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Increased supply of ambient nitrogen has minimal effect on salt marsh bacterial production

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Abstract

We examined the role of chronic low-level nutrient enrichment on the productivity of heterotrophic marsh bacteria via a marsh fertilization experiment in which we mimicked the conditions of widespread coastal eutrophication by enriching entire salt marshes to approximately 15× background nutrient concentrations. We measured the uptake of tritiated leucine, as a proxy for bacterial production, in both low and high marsh habitats in four salt marshes, two of which were enriched with nutrients. We hypothesized that adding nitrogen in these detritus-rich systems would directly stimulate bacterial decomposition of marsh peat. Contrary to our expectations, we found no response to added nutrients in high marsh habitats, where there is a significant supply of organic matter from marsh vegetation. Bacterial production did increase in the low marsh habitats, where fertilization increased the standing stock of benthic chlorophyll. Fertilization did not directly increase bacterial production by providing added nutrients that could be used to decompose organic matter derived from nutrient-poor marsh grasses. Rather, bacterial productivity was indirectly stimulated by the concomitant increase in labile benthic microalgae in low marsh habitats. Decomposition of salt marshes may therefore have a greater resilience to the threat of chronic eutrophication than has been previously recognized.

Salt marshes provide numerous ecosystem services and play host to a tremendous diversity of microorganisms, many of which play critical roles in regulating the flow of nutrients and carbon into salt marsh food webs (Valiela and Teal 1979; Howes et al. 1984; Blum et al. 2004). Carbon flow in salt marshes is influenced by organic matter inputs from two dominant primary producers, marsh macrophytes and benthic microalgae, both of which are typically limited by nitrogen (Van Raalte et al. 1976; Valiela and Teal 1979). Increasing the amount of nitrogen (N) available in salt marshes relieves nitrogen limitation of marsh macrophytes and consistently increases the biomass of marsh plants (Valiela and Teal 1974; Bertness et al. 2002; Deegan et al. 2007). Sullivan and Currin (2000) show that nitrogen addition to salt marshes also increased the biomass of benthic microalgae during the spring, but during summer the added nitrogen increased the macrophyte canopy and decreased light penetration, inhibiting the growth of benthic microalgae.

The different responses of primary producers to increased nitrogen supply will influence the amount and lability of carbon available to microbes and could have direct and cascading effects on salt marsh stability. Mineralization rates in salt marshes are among the highest recorded for any ecosystem (Boschker et al. 1999), but there has long been a debate as to the primary source of carbon that supports mineralization (Peterson and Howarth 1987; Boschker et al. 1999). Early research held that marsh vegetation, the primary component of marsh peat, was the largest carbon source supporting mineralization (Nixon 1980; Odum 1980); however, more recent isotopic evidence indicates that algal carbon may also be important (Peterson and Howarth 1987; Boschker et al. 1999; Tobias et al. 2003). Understanding the degree to which each of these carbon sources drives marsh metabolism and, hence, marsh elevation is imperative in light of projected sea level rise and coastal eutrophication (Kroer 1993; Sundareshwar et al. 2003).

The extent to which decomposition of the marsh platform, traditionally accomplished by a combination of fungi and bacteria, is responsive to changing carbon and nitrogen supplies remains unclear. Examples from the terrestrial literature indicate that decomposition of leaf litter was inhibited by fertilization when litter quality was low (Knorr et al. 2005) and that microbial nitrogen mining maintained higher rates of decomposition under nitrogen-limiting conditions than under nitrogen-replete conditions (Craine et al. 2007). Recently, McFarlin et al. (2008) demonstrated no effect of fertilization on fungal biomass after 1 yr of fertilization in a salt marsh. This is in contrast to a previous study indicating an increase in fungal biomass as a result of short-term fertilization experiments (Newell et al. 1996). The role of changing carbon and nitrogen supply on heterotrophic marsh bacteria has received even less attention, despite the realization that, at least in freshwater marshes, bacterial production can be significantly greater than fungal production (Buesing and Gessner 2006).

To better understand how chronic low-level nitrogen enrichment affects marsh decomposition we performed a 3-yr marsh fertilization experiment at the Plum Island Estuary Long Term Ecological Research Station in northeastern Massachusetts (Deegan et al. 2007). We enriched the water entering salt marshes to a concentration of 70 μmol L^{-1} NO_3^- (approximately 30 g nitrogen

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m^{-2} yr^{-1}) and 4 μmol L^{-1} PO_4^{-3} (15× over a background of <5 μmol L^{-1} NO^{-}_3; <1 μmol L^{-1} PO_4^{-3}) by adding dissolved nutrients to the flooding water on each tide throughout the growing season (Deegan et al. 2007). This approach differentiates our study from other marsh fertilization experiments, in which pellets of fertilizer are broadcast on small marsh plots (Valiela and Teal 1974; Pennings et al. 2002; Caffrey et al. 2007).

This whole-ecosystem approach will improve our understanding of salt marsh microbial dynamics in two ways. First, this approach mimics the expected concentrations and the mechanism for delivery of nutrients to marshes that would be expected from anthropogenic eutrophication of coastal waters. Most other marsh fertilization experiments enrich to much higher concentrations (20 to >1000 g N m^{-2} yr^{-1}) that are unlikely to be achieved from coastal eutrophication. Second, this approach maintains the spatial complexity of the marsh so that distantly separated microbial communities are exposed to the nutrient enrichment in proportion to their location within the marsh. These two features of our experimental design provide insight into how these marshes will actually respond to realistic environmental perturbations.

In light of the combined effects of sea level rise and coastal eutrophication, our variable of interest is actually the bacterially mediated decomposition of marsh peat. The best measure of this would be an assessment of both bacterial production and bacterial respiration; however, measuring in situ bacterial respiration rates in salt marsh sediments is difficult, as there are no nondestructive techniques that separate out respiration of marsh plants from respiration by the bacterial community. Bacterial production and respiration are related to one another by bacterial growth efficiency, that is, the quantity of bacterial biomass that is produced per unit of organic carbon assimilated. Bacterial growth efficiency is typically calculated from independent measurements of bacterial production and respiration, but estimates of bacterial growth efficiency in sediments are confounded by our inability to measure bacterial respiration.

It has been demonstrated that as the nutrient status of a system increases, so do both bacterial production and bacterial growth efficiency, although the relationship between the two is highly variable (del Giorgio and Cole 1988). The extent to which increasing nutrient availability alters bacterial growth efficiency is a subject of some debate, and as yet there are no conclusive data on how nutrient additions and substrate lability articulate to control bacterial growth efficiency in salt marsh sediments. As a result of this uncertainty we have made the assumption that bacterial growth efficiency varies proportionately with bacterial production, as demonstrated in a cross-system comparison reported by del Giorgio and Cole (1988), and we use bacterial production as a relative measure of the bacterially mediated decomposition of marsh peat.

We hypothesize two possible responses of marsh sediment microbial populations to nutrient enrichment. First, increased dissolved inorganic nitrogen could facilitate decomposition of the abundant organic matter derived from salt marsh vegetation. This carbon source, though plentiful, has low nitrogen content and is thus relatively recalcitrant (Valiela and Rietsma 1984). If, however, there is a direct supply of nitrogen in the overlying water, the microbes may be better able to use this carbon source. Under this 'direct stimulation hypothesis' added nitrogen would stimulate bacterial production across the entire marsh system, increase mineralization of marsh peat, and result in a lower marsh elevation.

A second possibility is that nitrogen additions would increase bacterial production indirectly by increasing labile carbon sources such as benthic microalgae that have a higher nitrogen content than does marsh vegetation (Van Raalte et al. 1976; Sullivan and Currin 2000). Under this 'indirect stimulation hypothesis' the sediment microbial community would take advantage of the increase in highly labile microalgal carbon and show a positive response to fertilization. We would therefore expect to see a differential response across the marsh platform, with an increase in microbial activity in low marsh habitats, where there is ample light to support the growth of benthic microalgae, and no change in rates of bacterial production in high marsh areas, where dense vegetation inhibits light penetration and, hence, microalgal growth (Van Raalte et al. 1976). Under this scenario any increases in bacterial production would not fundamentally alter marsh elevation, because bacteria would be responding largely to increases in benthic microalgae and would not decompose vegetation-derived marsh detritus that forms the physical structure of the marsh.

Methods

Experimental design—Our experiment was carried out in four salt marsh creeks located along the Rowley River, a tributary of Plum Island Sound in northeastern Massachusetts (Deegan et al. 2007). The four marsh creeks were divided into two pairs (pair 1: West and Sweeney; pair 2: Nelson and Clubhead) based on salinity, temperature, nutrients, vascular plant community composition, and benthic microalgal standing stock data. One creek per pair (Sweeney Creek, 2004–2006; Clubhead Creek, 2005 only) received fertilizer inputs during each flooding tide via a water flux–weighted delivery system that was calibrated (based on a hydrologic model estimation of volume of flooding water) to maintain a consistent nutrient flux of 70 μmol L^{-1} NO_3^- and 4 μmol L^{-1} PO_4^{-3} for approximately 150 d during the growing season.

Sample collection—Each experimental marsh system (8–10 ha per marsh) contained five marsh habitats along a gradient from the creek to the high marsh: creek mudflat (MF); filamentous algae on the creek bank wall (FA); tall-form *Spartina alterniflora* (TSA) at the creek edge; *Spartina patens* (SP), which forms most of the vegetation community on the marsh platform; and short-form *S. alterniflora* (SSA). Each habitat differed not only in dominant macrophyte but also in the standing stock of benthic chlorophyll and the stem density of the vegetation (Table 1). In each marsh 10 sediment cores (15 mm in

Table 1. Chlorophyll *a* concentrations (as a proxy for benthic microalgal biomass) and macrophyte stem density for each of the three dominant macrophytes in the reference (Ref.) marshes of each creek pair measured in 2005. Stem density data provided by R. Scott Warren (pers. comm.). All data are seasonal means ± standard error. ND = no data.

Habitat	Chlorophyll *a* (mg m^{-2})		Stem density (stems m^{-2})	
	Ref. No. 1	Ref. No. 2	Ref. No. 1	Ref. No. 2
Mudflat	73±13	88±11	Bare sediment	
Filamentous algae	131±36	87±51	Bare sediment	
Tall *Spartina alterniflora*	187±31	245±23	263±16	197±9
Spartina patens	54±10	21±4	6938±2356	5025±1314
Short *S. alterniflora*	60±10	ND	1336±60	1358±71

diameter, 10 mm deep) were taken from each habitat on approximately a monthly basis. They were then composited and homogenized in a sterile scintillation vial. The scintillation vials were sealed, stored in ambient water, and returned to the lab within 4 h. Subsamples (approximately 0.5 g) were placed into 2-mL centrifuge tubes for the bacterial production assay. The remaining sediments were weighed, dried at 60°C, reweighed to determine sediment bulk density, and ground for carbon and nitrogen content that was analyzed on a PerkinElmer (Waltham) model 2400 CHN analyzer.

Bacterial production assay—We used the incorporation of tritiated leucine (leu) as a proxy for bacterial production (Kirchman et al. 1985) modified for sediment slurries (Buesing and Gessner 2003). Leucine incorporation can be used to measure bacterial production if substrate saturation and isotope dilution are quantified (Fischer and Pusch 1999). Substrate saturation was determined by 90-min incubations of 0.5 g sediment in a slurry with a constant amount of ^{3}H-leucine and increasing amounts of unlabeled leu (0.03–99.5 μmol L^{-1} leu concentration, 6.40×10^{12}–1.85×10^9 Bq $mmol^{-1}$ specific activity; $n = 3 + 1$ killed control per concentration). The incubation was stopped with 50% trichloroloacetic acid to a final concentration of 5%, and the isotope was extracted and analyzed following the method of Buesing and Gessner (2003). Linearity in leu uptake was determined by holding the concentration and specific activity of leu constant and by varying the incubation time (15–175 min; $n = 3 + 1$ killed control per time point). The results of these experiments determined leu concentration and incubation duration for all marsh sediment bacterial production measurements.

Leucine incorporation into salt marsh sediment bacteria largely followed Michaelis–Menten uptake kinetics, with substrate saturation occurring around 50 μmol L^{-1} (Fig. 1, top panel). Using a Lineweaver–Burk plot (Fig. 1, inset) we calculated maximum uptake velocity (V_{max}) for this curve to be 38.5 pmol leu mL^{-1} h^{-1}. The ratio of V_{max} to V_{meas} at 50 μmol L^{-1} leu was 1.14, indicating that isotope dilution was negligible (Van Looij and Reiman 1993). Our time course results indicate that rates of leu uptake were linear through 175 min (Fig. 1, bottom panel). We therefore ran incubations using 50 μmol L^{-1} leu for not longer than 120 min.

The concentration at which the rate of leu incorporation into proteins becomes saturated varies over several orders of magnitude depending on the type of habitat. In pelagic marine systems substrate saturation occurs at leu concentration of 10–20 nmol L^{-1} (Simon and Azam 1989), whereas freshwater sediments are saturated at concentrations of roughly 50 μmol L^{-1} (Buesing and Gessner 2003; Buesing and Marxsen 2005). Substrate saturation on decaying vegetation ranges from 10 nmol L^{-1} to 50 μmol L^{-1} (Buesing and Gessner 2003; Gillies et al. 2006). Although other studies have used leu incorporation to measure bacterial production in salt marshes (Caffrey et al. 2007), this is the first study to report leu uptake kinetics in marine sediments. We found that the detritus-rich salt marsh sediments in our study have similar uptake kinetics to those measured in freshwater sediments (Buesing and Gessner 2003).

When concentrations of leu are in the micromolar range, there are added concerns that the high leu amounts might be incorporated into eukaryotic proteins (Buesing and Gessner 2003). Many bacterial production assays have demonstrated multiphasic uptake kinetics when examined over a broad range of substrate concentrations (Azam and Hodson 1981; Fischer and Pusch 1999). These multiple kinetic pathways could be a result of uptake by eukaryotic organisms (Wright and Hobbie 1966; Fischer and Pusch 1999) or of kinetic diversity within microbial populations (Azam and Hodson 1981). The effect of eukaryotic uptake at high concentrations of leu was examined with the use of eukaryotic inhibitors, and the results indicate little or no leu incorporation by eukaryotes up to a leu concentration of 200 μmol L^{-1} (Fischer and Pusch 1999; Buesing and Gessner 2003).

Data analysis

Habitat scale—The complex nature of whole-ecosystem manipulations confounds statistical interpretation because it could result in the misuse of replicates. In our experimental design we have no "within-habitat" replication for any particular sampling date because samples were composited and analyzed as subsamples. Two of the four marsh ecosystems were nutrient enriched; thus, differences in habitats in these manipulated marshes may arise from actual habitat differences or they may result from the treatment effect. As a result we examined within-habitat

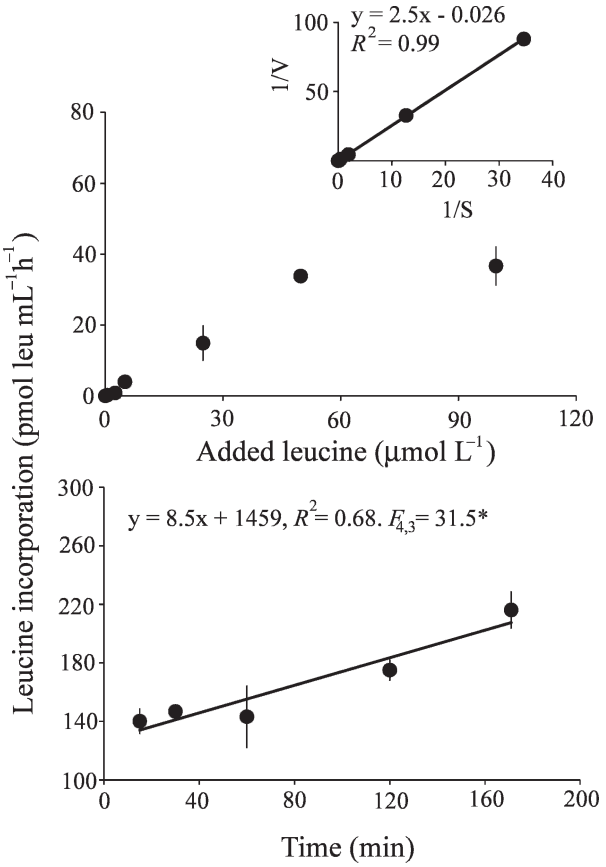

Fig. 1. Optimization parameters for bacterial production assay. Top panel: Substrate saturation curve indicating the increase in bacterial production with increasing leucine (leu) concentration. Inset: Lineweaver–Burk plot in which the inverse of the uptake rate (V) is plotted against the inverse of the substrate concentration (S). The y-intercept of the figure is equivalent to the inverse of the maximum uptake velocity for the curve depicted below. Bottom panel: Time course of leu incorporation through 3-h incubations. Asterisk indicates $p \leq 0.05$.

differences in leu incorporation using data from our two reference creeks in 2005 (Fig. 2) and plotted box plots of medians and quartiles using SPSS 11.0.

Temporal scale—Since there were no apparent differences among habitats in either of our reference ecosystems we used habitats as replicates to analyze temporal differences in rates of leu incorporation. Differences among months were analyzed on log-transformed data using a one-way ANOVA, and differences among years were analyzed using *t*-tests in SPSS 11.0. Climate data for the years 2004–2006 were recorded at a weather station at Governor's Academy, located within 10 km of the marsh creeks.

Effects of fertilization—We assessed the effects of fertilization by plotting the rates of leu incorporation for reference marshes vs. their paired fertilized marshes in each of the years of our experiment and for each of the five habitats sampled. Each figure was plotted against a one-to-one line, and we used the Wilcoxon signed rank test in

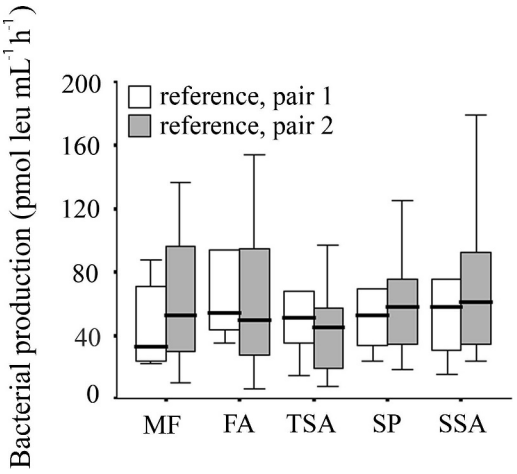

Fig. 2. Box plot of medians and quartiles of bacterial activity rates in the two reference marshes from each pair (pair 1: West Marsh; pair 2: Nelson Marsh). MF = mudflat; FA = filamentous algal wall; TSA = tall form of *Spartina alterniflora*; SP = *Spartina patens*; and SSA = short form of *S. alterniflora.*

SPSS to determine if significantly more points fell above the one-to-one line than fell below it. We expected that if there was an effect of fertilization on leu incorporation we would see more points skewed toward the abscissa (above the one-to-one line) than were skewed toward the ordinate.

Scaling up—To better understand the relative importance of different marsh habitats to marsh-wide bacterial production we converted our leu incorporation rates to total bacterial carbon (C) production using 1.44 kg C mol^{-1} leu incorporated (bacterial carbon production = 1.44 × mol leu; Buesing and Marxsen 2005). We then used data on the aerial coverage of each habitat (Drake et al. 2008) to extrapolate rates of bacterial carbon production to each habitat in our pair 1 marshes. The use of conversion factors such as this is subject to some debate, as it has been demonstrated that actual conversion factors can vary considerably from the theoretical conversion factor. There is evidence, however, that in nutrient-rich habitats the actual conversion factor is similar to the theoretical factor (Calvo-Díaz and Morán unpubl.). As salt marshes tend to be relatively enriched, we conclude that the theoretical conversion factor is a reasonable first approximation of bacterial carbon production.

Results

Temporal and spatial trends in bacterial production—In our control marshes rates of leu incorporation (as a proxy for bacterial production) among the different habitats were highly variable, and there were no significant differences when averaged over the 2005 growing season (Fig. 2). As there were no significant differences among habitats, we used the data from each habitat as replicates to examine seasonal and interannual differences in leu incorporation within each marsh.

There were significant differences in leu incorporation during the growing season in some of our sites, but these differences were not synchronous among the marshes examined (Fig. 3). Marsh pair 2 showed the expected seasonal pattern, with rates of leu incorporation that were low in the spring, that increased during the summer, and that decreased again in the fall. In the reference marsh of pair 2 (Fig. 3), mean rates of leu incorporation in July were higher than in other months, and this difference was significant between July and May, August, and September ($p \leq 0.01$). In the fertilized marsh in pair 2 (Fig. 3), the mean rates of leu incorporation were highest in June and July, and both months were significantly different than all other months examined ($p \leq 0.01$). The expected seasonal pattern that was evident in marsh pair 2 was not present in marsh pair 1. In marsh pair 1, rates of leu incorporation showed no differences throughout the growing season in the reference creek (Fig. 3). In the fertilized marsh, rates of leu incorporation were very high in the spring and summer and decreased significantly in September and October ($p \leq 0.01$).

Climate variability among years may have influenced annual rates of bacterial production (Table 2). In the northeastern United States, 2006 was among the wettest years in recorded history and was significantly wetter than in 2004 and 2005. On an annual basis the region received over 400 mm more rain in 2006, compared with the 1971–2000 climate normal rainfall of 1191 mm. During 2006, 68% of the total rainfall accumulated during the growing season, but this did not depress temperatures in the region; growing season temperatures in 2006 measured between those of 2004 and 2005 (Table 2). The extremely wet season in 2006 may have inhibited rates of bacterial production. We had multiyear data for our pair 1 marshes. In the fertilized marsh, leu incorporation rates in 2004 and 2005 were not significantly different from one another (Table 2; $t = -0.247$, $p = 0.8$), but both were significantly different from the 2006 rate ($t = 4.987$ [$p \leq 0.01$] and $t = 3.025$ [$p \leq 0.01$], respectively). In our reference marsh the average rates of leu incorporation in 2006 were also lower than in 2004 and 2005 (Table 2), but as a result of the high standard error in 2005, these differences were only significant between 2004 and 2006 ($t = 3.170$, $p \leq 0.01$).

Effects of fertilization—To directly examine the effects of fertilization on marsh bacterial production we plotted each rate of leu incorporation in the fertilized marsh against that site's paired control marsh for each month and habitat for the 3 yr of fertilization (Fig. 4). Overall, postfertilization leu incorporation had a skewed distribution in each year (Wilcoxon signed ranks tests; 2004: $p = 0.005$; 2005: $p = 0.001$; 2006: $p = 0.037$), with significantly more points above the one-to-one line than below it, indicating a positive response to fertilization (Fig. 4, closed circles). Moreover, rates of leu incorporation measured prior to the onset of fertilization in each year were randomly distributed around the one-to-one line (Fig. 4, open circles; 2004: $p = 0.394$; 2005: $p = 0.334$; 2006: $p = 0.730$), lending support to the hypothesis that fertilization increased the rates of bacterial production.

It is possible that the response to fertilization that we see in each year could be driven by a strong positive response in some marsh habitats and no response in other marsh habitats. We therefore used a similar approach to determine if the effects of fertilization were detectable within each of our salt marsh habitats (Fig. 5). A significantly greater number of points fell above the one-to-one line in the three low marsh habitats, including the MF ($p < 0.01$), the FA ($p = 0.021$), and the TSA ($p < 0.01$), indicating a positive response to fertilization. There was no significant response to fertilization in the two high marsh habitats, including the SSA ($p = 0.424$) and SP ($p = 0.538$).

Temporal and spatial differences in carbon production—The contribution of bacterial production to total carbon supply was determined more by habitat area than by differences in bacterial production rates. Our data indicate that there are relatively small differences in rates of leu uptake among different salt marsh habitats (Fig. 2) but that there are large differences in the area of each habitat, differences that translate into important differences in the quantity of carbon produced in different marsh regions (Table 3). Bacterial carbon production among habitats ranged from 3.4 to 5.5 g C m^{-2} $season^{-1}$ (Table 3). In both marshes bacterial carbon production was greatest in the high marsh, where there was no clear response to fertilization. *S. patens* habitats accounted for 69% and 72% of total bacterial carbon production in the reference and fertilized marshes, respectively. The short form of the *S. alterniflora* habitat accounted for 26% of bacterial carbon production in the fertilized marsh and for 24% in the reference marsh.

Discussion

In detritus-based systems such as salt marshes, increasing ambient nitrogen could have important effects on marsh structure. If salt marsh sediment microbes are nitrogen limited, as has been demonstrated in other organic-rich ecosystems (Findlay and Sinsabaugh 2003; Castillo et al. 2004), increasing the supply of ambient nitrogen may allow them to decompose nutrient-poor organic matter such as marsh peat, which is an essential component of the marsh platform. As decomposition of the peat progresses, it could lead to a decrease in marsh organic matter, subsidence, and a reduction in many of the ecological services of marshes.

The effect of nitrogen inputs on marsh elevation has been demonstrated in small heavily fertilized marsh plots (~15× the rate of fertilization of our plots) in South Carolina (Morris and Bradley 1999). These marshes showed a significant reduction in organic carbon in the top 5 cm of marsh sediment after several years of fertilization. These authors hypothesized that the increased nitrogen supply could alter the proportion of labile and refractory pools of carbon, making some heretofore inaccessible carbon available to degradation by microorganisms and decreasing the organic content of the sediments. Additional laboratory experiments in which soil

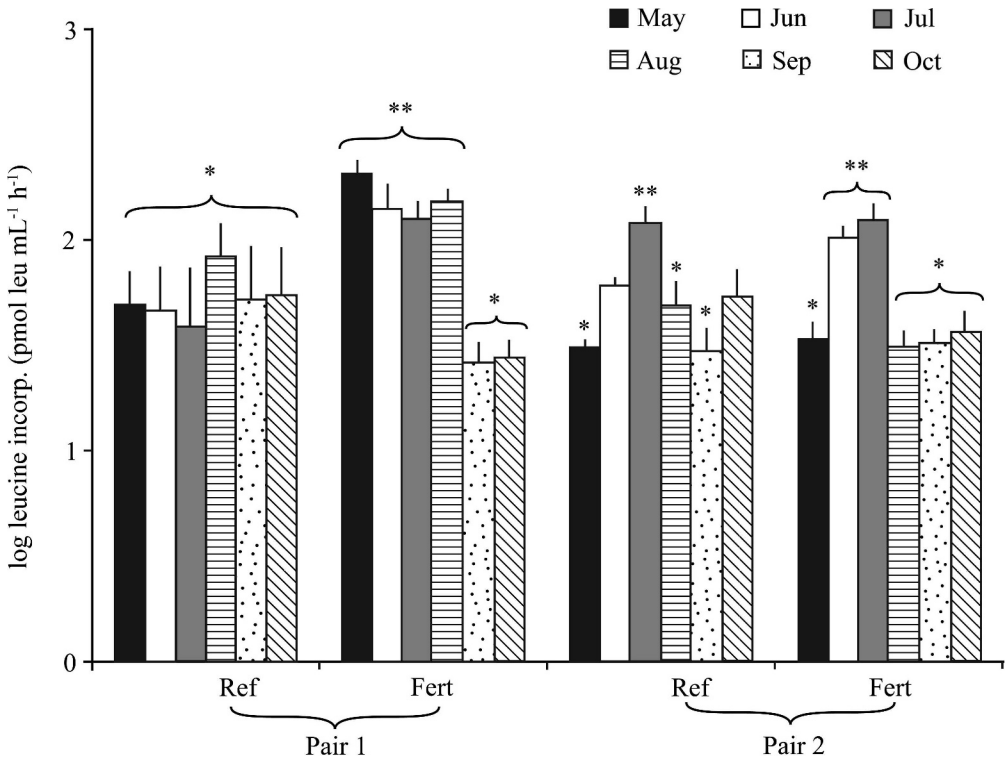

Fig. 3. Mean (±standard error) leucine incorporation rates for each month of the 2005 growing season in each of our two marsh pairs. Differences in rates of leucine incorporation within each marsh are indicated with asterisks; those months with two asterisks are statistically different ($p \leq 0.05$) from months with one asterisk.

cores were amended with inorganic nutrients demonstrated that, in fact, microbes could be directly stimulated by the additional nutrients and are not dependant on changes in the lability of the carbon pool to increase their activity (Morris and Bradley 1999).

Table 2. Growing season bacterial production (mean ± standard error) measured for each habitat in marsh pair one as well as growing season temperature (mean ± standard error) and precipitation.

Bacterial production (pmol leucine mL^{-1} h^{-1})	Year		
	2004	2005	2006
Fertilized marsh			
Mudflat	106±25	138±50	38±3
Filamentous algae	68±19	128±43	43±8
Tall *Spartina alterniflora*	97±15	121±37	34±3
Spartina patens	60±11	126±34	47±9
Short *S. alterniflora*	121±24	96±25	47±7
Reference marsh			
Mudflat	70±23	62±31	41±6
Filamentous algae	75±31	90±51	37±12
Tall *S. alterniflora*	60±12	78±51	30±8
S. patens	52±10	74±24	45±9
Short *S. alterniflora*	98±34	98±29	26±1
Mean growing season temperature (°C)	16.4±0.6	17.0±0.67	16.8±0.66
Total growing season precipitation (mm)	541	594	932

Our results provide evidence that when nutrients are delivered to salt marshes via a mechanism that mimics widespread coastal eutrophication, it is the supply of labile carbon, and not the direct supply of inorganic nitrogen, that affects rates of bacterial production in salt marsh sediments. Bacterial production increased in response to fertilization in creek and low marsh habitats (Fig. 5). These increases, however, only occurred within marsh habitats that had the highest standing stocks of benthic chlorophyll (Table 1) and that showed a positive response to fertilization by increased growth of benthic microalgae (Deegan et al. 2007).

Our hypothesis that the increases in bacterial production resulted from an increase in labile benthic microalgae rather than direct stimulation by added nitrogen is supported by the observed increase in microalgal production. We estimated microalgal production using a standard relationship between benthic chlorophyll biomass and microalgal production (Pinckney and Zingmark 1993). We applied this relationship to the change in the end-of-season biomass of benthic chlorophyll between 2003 (prior to the onset of fertilization) and 2005 (after 2 yr of fertilization) to obtain a rough estimate of the rates of microalgal production.

The increase in microalgal production can easily account for our observed increase in bacterial production. In the fertilized marshes microalgal production increased to 192 g C m^{-2} growing $season^{-1}$ (225% increase from 2003–2005) in the fertilized mudflat zone, while in the reference marsh algal production was only 93 g C m^{-2}

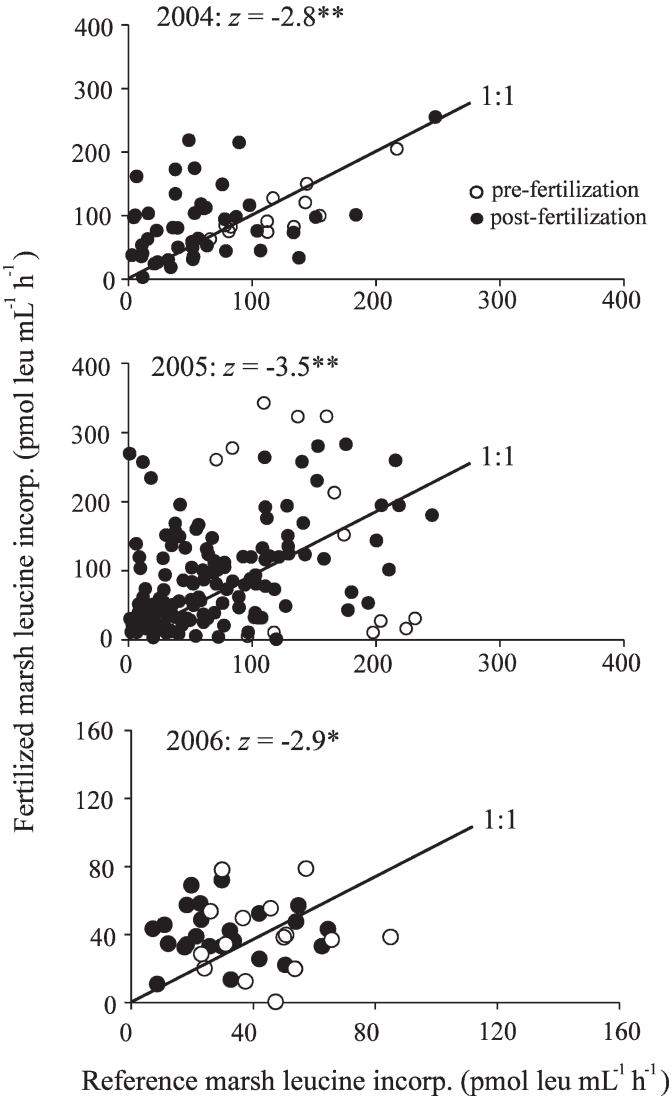

Fig. 4. Fertilized marsh bacterial production rates vs. the bacterial production rate from the paired reference marsh site for each of the 3 yr of the experiment. Solid line represents the one-to-one line of perfect fit. In all comparisons prior to the onset of fertilization (open circles) there were no differences between fertilized and reference plots. * indicates $p \leq 0.05$; ** indicates $p \leq 0.01$.

growing season^{-1} (16% increase from 77 g C m^{-2} growing season^{-1} in 2003). In the fertilized tall-form *S. alterniflora* zone microalgal production increased to 284 g C m^{-2} growing season^{-1} (46% increase from 195 g C m^{-2} growing season^{-1}), while the reference marsh increased only 7% (from 188 g C m^{-2} growing season^{-1} to 201 g C m^{-2} growing season^{-1}). Mean rates of bacterial production in all of our salt marshes fall within the range of reported values (Caffrey et al. 2007) and never exceeded 6 g C m^{-2} growing season^{-1}. This rough calculation demonstrates that there is a sufficient increase in benthic microalgal production to account for the increase in measured bacterial production. The effects of this increase in algal biomass on higher trophic levels is an area of active investigation (Deegan et al. 2007); however, grazing and decomposition of both microalgae and marsh vegetation limit the accumulation of biomass in these habitats.

Isotopic analysis of phospholipid-derived fatty acids (PLFA) provides another line of evidence regarding the carbon sources of salt marsh sediment bacteria. Boschker et al. (1999) found that in the Waarde Marsh the δ^{13}C signature of bacterial-specific fatty acids indicated that macrophyte-derived organic matter was only a small source of microbial carbon in both vegetated and unvegetated sediments. Instead, bacteria derived most of their energy from algal sources (Boschker et al. 1999). This marsh is located in the highly eutrophied Westerschelde Estuary and receives a significant supply of inorganic nitrogen and organic-rich silt that can sustain large populations of benthic microalgae with a δ^{13}C signature close to −20‰ (Boschker et al. 1999). In contrast, δ^{13}C of living *Spartina* tissue is typical of C4 plants and ranges between −12.5‰ and −13.5‰ (Boschker et al. 1999). As *Spartina* tissue decomposes, carbon isotopic signatures becomes lighter as the lignin fraction (δ^{13}C ~−17‰) of *Spartina* biomass becomes the dominant component of the decomposing vegetation (Benner et al. 1987). As a result, the actual isotopic signature of the *Spartina*-derived carbon that is available for decomposition by microbes will range between ~−12.5‰ and −17‰ depending on the stage of decomposition of the marsh peat (Benner et al. 1987).

Bouillon and Boschker (2006) found that sediment organic matter in four salt marsh sites in Plum Island Sound, near the site of our fertilization experiment, ranged from −21‰ to −16.5‰. The two samples that were isotopically heaviest (those above −17‰) were taken from within the densely vegetated *S. patens* habitat; all other sparsely vegetated and unvegetated sites had isotopically lighter signatures, indicating that the organic matter largely was derived from more depleted source materials, such as those found in algal carbon (Deegan and Garritt 1997). The bacterial δ^{13}C signatures of −14‰ to −20‰, based on PLFA analysis, were similar to those found in the sediment organic matter in that they were largely depleted in δ^{13}C relative to what would be expected if *Spartina* were the primary source of carbon fueling bacterial populations. The exception to this pattern was in the *S. patens* habitat, in which the bacterial PLFA signature more closely resembled the signature of highly degraded *Spartina*. These isotope data provide further evidence that benthic microalgae, rather than marsh grasses, are the primary carbon source that supports bacterial production.

Our results could be influenced by variability in the delivery rate of nutrients to the marsh surface. The spatial complexity of salt marshes implies that nutrients may be delivered in different quantities and for different durations during a tidal cycle. Analysis of the distribution of nutrients on the marsh surface demonstrated that flooding water carried similar concentrations of nutrients to all corners of the marsh (Deegan et al. 2007); however, the duration of time during which different marsh habitats were exposed varied, as would be expected in the case of widespread coastal eutrophication. Tall *S. alterniflora* habitats were submerged by nutrient-enriched water 35% of the time,

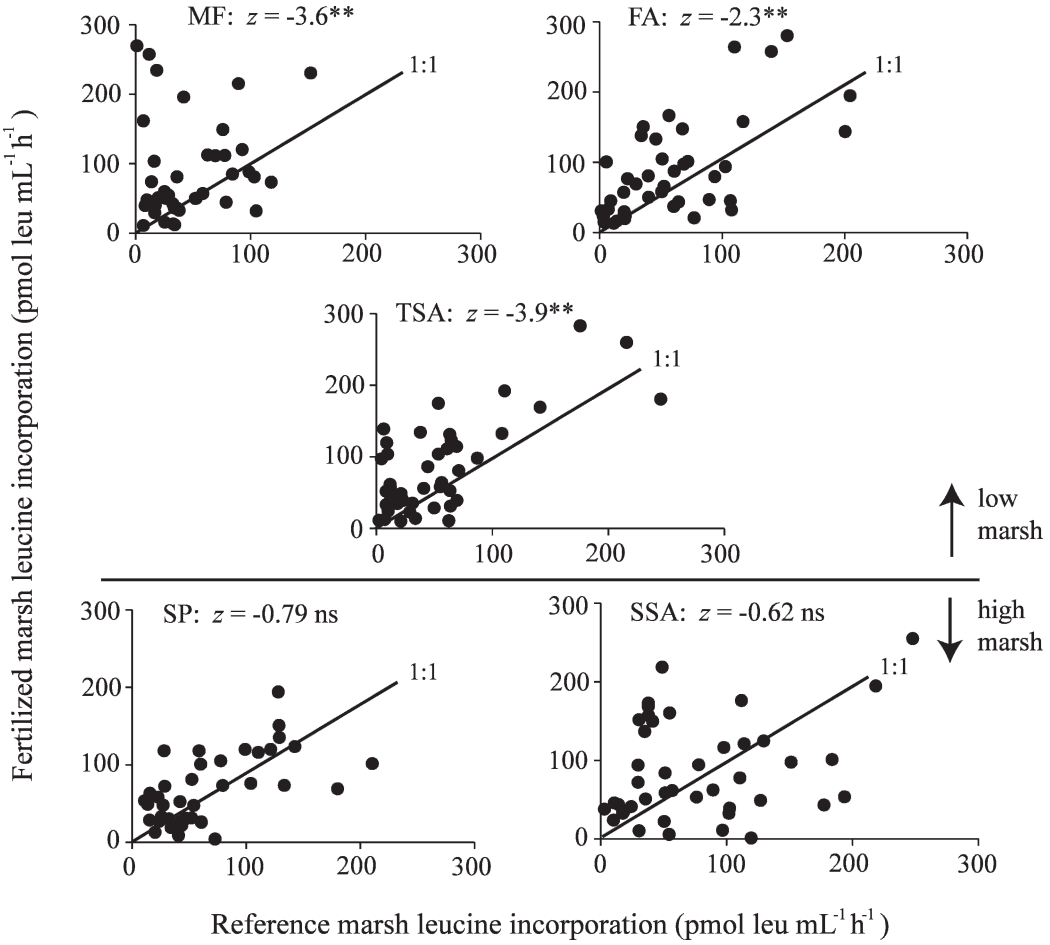

Fig. 5. Fertilized marsh bacterial production rates vs. bacterial production rates in their paired reference marsh for each of the five marsh habitats examined. The solid line represents the one-to-one line of perfect fit. MF = mudflat; FA= filamentous algal wall; TSA = tall form *Spartina alterniflora*; SP = *Spartina patens*; and SSA = short form *S. alterniflora*. * indicates $p \leq 0.05$; ** indicates $p \leq 0.01$.

whereas *S. patens* habitats were submerged only 12% of the time (Deegan et al. 2007). Despite the shorter duration of exposure, the enrichment of *S. patens* and the short form of *S. alterniflora* habitats was sufficient to modestly alter plant biology in the first 2 yr and was thus probably sufficient to meet microbial demands. Specifically, fertilization increased plant tissue nitrogen content in senescing leaves by 23% (short *S. alterniflora*) and 15% (*S. patens*) above reference marshes (Drake et al. 2008), and direct uptake of NO_3^- by *S. patens* was higher in fertilized marshes (Drake et al. 2008). It therefore seems unlikely that the neutral response in bacterial production rates in the high marsh were a result of a lack of exposure to the enriching nutrients.

Accounting for bacterial respiration will increase our estimates of bacterial carbon demand, and the extent to which bacterial growth efficiency increases nonlinearly with

Table 3. Total bacterial contribution to marsh carbon (C) cycling from each habit in our fertilized (Fert.) and reference (Ref.) marshes. Extrapolation based on mean rates measured during the growing seasons of 2004 through 2006. Data for habitat area from Drake et al. (2008).

	Area (m^2)		Mean bacterial production (g C m^{-2} $season^{-1}$)		Total marsh production (kg C)	
Habitat	Fert.	Ref.	Fert.	Ref.	Fert.	Ref.
Mudflat and filamentous	2480	1680	5.5	3.7	14	6
Tall *Spartina alterniflora*	2700	1700	4.8	3.4	13	6
Spartina patens	65,600	48,000	5.1	4.5	334	215
Short *S. alterniflora*	24,600	21,300	5.1	3.4	126	72
Total area (m^2)	95,380	72,680				
Mean bacterial production (g C m^{-2} $season^{-1}$)			5.1	3.7		
Total marsh production (kg C)					487	299

bacterial production will dictate the actual amount of decomposition of the marsh peat. Recent work in the Hudson River Estuary (del Giorgio et al. 2006) and in Monie Bay, Virginia (Apple and del Giorgio 2007), demonstrates the variability of bacterial growth efficiency. In the Hudson River study, deviations from mean bacterial growth efficiency coincided with local variations in concentrations of dissolved organic carbon (DOC) and seston. Both studies indicated that the quality of DOC may play a critical role in determining the proportion of total carbon mineralization that is partitioned into respiration and production. In our study, fertilization appears to alter the quality of carbon that is available to microbes and in turn to increase the rate of bacterial production in the low marsh habitats (less than 6% of the total marsh area). Long-term increases in the tissue N content of senescing leaves in the high marsh habitat has been shown to decrease the amount of remaining plant biomass in highly enriched salt marsh plots (Marinucci et al. 1983) through a combination of fungal and microbial activity. There is no evidence yet that a shift in litter quality is changing the production of the microbial community in our fertilization experiment, but given enough time, the enriched leaves may become a part of the detrital food web and as such could be another indirect mechanism of stimulating bacterial production and bacterial growth efficiency.

Our whole-marsh enrichment experiment provides an in situ approach to understanding how chronic low-level eutrophication of coastal waters will affect salt marsh processes. This experimental design has significant advantages over marsh plot fertilization experiments, because the nutrient delivery more closely mimics the delivery of nutrients by coastal eutrophication. Results of our marsh fertilization experiment demonstrate that over the first 3 yr of marsh enrichment bacterial production in detritus-rich sediments is more strongly controlled by the quality of the organic matter present than by the supply of inorganic nutrients in overlying water. In our experiment, bacterial production was enhanced by fertilization only in those habitats that also showed a positive response to fertilization by increasing quantities of highly labile benthic microalgae. Contrary to our assumptions, bacteria did not use this newly available nitrogen to decompose existing marsh peat. These results indicate a greater resiliency of the marsh platform to increased coastal enrichment than was previously assumed.

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