CHEMICAL REVIEWS

pubs.acs.org/CR

Review

Global Nitrogen Cycle: Critical Enzymes, Organisms, and Processes for Nitrogen Budgets and Dynamics

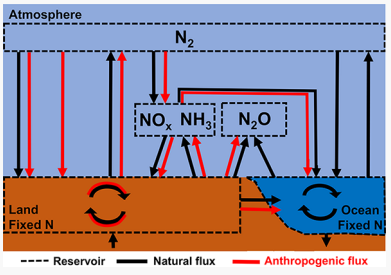
Xinning Zhang,* Bess B. Ward, and Daniel M. Sigman

Cite This: *Chem. Rev.* 2020, 120, 5308–5351

Read Online

ACCESS | Metrics & More | Article Recommendations

ABSTRACT: Nitrogen (N) is used in many of life's fundamental biomolecules, and it is also a participant in environmental redox chemistry. Biogeochemical processes control the amount and form of N available to organisms ("fixed" N). These interacting processes result in N acting as the proximate limiting nutrient in most surface environments. Here, we review the global biogeochemical cycle of N and its anthropogenic perturbation. We introduce important reservoirs and processes affecting N in the environment, focusing on the ocean, in which N cycling is more generalizable than in terrestrial systems, which are more heterogeneous. Particular attention is given to processes that create and destroy fixed N because these comprise the fixed N input/output budget, the most universal control on environmental N availability. We discuss preindustrial N budgets for terrestrial and marine systems and their modern-day alteration by N inputs from human activities. We summarize evidence indicating that the simultaneous roles of N as a required biomass constituent and an environmental redox intermediate lead to stabilizing feedbacks that tend to blunt the impact of N cycle perturbations at larger spatiotemporal scales, particularly in marine systems. As a result of these feedbacks, the anthropogenic "N problem" is distinct from the "carbon dioxide problem" in being more local and less global, more immediate and less persistent.



CONTENTS

1. INTRODUCTION

Nitrogen (N) is an essential element for life; it is the fourth most abundant element in biomass and is required in the most quintessential of biological macromolecules, including proteins, nucleic acids, and chlorophyll. Biologically available nitrogen (or "fixed" N) has been found to be the proximate limiting nutrient

Special Issue: Reactivity of Nitrogen from the Ground to the Atmosphere

Received: September 26, 2019
Published: June 12, 2020

https://dx.doi.org/10.1021/acs.chemrev.9b00613
Chem. Rev. 2020, 120, 5308–5351

in most Earth surface environments.[1−3] Nitrogen is also a participant in environmental redox chemistry. N acts as a reductant for oxygen (O_2) when present in chemical forms such as ammonium (NH_4^+) and nitrite (NO_2^-). When oxygen is scarce, N acts as an important oxidant for organic carbon and other reduced chemicals when present in species such as nitrite and nitrate (NO_3^-). Ammonium and nitrite can also participate in a mutually consuming biochemical process.

The biogeochemical processes involving N control the amount and form of N available for life's functions. The full range of interacting processes is usefully divided into two aspects. The first aspect is the input/output "budget" of fixed N in any given environment, composed of the processes that create and destroy fixed N. In this budget, the input is dominated by biological nitrogen (N_2) fixation, the focus of this volume, with minor natural augmentation by lightning, near-surface rocks, and an increasing contribution from human activities.[4−6] The second aspect is the "cycling" of fixed N, which is composed of the processes that transform fixed N among its different forms and transport it from one region to another, while neither creating nor destroying it and thus not altering the size of the fixed N reservoir. In most settings, the internal cycling of fixed N is representative of nutrient cycling in general; for example, it is often directly comparable to phosphorus in that both are assimilated into biomass for growth and released back to the environment upon organic matter decomposition. In contrast, the budget of fixed N is distinct from those of most other nutrients in that its dominant inputs and outputs are biological processes. Whereas most nutrients derive from uplift, weathering, or degassing of rocks on land, most fixed N derives from biological N_2 fixation. Whereas the removal of such nutrients as phosphorus, iron, and silicon from natural waters is by sedimentation, fixed N is removed by denitrification and associated microbial processes. Given the critical role of biology in shaping N budgets, N biogeochemistry provides an important case for understanding the role of biological feedbacks in determining Earth's environmental conditions.

Human activities, primarily related to the widespread use of N-rich fertilizers, the expansion of legume cultivation, and fossil fuel combustion have dramatically increased inputs of bioavailable N into terrestrial and coastal ecosystems.[4,7] This has led to a suite of environmental problems, the effects of which extend from local to global scales, from hotspots of aquatic eutrophication and regional air pollution to the impact of rising nitrous oxide (N_2O) concentrations on the greenhouse effect and on the stratospheric ozone layer.[8] The availability of N also has profound implications for the problem of anthropogenic carbon (C), as N is a major factor in determining the amount of atmospheric carbon dioxide (CO_2) naturally sequestered through biological productivity. Understanding of the processes that comprise the budget and cycling of fixed N, especially their controls and sensitivities to perturbation, will thus advance the broader goal of clarifying the future trajectory of Earth's biosphere and climate.

In this review of N in the global environment, we focus on the input/output budget of fixed N. There are two related motivations for this. First, the N budget has an overarching effect on N availability in the environment. Second, as mentioned above, humans are strongly affecting the environmental N budget, largely through the contribution to fixed N inputs, especially in the form of industrial and agricultural nitrogen fixation.[4,7] We introduce important reservoirs and processes affecting N in the environment, then discuss preindustrial and modern N budgets and their dynamics. We summarize evidence that the simultaneous roles of N as a required constituent of biomass and a redox intermediate in the environment can lead to strong stabilizing feedbacks that tend to blunt the impact of perturbations to the N cycle at larger spatial and temporal scales. Thus, with important caveats, the human-driven "N problem" is distinct from the "CO_2 problem" in being more local and less global, more immediate and less persistent.

1.1. Reservoirs and Forms of Nitrogen

The largest N reservoir at Earth's surface is dinitrogen (N_2) gas in the atmosphere[9,10] (~4×10^9 Tg N, Figure 1). There is also a large geological reservoir of non-N_2 nitrogen within the crust and ocean sediments, for example, as ammonium in silicate minerals and clays and as organic N in sediments and sedimentary rocks. The near-surface geological N reservoir in crust and deep ocean sediments is estimated to be ~2×10^9 Tg N[11−13] (Figure 1). However, the fluxes into and out of the geological N reservoir are far smaller than for N_2.[6,14] This makes it less important to the input/output budget of fixed N in the environment. Because N_2 gas can only be assimilated by a select and relatively rare group of prokaryotes termed diazotrophs

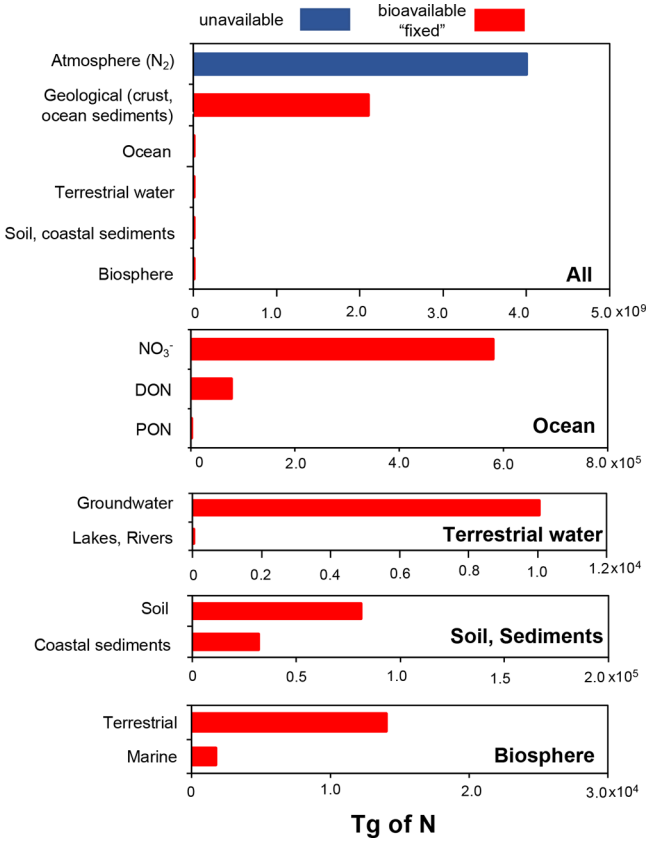


Figure 1. Estimated size of global N reservoirs. Atmospheric N_2, 4.0×10^9 Tg N;[10] Geological (continental crust, oceanic sediments), 2.1×10^9 Tg N;[10−13] Oceanic, 6.6×10^5 Tg N,[15] Terrestrial lakes, rivers, groundwater, 1.0×10^4 Tg N;[16,17] Soils and coastal sediments, 1.1×10^5 Tg N;[18,19] Biosphere, 1.6×10^4 Tg N;[9,13,18] Groundwater, 1.0×10^4 Tg N;[17] Lakes and rivers, 42 Tg N; Soils, 8.1×10^4 Tg N;[18,19] Coastal sediments, 3.2×10^4 Tg N,[15,20] Terrestrial Biosphere, 1.4×10^4 Tg N,[9,13,18] Marine Biosphere, 1.8×10^3 Tg N.[9,18] Global estimates of freshwater and groundwater N content are based on average nitrate concentrations in European freshwaters, median US continental nitrate concentrations,[16] and global water volumes.[21,22]

https://dx.doi.org/10.1021/acs.chemrev.9b00613
Chem. Rev. 2020, 120, 5308−5351

(Section 1.2.1), the fixed N budget can be viewed as a set of processes that transfer N between a large inert atmospheric N_2 pool and a much smaller pool of chemically diverse, biologically available, "fixed" N forms (Table 1) present in the biosphere, ocean, freshwaters, soils, and shallow sediments (Figure 1).

Table 1. N-Containing Species Important in the Global N Cycle[a]

form	molecular formula	redox state
Ammonium, ammonia	NH_4^+, NH_3	−3
Organic N	R-NH_3	−3
Hydrazine	N_2H_4	−2
Hydroxylamine	NH_2OH	−1
Dinitrogen*	N_2	0
Nitrous oxide*	N_2O	+1
Nitric oxide	NO	+2
Nitrite, nitrous acid	NO_2^-, HNO_2	+3
Nitrogen dioxide	NO_2	+4
Nitrate, nitric acid	NO_3^-, HNO_3	+5

[a]Asterisk symbol (∗) indicates a less reactive form that is largely inaccessible to organisms (i.e., a non-"fixed" form).

In the following discussion, the ocean is our focus for two reasons. First, the ocean contains much of the fixed N in the environment (Figure 1). Second, the concentrations of different N forms are better defined and easier to summarize and generalize for the ocean than in terrestrial and freshwater aquatic systems. This is in part due to the mixing of ocean waters, which tends to homogenize fixed N pools on small scales (e.g., of meters and less). Third, as a consequence of this situation, feedbacks among N cycle processes can be easier to identify in the ocean than in soils. Therefore, it is often convenient to first describe processes and feedbacks in the ocean and then consider their relevance for terrestrial systems.

The global environmental fixed N reservoir is primarily composed of dissolved inorganic and organic N in the ocean[24] (Figure 1). Nitrate (NO_3^-), an inorganic species, is by far the most abundant form of fixed N.[15] The concentration of nitrate varies greatly in the ocean (Figure 2), being much higher in deep waters than at the surface (i.e., the upper 100 m). This is a consequence of the internal cycling of the ocean's fixed N reservoir:[24] phytoplankton consume nitrate in sunlit surface waters for the construction of biomass (N_{org}, Figure 3). In less than a year of being generated from nitrate, typically within a few weeks, a significant fraction of organic N is exported to depth, largely as sinking particles[15,25] (green wavy arrow, Figure 3). There, the organic N is remineralized to ammonium, oxidized to nitrite, and then nitrate (black downward arrow, Figure 3; Section 1.2.2). Eventually, this nitrate in deep waters will be circulated back up into the surface ocean, where it will once again be available for phytoplankton assimilation (arced upward arrow, Figure 3). Because of the avidity of phytoplankton for fixed N, the concentration of nitrate in most open ocean surface waters is near the limit of detection (Figure 2A).

In ocean surface waters, there are spatial variations in the concentration of oceanic nitrate that both reflect and cause biogeochemical and ecological zonation (Figure 2A). Upwelling, mixing, and convection bring nitrate up to the surface in high latitude regions and along the equator and some coastlines, leading to concentrations in these environments that can be comparable to those observed in deep waters.[24] While the rapidity of nitrate supply is most important in causing these regions to be nitrate-rich, there are also constraints on phytoplankton growth that prevent complete nitrate consumption; the availabilities of light and iron have both been implicated.[26−28] In most tropical and subtropical ocean regions, there is little mixing, no upwelling, and even some downwelling.[24] With the slow rate of nitrate supply in these regions, nitrate is exhausted at the surface.

There is often a strong gradient in fixed N availability between the coastal zone and the open ocean, with greater availability in the former. The greater N availability of coastal zones can be driven by fixed N inputs from land or by the mixing or upwelling of high-nitrate subsurface waters onto the continental shelves.[24] Moreover, once nutrients enter the coastal zone, they are readily recycled. The sinking of organic matter is arrested by the shallow seafloor, where most of the organic matter is recycled into dissolved nutrients and carbon. Mixing can then quickly return the dissolved nutrients to the surface to fuel additional cycles of productivity and recycling. Because of the avidity of nutrient consumption by phytoplankton, this rapid resupply of nutrients may not be manifested as a significant elevation in the concentration of nitrate. We raise this issue here to indicate that, while global scale visualizations of surface nitrate concentration such as Figure 2A are a very useful indicator of N availability, they can be misleading in some cases, especially in more heterogeneous regions such as the coasts.

Gradients in nitrate concentration also exist within the ocean interior, largely due to ocean circulation (Figure 2B). For example, deep water flows from the North Atlantic to the Indian and Pacific, accumulating nitrate from the remineralization of sinking organic matter as it makes this journey. As a result, nitrate concentration increases from ∼20 μM in the deep North Atlantic to as high as 45 μM in the deep Pacific.[24]

Importantly, the gradients in nitrate concentration across the ocean are dominated by internal cycling (Section 1.2.2), with the input/output budget modifying the concentrations only modestly. For example, nitrate concentrations are often ∼30 μM or higher in the ocean's oxygen-deficient zones, which are hotspots of N loss. This situation reflects the greater N fluxes of the internal cycle, which accumulate nitrate in these regions, relative to those of the input/output budget. This difference in flux amplitudes between N cycling and the input/output budget derives from the requirements of the main fluxes in the input/output budget. These are (1) the need to consume all oxygen (and thus produce nitrate in oxic respiration) before reductive mechanisms of fixed N loss can begin and (2) the tendency of fixed N input by N_2 fixation to occur where N is scarce.

The inventory of ammonium (NH_4^+) in the ocean is 1000-times lower than nitrate.[15] Concentrations typically range from zero to a few hundred nanomolar, with average values in the sunlit "euphotic" and dark "aphotic" zones of 300 and 10 nM.[15,29] Despite its very low abundance, ammonium plays a crucial role in the biogeochemical function of the ocean.[24] While ammonium is produced relatively rapidly from organic matter degradation, its low inventory reflects the avidity with which it is consumed, for biosynthesis by photosynthesizers as well as for its reductive capacity by nitrifiers (Section 1.2.2). Ammonium can accumulate to hundreds of micromolar in dark, reducing environments, such as coastal, estuarine and saltmarsh sediments, where the lack of light and oxygen prevent both anabolic and respiratory demand. However, ammonium does not accumulate in ocean suboxic zones, which can be explained by the occurrence of anaerobic ammonium oxidation ("anam-

https://dx.doi.org/10.1021/acs.chemrev.9b00613
Chem. Rev. 2020, 120, 5308−5351

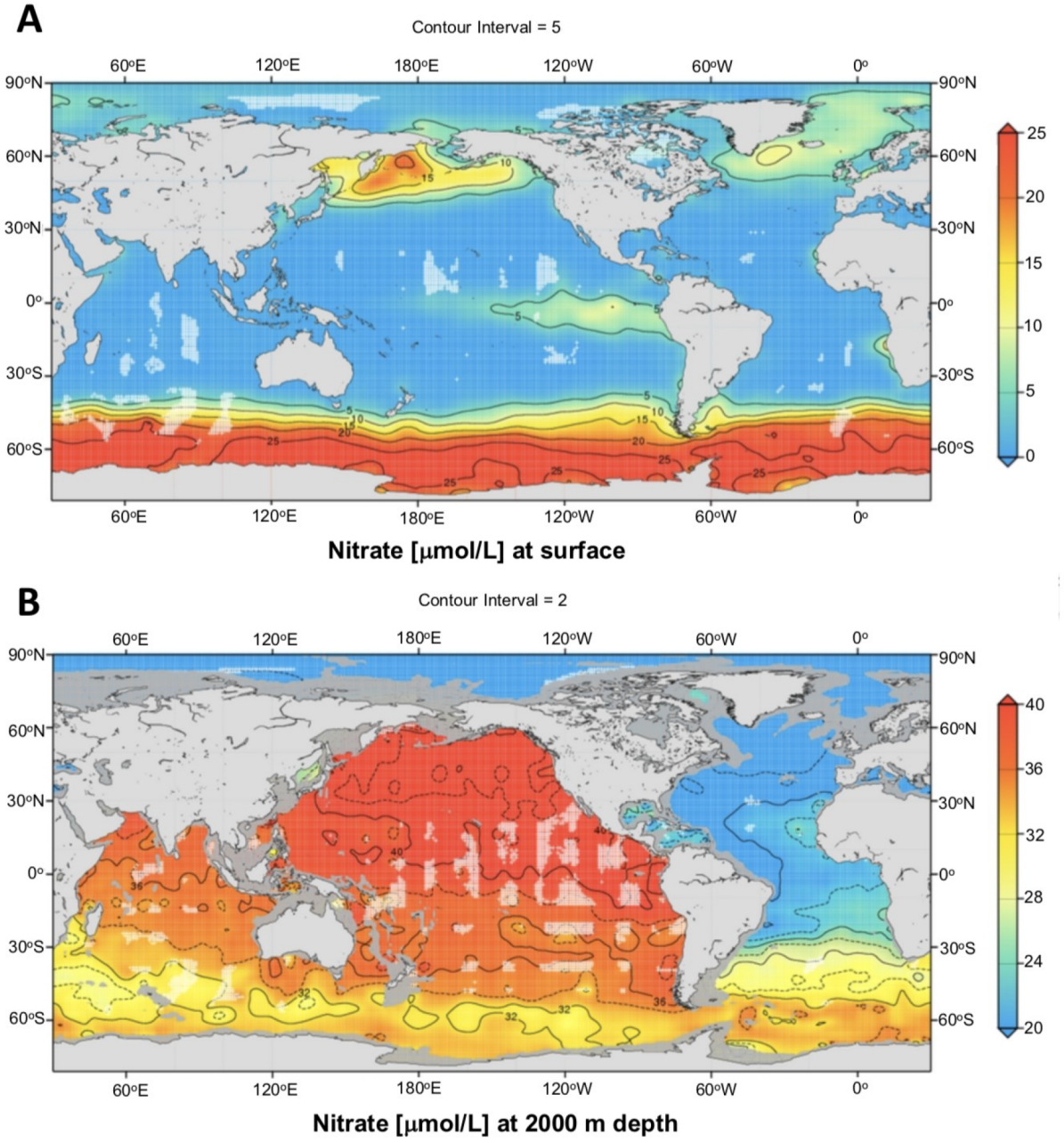


Figure 2. Nitrate (NO_3^-) concentrations for oceanic surface (A) and deep waters (B). Values are annual averages, a significant consideration only for surface waters (A). Based on Boyer et al.[23]

mox"), in which NH_4^+ is oxidized by nitrite, NO_2^-, to produce N_2 (Section 1.2.3).

Nitrite (NO_2^-) is another species that is important in the N cycle and yet has a small inventory. Average concentrations in the euphotic and aphotic zone of the ocean are 100 and 6 nM, respectively.[15] While nitrite can act as a nutrient for photosynthesizers in need of fixed N, its low concentration across a range of environments can be attributed to its role in redox processes.[24] Nitrite is an intermediate of oxidative and reductive processes such as nitrification and denitrification, and it is a substrate for anammox (Sections 1.2.2 and 1.2.3). The lack of nitrite accumulation in well-lit systems such as ocean surface waters is mostly a consequence of photosynthetic assimilators' consumption of ammonium,[30−32] the dominant proximal substrate for nitrite production in oxic systems, rather than the consumption of nitrite by these same assimilators. Nitrite is only observed to accumulate to significant levels when production and consumption processes become transiently uncoupled, such as (1) in oxygen-deficient zones (with concentrations up to 10 μM), (2) at the base of the euphotic zone in most regions (with 0.2 to 1.5 μM), and (3) in the surface waters of some polar regions (with ~0.25 μM).[24] That such cases of decoupling are so rare is a testament to the efficiency of biologically facilitated redox processes in the environment.

Dissolved organic nitrogen (DON) in the ocean is the second-most abundant form of fixed N[15,33] (Figure 1), with significant concentrations in the open ocean (i.e., > 4 μM in surface waters, decreasing to ~2 μM in deep water).[34] In subtropical surface waters, DON is by far the largest N pool. Despite its abundance, the chemical composition, sources, sinks, and bioavailability of DON are not well understood.[33,35] The significant concentration of DON in surface waters implies that

https://dx.doi.org/10.1021/acs.chemrev.9b00613
Chem. Rev. 2020, 120, 5308−5351

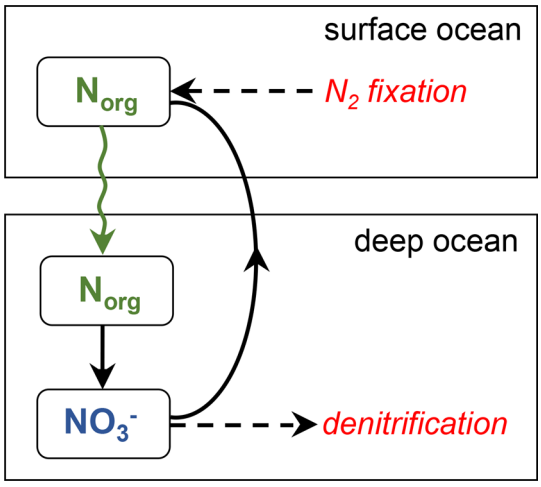


Figure 3. Simplified diagram of ocean nitrogen cycling that distinguishes between the ocean's internal N "cycle" (solid lines) and its input/output "budget" (dashed lines). N_2 fixation in the surface ocean and denitrification in the ocean interior and sediments are, respectively, the dominant input and output terms of the budget. These processes are described in detail in Sections 1.2.1.1 and 1.2.3.1. To maintain a constant size for the fixed N reservoir, the rates of the input and output terms must be equal, and feedbacks for achieving this balance are discussed in section 3. In the internal cycle, phytoplankton in surface waters obtain N for their biomass (N_{org}) by assimilating nitrate (NO_3^-), biological N_2 fixation occurring at a much lower rate. The resulting biomass organic N is eventually exported to the dark ocean interior (i.e., the deep ocean), mostly as sinking particles (green wavy arrow). Within the deep ocean, organic N is converted by microbial activities to nitrate (black downward arrow). This deep-water nitrate can be circulated back up into the surface ocean, where it once again supports phytoplankton growth (arced upward arrow). Oceanic fixed N, prior to its loss from the ocean by denitrification, undergoes roughly 5−10 iterations of the internal cycle shown in the diagram. Another important aspect of N cycling, not shown in this diagram, is the metabolism or degradation of N_{org} in surface waters, which produces ammonium (NH_4^+) that is then reassimilated by phytoplankton to remake N_{org}.

its composition leads it to be of limited bioavailability; it is often described as "chemically refractory".[36] Higher molecular weight DON exists predominantly as amides, amines, and N-acetylated sugars.[33,35,37,38] However, upon production in ocean surface waters, DON is eventually consumed mostly in the surface (the upper 100 m) or in the relatively shallow waters of the ocean thermocline (100−500 m depth).[39] The net DON production in some regions followed by its transport by circulation and then its net decomposition elsewhere may be an important mode of transporting fixed N among marine environments. For example, this process has been suggested to provide N to the nutrient-poor waters of the subtropical gyres.[34]

Freshwaters, soils, coastal sediments, and biota each contain much less N than the ocean environment (Figure 1). The majority of freshwater N is found in groundwaters, which are more voluminous than fresh surface waters and can accumulate leached N as nitrate.[22,40] The N in soil and sediment pools is mostly organically bound and of unknown composition and availability.[41] Young soils recently produced from igneous or metamorphic rock are typically N-poor.[42] As these soils age, they become more N-rich, in part because of a longer history of N additions and in part because organic N becomes more refractory as it ages.[42,43] Physical processes such as erosion, weathering, and leaching along with microbial denitrification can lead to N loss. However, phosphorus (P) is also lost from soils, so the aging of soil typically increases the availability of N relative to P.[42] Despite their lower abundances, the other N forms described above (nitrate, nitrite, ammonium, and DON) also occur in soils, where their roles are largely the same as in the ocean. One further consideration in soils is that the different N forms have additional mechanisms of mobility: nitrate is susceptible to hydrologic loss (i.e., as nitrate in runoff and into groundwater), while ammonium can be bound to clays and has the potential for volatilization as ammonia.[16]

1.2. Transformations of Nitrogen

Within natural and human-influenced terrestrial and marine ecosystems, N can undergo numerous biologically driven transformations between different chemical forms (Table 1). Because most of these transformations are uniquely catalyzed by microbes in support of their growth and energy requirements, the N cycle is often referred to as the microbial N cycle (Figure 4). In our description of the major processes below, we focus on

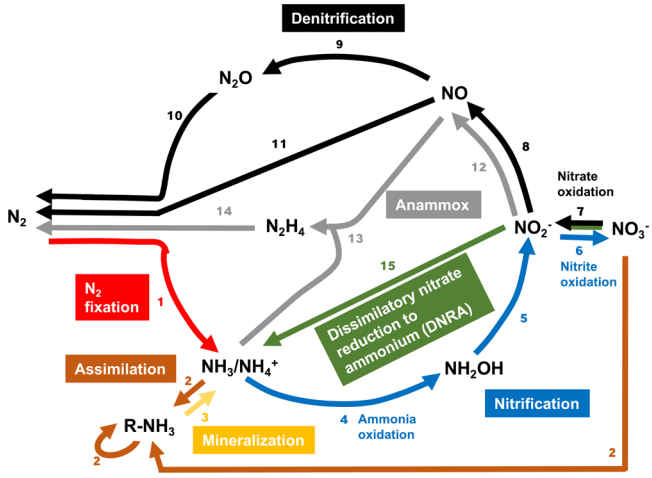


Figure 4. Cycle of biologically driven N transformations that occur in natural and human-influenced terrestrial and marine environments. Nitrogen (N_2) fixation (step 1) and N assimilation (from ammonium, nitrate, or organic N, step 2) are anabolic processes, whereas mineralization (step 3), nitrification (steps 4−6), DNRA (steps 7, 15), denitrification (steps 7−11), and anammox (anaerobic ammonium oxidation, steps 12−14) are (or are a consequence of) catabolic processes. Abiotic sources of fixed N enter the biological N cycle as ammonium and nitrate.

biological nitrogen fixation, denitrification, and anammox, as these are the dominant processes that add or remove N from the fixed N inventory and thus are the most generally important for ecosystem N availability. Abiotic sources of fixed N enter the biological N cycle as ammonium and nitrate. Other processes (assimilation, mineralization, nitrification, dissimilatory nitrate reduction) result in the internal cycling of N between different fixed forms, and they affect the spatial distribution of these forms; however, they do not directly affect integrated N reservoir sizes.

1.2.1. Input Processes. *1.2.1.1. Biological Nitrogen Fixation.* Biological nitrogen (N_2) fixation (step 1, Figure 4) is the only biological route for fixed N creation. It refers to the process by which specialized groups of prokaryotes termed "diazotrophs," or "N_2-fixers," reduce dinitrogen gas (N_2) to ammonia (NH_3) for the purpose of fulfilling their anabolic needs. The following provides an overview of the N_2 fixation

https://dx.doi.org/10.1021/acs.chemrev.9b00613
Chem. Rev. 2020, 120, 5308−5351

reaction, enzymes, and diazotrophic organisms, with the goal of highlighting the environmental sensitivities of this key process.

N_2 fixation enzymes and reaction. Because the triple bond of N_2 is very stable, diazotrophs exclusively use a large, structurally complex, iron-rich metalloenzyme called nitrogenase, along with significant amounts of ATP and reductant, to achieve the difficult conversion of N_2 to ammonia under ambient conditions.[44−46] This is in stark contrast to the extremely high temperatures and pressures needed for abiotic N_2 fixation with the Haber Bosch process, the source of fixed N used in fertilizers.[47] Nitrogenases, which can account for as much as 10% of total cell protein,[48] are multiprotein complexes that occur in three primary N_2-reducing isoforms, named based on the identity of a key active site metal atom: the molybdenum (Mo) nitrogenase, the vanadium (V) nitrogenase, and the iron (Fe)-only nitrogenase.[49−52] These forms are phylogenetically related and can be distinguished based on the amino acid sequence of the active site protein subunit.[53−55] All diazotrophs possess the Mo-nitrogenase; a limited subset also have the "alternative" V and Fe-only nitrogenases,[56] which they typically use under conditions of limited Mo availability (e.g., see refs 57−61).

The Mo nitrogenase is the best understood, most prevalent, and most ancient form of the enzyme.[46,54,55] It carries out the N_2 reduction reaction under optimal conditions with the following stoichiometry:[50]

$$N_2 + 8H^+ + 8e^- + 16ATP \rightarrow 2NH_3 + H_2 + 16ADP + 16P_i \quad (1)$$

A key feature of the reaction is the obligatory production of 1 H_2 per N_2 reduced; this reflects the mechanistically essential step of nitrogenase activation prior to N_2 reduction.[46,62] Recently, N_2 reduction reactions catalyzed by alternative nitrogenases were shown to have the same limiting stoichiometry[63] (eq 1), but because of kinetic variations related to cofactor structure, N_2 reduction by these isoforms is less catalytically efficient, resulting in more H_2 production on a molar basis compared to Mo-nitrogenases.[63,64] Interestingly, differences in the *in vivo* H_2 yields of various nitrogenase isoforms appear to be less substantial than *in vitro* yields.[64] Measurements of the *in vivo* fractionation of natural abundance stable isotopes between substrate and product also show isoform-specific variations.[65,66] The final product NH_3 is assimilated into the amino acid glutamate by a reductive reaction with α-ketoglutarate, a citric acid cycle intermediate, catalyzed by the enzyme glutamate dehydrogenase. It should be noted that nitrogenase can reduce several other multiply bonded substrates besides N_2,[50] and this wide substrate range has proved useful for studies of the mechanism and environmental role of nitrogenase. For example, the reduction of acetylene to ethylene forms the basis for a widely used assay of nitrogenase activity in the laboratory and in the environment[67] (Section 2.1.1). Different nitrogenase isoforms can be distinguished by their production of ethane as a minor byproduct of acetylene reduction[68] by the production of methane from CO_2 reduction.[69,70]

Nitrogenases share many structural and mechanistic similarities.[49,50,71] Critical for the function of all nitrogenases are the two proteins dinitrogenase reductase and dinitrogenase. Dinitrogenase reductase (i.e., the Fe protein) is a homodimer that accepts electrons from reduced ferredoxin or flavodoxin electron carrier proteins and transfers them one at a time in an ATP-dependent fashion to dinitrogenase (e.g., the MoFe protein in Mo nitrogenase), a heterotetrameric protein that contains the nitrogenase active site. The trace metal Fe, present within iron sulfur clusters distributed throughout the enzyme, plays a fundamental role in the reaction by participating in electron transfer between proteins and in catalysis. The surface exposure of Fe_4S_4 clusters results in nitrogenase being extremely sensitive to oxygen (O_2).[71,72] The molybdenum atom of Mo nitrogenase, another key metal, is present in the active site MoFe cofactor required for catalysis,[50] possibly aiding catalysis by tuning the cofactor's electronic structure.[63] Cofactors of the alternative V- and Fe-only nitrogenases most prominently differ from the MoFe form in the presence of a V or Fe atom in place of Mo and are termed the VFe and FeFe cofactors.[49,51,52,73]

Structural genes[49,74] encoding dinitrogenase reductase are *nif/vnf/anf H*, with *nif*, *vnf*, and *anf* denoting the Mo, V, and Fe nitrogenase isoforms, respectively. Those for the dinitrogenase protein are *nif/vnf/anf* D and *nif/vnf/anf* K. Alternative nitrogenases possess an additional structural subunit encoded by *vnf/anf* G.[49,52,74] Several other genes are required for N_2 fixation (e.g., *nif* ENB); these encode proteins involved in cofactor biosynthesis and assembly.[75,76]

Given the high metabolic cost of making, using, and maintaining such a complex and dominant cellular enzyme, it is not surprising that nitrogenase activity is strictly regulated. With regards to the global N cycle, the most important regulatory controls involve fixed N (i.e., ammonium), oxygen (O_2), energy, trace metals, and temperature, parameters which vary widely across different environments.[77−79] This control is enacted through complex regulatory cascades that can involve sensing and response at transcriptional through post-translational levels.[76]

Nitrogenase activity is modulated by fixed N availability in a wide diversity of diazotrophs (e.g., *Azotobacter*,[80−82] *Desulfovibrio*,[83] *Trichodesmium*,[84] *Klebsiella*,[85] *Clostridium*[86]). This regulation, best known in *Proteobacteria* species, occurs at the transcriptional level and, in some diazotrophs, also at the post-translational level.[76] Cellular N status is reflected by intracellular glutamine concentrations. Signal transduction proteins in the PII family (e.g., Gln *B* and Gln *K* ammonium sensors in *Proteobacteria*) communicate this signal, along with cellular energy (as ATP) and C status (as α-ketoglutarate), to regulatory target proteins, such as the master transcriptional regulator NifA, which directly control nitrogenase gene transcription.[76] For example, cellular glutamine levels could decrease in response to lower fixed N supply or to an increase in metabolic N demand. This would lead to a conformational change in PII proteins that inhibits their interaction with transcriptional regulators, allowing them to initiate gene transcription of nitrogen fixation genes. Once synthesized, nitrogenase activity can be controlled in response to ammonium and light by reversible covalent modification of the enzyme with ADP-ribose moieties.[76] This form of short-term regulation has been documented in a subset of diazotrophic bacteria and archaea.[58] Culture studies demonstrate that concentrations of ammonium which repress nitrogenase can range from below one to tens of micromolar, depending on the species.[80,83,84,87,88]

The oxygen regulation of nitrogenase transcription, also deciphered in *Proteobacteria*, relies on histidine kinases and transcription factors that together function as O_2/redox sensors.[76] The result of this regulatory cascade is the inhibition of gene transcription when intracellular O_2 levels are high. O_2 can also indirectly suppress nitrogenase synthesis by reducing the supply of C and reductant.[72] Once exposed to O_2, the

https://dx.doi.org/10.1021/acs.chemrev.9b00613
Chem. Rev. 2020, 120, 5308−5351

Table 2. Examples of Metabolic, Taxonomic, and Environmental Diversity for Diazotrophs

metabolism	example diazotroph (phylum, genus)	typical environment	O_2 protection strategy
Oxygenic phototrophy	*Cyanobacteria, Trichodesmium*	Marine	Time, spatial separation (?)
	Cyanobacteria, Anabaena	Freshwater	Heterocyst
	Cyanobacteria, Richelia	Endosymbiont of marine diatoms	Heterocyst
	Cyanobacteria, Atelocyanobacterium thalassa (UCYN-A)	Symbiont of marine algae	Unknown
	Cyanobacteria, Crocosphaera (UCYN-B)	Marine	Time
	Cyanobacteria, Cyanocethe (UCYN-C)	Marine	Time
Anoxygenic phototrophy	*Proteobacteria, Rhodopseudomonas*	Sediments, soils, microbial mats	Avoidance
	Chlorobi, Chlorobium	Freshwater	Avoidance
Aerobic heterotrophy	*Proteobacteria, Rhizobium*	Endosymbiosis	Physical
	Actinobacteria, Frankia	Endosymbiosis	Heterocyst-like vesicle
	Proteobacteria, Azotobacter	Soils	Respiration, conformational
Anaerobic heterotrophy	*Firmicutes, Clostridium*	Soils, sediments, animal guts	Avoidance
	Proteobacteria, Desulfovibrio	Soils, sediments	Avoidance
	Euryarchaeata, Methanosarcina	Soils, sediments, animal guts	Avoidance
Chemolithotrophy	*Proteobacteria, Acidothiobacillus*	soils, sediments, acid mine drainage	Avoidance

nitrogenase enzyme is quickly and irreversibly inactivated *in vivo*.[72] As a consequence of oxygen's deleterious effects, diazotrophs have evolved diverse mechanisms to protect existing enzyme from oxidative damage (e.g., see refs 89−93). We discuss these strategies in the following section on N_2-fixing organisms.

Mo nitrogenase is considered to be the most efficient isoform for N_2 reduction.[49,63] However, purified Mo and V nitrogenase can have similar activities at low temperatures[94] (e.g., ∼10 °C), and most recently, growth rates at 19 °C based on Mo and V-nitrogenase were found to be equivalent when a photoheterotrophic N_2-fixer was provided with a more reduced carbon substrate.[64] In certain diazotrophs (e.g., *Anabaena variabilis*, *Azotobacter vinelandii*, *Rhodobacter capsulatus*), the preferential use of the Mo enzyme under conditions of high Mo availability is based on the repression of alternative nitrogenase gene transcription by Mo.[60,82,95−97] Culture studies indicate that the threshold for Mo limitation is less than a few tens of nanomolar.[61,66,98] A decrease in temperature or the lack of high affinity Mo transporters can alter this dynamic by depressing Mo transport into cells.[97,99] The synthesis of alternative nitrogenases is also known to occur despite high Mo levels in N_2-fixers that cannot produce active Mo nitrogenase.[100,101] Molecular surveys indicate that alternative nitrogenase genes are ubiquitously distributed in terrestrial systems[55−57], and there is growing evidence for substantive non-Mo enzyme activity in environmental samples.[56,65,102,103] Whether such findings primarily reflect insufficient Mo availability or other reasons remains to be determined.

Iron is critical to all nitrogenase enzymes as well as most redox enzymes involved in respiration and photosynthesis.[104] Iron controls nitrogenase activity by affecting biosynthesis of iron sulfur clusters by NifS and NifU, proteins that incorporate intracellular Fe and S into cofactors.[105] Consistent with its central importance for redox metabolism, iron also exerts indirect control on N_2 fixation through its pervasive effects on growth (e.g., see refs 98, 106−108).

N_2-Fixing Organisms. Diazotrophy is limited to a subset of taxonomically and metabolically diverse prokaryotes, which typically account for a minor proportion of microorganisms in the environment.[109] Bioinformatic analysis of sequenced genomes for N_2 fixation genes suggests that ∼15% of prokaryotic species are known to or could potentially fix N_2.[75] These span at least 14 different bacterial and archaeal phyla, although most of those that have been studied experimentally belong to the phylum *Proteobacteria*.[75] In addition to their taxonomic diversity, diazotrophs utilize all forms of energy metabolism and are found in a variety of habitats within terrestrial and marine systems (Table 2). Molecular surveys of *nifH*, the marker gene for diazotroph ecology and diversity, show that soils harbor the greatest variety of N_2-fixers.[55,109,110]

Diazotrophs also use a diversity of strategies to solve the problem of nitrogenase damage by O_2 (Table 2). Some diazotrophs, such as *Clostridium*, avoid the issue entirely by inhabiting anaerobic environments. Oxygenic phototrophs, like the filamentous cyanobacterium *Anabaena*, spatially separate N_2 fixation from O_2 by enclosing nitrogenase in specialized heterotrophic cells, called heterocysts, which receive fixed C from nearby vegetative cells in exchange for fixed N. Within these cells, O_2-evolving photosystem II is inactive and the physical limitation of O_2 diffusion by thick cell walls and aerobic respiration keep intracellular O_2 levels sufficiently low.[111] The nonheterocystous marine cyanobacterial diazotroph, *Trichodesmium*, uses two strategies. It, like certain unicellular marine cyanobacteria,[112,113] can temporally separate N_2 fixation from O_2 evolution and may spatially separate nitrogenase into "diazocyte" cells.[92,114,115] The symbiotic N_2-fixing bacteria of legumes (e.g., *Rhizobium*) are aerobic heterotrophs that rely on the O_2-binding plant protein leghemoglobin to lower O_2 levels in the root nodules in which they reside.[116] Finally, the aerobic heterotroph *Azotobacter*, a common soil diazotroph, uses high respiration rates and proteins that cause nitrogenase to enter an O_2-tolerant conformation to solve the oxygen problem.[89,91,117] The metabolic cost of these strategies is considerable: over half of energy used for N_2 fixation is associated with O_2 protection.[118,119]

As expected based on the biochemistry and regulation of nitrogenase, external supplies of fixed N, O_2, energy (e.g., as light or organic C), trace metals (Fe, Mo, V), and temperature are important constraints on diazotroph activity in the environment (e.g., see refs 2, 79, 102, 120−127). Another important control is phosphorus availability, which indirectly affects N_2 fixation by constraining organism growth.[120,122−124,128−136] A high P requirement related to the large ATP demand of nitrogenase has been suggested to make N_2-fixers more prone to P limitation than nonfixers.[129] Inadequate moisture levels can also be

https://dx.doi.org/10.1021/acs.chemrev.9b00613
Chem. Rev. 2020, 120, 5308−5351

problematic for terrestrial diazotrophs.[129,137,138] Finally, ecological interactions (e.g., grazing of diazotrophs, competition with nonfixers over nutrients and light, narrower range of growth conditions) are also important in explaining the distribution of diazotrophy in nature.[124,129,139−142] Despite a variety of influencing factors, the dominant proximate control on diazotroph activity is fixed N availability (i.e., equivalent to supply minus demand). This dynamic is consistent with the genetic regulation, metabolic costs, and biological role of N_2 fixation.

Terrestrial systems host different diazotrophs from marine systems. The best studied soil diazotrophs are agriculturally important heterotrophic bacteria (e.g., *Rhizobia* and *Frankia*), which form symbiotic relationships with legumes and actinorhyzal plants, respectively, based on the exchange of fixed N for fixed C within root nodules. These types of symbiotic diazotrophs account for the majority of terrestrial N_2 fixation and are primarily found in low and midlatitude systems.[143,144] Consistent with fixed N control of symbiotic N_2 fixation in laboratory studies,[145,146] rates of legume N_2 fixation has been found to vary inversely with N richness in tropical soils.[147,148] Symbiotic N_2 fixation is also known to combat N deficiency associated with fast biomass accumulation during forest secondary growth.[149−151] However, high soil N supply does not always repress fixation.[152] This may be related to differences in light-constrained N demand,[153] an explanation consistent with the physiological role of N_2 fixation and its regulation by cellular N status. Interactions of nutrients with each other and biophysical factors are additional constraints on symbiotic diazotrophs. For example, global patterns of terrestrial symbiotic N_2 fixation can be reproduced by a biogeochemical model that incorporates the temperature dependency of nitrogenase activity and variable N costs of P acquisition in different environments.[79]

Asymbiotic diazotrophs also contribute to terrestrial N_2 fixation and can be more important than symbiotic diazotrophs in high latitude ecosystems.[143,144] These N_2-fixers account for the bulk of diazotroph diversity in nature[109] and comprise free-living species in soils (e.g., aerobic heterotroph *Azotobacter*) and freshwaters (e.g., heterocystous cyanobacterium *Anabaena*), associative diazotrophs in the rhizosphere of grasses (e.g., microaerophilic *Azospirillum*), as well as those that engage in mutualisms with nonvascular plants and lichens.[123,154] The vast majority remain uncultured, so our knowledge on the specific environmental sensitivities of different organisms is poor. However, nutrient addition experiments provide broad-scale insight on their environmental controls. A recent meta-analysis of such studies[125] indicates that N fertilization strongly suppresses free-living N_2 fixation, while Mo additions have a stimulatory effect. P limitation appeared to be confined to tropical forests. C limitation of heterotrophic N_2 fixation has also been suggested to be based on positive relationships between N_2 fixation rate, litter quality, and C/N ratios.[123,128,155,156] The importance of different controlling factors depends on the specific organism and environment. For example, free-living N_2 fixation in P-rich tropical soils is limited by Mo, but in P-depleted soils, fixation is Mo and P colimited.[131] However, Mo limitation of N_2 fixation could be relieved if diazotrophs produce strong Mo-binding molybdophores to facilitate Mo uptake[157] or utilize Mo-independent, alternative nitrogenases.[57−61]

In marine systems, the most important diazotrophs are pelagic cyanobacteria. These belong to the bloom-forming, filamentous genus *Trichodesmium*;[158,159] to heterocystous genera such as *Richelia*, which are endosymbionts of certain diatoms;[160] and to three groups of unicellular cyanobacteria, termed UCYN-A, UCYN-B, and UCYN-C, which consist of uncultured, non-photosynthetic symbionts (i.e., *Atelocyanobacterium thalassa*) of prymnesiophyte algae,[161] *Crocosphaera*, and uncultured *Cyanothece*-like species,[162] respectively. *Trichodesmium* species are typically found in the warm, N-poor waters of the subtropical and tropical ocean, where they can supply the N for a significant portion of produced and exported organic matter.[163,164] Field studies demonstrate that N_2 fixation by this organism is typically limited by P or Fe,[133−135] with Fe limitation depending on the prevalence of dust borne Fe flux to regional waters. For example, large inputs of dust from the Sahel and Sahara to the North Atlantic Ocean promote high rates of N_2 fixation that ultimately push the system toward P limitation.[135,162,165] In contrast, low dissolved Fe levels are correlated with suppressed N_2 fixation rates in the South Atlantic Ocean.[166] Culture studies demonstrate that under Fe deficient conditions, *Trichodesmium* reallocates cellular resources and upregulates Fe stress genes.[162,167,168] Under P limitation, *Trichodesmium* increases its maximal uptake rate of dissolved inorganic P, relies more heavily on dissolved organic P sources, and reduces its cellular P requirements.[162]

Compared to *Trichodesmium*, the ecophysiologies of other important marine diazotrophs are less understood. Diazotrophs associated with diatoms are widely distributed in warm oligotrophic waters, where they may contribute significantly to N_2 fixation[160,169] and play a role in exporting C to the deep ocean, due to the tendency of diatoms to be incorporated into sinking particles.[170] Phosphorus starvation has been suggested to limit *Richelia* activity in the ultraoligotrophic regions of the Mediterranean Sea.[171] In certain regions of the ocean, N_2 fixation by small unicellular cyanobacteria and bacterioplankton can equal or exceed those of larger diazotrophs like *Trichodesmium*.[172] Recent research has shown the unicellular uncultured cyanobacterium, *"Candidatus Atelocyanobacterium thalassa"* (also known as UCYN-A), to be a metabolically streamlined N_2-fixing algal symbiont that lacks the ability to perform oxygenic photosynthesis.[161] UCYN-A is present throughout low-, mid- and high-latitude waters.[161,173,174] An analyses of gene expression in response to nutrient additions suggested that UCYN-A might be P-limited in the tropical North Atlantic.[175] More recently, nutrient addition experiments revealed Fe limitation and possibly Fe and P colimitation of UCYN-A N_2 fixation in the subtropical eastern North Atlantic.[176] Like other marine diazotrophs, *Crocosphaera* species are most prevalent in warm, low-nutrient open ocean waters, where they are subject to limitation by Fe and P, depending on its particular oceanic habitat. To cope with Fe deficiency, *C. watsonii* lowers its cellular Fe requirement by reducing its Fe metalloprotein inventory.[106] To combat P limitation, *C. watsonii* can use a high affinity phosphate transporter to scavenge inorganic P as well as certain dissolved organic P sources.[177,178]

Marine systems host a wide variety and distribution of noncyanobacterial diazotrophs.[179−181] These organisms, typically detected with molecular surveys of the dinitrogenase reductase gene *nifH*,[180] are thought to be predominantly heterotrophic Proteobacteria. They have been identified in aphotic, nutrient-rich, and cold environments that are not normally associated with N_2 fixation (e.g., coastal and deep ocean waters and sediments[166,182−188]), as well as surface waters of the open ocean.[189] Recent studies suggest that organic matter particles could be important sites of heterotrophic N_2

https://dx.doi.org/10.1021/acs.chemrev.9b00613
Chem. Rev. 2020, 120, 5308−5351

fixation.[190,191] With regard to N flux, available data indicate that heterotrophic N_2 fixation rates are generally very low.[181] At this stage, without further information on the temporal and spatial distribution of heterotrophic N_2 fixation and its environmental controls, these diazotrophs are likely only a minor influence on N budgets.

1.2.1.2. Abiotic Sources of Fixed N. Fixed N in the form of ammonia (NH_3) or nitrogen oxides (NO_x = NO + NO_2) produced by natural and anthropogenic abiotic methods also contribute to environmental reservoirs of fixed N. Once deposited in terrestrial and marine systems, these compounds enter the microbial N cycle (Figure 4).

The production of NO_x by lightning in the atmosphere (5 ± 3 Tg N yr^{-1})[192] is an important source of fixed N. The high-temperature conditions associated with the lightning strike result in the dissociation of O_2 molecules and the formation of O radicals, which can react with N_2 to form NO (Zel'dovich mechanism[193]), which is subsequently stabilized by mixing with ambient air. Space-borne instruments show that most lightning activity occurs over land in deep-convective regions.[192] Volcanic activity is also accompanied by the release of fixed N originating from thermal fixation of atmospheric N_2.[194,195] Paulot et al. (2015)[29] estimated an emission of volcanic NH_3 of 0.9 Tg N yr^{-1} by scaling global SO_2 emissions (~13 Tg S yr^{-1}) using the NH_3:SO_2 molar ratio reported by Uematsu (2004).[196] Emission factors for oxidized N range from 0 to 0.1 kgN/kg(SO_2), with most estimates below 0.01 kgN/kg(SO_2). This suggests that volcanoes are likely to emit less than 0.2 Tg N yr^{-1} of oxidized nitrogen. Finally, mineral-catalyzed chemical reduction of N_2 to ammonium at the high temperatures and pressures observed in hydrothermal vents is a pathway for abiotic fixed N production that may have been particularly important early in Earth history.[197]

Recently, the release of fixed N from bedrock by physical and chemical weathering processes has been proposed to be a substantive source of fixed N for plants in certain high altitude and latitude terrestrial systems (~10 to 30 Tg N yr^{-1}).[6] The fixed nitrogen accumulated in rocks is ultimately derived largely from the atmosphere via biotic and abiotic N_2 fixation.[10] Thus, the fixed N released by rock weathering could be cast as an internal cycling term in the global fixed N cycle, which balances fixed N burial on long time scales.

Abiotic processes associated with human activities are large additional sources of new fixed N. The largest source of anthropogenic fixed N is the Haber Bosch process, which produces NH_3 for the synthesis of fertilizers and other chemical products[46] through the reaction of N_2 with H_2 using a ferrite catalyst under high temperature/pressure conditions. The modern rate of Haber Bosch fixed N production (~120 Tg N yr^{-1} in 2010 [5]) is similar to the rates of natural biological N_2 fixation on land and in the sea (Section 2.2). Fossil fuel combustion for power generation, heating, and transportation yields NO_x compounds as byproducts[198] either via the oxidation of the fuel N or through the reaction of atmospheric N_2 with oxygen and hydrocarbon radicals.[193,199] The release of fixed N from heavy-oil furnaces and coal-fired power plants is controlled by fuel N content. Radical mechanisms, favored by low-N fuel (e.g., gasoline, natural gas), high temperatures, and low air-to-fuel ratios, are responsible for the production of NO_x from transportation and natural gas-fired power plants.[200] As of 2010, fossil fuel use contributes ~40 Tg N yr^{-1}, primarily to terrestrial systems.[5] Increasing use of three-way catalytic converters, improvements in combustion engines, and the switch from coal to gas and renewable power sources[201] have led to dramatic reductions in NO_x emissions in the US and Europe,[202] and more recently in China.[203]

1.2.2. Internal Cycling. *1.2.2.1. Nitrogen Assimilation.* Nitrogen assimilation refers to the cellular production of organic nitrogen from external inorganic and organic nitrogen species (step 2, Figure 4). The assimilation of dissolved inorganic N as ammonium or nitrate is common in plants, phytoplankton, fungi, and microbes.[204−208] Ammonium assimilation into biomass typically involves ammonia diffusion or ammonium transport into the cell followed by incorporation of ammonia into amino acids by the enzymes glutamine synthetase and glutamate synthase.[208] Nitrate assimilation is more metabolically costly since nitrate must first be reduced to ammonia by nitrate and nitrite reductases before assimilating its N into biomolecules. When concentrations are low, ammonium is generally preferred over nitrate by phytoplankton as an inorganic fixed N source[207] as its N is already at the redox state of N in amino acids (Table 1). Plants show a preference for ammonium or nitrate, depending on plant functional type and environmental conditions (e.g., external ratios of ammonium/nitrate).[209−211] Because of its ease of assimilation into biomass, ammonium accumulation is rarely observed in the environment, except under dark, anoxic conditions when the absence of autotrophic growth or chemical oxidants limits its removal.

Organic N assimilation, traditionally associated with animals, has also been observed in plants, fungi, and microbes.[208,212,213] However, it is generally much less important for primary production than inorganic N[209] and is not discussed further.

1.2.2.2. Mineralization. The return of biomass-derived organic N (initially in the form of particulate organic nitrogen, PON) into the inorganic (mineral) form of ammonium is termed mineralization (or remineralization) (step 3, Figure 4). This process is associated with excretion by organisms during macromolecular recycling and the microbial degradation of organic matter. During mineralization, biomass PON may be first broken down into DON forms (e.g., by physical disintegration, solubilization, breakdown at C−C bonds), followed by deamination of protein and nucleotide macromolecules, ultimately resulting in the release of ammonium into the environment. Ammonium and small organic N compounds such as amino acids can be assimilated by organisms to make biomass. In the ocean, mineralization is mainly carried out by heterotrophic microbes, both prokaryotes and eukaryotes, while in terrestrial systems, fungi and macroorganisms such as earthworms are also important.[209]

1.2.2.3. Nitrification. The process of nitrification (steps 4−6, Figure 4), in which ammonium is oxidized with O_2 to nitrate, is typically carried out by specialized groups of chemoautotrophic nitrifying microbes that use ammonia or nitrite as a source of energy and as sources of biomass N. Under low O_2 availability, the greenhouse gas nitrous oxide (N_2O) is released during nitrification by multiple pathways.[214] Ammonia oxidizing bacteria (e.g., proteobacterium *Nitrosomonas*) and archaea (e.g., thaumarchaeon *Nitrosopumilus*) oxidize ammonia to nitrite with O_2 (steps 4 and 5, Figure 4). Nitrite oxidizing bacteria (e.g., proteobacteria *Nitrobacter*, *Nitrospina*) oxidize nitrite aerobically to nitrate with O_2 (molecular O_2 is not involved in the reaction) (step 6, Figure 4). Recently, a very restricted group of proteobacteria (Comammox) was found to be capable of completely oxidizing ammonia to nitrate.[215,216] Comammox proceeds by the same two steps, catalyzed by versions of the same two enzymes that are used separately by

https://dx.doi.org/10.1021/acs.chemrev.9b00613
Chem. Rev. 2020, 120, 5308−5351

ammonia- and nitrite-oxidizing nitrifiers. Diverse fungi and heterotrophic bacteria oxidize inorganic and organic reduced forms of N to nitrite and nitrate, in a process called heterotrophic nitrification.[217] Heterotrophic nitrification, generally less understood compared to autotrophic nitrification, may be an important nitrification process in soils.[217−219] The mechanism may be similar to that in autotrophic ammonia oxidizers or is linked, in some strains, to aerobic denitrification.[217,219,220]

1.2.2.4. Dissimilatory Nitrate Reduction to Ammonium. In dissimilatory nitrate reduction to ammonium (abbreviated DNRA), nitrate is used as an electron acceptor for anaerobic respiration of organic matter by chemoheterotrophs, being reduced first to nitrite, and then ammonium (steps 7 and 15, Figure 4). DNRA has been identified in a variety of prokaryotes and eukaryotic microbes.[221,222] In contrast to denitrification, which removes fixed N (discussed below), DNRA recycles fixed N within an ecosystem. DNRA is relatively less important in marine systems than in terrestrial systems, where it can dominate the fate of the nitrate pool.[223] Microbes performing DNRA and denitrification may be in competition for nitrate and organic matter; DNRA is favored at higher organic C/nitrate ratios, while lower ratios favor denitrification.[224] Higher C/nitrate ratio may also favor N_2O production by incomplete DNRA if nitrite is allowed to accumulate.[225] DNRA may be less sensitive to oxygen concentrations (both DNRA and denitrification are predominantly anaerobic), thus allowing for differential N retention versus loss as a function of redox conditions.[226]

1.2.3. Output Processes. *1.2.3.1. Denitrification.* Denitrification converts nitrate back into N_2 (steps 7−11, Figure 4) and is the primary route by which fixed N is lost from marine systems. Even in terrestrial ecosystems, where runoff is a major physical loss term for fixed N, denitrification can dominate total N loss over large scales.[227] Most denitrifying organisms are facultatively aerobic, heterotrophic bacteria that use nitrate as a terminal electron acceptor for respiration, when O_2 is no longer sufficiently available in their environment. Complete denitrifiers can perform the complete reduction of nitrate to N_2 using a sequence of intermediate reductions (nitrate to nitrite, then to nitric oxide NO, nitrous oxide N_2O, ending in N_2, eq 3). Aerobic denitrification, using the same pathway, has been documented in several bacteria, notably *Paracoccus denitrificans* (formerly *Thiosphaera pantotropha*, in which it was originally discovered by Robertson and Kuenen (1984)[228]). Aerobic denitrification is seen as a common variant among conventional denitrifiers and results from differential regulation of aerobic and anaerobic pathways, often under oscillating oxygen conditions. It is potentially widespread in both natural and industrial systems;[229] its contribution to total N loss is unknown, as most denitrification occurs under anoxic conditions. Incomplete denitrification can lead to the release of N_2O and is assumed to account for N_2O accumulations in anoxic environments.

For the purposes of calculating the net loss of fixed N for budgetary purposes (Section 2.2), it may be sufficient to understand the loss processes as simply organic nitrogen → N_2. Until the 1990s, the transformation of organic matter to N_2, via respiration of nitrogen oxides (i.e., denitrification), was the only process known to convert fixed N into N_2. Conventional denitrification involves the consumption of organic matter by heterotrophic bacteria, who respire nitrate in the absence of oxygen, yielding ammonium as the main remineralized N product, and N_2 as the final respiratory product (eq 2).

$$(CH_2O)_{106}(NH_3)_{16}H_3PO_4 + 94.4HNO_3 \rightarrow 106CO_2 + 39.2N_2 + 16NH_3 + 16HNO_2 + H_3PO_4 + 145.2H_2O \quad (2)$$

$$NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2 \quad (3)$$

Our understanding of denitrification has changed dramatically in the last 20 years, enlightened greatly by experiments with natural microbial assemblages and analysis of molecular biological data coupled to experimental manipulations.

Complete denitrification, the sequential reduction of nitrate to dinitrogen gas (eq 3), is performed by many bacteria, using the individual N oxides as terminal electron acceptors during anaerobic respiration. The terminal reductase enzymes were purified and characterized by the 1990s, and we refer to Zumft[230] for a comprehensive review of this process. The model organisms were often Pseudomonads, and that model system has dominated our understanding of the process for many years. It is worth briefly reviewing some of the basics, but the reader is referred to earlier reviews and original literature for the details.

1.2.3.2. Denitrification Enzymes. Nitrate reductases are Fe−Mo proteins. There are four major types:[231] EukNR is the assimilatory protein in eukaryotes, Nas is the prokaryotic assimilatory enzyme, Nap is the membrane-bound, periplasm-facing dissimilatory enzyme in prokaryotes, and Nar is the membrane-bound, cytoplasm-facing dissimilatory enzyme in prokaryotes. Nar is the enzyme typically associated with denitrification in bacteria, and its cytoplasmic orientation is consistent with generation of proton motive force for energy generation during denitrification. Although the mechanism of energy generation for Nap is less clear, Nap is linked to nitrate respiration and dissimilatory nitrate reduction to ammonium (DNRA) and, rarely, complete denitrification. Nap is the version of the enzyme found in microbes capable of aerobic denitrification. Both Nap and Nar are involved in nitrate reduction, the first step toward loss of fixed N as N_2O or N_2.

Nitrite reduction is performed by two structurally unrelated enzymes: cdNiR (NirS) is a cytochrome-containing Fe protein, while CuNir (NirK) contains both type-I and type-II copper (Cu) sites. The active site of the CuNir has high affinity for both NO and O_2, suggesting mechanisms for both enzyme activity regulation by product inhibition and for oxygen sensitivity of the reaction.[232]

The capability for NO reduction is found very widely in the biological world, due to both the toxicity of NO, and its short lifetime, the latter making it an ideal signaling molecule. The diversity of NO-reducing enzymes (NORs) thus makes it difficult to identify them and assign function in genome sequences and leads to continuing uncertainty about the completeness of denitrification pathways even in cultivated bacteria. Nevertheless, NORs occur in just two main types, distinguished by the source of the electrons that they use to reduce NO. qNOR obtains its electrons from membrane-bound quinones (quinone-oxidizing), while cNOR gets its electrons from soluble electron carriers, mostly cytochromes (cytochrome-oxidizing). Both are found in denitrifying pathways. cNOR is most common in bacteria, always cooccurring with Nar, the cytoplasmic-facing nitrate reductase. qNOR occurs in denitrifying haloarchaea, linked to the periplasmic Nap enzyme. NORs are Fe-containing enzymes, closely related to oxygen reductases such as cytochrome and quinol oxidases.[233] Several

https://dx.doi.org/10.1021/acs.chemrev.9b00613
Chem. Rev. 2020, 120, 5308−5351

other kinds of NO-reducing enzymes also produce N_2O as part of detoxification, rather than respiratory pathways.[234]

Nitrous oxide reductase (N_2OR) is a complex Cu enzyme, containing a unique "Z" form Cu−S moiety. Although widespread and diverse in terms of gene sequence, there is only one kind of N_2OR.[235] There are, however, two clades of N_2OR, which differ mainly in their signal peptides,[236] rather than in the functional core of the enzyme. Clade I consists almost entirely of Proteobacteria (plus a few divergent Archaea), while Clade II includes members of numerous phyla (including Archaea), many of which do not contain complete denitrification pathways.[236,237] The relative abundance of the two clades varies widely across terrestrial environments, but Clade II N_2OR is generally more abundant.[236] The distribution of Clade II N_2OR has not been quantified in the ocean, but it has been detected, with relatively higher abundances in surface waters.[238]

Together, these individual respiratory enzymes encode what is considered the complete canonical denitrification pathway. The complete pathway is found in many cultivated denitrifying bacteria, but evidence is accumulating that the functional pathway in natural assemblages is actually more modular than linear. That is, many different kinds of microbes possess only part of the pathway, sometimes only one of the enzymes. Graf et al.[239] investigated the co-occurrence patterns of denitrification genes across 652 microbial genomes in 18 phyla and found that the *nosZ* gene had a significantly higher frequency of co-occurrence with *nirS* than with *nirK*. Thus, *nirS*-type denitrifiers are more likely to be capable of complete denitrification. Thirty percent of the genomes that contained *nosZ* did not contain either nitrite reductase gene, which implies a respiratory niche related to N_2O reduction, independent of the other nitrogen oxides. The modularity of the pathway might be related to the availability of substrates under different redox conditions or to the relative favorability of the individual steps. Under standard conditions,[240] the last step, $N_2O \rightarrow N_2$, is most favorable, followed by $NO \rightarrow N_2O$, $NO_3^- \rightarrow NO_2^-$ and last, $NO_2^- \rightarrow NO$. Thermodynamics alone does not explain the distribution of each step among microbes, however, because the $NO_3^- \rightarrow NO_2^-$ is thought to be the most widely distributed, and it is not the most favorable. The high redox potential of $N_2O \rightarrow N_2$ might, however, be a factor in selection for this step, especially since neither product nor substrate is toxic, unlike other reactants in the complete pathway (i.e., NO and NO_2^-).

The modularity of the pathway in natural environments implies that microbial community structure is important in determining the fate of fixed N and the yield of N_2O during denitrification. It might also help explain the distribution of denitrification intermediates, especially N_2O and NO_2^-, in seawater. Both N_2O and NO_2^- often exhibit discrete maxima, especially in low oxygen waters. For example, N_2O consistently shows a strong maximum (>10-fold above atmospheric equilibrium[241]) in the upper oxycline of an oceanic oxygen-deficient zone (ODZ). Although the oxycline occurs in stratified waters, turbulent diffusion would quickly erode the maxima if they were not maintained by production and consumption.[242] The N_2O maximum in the oxycline could be explained by the greater sensitivity to oxygen of N_2OR, relative to NOR, such that complete denitrification is inhibited by the presence of even low levels of O_2, or by the assemblage at that depth being dominated by incomplete denitrifiers, that is, lacking the capability for N_2O reduction.

The secondary NO_2^- maximum is a consistent feature of ODZs, and usually occurs in the ODZ core, where O_2 concentrations are consistently essentially zero. This accumulation could be due to the relative dominance of microbes capable of reducing NO_3^- to NO_2^- but no further, such that the NO_2^- production rate exceeds the NO_2^- removal rate. The ultimate control over N reduction rates would be the supply of organic matter.[243,244] Greater supply of organic matter in the upper oxycline supports high rates of N loss (complete denitrification and anammox), while reduced organic matter supply at depth limits N removal and results in the formation of the secondary nitrite maximum.[245]

1.2.3.3. Denitrifying Organisms. Heterotrophic bacteria of diverse genera are the best known and most widely distributed denitrifiers. Gammaproteobacteria, such as in the genera *Pseudomonas* and *Marinobacter* are perhaps the best known complete denitrifiers, having been studied extensively in culture. An extensive analysis by next-generation sequencing of *nirS*, a traditional marker gene for denitrification, found that Proteobacteria were indeed important members of marine and sediment assemblages, but sequences closely related to *Marinobacter* occurred only in sediments[246] and Pseudomonads were not abundant in either sediments or water column samples. The vast majority of microbes containing *nirS* in the oceanic ODZs were not closely related to any cultivated denitrifiers, and saltmarsh sediments harbored even more diverse, and novel, denitrifiers.[246]

Perhaps the only clade of potentially complete denitrifying bacteria that has been documented to be common in oceanic ODZs is Marinimicrobia. Detected in one of the first 16S rRNA surveys of the ocean,[247] metagenomic investigations have now identified Marinimicrobia as a diverse clade with multiple metabolisms that may be of importance in low-oxygen waters.[248] With a relative abundance on the order of 10% of the total assemblage, this is clearly an important clade, but many others remain to be identified beyond their functional gene sequences.

Complete denitrification is also coupled to sulfur oxidation by autotrophic sulfide oxidizing bacteria. These organisms are thought to complete a cryptic sulfur cycle, in which simultaneous activities of sulfate-reducing and sulfide-oxidizing pathways are so closely coupled that locally produced sulfide from sulfate-reducing bacteria is immediately oxidized back to elemental sulfur or sulfate by sulfide-oxidizing bacteria using nitrate, such that dissolved sulfide is not detectable. The environmental presence of SUP05, a gammaproteobacterial sulfur oxidizer and the first described representative[249] of a group now proposed to be named *Thioglobus perditis*,[250] is taken as an indication of the potential for cryptic sulfur cycling. *T. perditis*/SUP05 is autotrophic, fixing CO_2 by the Calvin Cycle, and some clades are capable of complete denitrification (i.e., from NO_3^- to N_2). Gene sequences associated with the SUP05 clade have been found in sulfidic inner shelf waters of the Eastern Tropical South Pacific Ocean (ETSP)[251,252] and also in outer shelf waters of the ETSP and Eastern Tropical North Pacific Ocean (ETNP) where sulfide is undetectable.[253,254] Callbeck et al.[250] found SUP05 genes in highly productive nearshore eddies in the ETSP, which transported the microbes and associated sulfur chemistry as much as 80 km from the coast. The degree to which nitrate reduction linked to autotrophic sulfur oxidation contributes to the total fixed N loss in ODZs is debated,[255] but it is likely to be most important in the coastal and sediment-linked regimes.

It is likely that the net process of complete denitrification is performed by diverse groups that may carry out only one or two of the steps in the pathway. Evidence of modular denitrification

https://dx.doi.org/10.1021/acs.chemrev.9b00613
Chem. Rev. 2020, 120, 5308−5351

is found in the metatranscriptomic data of Ganesh et al.,[256] who found that the phylogenetic affiliations of the *nirS* and *nosZ* genes were quite different for the most highly expressed genes. For example, at 300 m in the ETNP, the *nar* genes were dominated by candidate phylum OP1, *nirK/S* by Gammaproteobacteria, *norB/Z* by OP1 and *nosZ* by uncultivated prokaryotes (i.e., so novel as to be unassignable to a phylum). This pattern suggests that a complex assemblage of interacting microbes accomplishes the complete pathway and that the intermediates must be passing between them.

One clade of the most abundant microbe on earth, the oligotrophic heterotroph *Pelagibacter ubique* (SAR11), is very abundant in the upper oxycline of ocean ODZs and is apparently responsible for NO_3^- reduction in that depth interval. Metagenomic and single amplified genome analysis did not detect any other of the denitrification pathway genes in the ODZ SAR11. The ODZ SAR11 clade contains diverse *nar* genes and constituted upward of 80% of the total cells in some samples from the ODZ, implying that SAR11 is responsible for much of the organic matter decomposition, coupled to NO_3^- respiration to NO_2^-, in the upper region of the ODZ.[257] Indeed, high rates of NO_3^- reduction to NO_2^- are observed in ODZs,[258,259] with these exceeding the rate of N_2 production from complete denitrification. Comparably high rates of NO_2^- oxidation are reported in the same samples, at rates much higher than can be accounted for by anammox bacteria.[259−261] Known NO_2^- oxidizing bacteria (NOB) are all obligate aerobes, although novel NOB have been detected in metagenomic investigations of anoxic waters.[262] This enigma remains to be resolved. One possibility is that the measured rates are overestimated due to a poorly understood N isotope exchange reaction between NO_2^- and NO_3^- across the enzyme nitrite oxidoreductase.[263] Coupled NO_3^- respiration and NO_2^- oxidation implicates NO_2^- as a pivotal molecule in regulating the fate of fixed N − if NO_2^- is reoxidized to NO_3^-, it is conserved in the biologically available pool, rather than being further reduced to N_2O or N_2 and lost from the fixed N pool.

Identification of novel N_2OR enzymes that occur in microbes not generally associated with complete denitrification (see above, references[236,237]) suggests that as of yet undiscovered microbes might be responsible for the consumption of N_2O produced by other modular denitrifiers. The novelty of *nosZ* genes found in the ETNP[256] motivates the search for N_2O consumers in both deep and surface ocean waters.

Denitrification was recently discovered in eukaryotic microbes[264] and rates attributed to foraminifera and other Rhizaria can equal those of bacterial denitrification in marine sediments.[265−267] Although complete denitrification ($NO_3^- \rightarrow N_2$) has been verified in the eukaryotic microbes, and proven not to be attributable to bacterial contaminants, the enzymes responsible for the pathway are still under investigation. Both nitrite reductase and NOR are found in the mitochondrion[268] and Nar and N_2OR are assumed to be present as well, but so far unidentified. The NiR in the foraminiferan *Globobulimina* is a novel NirK, only distantly related to the assimilatory NirK of eukaryotes or the dissimilatory NirK of prokaryotes.[268]

1.2.3.4. Anammox. Anaerobic ammonium oxidation (or "Anammox") is a second route of fixed N loss that occurs in low-O_2 settings. Anammox bacteria, all of which belong to the phylum *Planctomycetes*, oxidize NH_4^+ using NO_2^- as an electron acceptor, generating N_2 as a final product (steps 12−14, Figure 4). Unlike denitrifiers, anammox bacteria are autotrophic and depend on heterotrophic mineralization to provide their reduced N substrates. Anammox reactions are located inside a specialized intracellular membrane-bound compartment composed of unique ladderane lipids, which are thought to protect the rest of the cell from the toxic, highly reactive reaction intermediates, hydrazine and NO.[269]

Anammox was discovered as a biological process in enrichment cultures from wastewater treatment plants.[270,271] The net reaction[272] (eq 4) oxidizes ammonium using nitrite and results in the production of N_2 and NO_3^- under anaerobic conditions, supporting autotrophic CO_2 fixation by anammox bacteria:

$$1NH_4^+ + 1.146NO_2^- + 0.071HCO_3^- + 0.057H^+ \rightarrow 0.986N_2 + 0.161NO_3^- + 2.002H_2O + 0.071CH_{1.74}O_{0.31}N_{0.20} \quad (4)$$

In the proposed biochemical pathway (eqs 5−8), NH_4^+ and NO_2^- are converted to dinitrogen gas. Nitrite is reduced first to nitric oxide (NO) and then to hydroxylamine (NH_2OH), which is then combined with NH_4^+ to form hydrazine (N_2H_4, in eq 6). Hydrazine is then oxidized to dinitrogen (eq 7). The enzymes that catalyze the last two steps, hydrazine synthase and hydrazine dehydrogenase, are apparently unique to the pathway and thus provide useful signature genes for diagnosis and quantification:

Nitrite reductase

$$NO_2^- + 2H^+ + e^- \rightleftharpoons NO + H_2O \quad (5)$$

Hydrazine synthase

$$NO + NH_4^+ + 2H^+ + 3e^- \rightleftharpoons N_2H_4 + H_2O \quad (6)$$

Hydrazine dehydrogenase or *HAO*-like

$$N_2H_4 \rightleftharpoons N_2 + 4H^+ + 4e^- \quad (7)$$

Net reaction

$$NO_2^- + NH_4^+ \rightleftharpoons N_2 + 2H_2O \quad (8)$$

Although previously unknown in nature, the thermodynamic favorability of the net reaction had been noted.[273] Moreover, prior to its discovery in wastewater treatment enrichment cultures,[270,271] the net process had been proposed from the lack of an ammonium concentration maximum in the cores of oceanic ODZs and from chemical distributions and observed mineralization stoichiometries in suboxic marine sediments.[274] Anammox is now known to be responsible for a portion of the total fixed N loss previously attributed to denitrification. The anammox reaction depends on inorganic N substrates that are supplied from the remineralization of organic matter, which is performed by heterotrophic denitrifying or oxygen respiring microbes, and thus is indirectly but stoichiometrically dependent upon the supply of organic matter.[260] The global average contribution of denitrification (71%) and anammox (29%) to N_2 production should be dictated by the average composition of marine organic matter.[275] Interestingly, however, anammox and denitrification are frequently uncoupled, which may be a result of the constraints of the different lifestyles of heterotrophic denitrifiers and autotrophic anammox bacteria. The denitrifiers may be able to respond rapidly to organic matter input and can exhibit a bloom response,[276] resulting in episodic rates of highly variable magnitude.[277] The modularity of the denitrification process could enhance the flexibility of this response to changing substrate supply. Anammox bacteria by contrast, have a very low

https://dx.doi.org/10.1021/acs.chemrev.9b00613
Chem. Rev. 2020, 120, 5308−5351

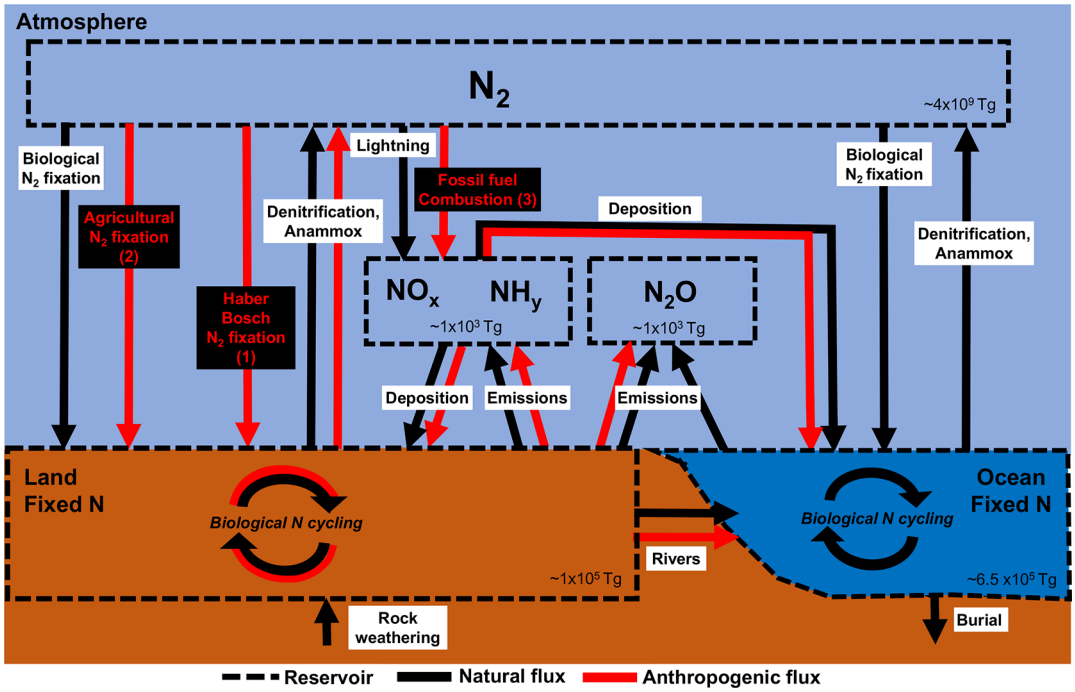


Figure 5. Global N cycling between the atmosphere, land, and ocean. N fluxes that result from natural processes are indicated by the black arrows; anthropogenically influenced fluxes are shown by the red arrows, with direct anthropogenic new N inputs indicated by the numbered arrows. Perturbations to coastal waters can be comparable to those of land; to draw a useful distinction, the ocean fluxes refer to the global (i.e., open) ocean. See Table 3 for estimated flux magnitudes.

energy yielding metabolism and cannot respond quickly to changing substrate concentrations—anammox thus proceeds at a more consistent rate than denitrification, occurring widely but with less dynamic range.

1.2.3.5. Anammox Bacteria. Five anammox genera have been described to date from environments including wastewater treatment plants, lakes, marine sediments, and the three large marine ODZs. The genera "*Candidatus Kuenenia*", "*Brocadia*", "*Jettenia*", and "*Anammoxoglobus*" have all been enriched from freshwater environments, mainly wastewater treatment plant outflows. The fifth genus, "*Candidatus Scalindua*", predominates in marine sediments and OMZs and was enriched from marine sediments.[278] Anammox bacteria have never been grown in pure culture, presumably due to obligate syntrophy with other microbes, especially ammonia oxidizing and denitrifying bacteria.

Anammox metabolism is autotrophic, fixing CO_2 using the Acetyl CoA pathway.[279] The ultimate source of reducing power for CO_2 fixation is the oxidation of NO_2^- to NO_3^-, catalyzed by an enzyme that is homologous with the nitrite oxidoreductase of aerobic nitrite oxidizing bacteria.

In a recent review, Oshiki et al.[272] described considerable physiological diversity leading to opportunities for niche differentiation and distinct biogeography of anammox species. Nonetheless, the fundamental metabolisms of anaerobic ammonium oxidation by the pathway involving hydrazine and of CO_2 fixation by the acetyl CoA pathway are shared among all known anammox bacteria. Within the marine genus *Candidatus Scalindua*, region-specific clades containing only within-species level sequence divergence, can be identified using the 16S rRNA gene,[272,280] but there is little information on ecologically significant differences in function or biogeochemistry among anammox species. This is in stark contrast to the diverse species and physiologies that comprise the "denitrifying" assemblage. On the basis of pyrosequencing to evaluate the diversity of denitrifiers and anammox bacteria in ODZs, the abundance of anammox genes is highly variable and *Ca. Scalindua*-like genes contributed from zero to 81% of the total *nirS* genes at different stations in the ETSP.[246]

1.3. Global Nitrogen Cycle and Its Anthropogenic Perturbation

The biotic and abiotic transformations of N along with its physical transport by the atmosphere and rivers result in the global cycling of N in multiple chemical forms between atmosphere, land, and ocean reservoirs (Figure 5).

Human activities related to food production and fossil fuel combustion have perturbed the global N cycle since the Industrial Revolution by introducing new N into terrestrial and marine fixed N inventories[7,47,281] (red arrows 1–3, Figure 5). The industrial scale production and widespread usage of N-rich fertilizers was enabled by invention of the Haber Bosch process for synthetic N_2 fixation to ammonium (red arrow 1, Figure 5) in the early 20th century. This has allowed agricultural yields, previously limited by the amount of naturally available fixed N, to expand to levels that support rapidly growing populations. Agriculture-associated biological N_2 fixation (e.g., legume cultivation) and fossil fuel combustion, which emits nitrogen oxides (NO_x), are other important human derived N sources (red arrows 2 and 3, respectively, Figure 5). Inefficiencies in food production and the high mobility of inorganic N lead to the loss of a significant fraction of anthropogenic N to surrounding soil, water, and atmosphere, which in turn leads to greater fluxes within the internal N cycle as well as the acceleration of N loss processes (red arrows for biological N cycling, emissions, denitrification/anammox, and transport, Figure 5).

The benefits of anthropogenic N for human society have not come without significant costs to the environment and human health. Galloway et al.[282] introduced the term "nitrogen

https://dx.doi.org/10.1021/acs.chemrev.9b00613
Chem. Rev. 2020, 120, 5308–5351

cascade" to highlight that a single atom of anthropogenic N can induce multiple environmental effects before denitrification removes it from the fixed N inventory. These effects, which we summarize below, include decreases in ecosystem biodiversity due to acidification and eutrophication, increases in greenhouse gases and ozone depletion, and the higher incidence of air-pollution-related illnesses.[8] The cascade concept was later extended to include "N footprints", which quantify the amount of N released at various stages in the human chain from fertilizer to grain to meat.[283]

1.3.1. Eutrophication. Excessive additions of anthropogenic nutrients (N and P) from fertilizer runoff and atmospheric deposition have promoted widespread eutrophication of fresh and coastal water bodies. Characteristic effects include blooms of harmful algal species, the development of O_2-depleted dead zones once aerobic bacteria draw down O_2 levels during their degradation of bloom biomass, associated fish kills and other losses of biodiversity, as well as acidification due to the release of CO_2 by vigorous decomposition.[284,285] A well-known example is the annually recurring hypoxic zone in the Gulf of Mexico,[286] which is a result of N and P added from agriculture and urbanization in the Mississippi River Basin, but there are now hundreds of such seasonally or episodic hypoxic zones worldwide.

Whole lake studies since the 1970s have shown that P control is key to combatting eutrophication in freshwater lakes,[287−289] since planktonic N_2-fixing cyanobacteria compensate for any reductions in N input by fixing additional N. However, control of both N and P appears to be necessary to mitigate coastal marine eutrophication,[290,291] as has most recently been shown for the Chesapeake Bay.[292] System-specific controls on the growth of N_2-fixing cyanobacteria, on the N:P of nutrient inputs, and on nutrient cycling are important in determining whether control strategies should focus on N, P, or both nutrients.[290,291]

While there is unambiguous evidence for the widespread reach of anthropogenic N into even the most remote watersheds of terrestrial systems,[293] the influence of anthropogenic N on the open ocean is less clear. Anthropogenic N on land that is not stored or denitrified is transported to the ocean by rivers, groundwater, or the atmosphere. Denitrification in coastal waters fueled by organic matter from coastal primary production appears to remove a significant fraction of riverine N inputs,[281,294−296] leaving the atmosphere as the main route for anthropogenic N input to the open ocean. Field observations show that N availability in the Northwestern Pacific marginal seas under the immediate atmospheric outflow of East Asia has increased, consistent with increasing N deposition.[297−299] Duce et al.[300] estimated that atmospheric N deposition could account for ∼10% of the ocean C sink for anthropogenic C. This estimate was recently downscaled by roughly half,[295] partly due to studies of the oligotrophic open North Atlantic Ocean indicating significantly lower anthropogenic N influence on the open ocean than originally proposed.[301,302] As described below, even this lower estimate fails to take into account downstream effects that render it an upper limit.

1.3.2. Effect on Soil pH and Biodiversity. N deposition, dominantly from anthropogenic N sources at biodiversity hotspots,[303] can cause soil acidification and associated major losses in biodiversity once N inputs exceed a critical load.[282,299,304−307] Although sulfur dioxide emissions have decreased since ∼1995−2000 across Europe, parts of North America, and China,[308,309] anthropogenic nitric acid deposition associated with NO_x emissions has accelerated.[310] Deposition-driven decreases in species richness, hypothesized to be important in global biodiversity loss by 2100,[311] is particularly prevalent in plants,[312] but has also recently been observed in soil microbes.[313] Differences in resource use traits appear to play a large role in shaping the form and function of species responses to N deposition.[314] A relatively long and variable time lag for the recovery of ecosystems to the biodiversity levels of their pre-N saturated states is suggested by recent forest studies.[315,316]

1.3.3. Effects on Climate and Stratospheric Ozone. Nitrogen cycling has a direct influence on climate through the production of atmospheric trace constituents that affect Earth's radiative energy budget.

Nitrous oxide (N_2O), the product of microbial nitrification and denitrification, is a long-lived (114-year-lifetime) and potent greenhouse gas that, per molecule, is ∼300 times stronger than CO_2.[317] It is currently the third most important greenhouse gas contributor to global warming, accounting for ∼10% of global radiative forcing.[318] Anthropogenic activities related to agriculture that stimulate microbial N_2O production have led to rising N_2O concentrations in the atmosphere.[319−321] Agriculture-related N_2O emissions account for 60−80% of the anthropogenic flux.[322] Global N_2O emissions have increased from ∼10 Tg N yr^{-1}[319] before the industrial era to 17.9 Tg yr^{-1} in the 2010−2015 period, as recently estimated by Thompson et al.[321] Once transported to the stratosphere, N_2O is involved in photochemical reactions that destroy stratospheric ozone (O_3),[323] an important shield against harmful ultraviolet radiation.

Nitrogen oxides (NO_x as NO and NO_2,) emitted by fossil fuel combustion have both warming and cooling effects on climate due their various chemical fates in the atmosphere. Over the short-term, NO_x emissions contribute to warming by promoting the production of tropospheric O_3, a strong greenhouse gas.[320] O_3, a strong oxidant, can also damage plant tissue by entering leaves through the stomata, which leads to a decrease in photosynthesis and terrestrial CO_2 sequestration.[324] On decadal time scales, NO_x can cool the climate by promoting methane oxidation, which leads to decreased O_3 formation.[320,325] The combined results of NO_x on O_3 depend strongly on where emissions occur.[320] However, it is thought that the net influence of NO_x on climate is likely to be a cooling effect.[320,326] Further negative radiative forcing (a tendency to cause cooling) originates from the reaction of ammonia (NH_3) with HNO_3, a product of NO_x oxidation, and sulfate (SO_4^{2-}), which produces ammonium nitrate (NH_4NO_3) and ammonium sulfate ($(NH_4)_2SO_4$). These aerosols raise Earth's reflectivity by scattering light and altering cloud properties.[320] The ultimate fate of aerosol N forms is deposition in surface environments, where they can cause unintended fertilization of marine and terrestrial ecosystems.[295,327,328]

As broached above, anthropogenic N can also indirectly affect climate through its influence on CO_2 sequestration by biological productivity. This topic is discussed in Section 4.

1.3.4. Reductions in Air Quality. Over large areas, particulate ammonium and nitrate burdens in the atmosphere have increased by factors of two to five relative to preindustrial conditions.[329−331] Ammonium nitrate has been identified as an important contributor to microscopic particulate matter (PM) in many polluted regions.[332−335] Fine PM (<2.5 μm diameter PM) has been associated with increased respiratory irritation, cardiovascular disease, and premature death.[336] In addition to PM production, nitrogen oxides also enhance the production of tropospheric O_3, a surface pollutant, which has been shown to

https://dx.doi.org/10.1021/acs.chemrev.9b00613
Chem. Rev. 2020, 120, 5308−5351

exacerbate chronic diseases like asthma[337] and cause ecosystem damage.[338,339] Globally, Lee et al.[340] estimated that a 10% reduction of NO_x and NH_x (i.e., NH_3+NH_4^+) would each prevent 22,000 premature deaths annually. A complete removal of agricultural emissions (primarily NH_3 emissions) may prevent as many as 800,000 deaths annually.[341] N-based air pollution also has hidden economic costs, as shown by Paulot et al.'s estimate that the premature mortality associated with fine PM produced from U.S. food export could amount to 50% of the gross food export value.[342]

At this stage, apart from the effects of anthropogenic N on Earth's radiative energy budget, substantial problems due to human perturbation of the global N cycle are largely restricted to land and coastal ecosystems. This conclusion is consistent with the estimated budgets of N for terrestrial and marine systems (Section 2).

2. NITROGEN BUDGETS AND THE SCALE OF HUMAN PERTURBATION

Quantifying the scale of anthropogenic perturbation of the global N cycle requires the comparison of global input/output budgets of fixed N between preindustrial and modern times.

2.1. Methods to Assess Budgets

Input/output budgets are composed of estimates of gross N input and losses from terrestrial and marine N reservoirs. Total rates of N input by N_2 fixation and N loss by denitrification and anammox are typically inferred by extrapolating data from incubation experiments and from geochemical signatures of N flux. We describe some common methods below.

Total anthropogenic N inputs by Haber Bosch industrial N_2 fixation, combustion, and agricultural N_2 fixation are estimated by compiling inventories from different source sectors; these rates are generally better quantified than those of natural processes.[4]

2.1.1. Incubation Methods. Incubation experiments provide estimates of N flux over small spatiotemporal scales. These can be categorized as direct or indirect tracers of N flow.

Direct methods to measure transformation rates involve the addition of ^{15}N-labeled substrate into a sample incubated under controlled conditions followed by measurement of the rate of ^{15}N transformation from substrate to product using mass spectrometry.

2.1.1.1. $^{15}N_2$ Tracer Method for Gross N_2 Fixation Rates. The rate of biological N_2 fixation in marine and terrestrial samples can be measured with the $^{15}N_2$ tracer method, which tracks the conversion of $^{15}N_2$ into ^{15}N-labeled particulate organic N.[343,344] If ^{15}N-enrichment of the dissolved organic N pool is negligible, then the measured rate represents the gross N_2 fixation rate.[345] In this method, the $^{15}N_2$ substrate for nitrogenase is introduced to a closed bottle containing the sample, followed by biomass collection over the time course, and the analyses of biomass ^{15}N content with elemental analyzer-isotope ratio mass spectrometry (IRMS).[344] An increase in the biomass ^{15}N upon label addition is the strongest evidence for N_2 fixation. Rates can be under- or overestimated depending on a range of factors.[343] Release of a significant amount of fixed N by N_2-fixing organisms to their surrounding environment[345] would result in underestimated rates, as would inadequate capture of small cells (<2 μm cell size) during filtration of aquatic samples for measurement of ^{15}N-particulate organic N.[190] Underestimated rates can also originate from insufficient equilibration of $^{15}N_2$ gas with the sample at the start of incubation,[346−348] but issues of equilibration can be minimized by adjusting the incubation time[348] or directly measuring the substrate enrichment. Incubations lasting longer than 12 h yield rates that are only negligibly underestimated due to disequilibrium of gas and dissolved phase $^{15}N_2$[348]. In addition to these issues, the use of $^{15}N_2$ gas stocks contaminated by $^{15}NH_4$ or $^{15}NO_x$ could contribute to overestimated rates.[349]

2.1.1.2. ^{15}N Tracer Methods for Gross N Loss Rates. The gross rate of N loss by denitrification can be directly assessed using the rate of $^{15}NO_3^-$ conversion to $^{15}N_2$.[350,351] Samples, typically amended such that the NO_3^- pool is 5−10 atom % ^{15}N, are incubated in closed bottles, with gas samples taken over the time course of incubation for quantification of product $^{15}N_2$ by gas chromatography-IRMS.[352] Similarly, N loss by anammox is directly measured by tracking the transformation of added $^{15}NO_2^-$ or $^{15}NH_4^+$ into $^{15}N_2$.[352] Production of N_2O is measured using separate incubations with $^{15}NO_3^-$, $^{15}NO_2^-$ or $^{15}NH_4^+$ to independently assess the reductive and oxidative reactions.[353] The consumption of N_2O can be quantified by measuring the production of $^{15}N_2$ from $^{15}N_2O$.[354]

2.1.1.3. Flux Chamber and Eddy Covariance for Net Transformation Rates. Net N_2 transformation rates reflect the balance of gross production through denitrification (and anammox) and consumption by N_2 fixation (i.e., net denitrification = gross denitrification−gross N_2 fixation). Such rates can be estimated by measuring N_2 concentrations within gastight chambers containing samples over time using mass spectrometry or gas chromatography, for example, see ref 351. Gas fluxes are calculated based on the rate of change in N_2 concentration, the ground area covered by the chamber, and the chamber volume for a static, closed cover chamber setup, or the air flow rate for a dynamic chamber setup. It is generally easier to measure N_2 measurements more precisely in aquatic systems than in terrestrial systems because background N_2 concentrations are lower in water and because gas exchange with the atmosphere is slower in saturated systems.[351] Measurement precision for N_2 can be increased by including a step that reduces the high background of N_2 prior to N_2 measurement. This is typically accomplished by flushing the chamber with a N_2-free inert gas, such as helium or argon (Ar). In aqueous samples, improved precision can also be obtained by tracking N_2:Ar ratios, since gas ratio data from mass spectrometers are more precise than concentration data. In the N_2:Ar method, net denitrification rates are obtained by correcting measurements of dissolved N_2:Ar ratios for the influence of air−liquid gas exchange using the conservative tracer Ar. For example, Kana et al.[355] estimated net denitrification by measuring N_2:Ar ratios in water flowing over sediment cores in a benthic flux chamber using membrane inlet mass spectrometry. Chamber methods can also be used to measure gross rates using ^{15}N tracer addition[356] or inhibitor methods[357,358] (see below).

Approaches that track N_2O concentration in flux chambers over time can be applied to estimate net N_2O production.[359] More recently, eddy covariance methods, which quantify trace gas fluxes between soil, vegetation and the atmosphere using high resolution measurements of gas concentration, wind direction and speed, have enabled estimates of net N_2O flux across natural, agricultural, and urban landscapes.[360−362]

Gross N_2 fixation and denitrification rates can also be estimated using indirect methods that typically rely on quantification of the rate of a proxy reaction.

2.1.1.4. Acetylene Reduction Assay for N_2 Fixation. Nitrogenase, the enzyme responsible for N_2 fixation, can reduce

https://dx.doi.org/10.1021/acs.chemrev.9b00613
Chem. Rev. 2020, 120, 5308−5351

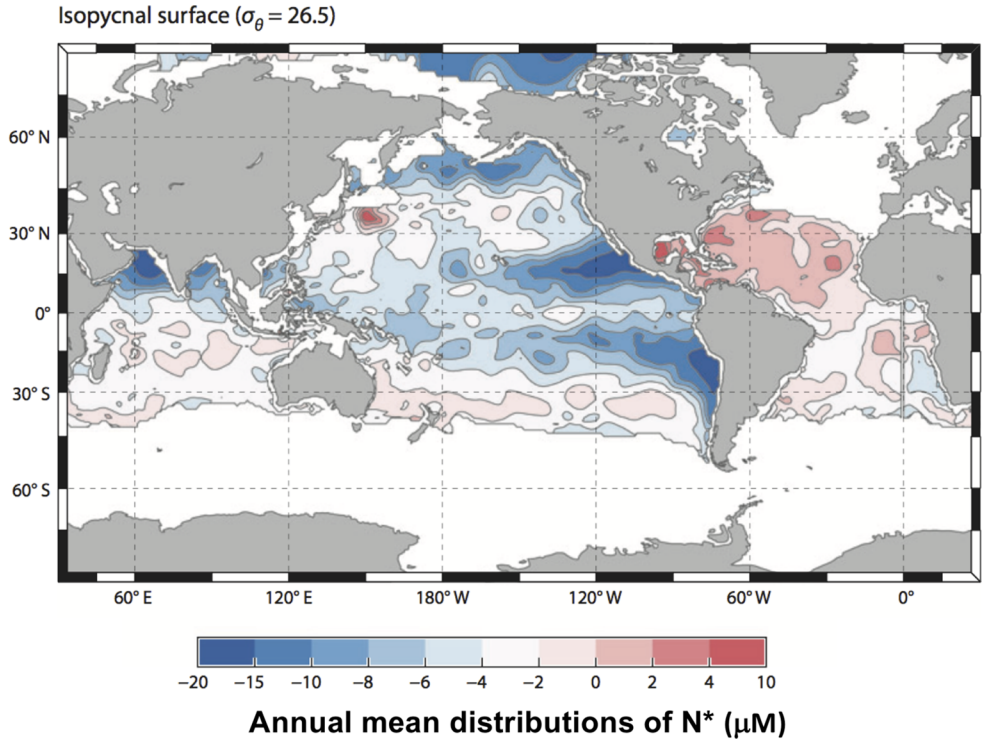


Figure 6. Global distribution of annual mean N* (in micromolar) along the isopycnal (constant density) surface, σ_θ = 26.5. N* (200−600 m depth), equivalent to $[NO_3^-] - 16^*[PO_4^{3-}] + 2.9\ \mu mol/kg$[377], quantifies the excess or deficit in nitrate relative to the phosphate concentration expected from production and remineralization of organic matter with the Redfield ratio of 16/1 N-to-P. Higher N*, as in the North Atlantic subtropical gyre, suggests N has been added by N_2 fixation. Lower N*, as found in suboxic zones of the Arabian Sea, and the Eastern Tropical North and South Pacific Oceans, suggest local or regional fixed N loss due to denitrification or anammox. Reprinted in part with permission from Deutsch and Weber, Nutrient Ratios as a Tracer and Driver of Ocean Biogeochemistry, *Annu. Rev. Mar. Sci.*, 2012, **4**, 113−141. Copyright 2012, Annual Reviews.

a variety of triple and double bonded molecules other than N_2. Its reduction of triple bonded acetylene (C_2H_2) to the double bonded gaseous molecule ethylene (C_2H_4) forms the basis for the "acetylene reduction assay" (ARA[67]), a simple, inexpensive, and widely applied method to quantify N_2 fixation rates. In ARA, acetylene gas is added to a closed container at a saturating concentration (typically 10% v/v) and the production of ethylene is monitored over time by gas chromatography. The acetylene reduction rate can be converted to an N_2 reduction rate by multiplication with the ratio of C_2H_2 to N_2 reduction (i.e., the R ratio), which is theoretically 3 for Mo nitrogenase, the most common nitrogenase isoform, but is lower for alternative nitrogenases (i.e., R = 1 or 2) and known to vary widely in environmental assays.[347,363] Thus, it is recommended that conversion ratios should be determined experimentally with parallel $^{15}N_2$ tracer incubations for different sample types, as described above. Another possibility involves the correction for variable R ratios, which can be made by directly measuring the contribution of alternative nitrogenases to acetylene reduction using natural abundance ^{13}C fractionation.[65] Aside from complications in converting results from this proxy assay to N_2 reduction rates, another important limitation of ARA is inhibitory effect of acetylene on certain microbes,[364] including N_2-fixing methanotrophs.[365]

2.1.1.5. Acetylene Block Method for Denitrification. The "acetylene block" technique is used to quantify N loss by denitrification[351,357,358,366,367] and is the most commonly used method for terrestrial denitrification rate estimates.[351] This sensitive method involves injecting acetylene (C_2H_2) to an incubation to inhibit the final step of denitrification (N_2O reduction to N_2 by nitrous oxide reductase) and induce accumulation of N_2O, which reflects the denitrification rate and can be easily quantified with gas chromatography. An important limitation of the method is the underestimation of denitrification rates in low combined NO_2^- and NO_3^- environments due to acetylene inhibition of nitrification (specifically, the enzyme ammonia monooxygenase[368]) and the tight coupling of nitrification and denitrification.[367] Other limitations, such as the removal of C_2H_2 inhibition of N_2O reductase by sulfide,[366] microbial C_2H_2 degradation,[369] and N_2O diffusion into low NO_3^- zones where N_2O is consumed,[370,371] can also lead to underestimated rates. An adaptation of the acetylene block method that includes protein synthesis inhibitors and amendments of nitrate and carbon substrates has been used to improve quantifications of maximal potential denitrification rate.[357,358] *In situ* denitrification rates estimated from acetylene-block based potential rates and the measured kinetic constants for natural denitrifying communities were similar to rates estimated using benthic flux nutrient stoichiometries.[372] Supplementation of the acetylene block method with direct measurements of N_2O reductase activity that track the disappearance of small amendments of N_2O can improve denitrification estimates in low NO_3^- settings.[373]

2.1.2. Geochemical Methods. Complementing the active experimental approaches described above, approaches have been developed for estimating net N input and loss (generally, N_2 fixation and denitrification) that are based on *in situ* (unaltered) properties in water collected from the environment. These properties include the ratios of nitrate to phosphate concentrations and of dissolved N_2 to Ar concentrations, as well

https://dx.doi.org/10.1021/acs.chemrev.9b00613
Chem. Rev. 2020, 120, 5308−5351

as the natural stable isotope ($^{15}N/^{14}N$) ratio of nitrate. These approaches provide estimates that integrate over larger spatial and temporal scales than the incubation- and experiment-based techniques, which, given the patchiness of the environment, is generally a benefit. Moreover, the lack of sample manipulation theoretically ensures that the resulting rates are not an artifact of non-natural incubation and experiment conditions. The geochemical methods have been applied primarily in marine settings, but stable isotope approaches are gaining broader use in terrestrial systems.[124,374,375]

2.1.2.1. Nitrate-to-Phosphate Ratio (N) Variations for Fixed N Input and Loss.* The deviation of the oceanic nitrate-to-phosphate ratio relative to that expected from the production and remineralization of organic matter with the "Redfield" N/P ratio of 16/1, ref 376, has been used to estimate rates of marine N_2 fixation and denitrification. Popular terms in the community such as N*, DINxs, and P*[377−379] each refer to a different way of quantifying the excess or deficit in nitrate relative to the phosphate concentration and the Redfield N/P ratio. Below, we refer solely to N*, which was first defined as $[NO_3^-] - 16\times[PO_4^{3-}] + 2.9$ μmol/kg, where the terms in the brackets are the nitrate and phosphate concentrations in the seawater sample, respectively, and the intercept of 2.9 μmol/kg yields a global mean N* of zero.[15,377,380] Key assumptions are that (1) phytoplankton uptake and organic matter remineralization consume and release N and P with the Redfield ratio of 16 and (2) N_2 fixation and denitrification (plus anammox and similar metabolisms) are the only processes that cause the nitrate-to-phosphate concentration of ocean water to substantially deviate from the Redfield ratio. These assumptions assume the negligibility of a range of processes that may affect ocean N:P in a non-Redfield manner, such as organic P degradation by microbial phosphatases,[381] phosphonate degradation by C−P lyase enzymes,[382] phosphate adsorption on particles,[383,384] and non-Redfieldian nutrient uptake by phytoplankton (see below). With these caveats, higher N*, such as that observed in the thermoclines of subtropical gyres (especially of the North Atlantic, Figure 6), indicates that nitrate that has been added by N_2 fixation and subsequent remineralization of the resulting organic N to nitrate. Lower N*, as found in the oxygen-deficient zones of the Arabian sea, and the Eastern Tropical North and South Pacific (Figure 6), indicates local or regional fixed N loss due to denitrification, anammox, or a related fixed N-consuming metabolism.

We caution that inappropriate extrapolation of this logic is common, mostly relating to the interpretation of N* as a rate as opposed to a concentration. For example, a region of high N* suggests that, at some time and in some region in communication with the measured sample, the remineralization/nitrification of newly fixed N has occurred.[379] However, it does not require that this process is currently ongoing. Such confusion aside, the spatial patterns in N*, when combined with data- or model-based constraints on ocean circulation, can indeed be used to map rates of N_2 fixation in the surface ocean[377,379,385] and denitrification in or near the oxygen-deficient zones in the shallow ocean interior.[380]

In the last two decades, it has become clear that there are large, environmentally systematic variations in the N/P (ratio) of organic matter produced in and exported from the upper ocean.[386−388] Phytoplankton in the low-productivity subtropical gyres produce organic matter with a N/P that is higher than the Redfield ratio of 16, while the N/P of the organic matter generated in more productive waters falls below the Redfield value. In N*-based approaches for estimating N_2 fixation and denitrification rates, the previous assumption of non-N_2-fixing biomass as having a constant N/P is now seen as problematic. For example, in early studies that used N* to track newly fixed N in the subtropical thermocline,[377,378] the assumption of constant N/P among non-N_2 fixing phytoplankton may have led to overestimation of regional N_2 fixation rates. Ignoring existing N/P variability in approaches based on the spatial convergence/divergence of N* in surface waters tends to cause inferred regions of N_2 fixation to be shifted eastward in ocean basins toward upwelling regions.[379,385] However, while non-Redfield biomass production and remineralization influence the internal cycling of N relative to P, they do not alter the global ocean N inventory. This introduces a degree of compensation between over- and underestimation of regional rates, reducing the error in the estimated rate of N_2 fixation at the basin scale or larger. In any case, the use of full ocean models for N*-based flux calculations[385,389] should be able to mitigate the uncertainty from variable biomass N/P.

2.1.2.2. Dissolved N_2/Ar Ratio Method for Fixed N Loss. The dissolved N_2 concentration in ocean interior waters rises as a consequence of fixed N loss, be it by canonical denitrification or anammox. This excess N_2 cannot be degassed to the atmosphere until the interior water returns to the surface; thus, the N_2 accumulates in the interior water, providing an integrated signal of net fixed N loss. The ratio of dissolved N_2 to Argon (N_2/Ar) is precisely measured by mass spectrometers and reflects N_2 concentration variations, and it partially corrects for variations in the N_2 concentration of the water prior to denitrification additions of N_2. Thus, as with N*, N_2/Ar has been used to quantify the fixed N loss in the oxygen-deficient zones.[390,391] N_2/Ar has theoretical benefits relative to N*, but the measurements are much more challenging, the data are still relatively sparse, and uncertain assumptions remain about the influences of various aspects of air/sea gas exchange on the "preformed" (initial) N_2/Ar ratio of ocean interior water.[392] Going forward, N_2/Ar is a particularly promising approach for quantifying fixed N loss in marine and other aquatic systems,[351] and this approach can be extended by isotopic ($^{15}N/^{14}N$ ratio) analysis of the dissolved N_2.[393]

2.1.2.3. Natural Abundance Stable Isotopes for Fixed N Input and Loss. Fixed N budgeting based on the stable isotope ratio of fixed N ($^{15}N/^{14}N$ ratio) has been deployed in both marine[394−396] and terrestrial systems.[124,374,375,396,397] Such methods have been most widely applied in marine systems to study N budgets at the scale of the global ocean and of individual ocean basins. The global budget has been used to provide a constraint on the ratio of fixed N loss that occurs in the water column, predominantly in the ocean's oxygen-deficient zones, versus in sediment porewaters. The basin budget approach has so far been applied to estimating N_2 fixation rates in the Atlantic basin. We describe each in turn.

2.1.2.4. Global Ocean N Isotope Budget. The ratio of denitrification occurring in the water column to that in the sediment is a primary determinant of the average $^{15}N/^{14}N$ ratio of ocean nitrate.[394] Below, in describing this dependency, we use "delta" terminology, where the $\delta^{15}N$ of a N sample or pool is the difference of its $^{15}N/^{14}N$ from the $^{15}N/^{14}N$ of atmospheric N_2, the universal reference, divided by the $^{15}N/^{14}N$ of atmospheric N_2: $\delta^{15}N$ (‰) = $(((^{15}N/^{14}N)_{sample}/(^{15}N/^{14}N)_{atmN2} - 1) \times 1{,}000$. When the ocean N budget is at steady state, the $\delta^{15}N$ of the fixed N removed through the combination of water column and sedimentary denitrification will equal the $\delta^{15}N$ of the fixed N

https://dx.doi.org/10.1021/acs.chemrev.9b00613
Chem. Rev. 2020, 120, 5308−5351

added (Figure 7). The $\delta^{15}N$ of oceanic newly fixed N appears to be in the range of −2 to 0‰ versus air N_2;[159] we will use a value of −1‰ here. The $\delta^{15}N$ of mean ocean nitrate is about + 5‰ versus air and thus ∼6‰ higher than that of N_2 fixation. This requires that a process is preferentially removing ^{14}N from the ocean, and that process is denitrification, in which nitrate consumption occurs with a strong isotopic fractionation. At steady state, denitrification must remove fixed N with a $\delta^{15}N$ of −1‰ (i.e., equivalent to the source). Therefore, on a global basis, the "net isotope effect" of oceanic denitrification (i.e., the isotopic fractionation of the entire process as it applies to the ocean as a whole, ε_d) is about +6‰ (*y*-axis difference between N_2 fixation and mean ocean nitrate, Figure 7). Here, we define the isotope effect, ε, as $(1- {}^{15}k/{}^{14}k) \times 1000$ in permil (‰), where ^{14}k and ^{15}k are effective rate coefficients for the consumption of ^{14}N- and ^{15}N-bearing substrate. Thus, this positive value for ε_d indicates preferential consumption of ^{14}N relative to ^{15}N. This net fractionation comprises the combined fractionation from both water column and sedimentary denitrification, weighted for their relative global rates.

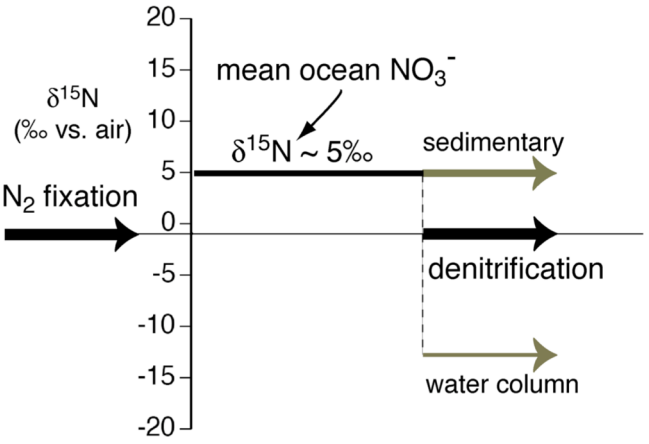


Figure 7. Whole ocean N isotope budget. The $\delta^{15}N$ of global mean ocean nitrate is controlled by the $\delta^{15}N$ of newly fixed N from N_2 fixation, the isotope effects of water column and sedimentary denitrification, and the proportion of total oceanic N lost through each route of denitrification. The $\delta^{15}N$ of a given flux or pool is indicated by the *y*-axis. The $\delta^{15}N$ of N produced by marine N_2 fixation, the dominant N input, is ∼ −1‰ ("N_2 fixation" arrow on the left). Mean ocean nitrate has a $\delta^{15}N$ of ∼ + 5‰. Water column denitrification removes nitrate with a low $\delta^{15}N$ ("water column" arrow; i.e., water column denitrification has a large isotope effect). Sedimentary denitrification removes nitrate with a $\delta^{15}N$ similar to that of mean ocean nitrate ("sedimentary" arrow; i.e., sedimentary denitrification has a small isotope effect). At steady state, the flux-weighted $\delta^{15}N$ of the total denitrification loss ("denitrification" arrow) must be equal to the $\delta^{15}N$ of the N input (∼ −1‰). This results in estimates of roughly two-thirds of total N loss by sedimentary denitrification. Limitations in this calculation include uncertainties in the isotopic fractionations (especially that of water column denitrification) and its neglect of the impacts of strong isotopic gradients that exist in some regions of the ocean (especially in the regions of water column denitrification). Reprinted in part with permission from Sigman et al., Ocean Process Tracers: Nitrogen Isotopes in the Ocean, *Encyclopedia of Ocean Sciences*., 2009. Copyright 2009, Elsevier.

Water column denitrification occurs in subsurface waters with a high nitrate concentration and consumes only a fraction of the NO_3^- available, and the NO_3^- remaining from the process is eventually circulated or mixed out of the denitrification zone. In this way, water column denitrification elevates the $\delta^{15}N$ of ocean NO_3^- at both regional and global scales. In the first global ocean N isotope budget seeking to partition N loss between water column and sedimentary denitrification,[394] the assumed isotope effect for water column denitrification (ε_{wcd}) was taken to be 25‰. In contrast, sedimentary denitrification occurs with much weaker net isotopic fractionation, because it consumes nearly all of the nitrate at the site where the process occurs, such that nearly no ^{15}N-rich nitrate is able to escape back into the overlying ocean water column.[394,398−402] For simplicity, we assume here that the isotope effect for sedimentary denitrification (ε_{sd}, the net isotope effect for the entire process of sedimentary N loss at the scale of the sediment/water interface) is 0‰. Assuming that the ocean's N budget approximates a steady state (i.e., fluxes in equal fluxes out), the partitioning between sedimentary and water column denitrification can be calculated from the following equation:

$$\varepsilon_d = X_{wcd} \cdot \varepsilon_{wcd} + X_{sd} \cdot \varepsilon_{sd} \quad (9)$$

where X_{wcd} and X_{sd} are the fractional contributions of water column and sedimentary denitrification to total ocean denitrification (i.e., $X_{wcd} + X_{sd} = 1$). A range of uncertainties, for example, in the water column denitrification isotope effect (ε_{wcd})[403,404] compromises this quantification. Nevertheless, even with such uncertainties, the calculation appears to require that the greater part of global ocean denitrification occurs in the sediments (Figure 7).[394] Coupling this estimation of the proportions of water column and sedimentary N loss with independent estimates of the water column N loss rate, a total N loss rate can then be calculated. Moreover, this approach can be applied to infer past changes in the relative importance of water column and sedimentary denitrification, with a higher mean ocean nitrate $\delta^{15}N$ implying a greater proportion of water column denitrification.[405] This overall approach of taking advantage of the distinct fractionations associated with different N loss processes has also been applied to N budgets in terrestrial systems.[124,374,375,397,406]

2.1.2.5. Regional N Isotope Budgeting. This strategy makes use of nitrate isotopic variations to estimate N input and loss rates, for example, see ref 407. As one version of this strategy, hydrographic depth sections across ocean basins are used to estimate the gross flows of water and nitrate at the different depths of each section. These results are combined with nitrate $\delta^{15}N$ data to calculate the $\delta^{15}N$ difference between gross nitrate transports in each direction across each section. These differences provide insight into N input and loss processes, with N_2 fixation adding low-$\delta^{15}N$ newly fixed N so as to depress nitrate $\delta^{15}N$ and water column denitrification removing low-$\delta^{15}N$ nitrate and thus raising the regional nitrate $\delta^{15}N$. This approach has been applied to a suite of oceanographic sections crossing the Atlantic Ocean basin at different latitudes to estimate the distribution and overall rate of N_2 fixation in the Atlantic.[404]

2.2. Nitrogen Budgets

The global N budget is best understood as being composed of two largely independent terrestrial and marine budgets that are linked due to the transfer of fixed N through the atmosphere and rivers (Figure 5). Natural and anthropogenic fluxes have been estimated by numerous studies over the past few decades. We provide a selection of these estimates (Tables 3 and 4), primarily taken from global budget studies that incorporate widely accepted values for global rates. Of particular note is Galloway

https://dx.doi.org/10.1021/acs.chemrev.9b00613
Chem. Rev. 2020, 120, 5308−5351

Table 3. Contemporary and Pre-industrial N Cycle Flux Estimates

flux	magnitude in Tg N yr^{-1}	ref
Natural New N Inputs		
Terrestrial N_2 Fixation		
	170 (modern), 195 (preindustrial)	Cleveland et al. (1999)[144]
	107 (ca. 1990s), 120 (ca. 1860), 128 (preindustrial)	Galloway et al. (2004)[281]
	110 (ca.1990s, preindustrial)	Gruber and Galloway (2008)[7]
	58 (modern, preindustrial)	Vitousek et al. (2013)[124], Schlesinger and Bernhardt (2013)[209]
	128 (modern)	Cleveland et al. (2013)[143]
	128 (modern, preindustrial)	Fowler et al. (2015)[5]
Marine N_2 Fixation		
	110 (modern)	Gruber and Sarmiento (1997)[377]
	132 (modern)	Codispoti et al. (2001)[441]
	110−330 (preindustrial)	Brandes and Devol (2002)[394]
	121 (modern, preindustrial)	Galloway et al. (2004)[281]
	137 (modern)	Deutsch et al. (2007)[379]
	100 (ca. 2000)	Duce et al. (2008)[300]
	140 (modern, preindustrial)	Gruber and Galloway (2008)[7]
	177 (modern)	Großkopf et al. (2012)[432]
	137 (modern)	Luo et al. (2012)[448]
	225 (preindustrial)	Somes et al. (2013)[435]
	140 (modern)	Voss et al. (2013)433, Fowler et al. (2015)[5]
	164[a] (ca. 1990s), 160[a] (preindustrial)	Jickells et al. (2017)[295]
	163 (modern)	Wang et al. (2019)[385]
Lightning	5 (modern, preindustrial)	Galloway et al. (2004)[281], Gruber and Galloway (2008)[7], Schlesinger and Bernhardt (2013)[209], Fowler et al. (2013, 2015)[4,5]
Rock weathering	14−34 (modern, preindustrial)	Houlton et al. (2018)[6]
Anthropogenic New N Inputs		
Haber Bosch Fertilizer Production		
	100 (ca. 1990s), 0 (ca. 1860, preindustrial)	Galloway et al. (2004)[281]
	100 (ca. 1990s), 0 (preindustrial)	Gruber and Galloway (2008)[7]
	136 (modern)	Schlesinger and Bernhardt (2013)[209]
	160 (ca. 2100), 120 (ca. 2010), 0(preindustrial)	Fowler et al. (2015)[5]
Fossil Fuel Combustion		
	25 (ca. 1990s), 0.6 (ca. 1860)	Galloway et al. (2004)[281]
	25 (ca. 1990s), 0 (preindustrial)	Gruber and Galloway (2008)[7], Schlesinger and Berhardt (2013)[209]
	40 (ca. 2010), 0 (preindustrial)	Fowler et al. (2015)[5]
Agricultural N_2 Fixation		
	32 (ca. 1990s), 15 (ca. 1860), 0 (preindustrial)	Galloway et al. (2004)[281]
	35 (ca. 1990s), 0 (preindustrial)	Gruber and Galloway (2008)[7]
	60 (ca. 2010), 0 (preindustrial)	Herridge et al. (2008)[411], Schlesinger and Bernhardt (2013)[209], Fowler et al. (2015)[5]
Outputs		
Terrestrial Denitrification (Land, Rivers)		
	115 (ca. 1990s), 100 (ca. 1860), < 100 (preindustrial)	Galloway et al. (2004)[281]
	115 (ca. 1990s), 100 (preindustrial)	Gruber and Galloway (2008)[7]
	28 (preindustrial, natural soils)	Houlton and Bai (2009)[397]
	44 (modern, soils), 27 (preindustrial, soils)	Schlesinger and Bernhardt[209]
	100 (ca. 2010)	Fowler et al. (2013)[4]
Pyrodenitrification	12−28 (modern)	Lobert et al.[424]
	37 (modern), 12 (preindustrial)	Schlesinger and Bernhardt (2013)[209]
Marine Denitrification		
	285 (modern)	Middleberg et al. (1996)[449]
	450 (150 water column, 300 sedimentary, modern)	Codispoti et al. (2001)[441]
	274−355 (75 water column, 200−280 sedimentary, preindustrial)	Brandes and Devol (2002)[394]
	322 (ca. 1990s), 301 (ca. 1860)	Galloway et al. (2004)[281]
	260 (70 water column, 190 sedimentary, preindustrial)	Deutsch et al.(2004)[395]
	240 (ca. 1990s), 220 (preindustrial)	Gruber and Galloway (2008)[7]

https://dx.doi.org/10.1021/acs.chemrev.9b00613
Chem. Rev. 2020, 120, 5308−5351

Table 3. continued

flux	magnitude in Tg N yr^{-1}	ref
Marine Denitrification		
	230 (66 water column, 164 sedimentary, modern)	Devries et al. (2012)[390]
	225 (76 water column, 149 sedimentary, preindustrial)	Somes et al. (2013)[435]
	100−280 (modern)	Fowler et al. (2013)[4]
	300 (modern)	Schlesinger and Bernhardt[209]
	212[a] (ca. 1990s), 209[a] (preindustrial)	Jickells et al. (2017)[295]
	201 (69 water column, 132 sedimentary, modern)	Wang et al. (2019)[385]
Marine Sediment Burial		
	25 (modern, preindustrial)	Codispoti et al. (2001)[441]
	25 (preindustrial)	Brandes and Devol (2002)[394]
	16 (ca. 1990s), 9 (ca. 1860)	Galloway et al. (2004)[281]
	25 (modern, preindustrial)	Gruber and Galloway (2008)[7]
	20 (modern, preindustrial)	Fowler et al. (2013)[4]
	58[a] (modern, preindustrial)	Jickells et al. (2017)[295]
Terrestrial N_2O Emissions		
	11 (ca. 1990s), 8 (ca. 1860), 7 (preindustrial)	Galloway et al. (2004)[281]
	12 (ca. 1990s), 8 (preindustrial)	Gruber and Galloway (2008)[7]
	13 (ca. 2010), 7 (preindustrial)	Fowler et al. (2013)[4]
Marine N_2O Emissions		
	6	Codispoti et al. (2001)[441]
	4 (modern and preindustrial)	Galloway et al. (2004)281, Gruber and Galloway (2008)[7]
	5.5 (ca. 2010), 2.5 (preindustrial)	Fowler et al. (2013)[4]
	2.1 [1] (ca. 1990s), 2.1 [1] (preindustrial)	Jickells et al. (2017)[295]
Transport between Reservoirs		
Atmospheric Emissions (NH_3, NO_X)		
	64 (ca. 1990s), 16 (ca. 1860)	Galloway et al. (2004)[281]
	70 (ca. 1990s), 20 (preindustrial)	Gruber and Galloway (2008)[7]
	116 (ca. 2000), 34 (ca. 1860)	Duce et al. (2008)[300]
	100 (ca. 2010), 25 (preindustrial)	Fowler et al. (2013)[4]
	104 (ca. 2005), 30 (ca. 1850)	Jickells et al. (2017)[295]
Atmospheric Deposition - Land (Net,[2] Reduced, Oxidized N)		
	49 (ca. 1990s), 12 (ca. 1860)	Galloway et al. (2004)[281]
	75 (ca. 1990s), 20 (preindustrial)	Gruber and Galloway (2008)[7]
	70 (ca. 2010)	Fowler et al. (2013)[4]
Atmospheric Deposition - Ocean (Net, Reduced, Oxidized N)		
	86 (modern)	Codispoti et al. (2001)[441]
	25 (preindustrial)	Brandes and Devol (2002)[394]
	33 (ca. 1990s), 8 (ca. 1860)	Galloway et al. (2004)[281]
	50 (ca. 1990s), 10 (preindustrial)	Gruber and Galloway (2008)[7]
	67 (ca. 2000), 20 (ca. 1860)	Duce et al. (2008)[300]
	54 (modern), 6 (preindustrial)	Schlesinger and Bernhardt (2013)[209]
	30 (ca. early 2000s)	Fowler et al. (2013)[4]
	39 (ca. 2005), 35[a] (ca. 1990s), 10−13[a] (ca. 1850)	Jickells et al. (2017)[295]
	26 (modern)	Wang et al. (2019)[385]
Rivers (DIN, DON, PON)		
	76 (modern)	Codispoti et al. (2001)[441]
	25 (preindustrial)	Brandes and Devol (2002)[394]
	48 (ca. 1990s), 27 (ca. 1860)	Galloway et al. (2004)[281]
	80 (ca. 1990s), 30 (preindustrial)	Gruber and Galloway (2008)[7]
	58 (modern), 27 (preindustrial)	Schlesinger and Bernhardt (2013)[209]
	80 (ca. 2010)	Fowler et al. (2013)[4]
	40−70 (modern)	Voss et al. (2013)[433]
	37 (ca. 2000), 19 (ca. 1900)	Beusen et al. (2016)[427]
	34 (total ocean), 16[a](open ocean, ca. 1990s)	Jickells et al. (2017)[295]
	11 (modern)	Wang et al. (2019)[385]
Groundwater	18 (modern), 0 (preindustrial)	Schlesinger,[16] Schlesinger and Bernhardt (2013)[209]

[a]Values are from Jickells et al. (2017)[295] Table 4 for the model run without suppression of N_2 fixation by enhanced atmospheric deposition.

https://dx.doi.org/10.1021/acs.chemrev.9b00613
Chem. Rev. 2020, 120, 5308−5351

Table 4. Terrestrial and Marine N Budgets[a]

	Galloway et al. (2004)[281]		Gruber and Galloway (2008)[15]		Schlesinger and Bernhardt (2013)[209]		Fowler et al. (2013, 2015)[4,5]	Codispoti et al. (2001)[441]	Brandes and Devol (2002)[394]	Jickells et al. (2017)[295]		Wang et al. (2019)[385]
Terrestrial												
Inputs												
Natural N_2 fixation	120	107	110	110	60	60	128	---	---	---	---	---
Agricultural N_2 fixation	15	32	0	35	0	60	60	---	---	---	---	---
Haber Bosch Fertilizer	0	100	0	100	0	136	120	---	---	---	---	---
Atm. deposition (net)	12	59	20	75	0	25	70	---	---	---	---	---
Rock weathering	---	---	---	---	20	20	---	---	---	---	---	---
Outputs								---	---	---	---	---
Denitrification	100	115	100	115	27	44	100	---	---	---	---	---
Pyrodenitrification	---	---	---	---	25	37	---	---	---	---	---	---
Emissions (NO_X, NH_3)	16	64	20	70	6	54	100	---	---	---	---	---
N_2O emission	8	11	8	12	---	---	13	---	---	---	---	---
Riverine flux	27	48	30	80	27	58	80	---	---	---	---	---
Groundwater	---	---	---	---	0	18	---	---	---	---	---	---
Combined inputs	147	298	130	320	85	306	378	---	---	---	---	---
Combined losses	151	238	158	277	85	211	293	---	---	---	---	---
Imbalance	-4	60	-28	43	0	95[b]	85	---	---	---	---	---
Marine												
Inputs												
Natural N_2 fixation	121	121	140	140	---	150	140	132	110-330	160	164	163
Atm. deposition (net)	9	33	10	50	---	67	30	86	25	13	35	26
Riverine flux	27	48	30	80	---	58	80	76	25	16	16	11
Outputs												
Denitrification	301	322	220	240	---	300	100-280	450	275-355	209	212	201
N_2O emission	4	4	4	4	---	---	6	6	---	2	2	---
Burial	9	16	25	25	---	10	20	25	25	58	58	---
Combined inputs	157	202	180	270	---	275	250	294	160 - 380	189	215	---
Combined losses	314	342	249	269	---	310	126-306	481	300 - 380	269	272	---
Imbalance	-158	-140	-69	1	---	-35	-56 - 124	-187	-200 - 0	-80	-57	-5.7[c]

[a]Grey and white columns contain preindustrial and modern flux estimates, respectively, in Tg N yr^{-1}. [b]Imbalance value includes an estimated biosphere N accumulation of 9 Tg N yr^{-1} and soil N accumulation of 48 Tg N yr^{-1}. [c]Most probable value in model run with slowly evolving disequilibrium.[385]

et al.'s comprehensive and quantitative analyses of human perturbations of the global N cycle based on budget constructions for early industrial (ca. 1860), modern (ca. 1990s), and future (ca. 2050) N cycles.[281] Gruber and Galloway[7] provided an updated view comparing industrial (ca. 1990s) and preindustrial fluxes. More recently, Fowler et al.[4,5] compiled global N cycle fluxes for the early 21st century by including new estimates of anthropogenic N_2 fixation. Uncertainties for individual fluxes can be substantial. Anthropogenic input fluxes from industrial fertilizer production, fossil fuel combustion, and agricultural N_2 fixation have estimated uncertainties of 10 to 30% [4]; errors for other processes typically range from 30% to 50%.[4,15] Quantifications of the preindustrial N cycle are the most uncertain; natural fluxes (i.e., process rates in unmanaged environments) for the modern era have been used as a first order estimate of such fluxes.

2.3.1. Terrestrial N Budget. The terrestrial N reservoir receives new bioavailable N from natural and anthropogenic N_2 fixation and rock weathering, and it loses N to the atmosphere by denitrification and emissions of reactive N gases and to the ocean by hydrologic (e.g., riverine) transport (Figure 5).

Prior to the industrial era, the only significant sources of new N were biological N_2 fixation,[4,16,124,209,281] the deposition of atmospheric NO_x produced from N_2 fixation by lightning,[4,16,209,281] weathering of fixed N from rocks,[6,209] with possible local augmentation from migratory organisms[408−410] or deposition of marine-derived atmospheric reactive N species. The global rate of natural terrestrial N_2 fixation has been estimated to fall within the range of 60 to 200 Tg N yr^{-1} (Table 3). Studies that have upscaled field-based measurements have suggested ~110 to 130 Tg N yr^{-1} inputs,[15,143,281] whereas one study that applied a $^{15}N/^{14}N$ isotope budgeting approach suggested a lower rate of terrestrial N_2 fixation (~60 Tg N yr^{-1} [124]). The majority of these inputs (~80%) originate within evergreen broadleaf forests and savannahs,[143] where symbiotic N_2 fixation by bacterial root nodule symbionts, which exchange fixed N for fixed C from their higher plant hosts, is the dominant form of fixation (Section 1.2.1.1). Asymbiotic N_2 fixation,

https://dx.doi.org/10.1021/acs.chemrev.9b00613
Chem. Rev. 2020, 120, 5308−5351

defined as comprising all forms of fixation independent of root nodules,[123] is thought to account for only ~20% of N_2 fixation globally, although it can be more important than symbiotic fixation in select biomes.[143] Compared to biological N_2 fixation, the amount of N_2 fixed by lightning is much smaller (~5 Tg N yr^{-1}, Table 3). Recently, fixed N released by rock weathering (~14 to 34 Tg N yr^{-1}, Table 3) was suggested to be a substantive of contributor to the terrestrial N budget, and particularly important in certain environments.[6]

Anthropogenic N_2 fixation has essentially doubled the global fixation rate over the last century. Estimates of anthropogenic N_2 fixation in the early 21st century[4,411] indicate industrial fertilizers and cultivation of naturally N_2-fixing crops add an additional ~120 Tg N yr^{-1} and ~60 Tg N yr^{-1}, respectively, to the land, while NO_x emissions from fossil fuel combustion add ~40 Tg N yr^{-1} to the atmosphere, most of which is rapidly deposited on land. Values in each category are ~15 to 20 Tg N yr^{-1} higher than estimates for the 1990s.[281] Human activities such as deforestation, peat and biomass burning, drainage of wetlands, and erosion[412] also increase N availability by freeing biologically stored N. The consensus of inventories thus far is that anthropogenic activities now outpace natural processes of N addition to terrestrial ecosystems.

Food production by both plants and animals is notoriously inefficient with respect to N utilization[322] and varies widely across the globe (~30 to 80% efficiency for crops, much less for livestock production[413]). N leakage results in a global average of 40 to 50% of total N inputs being lost to the natural system as reactive N.[322,414] Loss mechanisms include export through rivers, volatilization as ammonia, denitrification to NO, N_2O and N_2 in soils and surface waters, and accumulation in soils as organic N. Not only is this loss wasteful and expensive, but the lost "missing N" passes through the N cascade,[282] including N_2O emission to the atmosphere and N accumulation in soils. The relative importance of denitrification versus accumulation varies widely, depending on agricultural practices, crop varieties, manure handling procedures, soil type, weather, and other considerations. Recent metadata analyses have compiled results from large regions,[415,416] and they provide a consistent picture of the fate of reactive N in agricultural systems. In the Mississippi River Basin (the US corn belt), van Meter et al.[416] found that about 25% of the fixed N inputs (e.g., fertilizer, manure, N_2 fixation) accumulated as organic N in soils. Such estimates are likely to be highly dependent on time scale.

Within the terrestrial reservoir, natural and anthropogenic N can be transformed by the metabolism of plants, animals, and soil microbes (Figures 4 and 5, Section 1) and emitted to the atmosphere in reactive (NO_x, NH_3, organic N) or less reactive (N_2, N_2O) gaseous forms. Modern terrestrial emissions of reactive N (~100 Tg N yr^{-1} for ~2000s, Table 3), which are dominated by anthropogenic sources from livestock, agriculture, and fossil fuel burning,[4] are roughly four to five times higher than natural emissions from soil and vegetation. Ammonia emitted from fertilized soils and animal waste contributes over half of modern emissions.[4] With anticipated climate change and growth in food demand, anthropogenic N emissions are expected to increase.[281,417] A rise of 5 °C in global surface temperature is expected to result in a ~ 50% increase in ammonia emissions by 2100; higher demand for animal products would cause emissions to increase further.[5] The amount of added N that is volatilized as NH_3 to the atmosphere varies with crop type but can be as much as 50% of the N added through fixation and fertilizer.[322] Atmospheric deposition (of both NH_3 and NO_x) returns some of this N to terrestrial ecosystems, such that deposition is third in importance after fertilizer and biological N_2 fixation[418] (Table 3).

Permanent losses of N from land result from (1) the production of N_2 by microbial denitrification and anammox (Section 1.2.3) and pyrodenitrification (biomass burning); (2) the production of N_2O by nitrification and denitrification (Sections 1.2.2 and 1.2.3); and (3) the transport of fixed N from the land to the ocean by rivers, groundwaters, and the atmosphere (Figure 5). Of these routes, denitrification in hypoxic and anoxic environments (e.g., sediments and water logged soils) is the most important, removing ~30 to 115 Tg N yr^{-1} (Table 3), equivalent to roughly a tenth to a quarter of fixed N inputs to the land. Van Meter et al.[416] estimated the denitrification loss for the US corn belt at ~10% of fixed N inputs. Wang et al.[406] recently used natural N isotopes in a global mass-balance model to estimate a ~ 20% increase in soil denitrification N losses between 1860 and 2000 due to agricultural fertilizer use. This study along with others[7,281] which show a similar moderate increase in modern versus preindustrial denitrification fluxes indicate that denitrification in agrosystems, streams, and rivers serves as an incomplete sink for anthropogenic N.[281,419,420] However, we note that, given tremendous heterogeneity in environmental conditions and in N inputs across terrestrial systems, the rate of global terrestrial denitrification is likely to be the most uncertain component of the N budget.[281,421] Indeed, terms such as "hotspots" and "hot moments" are frequently used to describe the fact that small areas and brief periods of denitrification often account for much of the denitrification activity in both terrestrial and aquatic ecosystems.[421,422] Understanding the fundamental drivers for spatially and temporally inconsistent microbial N loss will be fundamental to better constraining N budgets at all scales.

Compared to denitrification, estimates of the N loss due to anammox, which accounts for a smaller absolute flux, are even less constrained. Most of the terrestrial estimates come from rice paddies, where anammox is estimated to range from ~0 to 37% of the loss conventionally attributed to denitrification.[423] On the basis of laboratory studies of biomass burning, Lobert et al.[424] suggested that tropical biomass burning could lead to a N loss of 12 to 28 Tg N yr^{-1}; an estimate consistent with that of Schlesinger and Bernhardt[209] (Tables 3 and 4).

Emissions of N_2O also deplete terrestrial fixed N stocks, as its ultimate fate is destruction in the stratosphere through photochemical reactions. Terrestrial sources of N_2O are microbial nitrification and denitrification in natural soils and agriculture (~50% and 40%, respectively) with the remainder derived from nonbiological sources (e.g., fossil fuel combustion, biomass burning, and industrial processes).[5] A global analysis by Fowler et al.[5] indicates that anthropogenic activities primarily related to increased fertilizer use and livestock production have caused terrestrial N_2O emissions to nearly double from preindustrial emission levels of ~7 Tg N yr^{-1} to reach ~13 Tg N yr^{-1} (ca. 2010).

Rivers are an important route for N removal from land, as they are sites for denitrification and also transport N to the ocean. Fixed N that is not lost from streams and rivers by denitrification (~30 to 70% is retained[419]) is delivered in dissolved and particulate forms to estuaries, where denitrification is estimated to remove up to 80% of the remaining N.[425,426] The fate of riverine N on the continental shelf is not well-known; a common assumption is that most of it is lost by denitrification in coastal shelf regions.[281,300] Current estimates[295,427,428] suggest that

https://dx.doi.org/10.1021/acs.chemrev.9b00613
Chem. Rev. 2020, 120, 5308−5351

human activities have roughly doubled N fluxes to the coastal ocean (Table 3), but most of this N appears to be lost locally to redox processes yielding N_2 and N_2O.

The sum of various input/output flux estimates produces a global terrestrial N budget for the preindustrial period[7,209,281] that is approximately balanced, within error (up to −28 Tg N yr^{-1} net imbalance, equivalent to 20% of inputs, Table 4). Given an average lifetime of terrestrial N of decades to a century,[5] it has been suggested that the N budget for the recent geologic past must have been balanced or nearly so[281] to avoid drastic swings in productivity. An analyses of N stable isotopes of lake sediments across the globe by McLauchlan et al.[429] suggested long-term declines in N availability for terrestrial ecosystems following the last deglaciation ~15,000 years ago, but relatively stable N availability during the past 500 years. The small apparent net N deficit in the preindustrial terrestrial N budget may be an artifact of underestimated rates of terrestrial N_2 fixation and of geologic N input. For example, recent findings of robust N_2 fixation in peat systems[430] and by geographically widespread cryptogamic species such as lichens, biological soil crusts, and mosses[431] and the use of alternative N_2-fixing enzymes[65,103] imply that terrestrial rates may be higher than current estimates. Fixed N liberated from rocks by *in situ* chemical weathering[6] may also help to reconcile apparent imbalances in the preindustrial N budget.

In contrast to the preindustrial budget, the modern N inventory is characterized by net N gain at a substantial rate[4,5,209,281] (~40 to 90 Tg N yr^{-1}, Table 4), requiring the storage of N in food, natural plant biomass, or soils. Some evidence of this sequestration is provided by observations of N-fertilization of plant and soil C storage in certain ecosystems.[16,327,328] Current projections indicate that new N inputs could rise to ~600 Tg N yr^{-1} to Earth systems by 2100 due to higher rates of natural biological N_2 fixation with global warming and of anthropogenic N_2 fixation driven by growth in food demand.[5,79] This is expected to be accompanied by greater emissions and atmospheric processing of fixed N. The long-term effects of anticipated rising anthropogenic N input on the terrestrial budget are not well constrained, but will depend greatly on the response of denitrification to continued N addition and global change.

2.3.2. Marine N Budget. The marine N budget is characterized by external inputs of fixed N from natural biological N_2 fixation, atmospheric deposition, and rivers and by N loss from denitrification/anammox and sediment burial (Figure 5).

The largest N input to marine systems is biological N_2 fixation (Table 3). The global marine N_2 fixation rate has been the subject of vigorous research. The extrapolation of sparse and highly variable direct measurements has suggested a global rate of 177 Tg N yr^{-1}.[432] This value falls within the range of other estimates, including those based on geochemical data,[377,379,385] which yield total marine N_2 fixation rates typically between 100 and 200 Tg N yr^{-1} (Table 3). A recent study[385] using inverse and prognostic models of marine N biogeochemistry has proposed ~160 Tg N yr^{-1} (with ± ~ 30% uncertainty) for the global rate, a value that is only slightly higher than a previous widely used estimate of 140 Tg N yr^{-1}.[4,7,433] Most global marine N_2 fixation is thought to occur in the surface of the low latitude open ocean, where it is carried out by the bloom-forming cyanobacterium genus *Trichodesmium*,[158] diatom-associated cyanobacteria,[160] and a discrete set of unicellular cyanobacteria and bacterioplankton[161,172] (Section 1.2.1.1). The remainder is attributed partly to benthic environments, including sediments, saltmarsh, mangrove and reef settings; early studies suggest 15 Tg N yr^{-1} for the total benthic N_2 fixation rate.[434]

At this point, it appears that anthropogenic activities on land have yet to strongly influence marine N_2 fixation. N budget reconstructions for the recent Holocene using N stable isotopes[394,435] report global N_2 fixation rates that are essentially equivalent to modern rates of N_2 fixation estimated using direct experimental and geochemical methods (Table 3). More recently, Jickells et al.[295] modeled the impact of rising atmospheric deposition due to anthropogenic activities on marine N_2 fixation rates and obtained only a small compensatory decrease (<10%) in the open ocean N_2 fixation rate for the 1990s (see Sections 1.2 and 3 for the feedback mechanism). However, a separate modeling study suggests that anthropogenic suppression of global marine N_2 fixation may be more substantial by year 2100, decreasing the rate by more than 10%.[436]

Hydrologic transport of terrestrial N into the ocean is a substantial source of external N. While estimates for modern riverine fluxes have varied over the years (e.g., from 11 to 76 Tg N yr^{-1}, Table 3), recent studies have converged on values in the low end of the range (~30 Tg N yr^{-1}). Groundwater N flux is less important − Voss et al.[433] estimated that the ocean receives less than 10% of the total hydrologic N flux from subsurface flows. It is thought that human activities have contributed to an approximate doubling of riverine N fluxes since the preindustrial period[295,427,428] (Table 3). However, the effect of riverine anthropogenic N on the open ocean is uncertain, as it depends on how much N is lost by sedimentary denitrification in estuaries and continental shelves, quantities that depend on denitrification rate and the residence time of a water parcel in these transitional environments.[420] Recent studies suggest that, for specific rivers, up to 75% of riverine dissolved inorganic N flux (or ~70% of total N flux) could reach the open ocean,[295,296] mostly in areas near the equator where the Coriolis force is weakest. However, these studies also suggest that variations between modern and preindustrial river fluxes to the open ocean are likely to be small, absent major changes in physical oceanographic factors.

The atmospheric transport and deposition of N derived from land sources to the ocean is thought to be the main route by which human perturbation of the N cycle reaches the open ocean. Estimates for contemporary N deposition to the total ocean vary by a factor of 3 (Table 3). A recent synthesis of anthropogenic impacts on the open ocean[295] suggests 39 Tg N yr^{-1} as the net atmospheric deposition flux for ~2005, with ~70% of this derived from human activities, consistent with findings based on independent data constraints.[301]

The most important route for permanent loss of N from the marine reservoir is denitrification in the water column and sediments of the ocean. Estimates of the total denitrification rate have varied widely from ~200 to 450 Tg N yr^{-1} (Table 3), primarily due to large uncertainties in sedimentary denitrification within estuary, continental shelf, and deep sea settings. The first N stable isotope budget for the ocean suggested sedimentary denitrification to be ~75% of the total rate.[394] However, consideration of uncertainties and details in the N isotope budget has subsequently lowered this estimate[389,395,403,404] (Section 2.1.2). Most studies propose a total denitrification rate between 200 and 280 Tg N yr^{-1} (Table 3). The degree to which human activities have increased total denitrification rates is not well-known. Some studies propose a

https://dx.doi.org/10.1021/acs.chemrev.9b00613
Chem. Rev. 2020, 120, 5308−5351

rise of ~10% for rates from preindustrial to modern times;[7,281] this value will depend strongly on the categorization criteria for riverine, estuarine, and marine environments. Marine denitrification may increase in response to climate warming, which is expected to generally reduce O_2 supply to the ocean and expand oxygen-deficient zones.[437−439] However, uncertainties in future atmosphere and ocean circulation as well as biological productivity make predictions difficult.[433,440]

Compared to denitrification, N removal through the burial of marine sediments and emissions of N_2O from marine sediments and oxygen-deficient zones account for minor losses from the marine reservoir. Burial rate estimates range from ~16 to 58 Tg N yr^{-1}, with many budget studies using a value of 25 Tg N yr^{-1} (Table 3). In contrast to the land emissions, it is currently unclear whether human activities have caused marine N_2O emissions, estimated at ~2−6 Tg N yr^{-1} (Table 3), to increase significantly.

On the basis of the above estimates of input and output fluxes, the marine N budget (Table 3) emerges as either balanced within the substantial error associated with estimates of N_2 fixation and denitrification or strongly imbalanced, with losses exceeding inputs by as much as ~200 Tg N yr^{-1} (Table 4). Budgets with severe net N losses (e.g., ~200 Tg yr^{-1}) have been suggested to reflect the stimulation of denitrification by anthropogenic N additions.[441] Early considerations suggested a possible decline in the marine fixed N reservoir since the last ice age ~20 thousand years ago.[442] Given the marine fixed N reservoir's apparent residence time of ~2,500−5,000 years, for example, see refs 395 and 443, the associated current imbalance in the N budget would be minor and difficult to detect.

To resolve suggestions of a measured deficit in the marine N budget, researchers are evaluating whether noncyanobacterial organisms and oceanic regions such as aphotic waters and sediments, traditionally not associated with N_2 fixation, could be important N sources. A recent study[444] which employed high resolution N_2 fixation measurements in the western North Atlantic ocean suggested that intensive N_2 fixation in coastal surface waters could be a source of ~17 Tg N yr^{-1} of new N. Extrapolation of limited observations showing low rates of aphotic N_2 fixation to the entire ocean yielded global estimates of dark N_2 fixation upward of 13 Tg N yr^{-1}.[179] Finally, studies of sediment N_2 fixation, for example, see refs 188, 445, and 446, have proposed that it may be a more significant contributor of fixed N than previously thought.

It is our view that the marine N budget is balanced over time scales of decades to centuries because of stabilizing feedbacks that result from the biochemical and environmental controls on N_2 fixation and denitrification (Sections 1.2 and 3). Human-driven increases in atmospheric CO_2 are causing the ocean to warm, acidify, become more stratified, and generally lose oxygen.[438,439,447] The combined effect of these factors on N cycling and the ocean's N input/output budget is uncertain, as each factor can lead to a variety of possible perturbations to ocean biogeochemistry. However, any trajectory must be considered in the context of system feedbacks. We next discuss the dynamics of the N cycle and the potential for human alteration of the fixed N budgets of land and ocean to enhance natural C sequestration and thus slow the anthropogenic rise in atmospheric CO_2 concentration.

3. DYNAMICS AND FEEDBACKS OF THE NITROGEN CYCLE

3.1. Fundamentals of Nutrient Limitation

Organisms require a suite of chemicals in addition to C for growth, and these elements, especially those with the potential to be scarce, are identified as "nutrients." The concept of nutrient limitation plays a central role in our understanding of how the N budget responds to perturbations. The typical organisms of interest are the primary producers, which synthesize organic matter from CO_2, mainly by photosynthesis, and comprise the base of the food web in terrestrial and marine systems. Accordingly, nutrient limitation has traditionally been interpreted in the context of Liebig's Law of the Minimum, which states that plant growth will be as great as that allowed by the least available resource, the "limiting nutrient" that sets the productivity of the system.[450] This view has been expanded to include the concept of "co-limitation," a condition in which productivity is controlled by multiple factors (e.g., multiple nutrients, energy) due to their interactions with each other. A good example comes from the polar ocean, where higher iron supply can increase the efficiency of light capture by phytoplankton living in these dimly lit environments.[28]

Elser et al.[1] performed a meta-analysis of over 27,500 studies of nutrient addition experiments designed to identify the limiting nutrient *sensu* Liebig. They found that marine systems were predominantly N-limited, while N and P limitation were equally prevalent in freshwater and terrestrial systems. In marine systems, Moore et al.'s[2] systematic review revealed two modes of phytoplankton nutrient limitation. Productivity is limited by N availability in the vast regions of the subtropical gyres of the Pacific and Atlantic Ocean, and in the equatorial Atlantic, where subsurface nitrate supply is slow. Where upwelling causes nitrate and phosphate to be plentiful in surface waters, as in high latitude ocean regions and in the equatorial Pacific, iron is limiting;[2,451] this can be understood as the consequence of iron being slowly scavenged out of deep waters.[452] Among terrestrial environments, temperate and high latitude forested ecosystems are the most strongly N-limited.[3,453] In contrast, N-richness (i.e., P limitation) is a typical feature of tropical forests.[3,148]

A frequently used rule of thumb to predict N limitation in a given ecosystem is to compare the N/P ratio of its nutrient reservoir to a canonical value for the N/P ratio of biomass (the "Redfield ratio" of 16 in the case of marine plankton), with a lower N/P ratio in the nutrient reservoir suggesting the potential for N limitation. However, both in the ocean and on land, the N/P ratio demanded for growth may vary for a range of reasons.[454,455] In particular, a lower N/P is expected for phytoplankton and land plants that must grow quickly because of any one of a diverse list of environmental drivers.[454,456] Given the evidence for variation in the N/P of plant and phytoplankton demand, patterns in N/P (or the related parameter N*; Section 2.1.2) cannot be interpreted solely in terms of N versus P limitation. On land, for example, one might be tempted to interpret the relatively low average value of leaf N/P at high latitudes (relative to the tropics)[457] as solely a reflection of N limitation in nontropical land ecosystems; yet, as a secondary contributor, the seasonality of growth has also been implicated as causing a lower biomass N/P.[458] Similarly, in the ocean, the subtropical gyres appear to develop a higher N/P partly due the biomass N/P of the cyanobacterial phytoplankton dominating in these regions.[386,388] An important implication of these examples is that regional variation in the N/P of biomass and

https://dx.doi.org/10.1021/acs.chemrev.9b00613
Chem. Rev. 2020, 120, 5308−5351

ecosystem nutrient reservoirs can be greater than the flexibility of a given ecosystem relative to this ratio. If so, feedbacks that stabilize the N/P of ecosystems may be stronger than imagined from regional N/P variation. As discussed in the following sections, stabilizing feedbacks dominate our understanding of the input/output budgets of fixed N.

3.2. Marine Feedbacks

In the discussions above, frequent reference has been made to feedbacks in the N cycle, in particular, with the regard to the input/output budget of fixed N, in the global ocean or in terrestrial ecosystems. The imagined feedbacks are "negative", or stabilizing. If an external pressure causes an imbalance in the N budget, then these feedbacks operate to restore balance. The stronger the feedback, the less the fixed N reservoir will change before balance is restored. Two feedbacks have been proposed (Figure 8). The first involves N_2 fixation, the dominant N input

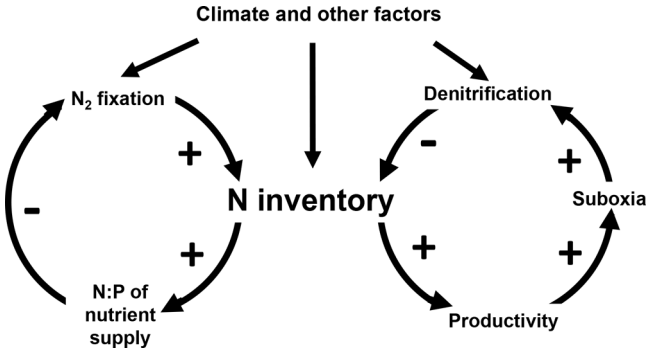


Figure 8. Hypothesized stabilizing feedbacks in the marine N budget. Natural and human-driven perturbations to the N inventory have been proposed to be stabilized by negative feedback cycles involving N_2 fixation or denitrification. In the N_2 fixation feedback, addition of N to the inventory increases the N/P of the nutrient supply to ocean surface waters, which reduces the competitive advantage of N_2-fixing organisms relative to nonfixing phytoplankton, resulting in a decrease in N_2 fixation rate that compensates for the initial rise in N inventory (left cycle). In the denitrification feedback, an increase in N input raises surface N supply, which leads to greater biological productivity, a greater flux of sinking organic matter into the ocean interior, greater respiratory consumption of O_2 in the ocean interior, greater volumes of oxygen-deficient (suboxic) water in the ocean interior, and ultimately higher rates of N loss by denitrification (right cycle). The two feedbacks have contrasting sensitivities. In particular, the N_2 fixation feedback is weakened by flexibility in the N/P of phytoplankton, whereas the denitrification feedback requires such flexibility. Reprinted in part with permission from Deutsch et al., Isotopic Constraints on Glacial/Interglacial Changes in the Oceanic Nitrogen Budget. *Global Biogeochem. Cycles*. 2004. Copyright 2004, John Wiley and Sons Inc.

in many systems with a standing N reservoir to be considered. The second involves denitrification, often the dominant N output (or loss). Below, we will focus on the discussion of these feedbacks as they have been considered in the ocean. However, the same feedbacks have been considered in terrestrial and freshwater systems. Indeed, some of the most compelling data come from freshwater lakes.[287−289]

The N_2 fixation feedback (left cycle, Figure 8) involves the competitive advantage of diazotrophic organisms over those that cannot fix N_2 when N limitation occurs.[139,459,460] If, for some reason, N becomes depleted relative to P, the N/P ratio of the nutrient supply to the sunlit ocean declines, and surface waters will tend toward limitation by N as opposed to P. Organisms capable of using the excess P by fixing their own N (i.e., the N_2-fixers) under conditions of low N availability find an expanded ecological niche, and inputs of newly fixed N increase, raising the N inventory. As the ocean's N/P ratio approaches that needed by phytoplankton, the energy- and iron-intensive process of N_2 fixation becomes disadvantageous since the N supply is sufficient to use the available P. Nonfixing phytoplankton once again becomes effective competitors for P, and N_2 fixation declines back to the initial rate. The net result is a stabilization of the N/P in the ecosystem's nutrient reservoir. This feedback assumes that non-N_2-fixing plankton do not simply alter the N/P ratio of their biomass (i.e., decrease their N needs relative to P) to match that of the nutrient supply from the subsurface. Such dynamics are consistent with the well-studied down-regulation of N_2 fixation under conditions of high ammonium and nitrate availability.[76,88]

In the ocean's denitrification feedback[395,461,462] (right cycle, Figure 8), a higher ocean nitrate reservoir drives higher biological productivity in the surface ocean and greater fluxes of sinking organic matter to the subsurface water column and the sediments. This increased flux of organic matter expands oxygen-deficient ("suboxic") environments in the subsurface ocean and in marine sediments. The increased flux also represents an increase in supply of reductant to these environments. Both effects work to increase the global rate of denitrification and associated redox processes (e.g., anammox) that remove fixed N from the ocean, compensating for the previous increase in the fixed N reservoir. The decline in ocean oxygen and the rise in the flux of organic matter to the seabed may also lead to an increase in organic N burial, but this loss term is minor compared to denitrification in the ocean. A critical assumption in this feedback is that phytoplankton and ecosystems can vary their N/P (and thus C/P) ratio, raising it as their N supply increases without a commensurate increase in the supply of P. This is counter to the assumption of an inflexible N/P of phytoplankton and ecosystems that underlies the N_2 fixation feedback.

These two distinct N budget feedbacks may be competitive, if not mutually exclusive. The more effectively any source/sink term acts to diminish perturbations to the N inventory, the smaller will be the effective perturbation to which other source/sink terms respond. For example, the N deficits generated by a hypothetical increase in denitrification might be rapidly and completely compensated by an increase in N_2 fixation. In this case, there will be no change in the nitrate reservoir or in the flux of organic matter to the ocean interior, and the denitrification feedback will not operate. On the other hand, if N_2 fixation responds only weakly to the N deficit, the flux of sinking organic matter out of the surface ocean will decrease, and a denitrification feedback will be the dominant mechanism for stabilizing the N reservoir.

The sensitivities of different feedbacks will determine their relative importance. In turn, the flexibility of the N/P ratio of non-N_2-fixing plankton appears to play a major role in these sensitivities.[463] If there is no flexibility in this ratio, then the N_2 fixation feedback will dominate; in contrast, if its flexibility is very high, then the denitrification feedback will dominate. In either case, the resulting change in the nitrate reservoir will depend on the combined sensitivities of all of the feedbacks.[395]

Before proceeding to explore these negative feedbacks, it should be recognized that other feedbacks may also be important, including feedbacks not yet identified, and these may be negative (stabilizing) or positive (amplifying). For example, there is the possibility of a feedback among global temperature, dust delivery, N_2 fixation, and atmospheric CO_2,[464]

https://dx.doi.org/10.1021/acs.chemrev.9b00613
Chem. Rev. 2020, 120, 5308−5351

which can be stabilizing or destabilizing depending on how dust delivery responds to climate.

Both process studies in the modern environment and investigations of past changes provide insight into the occurrence of feedbacks. While a far broader range of approaches is available for modern studies, the past provides the unique potential to identify the response of the N budget to a perturbation at the large scale.

At the small scale, laboratory studies and field incubations indicate that N_2-fixers will accelerate their rate of N_2 fixation when supplied with P alone as opposed to both N and P together.[133,465,466] At a somewhat broader scale, but still focused on *in situ* conditions for plankton, it is observed that P limitation, rather than N limitation, tends to occur in low-nutrient surface regions where dust-borne iron fluxes are high.[2] This suggests that, when iron availability is adequate, marine N_2-fixers consume even trace levels of excess P.

At a larger scale, studies of nitrate-to-phosphate ratio variation across the global ocean indicate that, in each of the ocean basins, N_2 fixation occurs at similar rates as denitrification.[379,385] This is consistent with the lowering of N/P by denitrification leading to a compensatory response from N_2 fixation, with this compensation occurring in response to relatively modest N/P variations. Thus, a strong negative feedback from N_2 fixation is implicated.

The Atlantic basin hosts no significant water column denitrification and only a modest rate of sedimentary denitrification. There, studies of the nitrate-to-phosphate ratio[467] and the N isotopes of nitrate[404,407,468,469] suggest that N_2 fixation occurs with a spatial distribution and basin-wide rate that are consistent with the process compensating for the relatively modest amount of excess P imported into the basin and upwelled into its surface waters. While the data allow for possible iron-limitation of N_2 fixation in the South Atlantic, this does not apply to the equatorial and North Atlantic. Thus, even if the rest of the global ocean had a substantial imbalance between denitrification and N_2 fixation, N_2 fixation in the North Atlantic would work to maintain the global ocean at a relatively constant N/P ratio as the global ocean's waters circulate through this region.[470] All totaled, the modern data provide strong evidence for the N_2 fixation-based stabilizing feedback.

Paleoceanographic data strengthen the case. Planktonic foraminifera are $CaCO_3$ shell-forming zooplanktonic protists that live throughout the ocean, and their shells are abundant in deep sea sediments. The organic N protected in the biomineral walls of planktonic foraminifera shells can be analyzed for its $^{15}N/^{14}N$, which tracks the $^{15}N/^{14}N$ of the subsurface nitrate supply.[471] In turn, the $^{15}N/^{14}N$ (or $\delta^{15}N$) of this nitrate declines with higher N_2 fixation in the basin. Thus, through the analysis of the $^{15}N/^{14}N$ of foraminifera shell organic matter (FB-$\delta^{15}N$, for foraminifera-bound $\delta^{15}N$) in deep sea sediment cores, N_2 fixation rates have been reconstructed for the tropical North Atlantic and the South China Sea.[299,471,472]

FB-$\delta^{15}N$ records from both regions over recent glacial/interglacial cycles indicate changes in N_2 fixation that are best explained as responses to changes in the N/P of the nutrient supply to surface waters. The record of FB-$\delta^{15}N$ from the South China Sea covers the last 800,000 years[473] (Figure 9). FB-$\delta^{15}N$ increases during ice ages (low sea level) and decreases into interglacials (high sea level). The FB-$\delta^{15}N$ changes are best explained as the consequence of sea level change, with which it shows the strongest correlation (Figure 9), in comparison to sea surface temperature and other relevant ocean properties.[473]

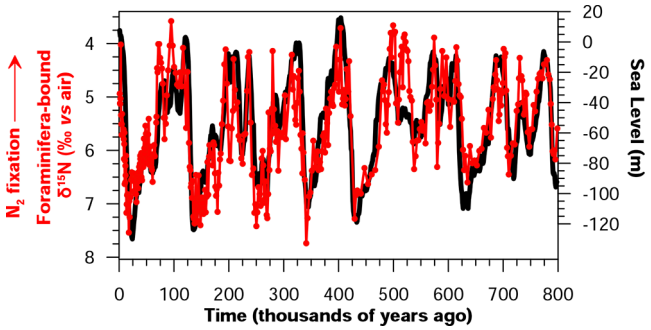


Figure 9. Foraminifera-bound $\delta^{15}N$ (FB-$\delta^{15}N$, red) from a sediment core in the South China Sea and a reconstruction of global sea level (black) covary due to the N_2 fixation feedback. A lower value for FB-$\delta^{15}N$ (upward on the left axis) implies a higher rate of N_2 fixation in the South China Sea. Sea level is in meters relative to modern sea level, and downward on the plot (a higher magnitude negative value) indicates a lower sea level. FB-$\delta^{15}N$ decreases (i.e., shifts upward on the plot) during interglacial periods, when sea level is high, and increases (i.e., shifts downward on the plot) during ice ages, when sea level is low. This can be explained by high sedimentary denitrification rates caused by flooding of the continental shelf regions during interglacials (high sea level stands), which produces a N deficit relative to P that promotes N_2 fixation, leading to a decrease in FB-$\delta^{15}N$. High FB-$\delta^{15}N$ signals reduced N_2 fixation rates during the ice ages, when the continental shelves are above sea level and thus do not host sedimentary denitrification. Data from Ren et al.[473]

During interglacial periods, the continental shelves of the region are flooded, leading to high rates of sedimentary denitrification. This appears to encourage N_2 fixation in the South China Sea, as signaled by lower FB-$\delta^{15}N$ and as expected from the N_2 fixation feedback. During the ice ages, sea level is low, there are no shelves to host sedimentary denitrification, and thus compensatory N_2 fixation rates are low, resulting in a high FB-$\delta^{15}N$.

In comparison to the N_2 fixation feedback, the denitrification feedback is far more difficult to document, in either the modern or the past ocean. With regard to isotopic approaches, the $\delta^{15}N$ of global mean ocean nitrate is sensitive not to the rates of water column and sedimentary denitrification alone, but rather to their ratio (Section 2.1.2). The denitrification feedback is expected to involve both water column and sedimentary denitrification, so mean ocean nitrate $\delta^{15}N$ does not provide a simple diagnostic for the feedback. Regional $\delta^{15}N$ elevation of nitrate accompanies the water column denitrification of the suboxic zones, and this nitrate $\delta^{15}N$ elevation is reflected in the sinking N above and nearby the suboxic zones.[474,475] Thus, the regional isotopic signal of water column denitrification could possibly be used to identify the denitrification feedback as executed through water column denitrification changes.

Deutsch et al.[395] used marine sedimentary N isotope changes since the last ice age and through the transition to the current interglacial to diagnose the importance of the feedbacks related to water column denitrification, sedimentary denitrification, and N_2 fixation. In this early numerical modeling effort, the available data were found to indicate a strong stabilization of the ocean's fixed N reservoir by the combined feedbacks, with the reservoir changing by less than 30%. However, given the limited data at the time, conclusions of significant confidence could not be made with regard to the relative feedback strength involving N_2 fixation versus denitrification or water column versus sedimentary denitrification. Information from new data

https://dx.doi.org/10.1021/acs.chemrev.9b00613
Chem. Rev. 2020, 120, 5308−5351

generation and numerical modeling efforts might allow for this next step.

Perhaps the strongest evidence for stabilizing feedbacks in the ocean N budget is the lack of dramatic trends in global ocean biological productivity and biogeochemical conditions over tens of thousands of years and longer, even though systematic regional oscillations are observed over glacial cycles.[476−478] As described above, changing the N inventory independently of the P reservoir would require that the N/P ratio of non-N_2-fixing plankton can vary with the N/P ratio of the nutrients supplied to surface waters (i.e., the lack of a conserved range of N/P in the global stock of phytoplankton). In this case, an increase in the N inventory would be expected to raise productivity in the low latitude ocean, where N often limits phytoplankton growth.

The residence time of oceanic fixed N (calculated by dividing the ocean N reservoir by the input or output) indicates how long it will take for a given imbalance between N input and output to materialize as a given proportional change in the N reservoir. For example, if the input was removed but the output remained constant, then the oceanic fixed N reservoir would be completely depleted in one residence time. The residence time of ocean fixed N is estimated to be 2,500 to 5,000 years, for example, see refs 394, 395, and 443 (Section 2.3.2). This is short relative to many periods of interest in Earth history, yielding the potential for large N reservoir changes in marine biological productivity if feedbacks do not push input and output toward a balance. For example, imbalances in the input/output budget could have dramatically changed the oceanic inventory of N during one of the ice ages of the last million years; each lasted for 20,000−100,000 years.

We consider just one of many possible dynamics: sea level lowering during ice ages due to the sequestration of water as ice on land. Most sedimentary denitrification in the modern ocean is believed to occur on the continental shelves, for example, see ref 479. During the ice ages, however, sea level dropped by up to ∼130 m (Figure 9), which would have exposed the shelves almost completely, such that sedimentary denitrification should have been substantially lower than in the modern ocean.[479,480] Even a modestly lower rate of sedimentary denitrification during the ice ages (e.g., by 30%), with all other fluxes constant, would have doubled the oceanic fixed N inventory within ∼7,500−15,000 years. The geologic record, however, provides no evidence of dramatic trends in global ocean productivity during recent ice ages, or on longer periods. Instead, the evidence suggests that the fertility of the ocean has varied over glacial/interglacial cycles on a regional basis but has been roughly stable within ice ages on a global basis.[476,477,481] The same appears to apply to ocean interior oxygen concentrations,[482] which are sensitive to the globally integrated rate of organic matter export to the ocean interior. The most straightforward explanation is that one or both of the negative feedbacks of Figure 8 regulate the N budget, stabilizing the size of the ocean N reservoir.[139,460,471−473,483,484] Similar arguments regarding the need for a long-term N input/output balance apply to the million-year time scale, when dramatic changes in ocean denitrification appear to have occurred in response to the tectonic evolution of ocean basins.[405]

3.3. Terrestrial Feedbacks

In the ocean, the distinction between N- and P-limited systems is typically subtle, not being immediately obvious from standard nutrient concentration measurements or the composition of the resident plankton. Nutrient addition experiments must be employed, and even these are somewhat uncertain due to the manipulations involved. This state of affairs indicates that the nutrient-poor tropical, subtropical, and temperate surface ocean is rarely far from colimitation by N and P,[2,451] as would be expected from a strong N_2 fixation feedback.

In comparison to marine and other aquatic systems, the feedbacks on the fixed N budget in terrestrial ecosystems appear to be weaker. For example, soil denitrification, while stimulated by anthropogenic fixed N additions, fails to remove all of the extra inputs, and declining N_2 fixation is similarly incapable of compensating for the N addition. As a result, fixed N accumulates in the ecosystem or is lost to rivers and the atmosphere (see Section 2.3.1).

The contrast in N status between tropical forests and higher latitude ecosystems further suggests that stabilizing feedbacks could be relatively weak on land. Elser et al.'s[1] meta-analyses of global nutrient limitation found that tropical forests responded more strongly to added P (and N + P) than to N, consistent with the long-held belief that P limitation (thus presumed N-richness) in tropical forests with highly weathered soils is widespread.[148,453] In tropical forests, atmospheric deposition and biological N_2 fixation succeed in generating widespread N richness at the ecosystem level, far surpassing the input rate needed to prevent N limitation.[148] One explanation for this finding relates to the inherent heterogeneity of terrestrial ecosystems in space, depth, and time.[148,485] For example, transient disturbance in a tropical forest by treefall, fire, clearing, or cultivation may remove the N_2 fixation inputs, while denitrification and hydrologic loss continues to remove N until N impoverishment is reached. This drives a burst of N input during early secondary succession by facultative N_2-fixing trees, which can adjust their N_2 fixation to soil N availability, leading to a subsequent accumulation of fixed N.[151,486] In concert, N_2 fixation in N-poor niches in the tree canopy and leaf litter, isolated from the N-richness in below-ground soils, can serve as additional ecosystem N inputs.[148] Finally, it has been proposed that N_2 fixation may be favored in low-P tropical systems because N_2-fixers are able to invest in N-rich phosphatase enzymes to increase local soil P availability.[79] This last proposal involves concepts of colimitation, while also implying a low barrier to N_2 fixation in tropical forests.

In contrast, N limitation appears to be widespread in tundra[1] and higher latitude (and altitude) forested ecosystems,[122,453] where soils are younger, generally less weathered, and more P-rich.[487] Like the question of the persistent N-richness of the tropics, the question of why N_2 fixation has yet to compensate for high latitude N impoverishment is longstanding. Deluca et al. suggested that a tightly regulated N_2 fixation feedback involving canopy throughfall N and N_2 fixation by cyanobacteria in the moss carpets of northern boreal forests stabilizes fixed N inputs in response to natural cycles of fire, succession, and human-driven N dynamics.[488] Nevertheless, boreal forests remain frequently N-limited.[489]

A range of possible constraints on high latitude terrestrial N_2 fixation have been considered. Energetic limitation of N_2 fixation is possible,[122,124] but light is abundant on at least a seasonal basis in most terrestrial ecosystems. Organic matter quality and substrate C:N stoichiometry are important constraints on N_2 fixation by asymbiotic heterotrophs;[128,156] however, these organisms are not typically as important to ecosystem N budgets as photosynthetic symbiotic counterparts.[123,143]

Micronutrient limitation of N_2-fixers is possible,[124,490] but iron, the most frequent driver of trace element limitation, is

https://dx.doi.org/10.1021/acs.chemrev.9b00613
Chem. Rev. 2020, 120, 5308−5351

abundant in most terrestrial environments (relative to marine ecosystems). In any case, mircoorganisms can secrete siderophores, strong Fe-chelators, to enhance iron bioavailability.[491] Furthermore, N_2-fixing trees have been recently found to mine minerals nutrients from bedrock.[492] The most abundant nitrogenase requires molybdenum, and Mo limitation has been documented in tropical as well as high latitude systems.[131,132,493,494] However, "alternative" nitrogenases that utilize vanadium or iron in place of Mo are available to overcome this limitation of N_2 fixation.[56,65,102,103]

Ecological arguments related to the biogeographical success of different N_2-fixer strategies (facultative N_2 fixation in the tropics versus obligate N_2 fixation in temperate systems) have also been suggested to explain N limitation in higher latitudes and its disappearance in tropical forests.[140] Below-ground N dynamics between symbiotic ectomycorrhizal fungi that exchange N for C from their host trees could shift in favor of greater fungal N retention when soil N availability declines, thus sustaining high latitude forest N deficits.[495]

Temperature constraints on nitrogenase activity may act alternatively or in addition to such ecological explanations: terrestrial nitrogenase activity is found to be substantially reduced at the lower temperatures that apply to N-limited high-latitude ecosystems.[79] Interestingly, the role of temperature in ocean N_2 fixation may be reduced by two previously undiscussed considerations. First, high latitude waters in the ocean are often nutrient-rich (in both N and P), such that they are not candidate regions for N_2 fixation. Second, any impairment of N_2 fixation in high latitude waters, such as in the coastal Artic ocean,[496] may be compensated by N_2 fixation at low latitudes and subsequent water exchange by circulation and mixing. In contrast, terrestrial N budgets are inherently local, so that such large scale compensation is not possible. We suggest that these distinctions contribute to explaining why, relative to the ocean, terrestrial systems are so much more variable in N- versus P-limitation.

Finally, relative to the ocean, any failure of terrestrial denitrification and N_2 fixation to compensate fully for anthropogenic N input can more easily change the terrestrial N reservoir. First, relative to the open ocean, land generally receives far more anthropogenic N, such that the anthropogenic perturbation of the N input is greater[4,7,281] (Table 4). Second, estimates of terrestrial fluxes and reservoirs (Figure 1, Table 3) suggest a natural residence time for fixed N on land (~500 yr) that is roughly an order of magnitude less than the fixed N residence time in the ocean (~2,500−5,000 yr). The greater anthropogenic perturbation and smaller N reservoir combine to make the terrestrial N reservoir more prone to alteration by anthropogenic effects.

4. IMPLICATIONS FOR ATMOSPHERIC CO_2

4.1. Role of Fixed N in the Carbon Cycle

An increase in the fixed N reservoir increases the quantity of C that may be removed from the atmospheric CO_2 reservoir and sequestered. On land, the additional sequestered C would be as living or dead biomass or as organic C in the soil. In the ocean, it would be largely in the form of dissolved inorganic C (dissolved CO_2, bicarbonate, and carbonate) in the ocean interior. This increase in C sequestration will only occur, however, if the N reservoir increase is accompanied by a stoichiometrically equivalent increase in P, or if the N/P ratio of the organic matter driving the C sequestration rises to account for the increase in the availability of N relative to P. The latter is more likely to occur in terrestrial ecosystems (specifically, in the currently N-limited temperate and high latitude ecosystems) than in the ocean. In the ocean, it is more likely that any rise in the N reservoir not accompanied by a rise in P would simply be removed by the stabilizing feedbacks described above.

It is helpful to relate changes in C storage to the frequently used terminology of biological productivity. Primary productivity refers to the biological synthesis of organic matter from CO_2, mainly by photosynthesis (Figure 10). The total rate of organic

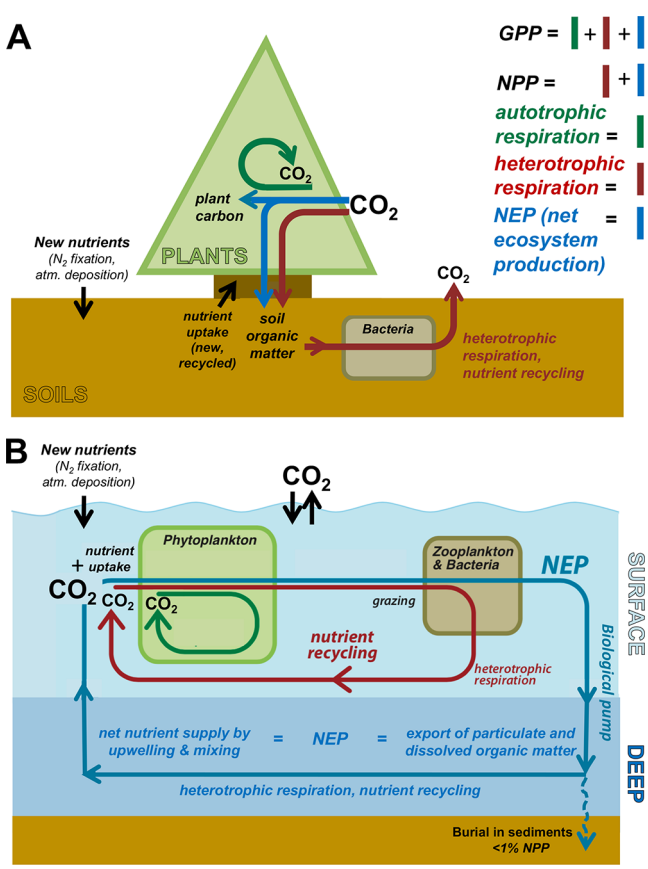


Figure 10. Biological productivity on land (A) and in the ocean (B) in relation to carbon and nutrient cycling. Gross primary productivity (GPP), representing gross photosynthesis, is the sum of primary productivity that (1) fuels plant or phytoplankton respiration (green arrow), (2) supports the respiration of heterotrophic organisms in the system (red arrow), and (3) yields organic C that can be sequestered in a long-lived terrestrial C reservoir or stored (mostly after oxidation to CO_2) in the deep ocean. The latter is also known as net ecosystem/export production (NEP, blue arrow). The sum of blue and red lines yield "net primary production" (NPP), which is equivalent to the primary production that forms the base of the food web. Land sequestration of biological C in living plant biomass and soil organic matter is due to NEP. In the ocean, NEP accounts for the biological C stored in the ocean interior. This is the result of the biological pump, which exports organic C from the surface ocean mostly as sinking particles, reducing the CO_2 concentration in the surface ocean and the atmosphere with which it equilibrates. The exported organic C is almost entirely decomposed back to CO_2 in the deep ocean by bacteria and other heterotrophs. The resulting excess CO_2 is sequestered for decades to thousands of years in the deep ocean before circulation returns it to the surface. The very small fraction of exported organic C that survives to be buried in marine sediments is held in these sediments for millions of years before they are re-exposed and weathered, releasing the C as CO_2 back to the atmosphere. Adapted from Sigman and Hain.[452]

https://dx.doi.org/10.1021/acs.chemrev.9b00613
Chem. Rev. 2020, 120, 5308−5351

C production by photosynthesis is termed "gross primary production" (GPP). "Net primary production" (NPP) is the rate at which the total metabolism of photoautotrophs produces biomass and is equivalent to GPP minus the organism's own rate of respiration. Land plants and single-celled marine phytoplankton each account for roughly half of global NPP.[497] "Net ecosystem production" (NEP) refers to GPP minus the total respiration of organisms in an ecosystem. Of these various terms, NEP is the one with a direct consequence for atmospheric CO_2 changes on interannual time scales and longer.

In terrestrial ecosystems, NEP leads to the accumulation of biomass dominantly in the above- or below-ground vegetation or in the soils (Figure 10A). This C will be stored until a change in the ecosystem, either gradual or abrupt, causes NEP to become negative, releasing CO_2 back into the atmosphere.[498] An increase in the fixed N reservoir (e.g., due to an increase in N input from N_2 fixation or atmospheric deposition) would allow NEP to be positive for a period of time, allowing the terrestrial C reservoir to rise. NEP would then decline to zero, indicating the return to a balance between photosynthetic C production and total ecosystem respiration, during which there would be neither net withdrawal from, nor net addition to, the atmospheric CO_2 reservoir. If the N reservoir were ever to decline, NEP would be negative for a time, until the sequestered C reservoir has adjusted to the new (lower) N reservoir. A very small fraction of terrestrial NEP may escape to be buried in a geologic repository such as a river delta or other continental margin sediments; this C will be sequestered until geologic processes expose it at the land surface.

In the ocean, NEP leads to export (mostly sinking) of organic C into the ocean interior, where most it is respired by microbes back to CO_2 (Figure 10B). Because the interior waters do not have direct access to the atmosphere, this C is then held as dissolved inorganic C for decades to roughly a thousand years before circulation returns it to the surface, where it has the opportunity to escape back to the atmosphere as CO_2. Rather than escaping, this NEP-derived CO_2 can be resequestered if (1) it emerges in a region of complete nutrient consumption where the biological pump (Figure 10B) operates efficiently (in the tropics, subtropics or temperate latitudes as opposed to the polar ocean) and (2) the fixed N allowing for its original sequestration is still present in the water. If the sinking organic C survives microbial processing in the water column or the biologically active upper layer of seafloor sediments, it is buried, sequestering the C for millions of years in sediments before they are re-exposed and weathered; however, as on land, this represents a very small fraction of NEP (less than 1%).[24]

4.2. Nitrogen Fertilization of the Terrestrial Carbon Sink

Anthropogenic N inputs are potentially important when considering changes in the terrestrial C reservoir. N additions from human activities to N-limited regions may stimulate photosynthesis and result in greater ecosystem C storage, helping to mitigate rises in anthropogenic CO_2, so long as rates of organic matter decomposition and N loss are not similarly enhanced. This appears to be the case, at least in select terrestrial ecosystems. Currently, enhancement of primary productivity on land (largely due to enhanced forest growth related to CO_2 fertilization, warming, and recovery from historical clearing[498]) removes about a third of anthropogenic CO_2 emissions from industrial activity and land use change.[498,499] Roughly ~10% of this increased land sink has been attributed to anthropogenic N deposition[418] incidentally fertilizing N-limited mid- and high-latitude forests.[327,328,500]

Plant productivity is often increased by N fertilization.[453,501] However, species-specific responses to N fertilization are diverse, with certain plant species showing biomass increases and others showing decreases within the same ecosystem.[314] There is strong evidence for the stimulatory effects of N deposition on above ground storage in forest biomes, for example, see refs 327, 328, and 502. N deposition can also induce greater levels of below-ground C storage as soil organic matter by stimulating the production of litter and fine roots, the production of more recalcitrant plant biomass, or altering soil microbial activities. Evidence for increased soil C in response to N addition was initially equivocal, as effects varied from positive, negative, to neutral, for example, see refs 327 and 503−505. However, it appears that recent studies[506−510] are reaching a consensus in support of a positive response. Soil fungi are thought to play a particularly important role in below-ground N and C dynamics.[506,510] Consistent with Chen et al.'s[509] meta-analysis on the effects of added N on lignin-modifying enzyme activity in soils, Zak et al.[510] recently demonstrated that N deposition was linked to the reduced activity of fungal peroxidase enzymes critical for lignin decay, slower organic matter mineralization, and higher soil C storage in temperate forest ecosystems.

The relationship between N input and C storage in nonforested systems is unclear. On the one hand, long-term N enrichment of Arctic tundra led to an increased decomposition rate that offset rises in above ground C sequestration due to plant growth.[511] Net C loss was also observed from peat bogs subject to N deposition.[512] On the other hand, mild C gains have been observed for wet sedge systems exposed to anthropogenic N.[513] Understanding how N fertilization affects the function of plant and microbes with different resource use traits will be essential to constraining the terrestrial C sink.[314]

Taken together, anthropogenic N has had a modest fertilizing effect in terrestrial ecosystems, slowing the accumulation of anthropogenic CO_2 in the atmosphere. Given predictions that insufficient nutrient availability will limit the continued function of the land C sink, particularly in boreal and temperate forests,[418,514] it is likely that anthropogenic N will continue to aid biological C sequestration in the 21st century.[515] However, this N, prior to its storage in biomass, can cause myriad problems for ecosystem and human health, and it is associated with activities that increase atmospheric N_2O concentrations. Thus, any beneficial fertilizing effects of anthropogenic N will probably be, in some fashion, offset by its negative environmental effects.

4.3. Nitrogen Fertilization of the Ocean

There are several considerations that make it highly unlikely that human additions of N will raise the ocean's biological sequestration of CO_2 to a degree that will significantly offset ongoing C emissions from fossil fuels and land use change. The first involves the circulation of water in the ocean subsurface. If anthropogenic fixed N is deposited on the low-nutrient waters of the tropical, subtropical, and temperate open ocean, it may well drive a rise in biological productivity and thus an increase in the flux of sinking organic matter out of the surface and into the ocean interior. Much of this additional organic matter flux will be decomposed back to CO_2 in the upper 1000 m of the ocean interior. On a time scale of decades, the water in which the added N and CO_2 is stored will flow or mix to the high latitude ocean regions, where the water will be exposed at the surface and

https://dx.doi.org/10.1021/acs.chemrev.9b00613
Chem. Rev. 2020, 120, 5308−5351

exchange gases with the atmosphere (the water's region of "ventilation"). In these regions, neither N nor P tends to be limiting; if nutrients are not limiting, the newly added N will not be reconsumed in the high latitude surface waters, and the CO_2 initially sequestered will escape back to the atmosphere. This high latitude "ventilation" occurs on a time scale of decades to a century, and it eventually undoes roughly half of the CO_2 sequestration that would result from anthropogenic N fertilization of the N-limited open ocean.[24,516] This series of events reduces the efficiency of any hypothetical N fertilization of the ocean.

Second, because of the feedbacks described in Section 3 (Figures 8 and 9), anthropogenic N additions to the global ocean are likely to be accommodated by compensatory changes in ocean N_2 fixation and denitrification. Indeed, there is evidence that many eutrophied coastal systems return to N-limitation,[517] which implies that denitrification is offsetting much of the anthropogenic N released into these systems. In the open ocean, the more important compensation is likely to be that of N_2 fixation, which is forecast to decrease as anthropogenic atmospheric N deposition grows.[436,518] Such considerations regarding feedbacks can alternatively be cast in terms of the flexibility of the N/P ratio of phytoplankton and ecosystem biomass. With the onset of anthropogenic N additions, N-limited ocean regions will experience an initial increase in NPP and NEP. However, N and P colimitation will develop quickly, and NPP and NEP will not be able to rise further unless the ecosystem shifts the N/P ratio of its nutrient demand. There is no evidence that, as a compensation for the shift away from N limitation and toward N and P colimitation, a shift in the N/P ratio of plankton demand would win out over a reduction in *in situ* N_2 fixation. Finally, while the stoichiometric and isotopic impact of rising anthropogenic atmospheric N deposition is identifiable in the western Pacific,[298,299] recent studies suggest that the global flux has been substantially overestimated.[301,302]

Relative to anthropogenic N inputs to the ocean, a climate-driven change in the ocean's internal N (and P) cycling has greater potential to impact the anthropogenic rise in atmospheric CO_2, for example, see ref 519. However, these changes could either reduce or enhance the rate of atmospheric CO_2 rise, and the considerations are complex, for example, see refs 520−522. One dynamic worth raising here is a tendency for natural and anthropogenic C fluxes to change in ways that compensate for one another. For example, consider the possible scenario in which warming and freshening of surface waters of the Southern Ocean (the ocean surrounding Antarctica) reduces the rate of surface-deep exchange in this ocean region and thus ventilation of the deep ocean by the region.[520] The Southern Ocean naturally vents biologically stored CO_2 to the atmosphere.[523] Thus, a decline in its surface-to-deep circulation would tend to reduce the venting of natural CO_2, even as the slowing of the circulation would reduce the rate at which fossil fuel-derived CO_2 is transported from surface waters into the deep ocean.[520] These offsetting effects would cause this scenario of Southern Ocean circulation change to have only a modest impact on the rate at which the ocean draws CO_2 out of the atmosphere. Moreover, it is possible that there will be multiple effects of climate on ocean circulation, each with their own implications for air/sea CO_2 fluxes.[522] In any case, these possible effects on the ocean's internal N and C cycling are separate from our focus here on anthropogenic N inputs to the environment.

5. SUMMARY AND OUTLOOK

In the ocean and freshwater systems such as lakes, the internal cycling of nitrogen (N) is representative of nutrient cycling in general. In contrast, the input/output budget for fixed N in these systems is distinct from other nutrients in being dominated by biological processes: biological N_2 fixation as the N input and denitrification as the N loss. Moreover, fixed N has the interesting duality of being a critical factor for both biosynthesis and redox cycling. The net result appears to be a budget that is highly regulated by feedbacks.

Because of these feedbacks, human impacts on the aquatic N cycle tend to be local and short-term. Consider, for example, an anthropogenic input of N to an aquatic system. If phosphorus (P) availability is high, excess N inputs tend to fuel denitrification. When P is scarce, excess N inputs depress N_2 fixation. In both cases, the tendency is to stabilize the fixed N reservoir. Of course, these responses occur only once the aquatic environment has been altered, so human impact is not avoided. Nevertheless, the situation is very different than for fossil fuel CO_2 emissions, the impacts of which are global and long-term.[524] In part because of this regulation by feedbacks, anthropogenic N inputs to aquatic systems are unlikely to fuel additional biological C fixation to a degree that will significantly mitigate the rise in atmospheric CO_2 due to fossil fuel CO_2 emissions.

While the same feedbacks apply to the input/output N budget of terrestrial systems, they appear to regulate the terrestrial N reservoir less strongly. This is most obviously suggested by (1) the incomplete removal of anthropogenic fixed N by soil and sediment denitrification and (2) the contrast of N richness in tropical terrestrial ecosystems with N scarcity in higher latitude systems. One contributor to this difference from aquatic systems may be the inherent lack of mechanisms to compensate for deviations from mean N/P across small spatial scales (i.e., the lack of mixing in terrestrial systems that occurs in aquatic systems). Other potentially complementary effects include differences in resource use strategies and the proposed impairment of N_2 fixation by temperature at high latitudes. On the one hand, the weaker regulation of the N reservoir in terrestrial systems makes anthropogenic N pollution a more pervasive concern than in aquatic systems (of course, the greater proximity of anthropogenic N sources to terrestrial systems is just as important). On the other hand, it implies that fertilization by anthropogenic N inputs can contribute more significantly to the terrestrial C sink that is currently mitigating anthropogenic CO_2 emissions.[498,499]

At this stage, the components of N cycling are relatively well-known. However, their interactions and consequences remain mysterious, with conceptual arguments currently playing an outsized role. To improve our mechanistic understanding, the field is expanding from a focus on culture experiments and field incubations to techniques that illuminate a broader range of scales in space and time. Biochemical, genomic, and metabolic studies improve our understanding of the sensitivities of key transformations. In parallel, geochemical budgets provide more robust, integrative estimates of environmental rates. This trajectory suggests that studies of the N cycle will continue to benefit from methodological innovations in diverse fields of science.

https://dx.doi.org/10.1021/acs.chemrev.9b00613
Chem. Rev. 2020, 120, 5308−5351

AUTHOR INFORMATION

Corresponding Author

Xinning Zhang − *Department of Geosciences and Princeton Environmental Institute, Princeton University, Princeton, New Jersey 08544, United States;* orcid.org/0000-0003-2763-1526; Email: xinningz@princeton.edu

Authors

Bess B. Ward − *Department of Geosciences and Princeton Environmental Institute, Princeton University, Princeton, New Jersey 08544, United States*

Daniel M. Sigman − *Department of Geosciences, Princeton University, Princeton, New Jersey 08544, United States*

Complete contact information is available at: https://pubs.acs.org/10.1021/acs.chemrev.9b00613

Notes

The authors declare no competing financial interest.

Biographies

Xinning Zhang received a B.S. in Biological and Environmental Engineering from Cornell University and a M.S. and Ph.D. in Environmental Science and Engineering from the California Institute of Technology. After joining the Princeton University community as a post doc, she is now a faculty member in the Department of Geosciences and the Princeton Environmental Institute. Her research aims at providing a mechanistic understanding of how microbial metabolism influences biogeochemical cycling and climate in modern and ancient environments.

Bess B. Ward received a B.S. in Zoology from Michigan State University and a M.S. and Ph.D. in Oceanography from the University of Washington. Following a post doc at Scripps Institution of Oceanography, she was a faculty member and department chair in Ocean Sciences at the University of California, Santa Cruz. She is now a faculty member in the Princeton Environmental Institute and the William J. Sinclair Professor and chair of the Department of Geosciences at Princeton University. Her research focuses on N cycling in the marine environment, understanding the microbial processes that control distributions and fluxes of N in the ocean.

Daniel M. Sigman received a B.S. in Geology from Stanford University in 1991 and a Ph.D. from the MIT/WHOI Joint Program in Oceanography in 1997. He came to Princeton in 1998 as a Harry Hess Postdoctoral Fellow in the Department of Geosciences, joined the faculty in 2000, and is currently the Dusenbury Professor of Geological and Geophysical Sciences. His research seeks to merge our understanding of the modern cycles of biologically important elements with their evolution over Earth history, including their roles in climate change.

ACKNOWLEDGMENTS

We thank three anonymous reviewers and Fabien Paulot for comments on the manuscript. X.Z. was funded by the Simons Foundation (Grant No. 622944), the National Science Foundation (Grant No. EAR1631814), and NASA (Grant No. 80NSSC17K0667) and D.M.S. by the National Science Foundation (Grant No. OCE 1736652) and ExxonMobil through the Andlinger Center for Energy and the Environment at Princeton University. D.M.S. thanks Haojia Ren and Alfredo Martinez-Garcia for providing Figure 9.

REFERENCES

(1) Elser, J. J.; Bracken, M. E. S.; Cleland, E. E.; Gruner, D. S.; Harpole, W. S.; Hillebrand, H.; Ngai, J. T.; Seabloom, E. W.; Shurin, J. B.; Smith, J. E. Global Analysis of Nitrogen and Phosphorus Limitation of Primary Producers in Freshwater, Marine and Terrestrial Ecosystems. *Ecol. Lett.* **2007**, *10*, 1135−1142.

(2) Moore, C. M.; Mills, M. M.; Arrigo, K. R.; Berman-Frank, I.; Bopp, L.; Boyd, P. W.; Galbraith, E. D.; Geider, R. J.; Guieu, C.; Jaccard, S. L.; et al. Processes and Patterns of Oceanic Nutrient Limitation. *Nat. Geosci.* **2013**, *6*, 701−710.

(3) Du, E.; Terrer, C.; Pellegrini, A. F. A.; Ahlström, A.; van Lissa, C. J.; Zhao, X.; Xia, N.; Wu, X.; Jackson, R. B. Global Patterns of Terrestrial Nitrogen and Phosphorus Limitation. *Nat. Geosci.* **2020**, *13*, 221−226.

(4) Fowler, D.; Coyle, M.; Skiba, U.; Sutton, M. A.; Cape, J. N.; Reis, S.; Sheppard, L. J.; Jenkins, A.; Grizzetti, B.; et al. The Global Nitrogen Cycle in the Twenty-First Century. *Philos. Trans. R. Soc., B* **2013**, *368*, 20130164.

(5) Fowler, D.; Steadman, C. E.; Stevenson, D.; Coyle, M.; Rees, R. M.; Skiba, U. M.; Sutton, M. A.; Cape, J. N.; Dore, A. J.; et al. Effects of Global Change During the 21st Century on the Nitrogen Cycle. *Atmos. Chem. Phys.* **2015**, *15*, 13849−13893.

(6) Houlton, B. Z.; Morford, S. L.; Dahlgren, R. A. Convergent Evidence for Widespread Rock Nitrogen Sources in Earth's Surface Environment. *Science* **2018**, *360*, 58−62.

(7) Gruber, N.; Galloway, J. N. An Earth-System Perspective of the Global Nitrogen Cycle. *Nature* **2008**, *451*, 293−296.

(8) Erisman, J. W.; Galloway, J. N.; Seitzinger, S.; Bleeker, A.; Dise, N. B.; Petrescu, A. M. R.; Leach, A. M.; de Vries, W. Consequences of Human Modification of the Global Nitrogen Cycle. *Philos. Trans. R. Soc., B* **2013**, *368*, 20130116.

(9) Sorai, M.; Yoshida, N.; Ishikawa, M. Biogeochemical Simulation of Nitrous Oxide Cycle Based on the Major Nitrogen Processes. *J. Geophys. Res.* **2007**, *112* (G1), 1.

(10) Johnson, B.; Goldblatt, C. The Nitrogen Budget of Earth. *Earth-Sci. Rev.* **2015**, *148*, 150−173.

(11) Wedepohl, H. K. The Composition of the Continental Crust. *Geochim. Cosmochim. Acta* **1995**, *59*, 1217−1232.

(12) Li, L.; Bebout, G.; Idleman, B. Nitrogen Concentration and $\delta^{15}N$ of Altered Oceanic Crust Obtained on ODP Legs 129 and 185: Insights into Alteration-Related Nitrogen Enrichment and the Nitrogen Subduction Budget. *Geochim. Cosmochim. Acta* **2007**, *71*, 2344−2360.

(13) Chamiedes, W.; Perdue, E. *Biogeochemical Cycles: A Computer-Interactive Study of Earth System Science and Global Change*; Oxford University Press: Oxford, 1997.

(14) Berner, R. A. Geological Nitrogen Cycle and Atmospheric N_2 over Phanerozoic Time. *Geology* **2006**, *34*, 413−415.

(15) Gruber, N. *Nitrogen in the Marine Environment*; Capone, D. G.; Bronk, D. A.; Mulholland, M. R.; Carpenter, E. J., Eds.; Elsevier: Amsterdam, The Netherlands, 2008.

(16) Schlesinger, W. H. On the Fate of Anthropogenic Nitrogen. *Proc. Natl. Acad. Sci. U. S. A.* **2009**, *106*, 203−208.

(17) Nolan, B. T.; Hitt, K. J.; Ruddy, B. C. Probability of Nitrate Contamination of Recently Recharged Groundwaters in the Conterminous United States. *Environ. Sci. Technol.* **2002**, *36*, 2138−2145.

(18) Lerman, A.; Mackenzie, F. T.; May Ver, L. Coupling of the Perturbed C-N-P Cycles in Industrial Time. *Aquat. Geochem.* **2004**, *10*, 3−32.

(19) Batjes, N. H. Total Carbon and Nitrogen in the Soils of the World. *European Journal of Soil Science* **2014**, *65*, 10−21.

(20) Emerson, S.; Stump, C.; Johnson, B.; Karl, D. M. In Situ Determination of Oxygen and Nitrogen Dynamics in the Upper Ocean. *Deep Sea Res., Part I* **2002**, *49*, 941−952.

(21) *Nutrients in freshwater in Europe*; European Environmental Agency, 2015. https://www.eea.europa.eu/data-and-maps/indicators/nutrients-in-freshwater/nutrients-in-freshwater-assessment-published-6.

(22) Shiklomonav, I. *Water in Crisis: A Guide to the World's Fresh Water Resources*; Gleick, P. H., Ed.; Oxford University Press: New York, 1993.

(23) Boyer, T. P.; Antonov, J. I.; Baranova, O. K.; Coleman, C.; Garcia, H. E.; Grodsky, A.; Johnson, D. R.; Locarnini, R. A.; Mishonov, A. V.; O'Brien, T. D. et al. *World Ocean Database 2013, NOAA NESDIS 72*; Technical Ed.: Silver Spring, MD, 2013.
(24) Sarmiento, J. L.; Gruber, N. *Ocean Biogeochemical Dynamics*; Princeton University Press: Princeton, NJ, 2006.
(25) Karl, D. M.; Lukas, R. The Hawaii Ocean Time-Series (HOT) Program: Background, Rationale and Field Implementation. *Deep Sea Res., Part II* **1996**, *43*, 129−156.
(26) Martin, J. H.; Fitzwater, S. E.; Gordon, R. M. Iron Deficiency Limits Phytoplankton Growth in Antarctic Waters. *Global Biogeochem. Cycles* **1990**, *4*, 5−12.
(27) Mitchell, B. G.; Brody, E. A.; Holm-Hansen, O.; McClain, C.; Bishop, J. Light Limitation of Phytoplankton Biomass and Macronutrient Utilization in the Southern Ocean. *Limnol. Oceanogr.* **1991**, *36*, 1662−1677.
(28) Sunda, W. G.; Huntsman, S. A. Interrelated Influence of Iron, Light and Cell Size on Marine Phytoplankton Growth. *Nature* **1997**, *390*, 389−392.
(29) Paulot, F.; Jacob, D. J.; Johnson, M. T.; Bell, T. G.; Baker, A. R.; Keene, W. C.; Lima, I. D.; Doney, S. C.; Stock, C. A. Global Oceanic Emission of Ammonia: Constraints from Seawater and Atmospheric Observations. *Global Biogeochem. Cycles* **2015**, *29*, 1165−1178.
(30) Zakem, E. J.; Al-Haj, A.; Church, M. J.; van Dijken, G. L.; Dutkiewicz, S.; Foster, S. Q.; Fulweiler, R. W.; Mills, M. M.; Follows, M. J. Ecological Control of Nitrite in the Upper Ocean. *Nat. Commun.* **2018**, *9* (1206), 1−13.
(31) Martens-Habbena, W.; Berube, P. M.; Urakawa, H.; de la Torre, J. R.; Stahl, D. A. Ammonia Oxidation Kinetics Determine Niche Separation of Nitrifying Archaea and Bacteria. *Nature* **2009**, *461*, 976−979.
(32) Smith, J. M.; Chavez, F. P.; Francis, C. A. Ammonium Uptake by Phytoplankton Regulates Nitrification in the Sunlit Ocean. *PLoS One* **2014**, *9*, e108173−e108173a.
(33) Aluwihare, L.; Meador, T. In *Nitrogen in the Marine Environment*, 2nd ed.; Capone, D. G.; Bronk, D. A.; Mulholland, M. R.; Carpenter, E. J., Eds.; Academic Press, 2008.
(34) Letscher, R. T.; Hansell, D. A.; Carlson, C. A.; Lumpkin, R.; Knapp, A. N. Dissolved Organic Nitrogen in the Global Surface Ocean: Distribution and Fate. *Global Biogeochem. Cycles* **2013**, *27*, 141−153.
(35) McCarthy, M.; Pratum, T.; Hedges, J.; Benner, R. Chemical Composition of Dissolved Organic Nitrogen in the Ocean. *Nature* **1997**, *390*, 150−154.
(36) Sipler, R. E.; Bronk, D. A. *Biogeochemistry of Marine Dissolved Organic Matter*, 2nd ed.; Hansell, D. A.; Carlson, C. A., Eds.; Academic Press: Boston, 2015.
(37) Aluwihare, L. I.; Repeta, D. J.; Pantoja, S.; Johnson, C. G. Two Chemically Distinct Pools of Organic Nitrogen Accumulate in the Ocean. *Science* **2005**, *308*, 1007−1010.
(38) Cao, X.; Mulholland, M. R.; Helms, J. R.; Bernhardt, P. W.; Duan, P.; Mao, J.; Schmidt-Rohr, K. A Major Step in Opening the Black Box of High-Molecular-Weight Dissolved Organic Nitrogen by Isotopic Labeling of *Synechococcus* and Multibond Two-Dimensional NMR. *Anal. Chem.* **2017**, *89*, 11990−11998.
(39) Follett, C. L.; Repeta, D. J.; Rothman, D. H.; Xu, L.; Santinelli, C. Hidden Cycle of Dissolved Organic Carbon in the Deep Ocean. *Proc. Natl. Acad. Sci. U. S. A.* **2014**, *111* (47), 16706−16711.
(40) Hansen, B.; Dalgaard, T.; Thorling, L.; Sørensen, B.; Erlandsen, M. Regional Analysis of Groundwater Nitrate Concentrations and Trends in Denmark in Regard to Agricultural Influence. *Biogeosciences* **2012**, *9*, 3277−3286.
(41) Schulten, H. R.; Schnitzer, M. The Chemistry of Soil Organic Nitrogen: A Review. *Biol. Fertil. Soils* **1997**, *26*, 1−15.
(42) Lambers, H.; Raven, J. A.; Shaver, G. R.; Smith, S. E. Plant Nutrient-Acquisition Strategies Change with Soil Age. *Trends Ecol. Evol.* **2008**, *23*, 95−103.
(43) Bingham, A. H.; Cotrufo, M. F. Organic Nitrogen Storage in Mineral Soil: Implications for Policy and Management. *Sci. Total Environ.* **2016**, *551*−*552*, 116−126.
(44) Burk, D. Azotase and Nitrogenase in *Azotobacter*. *Ergeb. Enzymforsch.* **1934**, *3*, 23−56.
(45) Bulen, W.; LeComte, J. The Nitrogenase System from *Azotobacter*: Two Enzyme Requirement for N_2 Reduction, ATP Dependent H_2 Evolution, and ATP Hydrolysis. *Proc. Natl. Acad. Sci. U. S. A.* **1966**, *56*, 979−986.
(46) Hoffman, B. M.; Lukoyanov, D.; Yang, Z. Y.; Dean, D. R.; Seefeldt, L. C. Mechanism of Nitrogen Fixation by Nitrogenase: The Next Stage. *Chem. Rev.* **2014**, *114*, 4041−4062.
(47) Smil, V. *Enriching the Earth: Fritz Haber, Carl Bosch, and the Transformation of World Food Production*; MIT Press: Cambridge, 2004.
(48) Dingler, C.; Kuhla, J.; Wassink, H.; Oelze, J. Levels and Activities of Nitrogenase Proteins in *Azotobacter vinelandii* Grown at Different Dissolved Oxygen Concentrations. *J. Bacteriol.* **1988**, *170*, 2148−2152.
(49) Eady, R. R. Structure-Function Relationships of Alternative Nitrogenases. *Chem. Rev.* **1996**, *96*, 3013−3030.
(50) Seefeldt, L. C.; Hoffman, B. M.; Dean, D. R. Mechanism of Mo-Dependent Nitrogenase. *Annu. Rev. Biochem.* **2009**, *78*, 701−722.
(51) Rees, J. A.; Bjornsson, R.; Schlesier, J.; Sippel, D.; Einsle, O.; DeBeer, S. The Fe-V Cofactor of Vanadium Nitrogenase Contains an Interstitial Carbon Atom. *Angew. Chem., Int. Ed.* **2015**, *54*, 13249−13252.
(52) Sippel, D.; Einsle, O. The Structure of Vanadium Nitrogenase Reveals an Unusual Bridging Ligand. *Nat. Chem. Biol.* **2017**, *13*, 956−960.
(53) Raymond, J.; Siefert, J. L.; Staples, C. R.; Blankenship, R. E. The Natural History of Nitrogen Fixation. *Mol. Biol. Evol.* **2004**, *21*, 541−554.
(54) Boyd, E.; Peters, J. W. New Insights into the Evolutionary History of Biological Nitrogen Fixation. *Front. Microbiol.* **2013**, *4*, 1−4.
(55) Zehr, J. P.; Jenkins, B. D.; Short, S. M.; Steward, G. F. Nitrogenase Gene Diversity and Microbial Community Structure: A Cross-System Comparison. *Environ. Microbiol.* **2003**, *5*, 539−554.
(56) McRose, D. L.; Zhang, X.; Kraepiel, A. M. L.; Morel, F. M. M. Diversity and Activity of Alternative Nitrogenases in Sequenced Genomes and Coastal Environments. *Front. Microbiol.* **2017**, *8*, 267.
(57) Betancourt, D. A.; Loveless, T. M.; Brown, J. W.; Bishop, P. E. Characterization of Diazotrophs Containing Mo-Independent Nitrogenases, Isolated from Diverse Natural Environments. *Appl. Environ. Microbiol.* **2008**, *74*, 3471−3480.
(58) Bishop, P. E.; Jarlenski, D. M. L.; Hetherington, D. R. Evidence for an Alternative Nitrogen-Fixation System in *Azotobacter vinelandii*. *Proc. Natl. Acad. Sci. U. S. A.* **1980**, *77*, 7342−7346.
(59) Masepohl, B. Regulation of Nitrogen Fixation in the Phototrophic Purple Bacterium *Rhodobacter capsulatus*. *J. Mol. Microbiol. Biotechnol.* **2002**, *4*, 243−248.
(60) Thiel, T.; Pratte, S. B. Regulation of Three Nitrogenase Gene Clusters in the Cyanobacterium *Anabaena variabilis* ATCC 29413. *Life* **2014**, *4*, 944−967.
(61) Bellenger, J. P.; Wichard, T.; Xu, Y.; Kraepiel, A. M. L. Essential Metals for Nitrogen Fixation in a Free-Living N_2-Fixing Bacterium: Chelation, Homeostasis and High Use Efficiency. *Environ. Microbiol.* **2011**, *13*, 1395−1411.
(62) Raugei, S.; Seefeldt, L. C.; Hoffman, B. M. Critical Computational Analysis Illuminates the Reductive-Elimination Mechanism That Activates Nitrogenase for N_2 Reduction. *Proc. Natl. Acad. Sci. U. S. A.* **2018**, *115*, E10521−E10530.
(63) Harris, D. F.; Lukoyanov, D. A.; Kallas, H.; Trncik, C.; Yang, Z.-Y.; Compton, P.; Kelleher, N.; Einsle, O.; Dean, D. R.; et al. Mo-, V-, and Fe-Nitrogenases Use a Universal Eight-Electron Reductive-Elimination Mechanism to Achieve N_2 Reduction. *Biochemistry* **2019**, *58*, 3293−3301.
(64) Luxem, K. L.; Kraepiel, A. M. L.; Zhang, L.; Waldbauer, J.; Zhang, X. Carbon Substrate Re-Orders Relative Growth of a Bacterium Using Mo-, V-, or Fe-Nitrogenase for Nitrogen Fixation. *Environ. Microbiol.* **2020**, *22*, 1397−1408.
(65) Zhang, X.; McRose, D. L.; Darnajoux, R.; Bellenger, J.-P.; Morel, F. M. M.; Kraepiel, A. M. L. Alternative Nitrogenase Activity in the

https://dx.doi.org/10.1021/acs.chemrev.9b00613
Chem. Rev. 2020, 120, 5308−5351

Environment and Nitrogen Cycle Implications. *Biogeochemistry* **2016**, *127*, 189−198.
(66) Zhang, X.; Sigman, D. M.; Morel, F. M. M.; Kraepiel, A. M. L. Nitrogen Isotope Fractionation by Alternative Nitrogenases and Past Ocean Anoxia. *Proc. Natl. Acad. Sci. U. S. A.* **2014**, *111*, 4782−4787.
(67) Hardy, R. W. F.; Holsten, R. D.; Jackson, E. K.; Burns, R. C. Acetylene-Ethylene Assay for N_2 Fixation - Laboratory and Field Evaluation. *Plant Physiol.* **1968**, *43*, 1185−1207.
(68) Dilworth, M. J.; Eady, R. R.; Robson, R. L.; Miller, R. W. Ethane Formation from Acetylene as a Potential Test for Vanadium Nitrogenase *in vivo*. *Nature* **1987**, *327*, 167−168.
(69) Zheng, Y.; Harris, D. F.; Yu, Z.; Fu, Y.; Poudel, S.; Ledbetter, R. N.; Fixen, K. R.; Yang, Z.-Y.; Boyd, E. S.; Lidstrom, M. E.; et al. A Pathway for Biological Methane Production Using Bacterial Iron-Only Nitrogenase. *Nature Microb* **2018**, *3*, 281−286.
(70) Luxem, K. E.; Leavitt, W. D.; Zhang, X. Large Hydrogen Isotope Fractionations Distinguish Nitrogenase-Derived Methane from Other Sources. *bioRxiv* **2020**, DOI: 10.1101/2020.04.10.036657.
(71) Burgess, B. K.; Lowe, D. J. Mechanism of Molybdenum Nitrogenase. *Chem. Rev.* **1996**, *96*, 2983−3012.
(72) Gallon, J. R. The Oxygen Sensitivity of Nitrogenase: A Problem for Biochemists and Micro-Organisms. *Trends Biochem. Sci.* **1981**, *6*, 19−23.
(73) Rees, J. A.; Bjornsson, R.; Kowalska, J. K.; Lima, F. A.; Schlesier, J.; Sippel, D.; Weyhermüller, T.; Einsle, O.; Kovacs, J. A.; DeBeer, S. Comparative Electronic Structures of Nitrogenase FeMoco and FeVco. *Dalton Trans* **2017**, *46*, 2445−2455.
(74) Zehr, J. P.; Turner, P. J. In *Methods in Microbiology*; Academic Press, 2001; Vol. *30*.
(75) Dos Santos, P. C.; Fang, Z.; Mason, S. W.; Setubal, J. C.; Dixon, R. Distribution of Nitrogen Fixation and Nitrogenase-Like Sequences Amongst Microbial Genomes. *BMC Genomics* **2012**, *13*, 162.
(76) Dixon, R.; Kahn, D. Genetic Regulation of Biological Nitrogen Fixation. *Nat. Rev. Microbiol.* **2004**, *2*, 621−631.
(77) Morel, F.; Hering, J. *Principles and Applications of Aquatic Chemistry*; 2nd ed.; John Wiley: New York, 1993.
(78) Brooks, S. J.; Collins, J. J.; Brill, W. J. Repression of Nitrogen Fixation in *Klebsiella pneumoniae* at High Temperature. *J. Bacteriol.* **1984**, *157*, 460−464.
(79) Houlton, B. Z.; Wang, Y.-P.; Vitousek, P. M.; Field, C. B. A Unifying Framework for Dinitrogen Fixation in the Terrestrial Biosphere. *Nature* **2008**, *454*, 327−330.
(80) Drozd, J. W.; Tubb, R. S.; Postgate, J. R. A Chemostat Study of the Effect of Fixed Nitrogen Sources on Nitrogen Fixation, Membranes and Free Amino Acids in *Azotobacter chroococcum*. *J. Gen. Microbiol.* **1972**, *73*, 221−232.
(81) Bühler, T. R.; Sann, U.; Monter, C.; Dingler, J.; Kuhla, J.; Oelze, J. Control of Dinitrogen Fixation in Ammonium-Assimilating Cultures of *Azotobacter vinelandii*. *Arch. Microbiol.* **1987**, *148*, 247−251.
(82) Jacobitz, S.; Bishop, P. E. Regulation of Nitrogenase-2 in *Azotobacter vinelandii* by Ammonium, Molybdenum, and Vanadium. *J. Bacteriol.* **1992**, *174*, 3884−3888.
(83) Postgate, J. R.; Kent, H. M. Derepression of Nitrogen Fixation in *Desulfovibrio gigas* and Its Stability to Ammonia or Oxygen Stress *in vivo*. *Microbiology* **1984**, *130*, 2825−2831.
(84) Ohki, K.; Zehr, J. P.; Falkowski, P. G.; Fujita, Y. Regulation of Nitrogen-Fixation by Different Nitrogen Sources in the Marine Non-Heterocystous Cyanobacterium *Trichodesmium* Sp. NIBB1067. *Arch. Microbiol.* **1991**, *156*, 335−337.
(85) Eady, R. R.; Issack, K. R.; Kennedy, C.; Postgate, J. R.; Ratcliffe, H. D. Nitrogenase Synthesis in *Klebsiella pneumoniae*: Comparison of Ammonium and Oxygen Regulation. *J. Gen. Microbiol.* **1978**, *104*, 277−285.
(86) Daesch, G.; Mortenson, L. E. Effect of Ammonia on the Synthesis and Function of the N_2-Fixing Enzyme System in *Clostridium pasteurianum*. *J. Bacteriol.* **1972**, *110*, 103−109.
(87) Kleiner, D. Quantitative Relations for the Repression of Nitrogenase Synthesis in *Azotobacter vinelandii* by Ammonia. *Arch. Microbiol.* **1974**, *101*, 153−159.
(88) Holl, C. M.; Montoya, J. P. Interactions between Nitrate Uptake and Nitrogen Fixation in Continuous Cultures of the Marine Diazotroph *Trichodesmium* (Cyanobacteria). *J. Phycol.* **2005**, *41*, 1178−1183.
(89) Robson, R. L.; Postgate, J. R. Oxygen and Hydrogen in Biological Nitrogen Fixation. *Annu. Rev. Microbiol.* **1980**, *34*, 183−207.
(90) Stal, L. J.; Krumbein, W. E. Oxygen Protection of Nitrogenase in the Aerobically Nitrogen Fixing, Non-Heterocystous Cyanobacterium *Oscillatoria* sp. *Arch. Microbiol.* **1985**, *143*, 72−76.
(91) Poole, R. K.; Hill, S. Respiratory Protection of Nitrogenase Activity in *Azotobacter vinelandii*—Roles of the Terminal Oxidases. *Biosci. Rep.* **1997**, *17*, 303−317.
(92) Berman-Frank, I.; Lundgren, P.; Chen, Y.-B.; Küpper, H.; Kolber, Z.; Bergman, B.; Falkowski, P. Segregation of Nitrogen Fixation and Oxygenic Photosynthesis in the Marine Cyanobacterium *Trichodesmium*. *Science* **2001**, *294*, 1534−1537.
(93) Schlesier, J.; Rohde, M.; Gerhardt, S.; Einsle, O. A Conformational Switch Triggers Nitrogenase Protection from Oxygen Damage by Shethna Protein II (FeSII). *J. Am. Chem. Soc.* **2016**, *138*, 239−247.
(94) Miller, R. W.; Eady, R. R. Molybdenum and Vanadium Nitrogenases of *Azotobacter chroococcum*. Low Temperature Favours N_2 Reduction by Vanadium Nitrogenase. *Biochem. J.* **1988**, *256*, 429−432.
(95) Kutsche, M.; Leimkühler, S.; Angermüller, S.; Klipp, W. Promoters Controlling Expression of the Alternative Nitrogenase and the Molybdenum Uptake System in *Rhodobacter capsulatus* are Activated by NtrC, Independent of Sigma54, and Repressed by Molybdenum. *J. Bacteriol.* **1996**, *178*, 2010−2017.
(96) Hamilton, T. L.; Ludwig, M.; Dixon, R.; Boyd, E. S.; Dos Santos, P. C.; Setubal, J. C.; Bryant, D. A.; Dean, D. R.; Peters, J. W. Transcriptional Profiling of Nitrogen Fixation in *Azotobacter vinelandii*. *J. Bacteriol.* **2011**, *193*, 4477−4486.
(97) Demtröder, L.; Narberhaus, F.; Masepohl, B. Coordinated Regulation of Nitrogen Fixation and Molybdate Transport by Molybdenum. *Mol. Microbiol.* **2019**, *111*, 17−30.
(98) Zerkle, A. L.; House, C. H.; Cox, R. P.; Canfield, D. E. Metal Limitation of Cyanobacterial N_2 Fixation and Implications for the Precambrian Nitrogen Cycle. *Geobiology* **2006**, *4*, 285−297.
(99) Walmsley, J.; Kennedy, C. Temperature-Dependent Regulation by Molybdenum and Vanadium of Expression of the Structural Genes Encoding 3 Nitrogenases in *Azotobacter vinelandii*. *Appl. Environ. Microbiol.* **1991**, *57*, 622−624.
(100) Lehman, L. J.; Roberts, G. P. Identification of an Alternative Nitrogenase System in *Rhodospirillum rubrum*. *J. Bacteriol.* **1991**, *173*, 5705−5711.
(101) Oda, Y.; Samanta, S. K.; Rey, F. E.; Wu, L.; Liu, X.; Yan, T.; Zhou, J.; Harwood, C. S. Functional Genomic Analysis of Three Nitrogenase Isozymes in the Photosynthetic Bacterium *Rhodopseudomonas palustris*. *J. Bacteriol.* **2005**, *187*, 7784−7794.
(102) Darnajoux, R.; Zhang, X.; McRose, D. L.; Miadlikowska, J.; Lutzoni, F.; Kraepiel, A. M. L.; Bellenger, J.-P. Biological Nitrogen Fixation by Alternative Nitrogenases in Boreal Cyanolichens: Importance of Molybdenum Availability and Implications for Current Biological Nitrogen Fixation Estimates. *New Phytol.* **2017**, *213*, 680−689.
(103) Darnajoux, R.; Magain, N.; Renaudin, M.; Lutzoni, F.; Bellenger, J.-P.; Zhang, X. Molybdenum Threshold for Ecosystem Scale Alternative Vanadium Nitrogenase Activity in Boreal Forests. *Proc. Natl. Acad. Sci. U. S. A.* **2019**, *116*, 24682−24688.
(104) da Silva, J. J. R. F.; Williams, R. J. *The Biological Chemistry of the Elements: The Inorganic Chemistry of Life*; Oxford University Press: Oxford, 2001.
(105) Hu, Y.; Ribbe, M. W. Biosynthesis of the Metalloclusters of Molybdenum Nitrogenase. *Microbiol. Mol. Biol. Rev.* **2011**, *75*, 664−677.
(106) Saito, M. A.; Bertrand, E. M.; Dutkiewicz, S.; Bulygin, V. V.; Moran, D. M.; Monteiro, F. M.; Follows, M. J.; Valois, F. W.; Waterbury, J. B. Iron Conservation by Reduction of Metalloenzyme

Inventories in the Marine Diazotroph *Crocosphaera watsonii*. *Proc. Natl. Acad. Sci. U. S. A.* **2011**, *108* (6), 2184−2189.
(107) Rueter, J. G.; Ohki, K.; Fujita, Y. The Effect of Iron Nutrition on Photosynthesis and Nitrogen Fixation in Cultures of *Trichodesmium* (Cyanophyceae). *J. Phycol.* **1990**, *26*, 30−35.
(108) Paerl, H. W.; Prufert-Bebout, L. E.; Guo, C. Iron-Stimulated N_2 Fixation and Growth in Natural and Cultured Populations of the Planktonic Marine Cyanobacteria *Trichodesmium*. *Appl. Environ. Microbiol.* **1994**, *60*, 1044−1047.
(109) Gaby, J. C.; Buckley, D. H. In *Biological Nitrogen Fixation*; de Bruijn, F. J., Ed.; John John Wiley & Sons, Inc: Hoboken, NJ, 2015.
(110) Gaby, J. C.; Buckley, D. H. *Database* **2014**, *2014*, bau001.
(111) Kumar, K.; Mella-Herrera, R. A.; Golden, J. W. Cyanobacterial Heterocysts. *Cold Spring Harbor Perspect. Biol.* **2010**, *2*, a000315−a000315a.
(112) Mohr, W.; Intermaggio, M.; Laroche, J. Diel Rhythm of Nitrogen and Carbon Metabolism in the Unicellular, Diazotrophic Cyanobacterium *Crocosphaera watsonii* WH8501. *Environ. Microbiol.* **2010**, *12*, 412−421.
(113) Wilson, S. T.; Aylward, F. O.; Ribalet, F.; Barone, B.; Casey, J. R.; Connell, P. E.; Eppley, J. M.; Ferrón, S.; Fitzsimmons, J. N.; Hayes, C. T.; et al. Coordinated Regulation of Growth, Activity and Transcription in Natural Populations of the Unicellular Nitrogen-Fixing Cyanobacterium *Crocosphaera*. *Nature Microb* **2017**, *2*, 17118.
(114) Bergman, B.; Sandh, G.; Lin, S.; Larsson, J.; Carpenter, E. J. *Trichodesmium*-a Widespread Marine Cyanobacterium with Unusual Nitrogen Fixation Properties. *FEMS Microbiol. Rev.* **2013**, *37*, 286−302.
(115) Ohki, K. Intercellular Localization of Nitrogenase in a Non-Heterocystous Cyanobacterium (Cyanophyte), *Trichodesmium* sp. NIBB1067. *J. Oceanogr.* **2008**, *64*, 211−216.
(116) Appleby, C. A. Leghemoglobin and Rhizobium Respiration. *Annu. Rev. Plant Physiol.* **1984**, *35*, 443−478.
(117) Robson, R. L. Characterization of an Oxygen-Stable Nitrogenase Complex Isolated from *Azotobacter chroococcum*. *Biochem. J.* **1979**, *181*, 569−575.
(118) Großkopf, T.; LaRoche, J. Direct and Indirect Costs of Dinitrogen Fixation in *Crocosphaera watsonii* WH8501 and Possible Implications for the Nitrogen Cycle. *Front. Microbiol.* **2012**, *3*, 236.
(119) Inomura, K.; Bragg, J.; Follows, M. J. A Quantitative Analysis of the Direct and Indirect Costs of Nitrogen Fixation: A Model Based on *Azotobacter vinelandii*. *ISME J.* **2017**, *11*, 166−175.
(120) Howarth, R. W.; Marino, R.; Cole, J. J. Nitrogen Fixation in Freshwater, Estuarine, and Marine Ecosystems. 2. Biogeochemical Controls[1]. *Limnol. Oceanogr.* **1988**, *33*, 688−701.
(121) Howarth, R. W.; Cole, J. J. Molybdenum Availability, Nitrogen Limitation, and Phytoplankton Growth in Natural Waters. *Science* **1985**, *229*, 653−655.
(122) Vitousek, P. M.; Howarth, R. W. Nitrogen Limitation on Land and in the Sea: How Can It Occur? *Biogeochemistry* **1991**, *13*, 87−115.
(123) Reed, S. C.; Cleveland, C. C.; Townsend, A. R. Functional Ecology of Free-Living Nitrogen Fixation: A Contemporary Perspective. *Annu. Rev. Ecol. Evol. Sys.* **2011**, *42*, 489−512.
(124) Vitousek, P. M.; Menge, D. N. L.; Reed, S. C.; Cleveland, C. C. Biological Nitrogen Fixation: Rates, Patterns and Ecological Controls in Terrestrial Ecosystems. *Philos. Trans. R. Soc., B* **2013**, *368*, 20130119.
(125) Dynarski, K. A.; Houlton, B. Z. Nutrient Limitation of Terrestrial Free-Living Nitrogen Fixation. *New Phytol.* **2018**, *217*, 1050−1061.
(126) Smercina, D. N.; Evans, S. E.; Friesen, M. L.; Tiemann, L. K. To Fix or Not to Fix: Controls on Free-Living Nitrogen Fixation in the Rhizosphere. *Appl. Environ. Microbiol.* **2019**, *85*, e02546−02518.
(127) Brear, E.; Day, D.; Smith, P. Iron: An Essential Micronutrient for the Legume-Rhizobium Symbiosis. *Front. Plant Sci.* **2013**, *4*, 359.
(128) Vitousek, P. M.; Hobbie, S. Heterotrophic Nitrogen Fixation in Decomposing Litter: Patterns and Regulation. *Ecology* **2000**, *81*, 2366−2376.
(129) Vitousek, P. M.; Cassman, K.; Cleveland, C.; Crews, T.; Field, C. B.; Grimm, N. B.; Howarth, R. W.; Marino, R.; Martinelli, L.; Rastetter, E. B.; et al. Towards an Ecological Understanding of Biological Nitrogen Fixation. *Biogeochemistry* **2002**, *57*, 1−45.
(130) Smith, V. H. Nitrogen, Phosphorus, and Nitrogen Fixation in Lacustrine and Estuarine Ecosystems. *Limnol. Oceanogr.* **1990**, *35*, 1852−1859.
(131) Wurzburger, N.; Bellenger, J. P.; Kraepiel, A. M. L.; Hedin, L. O. Molybdenum and Phosphorus Interact to Constrain Asymbiotic Nitrogen Fixation in Tropical Forests. *PLoS One* **2012**, *7*, No. e33710.
(132) Rousk, K.; Degboe, J.; Michelsen, A.; Bradley, R.; Bellenger, J.-P. Molybdenum and Phosphorus Limitation of Moss-Associated Nitrogen Fixation in Boreal Ecosystems. *New Phytol.* **2017**, *214*, 97−107.
(133) Sanudo-Wilhelmy, S. A.; Kustka, A. B.; Gobler, C. J.; Hutchins, D. A.; Yang, M.; Lwiza, K.; Burns, J.; Capone, D. G.; Raven, J. A.; Carpenter, E. J. Phosphorus Limitation of Nitrogen Fixation by *Trichodesmium* in the Central Atlantic Ocean. *Nature* **2001**, *411*, 66−69.
(134) Mills, M. M.; Ridame, C.; Davey, M.; La Roche, J.; Geider, R. J. Iron and Phosphorus Co-Limit Nitrogen Fixation in the Eastern Tropical North Atlantic. *Nature* **2004**, *429*, 292−294.
(135) Sohm, J. A.; Mahaffey, C.; Capone, D. G. Assessment of Relative Phosphorus Limitation of *Trichodesmium* spp. in the North Pacific, North Atlantic, and the North Coast of Australia. *Limnol. Oceanogr.* **2008**, *53*, 2495−2502.
(136) Moutin, T. B.; Van Den Broeck, N.; Beker, B.; Dupouy, C.; Rimmelin, P.; Le Bouteiller, A. Phosphate Availability Controls *Trichodesmium* spp. Biomass in the SW Pacific Ocean. *Mar. Ecol.: Prog. Ser.* **2005**, *297*, 15−21.
(137) Chapin, D. M.; Bliss, L. C.; Bledsoe, L. J. Environmental Regulation of Nitrogen Fixation in a High Arctic Lowland Ecosystem. *Can. J. Bot.* **1991**, *69*, 2744−2755.
(138) Rousk, K.; Pedersen, P. A.; Dyrnum, K.; Michelsen, A. The Interactive Effects of Temperature and Moisture on Nitrogen Fixation in Two Temperate-Arctic Mosses. *Theor. Exp. Plant Physiol.* **2017**, *29*, 25−36.
(139) Tyrrell, T. The Relative Influences of Nitrogen and Phosphorus on Oceanic Primary Production. *Nature* **1999**, *400*, 525−531.
(140) Menge, D. N. L.; Lichstein, J. W.; Ángeles-Pérez, G. Nitrogen Fixation Strategies Can Explain the Latitudinal Shift in Nitrogen-Fixing Tree Abundance. *Ecology* **2014**, *95*, 2236−2245.
(141) Reynolds, L. K.; Marino, R.; Muth, M. F.; McLenaghan, N.; Hayn, M.; Tyler, A. C.; McGlathery, K. J.; Howarth, R. W. Evidence of Grazer Control on Nitrogen Fixation by Eelgrass Epiphytes in a Temperate Coastal Bay. *Mar. Ecol.: Prog. Ser.* **2015**, *526*, 11−19.
(142) Marino, R.; Chan, F.; Howarth, R.; Pace, M.; Likens, G. E. Ecological Constraints on Planktonic Nitrogen Fixation in Saline Estuaries. I. Nutrient and Trophic Controls. *Mar. Ecol.: Prog. Ser.* **2006**, *309*, 25−39.
(143) Cleveland, C. C.; Houlton, B. Z.; Smith, W. K.; Marklein, A. R.; Reed, S. C.; Parton, W.; Del Grosso, S. J.; Running, S. W. Patterns of New Versus Recycled Primary Production in the Terrestrial Biosphere. *Proc. Natl. Acad. Sci. U. S. A.* **2013**, *110*, 12733−12737.
(144) Cleveland, C. C.; Townsend, A. R.; Schimel, D. S.; Fisher, H.; Howarth, R. W.; Hedin, L. O.; Perakis, S. S.; Latty, E. F.; Von Fischer, J. C.; Elseroad, A.; et al. Global Patterns of Terrestrial Biological Nitrogen N_2 Fixation in Natural Ecosystems. *Global Biogeochem. Cycles* **1999**, *13*, 623−645.
(145) Ingestad, T. Growth, Nutrition, and Nitrogen Fixation in Grey Alder at Varied Rate of Nitrogen Addition. *Physiol. Plant.* **1980**, *50*, 353−364.
(146) Fujikake, H.; Yashima, H.; Sato, T.; Ohtake, N.; Sueyoshi, K.; Ohyama, T. Rapid and Reversible Nitrate Inhibition of Nodule Growth and N_2 Fixation Activity in Soybean (Glycine Max (L.) Merr.). *Soil Sci. Plant Nutr.* **2002**, *48*, 211−217.
(147) Barron, A. R.; Purves, D. W.; Hedin, L. O. Facultative Nitrogen Fixation by Canopy Legumes in a Lowland Tropical Forest. *Oecologia* **2011**, *165*, 511−520.

https://dx.doi.org/10.1021/acs.chemrev.9b00613
Chem. Rev. 2020, 120, 5308−5351

(148) Hedin, L. O.; Brookshire, E. N. J.; Menge, D. N. L.; Barron, A. R. The Nitrogen Paradox in Tropical Forest Ecosystems. *Annu. Rev. Ecol. Evol. Sys.* **2009**, *40*, 613−635.
(149) Bormann, B. T.; Gordon, J. C. Stand Density Effects in Young Red Alder Plantations: Productivity, Photosynthate Partitioning, and Nitrogen Fixation. *Ecology* **1984**, *65*, 394−402.
(150) Gehring, C.; Vlek, P. L. G.; de Souza, L. A. G.; Denich, M. Biological Nitrogen Fixation in Secondary Regrowth and Mature Rainforest of Central Amazonia. *Agric., Ecosyst. Environ.* **2005**, *111*, 237−252.
(151) Batterman, S. A.; Hedin, L. O.; van Breugel, M.; Ransijn, J.; Craven, D. J.; Hall, J. S. Key Role of Symbiotic Dinitrogen Fixation in Tropical Forest Secondary Succession. *Nature* **2013**, *502*, 224−227.
(152) Menge, D. N. L.; Hedin, L. O. Nitrogen Fixation in Different Biogeochemical Niches Along a 120 000-Year Chronosequence in New Zealand. *Ecology* **2009**, *90*, 2190−2201.
(153) Taylor, B. N.; Menge, D. N. L. Light Regulates Tropical Symbiotic Nitrogen Fixation More Strongly Than Soil Nitrogen. *Nature Plants* **2018**, *4*, 655−661.
(154) Steenhoudt, O.; Vanderleyden, J. *Azospirillum*, a Free-Living Nitrogen-Fixing Bacterium Closely Associated with Grasses: Genetic, Biochemical and Ecological Aspects. *FEMS Microbiol. Rev.* **2000**, *24*, 487−506.
(155) Perez, C. A.; Carmona, M. R.; Armesto, J. J. Non-Symbiotic Nitrogen Fixation During Leaf Litter Decomposition in an Old-Growth Temperate Rain Forest of Chiloé Island, Southern Chile: Effects of Single Versus Mixed Species Litter. *Austral Ecology* **2010**, *35*, 148−156.
(156) Zheng, M.; Zhang, W.; Luo, Y.; Li, D.; Wang, S.; Huang, J.; Lu, X.; Mo, J. Stoichiometry Controls Asymbiotic Nitrogen Fixation and Its Response to Nitrogen Inputs in a Nitrogen-Saturated Forest. *Ecology* **2018**, *99*, 2037−2046.
(157) Bellenger, J. P.; Wichard, T.; Kustka, A. B.; Kraepiel, A. M. L. Uptake of Molybdenum and Vanadium by a Nitrogen-Fixing Soil Bacterium Using Siderophores. *Nat. Geosci.* **2008**, *1*, 243−246.
(158) Capone, D. G.; Burns, J. A.; Montoya, J. P.; Subramaniam, A.; Mahaffey, C.; Gunderson, T.; Michaels, A. F.; Carpenter, E. J. Nitrogen Fixation by *Trichodesmium* spp.: An Important Source of New Nitrogen to the Tropical and Subtropical North Atlantic Ocean. *Global Biogeochem. Cycles* **2005**, *19*, GB2024.
(159) Carpenter, E. J.; Harvey, H. R.; Fry, B.; Capone, D. G. Biogeochemical Tracers of the Marine Cyanobacterium *Trichodesmium*. *Deep Sea Res., Part I* **1997**, *44*, 27−38.
(160) Foster, R. A.; Kuypers, M. M. M.; Vagner, T.; Paerl, R. W.; Musat, N.; Zehr, J. P. Nitrogen Fixation and Transfer in Open Ocean Diatom-Cyanobacterial Symbioses. *ISME J.* **2011**, *5*, 1484−1493.
(161) Zehr, J. P.; Shilova, I. N.; Farnelid, H. M.; Muñoz-Marín, M. d. C.; Turk-Kubo, K. A. Unusual Marine Unicellular Symbiosis with the Nitrogen-Fixing Cyanobacterium UCYN-A. *Nature Microb* **2017**, *2*, 16214.
(162) Sohm, J. A.; Webb, E. A.; Capone, D. G. Emerging Patterns of Marine Nitrogen Fixation. *Nat. Rev. Microbiol.* **2011**, *9*, 499−508.
(163) Karl, D.; Letelier, R.; Tupas, L.; Dore, J.; Christian, J.; Hebel, D. The Role of Nitrogen Fixation in Biogeochemical Cycling in the Subtropical North Pacific Ocean. *Nature* **1997**, *388*, 533−538.
(164) Knapp, A. N.; Sigman, D. M.; Lipschultz, F. N. Isotopic Composition of Dissolved Organic Nitrogen and Nitrate at the Bermuda Atlantic Time-Series Study Site. *Global Biogeochem. Cycles* **2005**, *19*, GB1018.
(165) Wu, J.; Sunda, W.; Boyle, E. A.; Karl, D. M. Phosphate Depletion in the Western North Atlantic Ocean. *Science* **2000**, *289*, 759−762.
(166) Sohm, J. A.; Hilton, J. A.; Noble, A. E.; Zehr, J. P.; Saito, M. A.; Webb, E. A. Nitrogen Fixation in the South Atlantic Gyre and the Benguela Upwelling System. *Geophys. Res. Lett.* **2011**, *38*, L16608.
(167) Webb, E. A.; Moffett, J. W.; Waterbury, J. B. Iron Stress in Open-Ocean Cyanobacteria *Synechococcus, Trichodesmium*, and *Crocosphaera*: Identification of the IdiA Protein. *Appl. Environ. Microbiol.* **2001**, *67*, 5444−5452.
(168) Boatman, T. G.; Oxborough, K.; Gledhill, M.; Lawson, T.; Geider, R. J. An Integrated Response of *Trichodesmium erythraeum* IMS101 Growth and Photo-Physiology to Iron, CO_2, and Light Intensity. *Front. Microbiol.* **2018**, *9*, 624.
(169) Stenegren, M.; Caputo, A.; Berg, C.; Bonnet, S.; Foster, R. A. Distribution and Drivers of Symbiotic and Free-Living Diazotrophic Cyanobacteria in the Western Tropical South Pacific. *Biogeosciences* **2018**, *15*, 1559−1578.
(170) Subramaniam, A.; Yager, P. L.; Carpenter, E. J.; Mahaffey, C.; Björkman, K.; Cooley, S.; Kustka, A. B.; Montoya, J. P.; Sañudo-Wilhelmy, S. A.; Shipe, R.; et al. Amazon River Enhances Diazotrophy and Carbon Sequestration in the Tropical North Atlantic Ocean. *Proc. Natl. Acad. Sci. U. S. A.* **2008**, *105*, 10460−10465.
(171) Zeev, E. B.; Yogev, T.; Man-Aharonovich, D.; Kress, N.; Herut, B.; Béjà, O.; Berman-Frank, I. Seasonal Dynamics of the Endosymbiotic, Nitrogen-Fixing Cyanobacterium *Richelia intracellularis* in the Eastern Mediterranean Sea. *ISME J.* **2008**, *2*, 911−923.
(172) Montoya, J. P.; Holl, C. M.; Zehr, J. P.; Hansen, A.; Villareal, T. A.; Capone, D. G. High Rates of N_2 Fixation by Unicellular Diazotrophs in the Oligotrophic Pacific Ocean. *Nature* **2004**, *430*, 1027−1031.
(173) Harding, K.; Turk-Kubo, K. A.; Sipler, R. E.; Mills, M. M.; Bronk, D. A.; Zehr, J. P. Symbiotic Unicellular Cyanobacteria Fix Nitrogen in the Arctic Ocean. *Proc. Natl. Acad. Sci. U. S. A.* **2018**, *115*, 13371−13375.
(174) Zehr, J. P.; Bench, S. R.; Carter, B. J.; Hewson, I.; Niazi, F.; Shi, T.; Tripp, H. J.; Affourtit, J. P. Globally Distributed Uncultivated Oceanic N_2 Fixing Cyanobacteria Lack Oxygenic Photosystem II. *Science* **2008**, *322*, 1110−1112.
(175) Turk-Kubo, K.; Achilles, K.; Serros, T.; Ochiai, M.; Montoya, J.; Zehr, J. Nitrogenase (nifH) Gene Expression in Diazotrophic Cyanobacteria in the Tropical North Atlantic in Response to Nutrient Amendments. *Front. Microbiol.* **2012**, *3*, 386 DOI: 10.3389/fmicb.2012.00386.
(176) Krupke, A.; Mohr, W.; LaRoche, J.; Fuchs, B. M.; Amann, R. I.; Kuypers, M. M. M. The Effect of Nutrients on Carbon and Nitrogen Fixation by the UCYN-A-Haptophyte Symbiosis. *ISME J.* **2015**, *9*, 1635−1647.
(177) Dyhrman, S. T.; Haley, S. T. Phosphorus Scavenging in the Unicellular Marine Diazotroph *Crocosphaera watsonii*. *Appl. Environ. Microbiol.* **2006**, *72*, 1452−1458.
(178) Pereira, N.; Shilova, I. N.; Zehr, J. P. Use of the High-Affinity Phosphate Transporter Gene, *pstS*, as an Indicator for Phosphorus Stress in the Marine Diazotroph *Crocosphaera watsonii* (Chroococcales, Cyanobacteria). *J. Phycol.* **2019**, *55*, 752−761.
(179) Benavides, M.; Bonnet, S.; Berman-Frank, I.; Riemann, L. Deep into Oceanic N_2 Fixation. *Front. Mar. Sci.* **2018**, *5*, 108.
(180) Bombar, D.; Paerl, R. W.; Riemann, L. Marine Non-Cyanobacterial Diazotrophs: Moving Beyond Molecular Detection. *Trends Microbiol.* **2016**, *24*, 916−927.
(181) Moisander, P. H.; Benavides, M.; Bonnet, S.; Berman-Frank, I.; White, A. E.; Riemann, L. Chasing after Non-Cyanobacterial Nitrogen Fixation in Marine Pelagic Environments. *Front. Microbiol.* **2017**, *8*, 1736.
(182) Bentzon-Tilia, M.; Severin, I.; Hansen, L. H.; Riemann, L. Genomics and Ecophysiology of Heterotrophic Nitrogen-Fixing Bacteria Isolated from Estuarine Surface Water. *mBio* **2015**, *6*, 1−15.
(183) Bentzon-Tilia, M.; Traving, S. J.; Mantikci, M.; Knudsen-Leerbeck, H.; Hansen, J. L. S.; Markager, S.; Riemann, L. Significant N_2 Fixation by Heterotrophs, Photoheterotrophs and Heterocystous Cyanobacteria in Two Temperate Estuaries. *ISME J.* **2015**, *9*, 273−285.
(184) Gier, J.; Loscher, C. R.; Dale, A. W.; Sommer, S.; Lomnitz, U.; Treude, T. Benthic Dinitrogen Fixation Traversing the Oxygen Minimum Zone Off Mauritania (NW Africa). *Front. Mar. Sci.* **2017**, *4*, 390.
(185) Bonnet, S.; Dekaezemacker, J.; Turk-Kubo, K. A.; Moutin, T.; Hamersley, R. M.; Grosso, O.; Zehr, J. P.; Capone, D. G. Aphotic N_2 Fixation in the Eastern Tropical South Pacific Ocean. *PLoS One* **2013**, *8*, No. e81265.

https://dx.doi.org/10.1021/acs.chemrev.9b00613
Chem. Rev. 2020, 120, 5308−5351

(186) Hamersley, M. R.; Turk, K. A.; Leinweber, A.; Gruber, N.; Zehr, J. P.; Gunderson, T.; Capone, D. G. Nitrogen Fixation within the Water Column Associated with Two Hypoxic Basins in the Southern California Bight. *Aquat. Microb. Ecol.* **2011**, *63*, 193−205.
(187) Jayakumar, A.; Al-Rshaidat, M. M. D.; Ward, B. B.; Mulholland, M. R. Diversity, Distribution, and Expression of Diazotroph nifH Genes in Oxygen-Deficient Waters of the Arabian Sea. *FEMS Microbiol. Ecol.* **2012**, *82*, 597−606.
(188) Newell, S. E.; McCarthy, M. J.; Gardner, W. S.; Fulweiler, R. W. Sediment Nitrogen Fixation: A Call for Re-Evaluating Coastal N Budgets. *Estuaries Coasts* **2016**, *39*, 1626−1638.
(189) Delmont, T. O.; Quince, C.; Shaiber, A.; Esen, Ö. C.; Lee, S. T. M.; Rappé, M. S.; McLellan, S. L.; Lücker, S.; Eren, A. M. Nitrogen-Fixing Populations of Planctomycetes and Proteobacteria Are Abundant in Surface Ocean Metagenomes. *Nature Microb* **2018**, *3*, 804−813.
(190) Pedersen, J. N.; Bombar, D.; Paerl, R. W.; Riemann, L. Diazotrophs and N_2-Fixation Associated with Particles in Coastal Estuarine Waters. *Front. Microbiol.* **2018**, *9*, 2759.
(191) Farnelid, H.; Turk-Kubo, K.; Ploug, H.; Ossolinski, J. E.; Collins, J. R.; Van Mooy, B. A. S.; Zehr, J. P. Diverse Diazotrophs Are Present on Sinking Particles in the North Pacific Subtropical Gyre. *ISME J.* **2019**, *13*, 170−182.
(192) Schumann, U.; Huntrieser, H. The Global Lightning-Induced Nitrogen Oxides Source. *Atmos. Chem. Phys.* **2007**, *7*, 3823−3907.
(193) Zel'dovich, Y. B. The Oxidation of Nitrogen in Combustion Explosions. *Acta Physicochimica U.S.S.R.* **1946**, *21*, 577−628.
(194) Mather, T. A.; Pyle, D. M.; Allen, A. G. Volcanic Source for Fixed Nitrogen in the Early Earth's Atmosphere. *Geology* **2004**, *32*, 905−908.
(195) Sano, Y.; Takahata, N.; Nishio, Y.; Fischer, T. P.; Williams, S. N. Volcanic Flux of Nitrogen from the Earth. *Chem. Geol.* **2001**, *171*, 263−271.
(196) Uematsu, M.; Toratani, M.; Kajino, M.; Narita, Y.; Senga, Y.; Kimoto, T. Enhancement of Primary Productivity in the Western North Pacific Caused by the Eruption of the Miyake-Jima Volcano. *Geophys. Res. Lett.* **2004**, *31*, L06106.
(197) Brandes, J. A.; Boctor, N. Z.; Cody, G. D.; Cooper, B. A.; Hazen, R. M.; Yoder, H. S. Abiotic Nitrogen Reduction on the Early Earth. *Nature* **1998**, *395*, 365−367.
(198) Flynn, P. F.; Hunter, G. L.; Farrel, L.; Durrett, R. P.; Akinyemi, O.; Zur Loye, A. O.; Westbrook, C. K.; Pitz, W. J. The Inevitability of Engine-out NOx Emissions from Spark-Ignited and Diesel Engines. *Proc. Combust. Inst.* **2000**, *28*, 1211−1218.
(199) Fenimore, C. P. Formation of Nitric Oxide in Premixed Hydrocarbon Flames. *Symp. Combust., [Proc.]* **1971**, *13*, 373−380.
(200) Taylor, K. C. Nitric Oxide Catalysis in Automotive Exhaust Systems. *Catal. Rev.: Sci. Eng.* **1993**, *35*, 457−481.
(201) de Gouw, J. A.; Parrish, D. D.; Frost, G. J.; Trainer, M. Reduced Emissions of CO_2, NOx, and SO_2 from U.S. Power Plants Owing to Switch from Coal to Natural Gas with Combined Cycle Technology. *Earth's Future* **2014**, *2*, 75−82.
(202) Hoesly, R. M.; Smith, S. J.; Feng, L.; Klimont, Z.; Janssens-Maenhout, G.; Pitkanen, T.; Seibert, J. J.; Vu, L.; Andres, R. J.; Bolt, R. M.; et al. Historical (1750−2014) Anthropogenic Emissions of Reactive Gases and Aerosols from the Community Emissions Data System (CEDS). *Geosci. Model Dev.* **2018**, *11*, 369−408.
(203) Zheng, B.; Tong, D.; Li, M.; Liu, F.; Hong, C.; Geng, G.; Li, H.; Li, X.; Peng, L.; Qi, J.; et al. Trends in China's Anthropogenic Emissions since 2010 as the Consequence of Clean Air Actions. *Atmos. Chem. Phys.* **2018**, *18*, 14095−14111.
(204) Xu, G.; Fan, X.; Miller, A. J. Plant Nitrogen Assimilation and Use Efficiency. *Annu. Rev. Plant Biol.* **2012**, *63*, 153−182.
(205) Moreno-Vivián, C.; Flores, E. In *Biology of the Nitrogen Cycle*; Bothe, H.; Ferguson, S. J.; Newton, W. E., Eds.; Elsevier: Amsterdam, 2007.
(206) Fellbaum, C. R.; Gachomo, E. W.; Beesetty, Y.; Choudhari, S.; Strahan, G. D.; Pfeffer, P. E.; Kiers, E. T.; Bücking, H. Carbon Availability Triggers Fungal Nitrogen Uptake and Transport in Arbuscular Mycorrhizal Symbiosis. *Proc. Natl. Acad. Sci. U. S. A.* **2012**, *109*, 2666−2671.
(207) Glibert, P. M.; Wilkerson, F. P.; Dugdale, R. C.; Raven, J. A.; Dupont, C. L.; Leavitt, P. R.; Parker, A. E.; Burkholder, J. M.; Kana, T. M. Pluses and Minuses of Ammonium and Nitrate Uptake and Assimilation by Phytoplankton and Implications for Productivity and Community Composition, with Emphasis on Nitrogen-Enriched Conditions. *Limnol. Oceanogr.* **2016**, *61*, 165−197.
(208) Leigh, J. A.; Dodsworth, J. A. Nitrogen Regulation in Bacteria and Archaea. *Annu. Rev. Microbiol.* **2007**, *61*, 349−377.
(209) Schlesinger, W. H.; Bernhardt, E. S. *Biogeochemistry*, 3rd ed.; Academic Press: Boston, 2013.
(210) Wang, L.; Macko, S. A. Constrained Preferences in Nitrogen Uptake across Plant Species and Environments. *Plant, Cell Environ.* **2011**, *34*, 525−534.
(211) Xu, L.; Cheng, S.; Fang, H.; Xin, X.; Xu, X.; Tang, H. Soil Inorganic Nitrogen Composition and Plant Functional Type Determine Forage Crops Nitrogen Uptake Preference in the Temperate Cultivated Grassland, Inner Mongolia. *Soil Sci. Plant Nutr.* **2019**, *65*, 501−510.
(212) Näsholm, T.; Kielland, K.; Ganeteg, U. Uptake of Organic Nitrogen by Plants. *New Phytol.* **2009**, *182*, 31−48.
(213) Mulholland, M. R.; Lomas, M. W. In *Nitrogen in the Marine Environment*, 2nd ed.; Capone, D. G.; Bronk, D. A.; Mulholland, M. R.; Carpenter, E. J., Eds.; Academic Press: San Diego, 2008.
(214) Stein, L. Y. Insights into the Physiology of Ammonia-Oxidizing Microorganisms. *Curr. Opin. Chem. Biol.* **2019**, *49*, 9−15.
(215) van Kessel, M. A. H. J.; Speth, D. R.; Albertsen, M.; Nielsen, P. H.; Op den Camp, H. J. M.; Kartal, B.; Jetten, M. S. M.; Lücker, S. Complete Nitrification by a Single Microorganism. *Nature* **2015**, *528*, 555−559.
(216) Daims, H.; Lebedeva, E. V.; Pjevac, P.; Han, P.; Herbold, C.; Albertsen, M.; Jehmlich, N.; Palatinszky, M.; Vierheilig, J.; et al. Complete Nitrification by *Nitrospira* Bacteria. *Nature* **2015**, *528*, 504−509.
(217) Prosser, J. I. In *Encyclopedia of Soils in the Environment*; Hillel, D., Ed.; Elsevier: Oxford, 2005.
(218) Zhang, J.; Müller, C.; Zhu, T.; Cheng, Y.; Cai, Z. Heterotrophic Nitrification Is the Predominant No3−Production Mechanism in Coniferous but Not Broad-Leaf Acid Forest Soil in Subtropical China. *Biol. Fertil. Soils* **2011**, *47*, 533−542.
(219) Prosser, J. I. In *Biology of the Nitrogen Cycle*; Bothe, H.; Ferguson, S. J.; Newton, W. E., Eds.; Elsevier: Amsterdam, 2007.
(220) Wood, P. M. Autotrophic and Heterotrophic Mechanisms for Ammonia Oxidation. *Soil Use Manage.* **1990**, *6*, 78−79.
(221) Kuypers, M. M. M.; Marchant, H. K.; Kartal, B. The Microbial Nitrogen-Cycling Network. *Nat. Rev. Microbiol.* **2018**, *5*, 263−267.
(222) Kamp, A.; Høgslund, S.; Risgaard-Petersen, N.; Stief, P. Nitrate Storage and Dissimilatory Nitrate Reduction by Eukaryotic Microbes. *Front. Microbiol.* **2015**, *6*, 1492−1492a.
(223) Silver, W. L.; Herman, D. J.; Firestone, M. K. Dissimilatory Nitrate Reduction to Ammonium in Upland Tropical Forest Soils. *Ecology* **2001**, *82*, 2410−2416.
(224) King, D.; Nedwell, D. B. The Influence of Nitrate Concentration Upon the End-Products of Nitrate Dissimilation by Bacteria in Anaerobic Salt Marsh Sediment. *FEMS Microbiol. Lett.* **1985**, *31*, 23−28.
(225) Kelso, B.; Smith, R. V.; Laughlin, R. J.; Lennox, S. D. Dissimilatory Nitrate Reduction in Anaerobic Sediments Leading to River Nitrite Accumulation. *Appl. Environ. Microb.* **1997**, *63*, 4679−4685.
(226) Yang, W. H.; Traut, B. H.; Silver, W. L. Microbially Mediated Nitrogen Retention and Loss in a Salt Marsh Soil. *Ecosphere* **2015**, *6*, 1−15.
(227) Nevison, C.; Hess, P.; Riddick, S.; Ward, D. Denitrification, Leaching, and River Nitrogen Export in the Community Earth System Model. *J. Adv. Model. Earth Syst.* **2016**, *8*, 272−291.
(228) Robertson, L. A.; Kuenen, J. G. Aerobic Denitrification: A Controversy Revived. *Arch. Microbiol.* **1984**, *139*, 351−354.

https://dx.doi.org/10.1021/acs.chemrev.9b00613
Chem. Rev. 2020, 120, 5308−5351

(229) Ji, B.; Yang, K.; Zhu, L.; Jiang, Y.; Wang, H.; Zhou, J.; Zhang, H. Aerobic Denitrification: A Review of Important Advances of the Last 30 Years. *Biotechnol. Bioprocess Eng.* **2015**, *20*, 643−651.
(230) Zumft, W. G. Cell Biology and Molecular Basis of Denitrification. *Microbiol. Mol. Biol. Rev.* **1997**, *61*, 533−616.
(231) Stolz, J. F.; Basu, P. Evolution of Nitrate Reductase: Molecular and Structural Variations on a Common Function. *ChemBioChem* **2002**, *3*, 198−206.
(232) MacPherson, I. S.; Murphy, M. E. P. Type-2 Copper-Containing Enzymes. *Cell. Mol. Life Sci.* **2007**, *64*, 2887−2899.
(233) Ducluzeau, A.-L.; Schoepp-Cothenet, B.; van Lis, R.; Baymann, F.; Russell, M. J.; Nitschke, W. The Evolution of Respiratory O_2/NO Reductases: An out-of-the-Phylogenetic-Box Perspective. *J. R. Soc., Interface* **2014**, *11*, 20140196−20140196a.
(234) Stein, L. Y. In *Methods in Enzymology: Research on Nitrification and Related Processes*, 2011; Vol. *486*.
(235) Zumft, W. G.; Kroneck, P. M. H. In *Advances in Microbial Physiology*; Poole, R. K., Ed., 2007; Vol. *52*.
(236) Jones, C. M.; Graf, D. R. H.; Bru, D.; Philippot, L.; Hallin, S. The Unaccounted yet Abundant Nitrous Oxide-Reducing Microbial Community: A Potential Nitrous Oxide Sink. *ISME J.* **2013**, *7*, 417−426.
(237) Sanford, R. A.; Wagner, D. D.; Wu, Q.; Chee-Sanford, J. C.; Thomas, S. H.; Cruz-Garcia, C.; Rodriguez, G.; Massol-Deya, A.; Krishnani, K. K.; et al. Unexpected Nondenitrifier Nitrous Oxide Reductase Gene Diversity and Abundance in Soils. *Proc. Natl. Acad. Sci. U. S. A.* **2012**, *109*, 19709−19714.
(238) Sun, X.; Jayakumar, A.; Ward, B. B. Community Composition of Nitrous Oxide Consuming Bacteria in the Oxygen Minimum Zone of the Eastern Tropical South Pacific. *Front. Microbiol.* **2017**, *8*, 1−11.
(239) Graf, D. R. H.; Jones, C. M.; Hallin, S. Intergenomic Comparisons Highlight Modularity of the Denitrification Pathway and Underpin the Importance of Community Structure for N_2o Emissions. *PLoS One* **2014**, *9*, 1−20.
(240) Milazzo, G.; Caroli, S. *Tables of Standard Electrode Potentials*; Wiley & Sons: New York, 1978.
(241) Yamagishi, H.; Westley, M. B.; Popp, B. N.; Toyoda, S.; Yoshida, N.; Watanabe, S.; Koba, K.; Yamanaka, Y. Role of Nitrification and Denitrification on the Nitrous Oxide Cycle in the Eastern Tropical North Pacific and Gulf of California. *J. Geophys. Res.* **2007**, *112*, 1−15.
(242) Naqvi, S. W. A.; Noronha, R. J. Nitrous Oxide in the Arabian Sea. *Deep-Sea Res., Part A* **1991**, *38*, 871−890.
(243) Ward, B. B.; Tuit, C. B.; Jayakumar, A.; Rich, J. J.; Moffett, J.; Naqvi, S. W. A. Organic Carbon, and Not Copper, Controls Denitrification in Oxygen Minimum Zones of the Ocean. *Deep Sea Res., Part I* **2008**, *55*, 1672−1683.
(244) Babbin, A. R.; Ward, B. B. Controls on Nitrogen Loss Processes in Chesapeake Bay Sediments. *Environ. Sci. Technol.* **2013**, *47*, 4189−4196.
(245) Lam, P.; Jensen, M. M.; Kock, A.; Lettmann, K. A.; Plancherel, Y.; Lavik, G.; Bange, H. W.; Kuypers, M. M. M. Origin and Fate of the Secondary Nitrite Maximum in the Arabian Sea. *Biogeosciences* **2011**, *8*, 1565−1577.
(246) Bowen, J. L.; Weisman, D.; Yasuda, M.; Jayakumar, A.; Morrison, H. G.; Ward, B. B. Marine Oxygen-Deficient Zones Harbor Depauperate Denitrifying Communities Compared to Novel Genetic Diversity in Coastal Sediments. *Microb. Ecol.* **2015**, *70*, 311−321.
(247) Fuhrman, J. A.; McCallum, K.; Davis, A. A. Phylogenetic Diversity of Subsurface Marine Microbial Communities from the Atlantic and Pacific Oceans. *Appl. Environ. Microbiol.* **1993**, *59*, 1294−1302.
(248) Bertagnolli, A. D.; Ulloa, O. Hydrography Shapes Community Composition and Diversity of AmoA-Containing Thaumarchaeota in the Coastal Waters Off Central Chile. *Environ. Microbiol. Rep.* **2017**, *9*, 717−728.
(249) Walsh, D. A.; Zaikova, E.; Howes, C. G.; Song, Y. C.; Wright, J. J.; Tringe, S. G.; Tortell, P. D.; Hallam, S. J. Metagenome of a Versatile Chemolithoautotroph from Expanding Oceanic Dead Zones. *Science* **2009**, *326*, 578−582.
(250) Callbeck, C. M.; Lavik, G.; Ferdelman, T. G.; Fuchs, B.; Gruber-Vodicka, H. R.; Hach, P. F.; Littmann, S.; Schoffelen, N. J.; Kalvelage, T.; Thomsen, S.; et al. Oxygen Minimum Zone Cryptic Sulfur Cycling Sustained by Offshore Transport of Key Sulfur Oxidizing Bacteria. *Nat. Commun.* **2018**, *9* (1729), 1−11.
(251) Schunck, H.; Lavik, G.; Desai, D. K.; Grosskopf, T.; Kalvelage, T.; Loscher, C. R.; Paulmier, A.; Contreras, S.; Siegel, H.; et al. Giant Hydrogen Sulfide Plume in the Oxygen Minimum Zone Off Peru Supports Chemolithoautotrophy. *PLoS One* **2013**, *8*, No. e68661.
(252) Galan, A.; Faundez, J.; Thamdrup, B.; Santibanez, J. F.; Farias, L. Temporal Dynamics of Nitrogen Loss in the Coastal Upwelling Ecosystem Off Central Chile: Evidence of Autotrophic Denitrification through Sulfide Oxidation. *Limnol. Oceanogr.* **2014**, *59*, 1865−1878.
(253) Carolan, M. T.; Smith, J. M.; Beman, J. M. Transcriptomic Evidence for Microbial Sulfur Cycling in the Eastern Tropical North Pacific Oxygen Minimum Zone. *Front. Microbiol.* **2015**, *6* (334), 1−8.
(254) Stewart, F. J.; Ulloa, O.; DeLong, E. F. Microbial Metatranscriptomics in a Permanent Marine Oxygen Minimum Zone. *Environ. Microbiol.* **2012**, *14*, 23−40.
(255) Johnston, D. T.; Gill, B. C.; Masterson, A.; Beirne, E.; Casciotti, K. L.; Knapp, A. N.; Berelson, W. Placing an Upper Limit on Cryptic Marine Sulphur Cycling. *Nature* **2014**, *513*, 530−533.
(256) Ganesh, S.; Parris, D. J.; De Long, E. F.; Stewart, F. J. Metagenomic Analysis of Size-Fractionated Picoplankton in a Marine Oxygen Minimum Zone. *ISME J.* **2014**, *8*, 187−211.
(257) Tsementzi, D.; Wu, J. Y.; Deutsch, S.; Nath, S.; Rodriguez, L. M.; Burns, A. S.; Ranjan, P.; Sarode, N.; Malmstrom, R. R.; et al. SAR11 Bacteria Linked to Ocean Anoxia and Nitrogen Loss. *Nature* **2016**, *536*, 179−183.
(258) Lipschultz, F.; Wofsy, S. C.; Ward, B. B.; Codispoti, L. A.; Friedrich, G. J. W.; Elkins, J. W. Bacterial Transformations of Inorganic Nitrogen in the Oxygen-Deficient Waters of the Eastern Tropical South Pacific Ocean. *Deep-Sea Res., Part A* **1990**, *37*, 1513−1541.
(259) Babbin, A. R.; Peters, B. D.; Mordy, C. W.; Widner, B.; Casciotti, K. L.; Ward, B. B. Multiple Metabolisms Constrain the Anaerobic Nitrite Budget in the Eastern Tropical South Pacific. *Global Biogeochem. Cycles* **2017**, *31*, 258−271.
(260) Babbin, A. R.; Keil, R. G.; Devol, A. H.; Ward, B. B. Organic Matter Stoichiometry, Flux, and Oxygen Control Nitrogen Loss in the Ocean. *Science* **2014**, *344*, 406−408.
(261) Peng, X.; Fuchsman, C. A.; Jayakumar, A.; Oleynik, S.; Martens-Habbena, W.; Devol, A. H.; Ward, B. B. Ammonia and Nitrite Oxidation in the Eastern Tropical North Pacific. *Global Biogeochem. Cycles* **2015**, *29*, 2034−2049.
(262) Sun, X.; Kop, L. F. M.; Lau, M. C. Y.; Frank, J.; Jayakumar, A.; Lücker, S.; Ward, B. B. Uncultured *Nitrospina*-Like Species Are Major Nitrite Oxidizing Bacteria in Oygen Minimum Zones. *ISME J.* **2019**, *13*, 2391−2402.
(263) Kemeny, P. C.; Weigand, M. A.; Zhang, R.; Carter, B. R.; Karsh, K. L.; Fawcett, S. E.; Sigman, D. M. Enzyme-Level Interconversion of Nitrate and Nitrite in the Fall Mixed Layer of the Antarctic Ocean. *Global Biogeochem. Cycles* **2016**, *30*, 1069−1085.
(264) Risgaard-Petersen, N.; Langezaal, A. M.; Ingvardsen, S.; Schmid, M. C.; Jetten, M. S. M.; Op Den Camp, H. J. M.; Derksen, J. W. M.; Pina-Ochoa, E.; Eriksson, S. P.; Nielsen, L. P.; et al. Evidence for Complete Denitrification in a Benthic Foraminifer. *Nature* **2006**, *443*, 93−96.
(265) Piña-Ochoa, E.; Høgslund, S.; Geslin, E.; Cedhagen, T.; Revsbech, N. P.; Nielsen, L. P.; Schweizer, M.; Jorissen, F.; Rysgaard, S.; Risgaard-Petersen, N. Widespread Occurrence of Nitrate Storage and Denitrification among Foraminifera and *Gromiida*. *Proc. Natl. Acad. Sci. U. S. A.* **2010**, *107*, 1148−1153.
(266) Prokopenko, M. G.; Sigman, D. M.; Berelson, W. M.; Hammond, D. E.; Barnett, B.; Chong, L.; Townsend-Small, A. Denitrification in Anoxic Sediments Supported by Biological Nitrate Transport. *Geochim. Cosmochim. Acta* **2011**, *75*, 7180−7199.
(267) Bernhard, J. M.; Casciotti, K. L.; McIlvin, M. R.; Beaudoin, D. J.; Visscher, P. T.; Edgcomb, V. P. Potential Importance of Physiologically

https://dx.doi.org/10.1021/acs.chemrev.9b00613
Chem. Rev. 2020, 120, 5308−5351

Diverse Benthic Foraminifera in Sedimentary Nitrate Storage and Respiration. *J. Geophys. Res. Biogeosci.* **2012**, *117*, G03002.
(268) Woehle, C.; Roy, A. S.; Glock, N.; Wein, T.; Weissenbach, J.; Rosenstiel, P.; Hiebenthal, C.; Michels, J.; Schonfeld, J.; Dagan, T. A Novel Eukaryotic Denitrification Pathway in Foraminifera. *Curr. Biol.* **2018**, *28*, 2536−2543.
(269) Fuerst, J. A. Intracellular Compartmentation in Planctomycetes. *Annu. Rev. Microbiol.* **2005**, *59*, 299−328.
(270) Mulder, A.; van de Graaf, A. A.; Robertson, L. A.; Kuenen, J. G. Anaerobic Ammonium Oxidation Discovered in a Denitrifying Fluidized Bed Reactor. *FEMS Microbiol. Ecol.* **1995**, *16*, 177−183.
(271) van de Graaf, A. A.; de Bruijn, P.; Robertson, L. A.; Jetten, M. S. M.; Kuenen, J. G. Autotrophic Growth of Anaerobic Ammonium-Oxidizing Micro-Organisms in a Fluidized Bed Reactor. *Microbiology* **1996**, *142*, 2187−2196.
(272) Oshiki, M.; Satoh, H.; Okabe, S. Ecology and Physiology of Anaerobic Ammonium Oxidizing Bacteria. *Environ. Microbiol.* **2016**, *18*, 2784−2796.
(273) Broda, E. Two Kinds of Lithotrophs Missing in Nature. *Z. Allg. Mikrobiol.* **1977**, *17*, 491−493.
(274) Bender, M.; Jahnke, R.; Weiss, R.; Martin, W.; Heggie, D.; Orchardo, J.; Sowers, T. Organic Carbon Oxidation and Benthic Nitrogen and Silica Dynamics in San Clemente Basin, a Continental Borderland Site. *Geochim. Cosmochim. Acta* **1989**, *53*, 685−697.
(275) Ward, B. B. In *Encyclopedia of Ecology*, 2nd ed.; Fath, B., Ed.; Elsevier: Oxford, 2013.
(276) Jayakumar, A.; O'Mullan, G. D.; Naqvi, S. W. A.; Ward, B. B. Denitrifying Bacterial Community Composition Changes Associated with Stages of Denitrification in Oxygen Minimum Zones. *Microb. Ecol.* **2009**, *58*, 350−362.
(277) Dalsgaard, T.; Thamdrup, B.; Farías, L.; Revsbech, N. P. Anammox and Denitrification in the Oxygen Minimum Zone of the Eastern South Pacific. *Limnol. Oceanogr.* **2012**, *57*, 1331−1346.
(278) van de Vossenberg, J.; Rattray, J.; Geerts, W. J. C.; Kartal, B.; van Niftrik, L.; Donselaar, E. G.; Sinninghe-Damste, J.; Strous, M.; Jetten, M. Enrichment and Characterization of Marine Anammox Bacteria Associated with Global Nitrogen Gas Production. *Environ. Microbiol.* **2008**, *10*, 3120−3129.
(279) Kartal, B.; Maalcke, W. J.; de Almeida, N. M.; Cirpus, I.; Gloerich, J.; Geerts, W.; den Camp, H.; Harhangi, H. R.; Janssen-Megens, E. M.; et al. Molecular Mechanism of Anaerobic Ammonium Oxidation. *Nature* **2011**, *479*, 127−U159.
(280) Woebken, D.; Lam, P.; Kuypers, M. M. M.; Naqvi, S. W. A.; Kartal, B.; Strous, M.; Jetten, M. S. M.; Fuchs, B. M.; Amann, R. A Microdiversity Study of Anammox Bacteria Reveals a Novel *Candidatus* Scalindua Phylotype in Marine Oxygen Minimum Zones. *Environ. Microbiol.* **2008**, *10*, 3106−3119.
(281) Galloway, J. N.; Dentener, F. J.; Capone, D. G.; Boyer, E. W.; Howarth, R. W.; Seitzinger, S. P.; Asner, G. P.; Cleveland, C. C.; Green, P. A.; Holland, E. A.; et al. Nitrogen Cycles: Past, Present, and Future. *Biogeochemistry* **2004**, *70*, 153−226.
(282) Galloway, J. N.; Aber, J. D.; Erisman, J. W.; Seitzinger, S. P.; Howarth, R. W.; Cowling, E. B.; Cosby, B. J. The Nitrogen Cascade. *BioScience* **2003**, *53*, 341−356.
(283) Galloway, J. N.; Townsend, A. R.; Erisman, J. W.; Bekunda, M.; Cai, Z.; Freney, J. R.; Martinelli, L. A.; Seitzinger, S. P.; Sutton, M. A. Transformation of the Nitrogen Cycle: Recent Trends, Questions, and Potential Solutions. *Science* **2008**, *320*, 889−892.
(284) Sunda, W. G.; Cai, W.-J. Eutrophication Induced CO_2-Acidification of Subsurface Coastal Waters: Interactive Effects of Temperature, Salinity, and Atmospheric pCO_2. *Environ. Sci. Technol.* **2012**, *46*, 10651−10659.
(285) Cai, W.-J.; Hu, X.; Huang, W.-J.; Murrell, M. C.; Lehrter, J. C.; Lohrenz, S. E.; Chou, W.-C.; Zhai, W.; Hollibaugh, J. T.; Wang, Y.; et al. Acidification of Subsurface Coastal Waters Enhanced by Eutrophication. *Nat. Geosci.* **2011**, *4*, 766−770.
(286) Rabalais, N. N.; Turner, R. E. *Past and Present Water Column Anoxia*: Springer, Dordrecht, 2006; pp 225−245.
(287) Schindler, D. W. Evolution of Phosphorus Limitation in Lakes. *Science* **1977**, *195*, 260−262.
(288) Schindler, D. W.; Hecky, R. E.; Findlay, D. L.; Stainton, M. P.; Parker, B. R.; Paterson, M. J.; Beaty, K. G.; Lyng, M.; Kasian, S. E. M. Eutrophication of Lakes Cannot Be Controlled by Reducing Nitrogen Input: Results of a 37-Year Whole-Ecosystem Experiment. *Proc. Natl. Acad. Sci. U. S. A.* **2008**, *105*, 11254−11258.
(289) Schindler, D. W.; Carpenter, S. R.; Chapra, S. C.; Hecky, R. E.; Orihel, D. M. Reducing Phosphorus to Curb Lake Eutrophication Is a Success. *Environ. Sci. Technol.* **2016**, *50*, 8923−8929.
(290) Howarth, R. W.; Marino, R. Nitrogen as the Limiting Nutrient for Eutrophication in Coastal Marine Ecosystems: Evolving Views over Three Decades. *Limnol. Oceanogr.* **2006**, *51*, 364−376.
(291) Paerl, H. W. Controlling Eutrophication Along the Freshwater-Marine Continuum: Dual Nutrient (N and P) Reductions Are Essential. *Estuaries Coasts* **2009**, *32*, 593−601.
(292) Lefcheck, J. S.; Orth, R. J.; Dennison, W. C.; Wilcox, D. J.; Murphy, R. R.; Keisman, J.; Gurbisz, C.; Hannam, M.; Landry, J. B.; Moore, K. A.; et al. Long-Term Nutrient Reductions Lead to the Unprecedented Recovery of a Temperate Coastal Region. *Proc. Natl. Acad. Sci. U. S. A.* **2018**, *115*, 3658−3662.
(293) Holtgrieve, G. W.; Schindler, D. E.; Hobbs, W. O.; Leavitt, P. R.; Ward, E. J.; Bunting, L.; Chen, G.; Finney, B. P.; Gregory-Eaves, I.; Holmgren, S.; et al. A Coherent Signature of Anthropogenic Nitrogen Deposition to Remote Watersheds of the Northern Hemisphere. *Science* **2011**, *334*, 1545−1548.
(294) Seitzinger, S. P.; Kroeze, C. Global Distribution of Nitrous Oxide Production and N Inputs in Freshwater and Coastal Marine Ecosystems. *Global Biogeochem. Cycles* **1998**, *12*, 93−113.
(295) Jickells, T. D.; Buitenhuis, E.; Altieri, K.; Baker, A. R.; Capone, D.; Duce, R. A.; Dentener, F.; Fennel, K.; Kanakidou, M.; LaRoche, J.; et al. A Reevaluation of the Magnitude and Impacts of Anthropogenic Atmospheric Nitrogen Inputs on the Ocean. *Global Biogeochem. Cycles* **2017**, *31*, 289−305.
(296) Sharples, J.; Middelburg, J. J.; Fennel, K.; Jickells, T. D. What Proportion of Riverine Nutrients Reaches the Open Ocean? *Global Biogeochem. Cycles* **2017**, *31*, 39−58.
(297) Kim, T.-W.; Lee, K.; Najjar, R. G.; Jeong, H.-D.; Jeong, H. J. Increasing N Abundance in the Northwestern Pacific Ocean Due to Atmospheric Nitrogen Deposition. *Science* **2011**, *334*, 505−509.
(298) Kim, T.-W.; Lee, K.; Duce, R.; Liss, P. Impact of Atmospheric Nitrogen Deposition on Phytoplankton Productivity in the South China Sea. *Geophys. Res. Lett.* **2014**, *41*, 3156−3162.
(299) Ren, H.; Chen, Y.-C.; Wang, X. T.; Wong, G. T. F.; Cohen, A. L.; DeCarlo, T. M.; Weigand, M. A.; Mii, H.-S.; Sigman, D. M. 21st-Century Rise in Anthropogenic Nitrogen Deposition on a Remote Coral Reef. *Science* **2017**, *356*, 749−752.
(300) Duce, R. A.; LaRoche, J.; Altieri, K.; Arrigo, K. R.; Baker, A. R.; Capone, D. G.; Cornell, S.; Dentener, F.; Galloway, J.; Ganeshram, R. S.; et al. Impacts of Atmospheric Anthropogenic Nitrogen on the Open Ocean. *Science* **2008**, *320*, 893−897.
(301) Altieri, K. E.; Fawcett, S. E.; Peters, A. J.; Sigman, D. M.; Hastings, M. G. Marine Biogenic Source of Atmospheric Organic Nitrogen in the Subtropical North Atlantic. *Proc. Natl. Acad. Sci. U. S. A.* **2016**, *113*, 925−930.
(302) Wang, X. T.; Cohen, A. L.; Luu, V.; Ren, H.; Su, Z.; Haug, G. H.; Sigman, D. M. Natural Forcing of the North Atlantic Nitrogen Cycle in the Anthropocene. *Proc. Natl. Acad. Sci. U. S. A.* **2018**, *115*, 10606−10611.
(303) Paulot, F.; Jacob, D. J.; Henze, D. K. Sources and Processes Contributing to Nitrogen Deposition: An Adjoint Model Analysis Applied to Biodiversity Hotspots Worldwide. *Environ. Sci. Technol.* **2013**, *47*, 3226−3233.
(304) Likens, G. E.; Driscoll, C. T.; Buso, D. C. Long-Term Effects of Acid Rain: Response and Recovery of a Forest Ecosystem. *Science* **1996**, *272*, 244−246.
(305) Bowman, W. D.; Cleveland, C. C.; Halada, Ľ.; Hreško, J.; Baron, J. S. Negative Impact of Nitrogen Deposition on Soil Buffering Capacity. *Nat. Geosci.* **2008**, *1*, 767−770.

https://dx.doi.org/10.1021/acs.chemrev.9b00613
Chem. Rev. 2020, 120, 5308−5351

(306) Pardo, L. H.; Fenn, M. E.; Goodale, C. L.; Geiser, L. H.; Driscoll, C. T.; Allen, E. B.; Baron, J. S.; Bobbink, R.; Bowman, W. D.; Clark, C. M.; et al. Effects of Nitrogen Deposition and Empirical Nitrogen Critical Loads for Ecoregions of the United States. *Ecol. Appl.* **2011**, *21*, 3049−3082.
(307) Tian, D.; Niu, S. A Global Analysis of Soil Acidification Caused by Nitrogen Addition. *Environ. Res. Lett.* **2015**, *10*, 024019−11.
(308) Oulehle, F.; Evans, C. D.; Hofmeister, J.; Krejci, R.; Tahovska, K.; Persson, T.; Cudlin, P.; Hruska, J. Major Changes in Forest Carbon and Nitrogen Cycling Caused by Declining Sulphur Deposition. *Global Change Biol.* **2011**, *17*, 3115−3129.
(309) Fang, Y.; Wang, X.; Zhu, F.; Wu, Z.; Li, J.; Zhong, L.; Chen, D.; Yoh, M. Three-Decade Changes in Chemical Composition of Precipitation in Guangzhou City, Southern China: Has Precipitation Recovered from Acidification Following Sulphur Dioxide Emission Control? *Tellus, Ser. B* **2013**, *65*, 20213−16.
(310) Dentener, F.; Drevet, J.; Lamarque, J. F.; Bey, I.; Eickhout, B.; Fiore, A. M.; Hauglustaine, D.; Horowitz, L. W.; Krol, M.; Kulshrestha, U. C.; et al. Nitrogen and Sulfur Deposition on Regional and Global Scales: A Multimodel Evaluation. *Global Biogeochem. Cycles* **2006**, *20*, GB4003.
(311) Sala, O. E.; Stuart Chapin, F.; Armesto, J. J.; Berlow, E.; Bloomfield, J.; Dirzo, R.; Huber-Sanwald, E.; Huenneke, L. F.; Jackson, R. B.; et al. Global Biodiversity Scenarios for the Year 2100. *Science* **2000**, *287*, 1770−1774.
(312) Bobbink, R.; Hicks, K.; Galloway, J.; Spranger, T.; Alkemade, R.; Ashmore, M.; Bustamante, M.; Cinderby, S.; Davidson, E.; Dentener, F.; et al. Global Assessment of Nitrogen Deposition Effects on Terrestrial Plant Diversity: A Synthesis. *Ecol. Appl.* **2010**, *20*, 30−59.
(313) Zhang, T. a.; Chen, H. Y. H.; Ruan, H. Global Negative Effects of Nitrogen Deposition on Soil Microbes. *ISME J.* **2018**, *12*, 1817−1825.
(314) Wooliver, R.; Pellegrini, A. F. A.; Waring, B.; Houlton, B. Z.; Averill, C.; Schimel, J.; Hedin, L. O.; Bailey, J. K.; Schweitzer, J. A. Changing Perspectives on Terrestrial Nitrogen Cycling: The Importance of Weathering and Evolved Resource-Use Traits for Understanding Ecosystem Responses to Global Change. *Functional Ecology* **2019**, *33*, 1818−1829.
(315) Gilliam, F. S.; Burns, D. A.; Driscoll, C. T.; Frey, S. D.; Lovett, G. M.; Watmough, S. A. Decreased Atmospheric Nitrogen Deposition in Eastern North America: Predicted Responses of Forest Ecosystems. *Environ. Pollut.* **2019**, *244*, 560−574.
(316) Schmitz, A.; Sanders, T. G. M.; Bolte, A.; Bussotti, F.; Dirnböck, T.; Johnson, J.; Peñuelas, J.; Pollastrini, M.; Prescher, A.-K.; Sardans, J.; et al. Responses of Forest Ecosystems in Europe to Decreasing Nitrogen Deposition. *Environ. Pollut.* **2019**, *244*, 980−994.
(317) Prather, M.; Ehhalt, D.; Dentener, F.; Derwent, R.; Dlugokencky, E.; Holland, E.; Isaksen, I.; Katima, J.; Kirchhoff, V.; Matson, P. et al. In *Climate Change 2001: The Scientific Basis: Contribution of Working Group I to the Third Assessment Report of the Intergovernmental Panel on Climate Change*; Houghton, J. T., Ed.; Cambridge University Press: New York, 2001.
(318) Myhre, G.; Shindell, D.; Bréon, F. M.; Collins, W.; Fuglestvedt, J.; Huang, J.; Koch, D.; Lamarque, J. F.; Lee, D.; et al. In *Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change*; Stocker, T. F.; Qin, D.; Plattner, G. K.; Tignor, M.; Allen, S. K.; Doschung, J.; Nauels, A.; Xia, Y.; Bex, V.; Midgley, P. M., Eds.; Cambridge University Press: Cambridge, UK, 2013.
(319) Davidson, E. A. The Contribution of Manure and Fertilizer Nitrogen to Atmospheric Nitrous Oxide since 1860. *Nat. Geosci.* **2009**, *2*, 659−662.
(320) Pinder, R. W.; Bettez, N. D.; Bonan, G. B.; Greaver, T. L.; Wieder, W. R.; Schlesinger, W. H.; Davidson, E. A. Impacts of Human Alteration of the Nitrogen Cycle in the Us on Radiative Forcing. *Biogeochemistry* **2013**, *114*, 25−40.
(321) Thompson, R. L.; Lassaletta, L.; Patra, P. K.; Wilson, C.; Wells, K. C.; Gressent, A.; Koffi, E. N.; Chipperfield, M. P.; Winiwarter, W.; Davidson, E. A.; et al. Acceleration of Global N_2O Emissions Seen from Two Decades of Atmospheric Inversion. *Nat. Clim. Change* **2019**, *9*, 993−998.
(322) Coskun, D.; Britto, D. T.; Shi, W. M.; Kronzucker, H. J. Nitrogen Transformations in Modern Agriculture and the Role of Biological Nitrification Inhibition. *Nature Plants* **2017**, *3*, 17074−10.
(323) Portmann, R. W.; Daniel, J. S.; Ravishankara, A. R. Stratospheric Ozone Depletion Due to Nitrous Oxide: Influences of Other Gases. *Philos. Trans. R. Soc., B* **2012**, *367*, 1256−1264.
(324) Ainsworth, E. A.; Yendrek, C. R.; Sitch, S.; Collins, W. J.; Emberson, L. D. The Effects of Tropospheric Ozone on Net Primary Productivity and Implications for Climate Change. *Annu. Rev. Plant Biol.* **2012**, *63*, 637−661.
(325) Boucher, O.; Friedlingstein, P.; Collins, B.; Shine, K. The Indirect Global Warming Potential and Global Temperature Change Potential Due to Methane Oxidation. *Environ. Res. Lett.* **2009**, *4*, 044007.
(326) Wild, O.; Prather, M. J.; Akimoto, H. Indirect Long-Term Global Radiative Cooling from Nox Emissions. *Geophys. Res. Lett.* **2001**, *28*, 1719−1722.
(327) de Vries, W.; Solberg, S.; Dobbertin, M.; Sterba, H.; Laubhann, D.; van Oijen, M.; Evans, C.; Gundersen, P.; Kros, J.; Wamelink, G. W. W.; et al. The Impact of Nitrogen Deposition on Carbon Sequestration by European Forests and Heathlands. *For. Ecol. Manage.* **2009**, *258*, 1814−1823.
(328) Quinn Thomas, R.; Canham, C. D.; Weathers, K. C.; Goodale, C. L. Increased Tree Carbon Storage in Response to Nitrogen Deposition in the US. *Nat. Geosci.* **2010**, *3*, 13−17.
(329) Xu, L.; Penner, J. E. Global Simulations of Nitrate and Ammonium Aerosols and Their Radiative Effects. *Atmos. Chem. Phys.* **2012**, *12*, 9479−9504.
(330) Hauglustaine, D. A.; Balkanski, Y.; Schulz, M. A Global Model Simulation of Present and Future Nitrate Aerosols and Their Direct Radiative Forcing of Climate. *Atmos. Chem. Phys.* **2014**, *14*, 11031−11063.
(331) Lund, M. T.; Myhre, G.; Haslerud, A. S.; Skeie, R. B.; Griesfeller, J.; Platt, S. M.; Kumar, R.; Myhre, C. L.; Schulz, M. Concentrations and Radiative Forcing of Anthropogenic Aerosols from 1750 to 2014 Simulated with the Oslo CTM3 and CEDS Emission Inventory. *Geosci. Model Dev.* **2018**, *11*, 4909−4931.
(332) Franchin, A.; Fibiger, D. L.; Goldberger, L.; McDuffie, E. E.; Moravek, A.; Womack, C. C.; Crosman, E. T.; Docherty, K. S.; Dube, W. P.; Hoch, S. W.; et al. Airborne and Ground-Based Observations of Ammonium-Nitrate-Dominated Aerosols in a Shallow Boundary Layer During Intense Winter Pollution Episodes in Northern Utah. *Atmos. Chem. Phys.* **2018**, *18*, 17259−17276.
(333) Kuprov, R.; Eatough, D. J.; Cruickshank, T.; Olson, N.; Cropper, P. M.; Hansen, J. C. Composition and Secondary Formation of Fine Particulate Matter in the Salt Lake Valley: Winter 2009. *J. Air Waste Manage. Assoc.* **2014**, *64*, 957−969.
(334) Pant, P.; Shukla, A.; Kohl, S. D.; Chow, J. C.; Watson, J. G.; Harrison, R. M. Characterization of Ambient PM2.5 at a Pollution Hotspot in New Delhi, India and Inference of Sources. *Atmos. Environ.* **2015**, *109*, 178−189.
(335) Guo, S.; Hu, M.; Zamora, M. L.; Peng, J.; Shang, D.; Zheng, J.; Du, Z.; Wu, Z.; Shao, M.; et al. Elucidating Severe Urban Haze Formation in China. *Proc. Natl. Acad. Sci. U. S. A.* **2014**, *111*, 17373−17378.
(336) Anderson, J. O.; Thundiyil, J. G.; Stolbach, A. J. Clearing the Air: A Review of the Effects of Particulate Matter Air Pollution on Human Health. *J. Med. Toxicol.* **2012**, *8*, 166−175.
(337) Lippmann, M. Health Effects of Tropospheric Ozone: Review of Recent Research Findings and Their Implications to Ambient Air Quality Standards. *J. Expo. Anal. Environ. Epidemiol.* **1993**, *3*, 103−129.
(338) Fleming, Z. L.; Doherty, R. M.; von Schneidemesser, E.; Malley, C. S.; Cooper, O. R.; Pinto, J. P.; Colette, A.; Xu, X.; Simpson, D.; et al. Tropospheric Ozone Assessment Report: Present-Day Ozone Distribution and Trends Relevant to Human Health. *Elem. Sci. Anth.* **2018**, *6* (1), 12.

https://dx.doi.org/10.1021/acs.chemrev.9b00613
Chem. Rev. 2020, 120, 5308−5351

(339) Mills, G.; Pleijel, H.; Malley, C. S.; Sinha, B.; Cooper, O. R.; Schultz, M. G.; Neufeld, H. S.; Simpson, D.; Sharps, K.; Feng, Z.; et al. Tropospheric Ozone Assessment Report:Present-Day Tropospheric Ozone Distribution and Trends Relevant to Vegetation. *Elem. Sci. Anth.* **2018**, *6* (1), 47.
(340) Lee, C. J.; Martin, R. V.; Henze, D. K.; Brauer, M.; Cohen, A.; Donkelaar, A. v. Response of Global Particulate-Matter-Related Mortality to Changes in Local Precursor Emissions. *Environ. Sci. Technol.* **2015**, *49*, 4335−4344.
(341) Pozzer, A.; Tsimpidi, A. P.; Karydis, V. A.; de Meij, A.; Lelieveld, J. Impact of Agricultural Emission Reductions on Fine-Particulate Matter and Public Health. *Atmos. Chem. Phys.* **2017**, *17*, 12813−12826.
(342) Paulot, F.; Jacob, D. J. Hidden Cost of U.S. Agricultural Exports: Particulate Matter from Ammonia Emissions. *Environ. Sci. Technol.* **2014**, *48*, 903−908.
(343) White, A. E.; Granger, J.; Selden, C.; Gradoville, M. R.; Potts, L.; Bourbonnais, A.; Fulweiler, R. W.; Knapp, A. N.; Mohr, W.; et al. A Critical Review of the $^{15}N_2$ Tracer Method to Measure Diazotrophic Production in Pelagic Ecosystems. *Limnol. Oceanogr.: Methods* **2020**, *18*, 129−147.
(344) Montoya, J. P.; Voss, M.; Kahler, P.; Capone, D. G. A Simple, High-Precision, High-Sensitivity Tracer Assay for N_2 Fixation. *Appl. Environ. Microb.* **1996**, *62*, 986−993.
(345) Mulholland, M. R.; Bernhardt, P. W.; Heil, C. A.; Bronk, D. A.; O'Neil, J. M. Nitrogen Fixation and Release of Fixed Nitrogen by *Trichodesmium* spp. In the Gulf of Mexico. *Limnol. Oceanogr.* **2006**, *51*, 1762−1776.
(346) Mohr, W.; Grosskopf, T.; Wallace, D. W. R.; LaRoche, J. Methodological Underestimation of Oceanic Nitrogen Fixation Rates. *PLoS One* **2010**, *5*, No. e12583.
(347) Wilson, S. T.; Bottjer, D.; Church, M. J.; Karl, D. M. Comparative Assessment of Nitrogen Fixation Methodologies, Conducted in the Oligotrophic North Pacific Ocean. *Appl. Environ. Microbiol.* **2012**, *78*, 6516−6523.
(348) Wannicke, N.; Benavides, M.; Dalsgaard, T.; Dippner, J. W.; Montoya, J. P.; Voss, M. New Perspectives on Nitrogen Fixation Measurements Using $^{15}N_2$ Gas. *Front. Mar. Sci.* **2018**, *5*, 120.
(349) Dabundo, R.; Lehmann, M. F.; Treibergs, L.; Tobias, C. R.; Altabet, M. A.; Moisander, P. H.; Granger, J. The Contamination of Commercial $^{15}N_2$ Gas Stocks with ^{15}N-Labeled Nitrate and Ammonium and Consequences for Nitrogen Fixation Measurements. *PLoS One* **2014**, *9*, e110335.
(350) Dalsgaard, T.; Canfield, D. E.; Petersen, J.; Thamdrup, B.; Acuña-González, J. N_2 Production by the Anammox Reaction in the Anoxic Water Column of Golfo Dulce, Costa Rica. *Nature* **2003**, *422*, 606−608.
(351) Groffman, P. M.; Altabet, M. A.; Böhlke, J. K.; Butterbach-Bahl, K.; David, M. B.; Firestone, M. K.; Giblin, A. E.; Kana, T. M.; Nielsen, L. P.; Voytek, M. A. Methods for Measuring Denitrification: Diverse Approaches to a Difficult Problem. *Ecol. Appl.* **2006**, *16*, 2091−2122.
(352) Thamdrup, B.; Dalsgaard, T. Production of N_2 through Anaerobic Ammonium Oxidation Coupled to Nitrate Reduction in Marine Sediments. *Appl. Environ. Microbiol.* **2002**, *68*, 1312−1318.
(353) Ji, Q.; Babbin, A. R.; Jayakumar, A.; Oleynik, S.; Ward, B. B. Nitrous Oxide Production by Nitrification and Denitrification in the Eastern Tropical South Pacific Oxygen Minimum Zone. *Geophys. Res. Lett.* **2015**, *42* (10), 755−710.
(354) Ward, B. B.; Devol, A. H.; Rich, J. J.; Chang, B. X.; Bulow, S. E.; Naik, H.; Pratihary, A.; Jayakumar, A. Denitrification as the Dominant Nitrogen Loss Process in the Arabian Sea. *Nature* **2009**, *461*, 78−81.
(355) Kunu, T. M.; Sullivan, M. B.; Cornwell, J. C.; Groxzkowski, K. M. Denitrification in Estuarine Sediments Determined by Membrane Inlet Mass Spectrometry. *Limnol. Oceanogr.* **1998**, *43*, 334−339.
(356) An, S.; Gardner, W. S.; Kana, T. Simultaneous Measurement of Denitrification and Nitrogen Fixation Using Isotope Pairing with Membrane Inlet Mass Spectrometry Analysis. *Appl. Environ. Microbiol.* **2001**, *67*, 1171−1178.
(357) Joye, S. B.; Paerl, H. W. Contemporaneous Nitrogen Fixation and Denitrification in Intertidal Microbial Mats: Rapid Response to Runoff Events. *Mar. Ecol.: Prog. Ser.* **1993**, *94*, 267−274.
(358) Joye, S. B.; Paerl, H. W. Nitrogen Cycling in Microbial Mats: Rates and Patterns of Denitrification and Nitrogen Fixation. *Mar. Biol.* **1994**, *119*, 285−295.
(359) Butterbach-Bahl, K.; Willibald, G.; Papen, H. Soil Core Method for Direct Simultaneous Determination of N_2 and N_2O Emissions from Forest Soils. *Plant Soil* **2002**, *240*, 105−116.
(360) Famulari, D.; Nemitz, E.; Di Marco, C.; Phillips, G. J.; Thomas, R.; House, E.; Fowler, D. Eddy-Covariance Measurements of Nitrous Oxide Fluxes above a City. *Agric. For. Meteorol.* **2010**, *150*, 786−793.
(361) Wilkerson, J.; Dobosy, R.; Sayres, D. S.; Healy, C.; Dumas, E.; Baker, B.; Anderson, J. G. Permafrost Nitrous Oxide Emissions Observed on a Landscape Scale Using the Airborne Eddy-Covariance Method. *Atmos. Chem. Phys.* **2019**, *19*, 4257−4268.
(362) Molodovskaya, M.; Warland, J.; Richards, B. K.; Öberg, G.; Steenhuis, T. S. Nitrous Oxide from Heterogeneous Agricultural Landscapes: Source Contribution Analysis by Eddy Covariance and Chambers. *Soil Sci. Soc. Am. J.* **2011**, *75*, 1829−1838.
(363) Bellenger, J.-P.; Xu, Y.; Zhang, X.; Morel, F. M. M.; Kraepiel, A. M. L. Possible Contribution of Alternative Nitrogenases to Nitrogen Fixation by Asymbiotic N_2-Fixing Bacterial in Soils. *Soil Biol. Biochem.* **2014**, *69*, 413−420.
(364) Fulweiler, R. W.; Heiss, E. M.; Rogener, M. K.; Newell, S. E.; LeCleir, G. R.; Kortebein, S. M.; Wilhelm, S. W. Examining the Impact of Acetylene on N-Fixation and the Active Sediment Microbial Community. *Front. Microbiol.* **2015**, *6*, 418−418a.
(365) De Bont, J.; Mulder, E. Invalidity of the Acetylene Reduction Assay in Alkane-Utilizing, Nitrogen-Fixing Bacteria. *Appl. Environ. Microbiol.* **1976**, *31*, 640−647.
(366) Sørensen, J. Denitrification Rates in a Marine Sediment as Measured by the Acetylene Inhibition Technique. *Appl. Environ. Microbiol.* **1978**, *36*, 139−143.
(367) Dodsworth, J. A.; Hungate, B.; de la Torre, J. R.; Jiang, H.; Hedlund, B. P. In *Methods Enzymol.*; Klotz, M. G., Ed.; Academic Press, 2011; Vol. *486*.
(368) Hyman, M. R.; Daniel, A. Acetylene Inhibition of Metalloenzymes. *Anal. Biochem.* **1988**, *173*, 207−220.
(369) Brzezińska, M.; Rafalski, P.; Włodarczyk, T.; Szarlip, P.; Brzeziński, K. How Much Oxygen Is Needed for Acetylene to Be Consumed in Soil? *J. Soils Sediments* **2011**, *11*, 1142−1154.
(370) Christensen, P. B.; Nielsen, L. P.; Revsbech, N. P.; Sørensen, J. Microzonation of Denitrification Activity in Stream Sediments as Studied with a Combined Oxygen and Nitrous Oxide Microsensor. *Appl. Environ. Microbiol.* **1989**, *55*, 1234−1241.
(371) Seitzinger, S. P.; Nielsen, L. P.; Caffrey, J.; Christensen, P. B. Denitrification Measurements in Aquatic Sediments: A Comparison of Three Methods. *Biogeochemistry* **1993**, *23*, 147−167.
(372) Joye, S. B.; Smith, S. V.; Hollibaugh, J. T.; Paerl, H. W. Estimating Denitrification Rates in Estuarine Sediments: A Comparison of Stoichiometric and Acetylene Based Methods. *Biogeochemistry* **1996**, *33*, 197−215.
(373) Miller, L. G.; Oremland, R. S.; Paulsen, S. Measurement of Nitrous Oxide Reductase Activity in Aquatic Sediments. *Appl. Environ. Microbiol.* **1986**, *51*, 18−24.
(374) Bai, E.; Houlton, B. Z.; Wang, Y. P. Isotopic Identification of Nitrogen Hotspots across Natural Terrestrial Ecosystems. *Biogeosciences* **2012**, *9*, 3287−3304.
(375) Houlton, B. Z.; Sigman, D. M.; Hedin, L. O. Isotopic Evidence for Large Gaseous Nitrogen Losses from Tropical Rainforests. *Proc. Natl. Acad. Sci. U. S. A.* **2006**, *103*, 8745−8750.
(376) Redfield, A. C. *James Johnston Memorial Vol.*; Daniel, R. J., Ed.; Univ. Press of Liverpool: Liverpool, 1934.
(377) Gruber, N.; Sarmiento, J. L. Global Patterns of Marine Nitrogen Fixation and Denitrification. *Global Biogeochem. Cycles* **1997**, *11*, 235−266.
(378) Hansell, D.; Bates, N.; Olson, D. Excess Nitrate and Nitrogen Fixation in the North Atlantic Ocean. *Mar. Chem.* **2004**, *84*, 243−265.

https://dx.doi.org/10.1021/acs.chemrev.9b00613
Chem. Rev. 2020, 120, 5308−5351

(379) Deutsch, C.; Sarmiento, J. L.; Sigman, D. M.; Gruber, N.; Dunne, J. P. Spatial Coupling of Nitrogen Inputs and Losses in the Ocean. *Nature* **2007**, *445*, 163−167.
(380) Deutsch, C.; Gruber, N.; Key, R. M.; Sarmiento, J. L.; Ganachaud, A. Denitrification and N2 Fixation in the Pacific Ocean. *Global Biogeochem. Cycles* **2001**, *15*, 483−506.
(381) Duhamel, S.; Björkman, K. M.; Van Wambeke, F.; Moutin, T.; Karl, D. M. Characterization of Alkaline Phosphatase Activity in the North and South Pacific Subtropical Gyres: Implications for Phosphorus Cycling. *Limnol. Oceanogr.* **2011**, *56*, 1244−1254.
(382) Repeta, D. J.; Ferrón, S.; Sosa, O. A.; Johnson, C. G.; Repeta, L. D.; Acker, M.; DeLong, E. F.; Karl, D. M. Marine Methane Paradox Explained by Bacterial Degradation of Dissolved Organic Matter. *Nat. Geosci.* **2016**, *9*, 884−887.
(383) Sokoll, S.; Ferdelman, T. G.; Holtappels, M.; Goldhammer, T.; Littmann, S.; Iversen, M. H.; Kuypers, M. M. M. Intense Biological Phosphate Uptake onto Particles in Subeuphotic Continental Margin Waters. *Geophys. Res. Lett.* **2017**, *44*, 2825−2834.
(384) Herut, B.; Zohary, T.; Robarts, R. D.; Kress, N. Adsorption of Dissolved Phosphate onto Loess Particles in Surface and Deep Eastern Mediterranean Water. *Mar. Chem.* **1999**, *64*, 253−265.
(385) Wang, W.-L.; Moore, J. K.; Martiny, A. C.; Primeau, F. W. Convergent Estimates of Marine Nitrogen Fixation. *Nature* **2019**, *566*, 205−211.
(386) Weber, T.; Deutsch, C. Oceanic Nitrogen Reservoir Regulated by Plankton Diversity and Ocean Circulation. *Nature* **2012**, *489*, 419−422.
(387) Weber, T. S.; Deutsch, C. Ocean Nutrient Ratios Governed by Plankton Biogeography. *Nature* **2010**, *467*, 550−554.
(388) Martiny, A. C.; Pham, C. T. A.; Primeau, F. W.; Vrugt, J. A.; Moore, J. K.; Levin, S. A.; Lomas, M. W. Strong Latitudinal Patterns in the Elemental Ratios of Marine Plankton and Organic Matter. *Nat. Geosci.* **2013**, *6*, 279−283.
(389) DeVries, T.; Deutsch, C.; Rafter, P. A.; Primeau, F. Marine Denitrification Rates Determined from a Global 3-D Inverse Model. *Biogeosciences* **2013**, *10*, 2481−2496.
(390) DeVries, T.; Deutsch, C.; Primeau, F.; Chang, B.; Devol, A. Global Rates of Water-Column Denitrification Derived from Nitrogen Gas Measurements. *Nat. Geosci.* **2012**, *5*, 547−550.
(391) Chang, B. X.; Devol, A. H.; Emerson, S. R. Fixed Nitrogen Loss from the Eastern Tropical North Pacific and Arabian Sea Oxygen Deficient Zones Determined from Measurements of N_2:Ar. *Global Biogeochem. Cycles* **2012**, *26*, GB3030.
(392) Shigemitsu, M.; Gruber, N.; Oka, A.; Yamanaka, Y. Potential Use of the N_2/Ar Ratio as a Constraint on the Oceanic Fixed Nitrogen Loss. *Global Biogeochem. Cycles* **2016**, *30*, 576−594.
(393) Fuchsman, C. A.; Devol, A. H.; Casciotti, K. L.; Buchwald, C.; Chang, B. X.; Horak, R. E. A. An N Isotopic Mass Balance of the Eastern Tropical North Pacific Oxygen Deficient Zone. *Deep Sea Res., Part II* **2018**, *156*, 137−147.
(394) Brandes, J. A.; Devol, A. H. A Global Marine-Fixed Nitrogen Isotopic Budget: Implications for Holocene Nitrogen Cycling. *Global Biogeochem. Cycles* **2002**, *16*, 67−1−67−14.
(395) Deutsch, C.; Sigman, D. M.; Thunell, R. C.; Meckler, A. N.; Haug, G. H. Isotopic Constraints on Glacial/Interglacial Changes in the Oceanic Nitrogen Budget. *Global Biogeochem. Cycles* **2004**, *18*, GB4012.
(396) Sigman, D. M.; Fripiat, F. *Encyclopedia of Ocean Sciences*; 3rd ed.; Steele, J. H., Ed.; Academic Press, 2019.
(397) Houlton, B. Z.; Bai, E. Imprint of Denitrifying Bacteria on the Global Terrestrial Biosphere. *Proc. Natl. Acad. Sci. U. S. A.* **2009**, *106*, 21713.
(398) Brandes, J. A.; Devol, A. H. Isotopic Fractionation of Oxygen and Nitrogen in Coastal Marine Sediments. *Geochim. Cosmochim. Acta* **1997**, *61*, 1793−1801.
(399) Sigman, D. M.; Casciotti, K. L.; Andreani, M.; Barford, C.; Galanter, M.; Böhlke, J. K. A Bacterial Method for the Nitrogen Isotopic Analysis of Nitrate in Seawater and Freshwater. *Anal. Chem.* **2001**, *73*, 4145−4153.
(400) Sebilo, M.; Billen, G.; Grably, M.; Mariotti, A. Isotopic Composition of Nitrate-Nitrogen as a Marker of Riparian and Benthic Denitrification at the Scale of the Whole Seine River System. *Biogeochemistry* **2003**, *63*, 35−51.
(401) Lehmann, M. F.; Sigman, D. M.; Berelson, W. M. Coupling the $^{15}N/^{14}N$ and $^{18}O/^{16}O$ of Nitrate as a Constraint on Benthic Nitrogen Cycling. *Mar. Chem.* **2004**, *88*, 1−20.
(402) Lehmann, M. F.; Sigman, D. M.; McCorkle, D. C.; Granger, J.; Hoffmann, S.; Cane, G.; Brunelle, B. G. The Distribution of Nitrate $^{15}N/^{14}N$ in Marine Sediments and the Impact of Benthic Nitrogen Loss on the Isotopic Composition of Oceanic Nitrate. *Geochim. Cosmochim. Acta* **2007**, *71*, 5384−5404.
(403) Kritee, K.; Sigman, D. M.; Granger, J.; Ward, B. B.; Jayakumar, A.; Deutsch, C. Reduced Isotope Fractionation by Denitrification under Conditions Relevant to the Ocean. *Geochim. Cosmochim. Acta* **2012**, *92*, 243−259.
(404) Marconi, D.; Kopf, S.; Rafter, P. A.; Sigman, D. M. Aerobic Respiration Along Isopycnals Leads to Overestimation of the Isotope Effect of Denitrification in the Ocean Water Column. *Geochim. Cosmochim. Acta* **2017**, *197*, 417−432.
(405) Kast, E. R.; Stolper, D. A.; Auderset, A.; Higgins, J. A.; Ren, H.; Wang, X. T.; Martínez-García, A.; Haug, G. H.; Sigman, D. M. Nitrogen Isotope Evidence for Expanded Ocean Suboxia in the Early Cenozoic. *Science* **2019**, *364*, 386−389.
(406) Wang, C.; Houlton, B. Z.; Dai, W.; Bai, E. Growth in the Global N_2 Sink Attributed to N Fertilizer Inputs over 1860 to 2000. *Sci. Total Environ.* **2017**, *574*, 1044−1053.
(407) Knapp, A. N.; DiFiore, P. J.; Deutsch, C.; Sigman, D. M.; Lipschultz, F. Nitrate Isotopic Composition between Bermuda and Puerto Rico: Implications for N_2 Fixation in the Atlantic Ocean. *Global Biogeochem. Cycles* **2008**, *22*, GB3014.
(408) Schmidt, D. C.; Carlson, S. R.; Kyle, G. B.; Finney, B. P. Influence of Carcass-Derived Nutrients on Sockeye Salmon Productivity of Karluk Lake, Alaska: Importance in the Assessment of an Escapement Goal. *North American Journal of Fisheries Management* **1998**, *18*, 743−763.
(409) Chen, G.; Selbie, D. T.; Finney, B. P.; Schindler, D. E.; Bunting, L.; Leavitt, P. R.; Gregory-Eaves, I. Long-Term Zooplankton Responses to Nutrient and Consumer Subsidies Arising from Migratory Sockeye Salmon (Oncorhynchus Nerka). *Oikos* **2011**, *120*, 1317−1326.
(410) Schindler, D. E.; Scheuerell, M. D.; Moore, J. W.; Gende, S. M.; Francis, T. B.; Palen, W. J. Pacific Salmon and the Ecology of Coastal Ecosystems. *Frontiers in Ecology and the Environment* **2003**, *1*, 31−37.
(411) Herridge, D. F.; Peoples, M. B.; Boddey, R. M. Global Inputs of Biological Nitrogen Fixation in Agricultural Systems. *Plant Soil* **2008**, *311*, 1−18.
(412) Vitousek, P. M.; Aber, J. D.; Howarth, R. W.; Likens, G. E.; Matson, P. A.; Schindler, D. W.; Schlesinger, W. H.; Tilman, D. G. Human Alteration of the Global Nitrogen Cycle: Sources and Consequences. *Ecol. Appl.* **1997**, *7*, 737−750.
(413) Billen, G.; Lassaletta, L.; Garnier, J. A Biogeochemical View of the Global Agro-Food System: Nitrogen Flows Associated with Protein Production, Consumption and Trade. *Global Food Security* **2014**, *3*, 209−219.
(414) Liu, J. G.; You, L. Z.; Amini, M.; Obersteiner, M.; Herrero, M.; Zehnder, A. J. B.; Yang, H. A High-Resolution Assessment on Global Nitrogen Flows in Cropland. *Proc. Natl. Acad. Sci. U. S. A.* **2010**, *107*, 8035−8040.
(415) Gardner, J. B.; Drinkwater, L. E. The Fate of Nitrogen in Grain Cropping Systems: A Meta-Analysis of ^{15}N Field Experiments. *Ecol. Appl.* **2009**, *19*, 2167−2184.
(416) Van Meter, K. J.; Basu, N. B.; Veenstra, J. J.; Burras, C. L. The Nitrogen Legacy: Emerging Evidence of Nitrogen Accumulation in Anthropogenic Landscapes. *Environ. Res. Lett.* **2016**, *11*, 035014.
(417) Vuuren, D. P. v.; Bouwman, L. F.; Smith, S. J.; Dentener, F. Global Projections for Anthropogenic Reactive Nitrogen Emissions to the Atmosphere: An Assessment of Scenarios in the Scientific Literature. *Current Opinion in Environmental Sustainability* **2011**, *3*, 359−369.

https://dx.doi.org/10.1021/acs.chemrev.9b00613
Chem. Rev. 2020, 120, 5308−5351

(418) Zaehle, S. Terrestrial Nitrogen-Carbon Cycle Interactions at the Global Scale. *Philos. Trans. R. Soc., B* **2013**, *368*, 20130125−20130125a.
(419) Seitzinger, S. P.; Styles, R. V.; Boyer, E. W.; Alexander, R. B.; Billen, G.; Howarth, R. W.; Mayer, B.; van Breemen, N. Nitrogen Retention in Rivers: Model Development and Application to Watersheds in the Northeastern U.S.A. *Biogeochemistry* **2002**, *57*, 199−237.
(420) Seitzinger, S.; Harrison, J. A.; Böhlke, J. K.; Bouwman, A. F.; Lowrance, R.; Peterson, B.; Tobias, C.; Drecht, G. V. Denitrification across Landscapes and Waterscapes: A Synthesis. *Ecol. Appl.* **2006**, *16*, 2064−2090.
(421) Groffman, P. M. Terrestrial Denitrification: Challenges and Opportunities. *Ecol. Proc.* **2012**, *1*, 11.
(422) Groffman, P. M.; Butterbach-Bahl, K.; Fulweiler, R. W.; Gold, A. J.; Morse, J. L.; Stander, E. K.; Tague, C.; Tonitto, C.; Vidon, P. Challenges to Incorporating Spatially and Temporally Explicit Phenomena (Hotspots and Hot Moments) in Denitrification Models. *Biogeochemistry* **2009**, *93*, 49−77.
(423) Nie, S.; Lei, X.; Zhao, L.; Wang, Y.; Wang, F.; Li, H.; Yang, W.; Xing, S. Response of Activity, Abundance, and Composition of Anammox Bacterial Community to Different Fertilization in a Paddy Soil. *Biol. Fertil. Soils* **2018**, *54*, 977−984.
(424) Lobert, J. M.; Scharffe, D. H.; Hao, W. M.; Crutzen, P. J. Importance of Biomass Burning in the Atmospheric Budgets of Nitrogen-Containing Gases. *Nature* **1990**, *346*, 552−554.
(425) Seitzinger, S. P. Denitrification in Freshwater and Coastal Marine Ecosystems: Ecological and Geochemical Significance. *Limnol. Oceanogr.* **1988**, *33*, 702−724.
(426) Nixon, S. W.; Ammerman, J. W.; Atkinson, L. P.; Berounsky, V. M.; Billen, G.; Boicourt, W. C.; Boynton, W. R.; Church, T. M.; Ditoro, D. M.; et al. The Fate of Nitrogen and Phosphorus at the Land-Sea Margin of the North Atlantic Ocean. *Biogeochemistry* **1996**, *35*, 141−180.
(427) Beusen, A. H. W.; Bouwman, A. F.; Van Beek, L. P. H.; Mogollón, J. M.; Middelburg, J. J. Global Riverine N and P Transport to Ocean Increased During the 20th Century Despite Increased Retention Along the Aquatic Continuum. *Biogeosciences* **2016**, *13*, 2441−2451.
(428) Seitzinger, S. P.; Mayorga, E.; Bouwman, A. F.; Kroeze, C.; Beusen, A. H. W.; Billen, G.; Van Drecht, G.; Dumont, E.; Fekete, B. M.; et al. Global River Nutrient Export: A Scenario Analysis of Past and Future Trends. *Global Biogeochem. Cycles* **2010**, *24*, GB0A48.
(429) McLauchlan, K. K.; Williams, J. J.; Craine, J. M.; Jeffers, E. S. Changes in Global Nitrogen Cycling During the Holocene Epoch. *Nature* **2013**, *495*, 352−355.
(430) Vile, M. A.; Kelman Wieder, R.; Živković, T.; Scott, K. D.; Vitt, D. H.; Hartsock, J. A.; Iosue, C. L.; Quinn, J. C.; Petix, M.; et al. N_2-Fixation by Methanotrophs Sustains Carbon and Nitrogen Accumulation in Pristine Peatlands. *Biogeochemistry* **2014**, *121*, 317−328.
(431) Elbert, W.; Weber, B.; Burrows, S.; Steinkamp, J.; Büdel, B.; Andreae, M. O.; Pöschl, U. Contribution of Cryptogamic Covers to the Global Cycles of Carbon and Nitrogen. *Nat. Geosci.* **2012**, *5*, 459−462.
(432) Großkopf, T.; Mohr, W.; Baustian, T.; Schunck, H.; Gill, D.; Kuypers, M. M. M.; Lavik, G.; Schmitz, R. A.; Wallace, D. W. R.; LaRoche, J. Doubling of Marine Dinitrogen-Fixation Rates Based on Direct Measurements. *Nature* **2012**, *488*, 361−364.
(433) Voss, M.; Bange, H. W.; Dippner, J. W.; Middelburg, J. J.; Montoya, J. P.; Ward, B. The Marine Nitrogen Cycle: Recent Discoveries, Uncertainties and the Potential Relevance of Climate Change. *Philos. Trans. R. Soc., B* **2013**, *368*, 20130121.
(434) Capone, D. G. *Nitrogen Cycling in Coastal Marine Environments*; Blackburn, T. H.; Sorensen, J., Eds.; John Wiley & Sons Ltd, 1988.
(435) Somes, C. J.; Oschlies, A.; Schmittner, A. Isotopic Constraints on the Pre-Industrial Oceanic Nitrogen Budget. *Biogeosciences* **2013**, *10*, 5889−5910.
(436) Yang, S.; Gruber, N. The Anthropogenic Perturbation of the Marine Nitrogen Cycle by Atmospheric Deposition: Nitrogen Cycle Feedbacks and the 15N Haber-Bosch Effect. *Global Biogeochem. Cycles* **2016**, *30*, 1418−1440.
(437) Stramma, L.; Johnson, G. C.; Sprintall, J.; Mohrholz, V. Expanding Oxygen-Minimum Zones in the Tropical Oceans. *Science* **2008**, *320*, 655−658.
(438) Keeling, R. F.; Körtzinger, A.; Gruber, N. Ocean Deoxygenation in a Warming World. *Annu. Rev. Mar. Sci.* **2010**, *2*, 199−229.
(439) Gruber, N. Warming up, Turning Sour, Losing Breath: Ocean Biogeochemistry under Global Change. *Philos. Trans. R. Soc., A* **2011**, *369*, 1980−1996.
(440) Deutsch, C.; Berelson, W.; Thunell, R.; Weber, T.; Tems, C.; McManus, J.; Crusius, J.; Ito, T.; Baumgartner, T.; Ferreira, V.; et al. Centennial Changes in North Pacific Anoxia Linked to Tropical Trade Winds. *Science* **2014**, *345*, 665−668.
(441) Codispoti, L. A.; Brandes, J.; Christensen, J. P.; Devol, A.; Naqvi, S. W. A.; Paerl, H.; Yoshinari, T. The Oceanic Fixed Nitrogen and Nitrous Oxide Budgets: Moving Targets as We Enter the Anthropocene? *Sci. Mar.* **2001**, *65*, 85−105.
(442) McElroy, M. B. Marine Biological Controls on Atmospheric CO_2 and Climate. *Nature* **1983**, *302*, 328−329.
(443) Eugster, O.; Gruber, N. A Probabilistic Estimate of Global Marine N-Fixation and Denitrification. *Global Biogeochem. Cycles* **2012**, *26*, GB4013.
(444) Tang, W.; Wang, S.; Fonseca-Batista, D.; Dehairs, F.; Gifford, S.; Gonzalez, A. G.; Gallinari, M.; Planquette, H.; Sarthou, G.; Cassar, N. Revisiting the Distribution of Oceanic N_2 Fixation and Estimating Diazotrophic Contribution to Marine Production. *Nat. Commun.* **2019**, *10* (831), 1−10.
(445) Fulweiler, R. W.; Nixon, S. W.; Buckley, B. A.; Granger, S. L. Reversal of the Net Dinitrogen Gas Flux in Coastal Marine Sediments. *Nature* **2007**, *448*, 180−182.
(446) Gier, J.; Sommer, S.; Loscher, C. R.; Dale, A. W.; Schmitz, R. A.; Treude, T. Nitrogen Fixation in Sediments Along a Depth Transect through the Peruvian Oxygen Minimum Zone. *Biogeosciences* **2016**, *13*, 4065−4080.
(447) Takano, Y.; Ito, T.; Deutsch, C. Projected Centennial Oxygen Trends and Their Attribution to Distinct Ocean Climate Forcings. *Global Biogeochem. Cycles* **2018**, *32*, 1329−1349.
(448) Luo, Y. W.; Doney, S. C.; Anderson, L. A.; Benavides, M.; Berman-Frank, I.; Bode, A.; Bonnet, S.; Boström, K. H.; Böttjer, D.; Capone, D. G.; et al. Database of Diazotrophs in Global Ocean: Abundance, Biomass and Nitrogen Fixation Rates. *Earth Syst. Sci. Data* **2012**, *4*, 47−73.
(449) Middelburg, J. J.; Soetaert, K.; Herman, P. M. J.; Heip, C. H. R. Denitrification in Marine Sediments: A Model Study. *Global Biogeochem. Cycles* **1996**, *10*, 661−673.
(450) de Baar; Von, H. J. W. Liebig's Law of the Minimum and Plankton Ecology (1899−1991). *Prog. Oceanogr.* **1994**, *33*, 347−386.
(451) Moore, C. M. Diagnosing Oceanic Nutrient Deficiency. *Philos. Trans. R. Soc., A* **2016**, *374*, 20150290.
(452) Sigman, D. M.; Hain, M. P. The Biological Productivity of the Ocean. *Nature Education Knowledge* **2012**, *3*, 21.
(453) LeBauer, D. S.; Treseder, K. K. Nitrogen Limitation of Net Primary Productivity in Terrestrial Ecosystems Is Globally Distributed. *Ecology* **2008**, *89*, 371−379.
(454) Arrigo, K. R. Marine Microorganisms and Global Nutrient Cycles. *Nature* **2005**, *437*, 349−355.
(455) Geider, R.; La Roche, J. Redfield Revisited: Variability of C:N:P in Marine Microalgae and Its Biochemical Basis. *Eur. J. Phycol.* **2002**, *37*, 1−17.
(456) Klausmeier, C. A.; Litchman, E.; Daufresne, T.; Levin, S. A. Optimal Nitrogen-to-Phosphorus Stoichiometry of Phytoplankton. *Nature* **2004**, *429*, 171−174.
(457) McGroddy, M. E.; Daufresne, T.; Hedin, L. O. Scaling of C:N:P Stoichiometry in Forests Worldwide: Implications of Terrestrial Redfield-Type Ratios. *Ecology* **2004**, *85*, 2390−2401.
(458) Kerkhoff, A. J.; Enquist, B. J.; Elser, J. J.; Fagan, W. F. Plant Allometry, Stoichiometry and the Temperature-Dependence of Primary Productivity. *Glob. Ecol. Biogeogr.* **2005**, *14*, 585−598.
(459) Redfield, A. C.; Ketchum, B. H.; Richards, F. A. In *The Sea*, Vol. 2; Hill, M. N., Ed.; Interscience: New York, 1963.

https://dx.doi.org/10.1021/acs.chemrev.9b00613
Chem. Rev. 2020, 120, 5308−5351

(460) Haug, G. H.; Pedersen, T. F.; Sigman, D. M.; Calvert, S. E.; Nielsen, B.; Peterson, L. C. Glacial/Interglacial Variations in Production and Nitrogen Fixation in the Cariaco Basin During the Last 580 Kyr. *Paleoceanography* **1998**, *13*, 427−432.
(461) Codispoti, L. A. *Productivity of the Ocean: Past and Present*; Berger, W. H.; Smetacek, V. S.; Wefer, G., Eds.; John Wiley: Hoboken, NJ, 1989.
(462) Toggweiler, J. R.; Carson, S. *Upwelling in the Ocean: Modern Processes and Ancient Records*; Summerhayes, C. P. E. A., Ed.; John Wiley: Hoboken, NJ, 1995.
(463) Deutsch, C.; Weber, T. Nutrient Ratios as a Tracer and Driver of Ocean Biogeochemistry. *Annu. Rev. Mar. Sci.* **2012**, *4*, 113−141.
(464) Michaels, A. F.; Karl, D. M.; Capone, D. G. Element Stoichiometry, New Production and Nitrogen Fixation. *Oceanography* **2001**, *14*, 68−77.
(465) Knapp, A. The Sensitivity of Marine N_2 Fixation to Dissolved Inorganic Nitrogen. *Front. Microbiol.* **2012**, *3*, 374.
(466) Knapp, A. N.; Dekaezemacker, J.; Bonnet, S.; Sohm, J. A.; Capone, D. G. Sensitivity of *Trichodesmium erythraeum* and *Crocosphaera watsonii* Abundance and N_2 Fixation Rates to Varying NO_3^- and PO_4^{3-} Concentrations in Batch Cultures. *Aquat. Microb. Ecol.* **2012**, *66*, 223−236.
(467) Moore, C. M.; Mills, M. M.; Langlois, R.; Milne, A.; Achterberg, E. P.; La Roche, J.; Geider, R. J. Relative Influence of Nitrogen and Phosphorous Availability on Phytoplankton Physiology and Productivity in the Oligotrophic Sub-Tropical North Atlantic Ocean. *Limnol. Oceanogr.* **2008**, *53*, 291−305.
(468) Marconi, D.; Alexandra Weigand, M.; Rafter, P. A.; McIlvin, M. R.; Forbes, M.; Casciotti, K. L.; Sigman, D. M. Nitrate Isotope Distributions on the US Geotraces North Atlantic Cross-Basin Section: Signals of Polar Nitrate Sources and Low Latitude Nitrogen Cycling. *Mar. Chem.* **2015**, *177*, 143−156.
(469) Marconi, D.; Weigand, M. A.; Sigman, D. M. Nitrate Isotopic Gradients in the North Atlantic Ocean and the Nitrogen Isotopic Composition of Sinking Organic Matter. *Deep Sea Res., Part I* **2019**, *145*, 109−124.
(470) Marconi, D.; Sigman, D. M.; Casciotti, K. L.; Campbell, E. C.; Alexandra Weigand, M.; Fawcett, S. E.; Knapp, A. N.; Rafter, P. A.; Ward, B. B.; Haug, G. H. Tropical Dominance of N_2 Fixation in the North Atlantic Ocean. *Global Biogeochem. Cycles* **2017**, *31*, 1608−1623.
(471) Ren, H.; Sigman, D. M.; Meckler, A. N.; Plessen, B.; Robinson, R. S.; Rosenthal, Y.; Haug, G. H. Foraminiferal Isotope Evidence of Reduced Nitrogen Fixation in the Ice Age Atlantic Ocean. *Science* **2009**, *323*, 244−248.
(472) Straub, M.; Sigman, D. M.; Ren, H.; Martinez-Garcia, A.; Meckler, A. N.; Hain, M. P.; Haug, G. H. Changes in North Atlantic Nitrogen Fixation Controlled by Ocean Circulation. *Nature* **2013**, *501*, 200−203.
(473) Ren, H.; Sigman, D. M.; Martínez-García, A.; Anderson, R. F.; Chen, M.-T.; Ravelo, A. C.; Straub, M.; Wong, G. T. F.; Haug, G. H. Impact of Glacial/Interglacial Sea Level Change on the Ocean Nitrogen Cycle. *Proc. Natl. Acad. Sci. U. S. A.* **2017**, *114*, E6759−E6766.
(474) Altabet, M. A.; Pilskaln, C.; Thunell, R.; Pride, C.; Sigman, D.; Chavez, F.; Francois, R. The Nitrogen Isotope Biogeochemistry of Sinking Particles from the Margin of the Eastern North Pacific. *Deep Sea Res., Part I* **1999**, *46*, 655−679.
(475) Altabet, M. A. *Marine Organic Matter: Biomarkers, Isotopes and DNA. The Handbook of Environmental Chemistry*; Volkman, J. K., Ed.; Springer: Berlin, Heidelberg, 2005; Vol. *2N*.
(476) Sigman, D. M.; Haug, G. H. *Treatise on Geochemistry*; Holland, H. D.; Turekian, K. K., Eds.; Pergamon: Oxford, 2003. DOI: 10.1016/B0-08-043751-6/06118-1.
(477) Hain, M. P.; Sigman, D. M.; Haug, G. H. *Treatise on Geochemistry*, 2nd ed.; Holland, H. D.; Turekian, K. K., Eds.; Elsevier, 2014.
(478) Jaccard, S. L.; Hayes, C. T.; Martínez-García, A.; Hodell, D. A.; Anderson, R. F.; Sigman, D. M.; Haug, G. H. Two Modes of Change in Southern Ocean Productivity over the Past Million Years. *Science* **2013**, *339*, 1419−1423.
(479) Bianchi, D.; Dunne, J. P.; Sarmiento, J. L.; Galbraith, E. D. Data-Based Estimates of Suboxia, Denitrification, and N_2O Production in the Ocean and Their Sensitivities to Dissolved O_2. *Global Biogeochem. Cycles* **2012**, *26*, GB2009.
(480) Christensen, J. P. Carbon Export from Continental Shelves, Denitrification and Atmospheric Carbon Dioxide. *Cont. Shelf Res.* **1994**, *14*, 547−576.
(481) Blunier, T.; Barnett, B.; Bender, M. L.; Hendricks, M. B. Biological Oxygen Productivity During the Last 60,000 Years from Triple Oxygen Isotope Measurements. *Global Biogeochem. Cycles* **2002**, *16*, 3−1−3−13.
(482) Jaccard, S. L.; Galbraith, E. D.; Frölicher, T. L.; Gruber, N. Ocean (De)Oxygenation across the Last Deglaciation: Insights for the Future. *Oceanography* **2014**, *27*, 26−35.
(483) Ganeshram, R. S.; Pedersen, T. F.; Calvert, S.; François, R. Reduced Nitrogen Fixation in the Glacial Ocean Inferred from Changes in Marine Nitrogen and Phosphorus Inventories. *Nature* **2002**, *415*, 156−159.
(484) Eugster, O.; Gruber, N.; Deutsch, C.; Jaccard, S. L.; Payne, M. R. The Dynamics of the Marine Nitrogen Cycle across the Last Deglaciation. *Paleoceanography* **2013**, *28*, 116−129.
(485) Menge, D. N.; Levin, S. A. Spatial Heterogeneity Can Resolve the Nitrogen Paradox of Tropical Forests. *Ecology* **2017**, *98*, 1049−1061.
(486) Menge, D. N. L.; Chazdon, R. L. Higher Survival Drives the Success of Nitrogen-Fixing Trees through Succession in Costa Rican Rainforests. *New Phytol.* **2016**, *209*, 965−977.
(487) Reich, P. B.; Oleksyn, J. Global Patterns of Plant Leaf N and P in Relation to Temperature and Latitude. *Proc. Natl. Acad. Sci. U. S. A.* **2004**, *101*, 11001−11006.
(488) DeLuca, T. H.; Zackrisson, O.; Gundale, M. J.; Nilsson, M.-C. Ecosystem Feedbacks and Nitrogen Fixation in Boreal Forests. *Science* **2008**, *320*, 1181.
(489) Jarvis, P.; Linder, S. Constraints to Growth of Boreal Forests. *Nature* **2000**, *405*, 904−905.
(490) Winbourne, J. B.; Brewer, S. W.; Houlton, B. Z. Iron Controls over Di-Nitrogen Fixation in Karst Tropical Forest. *Ecology* **2017**, *98*, 773−781.
(491) Kraepiel, A. M. L.; Bellenger, J. P.; Wichard, T.; Morel, F. M. M. Multiple Roles of Siderophores in Free-Living Nitrogen-Fixing Bacteria. *BioMetals* **2009**, *22*, 573−581.
(492) Perakis, S. S.; Pett-Ridge, J. C. Nitrogen-Fixing Red Alder Trees Tap Rock-Derived Nutrients. *Proc. Natl. Acad. Sci. U. S. A.* **2019**, *116*, 5009−5014.
(493) Barron, A. R.; Wurzburger, N.; Bellenger, J. P.; Wright, S. J.; Kraepiel, A. M. L.; Hedin, L. O. Molybdenum Limitation of Asymbiotic Nitrogen Fixation in Tropical Forest Soils. *Nat. Geosci.* **2009**, *2*, 42−45.
(494) Jean, M.-E.; Phalyvong, K.; Forest-Drolet, J.; Bellenger, J.-P. Molybdenum and Phosphorus Limitation of Asymbiotic Nitrogen Fixation in Forests of Eastern Canada: Influence of Vegetative Cover and Seasonal Variability. *Soil Biol. Biochem.* **2013**, *67*, 140−146.
(495) Franklin, O.; Näsholm, T.; Högberg, P.; Högberg, M. N. Forests Trapped in Nitrogen Limitation-an Ecological Market Perspective on Ectomycorrhizal Symbiosis. *New Phytol.* **2014**, *203*, 657−666.
(496) Sipler, R. E.; Gong, D.; Baer, S. E.; Sanderson, M. P.; Roberts, Q. N.; Mulholland, M. R.; Bronk, D. A. Preliminary Estimates of the Contribution of Arctic Nitrogen Fixation to the Global Nitrogen Budget. *Limnol. Oceanogr. Lett.* **2017**, *2*, 159−166.
(497) Field, C. B.; Behrenfeld, M. J.; Randerson, J. T.; Falkowski, P. Primary Production of the Biosphere: Integrating Terrestrial and Oceanic Components. *Science* **1998**, *281*, 237−240.
(498) Keenan, T. F.; Williams, C. A. The Terrestrial Carbon Sink. *Annu. Rev. Environ. Resour.* **2018**, *43*, 219−243.
(499) Ciais, P.; Sabine, C.; Bala, G.; Bopp, L.; Brovkin, V.; Canadell, J.; Chhabra, A.; DeFries, R.; Galloway, J.; et al. In *Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change*; Stocker, T. F.; Qin, D.; Plattner, G.-K.; Tignor, M.; Allen, S. K.;

https://dx.doi.org/10.1021/acs.chemrev.9b00613
Chem. Rev. 2020, 120, 5308−5351

Boschung, J.; Nauels, A.; Xia, Y.; Bex, V.; Midgley, P. M., Eds.; Cambridge University Press: Cambridge, 2013.
(500) Nadelhoffer, K. J.; Emmett, B. A.; Gundersen, P.; Kjønaas, O. J.; Koopmans, C. J.; Schleppi, P.; Tietema, A.; Wright, R. F. Nitrogen Deposition Makes a Minor Contribution to Carbon Sequestration in Temperate Forests. *Nature* **1999**, *398*, 145−148.
(501) Harpole, W. S.; Ngai, J. T.; Cleland, E. E.; Seabloom, E. W.; Borer, E. T.; Bracken, M. E. S.; Elser, J. J.; Gruner, D. S.; Hillebrand, H.; Shurin, J. B.; et al. Nutrient Co-Limitation of Primary Producer Communities. *Ecol. Lett.* **2011**, *14*, 852−862.
(502) Erisman, J. W.; Galloway, J.; Seitzinger, S.; Bleeker, A.; Butterbach-Bahl, K. Reactive Nitrogen in the Environment and Its Effect on Climate Change. *Curr. Opin. Environ. Sustain.* **2011**, *3*, 281−290.
(503) Liu, L.; Greaver, T. L. A Global Perspective on Belowground Carbon Dynamics under Nitrogen Enrichment. *Ecol. Lett.* **2010**, *13*, 819−828.
(504) Vivanco, L.; Austin, A. T. Nitrogen Addition Stimulates Forest Litter Decomposition and Disrupts Species Interactions in Patagonia, Argentina. *Global Change Biol.* **2011**, *17*, 1963−1974.
(505) Magill, A. H.; Aber, J. D.; Currie, W. S.; Nadelhoffer, K. J.; Martin, M. E.; McDowell, W. H.; Melillo, J. M.; Steudler, P. Ecosystem Response to 15 Years of Chronic Nitrogen Additions at the Harvard Forest LTER Massachusetts, USA. *For. Ecol. Manage.* **2004**, *196*, 7−28.
(506) Treseder, K. K. A. Meta-Analysis of Mycorrhizal Responses to Nitrogen, Phosphorus, and Atmospheric CO_2 in Field Studies. *New Phytol.* **2004**, *164*, 347−355.
(507) Frey, S. D.; Ollinger, S.; Nadelhoffer, K. e. a.; et al. Chronic Nitrogen Additions Suppress Decomposition and Sequester Soil Carbon in Temperate Forests. *Biogeochemistry* **2014**, *121* (2), 305−316.
(508) Greaver, T. L.; Clark, C. M.; Compton, J. E.; Vallano, D.; Talhelm, A. F.; Weaver, C. P.; Band, L. E.; Baron, J. S.; Davidson, E. A.; et al. Key Ecological Responses to Nitrogen Are Altered by Climate Change. *Nat. Clim. Change* **2016**, *6*, 836−843.
(509) Chen, J.; Luo, Y.; van Groenigen, K. J.; Hungate, B. A.; Cao, J.; Zhou, X.; Wang, R.-w. A Keystone Microbial Enzyme for Nitrogen Control of Soil Carbon Storage. *Science Advances* **2018**, *4*, No. eaaq1689.
(510) Zak, D. R.; Argiroff, W. A.; Freedman, Z. B.; Upchurch, R. A.; Entwistle, E. M.; Romanowicz, K. J. Anthropogenic N Deposition, Fungal Gene Expression, and an Increasing Soil Carbon Sink in the Northern Hemisphere. *Ecology* **2019**, *100*, No. e02804.
(511) Mack, M. C.; Schuur, E. A. G.; Bret-Harte, M. S.; Shaver, G. R.; Chapin, F. S. Ecosystem Carbon Storage in Arctic Tundra Reduced by Long-Term Nutrient Fertilization. *Nature* **2004**, *431*, 440−443.
(512) Bragazza, L.; Freeman, C.; Jones, T.; Rydin, H.; Limpens, J.; Fenner, N.; Ellis, T.; Gerdol, R.; Hájek, M.; et al. Atmospheric Nitrogen Deposition Promotes Carbon Loss from Peat Bogs. *Proc. Natl. Acad. Sci. U. S. A.* **2006**, *103*, 19386−19389.
(513) Johnson, L. C.; Shaver, G. R.; Cades, D. H.; Rastetter, E.; Nadelhoffer, K.; Giblin, A.; Laundre, J.; Stanley, A. Plant Carbon-Nutrient Interactions Control CO_2 Exchange in Alaskan Wet Sedge Tundra Ecosystems. *Ecology* **2000**, *81*, 453−469.
(514) Wieder, W. R.; Cleveland, C. C.; Smith, W. K.; Todd-Brown, K. Future Productivity and Carbon Storage Limited by Terrestrial Nutrient Availability. *Nat. Geosci.* **2015**, *8*, 441−444.
(515) Fleischer, K.; Dolman, A. J.; van der Molen, M. K.; Rebel, K. T.; Erisman, J. W.; Wassen, M. J.; Pak, B.; Lu, X.; Rammig, A.; Wang, Y.-P. Nitrogen Deposition Maintains a Positive Effect on Terrestrial Carbon Sequestration in the 21st Century Despite Growing Phosphorus Limitation at Regional Scales. *Global Biogeochem. Cycles* **2019**, *33*, 810−824.
(516) Sigman, D. M.; McCorkle, D. C.; Martin, W. R. The Calcite Lysocline as a Constraint on Glacial/Interglacial Low-Latitude Production Changes. *Global Biogeochem. Cycles* **1998**, *12*, 409−427.
(517) Paerl, H. W. Why Does N-Limitation Persist in the World's Marine Waters? *Mar. Chem.* **2018**, *206*, 1−6.
(518) Krishnamurthy, A.; Moore, J. K.; Zender, C. S.; Luo, C. Effects of Atmospheric Inorganic Nitrogen Deposition on Ocean Biogeochemistry. *J. Geophys. Res.* **2007**, *112*, G02019.
(519) Studer, A. S.; Sigman, D. M.; Martínez-García, A.; Thöle, L. M.; Michel, E.; Jaccard, S. L.; Lippold, J. A.; Mazaud, A.; Wang, X. T.; Robinson, L. F.; et al. Increased Nutrient Supply to the Southern Ocean During the Holocene and Its Implications for the Pre-Industrial Atmospheric CO_2 Rise. *Nat. Geosci.* **2018**, *11*, 756−760.
(520) Sarmiento, J. L.; Hughes, T. M. C.; Stouffer, R. J.; Manabe, S. Simulated Response of the Ocean Carbon Cycle to Anthropogenic Climate Warming. *Nature* **1998**, *393*, 245−249.
(521) Bernardello, R.; Marinov, I.; Palter, J. B.; Sarmiento, J. L.; Galbraith, E. D.; Slater, R. D. Response of the Ocean Natural Carbon Storage to Projected Twenty-First-Century Climate Change. *J. Clim.* **2014**, *27*, 2033−2053.
(522) Ito, T.; Bracco, A.; Deutsch, C.; Frenzel, H.; Long, M.; Takano, Y. Sustained Growth of the Southern Ocean Carbon Storage in a Warming Climate. *Geophys. Res. Lett.* **2015**, *42*, 4516−4522.
(523) Sarmiento, J. L.; Toggweiler, J. R. A New Model for the Role of the Oceans in Determining Atmospheric pCO_2. *Nature* **1984**, *308*, 621−624.
(524) Solomon, S.; Plattner, G.-K.; Knutti, R.; Friedlingstein, P. Irreversible Climate Change Due to Carbon Dioxide Emissions. *Proc. Natl. Acad. Sci. U. S. A.* **2009**, *106*, 1704−1709.

https://dx.doi.org/10.1021/acs.chemrev.9b00613
Chem. Rev. 2020, 120, 5308−5351