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

JENNIFER L. BOWEN, ANNE E. GIBLIN, ANNA E. MURPHY, ASHLEY N. BULSECO, LINDA A. DEEGAN, DAVID S. JOHNSON, JAMES A. NELSON, THOMAS J. MOZDZER, AND HILLARY L. SULLIVAN

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, $\text{NO}_3^-$) and reduced forms (ammonium, $\text{NH}_4^+$) can relieve nutrient limitation and increase primary production. However, unlike $\text{NH}_4^+$, $\text{NO}_3^-$ can also be used as an electron acceptor for microbial respiration. We present results demonstrating that, in salt marshes, microbes use $\text{NO}_3^-$ 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 ($\text{NH}_4^+$) 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 ($\text{N}_r$). 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, Erismann et al. 2008), and therefore, increases in human population are tightly coupled with increases in fertilizer use (Erismann et al. 2008). Today, $\text{N}_r$ 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 $\text{N}_r$ from agricultural fields and other sources, through ecosystems, and into coastal waters is well documented (Galloway et al. 2003, Billen et al. 2013). Fertilizer $\text{N}_r$ 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 $\text{N}_r$ 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 $\text{N}_r$ 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 $\text{N}_r$ back to $\text{N}_2$ gas, essentially reversing the Haber–Bosch process, has limited industrial analogs, particular once $\text{N}_r$ enters coastal waters. Instead, to prevent eutrophication of the coastal zone, a series of microbial transformations of N must occur for the excess $\text{N}_r$ to be returned to inert $\text{N}_2$ 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 $\text{N}_r$ 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...
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.

The retention and loss of N$_2$ that are required to ultimately remove some fraction of anthropogenically derived N$_2$ delivered to the coastal zone; however, the amount of N$_2$ retained or removed varies widely, with wetlands in eutrophic systems retaining a smaller fraction of total N$_2$ than wetlands that receive lower N$_2$ 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$_2$ removal capacity (Valiela and Cole 2002).

The transformations of N$_2$ 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$_2$ gets delivered to the coastal zone. N$_2$ 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), with NH$_4^+$ readily binds to clay particles in soils), or can be directly discharged from treatment plants into estuarine and coastal waters. This N$_2$ is typically delivered to estuaries as NO$_3^-$ (Weller and Jordan 2020), with NH$_4^+$ often making up less than 10% of N$_2$ 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$_2$ 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$_2$ 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 (Gillian et al. 2019). The retention and loss of N$_2$ can occur through 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$_2$ (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$_2$ in the environment. Understanding the specific pathways involved in the processing of N$_2$ 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 (Bulresco et al. 2019).

When N\(_2\) 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\(_2\) 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\(_2\) is added in its reduced form, as NH\(_4^+\), the ecosystem scale outcome will depend on the balance between plant uptake and microbial nitrification. 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\(_2\) 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\(_2\).

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\(_2\) 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\(_2\) used in the experiment were clearly stated, N\(_2\) 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\(_2\) 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; NH\(_4^+\) \((n = 7)\), NH\(_4^+\)NO\(_3^–\) \((n = 4)\), other forms of NO\(_3^–\) \((n = 4)\), and organic N, either in the form of pelleted sewage sludge or organic fertilizer or as urea \((n = 7)\). We used the “escala” 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\(_2\) in the form of NO\(_3^–\) (excluding N\(_2\) that was added as NH\(_4^+\)NO\(_3^–\)) had a smaller effect size on plant biomass than other forms of N\(_2\) (figure 2a). When studies were aggregated together by the form of N\(_2\) 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\(_4^+\), \(p \leq .01\);
NO$_3^-$ versus NH$_4$NO$_3$, $p = .044$; 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 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$_2$ 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$_2$ 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 2. Results of meta-analysis that suggest response to N$_2$ 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$_2$ 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).
of added N\textsubscript{r} two different application methods (band, where N\textsubscript{r} was injected into the sediment, and broadcast, where N\textsubscript{r} was placed on the surface sediments), and two different N forms, (NH\textsubscript{4})\textsubscript{2}SO\textsubscript{4} and NaNO\textsubscript{3}. For both the short and tall ecotypes of *Spartina alterniflora* when N\textsubscript{r} was added as (NH\textsubscript{4})\textsubscript{2}SO\textsubscript{4} there was a greater increase in growth than when it was added as NaNO\textsubscript{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 (Mendelsohn 1979a). Our meta-analysis and Mendelsohn’s direct test indicate that *Spartina* biomass accumulation is lower with NO\textsubscript{3}\textsuperscript{−} enrichment, which could have consequences for the long-term sustainability of marshes in the face of rising sea levels.

**Whole creek $^{15}$N-NO\textsubscript{3} enrichment experiments support rapid uptake by microalgae and little NO\textsubscript{3}\textsuperscript{−} 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\textsubscript{r}. Benthic diatoms are capable of NO\textsubscript{3}\textsuperscript{−} uptake and storage (Lomas and Glibert 2000); however, responses to the addition of N\textsubscript{r} by benthic microalgae vary widely, depending on the study (Sullivan and Currrin 2002). Whole ecosystem additions of enriched $^{15}$N-NO\textsubscript{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\textsubscript{3}\textsuperscript{−}. However, the measured uptake kinetics of benthic microalgae were consistently higher when provided ammonium, compared with NO\textsubscript{3}\textsuperscript{−} in estuarine sediments of the Nakdong Estuary on the Korean Peninsula (Longphuirt et al. 2009). Our 13-year salt marsh NO\textsubscript{3}\textsuperscript{−} enrichment experiment, referred to as the TIDE project, showed that the addition of NO\textsubscript{3}\textsuperscript{−} 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\textsubscript{3}\textsuperscript{−} between salt marsh macrophytes, benthic microalgae, and microbial dissimilatory nitrate respiration pathways can partly be disentangled using whole marsh $^{15}$N-NO\textsubscript{3} addition experiments (Drake et al. 2009). As a part of the TIDE project, we performed a whole-creek marsh $^{15}$N-NO\textsubscript{3} addition experiment in a creek that was amended to 70 micromolar (µM) NO\textsubscript{3}\textsuperscript{−} dissolved into flooding tidal waters on every tide for 2 years and in reference creeks that had ambient (less than 5 µM) NO\textsubscript{3}\textsuperscript{−} concentrations. $^{15}$N-NO\textsubscript{3} was added to both creeks for 5 days in midsummer (23–28 July 2005). In the NO\textsubscript{3}\textsuperscript{−} enriched creek, enough $^{15}$N-NO\textsubscript{3} was added to maintain creek water at 650‰ enrichment while keeping the creek NO\textsubscript{3}\textsuperscript{−} 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\textsubscript{3}\textsuperscript{−} concentration (Drake et al. 2009). The reference creek was able to retain 98.8% of the added $^{15}$N-NO\textsubscript{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\textsubscript{3}\textsuperscript{−}. After all...
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 microbial 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
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 creks, 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 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.

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 microbial production, there did not appear to be a stimulation of

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

| Depth          | Nitrate reduction (mmol per cm$^3$) | DNF (mmol per cm$^3$) | DNRA (mmol per cm$^3$) | DNRA + DNF (mmol per cm$^3$) | Percentage of nitrate reduction |
|----------------|-------------------------------------|-----------------------|------------------------|-------------------------------|--------------------------------|
| Shallow (0–5 cm) | 87.5 (M) 7.4 (SD)                   | 70.6 (M) 1.7 (SD)     | 10.2 (M) 0.6 (SD)      | 80.9 (M) 1.8 (SD)             | 92                             |
| Mid (10-15 cm)  | 61.3 (M) 4.1 (SD)                   | 58.1 (M) 3.7 (SD)     | 4.8 (M) 1.4 (SD)       | 63 (M) 4.0 (SD)               | 102                            |
| Deep (20–25 cm) | 70.9 (M) 20.4 (SD)                  | 55.2 (M) 10.3 (SD)    | 3.6 (M) 0.9 (SD)       | 58.8 (M) 10.3 (SD)            | 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, cubic centimeters; mmol, millimoles.
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$_3^-$.

Conclusions

Reactive nitrogen enrichment of the coastal zone is well documented, but frequently, the form of N is overlooked. Our results support 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$_3^-$.

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).
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.

Acknowledgments

This work was supported by the following funding sources: National Science Foundation (NSF) grant no. DEB 1902712 to LAD, JLB, DSJ, and TJM; NSF grant no. DEB 1902695 to AEG; NSF grant no. DEB 1902704 to JAN; NSF grant no. DEB 1354214 to TJM; NSF grant no. DEB 1350491 to JLB; NSF grant no. OCE 1637630 to AEG and LAD; and additional funding from the Dorr Foundation, the Department of the Interior Northeast Climate Science Center (grant no. DOI G12AC00001), and a Bullard Fellowship (Harvard University) to JAN. Resources purchased with funds from the NSF Biological Field Stations and Marine Laboratories program (grant no. DBI 1722553, to Northeastern University) were used to generate the data for the manuscript. Initial conversations on the effects of nutrient enrichment in marshes with Scott Warren and Bruce Peterson were critical in informing the work described in the manuscript. Sam Kelsey and Jane Tucker contributed to much of the N cycling biogeochemistry; Caitlin Bauer, Frankie Leach, Paige Weber, Emily Geoghegan and Sophie Drew assisted with field work; and Joe Vineis assisted with metagenomic analysis. This is contribution 3941 from the Virginia Institute of Marine Science. The data were compiled from multiple published sources. Links to published data can be found here: https://pie-lter.ecosystems.mbl.edu/data. The sequence data used to derive figure 6 are publicly available on the MG-RAST website under project number mgp84173.

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
See page 1117 of BioScience for the full text.
Koop-Jakobsen, K, Giblin AE. 2010. The effect of increased nitrate loading on nutrient retention via denitrification and DNRA in salt marsh sediments. Limnology and Oceanography 55: 789–802.

Kroeger KD, Charette MA. 2008. Nitrogen biogeochemistry of submarrine 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, Gilbert PM. 2000. Comparisons of nitrate uptake, storage, and reduction in marine diatoms and flagellates. Journal of Physiology 36: 903–913.

Longhuirt 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 37: 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. Biogeochmistry 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.

Mendelsohn 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.

Mendelsohn 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, Sundberg K, Niechta CT, Kierfe B, Kahoon 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 pressures 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 KN, 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 chonic 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 BC. 2007. Effects of Elevation and Nutrient Availability on the Primary Production of Spartina 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 JW, Bennert 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 Virginia 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 AJ, 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 85–106 in Weinstein MP, Kreeger DA, eds. Concepts and Controversies in Tidal Marsh Ecology. Springer.

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

Tyler AC, Mastronikita 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 JI, Hughes J. 2003a. Processing watershed-derived nitrogen in a well-flushed New England estuary. Limnology and Oceanography 48: 1766–1788.

Tobias CR, Giblin AE, McClelland J, Tucker J, Peterson BJ. 2003b. Sediment DIN fluxes are preferentially recycling of benthic microbial 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, Joyce 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.

Vuichbauer 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 surprisingly 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.

1118 BioScience December 2020 / Vol. 70 No. 12

https://academic.oup.com/bioscience
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.

Jennifer Bowen (je.bowen@northeastern.edu) is an associate professor and Anna Murphy is a postdoctoral scholar at Northeastern University’s Marine Science Center, in Nahant, Massachusetts, and a senior scientist at INSPIRE Environmental, in Newport, Rhode Island. Linda Deegan is a senior scientist and Hillary Sullivan is a research assistant at the Woodwell Climate Research Center (formerly, the Woods Hole Research Center), in Falmouth, Massachusetts. Deegan leads the TIDE project, the long-term nutrient enrichment experiment from which much of these results derive. Anne Giblin is the director of the Plum Island Ecosystems LTER, on Plum Island, Massachusetts, and Ashley Bulseco was a postdoctoral scholar at the Marine Biological Laboratory, in Woods Hole, Massachusetts, and is now an assistant professor of Marine Science at Eckerd College, in St. Petersburg, Florida. David Samuel Johnson is an assistant professor at the Virginia Institute of Marine Science, at William and Mary, in Gloucester Point, Virginia. Thomas Mozdzer is an associate professor at Bryn Mawr College, in Bryn Mawr, Pennsylvania. James Nelson is an assistant professor at the University of Louisiana at Lafayette.