

Response of Salt Marsh Ponds to Eutrophication

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ABSTRACT: We examined the response of New England salt marsh ponds to nutrient loading via flooding tidal water as part of a larger, long-term whole ecosystem manipulation. Nitrate and phosphate concentration were added directly to two tidal creek in concentrations approximately 15x ambient for ~150 d during the growing season and 30x ambient for 3 d during the study period. During the summer, when fertilization was occurring, we measured a time series of nitrate and ammonium concentrations, water column algal content, the numerical abundance of benthic micro-algae, and the numerical abundance of protists. Additionally we measured benthic algae biomass, floating algae mat percent cover, and *R. maritima* percent cover. During the fall, sediment cores were collected and incubated in the lab where we obtained measurements of ammonium and nitrate fluxes, gross primary production, and bacterial production before and after the addition of nutrient (70 uM nitrate and 4uM phosphate). Our results indicate that gross nitrate processing in salt marsh ponds occurs at comparable rates to the rest of the marsh ecosystem. Additionally, our results indicate that primary producers in the ponds are limited by light and/or top-down grazer controls. Despite relatively high rate of production, are responsible for, at most, half of the initial processing of nitrate entering the ponds through flooding tidal water.

KEY WORDS: Salt marsh—Ponds—Pools—Nutrient loading—Eutrophication—Benthic microalgae—Amoebas—Bacterial production—Sediment metabolism—Top down controls

INTRODUCTION

Anthropogenic nitrogen loading to the coast has greatly increased in recent decades (Vitousek et al. 1997). Coastal ecosystems are typically nitrogen limited and as a result, cultural nitrogen enrichment of these waters lead to eutrophication, now a

widespread global environmental issue (Vitousek et al. 1997). Salt marshes act as nitrogen sinks (Valiela and J.M. Teal 1979), a valuable function in regards to dulling the extent of eutrophication (Gren 1999). Understanding the mechanisms by which salt marshes take up nutrients is necessary in order to predict critical loads, the capacity of a marsh to take up before a non-linear change to an alternate, less desirable state occurs (Verhoven 1983). Additionally, it is necessary to know how the relative importance of these mechanisms differs between micro-habitats contained in the marsh.

Here we examine nutrient processing in salt marsh ponds. In the Eastern US ponds are common features in the vegetated intertidal zones of salt marshes. Further, they are predicted to increase in abundance due to increased inundation of coastal marshes caused by a rate of sea level rise that is faster than the rate of organic matter and sediment accumulation (Craft et al. 2009). Attributed to rising sea levels, ponds within the Parker River estuary, where this experiment will take place, have increased in size and number since the 1950's (Cavatorta et al., 2003). Some studies have highlighted the use of salt marsh ponds as fish refuges and overwintering habitat (e.g. Raposa 2003, Smith and Able 2003, Rountree and Able 2007). Additionally, there is a long history of speculation by eco-morphologists on the initial mechanisms of pond formation (e.g. Redfield 1972 and more recently Miller et al 2009). However, despite their apparent (and growing) importance in the marsh landscape, very little is known about nutrient cycling in ponds (Deegan, personal communication; Figure 1).

To examine how salt marsh ponds respond to nutrient inputs we examined two related questions:

- 1) How are nutrients processed in nitrogen enriched ponds?
- 2) How does nutrient enrichment of ponds stimulate primary production relative to the stimulation of heterotrophic microbes?

To address these questions we carried out a combination of field and laboratory fertilization experiments, making measurements of: water chemistry; percent cover of submerged aquatic vascular plants; standing stocks, numerical abundance and gross primary production of benthic micro-algae; protist numerical abundance; and bacterial production.

METHODS

Experimental design and study area-This experiment on salt marsh ponds was carried out as a part of the TIDE project (Trophic Dynamics and Interacting Control Process in a Detritus based Ecosystem). TIDE is a long term whole ecosystem manipulation with the goal of quantify the effects of nutrient loading on salt marshes at large, realistic scales. The experiment takes place in 2 fertilized and 2 reference marshes located along the Rowley River. The Rowley River is a tributary of the Plum Island Sound, a 25-km long tidal estuary located in Northern Massachusetts. The treated marshes are fertilized for ~ 150 d during the growing season via the addition of 70 uM nitrate and 4uM phosphate directly to the tidal creeks. This method of fertilizer addition allows for a realistic gradient in fertilization to develop across the marsh surface. A detailed description of the experimental design and background data can be found in Deegan et al (2007).

The marshes consist of ~20% low marsh, where the dominant vegetation is *Spartina alterniflora* and ~80% high marsh were the dominant vegetation if *Spartina patens*. The low marsh is flooded on a daily basis. The high marsh, due fluxuations in maximum water height that occurs during the tidal cycle, is flooded only ~12% of the growing season during what are referred to as “spring tides”.

Ponds of varying sizes and shape are distributed throughout the high-marsh platform, varying in proximity from the tidal creeks. We examined 7 ponds in total, 4 located in fertilized marshes and 3 in reference marshes. The ponds range from ~6000-200 m² in area, ~25-50 cm in water depth, and ~20-50cm in soft sediment depth (Table 1). The soft sediment of the ponds is very amorphous, typically ~90% of the weight of the top 3 cm of sediment is water. Previous examination of the spatial gradient of nutrient concentrations during flooding of the marsh platform by spring tides indicates all of the ponds located on fertilized marshes receive nutrient concentrations elevated from the ambient (Figure 2).

We considered each pond a replicate although other experimental design strategies were also justifiable (e.g. paired analysis or consideration of each marsh as a replicate). Some of the fertilized ponds were located in the same marsh and therefore

received nutrients from the same initial source. Additionally, some ponds that appear separate on the surface of the marsh may actually be connected in the sub-surface (Wilson et al. 2009). Fertilized ponds located on the Clubhead marshes (Figure 2) were separated by ~2 m of marsh, a distance small enough to have some subsurface connections. All other ponds were spaced at least 50 m apart. Despite these potential issues, the ponds selected should be sufficiently independent that our design avoids the perils of pseudo-replication (Hulbert 1984).

Two tailed ttests (Excell 2007) were used to make comparisons between fertilized and reference ponds. Type 3 ttests were used when the variances were different in fertilized and reference ponds and/or when there was an uneven array. Measurements of benthic algae were log transformed and percent cover estimates were arcsine transformed before running ttests.

Field Sampling-We made measurements of salinity, water column nutrients (NO_3^- and NH_4^+), water column algal content, benthic micro-algae (BMA) numerical abundance, and protist numerical abundance in a ~month long time series during the growing season (Jun 29-Jul 30 2010). Measurements were made before and after the flooding of the high-marsh by a spring tide but not during the flooding (June 9-July 20). Isolation of the ponds was determined using bamboo rods painted with dyed glue that was washed away when flooding occurred. Salinity was determined with a YSI model 30 conductivity meter. Water samples were collected in 0.5 L acid washed Nalgene bottles, refrigerated, and filtered (combusted 47mm glass fiber filter [GFF]) within 24 hours of collection. The filter and filtrate were frozen until further analysis for nitrate, ammonium, and chlorophyll *a* concentrations (discussed below). BMA and protist numerical abundance were measured using glass slides as an artificial colonization substrate. Slides (3 per pond at each time point) were submerged in the ponds for 2-3 days, kept moist upon collection and preserved in 70% ethanol within 4 hours.

In addition to the time series measurements, we quantified benthic algae (BA) standing stock, floating algae mat percent cover, and *R. maritima* percent cover. Chlorophyll *a* (mg/m^2) was used as a proxy for algal biomass. Sediment cores to measure benthic chlorophyll *a* were collected in late-july and mid-november (cores were

2.75 cm in diameter and ~3 cm deep; 5 cores taken per pond at each time point). Excess water was drained from the sediment cores using a fine nitex mesh during the July sample collection, but not during the November sample collection. Floating algae mat percent cover was determined in mid-July based on the total area of the mats and ponds. The area of the mats was determined by measuring the distance that the mats extended from the shore of the ponds at 1 m increments. The total area of the ponds was determined based on their perimeter and assuming a circular shape. *R. maritima* percent cover was measured in late-july using a 1 m² quadrant placed every other meter across the long axis of each pond.

Sediment core incubations-Sediment cores were collected from 2 fertilized ponds (Sweeney-close and Clubhead-small) and 2 reference ponds (West and Shad) on 15 Nov 2010 (9.5 cm diameter and ~25cm deep; 2 per pond). We sampled areas with minimal *R. maritima* and fairly uniform BA cover. The cores were returned to the lab and bubblers were added to keep the overlying water oxygenated within 24 hours of collection. Two days after collection cores were placed in an incubation chamber (29 °C) and kept on a day night cycle (15 h light and 9 h dark). Although the air temperature in the incubation chamber was 29 C, the bottom half of the cores was placed in coolers and submerged in water; as a result the temperature of the overlying water in the cores never exceeded 24 °C. The intensity of the light reaching the sediments in the cores was ~300 $\mu\text{E m}^{-2} \text{s}^{-1}$, typically close to saturating light for BMA (MacIntyre et al 1996).

We commenced flux measurements on 18-Nov, 3 d after field collection. Immediately before starting measurements the water in the cores was replaced with seawater filtered through a 5 μM filter and diluted to a salinity of 29 ppt (~the same as the water in the ponds). Two different rounds of light-dark dissolved oxygen (DO), nitrate, and ammonium flux measurements were carried out. From Nov 18-19 measurements were made with non-nutrient enriched water overlying the sediment. On Nov 20, nitrate concentrations were increased by 70 μM and phosphate concentrations were increased by 4 μM in all of the cores (from both reference and fertilized ponds). Twenty-five hours after the addition of nutrients, from Nov 21-22, we commenced a second round of light-dark flux measurements.

The rate of change of oxygen, nitrate, and ammonium concentrations was determined using a linear regression. We considered a correlation coefficient (R^2) of 0.95 and acceptable degrees of correlation for the change in DO concentration and 0.85 acceptable for the change in nitrate and ammonium concentrations. Concentration changes that were acceptably linear were scaled to an areal flux ($\text{mmol m}^{-2} \text{h}^{-1}$) based on the volume of water overlying the sediment and area of the cores. Total respiration ($\text{mmol C m}^{-2} \text{h}^{-1}$) was determined assuming a respiratory quotient of 1. Gross primary production (GPP; $\text{mmol C m}^{-2} \text{h}^{-1}$) was calculated based on the difference between the DO flux in the light and dark, assuming a photosynthetic quotient of 1 and that respiration was the same in the light in the dark.

Additionally, we measured bacterial production in the sediments of the cores before and after the addition of nutrients (20-Nov and 22-Nov) using the leucine incorporation method (Kirchman et. al. 1985), adapted for the sediments of salt marsh ponds. To compensate for the amorphous, high water content of the sediments we created uniform suspension by centrifuging $\sim 400\mu\text{L}$ of sediment collected from each core at high speed for 5 min, decanting the overlying water, then re-diluting the sediment with water collected from the respective cores in order to create a suspension of a known concentration.

Incubations were carried out at 22 °C, adding 0.5 ml of the suspension to 0.5 ml of 50 μM leucine solution (specific activity = 0.0003 ci/mmol), resulting in an overall end concentration of 25 μM leucine. Samples were killed with 100% TCA, then a series of 3 centrifuging, vortexing and washing steps were carried out to remove leucine that was not incorporated by bacteria only absorbed by the sediment: (1) samples were centrifuged at high speed for 15 min, decanted, 0.5 ml of 5 % TCA was added, and then samples were vortexed for ~ 5 min to re-suspend the pellet in order to increase the effectiveness of the washing; (2) samples were again centrifuged at high speed for 15 min, decanted, 0.5 ml of 70 % ethanol was added, and samples were vortexed for ~ 5 min to re-suspend the pellet; (3) samples were vortexed on high for 15 min, decanted, let to dry for at least one hour, scintillation cocktail was added, and samples were vortexed for 10 min until the pellet was re-suspended. Radioactivity of the samples was measured in disintegrations per minute (DPM) on a scintillation counter.

We used weight to volume ratios of the sediment to estimate leucine incorporation rate per milliliter of actual core sediments. We assumed the process of creating a suspension did not significantly alter incorporation rates. Because sediment samples were taken based on volume only, not area, in order to scale the bacterial production measurement to an areal flux we assumed all the heterotrophic decomposers would be found distributed uniformly throughout the top 1 cm of the sediments only.

Leucine incorporation can only be used to quantify bacterial production if substrate saturation and isotope dilution are quantified. We examined uptake kinetics in sediments collected from a reference pond. Linearity of uptake was determined for up to 105 min incubations of suspension based on a time series of 2 incubations and 1 killed control at 25 uM (Figure 1, bottom panel). Saturation was determined by 95 min incubations of suspension ($n = 1$ sample + 1 killed control) with increasing concentration of leucine added (0.015uM-25uM, 0.01 ci/mmol). Leucine incorporation appeared to follow Michaelis-Menten uptake kinetics, although we did not add high enough concentrations of leucine to be certain (Figure 1, top panel). Using a Lineweaver-Burk plot (Figure 1, top panel inset) we calculated a maximum uptake velocity at 25 uM of $1429 \text{ pmol leu ml}^{-1} \text{ h}^{-1}$. The ratio of the maximum uptake velocity (V_{max}) to the measured uptake velocity (V_{meas}) at this concentration was 0.68, indicating a substantial amount of isotope dilution occurred and/or the sediment were not fully saturated at 25 uM concentration of added leucine. To compensate for this we used the Lineweaver-Burk plot to determine a half-saturation constant (k). We then used the Michaelis-Menton equation ($V_{\text{meas}} = V_{\text{max}} * [\text{leu}] / ([\text{leu}] + k$, where $[\text{leu}]$ is the concentration of leucine added) to model the leucine incorporation rate had we completely saturated the sediments.

We converted leucine incorporation to carbon production using the pseudo-constant $1.44 \text{ kg C mole}^{-1}$ leucine incorporated (Buesing and Marxsen 2005). Actual conversion factors can vary considerably from the theoretical conversion factors. However, evidence suggests the theoretical conversion factors are similar to the actual conversion factor in nutrient rich habitats, of which salt marshes could be included (Calvo-Diaz unpubl, cited through Bowen et al 2007).

At the end of sediment core incubations the top 1-3 cm of sediment was collected, homogenized and sub-sampled for chlorophyll *a*, and for the determination of wet:dry weight ratio and weight:volume ratios.

Sample processing- Nitrate concentrations were determined colorimetrically on a flow injection analyzer (Lachat Quick Chem 8000, Lachat Zellweger Instruments, Milwaukee, Wisconsin, USA) using the cadmium copper reduction method (Wood 1967) and ammonium concentrations were measured colorimetrically using the phenolypochlorite method modified for small sample sizes (Solarzano 1969). Water column chlorophyll *a* ($\mu\text{g/l}$) and benthic chlorophyll *a* (mg/m^2) were measured by spectrophotometric analysis using methods adapted from Lorenzen (1967). Ninety percent acetone was used for extraction of the water column chlorophyll-*a* from filters. One hundred percent acetone was added to sediment core samples which should have been diluted to ~90% due to sediment pore water. If necessary sediments were centrifuged for ~5 min at 2000RPM and excess overlying water was decanted before adding acetone.

RESULTS

Field measurements-Salinities were highly variable, ranging from ~27-32 PSU over the period of measurement (Figure 4). No consistent differences in salinity were observed between the fertilized and reference ponds or before and after flooding by spring tides. Nitrate concentrations in the fertilized ponds were higher than the reference ponds before flooding by spring tides (Figure 5, top panel). Less than 12 h after isolation from the final flooding tide nitrate concentrations in the fertilized ponds were well below 140 μM , the concentration of fertilizer added to the tidal creek. Within 3 d of isolation nitrate concentrations were below 2 μM in the fertilized ponds. The mean nitrate concentrations in the reference ponds remained below 2.5 μM at time although a slight increase occurring after flooding by spring tides. Ammonium concentrations ranged between 1 and 6 μM in both fertilized and reference ponds (Figure 5, bottom panel). Ammonium concentrations in the reference ponds were more variable than in the fertilized pond, no other consistent differences in were observed.

Water column algae content was consistently higher in the fertilized ponds and increased after flooding (Figure 6). The numerical abundance of colonizing BMA was also consistently higher in the fertilized ponds although they became more similar over time after flooding (Figure 7, top panel). There was no apparent increase of BMA in fertilized ponds after flooding. Colonizing amoeba outnumbered colonizing BMA at times (Fig 7, bottom panel; Figure 8). In reference ponds there was a significant negative correlation between the density of amoebas and the density of BMA, but only after adjusting for a delayed response by matching measurements of amoeba density with the prior measurement of BMA density (pearsons, $r = -0.9$, $p < 0.05$). There was no significant correlation in the fertilized ponds, even after adjusting for a delayed response (pearsons, $r = 0.4$, $p > 0.05$).

The mean standing stock biomass of BA was higher in the fertilized ponds during the fall but not during the spring, although the difference in the fall were not significant (type 3 2-tailed ttest with log transform, $p > 0.05$; Table 2). Additionally, the mean percent cover of ponds with floating algae mats was much higher in the fertilized ponds although the difference was not significant (type 3, 2-tailed t-test with arcsine transform, $p > 0.05$; Table 2). There was no difference between fertilized and reference ponds in *R. maritima* percent cover (type 3 2-tailed ttest with an arcsine transform, $p \gg 0.05$; Table 3).

Sediment core incubations-After removing outliers and cores with non-linear changes in nitrate concentrations ($r^2 < 0.85$) we found that the rate of decrease of nitrate during dark incubations ranged from -0.15 to -0.49 $\text{mmol NO}_3^- \text{ m}^{-2} \text{ d}^{-1}$, with no consistent differences between cores or treatments (Table 5). The change in nitrate conditions under light conditions did not show a enough strongly linear trends to determine a flux. The mean concentration of nitrate measured in the cores after the addition of nutrient was only slightly higher than the pre-nutrient addition measurements (Table 6).

The change in ammonium concentrations was not sufficiently linear to quantify a flux during the light-dark incubations only during the post nutrient addition incubation of a sediment core from the West reference pond. This core had the least BA of any collected. The flux of ammonium into the water column for this single core incubation

was $0.66 \text{ mmol NH}_4^+ \text{ m}^{-2} \text{ h}^{-1}$ during the dark incubation and $-1.1 \text{ mmol NH}_4^+ \text{ m}^{-2} \text{ h}^{-1}$ during the light incubation. The ratio of carbon mineralized to ammonium and nitrate mineralized (\sim DIN) was 9.9. The mean ammonium concentrations measured in all of the cores increased following the addition of nutrient, though the difference was only significant in the fertilized cores (ttest, 2 tailed, type 3, $p < 0.05$; Table 6).

There was a trend towards higher GPP in the sediment cores collected from fertilized pond sediments before the addition of nutrients (ttest, 2 tailed, type 1, $p = 0.08$; Table). Production per μg chlorophyll *a* was significantly higher in the sediment cores collected from reference ponds (ttest, 2 tailed, type 3, $p < 0.05$). GPP did not significantly increase in fertilized or reference sediment after the addition of nitrate and phosphate. Total respiration was higher from the sediment in fertilized ponds under both treatments, although there was high variability and no significant difference (2 tailed ttest, type 3, $p < 0.05$).

There was no significant difference in bacterial production between cores from fertilized and reference ponds and no significant changes after nutrient addition (2 tailed ttests, type 3, $p < 0.05$). Bacterial production was typically about 10% of gross primary production, although there was a large decrease in bacterial production in the fertilized cores after nitrate addition and in this case bacterial production was $\sim 3.5\%$ of GPP.

DISCUSSION

Question 1: How are nutrients processed in salt marsh ponds?

The capacity of salt marsh ponds to process nitrate appears roughly similar to other marsh habitats, although we cannot determine exact rates. During our field measurements nitrate concentrations in the ponds were reduced to ambient within ~ 48 hours of flooding from nutrient enriched waters. Although $140 \mu\text{M}$ nitrate was added to the flooding tidal creek we do not know the exact concentration of nitrate added to each pond. To determine this value we would need to know the amount of turnover of pond water that occurred during flooding as well as the exact amount of nitrate dilution that occurred due to distance from the point of addition.

The overlying water in the sediment cores was $\sim 25 \text{ cm}$, the same mean depth as many ponds. Fluxes observed in lab sediment core incubation should therefore be

roughly comparable to *in situ* fluxes. One difference is that the in lab sediment core incubation only account for nutrient processing in the sediments, as the overlying pond water was replace with filtered seawater.

During the sediment core incubations added concentrations of 70 uM nitrate were reduced to an average of 1.7 uM within 25 hours. Assuming nitrate uptake is linear this would require that nitrate was nitrate lost from the water column at a rate of $\sim 0.7 \text{ mmol NO}_3^- \text{ m}^{-2} \text{ h}^{-1}$. The actual rate of loss was probably much faster considering the consequences of Michealis-Menton uptakes; the actual nitrate fluxes we measured in the dark incubation after fertilization were much lower than $0.7 \text{ mmol NO}_3^- \text{ m}^{-2} \text{ h}^{-1}$, indicating the sediment were no longer saturated with nitrate. A previous TIDE project 15N tracer study found a gross nitrate-processing rate of 1.9-2.5 $\text{mmol NO}_3^- \text{ m}^{-2} \text{ h}^{-1}$ over an entire marsh fertilized with 84-96uM of nitrate (Drake et al 2009).

We observed no differences in the nitrate processing ability of fertilized and reference ponds. However, because nitrate concentrations in the overlying water were no longer saturating during our second round of flux measurements, more precise differences in processing rates are not known.

The effect of acute nitrate and phosphate additions on ammonium concentrations was different in the field an lab experiments. Ammonium concentrations measured in the field were typically below 4 uM in both fertilized and reference ponds with no change observed in the fertilized ponds after flooding. In contrasts, the mean ammonium concentrations measured in the light in fertilized cores increased by an order of magnitude after the addition of nutrients.

Question 2: How are primary producers stimulated relative to heterotrophic microbes?

There appears to some increased algal production in the fertilized ponds. Although few significant differences were found, most direct and indirect measurements of algae production and standing stock biomass were higher in the fertilized ponds. The clearest difference is the high concentrations of water column algae seen in the fertilized and reference ponds. Although some of the water column algal content may be from suspended BA, the delayed increase we observed after spring tides is consistent with a typical phytoplankton turnover time of 2-3 times days (Valiella 1995). The increase in

water column algal content is a useful indication of nutrient enrichment. However, given the low concentrations measured and the shallow depth of the ponds, the total biomass of water column algal content is negligible ($<10\text{mg/m}^2$) relative to total BA biomass. Water column algae is therefore unlikely to play an important role in nutrient processing.

Colonizing BMA numerical abundance was also consistently higher in the fertilized ponds than reference ponds. An overall increase in the number of colonizing BMA in fertilized ponds after flooding by spring tides did not occur. Examining each fertilized pond individually, it is apparent that BMA numerical abundance did increase in 2 of 4 ponds. In the other 2 fertilized ponds BMA numerical abundance decreased after flooding.

We did not find any significant difference in the measurement of BA standing stock or the percent cover of floating algae mats in fertilized ponds, although the average standing stock measured in fertilized ponds was much higher during the fall. BA biomass in the ponds was comparable to measurement made in the low marsh *Spartina alterniflora* (Deegan et al 2007). It is surprising how similar that standing stock was in the summer considering the consistently higher numerical abundance of colonizing BMA in fertilized ponds. However, the relationship between colonizing BMA numerical abundance and benthic micro-algae biomass and/or production is unclear.

The percent cover of floating algae mats was highly variable. However we measured no percent algae mat cover in reference ponds with floating algae mats that was greater than 4% cover where as some fertilized were 85% covered in algae mats. Algae mats are mostly found on the bottom of the ponds. However, when they become highly productive, gas becomes trapped in the mats and the float to the surface. It is possible, therefore, that the area of floating algae is an index of BA production.

Direct measurements of GPP made during sediment core incubations ranged from $8.2\text{-}10.7\text{ mmol C m}^{-2}\text{ d}^{-1}$ with a trend towards increased production in the fertilized ponds before the addition of nutrients. These are very high rates of production compared to measurements of micro-algae production made in other marsh habitats (Raalte et al 1976; Macintyre et al 1996; Sullivan and Currin 2002).

A suite of factors may explain the lack of a robust response of BA in ponds to fertilization. One possibility is that mineralization within the ponds may be the dominant

nutrient source driving benthic production rather than external input from flooding tides. This would require large amounts of allochthonous inputs into the ponds. One of the few other studies of BA in salt marsh ponds found a negative correlation between inundation time and BA biomass (Miller et al 2009).

Another explanation is that BMA is light limited rather than nutrient limited. Self-shading is an important control on algae production in other nutrient enriched habitats (Hillebrand 2005). Supporting this possibility, we observed during sediment core incubations that chlorophyll specific production ($\mu\text{mol C h}^{-1} \mu\text{g chl-a}^{-1}$) was much higher in the reference ponds. Additionally, an expansion of floating algae mats following flooding by spring tides could have also resulted in increased shading of the benthos (Sundback and McGlathery 2004). This may explain a substantial decline in BMA abundance observed in one of the fertilized ponds that was 85% covered in floating algae mats. Competition for light may also explain why *R. maritima* percent cover was no different in fertilized or reference ponds. *R. maritima* in fertilized ponds tended to have higher amounts of algal epiphytes (personal observation).

Finally, it is possible that BMA abundance and biomass is limited by top-down grazing control, shown to be substantial in other salt marsh habitats (Valiella 1995). A previous plot level fertilization experiment illustrated the importance of top-down control in limiting benthic micro-algae growth observing a difference in benthic algae biomass between fertilized and reference plots only during the fall when predation pressure was lower (Valiella 1995). Although the differences were not significant, we also observed higher benthic algae biomass in fertilized ponds during the fall only. An experiment using in situ salt marsh pond mesocosms found macro-fauna were important grazers in these systems (Stocks and Grassle 2001).

Although we do not have data on invertebrate abundance in the ponds, we did observe what appeared to be predator-prey relationships between BMA and amoebas. Little is known about amoeba feeding habits. Most studies have focused on bacterivorous amoebas, potentially an important part of the carbon cycle in the sea (Butler and Rogerson 1996; Decamp et al 1999; Butler and Rogerson 2007). However, algivorous amoebas are known to exist and may be an important part of estuarine carbon cycles (Rogerson 1991). Amoebas are typically sensitive to cold temperatures (Decamp

et al 1999); although amoebae abundance was not measured in the winter, a decline in amoeba grazing pressure could explain the increase in BA in the winter. We do not know if amoebas are truly abundant throughout the pond because they were only measured with artificial substrate. Other protists may have also colonized the slide but were not preserved well.

Amoebas may have contributed to a substantial amount of re-mineralization in the ponds, at least during the summer. Bacteria certainly also contribute to mineralization given bacterial production in the top 1 cm of the core was typically 10% of GPP. Some claim however that bacteria are relatively poor re-mineralizers compared eukaryotes due to their low C:N ratios (Valiella 1995). Measurements in the sediment cores showed that bacterial production in the top

We hypothesized that nutrient enrichment would stimulate bacterial production as they would be able to immobilize free nitrogen in order to decompose more refractory material. However, we found not evidence for this. Bacterial production was not high in the sediment cores collected from fertilized ponds, nor did bacterial production increase after the addition of nutrients. Previous work on the TIDE project that measured bacterial production in the high, low marsh and mudflat habitat also found no direct stimulation of bacterial production from fertilization (Bowen et al 2009). The conclusions reached in this study were that fertilization stimulated bacterial production only indirectly by increased BMA production. This indicates immobilization of water column nitrate by bacteria does not substantial contribute to the negative flux of nitrate out of the water column after nutrient addition.

The bacterial production rates we measured are ~4 time higher than the highest rates measured by Bowen (2009) in other micro-habitats on the same marshes. These higher rates might be attributed to high benthic micro-algae production in the ponds, supporting the conclusion of only indirect stimulation of bacterial production in the fertilized marshes dues to increased BMA production. An addition explanation is that the relatively high temperature of incubation resulted in higher bacterial production. Leucing incorporation rates by bacteria in marsh sediments are highly sensitive to incubation temperature (Bowen, personal communication).

Although bacterial immobilization of nitrate does not appear important to gross nitrate processing, the contribution of bacteria to nitrate procession through denitrification and DNRA probably is important. One study has shown that in salt marsh creeks benthic micro-algae initially outcompete denitrifiers for water column nitrate, although half of this nitrogen is eventually denitrified through mineralization of the algae and couple nitrification-denitrification (Hamersley and Howes 2003). However, this may only be the case when nitrate concentrations in the water column are relatively low. A previous study on the TIDE project found that fertilization increased denitrification of water column nitrate by approximately 1 order of magnitude, and coupled-nitrification denitrification by three fold in the tidal creek sediment; DNRA, measured in the surface sediment only, was of comparable magnitude to denitrification (Koop-Jakobsen and Giblin).

We did not measure denitrification or DNRA directly. However, by estimating algal nitrogen uptake, and using typical marsh nitrification rates we can perform a rough mass balance to infer the relative importance of denitrification and DNRA. Assuming net primary production (NPP) is 80% of GPP (Valiella 1995) and that the microalgae have a C:N ratio of 9.2 (a typical value to use: e.g. Sundback 2000), the potential nitrogen uptake by algae in the sediment cores after the addition of nutrients (based on a GPP of 10.5, the mean of the fertilized and reference cores after nutrient addition) should be $\sim 13.7 \text{ mmol NO}_3^- \text{ m}^{-2} \text{ d}^{-1}$. A previous TIDE project ^{15}N tracer study found nitrification rates were $\sim 12 \text{ mmol NO}_3^- \text{ m}^{-2} \text{ d}^{-1}$ (Drake et al 2009). We showed previously that to reach the nitrate concentration observed in the measured sediment cores 25 hours of after addition of 70uM nutrient would require a negative flux of nitrate in the water column of at least $16.8 \text{ mmol NO}_3^- \text{ m}^{-2} \text{ d}^{-1}$. Therefore, nitrification rates in the ponds are at all comparable to the rates observed of over the entire marsh, an algal uptake of nearly $30 \text{ mmol NO}_3^- \text{ m}^{-2} \text{ d}^{-1}$ would be require to account for the entire negative flux of nitrate out of the water colum, twice as high as the predicted demand. Further, this ignores BMA uptake of ammonium. Assuming that algal respiration is 20 % of GPP (Valiella 1995) and that the ratio of C:N mineralized is 6.625 (the redfield ratio) we estimate the potential mineratlization in the sediment cores collected from fertilized ponds is 12.7 mmol NH_4^+

$\text{m}^{-2} \text{d}^{-1}$ after the addition of nutrients. This value is similar to ammonium flux observed in the single analyzable core from a reference pond.

Based on these speculative calculations it appears that, despite the high GPP of BA in salt marsh ponds it, at most, accounts for half of the gross nitrogen processing. Light limitation and/or top down control by grazers may limit GPP and hence the ability of algae to out compete bacteria performing denitrification or DNRA for water column nitrate. Additionally, due to high water saturation of salt marsh pond sediment, the rate of nitrate diffusion into the anoxic portions of the sediment where denitrification occurs may be faster than in other habitats reducing the ability of BMA to compete with bacteria for water column nutrients.

CONCLUSIONS

Our study illustrates that the response of salt marsh ponds to nutrient enrichment is unique from other marsh habitats. Although nitrate-processing rates are grossly similar more precise measurements of nitrate processing need to be made to allow for better predictions of how the ability of salt marshes to take up nutrients will change as the abundance of ponds increases.

ACKNOWLEDGMENTS

The author would like to thank Linda A. Deegan, Anne Giblin, Sallie Sheldon, David Johnson, Kate Morkeski, Will Daniels, Jennifer Bowen, Joe Vallino, and Sam Safran for help with experimental design, data collection, and analysis.

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FIGURES AND TABLES

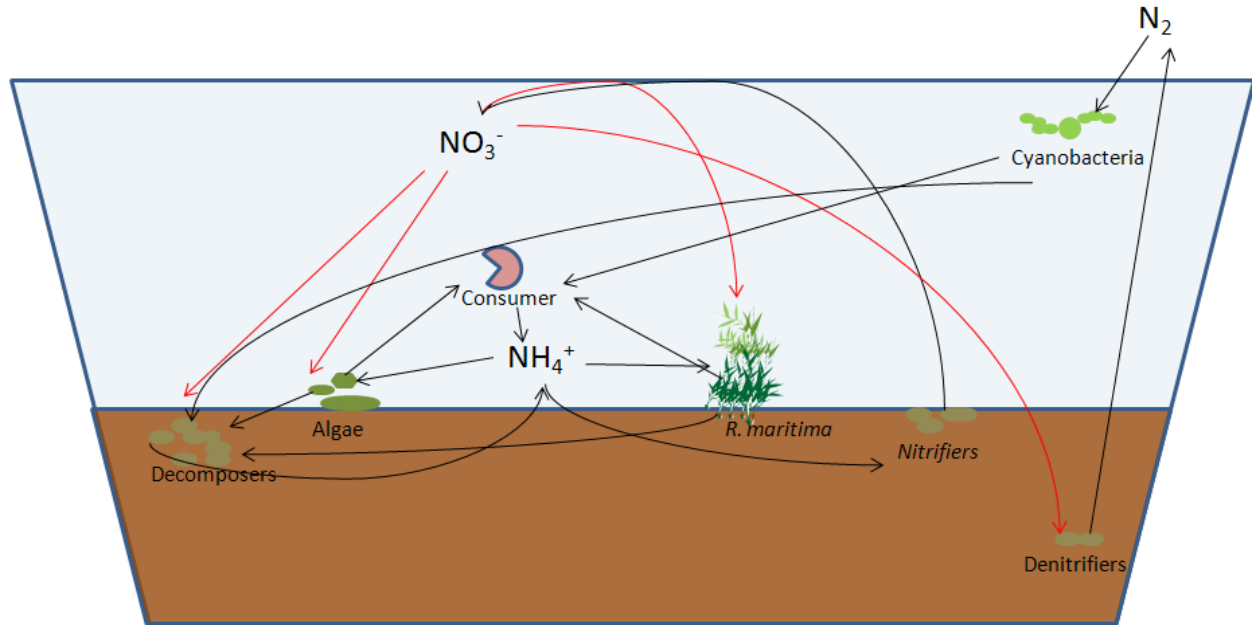


Figure 1. A simplified hypothetical schematic of nitrogen cycling in salt marsh pools. Although we now all of these flow paths should exist in pools, the relative importance of each has not been studied. Red arrows indicate the paths most affected by increased nitrate, the most prevalent form of anthropogenic nutrient pollution in coastal waters.

Table 1. Basic geomorphological characteristics of the ponds studied. Depth values are means.

	Pond	Area (m ²)	Water Column Depth (cm)	Soft Sediment Depth (cm)
Ref	NE	340	33	28
	SH	1,300	25	28
	WE	6100	31	21
Fert	SW.c	1,200	51	48
	SW.f	700	33	26
	CL.l	300	51	43
	CL.s	1,300	52	53

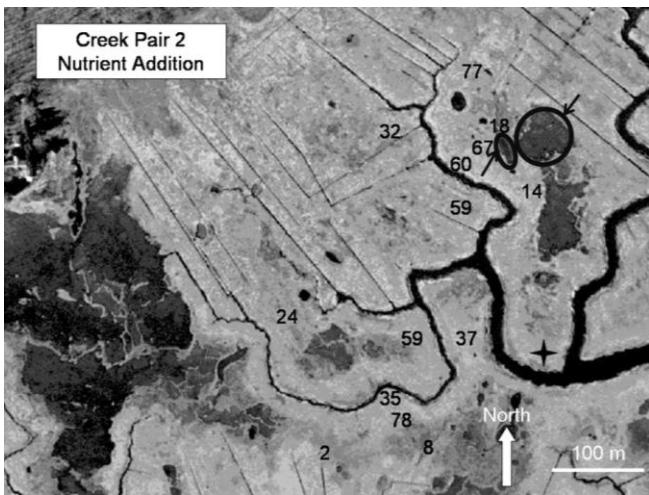
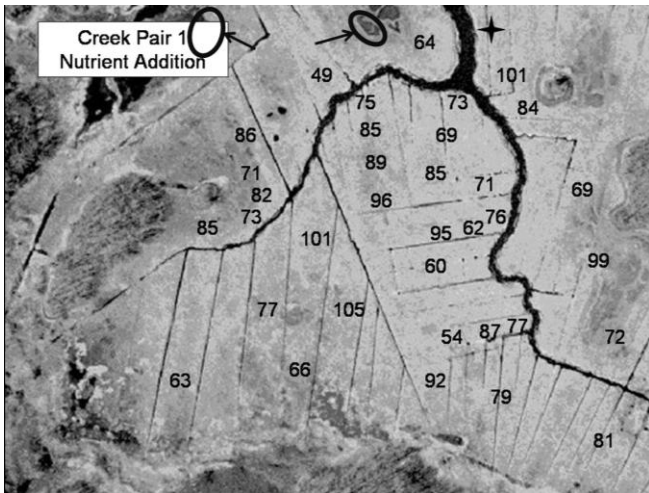


Figure 2. The location of the fertilized pond (black circle and arrow) in relation to the spatial gradient of fertilizer dilution measure at high tide on July 2005 (Adapted from Deegan et al 2007).



Plate 1. Collection of sediment cores in West pond during the fall (Left: author, center: David Dodge, Right: Anne Giblin). Photo credit: A. Ritter.

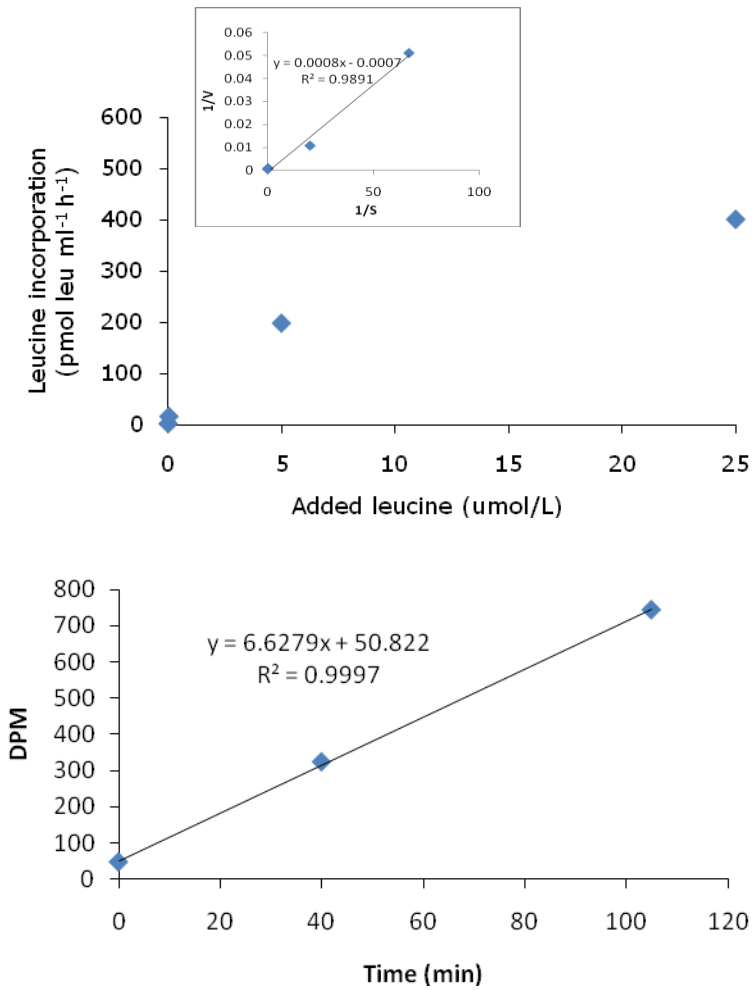


Figure 3. Optimization parameters for bacterial production assay. Top panel: substrate saturation curve indicating the increase in bacterial production with increasing leucine addition. Inset: Lineweaver-Burk plot in which the inverse of uptake (V) is plotted against the inverse of substrate concentration (S). The y-intercept of the figure is equivalent to the maximum uptake velocity for the curve depicted below. Bottom panel: time course of the increase in destructions per minute (DPM) indicating linearity of uptake for up to 105 min incubations.

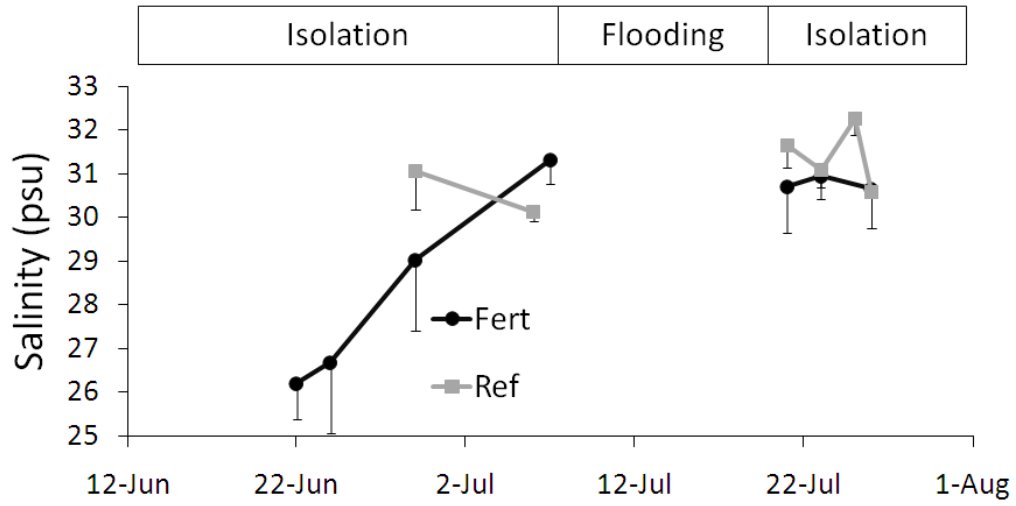
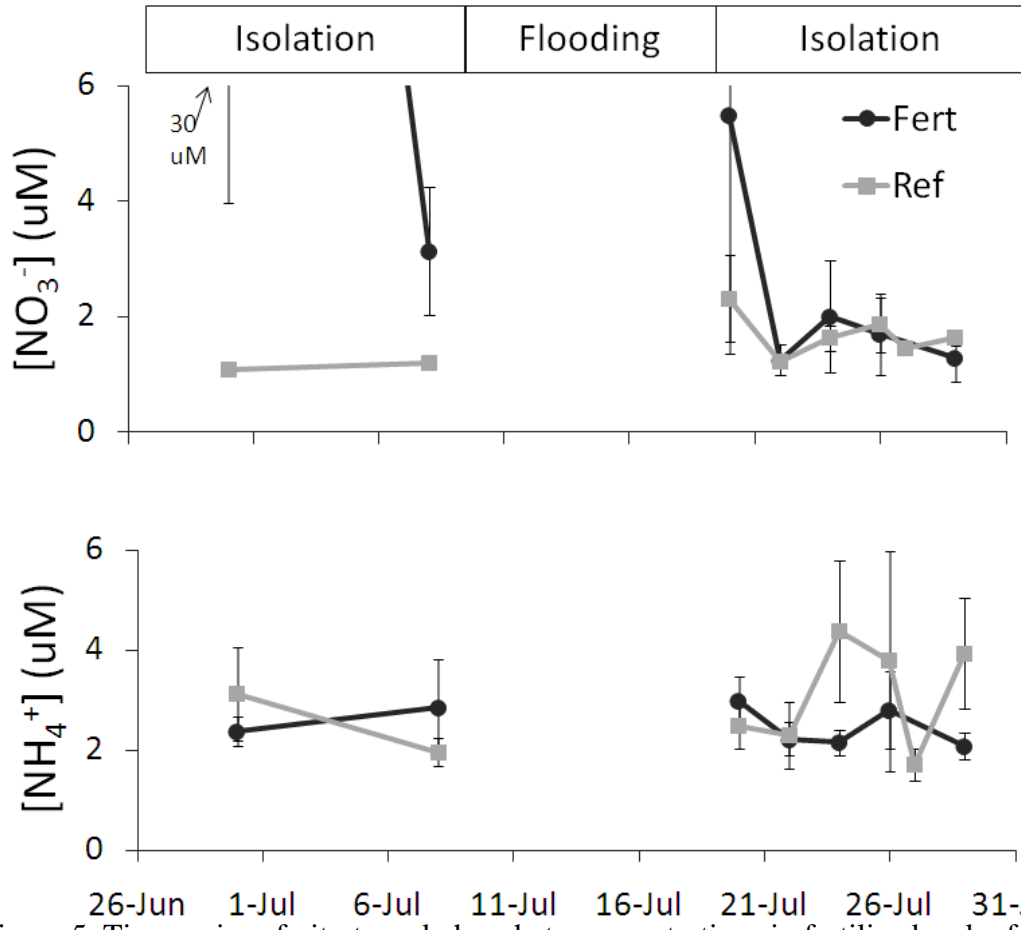


Figure 4. Time series of salinity measurements made in fertilized and reference ponds during the summer of 2010 before and after flooding of the ponds during spring tides.



26-Jun 1-Jul 6-Jul 11-Jul 16-Jul 21-Jul 26-Jul 31-Jul
 Figure 5. Time series of nitrate and phosphate concentrations in fertilized and reference ponds during the summer of 2010 before and after flooding of the ponds during spring tides.

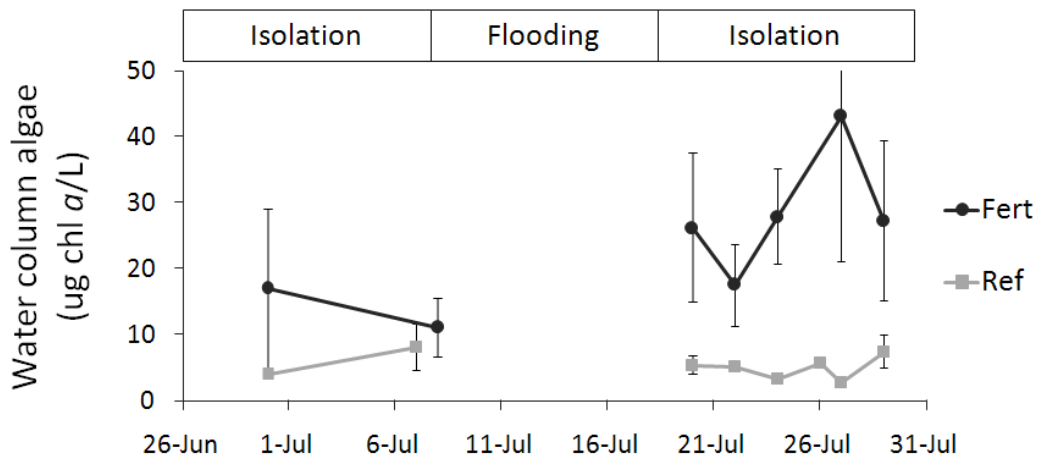


Figure 6. Time series of water column algae concentrations in fertilized and reference ponds during the summer of 2010 before and after flooding of the ponds during spring tides.

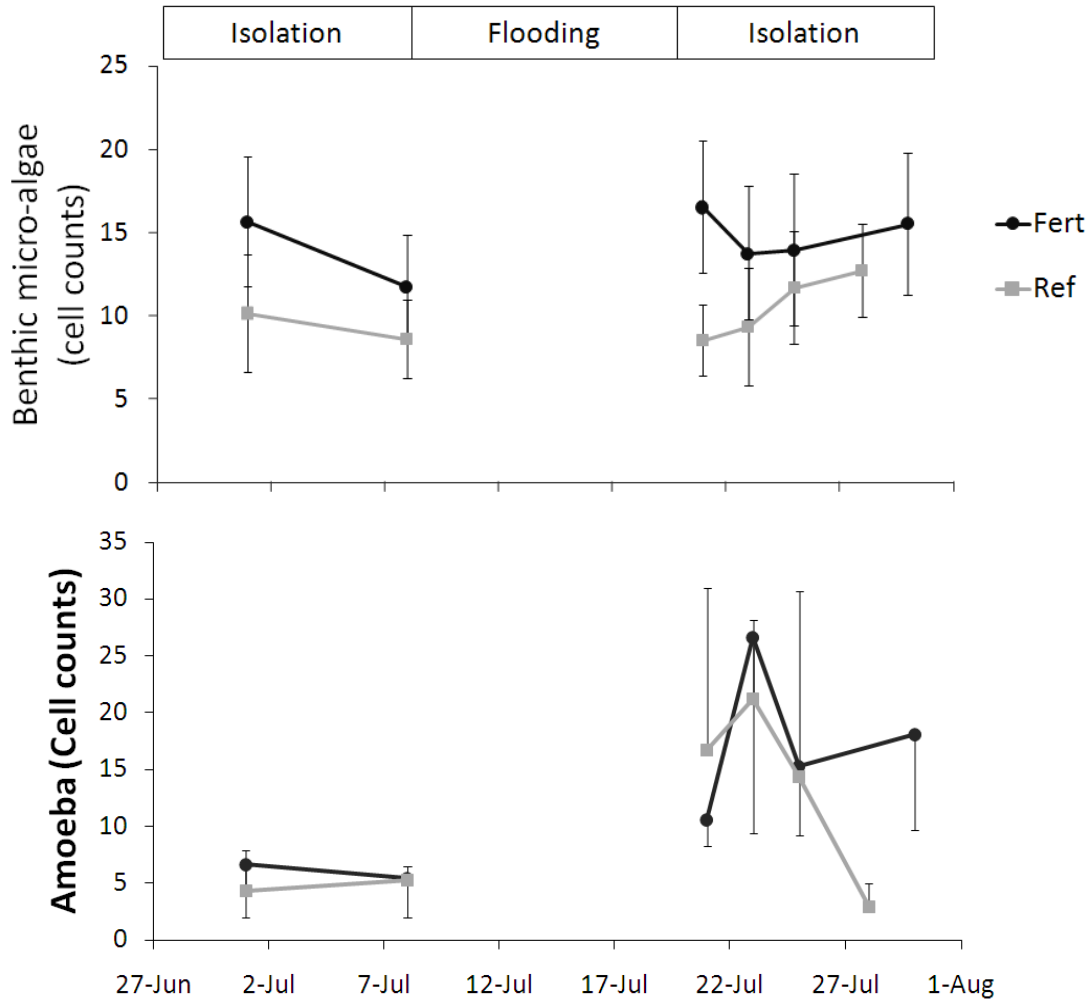


Figure 8. Time series of benthic micro-algae (top panel) and amoeba density (bottom panel) on colonized glass slide in fertilized and reference ponds during the summer of 2010 before and after flooding of the ponds during spring tides. Densities represent the number of cell per FOV on 40x magnification (exact scale not determined).



Figure 9. Amoebas and diatoms that colonized a glass slide (scale not determined).

Table 2. Percent cover of ponds with floating algae mats and benthic algae biomass in salt marsh ponds (---: no samples taken, bold values are means \pm SE). Floating algae mats were measured in late-july 2010. Benthic algae biomass was measured in late-July and mid-november.

		Floating algae cover (%)	Benthic algal biomass (mg chl <i>a</i> m ⁻²)	
			<u>Jul</u>	<u>Nov</u>
Ref	SH	3.5	151	115
	WE	2.3	132	56
	NE	1.2	124	---
	Mean	2.3 \pm 0.7	136 \pm 8	90 \pm 30
Fert	CL.s	6.1	135	216
	CL.l	34.3	51	---
	SW.c	88.7	152	202
	SW.f	1.1	190	---
	Mean	32.6 \pm 20	132 \pm 29.5	209 \pm 7.5

Table 3. Percent cover of ponds by *R. maritima*, measured in late-july 2010 (bold values are means \pm SE).

		<i>R. maritima</i> cover
	Pond	(%)
Ref	NE.p	69
	SH.p	40 %
	WE.p	85
	Mean	64 \pm 9.4
Fert	SW.f	15
	sw.c	100
	CL.s	44
	CL.l	83
	Mean	60 \pm 19.0

Table 4. Measurements made during incubations of sediments in cores collected from fertilized and reference salt marsh ponds in mid-November 2010 (n=2, means \pm SE. ----: no measurement made). Post-nutrient measurements commenced 25 hours increasing nitrate concentration by 70 μ M and phosphate concentrations by 4 μ M.

Parameter	Pre-nutrient addition		Post-nutrient addition	
	<u>Fert</u>	<u>Ref</u>	<u>Fert</u>	<u>Ref</u>
Chlorophyll <i>a</i> (mg/m ²)	----	----	486 \pm 113	250 \pm 59
GPP (mmol C m ⁻² h ⁻¹)	10.0 \pm 0.8	8.2 \pm 1.0	10.7 \pm 2.3	10.3 \pm 0.09
GPP/chl <i>a</i> (mmol C/mg chl <i>a</i>)	0.02 \pm .0035	0.037 \pm 0.0039	0.025 \pm 0.012	0.049 \pm 0.13
Respiration (mmol C m ⁻² h ⁻¹)	-7.3 \pm 1.9	-5.5 \pm 1.2	-6.1 \pm 0.5	-5.1 \pm 1.3
Bacterial Production (mmol C m ⁻² h ⁻¹)	1.0 \pm 0.6	1.2 \pm 0.4	0.37 \pm 0.06	1.3 \pm 0.5

Table 5. Nitrate flux during dark incubations of sediment cores (means \pm SE, N/A is written in place of a SE when fluxes could only be obtained for of a single pond) collected from fertilized and reference ponds. Negative values indicate a loss of nitrate from the overlying water column. Post nutrient addition fluxes began 25 hours nitrate concentrations were increased by 70 μ M and phosphate concentrations were increased by 4 μ M.

	Pre-nutrient addition		Post-nutrient addition	
	<u>Fert</u>	<u>Ref</u>	<u>Fert</u>	<u>Ref</u>
Nitrate flux (mmol NO ₃ ⁻ m ⁻² h ⁻¹)	-0.15 \pm N/A	-0.24 \pm 0.09	-0.40 \pm N/A	-0.16 \pm 0.01

Table 6. Concentrations of nitrate and ammonium during dark incubations water overlying sediment cores (n=2, means \pm SE). Post nutrient addition fluxes began 25 hours nitrate concentrations were increased by 70 μ M and phosphate concentrations were increased by 4 μ M.

	Pre-nutrient addition		Post-nutrient addition	
	<u>Fert</u>	<u>Ref</u>	<u>Fert</u>	<u>Ref</u>
Nitrate (mmol NO ₃ ⁻ m ⁻²)	1.4 \pm 0.07	1.2 \pm 0.1	2.7 \pm 1.6	2.1 \pm 0.7
Ammonium (mmol NH ₄ ⁺ m ⁻²)	3.4 \pm 0.3	2.7 \pm 0.4	13.6 \pm 4.5	11.7 \pm 5.8

