

Nonlinear responses in salt marsh functioning to increased nitrogen addition

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Abstract. Salt marshes provide storm protection to shorelines, sequester carbon (C), and mitigate coastal eutrophication. These valuable coastal ecosystems are confronted with increasing nitrogen (N) inputs from anthropogenic sources, such as agricultural runoff, wastewater, and atmospheric deposition. To inform predictions of salt marsh functioning and sustainability in the future, we characterized the response of a variety of plant, microbial, and sediment responses to a seven-level gradient of N addition in three Californian salt marshes after 7 and 14 months of N addition. The marshes showed variable responses to the experimental N gradient that can be grouped as neutral (root biomass, sediment respiration, potential carbon mineralization, and potential net nitrification), linear (increasing methane flux, decreasing potential net N mineralization, and increasing sediment inorganic N), and nonlinear (saturating aboveground plant biomass and leaf N content, and exponentially increasing sediment inorganic and organic N). The three salt marshes showed quantitative differences in most ecosystem properties and processes rates; however, the form of the response curves to N addition were generally consistent across the three marshes, indicating that the responses observed may be applicable to other marshes in the region. Only for sediment properties (inorganic and organic N pool) did the shape of the response differ significantly between marshes. Overall, the study suggests salt marshes are limited in their ability to sequester C and N with future increases in N, even without further losses in marsh area.

Key words: *Carpinteria Salt Marsh; coastal eutrophication; ecological thresholds; ecosystem services; methane flux; Morro Bay Estuary; pickleweed; Salicornia virginica; tidal wetlands; Tijuana River Estuary.*

INTRODUCTION

Salt marshes are tidal wetlands positioned at the interface between the land and the sea, where they provide important ecological services, such as storm protection for coastal cities and habitat for numerous species of fish, birds, and invertebrates (Zedler and Kercher 2005, Gedan et al. 2009). They are also valued as major carbon-storing ecosystems due to their high primary production, low decomposition, high sedimentation, burial through sea-level rise, and negligible rates of CH₄ release (Bartlett et al. 1987, Bridgham et al. 2006). Further, marshes are often cited for their ability to act as a pollution buffer, removing land-derived nutrients through aboveground plant production or denitrification, and thereby protecting coastal waters from eutrophication (Valiela and Cole 2002).

At the same time, recent evidence suggests that increased nutrient levels threaten coastal marshes

themselves (Howarth 2008). Nitrogen (N) enrichment to the coastal zone has increased at least tenfold over preindustrial levels and is projected to increase further over the next few decades (Galloway et al. 2008, Howarth 2008). Increased nutrients can decrease geomorphic stability leading to conversion of creek edge and bay edge into mud flats (Deegan et al. 2012). In addition, nutrient-driven shifts may favor lower-biomass plant species and accelerate elevation loss through reduced sediment deposition (Langley and Megonigal 2010). Such physical losses would exacerbate previous declines; up to 50% of original salt marshes have already been lost, mainly due to conversion to agricultural lands and urban development (Zedler and Kercher 2005, Pendleton et al. 2012).

In contrast to this focus on the loss of marsh area, here, we investigate how continued nutrient inputs will alter the functioning of remaining marshes. Specifically, we applied a seven-level gradient of N addition in three southern California salt marshes and then measured a variety of microbial processes and plant and sediment characteristics. This gradient design allowed us to

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quantify the shape of the marshes' response to increasing N, rather than to one particular N dose. In this way, we aimed to assay the capacity of marshes to continue to provide two important ecosystem services, nitrogen removal and carbon storage, under a range of future N-input scenarios.

Broadly speaking, N addition increases plant growth, plant tissue N content, and aboveground plant biomass (Xia and Wan 2008). These responses are not always linear with increased inputs, however. For instance, aboveground plant biomass often saturates at high N inputs in both terrestrial (Lu et al. 2011) and salt marsh ecosystems (Darby and Turner 2008). N addition also alters microorganisms and their activities, often in a predictable direction. Meta-analyses compiling a variety of studies across terrestrial ecosystems find that microbial processes, like N₂O emission, nitrification, and CH₄ emissions, tend to increase with N addition (Liu and Greaver 2009, Aronson and Helliher 2010, Lu et al. 2011), while microbial respiration decreases (Liu and Greaver 2009, Janssens et al. 2010). However, these broad scale relationships do not address the ability of an ecosystem, such as a particular salt marsh, to buffer future nitrogen influxes and maintain current levels of ecosystem services. The response of a particular ecosystem will depend largely on whether nutrient pools and processes increase linearly or saturate as N increases.

We conducted the N gradient experiment in three marshes, Morro Bay National Estuary (MBE), Carpinteria Salt Marsh Reserve (CSM), and Tijuana River Reserve Estuary (TRE), selected for their protected status and similarities in vegetation. Like others around the world, Californian marshes are in decline, having already lost 90% of their original area (Gedan et al. 2009). They are subject to nitrogen enrichment from industrial and municipal effluents, runoff from agricultural and residential use, and atmospheric deposition, with estimates ranging from 39 to 78% of estuaries showing signs of eutrophication (McLaughlin et al. 2014). Unlike those in the Gulf of Mexico or the North American Atlantic coast, Californian marshes are usually hypersaline and dominated by salt-tolerant succulent plant species that withstand seasonal drought during the summer and autumn (Zedler 1982).

To quantify the capacity of salt marshes to buffer additional nutrient inputs, we tested three hypotheses. First, we hypothesized that plant and microbial processes and properties would change nonlinearly with increasing N. For instance, we predicted that, as previously observed, aboveground plant biomass would level off at high N levels as other resources start to constrain plant growth (Darby and Turner 2008, Lambers et al. 2008). Similarly, we expected that microbial processes that depend on both N and plant carbon substrates, such as CO₂ production, CH₄ flux and net N mineralization (Chapin et al. 2002), would track the plant response; these processes might initially

be limited by N, but constrained by carbon substrates at high N levels when plant growth levels off. In addition, some microbial processes might respond nonlinearly because they integrate several underlying processes at once. N addition might impact CH₄ flux through synergistic effects, for example, by inhibiting methanotrophy (Schimel and Gulledge 1998) and reducing C limitation of methanogenesis (through plant exudates).

Second, we hypothesized that sediment inorganic N and organic (C and N) pools would differ in whether they responded to N addition. Because inorganic N in sediments follows immediate plant and microbial demands for N (Chapin et al. 2002), we predicted that it would show nonlinear responses similar to those of marsh plants and microorganisms. In contrast, we hypothesized that the organic C and N pools as well as sediment physical properties (e.g., bulk density, and moisture) would not be affected by N addition over the time frame of the experiment. The organic pools should take more time to change, because they are incorporated over a whole cycle of plant growth, senescence, and decay. In addition, such pools in sediments are generally large, making it difficult to detect small fluxes relative to the total pool size. It is further unlikely to detect changes in physical properties that directly depend on sediment organic content (Neubauer 2008).

Finally, because of intrinsic differences in the sites, we expected that the three marshes would vary in the degree to which they responded to N addition; that is, we expected an interactive effect of marsh and nitrogen level on the measured variables. For instance, the response of soil carbon respiration to N addition in forest ecosystems appears to depend on ambient rates of N deposition, as well as on their levels of primary production (Janssens et al. 2010). Specifically, we hypothesized that the plants and microorganisms in marshes with lower background N availability would be more N limited and, therefore, respond more strongly (i.e., exhibit a steeper slope of the response curve) to increasing levels of N addition.

METHODS

Study sites

We conducted the study in three salt marshes along the Californian coast in North America, Morro Bay National Estuary (MBE), Carpinteria Salt Marsh Reserve (CSM), and Tijuana River Reserve Estuary (TRE), selected for their protected status and similarities in vegetation. The marshes encompass intertidal estuarine wetlands with adjacent palustrine wetlands and deep channel sub-tidal deep-water habitat (natural and artificial channels). MBE (35°20'00" N, 120°49'51" W) is the northernmost marsh, with a mean annual precipitation of 463 mm and temperature of 15°C. This 930 ha estuary is at the terminus of a 19424 ha watershed that drains the Los Padres National Forest, agricultural fields, ranches, and parks. CSM is located 180 km south of MBE in Carpinteria, California

(34°24'03" N, 119°32'09" W) with a mean annual precipitation of 438 mm and a mean annual temperature of 15°C. At approximately 93 ha, CSM is the smallest salt marsh of our three study sites. The Carpinteria Creek watershed (3884 ha) includes open field agriculture, commercial nurseries, orchards, and densely populated residential areas and businesses that drain into CSM. TRE (32°32'59" N, 117°07'22" W) is the largest (1024 ha) and southernmost marsh site located just north of the Mexico–United States border in Imperial Beach, California (San Diego County). TRE has the lowest mean annual precipitation (274 mm) and the highest mean annual temperature (18°C) of the three marshes. The estuary is at the terminus of a 453 248 ha watershed that drains the Tijuana River Valley, three quarters of which is located in Mexico, and includes dense urban areas (the city of Tijuana and parts of San Diego County). Plant species composition within the experimental area of the three marshes was dominated by pickleweed (*Salicornia virginica*). Other plant species such as *Frankenia salina*, *Limonium californicum*, *Distichlis spicata* var. *spicata*, *Suaeda* spp., and *Cuscuta salina* were rare in the plots, accounting, on average, for less than 2% cover.

Experimental design

We established a nitrogen fertilization experiment with seven levels of N addition (0, 10, 20, 40, 80, 160, and 320 g N·m⁻²·yr⁻¹) in three study sites. These values span the range of natural and experimental values. The natural background flux of N off landscapes in regions with little human influence has been estimated at approximately 0.01 g N·m⁻²·yr⁻¹ (Howarth 2008), whereas this value can be as high as 100 and 900 g N·m⁻²·yr⁻¹ in natural and constructed treatment wetlands, respectively (Valiela and Cole 2002, Verhoeven et al. 2006). Fertilization rates used in wetland studies have ranged from 30 g N·m⁻²·yr⁻¹ (Bowen et al. 2009) to 400 g N·m⁻²·yr⁻¹ (Morris and Bradley 1999).

Plots were located in areas with vegetation entirely dominated by *Salicornia virginica* (pickleweed) at similar positions in the landscape with respect to the main channel, the ocean, and the tidal flooding regime (plots flooded during a >+1.6 m tide). Treatments were randomly assigned to 1-m² plots that were spaced 2 m apart (see Plate 1). Each fertilization treatment was replicated five times, totaling 105 plots (3 marshes × 7 treatment levels × 5 replicates/treatment). Nitrogen was delivered as slow-release urea in perforated (8 cuts, 4 cm long × 4 mm wide) 50-mL centrifuge tubes that were buried so that only the caps were exposed above the sediment. Nine tubes were placed in each plot in an evenly spaced grid (33 cm apart). The fertilizer was packaged in nylon mesh bags within the tubes to further slow its release. These fertilizer bags were replaced in the tubes every 10 weeks during the course of the experiment. Urea-free tubes were placed in unfertilized plots as disturbance controls.

Urea at a 10-week fertilization interval was chosen to provide a relatively constant input of N to the plots (we also tested another fertilizer, NH₄Cl, and shorter time intervals between fertilizer applications). There were two reasons to maintain a constant N input. First, large pulsed inputs might have moved outside of the experimental plots; the urea treatments appeared to be well contained, as indicated by clearly defined changes in plant growth in the plots (Appendix A). Second, because each marsh has different nutrient sources and input timing, there was not an obvious, realistic regime to mimic. All of the marshes receive year-round atmospheric N deposition 3–5 kg N·ha⁻¹·yr⁻¹ (Tonnesen et al. 2007), but each receives other N inputs from a variety of sources over different times. For example, N fluxes to coastal waters near TRE are an order of magnitude larger than annual N fluxes of those near CSM, mainly because of higher natural marine N upwelling in spring and early-summer and daily treated wastewater discharge (Howard et al. 2014). In contrast, storm water and agricultural runoff can be dominant N sources at other times of year. Of course, each of these different sources provides different proportions of N forms (NO₃, NH₄, and organic N). Thus, while constant urea addition does not mimic realistic N inputs, it provided a well-contained, consistent comparison of N addition across the three marshes.

We started applying the fertilizer in July 2008 (start of the summer). The first sampling date was seven months later, in February 2009 (end of the winter). The total amount of N delivered to the plots at the seven-month sampling was 0, 6, 12, 24, 48, 96, and 192 g N·m⁻². We sampled a second time for certain variables (see below) at 14 months after the beginning of the experiment, in September 2009 (end of the summer). At this time, just after the start of the second year of fertilization, the plots had received the yearly amount of 0, 10, 20, 40, 80, 160, and 320 g N/m². At both dates, we sampled each marsh over the course of two days and completed all three marshes within one week, although we were not able to collect every measurement for both dates due to limited resources and personnel (see details below).

Plant characteristics

Aboveground plant dry biomass (g/m²) was assessed by harvesting a 12-cm² subplot within each plot in February and in September 2009. In February, we harvested all plant standing biomass that was present in the subplot (aboveground plant biomass). In September 2009, we harvested all plant biomass that regrew in the previously harvested subplot (aboveground plant regrowth). Total carbon and nitrogen content of green leaves (percentage C and N) was determined by dry combustion with a Flash EA1112 Series NC Analyzer (CE Instruments, Hindley Green, Wigan, UK) from plants harvested in February.

Belowground plant biomass was assessed using root ingrowth chambers. Chambers consisted of perforated

50-mL centrifuge tubes (same as described for fertilizer delivery) containing a swimsuit-material casing filled with ion exchange resin (20 g resin/casing) placed in the tube. One chamber was buried in each plot and left undisturbed for seven months (between the February and September samplings). We removed the chambers by cutting the roots around the tube and gently lifting them from the sediment. The chambers were transported to the lab on ice and frozen (-20°C) until processing. The roots were extracted from the chambers, washed, dried (60°C), sorted as fine (<1 mm) or coarse (>1 mm), and weighed.

Sediment properties

To assay sediment properties, we took sediment cores of 25.4 cm diameter to a depth of 10 cm from each plot in February. The sediments were kept on ice until they were processed in the lab the same day of sampling. Before processing, each sample was well mixed by hand in a plastic bag and roots were removed. Sediment gravimetric water content was assessed by drying a subsample at 65°C until constant weight. To measure inorganic N, we extracted a 25-g subsample using 2 mol/L KCl. We measured NH_4^+ concentrations using a modified Berthelot-salicylate method (Weatherburn 1967) and NO_3^- concentrations with the reduction of vanadium (III) in acid solution (Doane and Horwath 2003). Both methods are colorimetric assays that were evaluated with a Synergy 4 Hybrid Multi-Mode Microplate Reader (BioTek Instruments, Winooski, Vermont, USA). Total carbon and nitrogen (percentage C and N) content (mostly organic) were determined by dry combustion, as described for the plant samples; pH was measured in a 1:1 mixture with distilled water. Sediment bulk density was assessed from five sediment cores of 7 cm diameter to a depth of 10 cm adjacent to the plots in each marsh.

Microbial processes

We estimated potential net N mineralization by measuring the rate of change in inorganic N concentration in laboratory incubations. An initial sample was used to determine soil inorganic N content, and a second sample was incubated in a plastic cup covered with parafilm at 20°C for 28 days in the dark (Robertson et al. 1999a). Sediments were kept at field moisture over the incubation period with the addition of a 35‰ NaCl solution, the same salinity of the pore water in the field sites. Potential net N mineralization and potential net nitrification were estimated as the difference in soil ammonium concentration and soil nitrate between the initial and the incubated sample, respectively. To determine potential sediment carbon mineralization, we incubated a 10-g subsample at 20°C , in a 40-mL vial with gas-tight septum caps. CO_2 production was measured after a 24-h incubation period with an infrared gas analyzer (PP Systems EGM-4, Amesbury, Massachusetts, USA).

Sediment respiration (CO_2 production) and CH_4 fluxes were assessed using PVC static gas chambers (16 cm high with a surface area of 104 cm^2) that were gently secured 2.5 cm deep into the sediment of each plot at the time of sampling. We clipped the plant biomass just above the sediment surface of this space at the beginning of the experiment to limit our measurements to microbial and root emissions and let the system stabilize for seven months before sampling. We sampled gases (5 mL) from the capped headspace by syringe. Each plot was sampled at four time points after securing the chamber (0, 30, 60, and 90 min). We analyzed CO_2 and CH_4 concentrations with a Shimadzu GC2014 gas chromatograph (Shimadzu, Kyoto, Japan) equipped with a thermal conductivity detector for measuring CO_2 connected in series to a flame ionization detector to measure CH_4 , and fitted with a 2-mL sample loop. We calculated CO_2 and CH_4 flux rates for each plot as the slope of the linear regression of gas concentration over time. The detection limits were 10 ppm for CO_2 and 0.7 ppm for CH_4 .

Data analysis

We compared baseline ecosystem properties and processes (from non-fertilized control plots) among the three marshes with a one-way ANOVA and a principal components analysis in the first sampling date (February 2009). To evaluate the effects of N addition on ecosystem processes and properties at both sampling dates (February and September 2009), we used a generalized linear model (GLM) with salt marsh as a categorical factor (marsh MBE, CSM, and TRE), N addition treatment as a continuous factor (N as 0, 10, 20, 40, 80, 160, and $320\text{ g N}\cdot\text{m}^{-2}\cdot\text{yr}^{-1}$), and the interaction $\text{marsh} \times \text{N}$. All a posteriori comparisons were performed by a Tukey HSD test (JMP7, SAS Institute, Cary, North Carolina, USA).

To test the shape of the ecosystem responses to the N addition gradient, we fitted each response parameter to five functions, linear ($y = ax + b$), power ($y = ax^b$), exponential with a horizontal asymptote at zero ($y = ae^{bx}$), a three-parameter exponential with a horizontal asymptote at c ($y = ae^{bx} + c$), and Monod ($y = a/(1 + bx^{-1})$), where x is N concentration, and a , b , and c are constants. We chose these functions because they can describe a variety of curve shapes. We also included the Monod function, as it is thought to be a biologically realistic model for nutrient uptake by organisms (Grover 1990). Because some CH_4 flux values were negative, for this parameter only, we fit $y = ax^b - 0.2$ for the power function and $y = a/(1 + bx^{-1}) - 0.2$ for the Monod function, allowing for a negative y -intercept (Fig. C1d). The regressions were carried out using the `lm` and `nls` functions in R (R Development Team 2008).

When the $\text{marsh} \times \text{N}$ interaction from the GLM analysis was significant, we tested for the best fit of the data for each marsh separately (averaging the five replicates at each N level). When the interaction was

TABLE 1. Biogeochemical properties and processes of three salt marshes along the Pacific coast in California, USA: MBE, Morro Bay Estuary; CSM, Carpinteria Salt Marsh; and TRE, Tijuana River Estuary.

Biogeochemical properties and processes	MBE	CSM	TRE
Plant characteristics			
Plant biomass (kg/m ²)	1.42 (0.19)	1.11 (0.18)	1.24 (0.09)
Leaf N content (%)	2.24 (0.30)	2.47 (0.22)	2.03 (0.27)
Leaf C content (%)	32.1 (0.9)	32.9 (0.6)	30.3 (1.3)
Microbial processes			
Methane flux (mg CH ₄ ·m ⁻² ·d ⁻¹)	-0.16 (0.26)	0.29 (0.18)	-0.27 (0.23)
Sediment respiration (g CO ₂ ·m ⁻² ·d ⁻¹)	4.14 ^a (1.11)	1.06 ^b (0.26)	1.91 ^{ab} (0.37)
Carbon mineralization (μg C·[g dry sediment] ⁻¹ ·d ⁻¹)	55.6 (6.1)	43.90 (7.4)	46.80 (7.2)
Potential net N mineralization (μg NH ₄ -N·[g dry sediment] ⁻¹ ·d ⁻¹)	-0.031 (0.011)	0.137 (0.213)	-0.011 (0.038)
Potential net nitrification (μg NO ₃ -N·[g dry sediment] ⁻¹ ·d ⁻¹)	0.084 (0.021)	0.079 (0.033)	0.085 (0.026)
Sediment characteristics			
Total C (kg/m ²)	2.96 ^a (0.16)	2.12 ^b (0.20)	1.32 ^c (0.12)
Total N (kg/m ²)	0.21 ^a (0.01)	0.16 ^a (0.02)	0.09 ^b (0.01)
Extractable ammonium (μg NH ₄ -N/g dry sediment)	2.86 (0.59)	6.23 (0.71)	5.38 (1.52)
pH	7.10 ^a (0.04)	6.82 ^b (0.04)	7.08 ^a (0.04)
Gravimetric water content (%)	104 (3)	138 (5)	134 (3)
Bulk density (g/cm ³)	0.62 ^a (0.02)	0.50 ^{ab} (0.08)	0.41 ^b (0.01)

Notes: Values are means ($n = 5$ replicates; SE in parentheses) of control plots. Different superscript letters indicate significant differences among the marshes ($P < 0.05$). See *Study site* for salt marsh descriptions.

not significant, we averaged observations across all marshes ($n = 15$ observations). To infer the preferred model for each dataset, we used Akaike's Information Criterion (AIC function in R), corrected for finite sample size (AIC_c), and calculated the Akaike weights (Burnham and Anderson 2002).

RESULTS

Baseline comparisons among three Californian salt marshes

The baseline biogeochemical processes and properties differed significantly among the three marshes. In the unfertilized, control plots, several characteristics differed in at least one marsh in the first sampling date (February 2009), including those related to C and N cycling (Table 1). In general, CSM and TRE were more similar to one another than to MBE in terms of sediment C and N pools and C cycling as indicated by the eigenvector scores in the first principal component analysis (PCA) axes that account for 24% of variance (Fig. 1; Appendix B: Table B1). Specifically, total sediment C pool (mostly organic) in MBE was 40% higher than in CSM and 100% higher than in TRE, and sediment respiration rates in MBE were 200–300% higher than in the other marshes (Table 1). There were also differences in the total sediment N pool (mostly organic), where TRE showed almost 50% lower values than MBE and CSM (Table 1). Inorganic N content in the sediment was principally in the form of ammonium in all three marshes, whereas nitrate was below the detection limit ($0.03 \mu\text{g N-NO}_3^-/\mu\text{L}$). Nitrogen cycling, as measured by potential net N mineralization and potential net nitrification, was similar among marshes (Table 1). Finally, sediments were water-saturated and pH was approximately neutral in all three marshes, although CSM showed slightly more acidic sediments (Table 1).

Sediment bulk density (sediment mass per unit of volume) was also higher in MBE than in TRE (Table 1).

Responses to the N gradient

A variety of plant characteristics, microbial processes, and sediment characteristics responded to the experimental N gradient. While four of the eight significant responses were linear (Table 2, Fig. 1c), the other four responses fit a three-parameter exponential model, yielding a saturating curve (in the case of plant biomass at 14 months and leaf N content; Fig. 2c) or an exponentially increasing curve (sediment total N and NH₄-N, Table 2, Fig. 4). However, support for our first

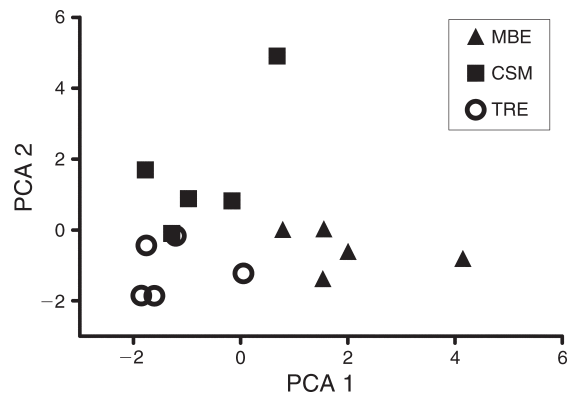


FIG. 1. Principal component ordination of three salt marshes along the Pacific coast in California, USA, on the basis of 13 biogeochemical properties and processes (see Table 1) under unfertilized conditions (control plots) at seven months of fertilization. Eigenvector scores of all biogeochemical properties and processes along principal component analysis (PCA) axes 1–3 are in Appendix B: Table B1. PCA 1 accounts for 24.5% of variance; PCA 2 accounts for 21.9% of variance. MBE is Morro Bay Estuary, CSM is Carpinteria Salt Marsh, and TRE is Tijuana River Estuary.

TABLE 2. Results of the generalized linear model (GLM) used to test the effects of salt marsh and N addition gradient and their interaction for several biogeochemical variables measured after seven and 14 months of fertilization.

Biogeochemical properties and processes	Time exposure to N addition (months)	P value from GLM			Shape of response
		Salt marsh (SM)	N addition (N)	SM × N	
Plant characteristics					
Aboveground plant biomass	7	<0.0001	0.001	0.1627	linear increase
Aboveground plant regrowth	14	<0.0001	<0.0001	0.7804	saturation
Leaf N content	7	<0.0001	0.0001	0.2954	saturation
Leaf C content	7	0.0005	0.7535	0.6209	
Root biomass					
Total	14	<0.0001	0.6677	0.2031	
Coarse roots	14	0.0503	0.4567	0.4351	
Fine roots	14	<0.0001	0.7766	0.2691	
Microbial processes					
Methane flux	7	0.1445	0.0252	0.7542	linear increase
Methane flux	14	0.0046	0.3205	0.711	
Sediment respiration	7	<0.0001	0.7062	0.2241	
Sediment respiration	14	<0.0001	0.3801	0.8668	
Carbon mineralization	7	<0.0001	0.0868†	0.6186	
Potential net N mineralization	7	0.4335	0.0005	0.1041	linear decrease
Potential net nitrification	7	0.0419	0.4933	0.9239	
Sediment characteristics					
Total C	7	<0.0001	0.8116	0.4904	
Total N	7	<0.0001	<0.0001	<0.0001	
CSM					
MBE					
TRE					exponential increase
Extractable ammonium	7	<0.0001	<0.0001	<0.0001	
CSM					linear increase
MBE					
TRE					exponential increase
Gravimetric water content	7	<0.0001	0.3389	0.025	
Bulk density	7	<0.0001	0.9056	0.9681	

Notes: The shape of the response to the N addition gradient is shown after choosing a model following the Akaike criterion (see Appendix C: Table C1 for model selection details). Linear responses follow the model $Y = kX$, where k is the slope expressed in units of the [biogeochemical variable]/g N·m⁻²·yr⁻¹. Exponential responses follow the model $Y = \exp(kX)$, where k is the rate constant, expressed in kg N·m⁻²·yr⁻¹.

† Although marginally significant, neither of the models tested fit significantly (see Fig. 3a).

hypothesis was mixed. As expected, aboveground plant responses were generally saturating, but, contrary to expectations, the responses of microbial processes were linear or neutral (Fig. 3). Our second hypothesis, that the inorganic N pool would respond to N addition while the organic N pool would not, was generally supported (Fig. 4). Finally, the shape of the plant and microbial responses were generally consistent among marshes in six of the eight cases of an N response, there was no marsh × N interaction (Table 2), and marshes often differed in their mean values (a significant marsh effect).

Plant responses to N addition.—Aboveground plant biomass increased linearly with N addition after seven months of fertilization (Fig. 2a; see Fig. A1 in Appendix A and Table C1 in Appendix C). For each 1 g N·m⁻²·yr⁻¹ applied to the marsh, there was an increase of 1 g plant biomass. The three marshes showed similar linear responses, although, on average, MBE produced significantly more plant biomass than CSM and TRE (Table 2). However, plant biomass showed a saturating response with N addition by 14 months of fertilization, leveling off above 160 g N·m⁻²·yr⁻¹ (Fig. 2c; Appendix

C: Table C1). Note that the seven- and 14-month results are not directly comparable, because we measured plant regrowth at 14 months vs. total harvest at seven months (see *Methods*). Leaf N content also showed a saturating response to N addition, leveling off around 80 g N·m⁻²·yr⁻¹ at seven months' fertilization (Fig. 2b; Appendix C: Table C1). Like aboveground plant biomass, the shape of the leaf N content response was similar among marshes, although average leaf N content was higher in CSM plants than MBE and TRE plants.

In contrast to the aboveground responses, root growth (total, coarse, and fine roots) did not respond to N addition in any of the salt marshes after 14 months of fertilization (Fig. 2d). However, total root growth differed among salt marshes; root biomass was significantly lower in CSM than MBE and TRE, primarily due to fewer finer roots (Table 2).

Microbial responses to N addition.—Nitrogen addition did not affect CO₂ emissions from the salt marsh sediments at either of the sampling dates or any of the study sites. Both C mineralization, which indicates total potential microbial respiration, and field sediment

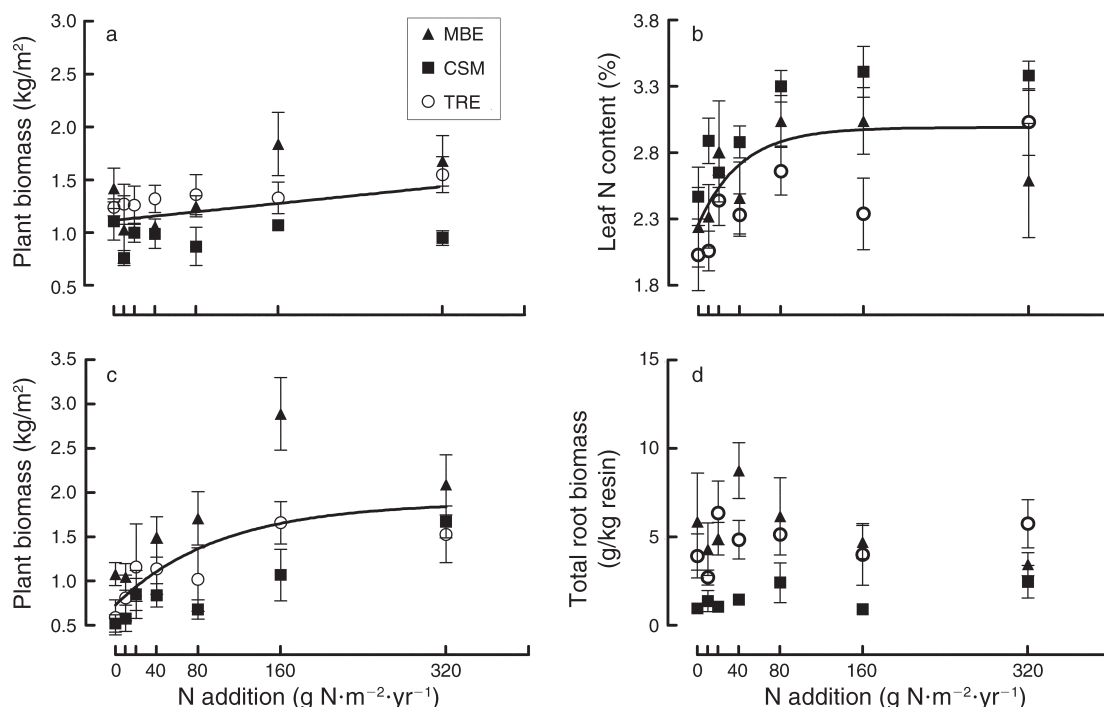


FIG. 2. Plant responses to a nitrogen addition gradient in three California salt marshes (key as in Fig. 1). Significant regressions are given for all marshes pooled, with a level of significance of $P < 0.05$. (a) Aboveground plant biomass after seven months of fertilization; $y = 0.001x + 1.117$, $R^2 = 0.58$. (b) Leaf N content after seven months of fertilization; $y = -0.73 \times \exp(-0.024x) + 2.99$. (c) Aboveground plant biomass regrowth after 14 months of fertilization (see *Methods* for details); $y = -1.16 \times \exp(-0.01x) + 1.89$. (d) Total root biomass after 14 months of fertilization. Symbols and error bars are the mean \pm SE for each site ($n = 5$ replicates). Model details for (a–c) are in Table 2. In all cases, *Salicornia virginica* was the single dominant plant species.

respiration, which measures microbial and root respiration, indicated that CO_2 production was unaffected by N fertilization (Fig. 3a, b, Table 2). Like the plant characteristics, the three marshes differed in their average respiration and C mineralization rates (Table 2). At seven months of fertilization, MBE showed higher rates of C mineralization ($72 \mu\text{g C/g dry sediment}$) and field sediment respiration ($3.58 \text{ g CO}_2\cdot\text{m}^{-2}\cdot\text{d}^{-1}$), compared to those of CSM and TRE ($52 \mu\text{g C/g dry sediment}$ and $1.91 \text{ g CO}_2\cdot\text{m}^{-2}\cdot\text{d}^{-1}$ for C mineralization and sediment respiration, respectively). At 14 months, the order changed for field respiration; TRE showed the highest rates ($5.56 \text{ g CO}_2\cdot\text{m}^{-2}\cdot\text{d}^{-1}$), followed by CSM ($2.70 \text{ g CO}_2\cdot\text{m}^{-2}\cdot\text{d}^{-1}$), and MBE ($1.03 \text{ g CO}_2\cdot\text{m}^{-2}\cdot\text{d}^{-1}$; Table 2).

When marsh microbial processes responded to N addition, they did so linearly. For instance, methane flux increased linearly with N addition at seven months of fertilization (Fig. 3c, Table 2; Appendix C: Table C1, Fig. C1d). As previously reported (Irvine et al. 2012), CH_4 flux increased by $1.23 \mu\text{g CH}_4\cdot\text{m}^{-2}\cdot\text{d}^{-1}$ for each $1 \text{ g N}\cdot\text{m}^{-2}\cdot\text{yr}^{-1}$ applied, and all marshes showed similar rates of CH_4 production (Table 2). At 14 months of fertilization, average CH_4 flux was similar to the previous sampling date ($P > 0.05$). However, we no longer observed an effect of N addition on this process, and CSM showed higher rates of CH_4 production than

the other two salt marshes ($0.45 \text{ mg CH}_4\cdot\text{m}^{-2}\cdot\text{d}^{-1}$ and $0.13 \text{ mg CH}_4\cdot\text{m}^{-2}\cdot\text{d}^{-1}$, respectively; Table 2).

Potential net N mineralization and potential net nitrification were differentially affected by fertilization. Potential net N mineralization declined linearly with N addition at seven months of fertilization, and all marshes showed similar responses (Fig. 3d, Table 2; Appendix C: Table C1, Fig. C1e). In contrast, potential net nitrification did not respond to N addition (not shown), but differed significantly among salt marshes (Table 2). TRE showed significantly higher nitrification rates ($0.09 \mu\text{g NO}_3\text{-N}\cdot[\text{g dry sediment}]^{-1}\cdot\text{d}^{-1}$) than CSM ($0.06 \mu\text{g NO}_3\text{-N}\cdot[\text{g dry sediment}]^{-1}\cdot\text{d}^{-1}$), and MBE had intermediate levels of nitrification rates ($0.085 \mu\text{g NO}_3\text{-N}\cdot[\text{g dry sediment}]^{-1}\cdot\text{d}^{-1}$).

Sediment responses to N addition.—Sediment inorganic N content changed with N addition. In contrast to the plant and microbial responses, the shape of this response differed by marsh (Table 2), supporting the third hypothesis (marsh-by-N addition interactions). Levels of extractable $\text{NH}_4\text{-N}$ did not change along the fertilization gradient at MBE, but showed a linear and exponential increase at CSM and TRE respectively (Fig. 4a, Table 2; Appendix C: Table C1, Fig. C1f, g). At the same time, sediment total N only responded to N addition in one of the marshes (TRE), where it increased exponentially (Fig. 4b, Table 2; Appendix C: Table C1,

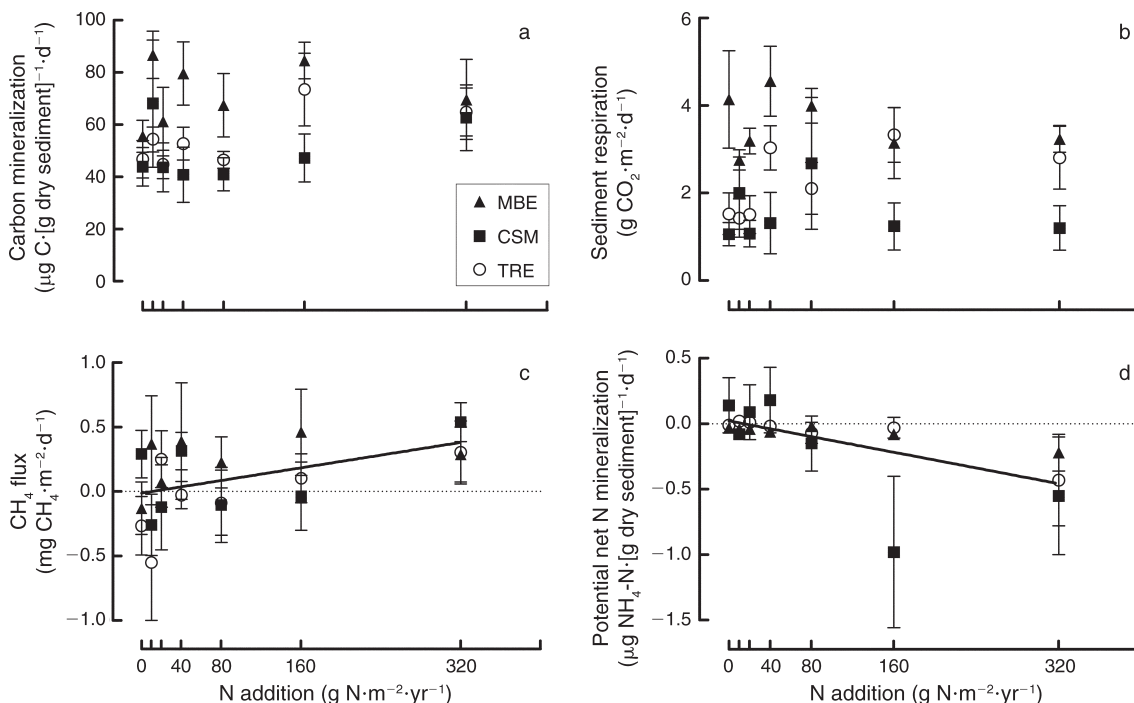


Fig. 3. Microbial responses to a nitrogen addition gradient in three Californian salt marshes after seven months of fertilization. Significant regressions are given for all marshes pooled. (a) Potential carbon mineralization; (b) sediment respiration; (c) methane production; $y = 0.0012x - 0.012$, $R^2 = 0.23$ (from Irvine et al. 2012); and (d) potential net N mineralization; $y = -0.0015x + 0.022$. Symbols and error bars are the mean \pm SE for each site ($n = 5$ replicates). Model details for (a–c) are in Table 2 and Appendix C: Table C1.

Fig. C1h). Sediment total C did not change with N addition, but the mean value differed significantly among marshes (3.1 kg/m^2 in MBE, 2.1 kg/m^2 in CSM, and 1.4 kg/m^2 in TRE). The pH in the sediment did not change significantly along the N fertilization gradient for any of the salt marshes; average sediment pH value was 6.9 across all marshes and N treatments.

DISCUSSION

A large unknown in predicting the consequences of human-driven environmental changes is how often ecosystem functioning will exhibit thresholds and other nonlinear responses to such changes (Scheffer et al. 2001, Rockström et al. 2009). This knowledge gap directly pertains to salt marsh functioning and sustainability, as the shapes of these responses will determine the capacity of these ecosystems to buffer future inputs while maintaining current levels of other processes. To begin to address this gap, we established gradient experiments in three study sites to test whether Californian salt marshes respond predictably to N addition. Previous studies have typically focused on the effect of a single N dose limiting the ability to make quantitative predictions for N-enrichment scenarios.

Our experimental gradients of N addition revealed two major findings. First, the ability of the three salt marshes to retain C and N is impaired by increasing levels of N. Aboveground plant biomass, which ac-

counts for most of the C and N retention in these highly productive ecosystems, saturated at high levels of N addition. At the same time, CH_4 emissions, although generally low, were linearly stimulated by N addition and converted the marshes into a source of the potent greenhouse gas. Further, the salt marshes' ability to reduce the amount of N running off into coastal waters was diminished as sediment inorganic N increased linearly or exponentially in two of these marshes.

The second major finding is that the shape of the responses (form and/or slope) to N addition was remarkably consistent across marshes with the exception of sediment N content, despite quantitative differences in a variety of plant, microbial, and sediment properties and processes. Extensive marsh differences were apparent in both the control plots (Table 1) and the broader N addition experiment where significant marsh effects were found for the majority of the measured variables. The consistency in marsh responses indicates that the qualitative trends observed here may be transferable to other marshes in the region. This result contrasts with reports from studies in grassland, forests, and tundra ecosystems where the magnitude of N effects depended on background conditions, such as atmospheric nitrogen deposition (Knorr et al. 2005, Janssens et al. 2010).

We emphasize, however, that these conclusions are based on a relatively short-term (14-month) experiment, whereas the direction or strength of the responses to N

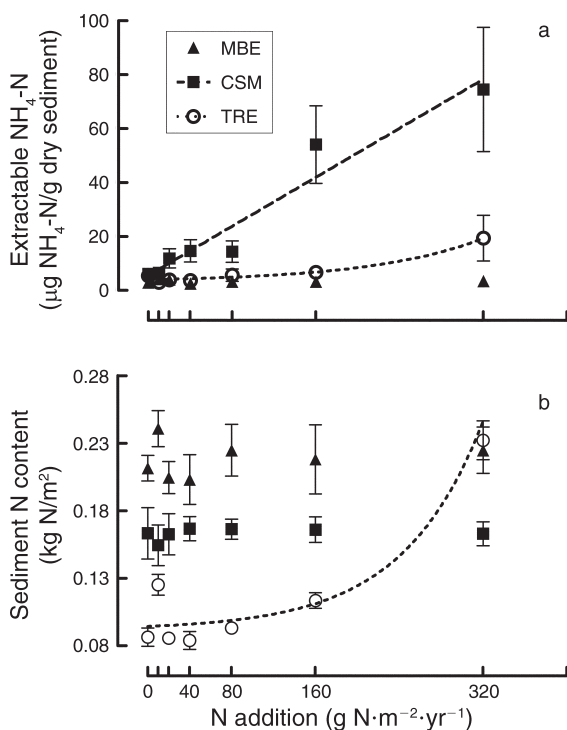


FIG. 4. Sediment nitrogen content along a nitrogen addition gradient in three Californian salt marshes after seven months of fertilization. (a) Extractable ammonium; significant regressions are shown for CSM ($y = 0.23x + 5.59$, $R^2 = 0.94$) and TRE ($y = 0.82 \times \exp(0.0093x) + 3.19$). (b) Total N in sediment; the significant regression is shown for TRE ($y = 0.0024 \times \exp(0.013x) + 0.092$). Symbols and error bars are the mean \pm SE for each site ($n = 5$ replicates). Model details are in Table 2 and Appendix C: Table C1.

addition might change over longer periods of time. For example, in a grassland experiment, N addition stimulated aboveground primary production, but the strength of this positive response was tempered over 13 years (Isbell et al. 2013). Notably, this diminishing effect on productivity was associated with plant species loss, whereas the marshes in our study have already lost much of their plant diversity, presumably due to chronic nutrient loading in the past (Ryan and Boyer 2012).

Marsh responses to N addition.—Support for our first hypothesis, that plant and microbial properties and processes would change nonlinearly with increasing N, was mixed. As expected, the marsh plants responded to N addition by increasing biomass and regrowth. Linear increases of aboveground standing biomass over the summer and autumn (after seven months' fertilization) suggest that N stimulates growth even outside the spring peak growth season. However, nonlinear aboveground regrowth during the following spring and summer (after 14 months' fertilization) demonstrated that N addition does not stimulate plant growth indefinitely. N saturation of plant growth generally occurs above $10 \text{ g N}\cdot\text{m}^{-2}\cdot\text{yr}^{-1}$ in terrestrial ecosystems (Lu et al. 2011), whereas this study and others suggests a higher plateau

in salt marshes ($60 \text{ g}\cdot\text{m}^{-2}\cdot\text{yr}^{-1}$ in this study; $240 \text{ g}\cdot\text{m}^{-2}\cdot\text{yr}^{-1}$ in a Louisiana salt marsh; Darby and Turner 2008). Higher saturation levels indicate that salt marsh plants are more limited by N in relation to other resources, or that marshes are able to deplete incoming excess of N (i.e., via denitrification) more efficiently before it can be used by plants. In contrast, root growth was unaffected by any level of N addition in agreement with previous reports (Darby and Turner 2008, Anisfeld and Hill 2012), but in contrast to mesocosm or longer-term field studies (Langley et al. 2009, Deegan et al. 2012). Both neutral and suppression effects on belowground growth indicate that salt marsh plants change their biomass allocation patterns to maximize capture of the growth-limiting resource (i.e., light when N limitation is ameliorated) as suggested by the resource optimization hypothesis (Bloom et al. 1985).

Contrary to our expectations, the responses of microbial processes to N addition were either neutral or linear. Neutral effects of N addition on microbial CO_2 production suggest that, unlike plant growth, aerobic decomposition of organic matter is not limited by N. Rather, organic carbon limitation might explain why Californian salt marshes responded differently than North American east coast marshes, where single doses of N stimulated CO_2 production (Morris and Bradley 1999, Anisfeld and Hill 2012). Indeed, sediment carbon content is lower in these Californian salt marshes (3–5%) than in those marshes (6–10% in South Carolina and 5–15% in Connecticut; Craft 2007). Specific differences among our Californian salt marshes also provide support for this hypothesis of carbon limitation. For example, MBE had more than twice the sediment C content than TRE, and showed higher sediment respiration as well as carbon mineralization rates accordingly. Although conclusive evidence of carbon limitation for aerobic microbial respiration is lacking, differences in sediment C content between east coast marshes and within Californian marshes provide a plausible explanation for neutral N effects on CO_2 production.

In contrast to CO_2 production, net methane flux increased linearly with N addition at seven months of fertilization. Methane flux increased from low or nearly negative to net positive values above $\sim 10 \text{ g N}\cdot\text{m}^{-2}\cdot\text{yr}^{-1}$, showing that the effect of added N on methanogenesis, the final step in anaerobic decomposition, offset any increase in methanotrophy. Differential effects of N on both methanogenesis and methanotrophy have been documented in other ecosystems (Liu and Greaver 2009, Irvine et al. 2012). In a microcosm experiment with salt marsh sediments from Tijuana River Estuary, N and C addition independently enhanced methanogenesis, but did not affect methanotrophy (Irvine et al. 2012). Thus, we hypothesize that CH_4 fluxes increased with N addition because methanogenesis was stimulated directly and, perhaps, indirectly through increased C availability, due to increased root exudates. After 14 months

of fertilization, however, N addition had no effect on CH_4 flux, although methanogenesis was still stronger than methanotrophy. It is unclear whether this difference between sampling dates is due to seasonal variation or the length of N exposure. We hypothesize that the quantity and/or quality of root exudates may have better supported methanogenesis at the seven-month sampling in February during the spring rejuvenation of the pickleweed, whereas, at 14 months (September), pickleweed was entering dormancy. Together, neutral effects of N on microbial aerobic respiration and stimulating effects on anaerobic respiration indicate that the microorganisms involved in these processes differ in their sensibility to N enrichment.

Our second hypothesis, that the inorganic N pool would respond to N addition while the organic N pool would not, was generally supported. Net N mineralization responded linearly, decreasing with N addition, and thereby reducing the net microbial contribution of NH_4^+ to the marsh sediments. Decreasing levels of net N mineralization could result either from a decrease in NH_4^+ production (gross N mineralization) or from an increase of NH_4^+ consumption (microbial immobilization, nitrification, and/or denitrification; Robertson et al. 1999b). Our laboratory assay did not allow us to disentangle which of these NH_4^+ pathways were altering net N mineralization. However, we speculate that microbial NH_4^+ consumption was relatively more stimulated than NH_4^+ production, as organic matter decomposition (measured by respiration) was unaffected by N addition. Thus, part of the added N was immobilized and used for coupled nitrification–denitrification, and another part stayed in sediment as inorganic N (Fig. 4a). This result is opposite to the stimulation of net N mineralization often observed in terrestrial ecosystems. Notably, these increases are usually associated with slight increases in total soil N content and decreases in soil C:N ratio (Lu et al. 2011), which we did not detect in two of the salt marshes.

The effects of N addition on sediment N pools were site-specific, supporting our third hypothesis and in contrast to the unified shape of the plant and microbial responses. We propose that background differences in C and N pools and cycling among the marshes explain these differential sediment responses. MBE, the most productive marsh with the largest pools of C and N and the fastest C cycling (Table 1), showed no changes in inorganic N pool due to high plant and microbial demands, and no change in sediment total N pool. In contrast, CSM with the smallest pool of C in plant biomass and the slowest C cycling (Table 1), showed linear increases in inorganic N content because of much lower plant demand, and no consequent contribution to total N pool in sediments. Finally, TRE, with intermediate levels of C pools and cycling, showed an intermediate response in inorganic N, but an unexpected increase in total N, possibly due to a small baseline



PLATE 1. Light colored flags demarcate areas showing plant biomass increase with nitrogen addition at Tijuana River Estuary, California, USA. A color version of this photo (with red flags) is available in Appendix A. Photo credit: Irina C. Irvine.

organic N pool, where it is easier to detect changes than in a large pool, such as MBE.

Implications for salt marsh sustainability.—Ecosystem properties and processes are intimately involved in the maintenance of marsh vertical elevation and, thus, the ability of marshes to resist sea-level rise (Kirwan and Megonigal 2013). Plants promote increases in marsh elevation by trapping sediments and adding organic matter above- and belowground. In contrast, microbial decomposition of organic matter, along with erosion and compaction, promotes elevation loss. A nine-year study of one N addition dose to tidal creeks showed that it increased aboveground biomass, decreased belowground biomass, and increased microbial decomposition. These changes resulted in creek-bank collapse and reduced marsh area in Northeast *Spartina*-dominated salt marshes (Deegan et al. 2012). Our experiment, carried out on sediments rather than creek waters, suggests that N addition would not alter marsh elevation in *Salicornia*-dominated California marshes. The aboveground plant response saturated at high N addition, while N did not appear to stimulate belowground plant biomass or microbial processes associated with decomposition, such as sediment respiration and carbon mineralization. However, accurate predictions of tidal wetland stability require an integration of climatic,

geomorphic, and socioeconomic processes that operate on a landscape scale and over much longer than the time scale of our experiment (Kirwan and Megonigal 2013).

Conclusions

Salt marshes are threatened worldwide by many factors, including sea-level rise, human development, invasive species, and nitrogen enrichment (Bertness et al. 2002, Gedan et al. 2009). Understanding how different levels of these stressors affect marsh functioning will be crucial for determining their capacity to provide ecosystem services under continuing environmental change. This study demonstrates the power of experimental gradients for predicting future changes to ecosystem functioning; half the observed responses were nonlinear in the form of saturating or exponentially increasing curves. In such cases, an experimental gradient provides a better basis for prediction under future N scenarios than a single-dose experiment. Our specific results revealed that the capacity of remaining Californian marshes to remove land-derived N is limited and provides insights into the quantitative thresholds of specific processes. Further, the responses to N addition were not idiosyncratic to location and, therefore, likely applicable at a regional level. However, while short-term marsh responses have implications for marsh stability, our conclusions are restricted to intact marshes. Additional losses of marsh area, through reduced sediment deposition, creek bank collapse, or habitat conversion, will ultimately limit the delivery of marsh services.

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SUPPLEMENTAL MATERIAL

Ecological Archives

Appendices A–C are available online: <http://dx.doi.org/10.1890/13-1983.1.sm>