Taylor et al. 2003).

In addition, nitrogen content from the collected roots and rhizomes in 2004 is consistent with the belowground production results. No significant differences were seen in any of the community types between the fertilized and unfertilized creeks. Nitrogen content from 2005 for both aboveground and belowground production, which has yet to be analyzed, will be important for providing a more vivid picture.

Vegetation

Vegetation data from transects and GIS maps, shows that each creek exhibits plant zonation patterns classic to New England salt marshes (Miller and Egler 1950, Niering and Warren 1980, Bertness and Ellison 1987). Tall *S. alterniflora* dominates the intertidal zone, while *S. patens* is most abundant on the high marsh, followed by short *S. alterniflora* and *D.*

spicata (Figs. 5, 6, & 7). The vegetation data also supports the creek pairings (Sweeney-West & Clubhead-Nelson). Table 3 shows that creeks within each pairing are more similar to each other than to creeks across pairings. The results also document similar community composition between branches of each creek, supporting the assumption that the branches are reasonable replicates of each other.

Furthermore, the vegetation maps from Sweeney and West indicate transects to be representative samples of each creek (Fig. 10). GPS boundaries of plant communities were walked in the field. Map data from Sweeney and West (Fig. 10) shows *S. patens* to be the most abundant species, followed by short *S. alterniflora*, and *D. spicata*. With the exception of *D. spicata*, which was less dominant near transects, the similarities between the two data sources indicate transects do reasonably sample the creekshed. The support for each of the project’s sampling assumptions allows for treatment comparisons between branches (predator removal) and creeks (fertilization) to be made with confidence.

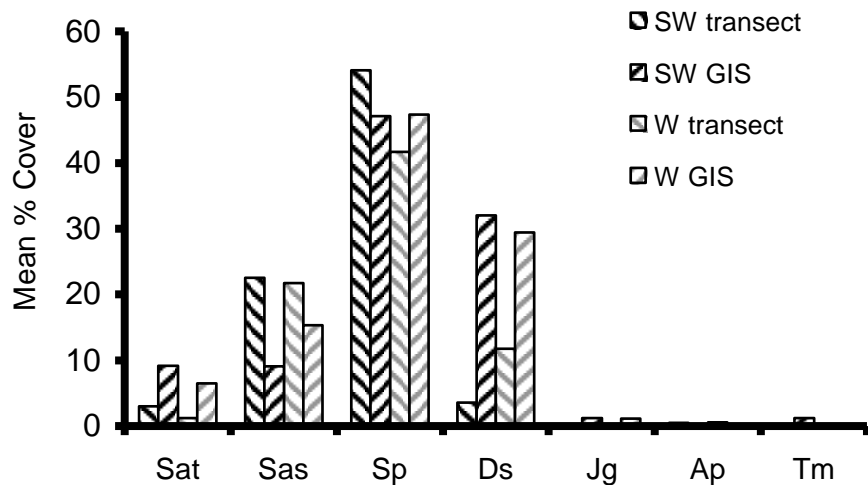


Fig. 10. Mean cover (2004) along transects and in vegetation maps (GIS) for Sweeney and West. Tall *S. alterniflora* (SAT), short *S. alterniflora* (SAS), *S. patens* (SP), *D. spicata* (DS), *J. gerardii* (JG), *A. patula* (AP), *T. maritima* (TM).

As expected, there have been no changes in community composition with just 2 years of nutrient enrichment (Table 4). Abundances of the plants most vulnerable to change such as, *S. alterniflora*, *S. patens*, and *D. spicata*, did not significantly change in either of the fertilized creeks (Table 4). As previously noted, Levine *et al.* (1998) observed community changes after only two years at annual loadings of 30 g N/m²/y. Although loading amounts between the studies are similar, the method of nutrient application is different. Levine *et al.* (1998) used a dry fertilizer applied directly to the marsh surface, therefore, each community type saw actual loads of 30 g N/m²/y. In the TIDE study, however, frequency and duration of flooding dictates actual loading amounts to the communities. As a result, even though the average loading to the creekshed was 30 g N/m²/y, recorded levels in high marsh habitats, where changes are most likely occur, were substantially lower (Deegan et al. 2006). With these low loading rates, any changes to community composition in this system are likely to occur over a longer time frame than the 2 years of this study.

Mean transect elevation data (MLLW) collected in 2005 further supports the creek pairings of Sweeney/West and Clubhead/Nelson. Mean elevations revealed that West and Sweeney were the lowest creeks, while Nelson and Clubhead were both significantly higher (Fig. 8). The results suggest that perhaps the similar trends in elevation are at least partially responsible, along with position in the estuary, for the similarities found in community composition (Adam 1990, Howes et al. 1981).

Mean species elevations are also significantly different among the 4 creeks (Table 5). Mean elevations of SP, SAS, SAT, and DS all varied significantly between creeks, with individual mean species elevations highest in Clubhead and lowest in West. Differences in SP elevations were significant among each of the creeks, and SAS elevations in West and

Sweeney were both significantly lower than Clubhead and Nelson. DS mean elevations were also significantly lower in West compared to Clubhead and Nelson, however, Sweeney differences were significant only from Clubhead.

The 2005 hypsographic curves (Fig. 9, A & B), illustrate species distribution patterns of SP, SAS, SAT, and DS across marsh elevation. The SAT curve, although variable, indicates SAT occurs over a vertical range of 1.5 m; it is not recorded below 1.6 m or above 3.2 m (Fig. 9, A). Cover for SP, SAS, and DS is highest ca. 3.0 m, but drops rapidly within a cm in either direction. Interestingly however, despite the tendency to inhabit poorly drained depressions, SAS cover is greatest at higher elevations and is greater than both SP and DS cover (Fig. 9, B). Table 6 shows that the combined mean elevation of SAS (3.02) is significantly greater than SP (2.95) and DS (2.92 m). This is contrary to past studies that found SAS to be lower in elevation than SP (Warren et al. 1993). This suggests that, in this system at least, absolute elevation is not driving plant species distribution, but rather local surface topography. Evidence of this has been previously recorded in the Plum Island Sound estuary.¹

Changes in marsh community composition and structure were not detected among creeks after 2 years of nutrient enrichment. However, small, but significant increases in *S. alterniflora* and *S. patens* stem height (cm) and g cm^{-1} shoot height were recorded in 2004 and 2005. These limited responses argue that these salt marsh creeksheds have the capacity to absorb low-level nutrient enrichment, over the short-term, with relatively little impact on plant community structure and function. Determining the impacts on marsh angiosperms to chronic nutrient loading are not possible from this study, but the groundwork has been set for continuous monitoring of productivity, composition, and elevation over the long-term.

¹ Personal communication; Vinton Valentine, Marine Biological Laboratory, Woods Hole, MA.

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