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Not All Nitrogen Is Created Equal: Differential Effects of Nitrate and Ammonium Enrichment in Coastal Wetlands

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Excess reactive nitrogen (N) flows from agricultural, suburban, and urban systems to coasts, where it causes eutrophication. Coastal wetlands take up some of this N, thereby ameliorating the impacts on nearshore waters. Although the consequences of N on coastal wetlands have been extensively studied, the effect of the specific form of N is not often considered. Both oxidized N forms (nitrate, NO_3^-) and reduced forms (ammonium, NH_4^+) can relieve nutrient limitation and increase primary production. However, unlike NH_4^+, NO_3^- can also be used as an electron acceptor for microbial respiration. We present results demonstrating that, in salt marshes, microbes use NO_3^- to support organic matter decomposition and primary production is less stimulated than when enriched with reduced N. Understanding how different forms of N mediate the balance between primary production and decomposition is essential for managing coastal wetlands as N enrichment and sea level rise continue to assail our coasts.

Keywords: reactive nitrogen, salt marshes, PIE LTER, nitrogen cycling, carbon cycling

The course of human history was dramatically changed when, in 1908, Fritz Haber filed a patent for ammonium (NH_4^+) production and his contemporary, Carl Bosch, industrialized the process to increase the scale of production. During the tumultuous first half of the twentieth century, this discovery facilitated arms manufacturing, which required extensive supplies of reactive nitrogen (N_r). Even more transformative was its value in fertilizer production, particularly after World War II, which enhanced food availability across the globe. Today, 40%–50% of the human population depends on food grown with fertilizers resulting from the Haber–Bosch process (Smil 2004, Stewart et al. 2005, Erisman et al. 2008), and therefore, increases in human population are tightly coupled with increases in fertilizer use (Erisman et al. 2008). Today, N_r derived via the Haber–Bosch process has more than doubled the annual supply of biologically available nitrogen entering the biosphere (Fowler et al. 2013).

The cascade of N_r from agricultural fields and other sources, through ecosystems, and into coastal waters is well documented (Galloway et al. 2003, Billen et al. 2013). Fertilizer N can be lost from agricultural land through volatilization to the atmosphere that is later deposited as wet or dry deposition (Paerl 1995, Hinga et al. 1991), through leaching into surficial water bodies and into groundwater (Forster et al. 1982), and through harvest and subsequent movement of N_r around the planet in the form of food and feed stocks (Galloway et al. 2008) that ultimately enters wastewater streams. Much of this human-derived N_r eventually finds its way to the N-limited coastal zone, where it increases primary production and, in excess, can lead to eutrophic conditions and anoxia (Nixon 1995, Diaz and Rosenberg 2008). Unfortunately, conversion of N_r back to N_2 gas, essentially reversing the Haber–Bosch process, has limited industrial analogs, particular once N_r enters coastal waters. Instead, to prevent eutrophication of the coastal zone, a series of microbial transformations of N must occur for the excess N_r to be returned to inert N_2 gas that is then removed from the ecosystem.

Coastal emergent wetlands—principally, salt marshes in the temperate zone—sit between the land and the sea and intercept N_r before it enters open coastal waters (Valiela et al. 2002, Verhoeven et al. 2006, Sousa et al. 2008, Brin et al. 2010, Nelson and Zavaleta 2012). Coastal wetlands can

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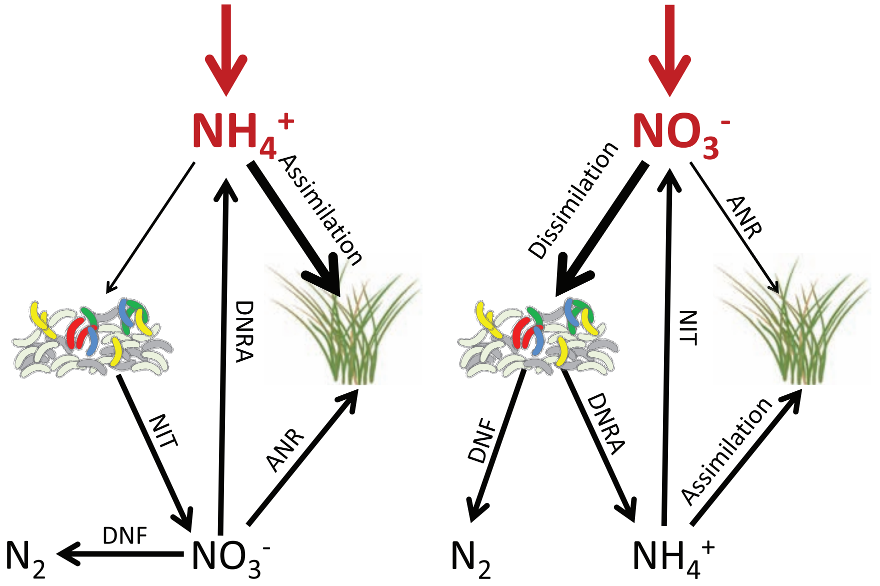

Figure 1. Conceptual diagram illustrating different possible fates of added nitrogen to salt marshes. (a) nitrogen is added in the reduced form of ammonium (NH_4^+). (b) nitrogen is added in the oxidized form of nitrate (NO_3^-). We propose that when nitrogen is added as NO_3^-, it will be predominantly used as an electron acceptor to fuel dissimilatory bacterial respiration rather than to stimulate primary production. ANR = assimilatory nitrate reduction, NIT = nitrification, DNF = denitrification, DNRA = dissimilatory nitrate reduction to ammonium.

retain or remove some fraction of anthropogenically derived N_r delivered to the coastal zone; however, the amount of N_r retained or removed varies widely, with wetlands in eutrophic systems retaining a smaller fraction of total N_r than wetlands that receive lower N_r inputs (Valiela and Cole 2002). The loss of seagrass beds is considered a sentinel indicator of eutrophic conditions (Orth et al. 2006) and estuaries with large areas of emergent wetlands have greater extents of seagrass beds than similar estuaries with no emergent wetlands because of wetlands' N_r removal capacity (Valiela and Cole 2002).

The transformations of N_r that are required to ultimately remove N from ecosystems depend on the initial form of the N and on the geochemical conditions present in the environment. There are several mechanisms by which anthropogenic N_r gets delivered to the coastal zone. N_r derived from sewage waste and fertilizers can be transported to estuaries via river (Peierls et al. 1991, Caraco and Cole 1999) and groundwater flows (Valiela et al. 1990, Caraco and Cole 1999, Bowen et al. 2007, Kroeger and Charette 2008), or can be directly discharged from treatment plants into estuarine and coastal waters. This N_r is typically delivered to estuaries as NO_3^- (Weller and Jordan 2020), with NH_4^+ often making up less than 10% of N_r as a result of oxygen-dependent nitrification, which converts reduced NH_4^+ to NO_3^- through a series of microbially mediated oxidation steps (Ward et al. 2011). Enhanced mobility of NO_3^- compared with NH_4^+ and the preferential uptake of NH_4^+ compared with NO_3^- by macrophytes, underscores the importance of separately identifying the fates of each form of N_r in coastal systems. Reactive N can also be delivered to coastal systems via atmospheric deposition both directly to the estuarine water body and via deposition on land and subsequent delivery to the estuary via river and groundwater flows. Atmospheric deposition by these mechanisms can account for 10%–40% of total N_r inputs to the coastal zone (Fisher and Oppenheimer 1991, Paerl 1995, Paerl et al. 2002). Historically, oxidized N deposition dominated this flux in eastern North America; however, in recent years, the flux of N oxides decreased as a result of emissions reduction strategies adopted by industrial processes (Lloret and Valiela 2016), whereas the flux of reduced N either increased (largely because of volatilization from animal wastes and fertilizers) or remained constant (Gilliam et al. 2019).

The retention and loss of N_r can occur via multiple pathways (figure 1), including uptake into plant biomass and storage in salt marsh sediments (Valiela and Teal 1979, Drake et al. 2009) and through microbial denitrification, which converts N in its most oxidized form, NO_3^-, to N_2 gas through stepwise reduction (Zumft 1997). This process is largely anaerobic (requiring low oxygen conditions) and heterotrophic (requiring organic carbon as a carbon source; Burgin and Hamilton 2007). Autotrophic (fixing carbon) denitrification also exists, often coupled with reduced iron or sulfur compounds, although its quantitative importance in coastal wetlands is unclear (Rivett et al. 2008). In addition to these denitrification pathways, NO_3^- can also be converted to NH_4^+ via microbes that are capable of dissimilatory nitrate reduction to ammonium (DNRA) or anaerobic ammonia oxidation to N_2 (annamox). DNRA recycles N within the environment, rather than returning this N to the atmosphere as N_2 gas (An and Gardner 2002, Giblin et al. 2013). Organisms capable of DNRA can also be either autotrophic or heterotrophic (Burgin and Hamilton 2007). Annamox also results in loss of N_r (Dalsgaard et al. 2005); however, its importance in coastal wetlands appears limited (Koop-Jakobsen and Giblin 2009). This complex combination of processes, some autotrophic and requiring oxygen and others heterotrophic and requiring anoxic conditions, coupled with different ionic interactions with soil particles (e.g., NH_4^+ readily binds to clay particles in soils), ultimately dictates the dominant form of N_r in the environment. Understanding the specific pathways involved in the processing of N_r in the coastal zone is therefore essential for predicting the stability of coastal wetlands because shifts in

N cycle processes can have simultaneous implications for carbon cycling in these critical carbon-rich habitats (Bulseco et al. 2019).

When N_r enters coastal salt marshes as NO_3^-, unlike its reduced forms, it can play two distinct roles (figure 1) with very different consequences at the ecosystem scale. As with NH_4^+ and organic based fertilizers (figure 1a), NO_3^- can be used to relieve nutrient limitation and stimulate primary production of vascular plants, benthic algae, and phytoplankton. However, unlike these other forms of N, in the absence of oxygen, it can also be used as an electron acceptor to fuel microbial respiration through denitrification or DNRA (figure 1b). These two opposite outcomes—stimulation of primary production and enhanced decomposition—make it essential that we understand how the forms of N_r entering our coastal waters are being used. If the added NO_3^- relieves nutrient limitation and supports primary production, it will increase plant biomass, which will facilitate sediment trapping. This increased trapping of sediment, combined with increased peat build up through production of roots and rhizomes, increases the marsh's ability to keep pace with sea level rise (Kirwan et al. 2010, Kirwan and Megonigal 2013, Morris et al. 2013). However, if NO_3^- is primarily being used as an electron acceptor by the microbial community, this could stimulate microbial respiration and potentially decrease the rate of wetland carbon storage and destabilize marsh creeks (Deegan et al. 2012). By contrast, if the N_r is added in its reduced form, as NH_4^+, the ecosystem scale outcome will depend on the balance between plant uptake and microbial nitrificiation. If microbes are able to use the NH_4^+ in nitrification to produce NO_3^- that is coupled to denitrification, then increased NH_4^+ should also ultimately stimulate microbial N_2 production through coupled nitrification and denitrification; however, if the plants have a higher affinity for available NH_4^+, then we would expect increased primary production by marsh macrophytes.

Below, we synthesize multiple lines of evidence demonstrating that, when NO_3^- supply is abundant, primary production rates are lower than are achieved by the addition of comparable supplies of NH_4^+ and that microbial decomposition is stimulated by NO_3^- additions. Therefore, it is imperative that we consider not just the quantity but also the form of N_r entering coastal systems to properly manage and mitigate its downstream consequences.

NO_3^- additions have a smaller effect on primary production of the foundation species *Spartina* than other forms of N_r

The salt marsh vegetation throughout the East and Gulf Coasts of the United States and in Europe is generally N limited (Valiela and Teal 1979, Kiehl et al. 1997, Tyler et al. 2003) and the addition of exogenous N, particularly when added as NH_4^+ or organic based fertilizers, typically increases primary production of marsh grasses (figure 2). The foundation species along the East Coast of the United States, *Spartina alterniflora* and *Spartina patens*, are both capable of taking up NO_3^- through their roots (Smith and McLachlan 1979, Mendelssohn 1979b, Cott et al. 2018). *S. alterniflora* can also take up NO_3^- through its shoots (Mozdzer et al. 2011), although we could find no studies that examined NO_3^- uptake via shoots in *S. patens*. In a field study in North Carolina, nitrate reductase activity was much lower than glutamate dehydrogenase activity (induced by NH_4^+ availability) across all growth forms of *Spartina alterniflora* in both the shoots and the roots (Mendelssohn 1979b), suggesting that NH_4^+ was the preferred nutrient for *S. alterniflora* growth. A nutrient enrichment experiment indicated that NO_3^- addition did increase the expression of nitrate reductase in *S. alterniflora*; however, activity under high ambient NO_3^- concentrations was still dramatically lower than activity of glutamate dehydrogenase (Mendelssohn 1979b). In addition, foliar uptake of *S. alterniflora* was around 70% higher for NH_4^+ and glycine than for NO_3^- (Mozdzer et al. 2011). *S. patens*, similarly showed uptake rates of NH_4^+ that were an order of magnitude higher than NO_3^- across a range of nutrient concentrations (Cott et al. 2018), further suggesting that, although these *Spartina* species are capable of NO_3^- uptake, they prefer N in the form of NH_4^+.

We performed a meta-analysis of field studies focusing on *Spartina* marsh nutrient enrichment to assess whether the form of N_r used in an experiment affected measured outcomes. We included in our study any salt marsh nutrient enrichment experiments where the enriched habitat consisted of predominantly *S. alterniflora* or *S. patens*, both the form and quantity of N_r used in the experiment were clearly stated, N_r addition results were presented relative to an unenriched reference, and standard deviation or standard error of the mean and sample size for the response variable were reported. There were too few studies documenting the response of belowground vegetation so we focused on the response of aboveground *Spartina* biomass to N_r enrichment (figure 2).

Twenty-two studies from the Western Atlantic ($n = 17$), the Gulf of Mexico ($n = 3$) in the United States, and China ($n = 2$) clustered into four different types of added N_r: NH_4^+ ($n = 7$), NH_4NO_3 ($n = 4$), other forms of NO_3^- ($n = 4$), and organic N, either in the form of pelletized sewage sludge or organic fertilizer or as urea ($n = 7$). We used the "escalc" function in the metafor v.2.1–0 R package (Viechtbauer 2010) to calculate the mean and variance in effect size using ROM, the log transformed ratio of the means (Hedges et al. 1999, Lajeunesse 2011), as the effect size measure while specifying the means, standard deviations, and sample sizes from each study.

We found that added N_r in the form of NO_3^- (excluding N_r that was added as NH_4NO_3) had a smaller effect size on plant biomass than other forms of N_r (figure 2a). When studies were aggregated together by the form of N_r added (figure 2b) the mean effect size was significantly less (ANOVA, $F(3,40) = 9.89$, $p < .01$) in the NO_3^- only additions (Tukey's posthoc test: NO_3^- versus NH_4^+, $p \leq .01$;

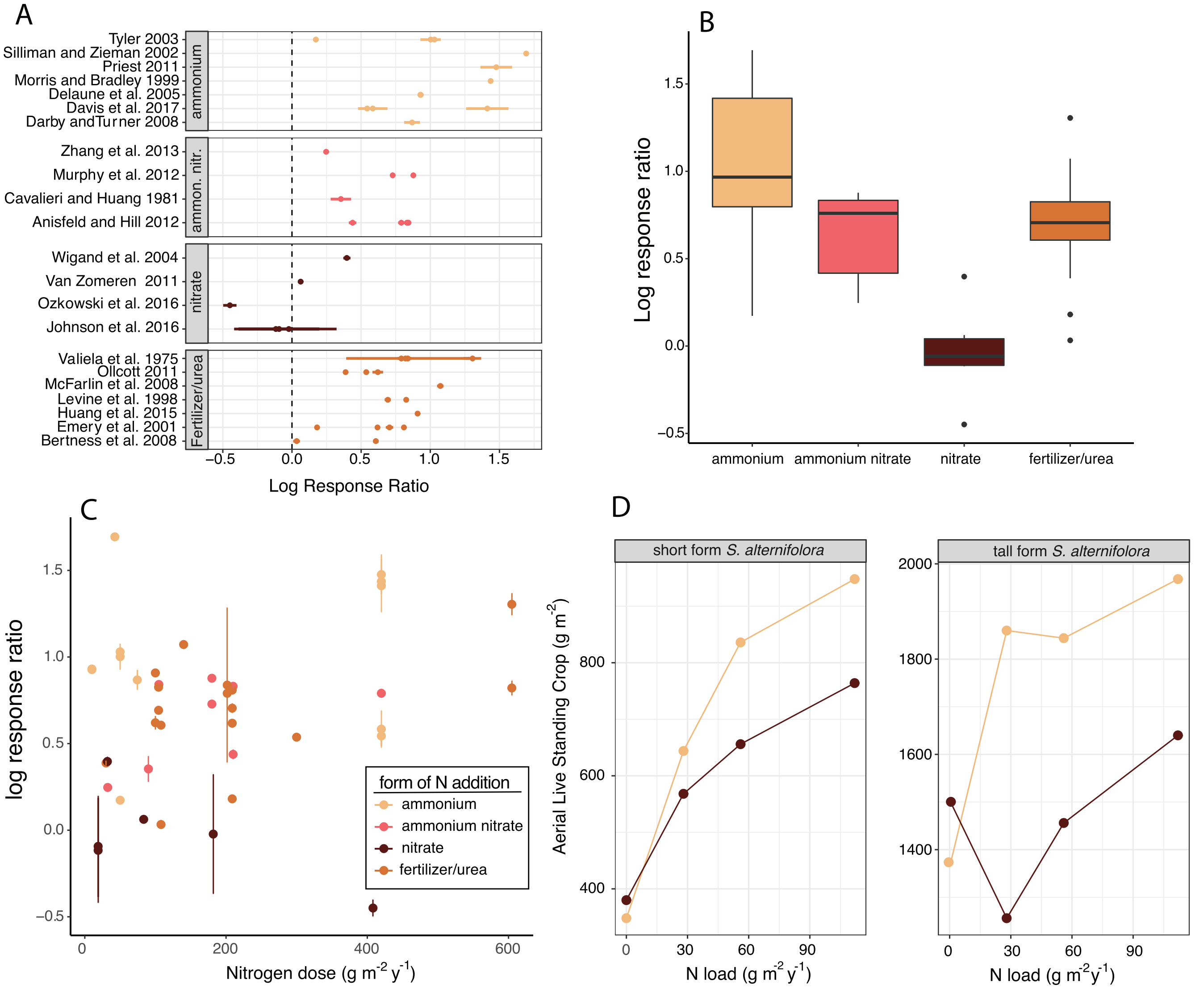

Figure 2. Results of meta-analysis that suggest response to N_r additions in the form of NO_3^- leads to a smaller positive effect on aboveground biomass of* Spartina *plants than when other forms of nitrogen enrichment are added. (a) Log response ratios of studies that report response to N_r in enriched compared with reference salt marsh samples separated by the form of N added. (b) Box and whisker plot of the log response ratios reported in panel (a). The center line represents the median response, upper and lower edges of the box represent the upper and lower quartiles, respectively, and the whiskers indicate the highest and lowest values. (c) Log response ratio plotted as a function of the amount of N added. (d) Aboveground biomass by different* Spartina *ecotypes to the direct addition of NO_3^- (brown) and NH_4^+ (yellow) under identical experimental conditions. Source: The data were plotted from table 1 in Mendelssohn (1979a).

NO_3^- versus NH_4NO_3, $p = .044$; NO_3^- versus organic fertilizer N or urea, $p \leq .01$). There was no relationship between the quantity of N added and the effect size across all treatments (figure 2c), suggesting that the muted responses observed in NO_3^- addition experiments were not the result of a lower overall amount of N_r added in the enrichment experiments, but rather, it was the form of N that played a consequential role.

Mendelssohn (1979a) performed the only study that directly compared the change in *Spartina alterniflora* aboveground biomass when plants were grown separately with NO_3^- or NH_4^+ as the added N_r source. This study was not included in our meta-analysis because no error was reported; however, the results are consistent with our hypothesis of a muted response by *S. alterniflora* to N_r enrichment when it is added as NO_3^- (figure 2d). Mendelssohn measured live standing crop of both the tall ecotype and the short ecotype of *S. alterniflora* from the East Coast (Walden Creek Marsh, North Carolina) under different nutrient regimes. The experiment used a 4 × 2 × 2 factorial design with four N application rates ranging from 0 to 112 grams per square meter per year

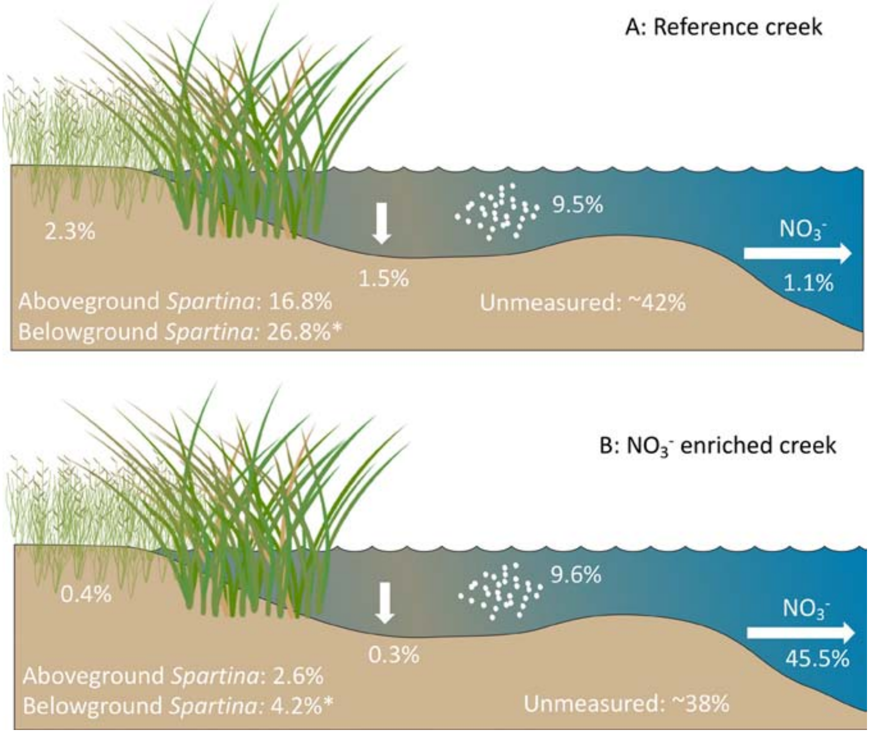

Figure 3. Mass balance of $^{15}NO_3^-$ added in low dose to an unenriched reference salt marsh creek (a) and in high dose to a NO_3^-enriched salt marsh creek (b) during a paired ^{15}N isotope enrichment experiment (Drake et al. 2009). The reference marsh retained 98.8% of added NO_3^-, compared with 54.5% in the enriched marsh. The percentages are the percentage of added NO_3^- found in each marsh pool after 5 days of dosing. NO_3^- that was unaccounted for was assumed to be lost via dissimilatory microbial processes such as denitrification. Asterisk indicates that the value was estimated and not directly measured. More details on the experimental design and measurements can be found in Drake and colleagues (2009). Source: The images of marsh vegetation were compiled from the IAN symbol repository.

of added N_r, two different application methods (band, where N_r was injected into the sediment, and broadcast, where N_r was placed on the surface sediments), and two different N forms, $(NH_4)_2SO_4$ and $NaNO_3$. For both the short and tall ecotypes of *Spartina alterniflora* when N_r was added as $(NH_4)_2SO_4$ there was a greater increase in growth than when it was added as $NaNO_3$ (figure 2d) We show only the results of band delivery in the present article but the response to the broadcast delivery method were comparable (Mendelssohn 1979a). Our meta-analysis and Mendelssohn's direct test indicate that *Spartina* biomass accumulation is lower with NO_3^- enrichment, which could have consequences for the long-term sustainability of marshes in the face of rising sea levels.

Whole creek ^{15}N-NO_3^- enrichment experiments support rapid uptake by microalgae and little NO_3^- uptake by *Spartina*

In unvegetated areas of salt marshes and even in understory areas of the marsh where there is sufficient light penetration, benthic microalgae, along with macrophytes and denitrifying bacteria (Sundback and Miles 2000), use N_r. Benthic diatoms are capable of NO_3^- uptake and storage (Lomas and Glibert 2000); however, responses to the addition of N_r by benthic microalgae vary widely, depending on the study (Sullivan and Currin 2002). Whole ecosystem additions of enriched ^{15}N-NO_3^- in saltmarshes quickly and consistently result in highly labeled benthic algae (Hughes et al. 2000, Tobias et al. 2003a, Tobias et al. 2003b, Drake et al. 2009, Galván et al. 2011), suggesting uptake of NO_3^-. However, the measured uptake kinetics of benthic microalgae were consistently higher when provided ammonium, compared with NO_3^- in estuarine sediments of the Nakdong Estuary on the Korean Peninsula (Longphuirt et al. 2009). Our 13-year salt marsh NO_3^- enrichment experiment, referred to as the TIDE project, showed that the addition of NO_3^- did not appreciably increase standing stock biomass of benthic microalgae (Deegan et al. 2007), although high grazing could have masked an increase in algal productivity (Galván et al. 2011, Pascal and Fleeger 2013).

The partitioning of NO_3^- between salt marsh macrophytes, benthic microalgae, and microbial dissimilatory nitrate respiration pathways can partly be disentangled using whole marsh ^{15}N-NO_3^- addition experiments (Drake et al. 2009). As a part of the TIDE project, we performed a whole-creek marsh ^{15}N-NO_3^- addition experiment in a creek that was amended to 70 micromolar (μM) NO_3^- dissolved into flooding tidal waters on every tide for 2 years and in reference creeks that had ambient (less than 5 μM) NO_3^- concentrations. ^{15}N-NO_3^- was added to both creeks for 5 days in midsummer (23–28 July 2005). In the NO_3^- enriched creek, enough ^{15}N-NO_3^- was added to maintain creek water at 650‰ enrichment while keeping the creek NO_3^- concentration at approximately 70 μM. In the reference creek we maintained a target enrichment of 1000‰, which resulted in a 3%–11% increase in the creek NO_3^- concentration (Drake et al. 2009). The reference creek was able to retain 98.8% of the added ^{15}N-NO_3^- (figure 3a). Aboveground plant biomass accumulated approximately 17% of the isotope label and belowground biomass was estimated to take up an additional approximately 25%, although belowground accumulation was not directly measured. The sediment pool, including benthic microalgae, took up less than 2% of the added NO_3^-. After all

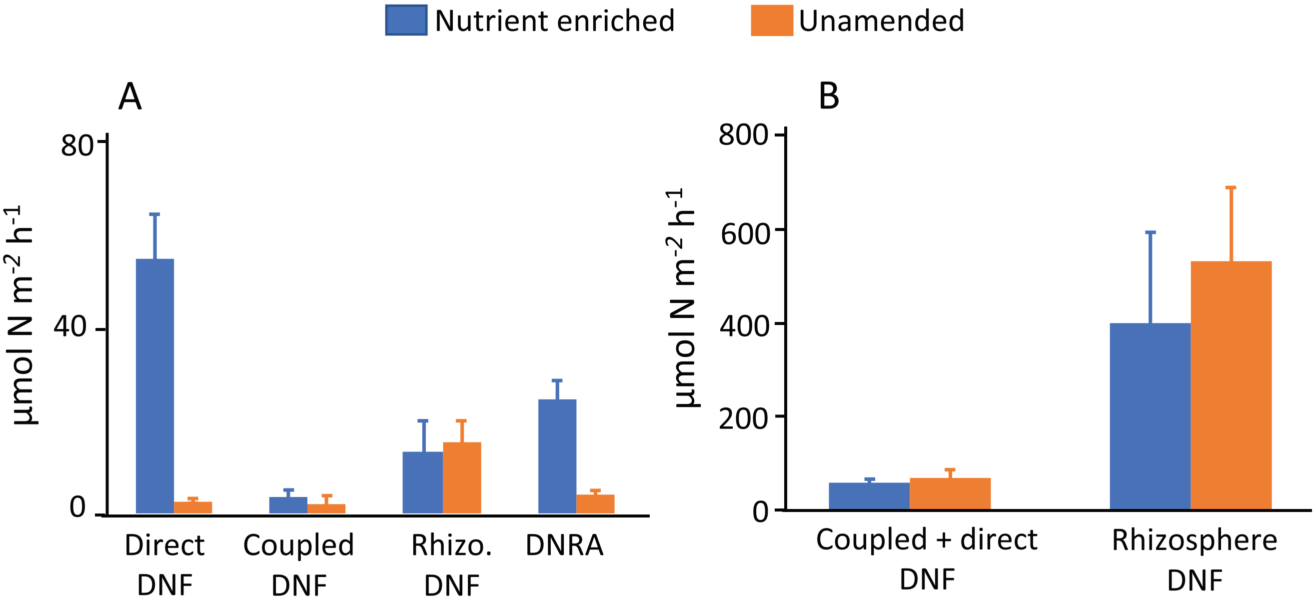

Figure 4. Field- and lab-based assessments of the fate of added NO_3^- in salt marsh sediments. (a) Direct denitrification, coupled denitrification, rhizosphere (rhizo.) associated denitrification and dissimilatory reduction of nitrate to ammonium (DNRA) rates measured on the marsh platform in NO_3^- enriched and unamended marshes (adapted from Koop-Jakobsen and Giblin 2010). (b) rates of coupled plus direct denitrification and rhizosphere denitrification in cores collected from NO_3^- and unamended marshes when exposed to comparable supplies of exogenous NO_3^-.

the measured pools were accounted for, 42% of the added NO_3^- in the reference marsh remained unaccounted for and was assumed to be lost via microbial denitrification. In the NO_3^- enriched marsh, 54.5% of added NO_3^- was retained in the plant and sediment pools. Although the percentage retention in the enriched marsh was lower than in the reference marsh, the total mass of NO_3^- retained in the system was higher, because of an overall larger mass of NO_3^- added during the experiment. In the enriched creek, the plants and benthic microalgae retained less than 10% of the added NO_3^- and approximately 38% was unaccounted for and assumed to be lost via microbial denitrification. The remainder was exported from the creek in the tidal waters. These enrichment experiments occurred toward the latter part of the growing season of *Spartina*, when biomass accumulation had slowed. It remains to be seen whether similar partitioning of added NO_3^- would be observed during times of peak *Spartina* growth. These results further support our hypothesis that salt marsh vegetation and the benthic microalgal communities use a small amount of added NO_3^- compared with presumed dissimilatory NO_3^- respiration performed by the microbial community.

Both field and controlled laboratory experiments support microbial use of added NO_3^-

The whole creek ^{15}N- NO_3^- isotope enrichment experiment, both in enriched and reference creeks, had high proportions of ^{15}N-NO_3^- that were unaccounted for and that were assumed to be lost via microbial denitrification (Drake et al. 2009); however, denitrification was not directly measured. Additional work by Koop-Jakobsen and Giblin (2010) measured the rates of DNRA, denitrification that is coupled with nitrification, direct denitrification, and rhizosphere associated denitrification in specific subhabitats (creek sediments and high marsh platform) within nutrient enriched and unamended creeks. During flooding tides, the addition of NO_3^- stimulated direct denitrification, which was nearly twentyfold higher on the fertilized marsh platform than at the reference site (figure 4a). DNRA on the fertilized platform was also stimulated although it was only a fourfold stimulation. In contrast, there was no stimulation of coupled denitrification in either the surface sediments or the rhizosphere (figure 4a).

The major reason for this increase in direct denitrification appears to be a direct and rapid response to added NO_3^-. Pore water measurements showed that the flooding NO_3^- did not reach the rhizosphere in the high marsh and therefore rates were similar between reference and enriched sites. When sediments collected from reference sites were amended with NO_3^- in whole core incubations, denitrification rates increased and did not differ from sediments in enriched sites when both treatments received comparable amounts of NO_3^- (figure 4b). Similarly, when NO_3^- was added to the rhizosphere of sediments from the reference and the enriched sites, there was a large stimulation in denitrification rates in sediments from both sites. Therefore, in all cases when NO_3^- was directly added to

Table 1. Total nitrate reduction, denitrification (DNF) and dissimilatory nitrate reduction to ammonium (DNRA) integrated across the duration of the flow through reactor experiment.

	Nitrate reduction (mmol per cm^3)		DNF (mmol per cm^3)		DNRA (mmol per cm^3)		DNRA + DNF (mmol per cm^3)		
Depth	**Mean (M)**	**Standard deviation (SD)**	**M**	**SD**	**M**	**SD**	**M**	**SD**	**Percentage of nitrate reduction**
Shallow (0–5 cm)	87.5	7.4	70.6	1.7	10.2	0.6	80.9	1.8	92
Mid (10–15 cm)	61.3	4.1	58.1	3.7	4.8	1.4	63	4.0	102
Deep (20–25 cm)	70.9	20.4	55.2	10.3	3.6	0.9	58.8	10.3	82

Note: The sum of DNF and DNRA accounted for between 82%–102% of the total amount of nitrate reduced during the experiment. The data were derived from Bulseco and colleagues (2019). Abbreviations: cm^3, cubic centimeters; mmol, millimoles.

marsh sediments, both to the rhizosphere and to the overlying water in intact cores, there was a rapid increase in microbial respiration of NO_3^- through denitrification. *In situ* measurements of ecosystem respiration (Geoghegan et al. 2018) and soil respiration (Wigand et al. 2018) were also significantly higher in the nutrient enriched creeks, providing further evidence of NO_3^- stimulated microbial processes. This suggests that salt marsh sediments have populations of microbes capable of using NO_3^- within hours of it being added and implies that there is a sufficient supply of biologically available salt marsh organic matter to support this NO_3^- respiration.

To explore the capacity of salt marsh microbes to use NO_3^- to decompose organic matter over longer time periods, we used a flow through reactor (FTR) approach (Bulseco et al. 2019). In an FTR, seawater flows through reactors filled with homogenized marsh sediment and peat, half of which were enriched for approximately 90 days with ^{15}N-NO_3^-, and we measured total NO_3^- reduction on the basis of the difference between the NO_3^- concentration entering the reactor and the concentration leaving the reactor. Simultaneously, we measured production of $^{30}N_2$ (denitrification) and production of $^{15}NH_4$ (DNRA) in sediments collected from the top 5 centimeters (cm), 10–15 cm, and 20–25 cm depth from a *Spartina* marsh (table 1). Regardless of the depth from which the sediments were collected, NO_3^- addition resulted in a stimulation of decomposition (Bulseco et al. 2019). When we added enough NO_3^- that it was never limiting, denitrification was the dominant NO_3^- loss process (table 1). The combined rates of denitrification and DNRA accounted for the total amount of NO_3^- that was consumed by the reactors (table 1), further supporting our supposition that seemingly biologically unavailable salt marsh peat can support substantial rates of denitrification.

NO_3^- respiration liberates more free energy for microbes than respiration via sulfate reduction or fermentation, two of the most important organic matter decomposition processes in salt marsh sediments (Howarth 1984). Therefore, we hypothesized that the addition of NO_3^- would not only stimulate additional decomposition, but also that there would be a pool of complex organic matter that could be decomposed in the presence of NO_3^- that would not be decomposed by microbes using less energetically favorable electron acceptors, such as sulfate (Bulseco et al. 2019). Indeed, deeper sediments showed less stimulation of dissolved inorganic carbon (DIC) production than surface sediments, indicating that the quality of the carbon in salt marsh sediments does differ; however, at all depths, respiration was stimulated by NO_3^- addition (Bulseco et al. 2019).

We also measured the byproducts of respiration in the flow through reactor experiment and calculated the ratio of DIC:NH_4^+ produced over time (figure 5). We found that in surface sediments, where newly deposited biologically available carbon is abundant, there was little difference in the DIC:NH_4^+ ratio between reactors that received NO_3^- and those that did not. However, in the mid-depth and deep sediments, where the organic matter is likely less biologically available and more complex, there was an increase in the DIC:NH_4^+ ratio when NO_3^- was added compared with the unamended reactors. This suggests that the addition of NO_3^- allowed the microbes to access a pool of organic matter that was not accessed in the unamended reactors. In the absence of added NO_3^-, this carbon would remain stable, which has implications for our understanding of carbon storage in eutrophic systems. If excess NO_3^- in coastal systems allows for enhanced decomposition of more complex carbon it could slow the rate of carbon storage in these systems, relative to systems that do not receive excess NO_3^- supply.

To confirm the microbial role in NO_3^- use via enhanced denitrification or DNRA and to test our supposition that microbes exposed to high concentrations of NO_3^- are able to decompose more complex organic matter, we used metagenomics to link our rate measurements to their underlying microbial mechanisms. Analysis of sediment metagenomics is complex because the tremendous diversity of sediment microbes makes deciphering patterns in genetic changes challenging. Previous research on the microbial community structure in the TIDE project indicated that although NO_3^- increased microbial leucine uptake (a proxy for microbial production) in bare sediment that receives sufficient light to promote increased microalgal production, there did not appear to be a stimulation of

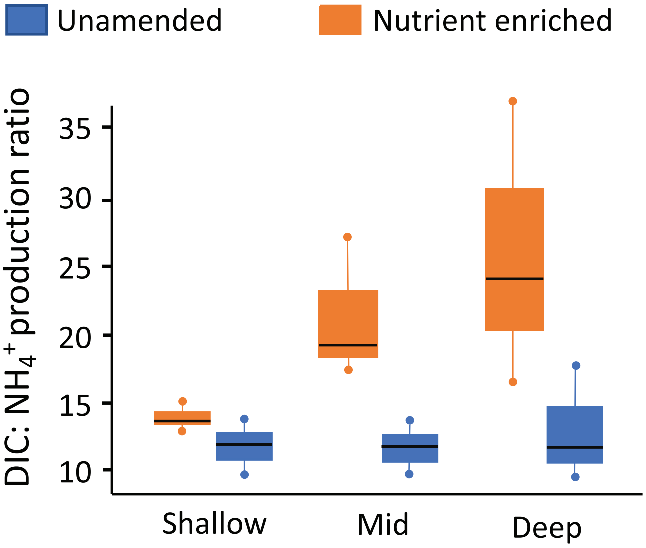

Figure 5. DIC:NH_4^+ production ratio, as a proxy for the complexity of the organic matter being decomposed. A high ratio indicates a more complex carbon source. Horizontal black bars indicate the median values and whiskers represent the upper and lower quartiles. Source: Adapted from Bulseco and colleagues (2019).

microbial productivity in more heavily vegetated regions of the marsh (Bowen et al. 2009a). Even in regions where leucine uptake was enhanced, the increase in leucine uptake did not translate into shifts in the overall microbial community structure, which was remarkably consistent over a decade of nutrient enrichment (Bowen et al. 2009b, 2011). We did observe large shifts in the active bacterial community, including shifts in bacterial taxa known to denitrify (Kearns et al. 2016), and shifts in putative fungal denitrifiers (Kearns et al. 2019), which suggests that microbial communities using NO_3^- as an electron acceptor may be able to increase respiration using NO_3^-, without leading to a whole scale shift in the community structure.

In light of these results, it is not surprising that we were unable to detect a strong signal of nutrient enrichment in the microbial metagenomes from our field experiment (figure 6a). In the FTR experiments, however, where NO_3^- was added at higher concentrations and in a continuous manner, we saw broad shifts in the metagenomes (figure 6b) that are consistent with the biogeochemical rate measurements (Bulseco et al. 2020). Many genes involved in central carbon metabolism were enhanced in the NO_3^- addition (figure 6c, supplemental table S1), including increases in the Entner–Doudoroff pathway for the generation of pyruvate from glucose, and decarboxylates that harness that pyruvate for cellular respiration, supporting the hypothesis that the addition of NO_3^- allows the microbial community to access otherwise inaccessible organic matter.

Surprisingly, we also observed an increase in genes associated with CO_2 fixation as a result of nutrient enrichment, suggesting that added NO_3^- could also promote autotrophic denitrification pathways (Bulseco et al. 2020). Many of the genes responsible for the cycling of nitrogen, including all the genes found in the denitrification pathway, were also significantly higher in the NO_3^- amended FTRs, compared with the unamended reactors (figure 6d, table S1). By contrast, subsystems involved in fermentation and other low energy producing metabolisms were more abundant in the unamended treatment (figure 6c) as were genes responsible for N fixation (figure 6d). The metagenomics findings from our FTR experiment are consistent with the biogeochemical rates we measured (Bulseco et al. 2020) and provide a mechanistic link that connects microbial genetics to the NO_3^- loss and DIC production we observed under NO_3^- addition.

Conclusions

Reactive nitrogen enrichment of the coastal zone is well documented, but frequently, the form of N is overlooked. Our results suggest that understanding the form of N that is biologically available in coastal systems is critically important for managing coastal resources. Most anthropogenically derived N delivered to the coastal zone is in its oxidized form, as NO_3^-. Our rate measurements, both in the field and in the laboratory, show that NO_3^- is being used as an electron acceptor to fuel decomposition, and that this overshadows its role as a nutrient to support growth of plant biomass. Our results suggest that microbes can access available NO_3^- and use it as an electron acceptor to respire organic matter that might have otherwise been stored. This process could potentially decrease the carbon sink capacity of marshes because of enhanced decomposition and limit effects on primary production needed to facilitate sediment trapping and offset increased decomposition.

In all likelihood, the use of NO_3^- in coastal marsh sediments is not a winner take all scenario, where 100% of the resource goes either to the microbes or to the plants, but rather, the ecosystem outcome is a function of the relative responses of the two groups of organisms. Several unknowns need resolution before predictive models (e.g., the Marsh Equilibrium Model, Morris and Bowden 1986, Morris et al. 2002) can accurately incorporate nutrient supply into their algorithms to predict marsh persistence relative to sea level rise. First, with a carbon to N ratio of around 30, marsh vegetation requires a fairly small amount of N to fix a fairly large amount of carbon and it remains to be seen whether losing a portion of that NO_3^- to the microbial community materially alters the productivity or annual storage by vascular plants. Second, we need to better parameterize whether variations in the timing, supply, and duration of use of NO_3^- or NH_4^+ by plants, microalgae, and microbes has long-term consequences for marsh carbon storage. In particular, the variation in growing season length and phenology, which may drive plant nutrient uptake, varies with latitude and could affect response to increasing supply of NO_3^- and NH_4^+. Third, we need a better understanding of how aboveground

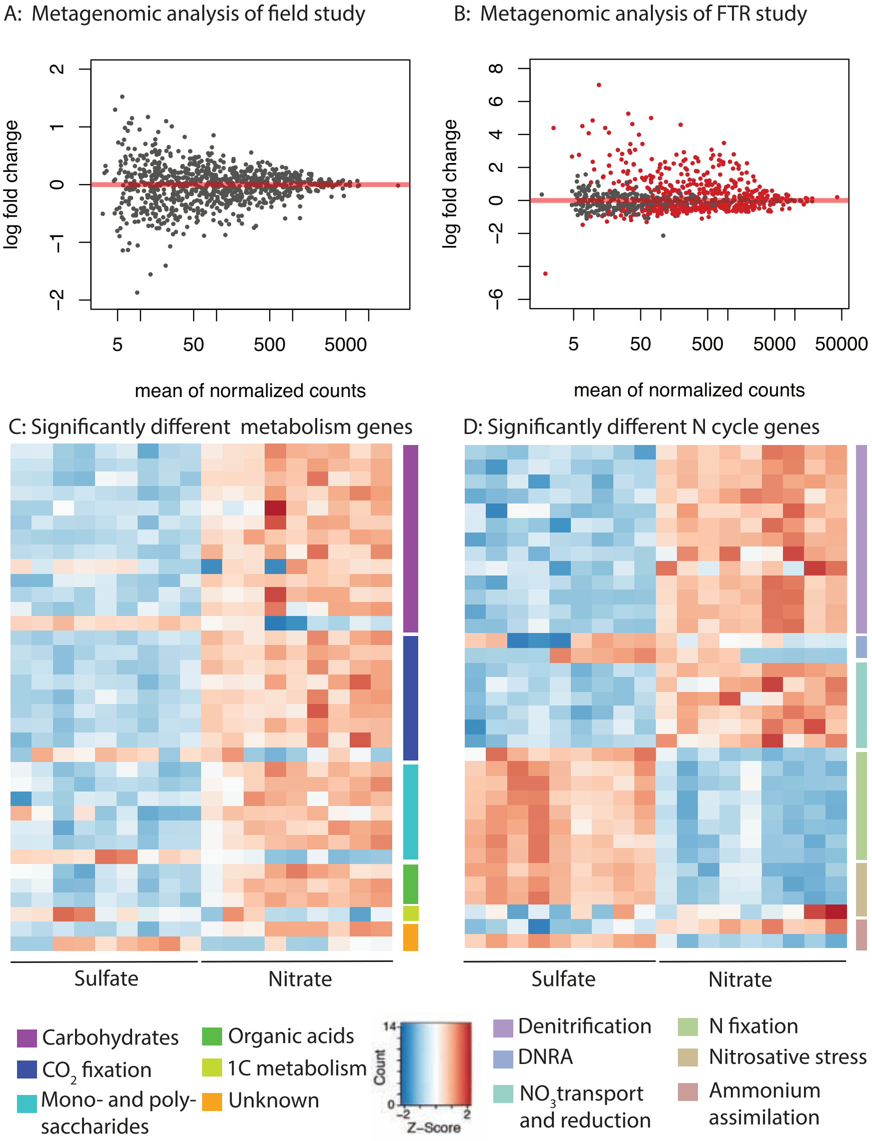

Figure 6. Metagenomic evidence for increased microbial respiration during NO_3^- addition in field and laboratory experiments. (a) Log-fold differences in gene abundance between NO_3^- enriched and unenriched salt marsh surface sediments collected from the TIDE nutrient enrichment experiment. (b) Log-fold differences in gene abundance in FTRs between NO_3^- amended reactors and reactors receiving only seawater. Red points indicate genes that are significantly different between the two treatments. (c) Heat map indicating the 35 biggest differences between amended and unamended FTRs for genes involved in carbon metabolism. (d) Heat map indicating the 35 biggest differences between amended and unamended FTRs for genes involved in the nitrogen cycle. Source: The data used in panels (c) and (d) were adapted from Bulseco and colleagues (2020).

and belowground biomass is partitioned and how these factors feedback on sediment trapping and storage when N is supplied as NO_3^- rather than as NH_4^+. Finally, our genomic data suggest that chemoautotrophic metabolisms could play a larger than expected role in carbon fixation in nutrient enriched marshes; however, the extent of this needs to be better parameterized. Understanding these critical unknowns will be essential for predicting marsh carbon storage in a high nutrient world.

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Supplemental material

Supplemental data are available at *BIOSCI* online.

References cited

An S, Gardner WS. 2002. Dissimilatory nitrate reduction to ammonium (DNRA) as a nitrogen link, versus denitrification as a sink in a shallow estuary (Laguna/Madre/Baffin Bay, Texas). Marine Ecology Progress Series 237: 41–50.

Anisfeld SC, Hill TD. 2012. Fertilization effects on elevation change and belowground carbon balance in a Long Island Sound tidal marsh. Estuaries and Coasts 35: 201–211.

Bertness MD, Crain C, Holdredge C, Sala N. 2008. Eutrophication and consumer control of New England salt marsh primary productivity. Conservation Biology 22: 131–139.

Billen G, Garnier J, Lassaletta L. 2013. The nitrogen cascade from agricultural soils to the sea: Modelling nitrogen transfers at regional watershed and global scales. Philosophical Transactions of the Royal Society B 368: 20130123.

Bowen JL, Kroeger KD, Tomasky G, Pabich WJ, Cole ML, Carmichael RH, Valiela I. 2007. A review of land-sea coupling by groundwater discharge of nitrogen to New England estuaries: Mechanisms and effects. Applied Geochemistry 22: 175–191.

Bowen JL, Crump BC, Deegan LA, Hobbie JE. 2009a. Increased supply of ambient nitrogen has minimal effect on salt marsh bacterial production. Limnology and Oceanography 54: 713–722.

Bowen JL, Crump BC, Deegan LA, Hobbie JE. 2009b. Salt marsh sediment bacteria: Their distribution and response to external nutrient inputs. ISME Journal 3: 924–934.

Bowen JL, Ward BB, Morrison HG, Hobbie JE, Valiela I, Deegan LA, Sogin ML. 2011. Microbial community composition in sediments resists perturbation by nutrient enrichment. ISME Journal 5: 1540–1548.

Brin LD, Valiela I, Goehringer D, Howes B. 2010. Nitrogen interception and export by experimental salt marsh plots exposed to chronic nutrient addition. Marine Ecology Progress Series 400: 3–17.

Bulseco AN, Giblin AE, Tucker J, Murphy AE, Sanderman J, Hiller-Bittrolff K, Bowen JL. 2019. Nitrate addition stimulates microbial decomposition of organic matter in salt marsh sediments. Global Change Biology 25: 3224–3241.

Bulseco AN, Vineis JH, Murphy AE, Spivak AC, Giblin AE, Tucker J, Bowen JL. 2020. Metagenomics coupled with biogeochemical rates measurements provide evidence that nitrate addition stimulates respiration in salt marsh sediments. Limnology and Oceanography 65: S321–S339.

Burgin AJ, Hamilton SK. 2007. Have we overemphasized the role of denitrification in aquatic ecosystems? Frontiers in Ecology and the Environment 5: 89–96.

Caraco N, Cole JJ. 1999. Human impact on nitrate export: An analysis using major world rivers. Ambio 28: 167–170.

Cavalieri AJ, Huang AHC. 1981. Accumulation of proline and glycine betaine in *Spartina alterniflora* Loisel. in response to NaCl and nitrogen in the marsh. Oecologia 49: 224–228.

Cott GM, Caplan JS, Mozdzer TJ. 2018. Nitrogen uptake kinetics and saltmarsh plant responses to global change. Scientific Reports 8: 5398.

Dalsgaard T, Thamdrup B, Canfield DE. 2005. Anaerobic ammonium oxidation (anammox) in the marine environment. Research in Microbiology 156: 457–464.

Darby FA, Turner RE. 2008. Below- and aboveground biomass of *Spartina alterniflora*: Response to nutrient addition in a Louisiana salt marsh. Estuaries and Coasts 31: 326–334.

Davis J, Currin C, Morris JT. 2017. Impacts of fertilization and tidal inundation on elevation change in microtidal, low relief salt marshes. Estuaries and Coasts 40: 1677–1687.

Deegan LA, *et al.* 2007. Susceptibility of salt marshes to nutrient enrichment and predator removal. Ecological Applications 17: S42–S63.

Deegan LA, Johnson, DS, Warren RS, Peterson BJ, Fleeger JW, Fagherazzi S, Wollheim WM. 2012. Coastal eutrophication as a driver of salt marsh loss. Nature 490: 388–392.

DeLaune RD, Pezeshki RD, Jugsujinda A. 2005. Impact of Mississippi River freshwater reintroduction on *Spartina patens* marshes: Response to nutrient input and lowering of salinity. Wetlands 25: 155–161.

Diaz, RJ, Rosenberg R. 2008. Spreading dead zones and consequences for marine ecosystems. 2008. Science 321: 926–929.

Drake DC, Peterson BJ, Galván KA, Deegan LA, Hopkinson C, Johnson JM, Koop-Jakobsen K, Lemay LE, Picard C. 2009. Salt marsh ecosystem biogeochemical responses to nutrient enrichment: A paired ^{15}N tracer study. Ecology 90: 2535–2546.

Emery NC, Ewanchuk PJ, Bertness MD. 2001. Competition and salt marsh plant zonation: Stress tolerators may be dominant competitors. Ecology 82: 2471–2485.

Erisman JW, Sutton MA, Galloway J, Klimont Z, Winiwarter, W. 2008. How a century of ammonia synthesis changed the world. Nature Geoscience 1: 636–639.

Fisher DC, Oppenheimer M. 1991. Atmospheric nitrogen deposition and the Chesapeake Bay estuary. Ambio 20: 102–108.

Forster SSD, Cripps AC, Smith-Carington A. 1982. Nitrate leaching to groundwater. Philosophical Transactions of the Royal Society B 296: 21. http://doi.org/10.1098/rstb.1982.0021.

Fowler D, et al. 2013. The global nitrogen cycle in the twenty-first century. Philosophical Transactions of the Royal Society B 368: 20130164.

Galloway JN, Aber JD, Erisman JW, Seitzinger SP, Howarth RW, Cowling EB, Cosby BJ. 2003. The Nitrogen Cascade. BioScience 53: 341–356.

Galloway JN, Townsend AR, Erisman JW, Bekunda M, Cai Z, Freney JR, Martinelli LA, Seitzinger SP, Sutton MA. 2008. Transformations of the nitrogen cycle: Recent trends, questions, and potential solutions. Science 320: 889–892.

Galván K, Fleeger JW, Peterson B, Drake D, Deegan LA, Johnson DS. 2011. Natural stable isotopes and dual isotope tracer additions help to resolve resources supporting a saltmarsh food web. Journal of Experimental Marine Biology and Ecology 410: 1–11.

Geoghegan EK, Caplan JS, Leech FN, Weber PE, Bauer CE, and Mozdzer TJ. 2018. Nitrogen enrichment alters carbon fluxes in a New England salt marsh. Ecosystem Health and Sustainability 4: 277–287.

Giblin AE, Tobias CR, Song B, Weston N, Banta GT. 2013. The importance of dissimilatory nitrate reduction to ammonium (DNRA) in the nitrogen cycle of coastal ecosystems. Oceanography 26: 124–131.

Gilliam FS, Burns DA, Driscoll CT, Frey SD, Lovett GM Watmough SA. 2019. Decreased atmospheric deposition in eastern North America: Predicted responses of forest ecosystems. Environmental Pollution 244: 560–574.

Hedges LV, Gurevitch J, Curtis PS. 1999. The meta-analysis of response rations in experimental ecology. Ecology 80: 1150–1156.

Hinga KE, Keller AA, Oviatt CA. 1991. Atmospheric deposition and nitrogen input to coastal waters. Ambio 20: 256–260.

Howarth RW. 1984. The ecological significance of sulfur in the energy dynamics of salt marsh and coastal marine sediments. Biogeochemistry 1: 5–27.

Huang J, Xu X, Wang M, Nie M, Qiu S, Wang Q, Quan Z, Xiao M, Li B. 2015. Responses of soil nitrogen fixation to *Spartina alterniflora* invasion and nitrogen addition in a Chinese salt marsh. Scientific Reports 6: 20384.

Hughes JE, Deegan LA, Peterson BJ, Holmes RM, Fry B. 2000. Nitrogen flow through the food web in the oligohaline zone of a New England estuary. Ecology 81: 433–452.

Johnson DJ, Warren RS, Deegan LA, Mozdzer TJ. 2016. Saltmarsh plant responses to eutrophication. Ecological Applications 26: 2649–2661.

Kearns PJ, Angell JH, Howard EM, Deegan LA, Stanley RHR, Bowen JL. 2016. Nutrient enrichment induces dormancy and decreases diversity of active bacteria in salt marsh sediments. Nature Communications 7: 12881.

Kearns PJ, Bulseco AN, Hoyt H, Angell JH, Bowen JL. 2019. Nutrient enrichment alters salt marsh fungal communities and promotes putative fungal denitrifiers. Microbial Ecology 77: 358–369.

Kiehl K, Esselink P, Bakker JP. 1997. Nutrient limitation and plant species composition in temperate salt marshes. Oecologia 111: 325–330.

Kirwan ML, Megonigal JP. 2013. Tidal wetland stability in the face of human impacts and sea-level rise. Nature 504: 53–60.

Kirwan ML, Guntenspergen GR, D'Alpaos A, Morris JT, Mudd SM, Temmerman S. 2010. Limits on the adaptability of coastal marshes to rising sea level. Geophysical Research Letters 37: L23401.

Koop-Jakobsen K, Giblin AE. 2009. Annamox in tidal marsh sediments: The role of vegetation, salinity, nitrogen loading and marsh vegetation. Estuaries and Coasts 32: 238–245.

Koop-Jakobsen, K, Giblin AE. 2010. The effect of increased nitrate loading on nitrate reduction via denitrification and DNRA in salt marsh sediments Limnology and Oceanography 55: 789–802.

Kroeger KD, Charette MA. 2008. Nitrogen biogeochemistry of submarine groundwater discharge. Limnology and Oceanography 53: 1025–1039.

Lajeunesse MJ. 2011. On the meta-analysis of response ratios for studies with correlated and multi-group designs. Ecology 92: 2049–2055.

Levine JM, Brewer JS, Bertness MD. 1998. Nutrients, competition and plant zonation in a New England salt marsh. Journal of Ecology 86: 285–292.

Lomas MW, Glibert PM. 2000. Comparisons of nitrate uptake, storage, and reduction in marine diatoms and flagellates. Journal of Phycology 36: 903–913.

Longphuirt SN, Lim J-H, Leynaert A, Claquin P, Choy E-J, Kang C-K, An S. 2009. Dissolved inorganic nitrogen uptake by intertidal microphytobenthos: Nutrient concentrations, light availability and migration. Marine Ecology Progress Series 379: 33–44.

Lloret J, Valiela I. 2016. Unprecedented decrease in deposition of nitrogen oxides over North America: The relative effects of emission controls and prevailing air-mass trajectories. Biogeochemistry 129: 165–180.

McFarlin CR, Brewer JS, Buck TL, Pennings S. 2008. Impact of fertilization on a salt marsh food web in Georgia. Estuaries and Coasts 31: 313–325.

Mendelssohn IA. 1979a. The influence of nitrogen level, form, and application method on the growth response of *Spartina alterniflora* in North Carolina. Estuaries 2: 106–112.

Mendelssohn IA. 1979b. Nitrogen metabolism in the height forms of *Spartina alterniflora* in North Carolina. Ecology 60: 574–584.

Morris JT, Bowden WB. 1986. A mechanistic numerical model of sedimentation, mineralization, and decomposition for marsh sediments. Soil Science Society of America Journal 50: 96–105.

Morris JT, Sundareshwar PV, Nietch CT, Kjerfve B, Cahoon DR. 2002. Response of coastal wetlands to rising sea level. Ecology 83: 2869–2877.

Morris JT, Sundberg K, Hopkinson CS. 2013. Salt marsh primary production and its responses to relative sea level and nutrients in estuaries at Plum Island, Massachusetts, and North Inlet, South Carolina USA. Oceanography 26: 78–84.

Mozdzer TJ, Kirwan M, McGlathery KJ, Zieman JC. 2011. Nitrogen uptake by the shoots of smooth cordgrass *Spartina alterniflora*. Marine Ecology Progress Series 433: 43–52.

Murphy SM, Wimp GM, Lewis D, Denno RF. 2012. Nutrient presses and pulses differentially impact plants, herbivores, detritivores and their natural enemies. PLOS ONE 7: e43929.

Nelson JL, Zavaleta ES. 2012. Salt marshes as a coastal filter for the oceans: Changes I function with experimental increases in nitrogen loading and sea-level rise. PLOS ONE 7: e38558.

Nixon SW. 1995. Coastal marine eutrophication: A definition, social causes and future concerns Ophelia 41: 199–219.

Olcott CA. 2011. Impacts of Nitrogen Addition on the Monthly Above- and Belowground Production of *Spartina alterniflora* in a Virginia Marsh. Master's thesis. University of Virginia, Charlottesville, Virginia.

Oczkowski A, Wigand C, Hanson A, Markham E, Miller KM, Johnson R. 2016. Nitrogen retention in salt marsh systems across nutrient-enrichment, elevation, and precipitation regimes: A multiple-stressor experiment. Estuaries and Coasts 39: 68–81.

Orth RJ, *et al.* 2006. A global crisis for seagrass ecosystems. BioScience 56: 987–996.

Paerl HW. 1995. Coastal eutrophication in relation to atmospheric nitrogen deposition: Current perspectives. Ophelia 41: 237–259.

Paerl HW, Dennis RL, Whitall DR. 2002. Atmospheric deposition of nitrogen: Implications for nutrient over-enrichment of coastal waters. Estuaries 25: 677–693.

Pascal P-Y, Fleeger JW. 2013. Diverse dietary responses by saltmarsh consumers to chornic nutrient enrichment. Estuaries and Coasts 36: 1115–1124.

Peierls BL, Caraco NF, Pace ML, Cole JJ. 1991. Human influence on river nitrogen. Nature 350: 386–387.

Priest B. 2007. Effects of Elevation and Nutrient Availability on the Primary Production of *Spartna alterniflora* and the Stability of Southeastern Coastal Salt Marshes Relative to Sea Level Rise. Master's thesis, University of South Carolina, Columbia, South Carolina.

Rivett MO, Buss SR, Morgan P, Smith JWN, Bemment CD. 2008. Nitrate attenuation in groundwater: A review of biogeochemical controlling processes. Water Research 42: 4215–4232.

Silliman BR, Zieman JC. 2002. Top-down control of *Spartina alterniflora* production by periwinkle grazing in a Virgina salt marsh. Ecology 82: 2830–2845.

Smil V. 2004. Enriching the Earth: Fritz Haber, Carl Bosch, and the Transformation of World Food Production. MIT Press.

Smith DL, McLachlan J. 1979. Nitrogen fixation, as determined by acetylene reduction, in two salt marshes of Minas Basin. Proceedings of the Nova Scotian Institute of Science 29: 381–392.

Sousa AI, Lillebø AI, Caçador I, Pardal MA. 2008. Contribution of *Spartina maritima* to the reduction of eutrophication in estuarine systems. Environmental Pollution 156: 628–635.

Stewart, WM, Dibb DW, Johnston AE, Smyth TJ. 2005. The contribution of commercial fertilizer nutrients to food production. Agronomy Journal 97: 1–6.

Sullivan MJ, Currin CA. 2002. Community structure and functional dynamics of benthic microalgae in salt marshes. Pages 81–106 in Weinstein MP, Kreeger DA, eds. Concepts and Controversies in Tidal Marsh Ecology. Springer.

Sundback K, Miles A. 2000. Balance between denitrification and microalgal incorporation of nitrogen in microtidal sediments, NE Kattegat. Aquatic Microbial Ecology 22: 291–300.

Tyler AC, Mastronicola TA, McGlathery KJ. 2003. Nitrogen fixation and nitrogen limitation of primary production along a natural marsh chronosequence. Oecologia 136: 431–438.

Tobias CR, Cieri M, Peterson BJ, Deegan LA, Vallino J, Hughes J. 2003a. Processing watershed-derived nitrogen in a well-flushed New England estuary. Limology and Oceanography 48: 1766–1788.

Tobias CR, Giblin AE, McClelland J, Tucker J, Peterson BJ. 2003b. Sediment DIN fluxes are preferentially recycling of benthic microalgal nitrogen in a shallow macrotidal estuary. Marine Ecology Progress Series 257: 25–36.

Valiela I, Cole ML. 2002. Comparative evidence that salt marshes and mangroves may protect seagrass meadows from land-derived nitrogen loads. Ecosystems 5: 92–102.

Valiela I, Teal JM. 1979. The nitrogen budget of a salt marsh ecosystem. Nature 280: 652–656.

Valiela I, Teal JM, Sass WJ. 1975. Production and dynamics of salt marsh vegetation and the effects of experimental treatment with sewage sludge: Biomass, production and species composition. Journal of Applied Ecology 12: 973–981.

Valiela I, Costa J, Foreman K, Teal JM, Howes B, Aubrey D. 1990. Transport of groundwater-borne nutrients and their effects on coastal waters. Biogeochemistry 10: 177–197.

Valiela I, Cole ML, Mcclelland J, Hauxwell J, Cebrian J, Joye SB. 2002. Role of salt marshes as part of coastal landscapes. Pages 23–36 in Weinstein MP, Kreeger DA, eds. Concepts and Controversies in Tidal Marsh Ecology. Springer.

Van Zomeren CM, White JR, DeLaune RD. 2011. Fate of nitrate in vegetated brackish coastal marsh. Soil Science of America Journal 76: 1919–1927.

Verhoeven JT, Arheimer B, Yin C, Hefting MM. 2006. Regional and global concerns over wetlands and water quality. Trends in Ecology and Evolution 21: 96–103.

Viechtbauer W. 2010. Conducting meta-analyses in R with the metaphor package. Journal of Statistical Software 36: 1–48.

Ward BB, Arp DJ, Klotz MG. 2011. Nitrification. American Society for Microbiology Press.

Weller DE, Jordan TE. 2020. Inexpensive spot sampling provides unexpectedly effective indicators of watershed nitrogen status. Ecosphere 11: e03224.

Wigand C, Thursby GB, McKinney RA, Santos AF. 2004. Response of *Spartina patens* to dissolved inorganic nutrient additions in the field. Journal of Coastal Research 45: 134–149.

Wigand C, Watson EB, Martin R, Johnson DS, Warren RS, Hanson A, Davey E, Johnson R and Deegan LA. 2018. Discontinuities in soil strength contribute to destabilization of nutrient-enriched creeks. Ecosphere 9: e02329.

Zhang Y, Wang L, Xie X, Huang L, Wu Y. 2013. Effects of invasion of *Spartina alterniflora* and exogenous N deposition on N2O emissions in a coastal marsh. Ecological Engineering 58: 77–83.

Zumft W. 1997. Cell biology and molecular basis of denitrification. Microbiology and Molecular Biology Reviews 61: 533–616.

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