

CURRENT EVIDENCE

Nitrogen fixation: A poorly understood process along the freshwater-marine continuum

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Scientific Significance Statement

Nitrogen fixation, the conversion of di-nitrogen (N₂) gas to reactive nitrogen (N) by specialized microbes, plays an essential role in Earth's nitrogen cycle. Research in marine ecosystems has shown that the organisms who carry out this process are widely distributed. Yet, the rates and controls of N₂ fixation in other aquatic ecosystems (i.e., lakes, rivers, streams, wetlands, and estuaries) have been vastly understudied and their ecological roles are poorly understood. We seek to promote new research to advance and transform understanding of the biodiversity, ecological controls, and cumulative contributions of N₂ fixation across inland and coastal aquatic ecosystems. Addressing N₂ fixation along the freshwater to marine continuum is necessary to understand and predict the future of global N cycling.

Abstract

N₂ fixation is a major component of the global N cycle and has been extensively studied in open-ocean and terrestrial ecosystems. Yet rates and ecological dynamics remain virtually unknown for the inland and coastal aquatic ecosystems (lakes, wetlands, rivers, streams, and estuaries) that connect terrestrial and marine biomes. This is due to the diversity of these habitats as well as the traditional paradigm that N₂ fixation rates were low to nonexistent, and therefore not important, in these ecosystems. We identify three major research themes to advance understanding of aquatic N₂ fixation: (1) the biological diversity of diazotrophs and variability of N₂ fixation rates, (2) the ecological stoichiometry of N₂ fixation, and (3) the upscaling of N₂ fixation rates from genes to ecosystems. Coordinating research across these areas will advance limnology and oceanography by fully integrating N₂ fixation into ecological dynamics of aquatic ecosystems from local to global scales.

Biological N₂ fixation, the microbial conversion of di-nitrogen (N₂) gas to biologically reactive ammonium, is a fundamental biogeochemical process that facilitated the great

evolutionary events from which all modern biodiversity evolved. The availability of biologically reactive N limits productivity in ecosystems across the globe, as it likely has since

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the earliest development of life on earth (Stüeken et al. 2016). Biological N_2 fixation evolved ca. 3.2 billion years ago (bya) (Stüeken et al. 2015), prior to the evolution of oxygenic photosynthesis 2.95 bya and the Great Oxidation Event 2.5–2.3 bya (Lyons et al. 2014), and likely helped to overcome constraints to carbon fixation and biomass production for the earliest microbial producers on earth. The origin of the nitrogenase enzyme which catalyzes N_2 fixation largely remains a mystery (Boyd and Peters 2013), but its structure has been highly conserved. Evidence suggests that molybdenum-nitrogenase existed 3.2 bya (Stüeken et al. 2015), with vanadium (V) and iron (Fe)-based nitrogenases emerging later (Boyd and Peters 2013). Although each form of nitrogenase has different activation energies and efficiencies, all organisms capable of N_2 fixation require substantial energy to carry out the process (Hoffman et al. 2014), and they must protect nitrogenase from oxygen inhibition. Today, the nitrogenase enzymes are limited to specialized groups of prokaryotes (Zehr et al. 2003), collectively referred to as diazotrophs. Dozens of bacterial genera have the genetic coding to equip them to fix N_2 , and they typically can be classified as either symbionts with vascular plants or algae (Prechtel et al. 2004; Brouwer et al. 2017; Zehr and Capone 2020), heterocytous or non-heterocytous cyanobacteria (Whitton 2012), or free-living heterotrophic bacteria (Smercina et al. 2019).

Biological N_2 fixation is a key control point in the modern global N cycle. Currently, N_2 fixation represents the largest natural source of fixed N globally, and only in the past few decades has anthropogenic N_2 fixation via the Haber-Bosch processes grown to represent 45% of the total N fixed globally (Canfield et al. 2010; Schlesinger and Bernhardt 2013). Importantly, it appears N_2 fixation can occur at rates high enough to impact ecosystem N budgets at local to global scales (Fulweiler

et al. 2007; Zehr and Capone 2020). Yet, because N_2 fixation influences the availability of a key limiting nutrient for biological activity (Vitousek et al. 2010; Zehr and Capone 2020), even low rates of this process can directly and/or indirectly influence ecological interactions throughout the biosphere. N_2 fixation controls the nutrient limiting primary producers at individual to ecosystem scales (Marcarelli and Wurtsbaugh 2006; Welter et al. 2015; Higgins et al. 2017), supports coevolved microbial consortia that couple cycling of carbon (C), N, sulfur (S), and other key elements (Bertics et al. 2010; Fulweiler et al. 2013; Dekas et al. 2018), and contributes to ecological interactions and symbioses among diazotrophs and a myriad of other organisms across all domains of life (Chapin et al. 1994; Foster and Zehr 2019; Zilius et al. 2020). We are only starting to understand the ways that diverse diazotrophs contribute to biogeochemical and ecological dynamics across global ecosystems, and the factors that control this process in space and time. Research in ocean ecosystems in particular has identified the important role of (1) the biological diversity of both autotrophic and heterotrophic microbes that fix N_2 (Zehr et al. 2003; Moisaner et al. 2010, 2017), (2) the stoichiometric constraints and regulation of N_2 fixation (Knapp 2012; Inomura et al. 2018; Wang et al. 2019), and (3) the importance of appropriate quantitative approaches for scaling N_2 fixation between local, regional, and global scales (Landolfi et al. 2018).

Despite the explosion of recent understanding of rates and controls of N_2 fixation on land and in the sea, the aquatic landscapes or aquascapes (e.g., lakes, wetlands, streams, rivers, and estuaries) in between have largely gone ignored. Oceanographers and terrestrial ecologists have studied the role of N_2 fixation in modern ecosystems more intensively than inland and coastal aquatic scientists (Fig. 1). The inequity of research across inland and coastal aquatic ecosystems may stem from their tremendous physical and ecological diversity compared to the open oceans and continents, which makes generalizing across these environments challenging. It may also be a product of early studies and syntheses reporting low N_2 fixation rates in these ecosystems (Howarth et al. 1988)—which is likely due to methodological issues as well as high spatial and temporal variability within and across aquatic ecosystems. Essentially, a generation of aquatic scientists was told N_2 fixation was not important and thus it need not be measured. Research on N_2 fixation in lakes, streams, and wetlands has been dominated by measuring how autotrophic N_2 fixation rates depend on the stoichiometric imbalance of N and phosphorus (P) (Maranger et al. 2018). Observations from microcosm to whole-lake enrichment experiments (Schindler et al. 2008; Paerl et al. 2016) and along natural gradients of N and P availability and/or enrichment (Eberhard et al. 2018; Scott et al. 2019) have been used to build conceptual models of where and when N_2 fixation may be important. Yet, this research lacks a fully developed theoretical framework on the underlying controls of N_2 fixation, and does not reflect the nutrient regeneration-uptake regime that dominates most natural ecosystems (Paerl et al. 2016). Therefore, we cannot answer

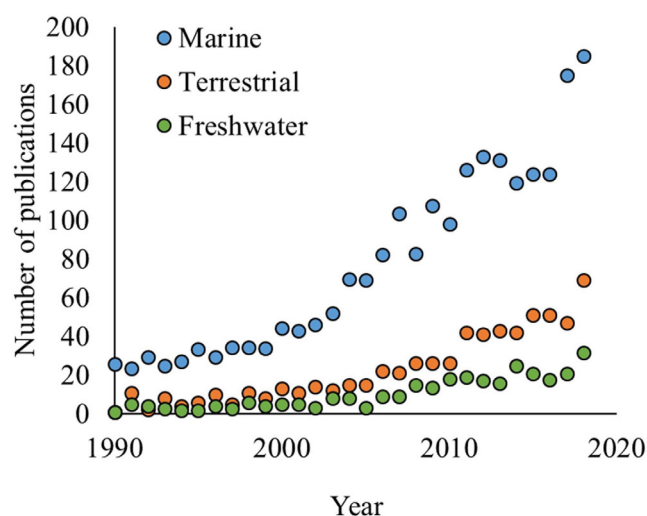


Fig. 1. Twenty-eight-year publication history on N_2 fixation in marine, terrestrial and freshwater environments derived from Web of Science with the topical search string “nitrogen fixation” * “marine,” “terrestrial,” or “freshwater.”

basic questions about the role of N_2 fixation in these waters, such as how this process supports spatial and temporal variability in primary and secondary production, when and where autotrophic and heterotrophic diazotrophs contribute to the N cycle in inland waters, and how rates may be extrapolated to regional and global scales.

We are poised now to ask how N_2 fixation varies among aquatic habitats across the aquascape due to patterns of biodiversity, nutrient delivery and transport, recycling and uptake affinity, and nutrient removal processes (Knapp 2012; Fulweiler et al. 2013; Eberhard et al. 2018). In order to reframe the paradigm of N_2 fixation in aquatic ecosystems, we outline here current understanding and future opportunities across three major research themes: (1) the biological diversity of N_2 -fixers and the environmental controls on N_2 fixation, (2) the ecological stoichiometry of N_2 fixation, and (3) scaling N_2 fixation from genes to ecosystems. Addressing these critical research themes would transform our understanding of aquatic primary and secondary production, biogeochemical cycling, and provide a mechanism for upscaling rates and forecasting for ecosystem change.

Biological diversity of diazotrophs and variability of N_2 fixation rates

Recent decades of research in marine ecosystems have demonstrated that both autotrophic and heterotrophic N_2 fixation are more widespread than traditionally thought and are occurring under varied and sometimes unexpected environmental conditions (Benavides et al. 2018; Zehr and Capone 2020). Cyanobacteria have been the primary focus of N_2 fixation research across the freshwater–marine continuum and currently are thought to account for most of the N_2 fixation occurring in marine (Montoya et al. 2004; Shiozaki et al. 2014) and freshwater environments (Oliver et al. 2012). However, it has long been hypothesized that heterotrophic bacteria evolved the ability to fix N_2 prior to the evolution of oxygenic photosynthesis (Raymond et al. 2004; Schlesinger and Bernhardt 2013), and heterotrophic prokaryotes have recently been shown to account for substantial proportions of N_2 fixation in the ocean (Benavides et al. 2018; Dekas et al. 2018). Couple this with the increasing evidence for the importance of heterotrophic N_2 fixation in both oxygen-depleted and oxygen-replete water columns (Fernandez et al. 2011; Bentzon-Tilia et al. 2015) as well as aquatic sediments (Fulweiler et al. 2007; Grantz et al. 2012; Dekas et al. 2018), and it becomes clear that we have a tremendous amount to learn about the phylogenetic diversity and functional importance of heterotrophic diazotrophs.

Over the last two decades, studies across a range of aquatic habitats have employed new techniques and linked microbial community composition and activity to rate measurements to elucidate the environmental and ecological controls on N_2 fixation rates and their contributions to ecosystem N budgets

(Zehr and Capone 2020). Quantification of *nif* gene abundance and expression has demonstrated that widely distributed unicellular cyanobacteria fix N_2 at high rates (Zehr and Turner 2001; Montoya et al. 2004; Moisander et al. 2010). Molecular techniques have also highlighted the potential importance of noncyanobacterial diazotrophs in the pelagic ocean and in coastal sediments (Brown and Jenkins 2014; Newell et al. 2016). Methodological issues related to gas dissolution and contamination have plagued ^{15}N measurements and the acetylene reduction assay (ARA), resulting in inaccurate, often underestimated rates of N_2 fixation (Mohr et al. 2010; Wilson et al. 2012). Moreover, acetylene toxicity alters heterotrophic sediment communities, including diazotrophs, suggesting that ARA may be an especially poor method for measuring N_2 fixation rates in benthic environments (Fulweiler et al. 2015). It is also important to note that these techniques may report net, actual, or potential rates depending on the incubation and measurement techniques, which complicates efforts to compare rates across habitats (Fig. 2a).

An additional challenge with understanding the distribution of N_2 fixation in the environment is that rates are typically measured over minutes to hours and in small-scale enclosures, and rates can be highly variable depending on the temporal and spatial scale of study. Spatial heterogeneity and/or “hot spots” where ideal environmental conditions for the process occur as have been observed to be caused by animal burrows (Bertics et al. 2010), cyanobacterial blooms, benthic mats and biofilms (Grimm and Petrone 1997; Eberhard et al. 2018), and animal microbiomes (Zilius et al. 2020; Fig. 2b). Temporal heterogeneity in N_2 fixation emerges as physiological responses of diazotrophs to environmental drivers occur in minutes to hours, while population-scale variation occurs across days to weeks (Marcarelli and Wurtsbaugh 2006; Masuda et al. 2018), and community and ecosystem variation occurs over seasons and among years (Lee and Joye 2006; Olofsson et al. 2020). N_2 fixation rates may show diel cycles that peak during the day or at night, depending on the energetics of the diazotroph and the adaptations they have evolved to protect nitrogenase from deactivation by oxygen (Grimm and Petrone 1997; Frischkorn et al. 2018; Masuda et al. 2018; Fig. 2c). Because of the short time scale of most measurements and the high variability in rates, comparing rates among studies and across habitats requires substantial environmental data and/or numerous assumptions. This issue is exemplified in the review by Howarth et al. (1988) where they report average annual N_2 fixation rates (as $g\ N\ m^{-2}\ y^{-1}$) from approximately 75 studies published between 1970 and 1987 and required almost 2000 words in table footnotes to explain the assumptions for the different scaling approaches. Some of these assumptions were as simple as assuming a relevant duration for an hourly areal rate, while others required greater extension in converting volumetric to areal rates.

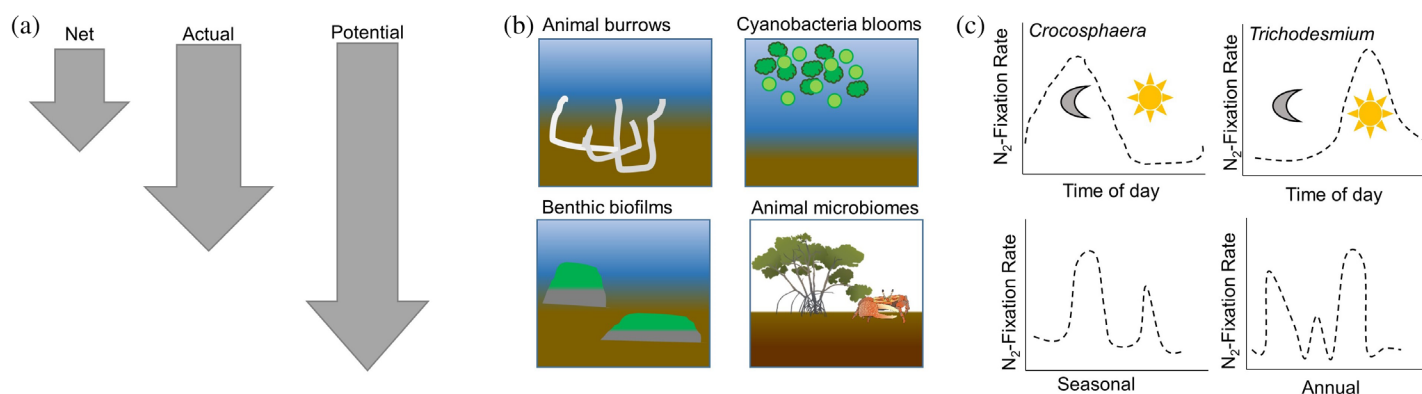


Fig. 2. High variability in reported rates is likely the result of a variety of factors, none of which are mutually exclusive. Variability may arise from differences in (a) measurement techniques which report net (e.g., membrane-inlet mass spectrometry), actual (e.g., direct ¹⁵N tracer techniques) or potential rates (e.g., ARAs); (b) spatial heterogeneity from “hot spots”; and (c) temporal variability from days to years (crab image from <https://ian.umces.edu/imagelibrary/displayimage-6420.html>; mangrove image from: <https://ian.umces.edu/imagelibrary/displayimage-4579.html>).

Advancing understanding of N₂ fixation across aquatic habitats requires a comprehensive categorization of the organisms who carry out the processes, their energetic and ecological strategies, and their sensitivities to environmental constraints. Synthesis focused on the drivers and controls of aquatic N₂ fixation needs to account for methodological differences, as well as spatial and temporal variability in the reported N₂ fixation rates (Fig. 2). Future research should involve integrating rate measurements with metagenomics and metatranscriptomic information that links this biogeochemical process to specific microbial populations or communities. Such understanding is essential to advance the next research theme, enabling individual to population-level modeling of N₂ fixation using ecological stoichiometry.

Ecological stoichiometry of N₂ fixation

Ecological stoichiometry integrates organismal nutrient requirements with the distribution, supply, and recycling of nutrients in their environment (Stern and Elser 2002). This discipline can help elucidate nutrient limitation and control of organismal growth rates and life history patterns (Elser et al. 2003), and provide insights into the dynamic composition and function of communities through time and space (Evans-White and Halvorson 2017). The strength of ecological stoichiometry lies in a common framework for integrating energy and material constraints, which is necessary to understand and model patterns of N₂ fixation in the environment (Welter et al. 2015). To achieve this goal, we must first build a conceptual model that moves beyond the stoichiometric imbalance of N : P to integrate the full suite of nutrients that could control and constrain the organisms that fix N₂ (Table 1). Here, we consider three different types of nutrient interactions with N₂ fixation: synthesis of the nitrogenase enzyme, energetic supply to carry out the N₂ fixation process, and supply of nutrients to build biomass using fixed N.

Nitrogenase is a two-component enzyme, with both components featuring active clusters or co-factors that are built around trace metals (Hoffman et al. 2014). The most common form of nitrogenase consists of a molybdenum (Mo)-Fe protein and an electron-transfer Fe protein. Therefore, synthesis of nitrogenase relies on supply of Mo and Fe, as well as C, N, and S, which form the base structure of the enzyme. Unsurprisingly, Mo has been identified as a limiting factor for N₂ fixation in the open and coastal ocean, and the supply of Fe has been identified as a key factor controlling the spatial pattern and overall contributions of N₂ fixation to lake, marine, and global ocean budgets (Mills et al. 2004; Glass et al. 2010). More recently, Fe has been linked to abundance of both cyanobacteria and nonphotosynthetic bacteria in streams (Larson et al. 2018). Access to Fe is determined by the environmental redox conditions that control the solubility and biological uptake rates (Ginn et al. 2017), as well as the availability of chelators including certain forms of dissolved organic matter (Nagai et al. 2006). The less common alternate forms of nitrogenase substitute either Fe or V for Mo in the MoFe protein, coupled with the same electron-transfer Fe protein (Hoffman et al. 2014), and with all three forms of nitrogenase exhibiting similar mechanisms and requirements for reactivity (Eady 1996). Therefore, it is reasonable to assume that organisms using these forms of nitrogenase would be similarly limited by environmental V and Fe supply. Nitrogenase may have high turnover rates in cells, with synthesis, inactivation, and degradation happening over periods as short as 24 h (Capone et al. 1990). Therefore, nitrogenase synthesis should also have a high P demand, due to amino acids required to carry out transcription and translation (Stern and Elser 2002) from the suite of six or more *nif* genes that encode for the various components of nitrogenase (Boyd and Peters 2013).

N₂ fixation is energy-intensive, requiring 16 ATP for reduction of N₂ to two NH₃ molecules (Schlesinger and Bernhardt 2013; Hoffman et al. 2014); therefore, N₂ fixation is also dependent on

Table 1. A summary of elemental roles in N₂ fixation.

Element	Nitrogenase synthesis	Energetic constraints	Biomass synthesis
Fe	Fe and MoFe protein in all forms of nitrogenase (Mo-, Fe-, V-)	Photosynthetic electron transport (photosystem I and II); energetics of Fe reducers	—
Mo	MoFe protein in Mo-type nitrogenase	—	—
V	VFe protein in V-type nitrogenase	—	—
P	Transcription/translation of nitrogenase enzyme	ATP hydrolysis; necessary for substrate reduction	Structural component - nucleic acids; biomass building and growth
C	Structural component	Energetics - photosynthesis for autotrophs, organic matter supply for heterotrophs	Structural component; heterocytes have extra glycolipid and polysaccharide layer; secondary metabolites – toxin production
S	Structural component	Energetics of S chemoautotrophs	Structural component – amino acids
N	Structural component	Chlorophyll <i>a</i> synthesis for photoautotrophs	Structural component – amino acids, nucleic acids; Secondary metabolites – toxin production

biochemical processes and energetic nutrient transformations. Most directly, N₂ fixation will be limited by the supply of P for ATP hydrolysis as part of the nitrogenase mechanism. Indirectly, N₂ fixation rates will be dependent on whatever energetic pathways the diazotroph uses to fuel its metabolism. For autotrophs, this means that any nutrients that limit primary productivity (e.g., N, P, Fe, and other trace metals) may indirectly limit N₂ fixation. Although not a nutrient, this also means that N₂ fixation by autotrophic diazotrophs may be inextricably linked to diel and seasonal patterns in light availability. Heterotrophic diazotroph metabolism is not just dependent on the supply of organic matter but also the quality (e.g., composition and biodegradability) of the organic material (Fulweiler et al. 2007). Furthermore, chemotrophic diazotrophs may couple nutrient cycles by using alternate substrates or terminal electron acceptors like SO₄ or Fe (Fulweiler et al. 2013; Dekas et al. 2018).

Finally, like all organisms, diazotrophs need varying compositions of elements to build their biomass, and those needs will vary based on their specific life-history strategies (Sterner and Elser 2002). Although diazotrophs are often assumed to exhibit stoichiometric homeostasis, particularly with regard to biomass N, they are also autotrophs which typically exhibit remarkable stoichiometric plasticity (Osburn et al. 2021). Similarly, aquatic heterotrophic microbes can be unexpectedly flexible in their P content, leading to a wide range of C : P stoichiometries across a range of taxa (Godwin and Cotner 2015). Among cyanobacteria, the various strategies and adaptations they have evolved to protect nitrogenase from oxygen deactivation and to deter grazers may also influence their biomass stoichiometry. For example, heterocyte cell walls have extra glycolipid and polysaccharide layers to reduce gas permeability

(Flores and Herrero 2005), which will increase the C content and/or demand of those cells. Other cyanobacteria produce toxic secondary metabolites, which require both C and N for production (Van der Waal et al. 2009).

Building a mechanistic, stoichiometric understanding of diazotrophs can provide a path toward more accurate upscaling of rates in aquatic ecosystems. Zheng et al. (2019) recently demonstrated the power of stoichiometric imbalance for predicting N₂ fixation rates using ecosystem-scale manipulations of terrestrial forests. Similar models have not been developed for the diverse autotrophic and heterotrophic diazotrophs of aquatic ecosystems, but developing them is fundamental to the next research theme of scaling N₂ fixation from genes to ecosystems.

Upscaling N₂ fixation from genes to ecosystems

Decades of research on Earth's oceans and terrestrial habitats has led to the development of scalable models to derive spatially explicit global estimates of N₂ fixation (Xu-Ri and Prentice 2017; Wang et al. 2019). In both cases, these models are based on: (1) the environmental conditions supporting the growth of diazotrophs using trait-based approaches linked to their habitats, and (2) the stoichiometric imbalance between C, N, P, and micronutrient availability and the relative biological demand for these resources for primary production. Models have yielded spatially explicit estimates of N₂ fixation that range from 0–5 g N m⁻² yr⁻¹ in the open ocean to 0–10 g N m⁻² yr⁻¹ in terrestrial ecosystems (Fig. 3a). However, these efforts to synthesize global and regional N cycling generally ignore N₂ fixation in freshwaters and the coastal

ocean because we lack the understanding of their spatial distribution and variability in rates, as well as the conceptual understanding and modeling tools necessary for accurate scaling.

The rates and spatial variability in N_2 fixation on the continents may be drastically underestimated by ignoring aquatic ecosystems. Global scaling of terrestrial N_2 fixation rates by Xu-Ri and Prentice (2017) results in relatively homogenous rates over continents, with low rates over much of the temperate, boreal, desert, and polar regions and higher rates in the tropics and sub-tropics (Fig. 3a). Yet, N_2 fixation rates in the aquatic ecosystems that are embedded in the terrestrial landscape, while spatially and temporally variable, can be quite high (e.g., desert stream Sycamore Creek: $8.4 \text{ mg N m}^{-2} \text{ h}^{-1}$, Grimm and Petrone 1997; coastal temperate Narragansett Bay: $3.5 \text{ mg N m}^{-2} \text{ h}^{-1}$, Fulweiler et al. 2007; warm temperate Lake Fayetteville: $5.1 \text{ mg N m}^{-2} \text{ h}^{-1}$, Grantz et al. 2012). Inland

water bodies cumulatively comprise substantial portions of the continental area (Fig. 3b). Globally, lakes and reservoirs $> 0.002 \text{ km}^2$ in area comprise $5 \times 10^6 \text{ km}^2$ (ca. 3%) of the Earth's nonglaciated land area, while rivers and streams comprise $7.7 \times 10^5 \text{ km}^2$ (ca. 0.6%), with lakes disproportionately distributed in the northern temperate and boreal zones, and rivers most abundant in tropical and northern boreal zones (Verpoorter et al. 2014; Allen and Pavelsky 2018). Coastal estuaries comprise another $1 \times 10^6 \text{ km}^2$, and the total continental shelf may be as much as 25 times that area (Rosentreter et al. 2021). Cumulatively, the contribution of diazotrophs in these habitats to global N_2 fluxes could be substantial even if rates are low and highly variable across those habitats, as has been shown for carbon dioxide and methane emissions (Raymond et al. 2013; Rosentreter et al. 2021).

Barriers to scaling of rates from small-scale measurements to global contributions are the extreme spatial diversity of inland and coastal aquatic habitats and resulting variability in environmental controls, as well as the diversity of organismal characteristics and ecological interactions outlined above. The aquatic habitats that select for diazotrophs are spatially diverse and dynamic, including the water column of lakes and estuaries, as well as the hard and soft benthic environments of streams, wetlands, lakes, and estuaries. For example, isolated freshwater wetlands can host free-living heterotrophic diazotrophs in their sediments that interact with diverse plant species (Rejmánková et al. 2018). These same wetlands can also be dominated by sediment-associated or floating cyanobacterial mats that include diazotrophs (Scott and Marcarelli 2012). The dominance of one condition or the other is controlled by the nutrient loading rates to the wetlands that constrain colonization by rooted aquatic macrophytes (Šantrůčková et al. 2010). Likewise, N_2 fixation may dynamically control other ecosystem processes. For example, N_2 fixation can be a major source of N to the biological communities of small streams (Marcarelli et al. 2008), and light availability controlled by terrestrial shading can control N inputs from N_2 fixation, leading to increasing concentrations of DON with increasing stream size (Finlay et al. 2011). Welter et al. (2015) showed that stream warming increased N_2 fixation which amplified the temperature dependence of primary production and ecosystem respiration by decreasing autotrophic N limitation. Thus, the ability to scale N_2 fixation is critical for predicting how ecosystems will respond to climate change and other anthropogenic forcing factors.

Numerous processes including atmospheric interactions, upland soil–water connections, hydrologic connectivity, water residence time, and internal nutrient cycling influence the stoichiometric imbalance of macronutrients and micronutrients available to diazotrophs across the aqulandscape. Moreover, the N biogeochemistry of these ecosystems is extremely complex and difficult to generalize. For example, $\geq 50\%$ of lakes experience a N deficit (relative to P) in the range of $0\text{--}80 \text{ g N m}^{-2} \text{ yr}^{-1}$, but $\leq 20\%$ of lakes achieve a net N_2 fixation rate as high as $0\text{--}10 \text{ g N m}^{-2} \text{ yr}^{-1}$, and N_2 fixation in most lakes never exceeds

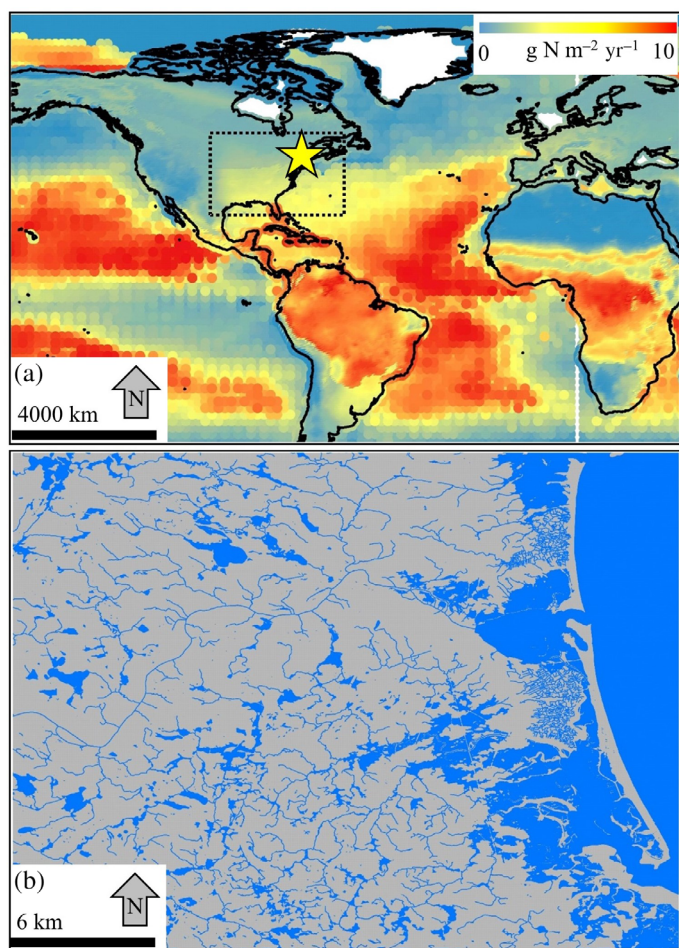


Fig. 3. (a) Spatially explicit global N_2 fixation rates adapted from Xu-Ri and Prentice (2017) for terrestrial ecosystems and Wang et al. (2019) for oceans; and (b) a regional scale example of the landscape-scale heterogeneity of inland and coastal habitats in the northeastern United States (located at star in a).

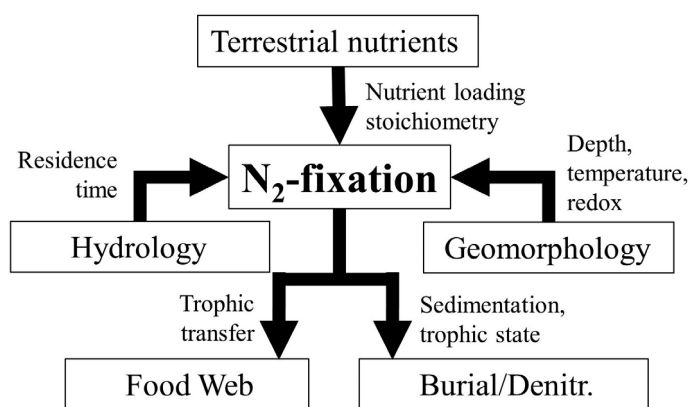


Fig. 4. Conceptual model for scaling N_2 fixation across aquascapes adapted from Seitzinger et al. (2006).

$2 \text{ mg N m}^{-2} \text{ yr}^{-1}$ (Scott et al. 2019). Instead, most lakes appear to be net sources of N_2 to the atmosphere through denitrification, which has been recently observed for a large number of lakes in the upper mid-western United States (Loeks-Johnson and Cotner 2020). Although some evidence has suggested the importance of aquatic food webs in the fate of fixed N_2 (Patoine et al. 2006), burial or denitrification may remove more N than is added annually through N_2 fixation (Higgins et al. 2017) and drive ecosystems toward perpetual N deficiency. But, these phenomena remain drastically understudied and require targeted research to address both temporal and spatial variability at the ecosystem scale.

These examples highlight the role of N_2 fixation as an ecological process carried out by microorganisms interacting with their environment and other life. Thus, scaling N_2 fixation requires information on diazotroph presence and identity across habitats, and their activity driven through ecological interactions which are driven by complex physical, chemical, and biological conditions (Fig. 4). Stoichiometric models, similar to those applied for marine and terrestrial habitats, or biodiversity scaling models (Locey and Lennon 2016) may be useful for estimating diazotroph presence or absence at large spatial scales. But, discerning variability in N_2 -fixation activity will also require a greater focus on selection and dispersal mechanisms controlled by physical drivers such as hydrology and geomorphology. These processes determine the conditions to favor N_2 fixation, but may not control the fate of the fixed N. Long-term storage of fixed N or its transformation back to atmospheric N_2 creates continued demand for new N_2 fixation.

Next steps

The common view of N_2 fixation as only a biogeochemical flux contributing to nutrient budgets has hamstrung our understanding of the potential for this process to influence ecosystem dynamics. Moreover, the proximate and ultimate

fate of fixed N_2 has not been constrained for diverse habitats across the aquascope. We suggest that the net effect of N_2 fixation should be considered in an ecological framework as a novel input of N carried out by a specialized group of organisms that reside in ecological communities. In this framework, the imbalance between the variable biological stoichiometry of diverse diazotrophs and the stoichiometry of environmental nutrient supply control N_2 fixation rates, but the fates of fixed N can change ecological interactions and ecosystem trajectories. Moreover, focusing on both the rate and fate of fixed N will more accurately constrain the process and will be more constructive for scaling processes from population-level sensitivities through community and ecosystem interactions and up to global fluxes. Together, we argue that this ecological framework will transform our understanding of N cycling by elucidating the fundamental role of N_2 fixation in aquatic ecosystems.

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