

New Developments in the Marine Nitrogen Cycle

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1. Introduction

Nitrogen, as a building block in the structures of nucleic acid and amino acids, porphyrins, and amino sugars, is a fundamental player in many biogeochemical cycles.¹ It also shares with many elements a role in reduction–oxidation reactions in the marine environment.^{2,3} Additionally, nitrogen is strongly impacted by anthropogenic activities.^{4–6} Most nitrogen in marine environments is present in five forms: N₂, a quite stable molecule that requires specialized enzymatic systems to break and use; nitrate, the most oxidized form of nitrogen and the dominant biologically utilizable form of N within oxic environments; ammonium, the most reduced natural form of N and the dominant biologically available form found in anoxic environments; particulate nitrogen, predominant within sediments and primarily in the form of organic N, and dissolved organic N (DON), a complex mixture of compounds with a wide range of compositions.^{7–9} Nitrate, nitrite, ammonium, and organic nitrogen are typically grouped together as “fixed N” in discussions of nitrogen availability, although each form has a different level of reactivity. A complex web of reactions links these different compounds in ways that are still being determined. In the simplest sense, these reactions, together

with major flux terms, describe a marine nitrogen cycle reduced to six terms: N₂ fixation, riverine inputs, atmospheric fallout, sediment organic matter burial, and water column and sedimentary denitrification (conversion of fixed N to N₂).^{6,10–12} Our understanding of the relative and absolute importance of each process has changed dramatically over the past 40 years.

Early marine nitrogen studies focused on the role of N as a primary productivity-limiting element. The advent of the “Redfield Ratio (RR)”^{13,14} provided a simple metric to determine whether nitrogen or phosphorus would limit overall levels of primary productivity in a particular ecosystem. Simply put, the RR hypothesis posits that all marine organic matter consists of material with roughly 16 N for every one P. One can thus use this assumption to both predict the usage ratios and remineralization ratios of inorganic N and P within the water column. Global studies of dissolved nutrient patterns show strong correlations between the abundances of PO₄^{3–} and NO₃[–] that would be expected if “Redfieldian” organic matter was being remineralized.¹⁵ One can thus use this assumption to predict the usage ratios and remineralization ratios of N and P. Deviations in the N/P stoichiometry of dissolved nutrient concentrations,¹⁶ defined by the tracer N* (N* = [NO₃] – 16[PO₄] + 2.9),¹⁷ therefore reflect non-Redfield biological nutrient inputs, such as N₂ fixation, which causes N* to increase, and losses such as denitrification, which reduces N*. In suboxic water columns, such as occur in the eastern tropical Pacific (ETP) Ocean and the northern Arabian Sea (AS), N* values indicated the loss of NO₃[–] as (unmeasured) N₂ via the process of denitrification. An earlier version of the N* relationship was used to estimate the difference between observed and calculated fixed N levels.¹⁶ This result, together with residence time estimates, was then used to estimate the fixed nitrogen loss from the ETP and subsequently expanded to cover other suboxic and anoxic regions.^{18–21}

While water column N losses generate observable imprints on ocean chemistry, sedimentary N losses are more difficult to quantify because rates depend on direct flux measurements and sediments exhibit wide variations in N/P fluxes.^{22,23} Initial efforts to quantify fixed nitrogen losses resulted in underestimates because only fluxes from the water column were considered. The advent of direct measurements of N₂ fluxes from sediments provided more reliable estimates,²⁴ but the combination of making difficult measurements against a large dissolved N₂ background and the sparse coverage of sediment respiration measurements has led to wide uncertainties in the values assigned to sedimentary fixed nitrogen losses. The most striking aspect of the sedimentary denitrification literature has been a marked increase in global flux estimates as measurements are conducted in more regions

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and with better techniques.^{6,25} For example, up until the late 1980s, it was often assumed that marine sedimentary denitrification was around 85 Tg of N per year (1 Tg of N = 1×10^{12} g of N).^{10,26} However, a dramatic increase in such estimates has occurred over the past 2 decades; using a variety of different techniques, investigators have arrived at values of between 200 and 300 Tg of N year for sedimentary denitrification.^{6,11,25,27} Also striking has been the discovery of new processes, primarily suboxic in nature, that remove fixed N from sedimentary and some water column environments in ways quite different from "classic" or "canonical" denitrification.^{28–31} The influence of these new



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processes is still being debated, but in some environments, they can dominate the loss of fixed N.³²

A logical consequence of the increase in denitrification estimates has been to create difficulties in achieving balanced marine fixed nitrogen budgets, which would require higher globally integrated nitrogen fixation rates.^{6,11,12,25} The focus on these two terms remains because other terms are either relatively well constrained (sedimentary N burial can be estimated from a wealth of organic matter studies) or cannot be logically increased by 2–3 times (e.g., riverine and atmospheric inputs). However, a number of N fixation studies indicate that N₂ fixation both is more widespread and involves a much larger number of organisms than previously assumed. Thus, nitrogen fixation rates may be sufficient to generate a balanced marine N budget.

Several overviews of the marine N cycle have been published over the past few years.^{6,33,34} This work will focus on the frontiers of this field, with special attention to three areas: new processes leading to nitrogen losses, sites of nitrogen fixation, and an assessment to balance the pre-industrial marine N budget.

2. Denitrification and the Global Marine N Cycle

2.1. Canonical Denitrification

Two decades ago, a relatively simple diagram of the marine nitrogen cycle was adequate to explain all known processes (Figure 1, based on ref 35). Biologically available nitrogen, whether generated on land or sea, was converted from N₂ by nitrogen-fixing bacteria. This fixed N made its way into the total biologically available N pool by remineralization of organic matter and subsequent bacterial nitrification of ammonium to nitrate in oxic environments. Where intermediary species, such as nitrite and N₂O, were present, they were considered to be ephemeral indicators of robust N cycling between the major end members of N₂, nitrate, ammonium, or organic nitrogen.^{36–39} Denitrification was considered to be a simple heterotrophic process whereby nitrate was used as the terminal electron acceptor in the oxidation of organic matter after dissolved oxygen was exhausted, and this reaction was assumed to be conducted by facultative anaerobic organisms.^{2,6,40,41} Thus, denitrifica-

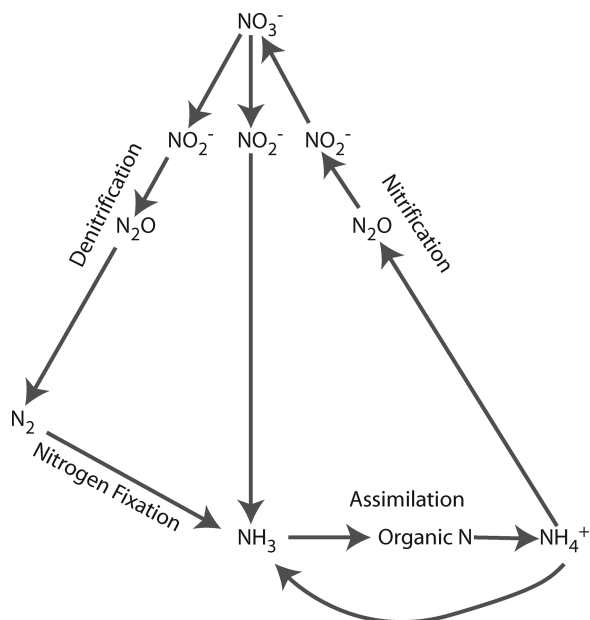


Figure 1. Diagram of the marine nitrogen cycle, based on ref 35. Arrows represent the direction of named reactions.

tion, the only then known loss route of fixed N to N_2 gas, was confined to sediments and water columns with $<2\text{--}4\ \mu\text{M}$ dissolved O_2 concentrations, that is, suboxic environments. These assumptions were based upon observation of patterns in suboxic water columns, and denitrification under these limitations is described as “canonical” denitrification.

Suboxic conditions occur in marine sediments because supply of oxygen to the sediments is limited by molecular diffusion from the overlying water (muddy sediments), and oxygen demand is high due to accumulation of sedimenting detritus. In general, continental shelf and upper slope waters (less than $\sim 1000\text{ m}$ water depth) have oxygen penetration depths less than 1 cm .⁴² Suboxic conditions also occur in the water column of the pelagic ocean in several locations, namely, the eastern tropical north and south Pacific and the Arabian Sea. Water column suboxia occurs over continental shelves, either as a natural phenomenon as on the Benguelian shelf⁴³ or due to anthropogenic influences such in the northwestern Gulf of Mexico⁴⁴ or off the western coast of India.⁴⁵

Ammonium entering suboxic systems, by remineralization of organic matter within such systems or by diffusive transport from underlying anoxic waters/sediments, was assumed to be oxidized to nitrate and then denitrified. This process, termed coupled nitrification–denitrification, explained N_2 fluxes from sediments that were too large to be supported by NO_3^- diffusion supply alone.²⁴ However, the lack of a buildup of ammonium in suboxic waters remained a problem (see discussion below). Ammonium oxidation was believed to produce only oxides and not N_2 directly, while N_2 was thought to be the only end product of heterotrophic nitrate reduction. These concepts are summarized by the processes illustrated on the outside of the circle of nitrogen species in Figure 2. Beginning in the late 1980s and accelerating in the 1990s, a host of new processes were discovered, generally in nonmarine environments, that led to the pathways described within the circle of N species in Figure 2. These processes are marked by either (1) the lack of a requirement for the participation of oxygen per se or of nitrification to nitrate (anaerobic ammonia oxidation or anammox and oxygen-limited autotrophic nitrification–

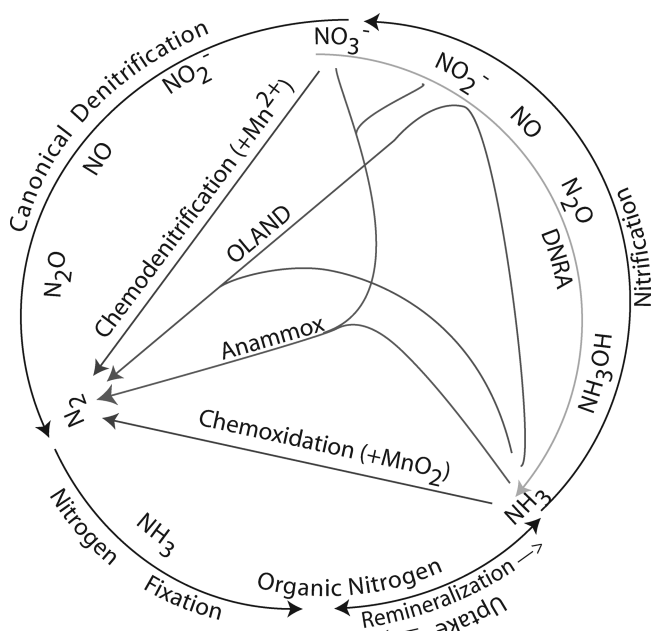


Figure 2. Diagram of the nitrogen cycle as it is understood today. Processes given on the outside of the circle of nitrogen compounds are redrawn from Figure 1. Processes on the inside of the circle are those discovered or identified in the last 15 years. Arrows represent the direction of reactions. Chemonitrification and chemodenitrification reactions are listed with their respective manganese species used as a redox pair. The reduction of NO_3^- to NH_3 during assimilation by photosynthetic organisms is not drawn for clarity.

denitrification or OLAND) or (2) the bypassing of N_2 as a sink and the production of NH_3 from oxidized species (dissimilatory nitrate reduction to ammonia, DNRA). Each of these will be described in turn below.

2.2. Anaerobic Ammonia Oxidation (Anammox)

As a heterotrophic process, canonical denitrification should be accompanied by the liberation of the ammonium from the organic matter being respired. However, it was noticed by Richards⁴⁶ that this build up of ammonium did not occur. This observation lead Richards⁴⁶ and Cline and Richards⁴⁰ to propose that a Van Slyke-like reaction was responsible for the anaerobic oxidation of ammonium. In the Van Slyke reaction,⁴⁷ organic amines react with nitrite under mildly acidic conditions to produce N_2 gas. Richards suggested a similar reaction but with nitrate and ammonium as the reactants. Since then others have suggested anaerobic oxidation of ammonium to N_2 was occurring in suboxic environments based on chemical distributions of ammonium and nitrate.^{16,40,48–51} Perhaps the best example of these types of distributions is from the Black Sea, where oxygen is depleted at a depth of about 60 m ($\sigma_\theta = 15.7$) and nitrate is not depleted until a depth of about 80 m ($\sigma_\theta = 15.95$), where ammonium, which diffuses upward from the resulting sulfate reduction below, is also depleted. Measurable nitrite concentrations are also present in this depth zone (Figure 3). These profiles strongly suggest diffusion of both nitrate and ammonium into a common reaction zone where they are both consumed. Despite this strong geochemical evidence for anaerobic oxidation of ammonium to N_2 , at the time of these studies, an organism that could carry out this energetically favorable reaction was “missing in Nature”.⁵²

It was not until 1995 that the “anammox” reaction ($NH_3 + NO_2^- \rightarrow N_2$) was discovered in a fluidized bed reactor by

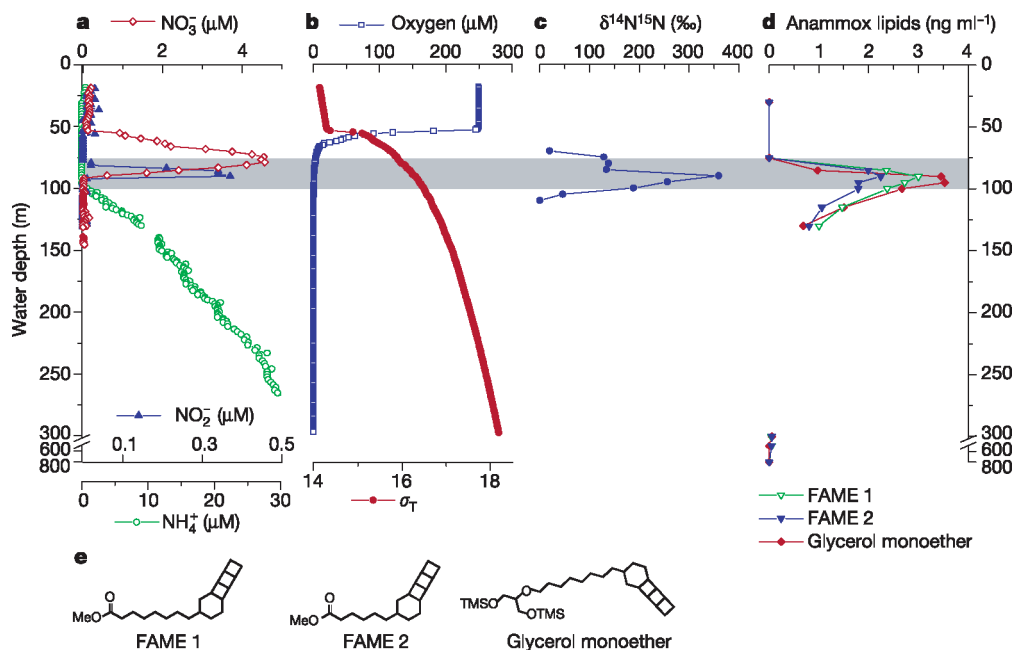
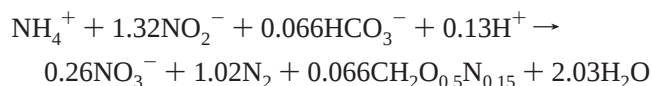


Figure 3. Anammox in the suboxic transition of the Black Sea: (a) Nitrate (red), nitrite (blue), and ammonia (green) concentrations; (b) O_2 concentrations (blue) and water density (red); (c) vertical distribution of $^{15}N^{14}N$ produced in incubation experiments; (d) distributions of the three ladderane lipids, FAME 1, FAME 2, and glycerol monoether; (e) structures of the ladderane lipids as analyzed. The suboxic transition zone is shown as the gray shaded area. Reprinted by permission from Macmillan Publishers Ltd: *Nature* (<http://www.nature.com>), ref 55, copyright 2003.

the observation that nitrite and ammonium disappeared simultaneously with the production of N_2 gas.⁵³ Four years later a *Planctomycetes* microbe capable of the anammox reaction was isolated from a similar fluidized bed reactor.⁵⁴ The marine occurrence of the anammox process was first discovered in sediments using the isotope pairing technique.²⁸ The first anammox bacteria, “*Canadidatus Scalindua sorokinii*”, was identified from phylogenetic analysis of 16S rRNA isolated from the Black Sea,⁵⁵ and similar bacteria have been isolated from sediments of a shallow estuary in Denmark.⁵⁶

Anammox bacteria are thought to be strictly anaerobic chemoautotrophic bacteria that fix CO_2 using NO_2^- as the electron donor. Oxygen concentrations as low as $1.1 \mu M$ appear to completely inhibit anammox.⁵⁷ The overall reaction for anammox has been suggested to be⁵⁸



Anammox bacteria belong to the order Planctomycetes, and to date three genera of anammox bacteria have been identified, “*Candidatus Brocadia*”, “*Candidatus Kuenenia*” and “*Candidatus Scalindua*”, although none has been isolated in pure culture yet.^{59,60} The first marine anammox organism identified was of the genus *Scalindua* and was found in the Black Sea,⁵⁵ and all subsequent marine isolates are also *Scalindua*. All anammox organisms appear to have evolved a membrane-bound intracytoplasmic compartment called the anammoxosome. The membrane of the anammoxosome is composed of unusual structurally rigid lipids, called ladderanes after their ladder-like structure,⁶¹ that are apparently unique to anammox bacteria (Figures 3 and 4). In the proposed model for the anammox reaction (Figure 5), nitrite is reduced to hydroxylamine by a nitrite-reducing enzyme (NIR). The hydroxylamine is then combined with ammonium to form hydrazine by the enzyme hydrazine hydrolase (HH).

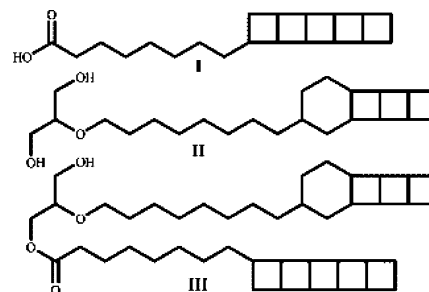


Figure 4. Three different anammox lipids containing the characteristic anammox ring structures. Ring system Y and X are shown in I and II, respectively, while III contains both ring systems. Reprinted with permission from ref 60. Copyright 2004 Blackwell Publishing.

The hydrazine is finally oxidized by a hydrazine-oxidizing enzyme (HZO) to N_2 , with the concomitant liberation of protons.⁶⁰ It is within the anammoxosome that the anammox reaction is proposed to take place. The rigid ladderanes membrane is thought to act as a diffusion barrier that confines the toxic intermediates of the anammox reaction within the anammoxosome, while leaving the relatively permeable cytoplasmic membrane (Figure 5) available for functions such as solute transport and osmotic regulation. The ladderane lipid membrane also allows the generation of the proton motive force required for ATP production.⁶⁰

Because anammox and canonical denitrification occur in suboxic environments, incubations with ^{15}N -labeled substrates are commonly used to distinguish the two processes. Typically, additions of $^{15}NH_4^+$, $^{15}NH_4^+ + ^{14}NO_3^-$, and $^{15}NO_3^-$ are made to anaerobic samples, which are then incubated for hours to several days. The incubations are then terminated and the isotopic composition of N_2 is determined. Production of $^{29}N_2$ during the incubations with added $^{15}NH_4^+$ indicates anammox, whereas formation of $^{30}N_2$ in the $^{15}NO_3^-$ treatment is a clear signal of canonical denitrification. The treatment with both $^{15}NH_4^+ + ^{14}NO_3^-$ is used to detect

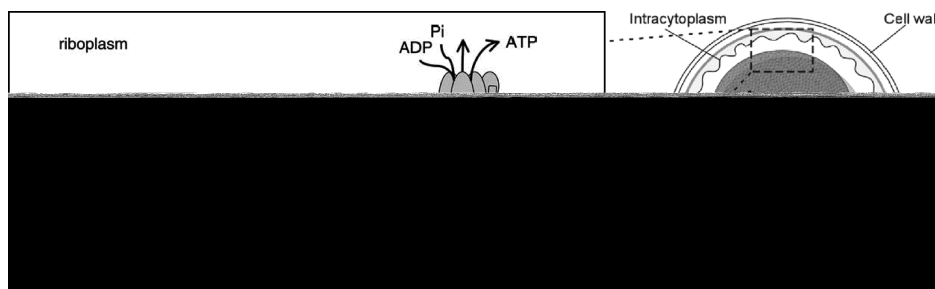
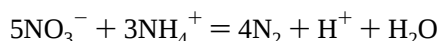
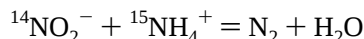


Figure 5. (right) Schematic depiction of anammox cell showing the anammoxozome and nucleoid and (left) postulated pathway of anaerobic ammonium oxidation coupled to the anammoxosome membrane resulting in a proton motive force and ATP synthesis via membrane-bound ATPases. HH, hydrazine hydrolase; HZO, hydrazine oxidizing enzyme; NIR, nitrite reductase. (Redrawn from refs 60 and 55 and modified to account for recent NirS gene sequences in anammox community genome¹⁷⁴). Adapted with permission from ref 60. Copyright 2004 Blackwell Publishing.

anammox in samples with little or no ambient NO_3^- or in samples that have been preincubated to remove traces of O_2 and NO_3^- . The actual oxidant for NH_4^+ , NO_2^- or NO_3^- , has been determined from the isotopic composition of N_2 at the end of the experiment. With $^{15}\text{NO}_3^-$ as the electron acceptor, the reaction stoichiometry would be



whereas with $^{15}\text{NH}_4^+$ and $^{14}\text{NO}_2^-$ as the electron acceptor, the stoichiometry would be



The former stoichiometry would yield 75% $^{29}\text{N}_2$ and 25% $^{30}\text{N}_2$, while the latter would yield 100% $^{29}\text{N}_2$. Nitrite appears to be the oxidant for anammox because only $^{29}\text{N}_2$ product is typically found experimentally in relatively pure anammox cultures.⁵⁹

The discovery of anammox in the marine environment was made by Thamdrup and Dalsgaard²⁸ in sediments of the Skagerrak. Nitrogen isotope pairing experiments such as those described above and relative yields of $^{29}\text{N}_2$ and $^{30}\text{N}_2$ in the different incubations suggested that 24% and 67% of the total N_2 produced at two continental margin sites (Skagerrak) was attributable to anammox. Since its initial discovery anammox has been reported for a wide variety of coastal and pelagic marine environments including sediments,^{56,62–65} the water column,^{43,55,66} mangrove sediments,⁶⁷ and even Arctic Sea ice.⁶⁸

The Black Sea is perhaps the classic example of an anammox environment. As mentioned above the geochemical evidence of a zone in which NO_3^- , NH_4^+ , and NO_2^- all disappear is very clear-cut, as pointed out by Murray et al.² A combined microbiological and biogeochemical investigation was conducted to determine whether the disappearance of combined nitrogen in the suboxic zone was due to anammox.⁵⁵ The isotope pairing technique was used to determine the depth distribution of anammox and canonical denitrification. A clear peak in anammox activity, $^{29}\text{N}_2$ production during $^{15}\text{NH}_4^+$ incubation amendments, was found within in the suboxic zone, but no anammox activity was found outside of the suboxic zone (Figure 3). As an additional indication of anammox, the ladderane lipid content of suspended particulate matter was also analyzed. The depth distribution of the ladderane lipids was very similar to that of $^{29}\text{N}_2$ incubations indicating that anammox bacteria could be the agents of the ammonium oxidation to N_2 . Primers specific for Planctomycetes bacteria were used to amplify

the 16S rRNA gene sequences from the zone of apparent anammox activity, which were then cloned and sequenced. The sequences were closely related to known anammox bacteria with 87.9% and 87.6% similarity to *Kuenenia* and *Brocadia*, respectively. The Planctomycetes from the Black Sea suboxic zone were tentatively named “*Candidatus Scalundua sorokinii*”. Finally FISH (fluorescence in situ hybridization) probes were designed from their sequences that gave a positive signal for an unusual doughnut-shaped bacteria found in the suboxic zone. The doughnut shape of the bacteria was also characteristic for anammox bacteria found in bioreactors.

Since its first discovery in marine sediments, anammox has been found in many of the sediments that were investigated. Anammox in sediments accounted for 0–80% of the total N_2 production, and the range of rates reported by Engström et al.,⁶² 0.14 and 16 $\mu\text{M N}_2 \text{ h}^{-1}$, more or less brackets the entire range reported in the literature. Anammox appears to contribute progressively more to total N_2 production as water depth increases (Figure 6), and it appears that this variation may be a function of the rate of overall sedimentary carbon oxidation.^{28,62} Engström et al.⁶² observed a negative correlation between the sedimentary carbon mineralization rate, as indicated by ammonium production or sedimentary chlorophyll content, and the relative importance of anammox (Figure 7). This relationship is not linear, however, because while the absolute rate of denitrification increased with increasing remineralization rate, the rate of anammox reached a plateau at an intermediate rate of remineralization. A positive correlation of anammox with carbon content has been observed in the Thames estuary.⁶⁹

Anammox bacteria appear to be robust. They can tolerate exposure to oxygen and resume anammox activity quickly upon re-establishment of suboxic conditions.⁵⁷ Under conditions of intermittent oxygenation, the rate of anammox after oxygenation was the same as before oxygenation.^{70,71} Anammox organisms are active over a temperature range of at least -1 to 24°C in the environment^{67,68} and at temperatures of 37°C for the wastewater reactor organisms,⁷² and anammox activity has even been found in brine pockets in sea ice from the Greenland Sea.⁶⁸

Until the discovery of anammox in the oceans, canonical denitrification was thought to be the only substantial sink of combined nitrogen in the marine combined nitrogen budget. It now seems clear that anammox is a second important sink. N_2 gas production in the three major oxygen deficient zones (ODZ) of the ocean accounts for 30–50% of the total marine denitrification. If canonical denitrifying bacteria are responsible for the heterotrophic oxidation of

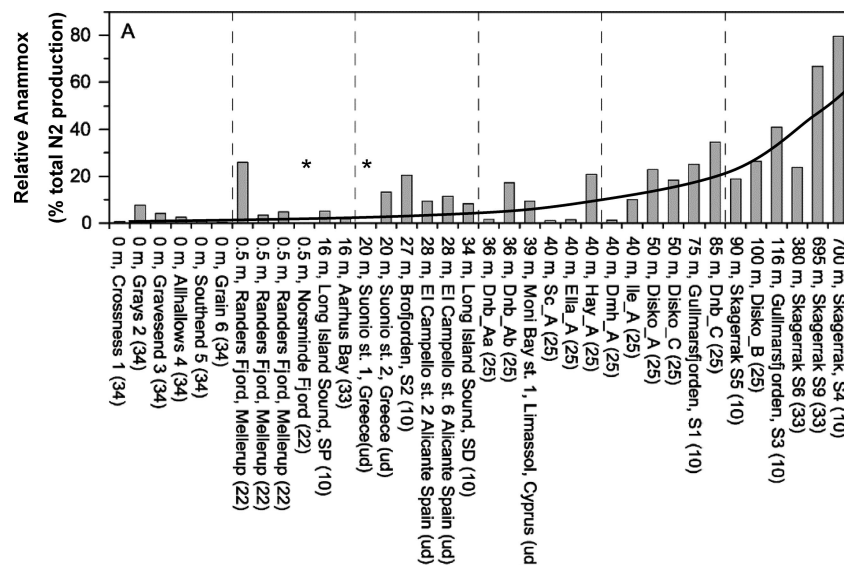


Figure 6. Relative rate of N_2 production from anammox as a percentage of the total N_2 production rate as determined from ^{15}N incubation experiments described in text. An * indicates rates below the limit of detection. Although the relative rate of anammox increases with water depth, the absolute rate of N_2 production generally decreases with increasing water depth. Reprinted from ref 59, Copyright 2005, with permission from Elsevier.

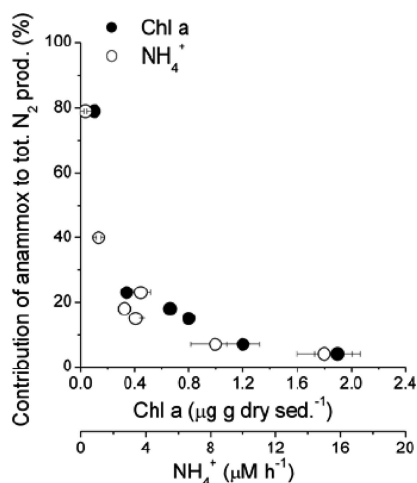


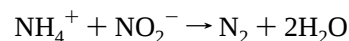
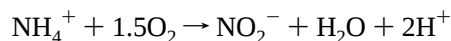
Figure 7. Percentage of anammox relative to total N_2 production rate as a function of sedimentary chlorophyll content and sedimentary ammonium production rate. Reprinted from ref 62, Copyright 2005, with permission from Elsevier.

organic matter in these zones and anammox bacteria are responsible for the oxidation of the remineralized ammonium, which seems likely, anammox bacteria would account for 29% of the N_2 production (assuming Redfield stoichiometry). The 29% may also be an underestimate of the importance of anammox in ODZs. Amino acids are preferentially consumed in the eastern tropical north Pacific ODZ,⁷³ which would increase NH_4^+ production per organic matter oxidized, thus increasing the importance of anammox. Codispoti et al.⁶ have also suggested that there is a discrepancy between the excess N_2 gas and the amount of denitrified nitrate in the Arabian Sea oxygen deficient zone. One possible source of this extra N_2 could be anammox. Anammox as a source of N_2 production in sediments has also been shown to occur in most of the sedimentary environments investigated. Within the various sedimentary studies, the importance of anammox relative to canonical denitrification as a N_2 production pathway varied from 0% to 80% with the anammox contribution increasing with increasing water depth. Although Figure 6 is still preliminary, anammox appears to be

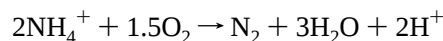
responsible for something like 25% of the N_2 production in the depth range 50–300 m, where much of the sedimentary denitrification takes place. As a conservative first estimate, anammox appeared to account for a minimum of about 25–30% of marine denitrification. However, the study of anammox in the marine environment is in its infancy, and undoubtedly surprises are ahead that will alter our current thinking.

2.3. Oxygen-Limited Autotrophic Nitrification–Denitrification (OLAND)

OLAND is another process discovered in the wastewater treatment field in the late 1990s.^{30,74,75} It differs from the anammox process in two critical aspects: nitrite only is the oxidant, and this nitrite is presumed to be the result of locally produced OLAND, and thus OLAND is not strictly an anaerobic process. This oxidation is presumably carried out within a consortium of nitrifiers associated with ammonium oxidizers within sediments.⁷⁴ The reactions can be described as



The combination of these two reactions yields

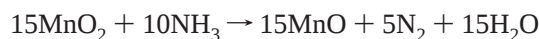
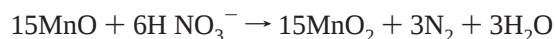


In OLAND, low amounts of dissolved O_2 are thought to limit the oxidation of NO_2^- to NO_3^- , and the oxidation of ammonium is closely tied to nitrite reduction. Higher levels of O_2 availability shift the balance of reactions 1 and 2 toward nitrite formation.⁷⁶ It is unclear how important this process is in the natural environment, or how much nitrogen cycling attributed to anammox might be from OLAND.

2.4. Chemodenitrification

Several possible reactions with inorganic species have been proposed that lead to the conversion of fixed nitrogen to N_2 .

The most prominent of these has been the possible reaction of manganese species with nitrate or ammonium.^{31,77} This interaction was originally proposed after water column and sediment profiles of these species suggested that Mn was playing a role in N cycling at oxic–anoxic interfaces. Luther et al.^{31,78} have proposed two reactions with manganese that result in denitrification:



The catalytic nature of this mechanism becomes apparent if the two reactions are coupled:



Luther et al.,³¹ have shown that the first reaction can proceed abiotically, but any such reactions in the natural environment are likely to be microbially catalyzed. MnO_2 may also oxidize ammonium to nitrate.³ Under more extreme conditions other fixed nitrogen losses are possible. The Van Slyke reaction⁴⁷ noted above between nitrite and amines will form N_2 under acidic conditions. Nitrate and nitrite both can be converted to N_2 in contact with minerals under hydrothermal conditions, although the degree of loss is dependent upon the temperature and mineral species involved.^{79,80} Nitrite will also react with ammonium to produce N_2 under acidic conditions.⁸¹ However these loss routes are presumably minor when compared with other biologically catalyzed reactions.

2.5. Dissimilatory Nitrate Reduction to Ammonium (DNRA)

DNRA has gained importance in recent years as an environmentally relevant reaction within both terrestrial and marine ecosystems. The reaction has been reported for anoxic sediments^{82–84} and sediments with substantial free sulfide, possibly due to sulfide inhibition of nitrification and denitrification.^{85,86} The most notable nitrate-fermenting organisms are *Thioploca* and *Thiomargarita*, found in sediments underlying the major suboxic denitrifying water columns of the Arabian Sea, eastern tropical Pacific, and Namibia.^{87–91} These organisms can couple the reduction of NO_3^- to ammonium with the oxidation of reduced sulfur compounds. Both *Thioploca* and *Thiomargarita* are able to concentrate NO_3^- at up to 0.5 M concentrations within large vacuoles within their cells for subsequent sulfide oxidation.^{91,92} The fate of ammonium produced by DNRA is not well understood at this time. In environments where high sulfide levels inhibit conventional nitrification or denitrification,⁹³ DNRA may serve as a “short circuit” to the N cycle, preserving fixed N within such environments and supporting higher productivity levels than would otherwise be expected.^{86,94,95} Conversely, active transport and reduction of nitrate by *Thioploca* and *Thiomargarita* may enhance ammonium fluxes, as well as reducing sulfide fluxes to the oxic/anoxic interface,⁹⁶ but this material may still be lost to N_2 via anammox⁴³ or coupled nitrification–denitrification at the sediment oxic–suboxic interface.^{97–99}

2.6. Interactions with “Canonical” Denitrification

Canonical denitrification is defined as a heterotrophic process that reduces NO_3^- (and the intermediaries NO_2^- and

N_2O) to N_2 under conditions of very low dissolved O_2 content. However this is not the only source of N_2 in the marine environment, as has been shown above. It has been known for nearly 2 decades that the flux of nitrate to sediments cannot account for the total N_2 flux from those sediments, and it is becoming apparent that the same phenomenon may be occurring in certain suboxic water columns.⁶ While the discovery of the anammox process led to a flurry of excitement and speculation about the importance of this process in relation to canonical denitrification, the few studies that have examined sediment N cycling in detail using targeted stable isotopic tracer techniques have tended to find that anammox is a not the dominant process (see discussion above). Where anammox and other alternative processes come into play in the global N cycle is in explaining the efficiency of N_2 production within and around redox boundaries. Removing NH_3 without prior oxidation to nitrate allows for steeper nitrate gradients within sediments and therefore greater fluxes. In addition, the possibility that NH_3 can be oxidized and then reduced under O_2 -limiting conditions (OLAND) opens up alternative explanations for the absence of NH_3 in the suboxic waters. And the Mn-catalyzed removal of fixed N at the oxic–anoxic boundaries within the Black Sea (and other anoxic basins) may shift the overall flux of N within such regions. All of these processes have the effect of increasing overall fixed N losses over those calculated by methods that assume canonical denitrification (Devol et al., in review).

3. New Developments in Understanding Marine Nitrogen Fixation

The most recent estimates of the global oceanic N_2 fixation rate are ~ 100 Tg of N per year or higher (1 Tg of N = 10^{12} g of N),^{11,17,100,101} nearly an order of magnitude greater than those in earlier studies.^{102,103} The biological fixation of N by diazotrophic organisms is now therefore considered to be the dominant source of fixed N in the ocean. In oligotrophic regions of the world’s oceans, N_2 fixation is believed to supply roughly half of the N needed to support the export of organic matter out of the ocean surface.¹⁰⁴ The fundamental sensitivity of N_2 fixation to the abundance of Fe confers a great significance to this process over geological time scales. For these reasons, N_2 fixation has become a central focus of investigation into the marine N cycle.

A complete understanding of N_2 fixation in the marine environment must strive to link this biochemical process at the cellular scale with its role in global biogeochemical cycles. We therefore begin this section with a brief overview of some relevant biochemical characteristics of N_2 fixation. We then review what is known about the distribution of N_2 fixation in the ocean, since this information may shed light on the environmental controls relevant to the long-term N budget. The distribution of N_2 fixation can then be integrated to provide a global rate of N_2 fixation, which is central to establishing the degree to which the ocean N budget is in balance.

3.1. Sensitivity—The Cellular Scale

The enzyme nitrogenase, which is responsible for breaking the strong triple bonds of N_2 required for the formation of fixed N, is found among a diverse array of microorganisms. Among these, a genus of cyanobacteria, *Trichodesmium*, has long served as a model for the study of N_2 fixation because

it is commonly observed in the warm surface waters of the tropical and subtropical oceans. Much of what is known about marine N_2 fixation at the cellular scale has been established on the basis of this conspicuous subset of diazotrophs. Given the highly conserved nature of the nitrogenase system, inferences about the nature of marine N_2 fixation based on *Trichodesmium* may be justified. However, a growing body of research has revealed a wide diversity of N_2 -fixing organisms, and an understanding of N_2 fixation derived from *Trichodesmium* should be regarded as tentative. Here we summarize some of the physiological aspects of N_2 fixation that may ultimately govern the large-scale distribution and sensitivity of N_2 fixation to environmental factors. For a comprehensive review of N_2 fixation from an organismal perspective, the reader is referred to the review by Karl et al.¹⁰¹

The evolutionary history of diazotrophy provides several basic biochemical constraints that may limit the distribution and magnitude of N_2 fixation in the ocean.¹⁰⁵ Among the most fundamental is the inhibition of nitrogenase activity in the presence of O_2 . This necessity presents a problem for the majority of diazotrophs, for whom photosynthesis requires well-lit surface water habitats, where dissolved O_2 gas is also found in high concentrations. In ironic contrast to denitrification, a process that does not require anoxia but is generally restricted to anoxic environments, N_2 fixation is a strictly anaerobic process found primarily in surface waters awash in dissolved O_2 . This implies the existence of diverse strategies for protecting the active site of nitrogenase from O_2 and reveals an important decoupling between the fundamental biochemical constraints on diazotrophs from the large-scale distribution of bulk water properties in which they thrive.

Ambient concentrations of the major macronutrients, NO_3 and PO_4 , are also potentially important factors for N_2 fixation. Numerous studies have examined the effect of the presence of fixed N substrates on rates of N_2 fixation in *Trichodesmium* cultures.^{106,107} These studies generally find that when fixed N is present, N_2 fixation is inhibited, since *Trichodesmium* can assimilate most forms of fixed N (NH_4 , NO_3 , DON) commonly found in seawater.¹⁰⁷ The degree of inhibition depends on the form of fixed N available. While the presence of fixed N may suppress N_2 fixation, the availability of P is essential and therefore potentially limiting.¹⁰⁸ Diazotrophs appear to have evolved clever strategies for meeting their P needs because PO_4 is extremely depleted in most of the surface ocean. For example, *Trichodesmium* have been hypothesized to be able to “mine” P from depths of the water column by regulating their buoyancy.^{101,109} Recent evidence also shows an ability to use certain forms of DOP.¹¹⁰ In addition to macronutrients, trace metals have been emphasized as potential limiting factors for N_2 fixation. At the cellular level, this is because nitrogenase has been reported to require more Fe than other common enzyme systems, although the magnitude of the Fe quota is uncertain.^{111–113}

3.2. Global Distribution of Marine N_2 Fixation

In principle, the distribution of N_2 fixation in the surface waters of the global ocean may provide insight into the environmental conditions under which the process is favored and therefore its sensitivity to changes in those conditions. In practice, it has proved difficult to determine the time-averaged distribution of N_2 fixation at a basin scale, let alone

globally. Shipboard observations of in situ rates of N_2 fixation provide the most direct avenue for mapping the distribution of N_2 fixation. Geochemical tracer approaches that exploit the integrated signature of N_2 fixation on the chemical composition of seawater have also been pursued. Both approaches entail unique methodological difficulties. Here we describe the contributions of both the biological and geochemical approaches to our understanding of the distribution of marine N_2 fixation.

Observations of the abundance of *Trichodesmium* in surface waters of the world's oceans, accumulated over the past several decades, provide a qualitative picture of its large-scale biogeography.^{114,115} Two important conclusions can be drawn from these observations. First, the geographic distribution of *Trichodesmium* is limited to the warm waters ($>20^\circ C$) of the tropical and subtropical oceans.^{100,101} Whether temperature exerts a direct physiological control on *Trichodesmium* is not known, but it has been proposed that temperature governs N_2 fixation indirectly through its effect on respiration rates and O_2 solubility,¹¹⁶ and their geographic confinement has led to an understanding of N_2 fixation as a warm-water process. Second, within the low-latitude surface ocean, *Trichodesmium* biomass is highly variable in both space and time and the associated inputs of newly fixed N are likewise patchy and episodic.^{115,117} The frequency and spatial density of shipboard sampling is inherently limited, and estimating the distribution of N_2 fixation by *Trichodesmium* therefore presents a formidable challenge.

A major effort to observe N_2 fixation across a swath of the tropical North Atlantic in all seasons has recently been concluded to address this problem.¹¹⁵ Six cruises were conducted comprising the most exhaustive study of N_2 fixation in any ocean basin. Rates of N_2 fixation measured with a variety of techniques showed a remarkable degree of consistency and resulted in an estimated mean annual rate of N_2 fixation of $87 \text{ mmol}/(\text{m}^2 \cdot \text{year})$. Despite the comprehensive coverage of this study, extending the results to the entire North Atlantic or even across the subtropical gyre relies on an extrapolation of measurements over an uncertain domain. Importantly however, this study brings the directly measured rates of N_2 fixation in the North Atlantic within the range of estimates based on geochemical tracers (see below).

The development of satellite-based observations of ocean color has become a powerful tool to ameliorate the under-sampling of ocean biological processes. Using unique properties of light scattering by the gas vacuoles in *Trichodesmium*, algorithms are now being used to detect the presence of *Trichodesmium* blooms in satellite ocean color data.^{118–120} These studies have confirmed tropical and subtropical latitudes as the dominant habitat of *Trichodesmium* and produce greater detail about its distribution among different regions and ocean basins. Although long-term bloom statistics are not yet available, in boreal waters winter tropical blooms were detected across the Pacific from the margins of North and South America to Oceania and with great intensity in the Arabian and Caribbean Seas.¹¹⁸

Intensive ship-based sampling and satellite observations both aim to better resolve the relevant temporal and spatial scales of variability. Recent research has suggested that the diversity of organisms capable of fixing N_2 has also been undersampled. Marine microbes other than *Trichodesmium* may contribute substantial inputs of newly fixed N that would not be represented in any previous biological estimates.^{106,121}

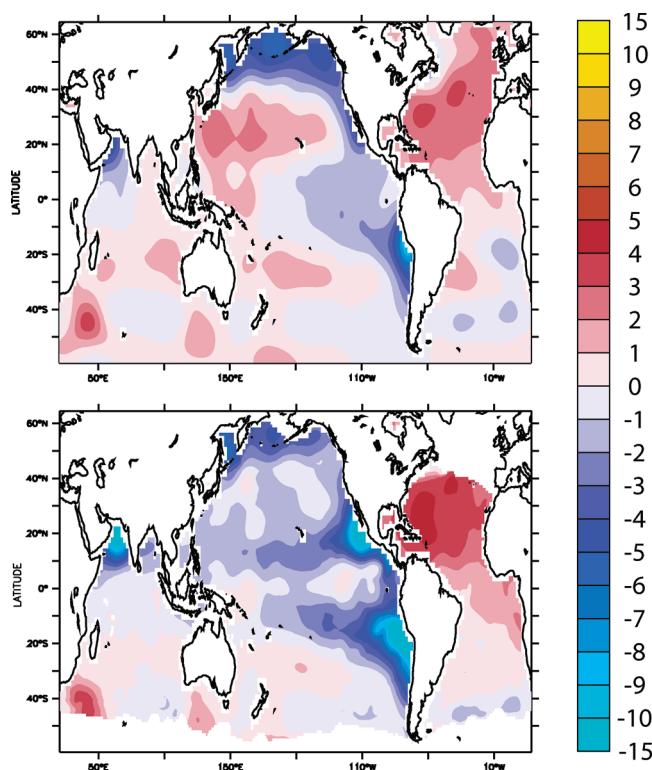


Figure 8. Global distribution of N^* ($N^* = [\text{nitrate}] - 16[\text{phosphate}] + 2.9$) in the surface ocean (0–100 m, panel a) and along a surface of constant density (1026.6 kg/m^3 , panel b), based on data from the World Ocean Circulation Experiment.

The contribution of unicellular diazotrophs was found to be substantial ($\sim 150 \text{ mmol of N/(m}^2\cdot\text{yr)}$) across the North Pacific at several locations along 30° N ,¹⁰⁶ whereas *Trichodesmium* N_2 fixation was relatively small. The overall contribution of unicellular N_2 fixers, while potentially important, remains unknown.¹²²

The spatial heterogeneity, episodic nature, and taxonomic diversity of marine N_2 fixation motivated the use of geochemical tracers to infer spatial distributions and rates of N_2 fixation. Geochemical estimates of N_2 fixation have relied on the distributions of the major macronutrients, NO_3 and PO_4 , which have been measured throughout the world ocean. Assuming that N_2 fixation and denitrification are the dominant causes of non-Redfield biotic N and P fluxes, the physical transport and mixing of N^* (see Introduction) can be quantitatively related to the net rate of N_2 fixation (F) and denitrification (D):^{17,18}

$$\frac{dN^*}{dt} + \text{diffusion}(N^*) = a_1 F + a_2 D$$

where d/dt is the time derivative following a water parcel and a_1 and a_2 are constants whose values depend on the stoichiometric ratios but are roughly one.^{17,18}

Because the broad distribution of N^* is well-known (Figure 8), the pattern of N_2 fixation (or denitrification) can in theory be estimated by computing the rates of transport and mixing of N^* . This basic approach has been used to estimate integrated rates of N_2 fixation spatially and temporally in thermocline waters of the North Atlantic, where denitrification can be assumed to be negligible. In studies by Gruber and Sarmiento¹⁷ and Hansell,¹²³ the rate of N^* increase along a flow path is estimated via the correlation between the N^* and water mass age anomalies. Such

correlations, which hold at the basin scale, allow only limited spatial information. Determining the area over which the rate is to be attributed presents a substantial uncertainty in this approach, however, accounting for a large difference in estimates of these two studies (see below). In addition, the coefficient a_1 can vary by up to 50% across the range of observed N/P ratios in the biomass of N_2 -fixing organisms. Finally, this approach is limited to water masses that are simple mixtures without the counteracting influence of denitrification¹⁸

N_2 fixation also acts as a source of N^* in surface waters due to the uptake of PO_4 by N_2 -fixing organisms. While nitrogen fixers can satisfy their N requirement by fixing N_2 , they must consume PO_4 from the surface reservoir. Uptake of PO_4 without uptake of NO_3 produces an elevated surface N^* anomaly, so the distribution of surface N^* will record the influence of N_2 fixation (Figure 8). Using global climatologies of NO_3 and PO_4 concentrations in the upper water column in conjunction with water mass transport from a general circulation model (GCM), Deutsch et al.¹²⁴ diagnosed the geographical patterns and rates of N_2 fixation implied by the observed N^* distribution in surface waters. They infer a distribution of N_2 fixation that is broadly consistent with the observed biogeography of *Trichodesmium* observed from ships¹²⁵ and satellites.¹¹⁸ However, the diagnosed rates of N_2 fixation are nearly twice as large in the Pacific as in the Atlantic, with intermediate rates in the Indian Ocean. The differences in N_2 fixation rates between these basins contrast with the differences in Fe deposition to the ocean surface waters, suggesting that the atmospheric Fe supply may not govern the large-scale distribution of N_2 fixation.

A complementary tracer of N_2 fixation is provided by the $^{15}\text{N}/^{14}\text{N}$ ratio of NO_3 . Because N_2 fixation produces organic N derived from atmospheric N_2 with little isotopic discrimination, the oxidation of newly fixed N adds NO_3 with a N isotope ratio that is lower than that of the mean ocean. Although measurements of marine N isotopes are sparse in comparison to macronutrient concentrations from which N^* is derived, they have been successfully used as both qualitative and quantitative indicators of the regional importance N_2 fixation.

In the northwest Pacific along the Kuroshio current, Liu et al.¹²⁶ reported an isotopically light pool of NO_3 (low $^{15}\text{N}/^{14}\text{N}$ ratio) indicating a large input of newly fixed N in the western subtropical gyre. In the eastern tropical Pacific and in the Arabian Sea, Brandes et al.¹²⁷ found that the upward decrease in the $^{15}\text{N}/^{14}\text{N}$ of NO_3 could not be explained by lateral mixing with surface waters from outside the suboxic water column. Instead, they argued that it required an isotopically light source of new N from local N_2 fixation. Their analysis of the N isotope mass balance led these authors to infer a large rate of N_2 fixation is surface waters overlying these major denitrification zones. An additional constraint on the source of NO_3 comes from its $^{18}\text{O}/^{16}\text{O}$ ratio.¹²⁸ Combined $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ profiles for NO_3 are consistent with a large input of newly fixed N in water masses with active denitrification. This finding is also supported by the distribution of N_2 fixation rates diagnosed from surface nutrients. Thus, several lines of evidence now point to a close spatial coincidence of denitrification and N_2 fixation.

3.3. Integrated Rates

Both the direct measurement of in situ rates of biological N_2 fixation and geochemical tracer techniques have been used

to derive estimates of global marine N_2 fixation. Each of these approaches is beset by unique problems. Biological rate measurements made over short periods at specific locations must be extrapolated in space and time to arrive at an annual global N input.¹²⁹ Geochemical approaches, which integrate over broad spatial scales, often provide little spatial or temporal resolution of the rates of interest.^{11,17,18,123,130} In principle, the two methods used together may provide robust integrated rates of N_2 fixation while also characterizing the relevant scales of temporal and spatial variability.

Until recently, the biological estimates of N_2 fixation have been consistently lower than geochemical estimates, by at least a factor of 2. However, more recent geochemical estimates for the North Atlantic of $\sim 2\text{--}7$ Tg of N per year¹²³ are in line with many of the earlier biological rate estimates,¹³¹ while the most recent biological estimate of $22\text{--}34$ Tg of N per year¹¹⁵ is in the same range as previous geochemical estimates.¹⁷ On a global basis, the two approaches are also converging, with extrapolations of direct biological rate estimates of $80\text{--}140$ Tg of N per year^{12,129} covering a similar range to global geochemically based estimates of $110\text{--}150$ Tg of N per year.¹⁷ There remains, however, a considerable range of estimates, none of which is able to resolve the long-standing question of whether the marine N budget is in balance.¹⁰¹

4. The Marine Fixed Nitrogen Budget in Light of These New Processes

Although much of the cutting-edge research in the nitrogen cycle community in recent years has focused on the alternative pathways and locations of sources and losses noted above, the integrated rates for each term are what matters in the global view. Much of the discussion among members of the nitrogen community has centered on the rate of N loss from sediments. As recently as the mid-1990s, the sedimentary denitrification rate was assumed to be on the order of 100 Tg of N per year.¹⁷ While this value continues to be used in some studies, particularly as a preanthropogenic value,^{4,12} the weight of both *in situ* measurements and modeling studies favors a rate 2–3 times higher.^{11,27} The focus of sedimentary denitrification studies has been on shelf environments,^{22,24,132–134} particularly fine grained sedimentary environments where the combination of shallow water columns and high surface primary productivity leads to very high fixed nitrogen losses. Denitrification rates may be quite significant even in coarse sands found over wide areas of continental shelves.^{135–138} In addition, hemipelagic sediments found in deeper environments may also be more important as sinks than commonly assumed. A modeling study by Middelburg et al.²⁷ found that fixed nitrogen losses were greater in slope and deep-sea sediments than in shelf sediments. This prediction is supported by a few other studies. Lehmann et al.¹³⁹ found notable nitrogen deficits in the deep Bering Sea, and calculated a fixed N loss of 1.27 Tg of N per year for that basin alone. Overall sedimentary respiration rates in the Bering Sea were around 3 times higher than those predicted for sediments found in the deep sea. Many studies report higher sediment respiration rates in deep sea sediments located near oceanic margins.^{140–146} Direct evidence for shelf-derived carbon transport to the deep sea has also been found.¹⁴⁷ This carbon export process has been examined in a variety of locations in recent years, and global marine benthic respiration rate models have used this phenomena to explain higher deep sea fluxes.¹⁴⁴ Local

sedimentary depocenters can have still higher values.¹⁴⁸ Also, nearly all studies of slope and deep sea respiration have determined denitrification rates using nitrate profiles, missing the contribution of reduced N species, for example, NH_3 , to the total N_2 flux.⁶ Even when N_2 is accounted for, methodological problems can significantly underestimate fluxes.¹⁴⁹ Thus older estimates of benthic denitrification may significantly underestimate the value of this term. The addition of anammox as a substantial process in water column oxygen minimum zones⁴³ suggests that water column fixed nitrogen losses are also presently underestimated, as noted above.

What, then, are the consequences of a sedimentary denitrification term of at least $200\text{--}250$ Tg of N per year, assuming that the global denitrification models are correct? Leaving aside any anthropogenic effects, the other major N loss terms, water column denitrification ($\sim 80\text{--}100$ Tg of N per year, conservatively) and burial (~ 25 Tg of N per year) when combined with a sedimentary denitrification rate of $175\text{--}225$ Tg of N per year result in a total removal rate of something on the order of $300\text{--}350$ Tg of N per year (see Codispoti et al.⁶ for a detailed discussion of these rates). Fixed nitrogen sources other than biological fixation total about $100\text{--}150$ Tg of N per year (also Codispoti et al.⁶). This leaves a deficit of $150\text{--}250$ Tg of N per year to be filled by marine N_2 fixation. As discussed above, the upper estimates of N_2 fixation fall into the low end of this range.¹⁰¹ Thus a balanced budget is possible. However it is likely that both estimates will be revised upward as alternative N loss pathways and N_2 fixation patterns are examined in more detail.

The concept of an imbalanced marine fixed N budget has been examined in both modern and paleo climates. A continuing discussion in the N community about the status of the marine N budget occurred in the late 1980s to the end of the 1990s, with some camps arguing for an imbalanced modern budget.²⁵ This became especially true after the publication of studies suggesting a diminution of water column denitrification rates in the marine suboxic regions during glacial periods,^{150–153} as well as possible decreases in sedimentary denitrification rates during concurrent sea level low stands.¹³⁴ Thus, according to this theory, the oceans changed from a high N/P regime during glacial periods to low N/P regimes during interglacials. The importance of hemipelagic sediments for sedimentary denitrification partially mitigates this last process, however, especially considering that such sediments would have received increased inputs of labile carbon during sea level low stands.^{154–156}

However, several lines of evidence suggest that the marine N and P budgets are more tightly coupled than predicted. Stable isotopic evidence from outside of the oxygen minimum zones indicates that global fixed N isotopic values did not shift significantly from interglacial to glacial periods.¹⁵⁷ N_2 fixation rates may have declined during glacial periods.¹⁵⁸ Furthermore, studies of nitrate use in the southern ocean^{159,160} also do not indicate strong global changes in glacial period up-welled nitrate concentrations. A continual N loss from the oceans due to large scale imbalances must be countered by a similar C release to the atmosphere, because less CO_2 can be fixed overall. Such a loss would be large enough to have been recorded in the atmospheric CO_2 record.¹¹ Although some evidence suggests short-term nitrogen budget imbalances,¹⁶¹ especially within basins, the weight of the scientific evidence so far supports a long-term balanced N budget.^{11,162} If future studies of sedimentary and water

column denitrification result in further increased estimates (as appears likely with the inclusion of anammox and other alternative NH_3 -oxidizing processes), where might corresponding increases in N_2 fixation be found?

The discussion above on N_2 fixation patterns provides a possible answer to this question. It appears likely that biological fixation and denitrification are far more closely spatially aligned than previously thought. The majority of marine nitrogen fixation studies to date have taken place in regions far from the influence of denitrification, particularly the North and Central Atlantic Ocean.¹¹⁵ In the Atlantic, the absence of water column denitrification provides a clear backdrop for both stable isotopic and N^* calculation patterns supporting the influence of biological N fixation (Figure 8). This finding has led to a focus on this region as perhaps being the most important basin for biological N_2 fixation. In the Pacific, the influence of the suboxic zones in the eastern basin confounds such calculations by generating N^* and stable isotopic signals in opposition to those generated by biological fixation (Figure 8). Biological fixation, enhanced “downstream” of denitrification zones,^{118,163} provides a clear indication that the two processes are closely linked, and the notion that one process, denitrification, can ebb and flow without a concomitant change in the other is unlikely. Thus, although water column denitrification may have changed between climatic periods, it is likely that biological fixation followed suit.¹⁵⁸ Taking this line of reasoning to its logical conclusion, there is therefore little reason to believe that marine N budgets prior to the Anthropocene were out of balance, and therefore biological N_2 fixation is higher (or at least at the extreme upper bounds) than presently thought. This conclusion also implies that fixation should be most important where denitrification is most influential on surface waters. Current studies along river-influenced coastlines and basins,^{118,164} downstream of suboxic zones,¹⁶⁵ as well as the emerging understanding of the importance of N_2 fixing organisms other than *Trichodesmium* spp.,^{121,166,167} support the assertion that the interplay between sources and sinks in the marine N cycle is still poorly understood. Indeed, one of the ecological concepts that may be most applicable to N cycling studies is that of “hot spots” and “hot moments”.¹⁶⁸ The concept that fluxes of material can be concentrated within small regions and time scales is common in terrestrial biogeochemistry and is becoming more important in marine biogeochemistry. Strong evidence exists that both water column denitrification^{26,45} and N_2 fixation^{118,169} are spatially and temporally variable. Estimates made in heterogeneous systems from “snapshots” of activity nearly always underestimate total fluxes.^{170–173} Therefore future advances in constraining the marine fixed N budget may come from higher resolution studies that capture the intrinsic variability in marine systems. It is clear that processes existing at the margins of oxic waters are likely to be the focus of such variability.

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