Global Biogeochemical Cycles

ADVANCING EARTH AND SPACE SCIENCES

COMMENTARY
10.1029/2023GB007777

Key Points:

- Coastal sediments provide ideal conditions for nitrogen fixation
- Sediment nitrogen fixation may take place for reasons unrelated to nitrogen limitation, such as allowing microbes to maintain intracellular redox balance
- Niche partitioning and microbial community relationships may provide helpful lenses in which to understand sediment nitrogen fixation

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Citation:
Fulweiler, R. W. (2023). More foxes than hedgehogs: The case for nitrogen fixation in coastal marine sediments. *Global Biogeochemical Cycles*, *37*, e2023GB007777. https://doi.org/10.1029/2023GB007777

Received 22 MAR 2023
Accepted 3 AUG 2023

More Foxes than Hedgehogs: The Case for Nitrogen Fixation in Coastal Marine Sediments

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Abstract Nitrogen fixation is an important process connecting the vast atmospheric pool of di-nitrogen gas to the biosphere. Nitrogen fixation is an energy intensive process, and it is historically thought to occur only to meet nitrogen demands. However, over the last two decades, research has demonstrated that sediment nitrogen fixation occurs in a variety of coastal environments, including those where reduced nitrogen is abundant. This can be met with skepticism when nitrogen fixation is viewed solely as a nitrogen limitation relief mechanism. Here, I propose that coastal sediments are actually ideal environments for nitrogen fixation and synthesize ideas on why this is the case. My goal is to help the community embrace a new paradigm for sediment nitrogen fixation and to see it as an important and even expected process. In doing so, I hope to motivate future research on the spatial and temporal rate dynamics of sediment nitrogen fixation as well as on the sediment nitrogen fixation community composition and activity.

Plain Language Summary Nitrogen fixation is a microbially driven process that transforms gaseous di-nitrogen gas into a bioavailable form of nitrogen that can promote growth. Typically, this process is thought to take place to help organisms meet their nitrogen demands in nitrogen deplete environments. Thus, in coastal sediments that are often rich in ammonium, it is thought that nitrogen fixation should not occur given the energetic costs. However, advances in methods have demonstrated that nitrogen fixation does occur in coastal sediments. Understanding the how and why of coastal sediment nitrogen fixation will help us better understand nitrogen cycling in general.

1. Main Text

Biological nitrogen fixation (or diazotrophy) is a microbially driven process that converts di-nitrogen gas (N_2) into ammonia, providing a bioavailable source of nitrogen for synthesizing proteins and nucleic acids. Nitrogen fixation can relieve nitrogen limitation and help support marine primary production. Over the last decade, we have made numerous advances in our understanding of the biodiversity, geochemical controls, and rates of marine nitrogen fixation (Zehr & Capone, 2020). However, the focus of much of these advances and subsequent reviews are on water column nitrogen fixation. Yet there is mounting evidence from rate measurements (e.g., Hou et al., 2018; Jabir et al., 2020) and molecular data (e.g., Kapili et al., 2020; Liesirova et al., 2023; Newell, McCarthy, et al., 2016) that benthic nitrogen fixation is an important process in marine systems with rates on par or even exceeding rates of denitrification (the microbial removal of bioavailable nitrogen). Despite this, our acceptance and understanding of benthic nitrogen fixation remain elusive. There is still significant disconnect between theory—when, why, and where benthic nitrogen fixation should take place, and reported observations. There are numerous reasons for this disconnect, including long-standing methodological issues, logistical and financial constraints which limit spatial and temporal rate measurements, a bias toward studying cyanobacteria, and a reticence to reject historical thinking (Marcarelli et al., 2022). We tend to think that nitrogen fixation is so energetically expensive that it would only be done to meet organismal nitrogen demands. But here, I argue that such thinking is limiting our full understanding of this process.

There is a parable attributed to the fourth century soldier and poet, Archilochus that describes the different tactics of survival employed by the fox and hedgehog: "*The fox knows many things, but the hedgehog knows one big thing*." I think this parable is useful in considering nitrogen fixation in marine sediments. It can be applied to both the researcher and the nitrogen fixer.

The researcher because we often look at the topic we study with one lens. In the case of nitrogen fixation, we default to the *one big thing*—nitrogen fixation occurs because nitrogen is limiting. This is what I call hedgehog-like

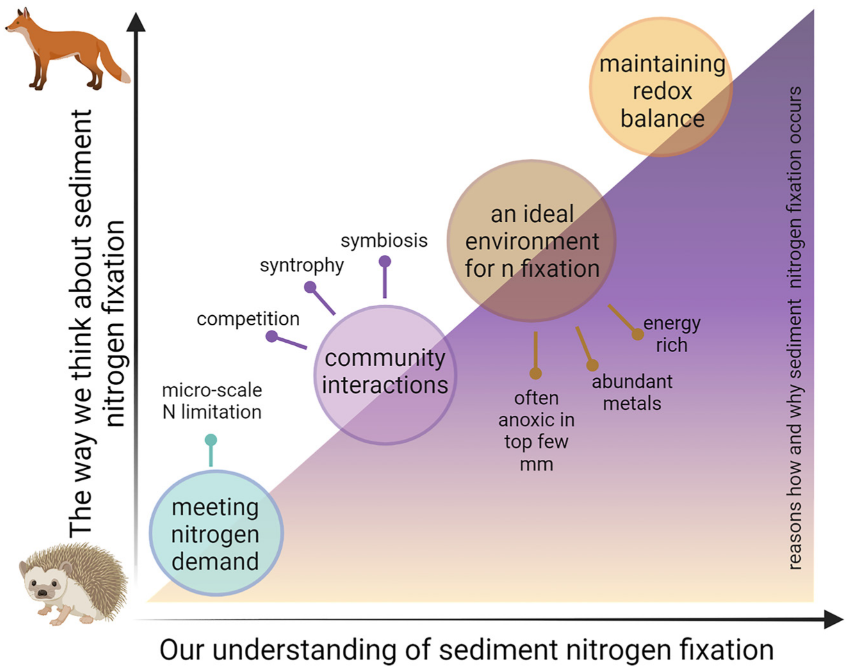

Figure 1. If sediment nitrogen fixation is viewed narrowly as taking place solely to relieve nitrogen demands (i.e., hedgehog thinking), then we limit our ability to understand why and when this process takes place. Alternatively, if we expand the scope beyond focusing on nitrogen availability as the primary driver of this process (i.e., fox thinking), we will improve our understanding of nitrogen fixation. Importantly, sediment nitrogen fixation may take place for a variety of co-occurring and not mutually exclusive reasons (shown here as a gradient within the triangle as well as circles with the ideas presented in the commentary). Created with BioRender.com.

thinking of the researcher. Instead, I want us to broaden our perspective and work to see nitrogen fixation through multiple lenses. This is what I call fox-like thinking of the researcher.

This parable applies to nitrogen fixers too. Of course, they do not "know" in the classic definition of the word. But if we think of "know" in terms of the reasons why a diazotroph fixes nitrogen, then some may indeed only fix nitrogen when nitrogen is limited. In this metaphor, those types of diazotrophs are the hedgehogs. Alternatively, other diazotrophs may fix nitrogen for many other reasons—these are the foxes.

Perhaps for too long we have been hedgehogs: imagining that nitrogen fixers are hedgehogs too. And while there may be hedgehogs amongst the diazotrophic community, I propose there are many more foxes. The purpose of this commentary is to help us shift our view, to urge us to think like foxes instead of hedgehogs, and in doing so to see nitrogen fixers as foxes instead of just hedgehogs (Figure 1). My hope then is that we can question and move beyond the common assumptions made about why it is surprising to observe sediment nitrogen fixation.

1.1. Coastal Sediments Provide Ideal Conditions for Nitrogen Fixation

Nitrogen fixation is an energy intensive process requiring at least 16 molecules of ATP and 8 electrons to reduce di-nitrogen gas to two molecules of ammonia (NH_3; Welsh, 2000; Sohm et al., 2011). However, the energetic cost ultimately becomes important when energy is limiting. This is a good example of how our hedgehog like approach to understanding nitrogen fixation has limited our understanding of where and when this process might occur. It is also a good example of our bias toward applying what we know about cyanobacteria to all nitrogen fixers. For example, cyanobacteria meet the energy demands of nitrogen fixation with sunlight and thus nitrogen fixation can be limited by light availability (Stal, 2015). In coastal sediments, however, it is unlikely that energy is limiting for non-cyanobacteria diazotrophs. For example, heterotrophic nitrogen fixation could be supported by high inputs of sediment organic matter. Being close to land, coastal sediments receive terrestrial sources of organic carbon as well as detritus from vegetated coastal ecosystems such as salt marshes, mangroves, and seagrasses. The shallowness of coastal systems also allows tight benthic-pelagic coupling—where primary production from the surface water is deposited to the sediments. Organic matter deposition is often enhanced by active filtration from coastal organisms such as shellfish. Further, organic matter production from microphytobenthos can be a new source of labile carbon to the sediments (Gattuso et al., 2006).

Importantly, the lability of organic matter might not ultimately matter to nitrogen fixers—as they can use a variety of organic matter sources. This is another good example of diazotrophs being more like foxes, that is, some are flexible in terms of what kind of carbon they can use and even where they derive their energy. For instance, active nitrogen fixation has been found to be associated with pulp and paper mill effluent (e.g., Gauthier et al., 2000), low-quality tree litter (Perez et al., 2010), and recalcitrant macroalgae (Raut & Capone, 2021). In both field observations and experimental manipulations, we saw higher rates of nitrogen fixation in sediments with higher C:N values, indicating lower quality organic matter (Fulweiler et al., 2007, 2013). Organic matter rich coastal sediments may also support fermentative nitrogen fixing (Gandy & Yoch, 1988; Huang et al., 2021; Streicher & Valentine, 1973). And finally, sediments are often rich in other elements (e.g., methane, reduced iron, sulfide, and hydrogen) that can support chemoautotrophic nitrogen fixation, a process now reported in numerous habitats including marine ecosystems (Desai et al., 2013; Zehr & Capone, 2020).

In addition to being rich in organic matter, sediments also contain abundant metals. This availability of metals is important for a variety of cellular processes, including the composition of nitrogenase (the enzyme that catalyzes nitrogen fixation). Nitrogenase is found in three homologous forms—each with a different metal in the active-site cofactor: Molybdate or Mo-nitrogenase, Vanadium or V-nitrogenase, and Iron or Fe-nitrogenase (Garcia et al., 2020 and references therein). To date, most environmental research has focused on the distribution of Mo-nitrogenases as it is thought that this isozyme is more efficient than the others (Zhang et al., 2014, 2016). Recent efforts, however, have highlighted that these "alternative" nitrogenases may be important in coastal environments (McRose et al., 2017). The availability of metals is thus a key factor controlling nitrogen fixation. Marine sediments typically have higher concentrations of metals, including those required for nitrogenase, than the water column. The high concentrations of organic matter and the reducing conditions typically found in sediments would also favor the availability of metals such as molybdenum and iron (Howarth et al., 1988).

Finally, the high concentrations of organic matter in coastal sediments also help relieve another key constraint on nitrogen fixation—the protection of nitrogenase from oxygen, which is irreversibly inactivated by oxygen. Diazotrophs have developed numerous resource demanding mechanisms to protect nitrogenase from oxygen. For example, some cyanobacteria form specialized cells called heterocysts, where nitrogen fixation can occur in a microanaerobic environment. Other cyanobacteria complete oxygenic photosynthesis during the day, and nitrogen fixation at night. Still others decrease intracellular oxygen concentrations by increasing respiration rates or hinder oxygen diffusion by producing extracellular polymeric substances. These strategies often result in reduced growth rates and/or efficiency (Dutkiewicz et al., 2012; Staal et al., 2007). In an elegant modeling study for the soil bacterium, *Azotobacter vinelandii*, Inomura et al. (2017) determined that the direct energetic cost of nitrogen fixation was small compared to the cost of maintaining intracellular oxygen concentrations.

In coastal marine sediments, however, the cost of managing oxygen inhibition of nitrogenase is non-existent because oxygen is depleted within the top ∼1 cm or even top few mm (Cai & Sayles, 1996; Soetaert & Middelburg, 2009). In fact, this is not a new idea—where foundational papers on aquatic nitrogen fixation (e.g., Capone, 1983; Howarth et al., 1988) and even widely read textbooks (e.g., Brock biology of microorganisms, Madigan et al., 2008) highlight that oxygen regulation of nitrogen fixation is "no problem" for anaerobes. Yet, oddly—we sometimes forget this when it comes to explaining nitrogen fixation in sediments.

1.2. Why Fix Nitrogen?

Taken together—the energy and metal rich and oxygen deplete environment of coastal marine sediments appears to provide an ideal environment for nitrogen fixation. However, while these conditions address the "how" it is possible, they do not address the "why"? And it is not satisfactory to simply say because they can. Here again—let us use our fox-like thinking to imagine that the sediment diazotrophic community is composed of foxes and not just hedgehogs. If we see them in this way, we can begin to open our minds to a variety of reasons why they might fix nitrogen.

The classic argument would be that they fix nitrogen because they are nitrogen limited. Data supporting this idea points to the inhibition of nitrogen fixation when exposed to inorganic nitrogen sources. There have been two mechanisms proposed to describe how sediment diazotrophs respond to ammonium. The first is the repression of nitrogenase synthesis; thus, concentrations of ammonium regulate the actual production of the key enzyme in nitrogen fixation. The second is the ammonium switch-off mechanism of the nitrogenase enzyme itself.

Marine sediments, especially coastal to continental shelf sediments, typically have high concentrations of ammonium (sometimes exceeding 1 mM) because of the degradation of organic matter. Despite this, the literature provides no clear pattern on the response of benthic diazotrophs to ammonium. For example, in a classic study by Postgate and Kent (1984), ammonium additions to a pure culture of *Desulfovibrio gigas* rapidly shut down nitrogen fixation as measured by the acetylene reduction assay. In another study, Capone and Carpenter (1982a, 1982b) found that nitrogenase activity increased as porewater ammonium was depleted. Some studies of environmental samples have also found decreased rates of nitrogen fixation in the presence of combined ammonium and nitrate (Dicker & Smith, 1980). In contrast, others have reported significant nitrogen fixation rates in ammonium-rich sediments, like those found in seagrass beds (Aoki & McGlathery, 2019: 388 μmol N_2 m^{-2} h^{-1} $^{30}N_2$ label push-pull technique) or subtidal coastal sediments (Fulweiler et al., 2007: −25 to −650 μmol N_2-N m^{-2} h^{-1} (N_2/Ar technique), Newell, McCarthy, et al., 2016: −124 μmol N_2-N m^{-2} h^{-1} (N_2/Ar technique)). Remarkably, in sediments underneath the Peruvian oxygen minimum zone, ammonium concentrations exceed 2 mM with no inhibition of nitrogen fixation (Gier et al., 2016)! In a review of the role of inorganic nitrogen on nitrogen fixation, Knapp (2012) concluded that some benthic diazotrophs are simply less sensitive to inorganic nitrogen than others.

This lack of consistent relationship between ammonium concentration and nitrogen fixation rates should drive us to think more deeply about how we think about this process and highlights just how rich and interesting it is. One easy explanation for the disconnect between ammonium concentration and nitrogen fixation rates was proposed by Yoch and Whiting (1986), who suggested that there are microenvironments within the sediment where nitrogen does become limiting and that is where the fixation occurs. If this is the case, our sampling efforts must miss these environments and thus the ammonium we measure is not the ammonium the nitrogen fixers see. Microzones were recently used to help explain how nitrogen fixation occurs on marine particles (Chakraborty et al., 2021).

Additionally, a lack of inorganic nitrogen inhibition also reveals that some diazotrophs may fix nitrogen for reasons unrelated to nitrogen requirements. Sediments rich in organic matter and high in reduced substrates provide an unusual conundrum for anaerobic organisms— the excess electrons can damage cells and there is a need to regenerate electron carriers. I first read about this idea in Bertics et al. (2013) who proposed it as the mechanism driving their observed rates of sediment nitrogen fixation under high ammonium concentrations. The idea is based on research in nonsulfur purple photosynthetic bacteria that demonstrated when the Calvin-Benson Cycle was absent, the manipulated strain could not use CO_2 as an electron sink (Joshi & Tabita, 1996). Instead, they state "some alternative redox system for the dissipation of the large amount of reducing power must be employed" and from their study they showed the nitrogenase complex fulfilled this role (Joshi & Tabita, 1996). Nitrogen fixation requires a lot of reducing power and ~25% of the electron demand is used to reduce protons to molecular hydrogen that can be released from cells under anoxia. Thus, nitrogen fixation may be a mechanism to maintain an ideal intracellular redox state (Bombar et al., 2016).

Finally, it is helpful to remember the diverse array of organisms capable of nitrogen fixation and that they are part of a larger community. At the microbial level, niche partitioning (i.e., where competing species use the environment differently allowing co-existence) within the sediment may compel microbes to adapt different metabolic strategies or enhanced metabolic flexibility to survive (Sayavedra et al., 2021). Microbes are fantastically diverse and metabolically flexible. For example, some sediment microbes such as *Pseudomonas stutzeri* and *Bradyrhizobium denitrificans* can both denitrify (i.e., convert bioavailable N to di-nitrogen gas) and fix nitrogen (Newell, Pritchard, et al., 2016). In fact, co-occurring denitrification and nitrogen fixation have been observed in a variety of sediment studies (e.g., Bertics et al., 2010, 2012; Brown & Jenkins, 2014; Newell, Pritchard, et al., 2016). Potentially, denitrification and nitrogen fixation are coupled producing a "cryptic" nitrogen cycle that has largely been overlooked. Nitrogen fixation may also be a product of syntrophic relationships, which is a common strategy for microbes in anoxic environments (Schink, 2002). A well-known example is the partnership between anaerobic methane oxidizing archaea (ANME) and sulfur-reducing bacteria in deep sea sediment, which have been shown to fix nitrogen (Dekas et al., 2009; Metcalfe et al., 2021; Pernthaler et al., 2008). Rather than cooperative, nitrogen fixation may confer a competitive advantage, where nitrogen fixers are able to outcompete denitrifiers for low-quality organic matter (Fulweiler et al., 2013).

At the organismal level, chemosynthetic nitrogen fixing symbionts have been found to be active in wood boring bivalves (Carpenter & Culliney, 1975; Lechene et al., 2007), zebra mussels (Marzocchi et al., 2021), deep-sea

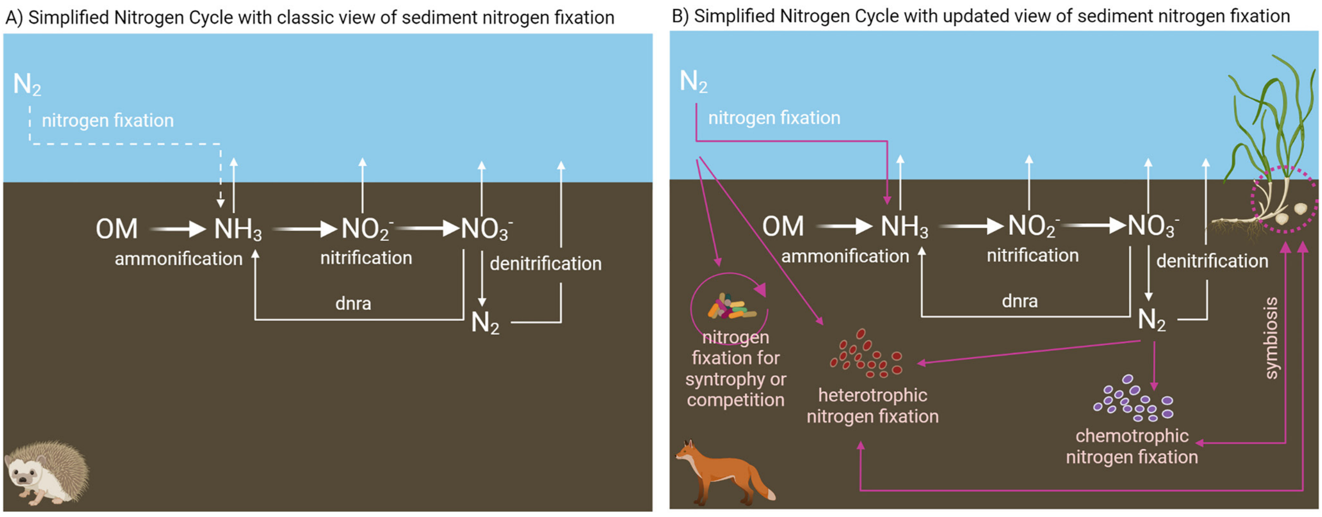

Figure 2. (a) Schematic of a simplified sediment nitrogen cycle if we think like hedgehogs and see nitrogen fixers as hedgehogs too. In this case, nitrogen fixation is unimportant (dashed line). (b) Schematic of a simplified sediment nitrogen cycle if we think like foxes and see nitrogen fixers as foxes too. In this case, we see nitrogen fixation (purple solid line) is more important than previously recognized and likely occurs for a variety of reasons and through different mechanisms (e.g., symbiosis, syntrophy) and different pathways (e.g., heterotrophic, chemotrophic). Note that OM is organic matter. Created with BioRender.com.

mussels (Ansorge et al., 2019; Ikuta et al., 2016), lucinid clams (Petersen & Yuen, 2021), marine nematodes (Paredes et al., 2021), and crab holobionts (Zilius et al., 2020). Additionally, the nitrogen fixing bacteria (*Candidatus* Celerinatantimonas neptuna) has recently been shown to live symbiotically within the root tissue of a seagrass (*Posidonia oceanica*; Mohr et al., 2021). The nitrogen provided through symbiotic nitrogen fixation is not only relevant to the organism but also to ecosystem functions and services (e.g., Cardini et al., 2019). Together, these examples highlight the critical role nitrogen fixers play in sediment communities.

2. Conclusion

If all sediment nitrogen fixers were hedgehogs, then it would be easier to understand why and when they fix nitrogen—it would be to meet their nitrogen demands. However, there is much the community needs to learn about nitrogen fixation and a myriad of data that provides convincing evidence that nitrogen fixation commonly occurs in marine sediments. Our job now is to think of them like foxes—so that we may uncover the mechanisms that drive this process, and the role sediment nitrogen fixation plays in marine ecosystems (Figure 2).

Understanding nitrogen fixation in terms of budgets has important implications at both local and global scales. At the global scale, the marine nitrogen budget is an area of continued debate, with so estimates demonstrating that denitrification greatly exceeds nitrogen fixation (e.g., Codispoti, 2007; Codispoti et al., 2001). But what if these estimates are incorrect because we have long ignored the role of sediment nitrogen fixation? Balancing the marine nitrogen budget also has important implications for global ocean primary productivity, higher trophic level production, and greenhouse gas cycling (Jickells et al., 2017). While some nitrogen budgets suggest there may be little to no imbalance (e.g., Carpenter and Capone, 1982a, 1982b; Gruber, 2008), these estimates have large uncertainties and typically use a sediment nitrogen fixation rate from over four decades ago.

At the local level, nitrogen fixation may add a significant source of nitrogen to an ecosystem which is not included in system specific nitrogen budgets. For example, in Narragansett Bay summer benthic nitrogen fixation could increase the total N load to the system by up to 30% (Fulweiler & Heiss, 2014). And in Waquoit Bay, the mean rate of nitrogen fixation when extrapolated annually was the same order of magnitude as the total N load to the system (Newell, Pritchard, et al., 2016). Overlooking sediment nitrogen fixation as a source of nitrogen to a system may mean we are working with an inaccurate budget, and therefore incorrectly managing anthropogenic nitrogen inputs (e.g., sewage discharge, runoff, etc.).

Yet, even if nitrogen fixation is an unimportant term in local or global nitrogen budgets, there is still great value in documenting where and when and by whom it happens. For nitrogen fixation may play a key role in establishing microbial communities, providing plants and other organisms nitrogen, or helping microbes survive in reduced conditions. Importantly, there is also no reason to assume that the reasons for why nitrogen fixers fix nitrogen provided here are mutually exclusive or the only reasons. Future research may highlight yet unexplored purposes for nitrogen fixation.

Examining the role of nitrogen fixation within an ecosystem context provides a richer and potentially more accurate view of nitrogen cycling. To further expand our understanding of marine sediment diazotrophy, we should encourage quantifying spatial and temporal rates of nitrogen fixation and relevant environmental parameters in a variety of locations and over different time scales to better describe the variability of this process. Combining isotope labeling studies, advanced mass spectrometry imaging techniques (e.g., NanoSIMS), and microbial community composition and activity data may help us gain a better understanding of finer scale sediment nitrogen fixation dynamics. I think there is also much to be learned about the different types of nitrogenases and understanding where and when they are used will likely also shed light on sediment nitrogen fixation. Finally, I would encourage us to think holistically about sediment nitrogen fixation and to better describe how these diazotrophs alter their geochemical environment and how they interact with microbes and multicellular organisms through syntrophy, symbiosis, predation, and competition (e.g., Liau et al., 2022). As the community continues to pursue this interesting nitrogen cycling pathway, I hope we can remember to approach it with the mindset of a fox, and not a hedgehog. And to remember that not all nitrogen fixers are hedgehogs, many (most?) might be foxes.

Data Availability Statement

Any data cited here are available in the appropriate citation in the reference list. No software was used to analyze data for this commentary. Figures were made in Biorender.

Acknowledgments
Thank you to Isaac Santos for inviting me to write this commentary. Many thanks to all those who study nitrogen fixation and whose papers have helped stimulate the ideas presented here. My thinking about nitrogen fixation has been enriched by conversations about nitrogen cycling over the years with many colleagues, in particular Anne Dekas, Sarah Foster, Anne Giblin, Pete Girguis, Silvia Newell, Scott Nixon, Nick Ray, Robert Twilley, Jane Tucker, and Amanda Vieillard. Special thanks to Amy Marcarelli and Thad Scott, who share a love of nitrogen fixation and an eagerness to discuss it no matter what the occasion. Our weekly nitrogen fixation meetings maintain my motivation and sanity. Extra thanks to Amy Marcarelli who provided helpful edits and discussion on this commentary. Thanks also to Todd Kana without whom I would never have measured sediment nitrogen fixation. This work was funded by a grant to RWF from RI Sea Grant and by the National Science Foundation (award number 2015825).

References

Ansorge, R., Romano, S., Sayavedra, L., Porras, M. Á. G., Kupczok, A., Tegetmeyer, H. E., et al. (2019). Functional diversity enables multiple symbiont strains to coexist in deep-sea mussels. *Nature Microbiology*, *4*(4), 2487–2497. https://doi.org/10.1038/s41564-019-0572-9

Aoki, L. R., & McGlathery, K. J. (2019). High rates of N fixation in seagrass sediments measured via a direct ^{30}N2 push-pull method. *Marine Ecology Progress Series*, *616*, 1–11. https://doi.org/10.3354/meps12961

Bertics, V. J., Löscher, C. R., Salonen, I., Dale, A. W., Gier, J., Schmitz, R. A., & Treude, T. (2013). Occurrence of benthic microbial nitrogen fixation coupled to sulfate reduction in the seasonally hypoxic Eckernförde Bay, Baltic Sea. *Biogeosciences*, *10*(3), 1243–1258. https://doi.org/10.5194/bg-10-1243-2013

Bertics, V. J., Sohm, J. A., Magnabosco, C., & Ziebis, W. (2012). Denitrification and nitrogen fixation dynamics in the area surrounding an individual Ghost shrimp (*Neotrypaea californiensis*) Burrow system. *Applied and Environmental Microbiology*, *78*(11), 3864–3872. https://doi.org/10.1128/aem.00114-12

Bertics, V. J., Sohm, J. A., Treude, T., Chow, C. E. T., Capone, D. G., Fuhrman, J. A., & Ziebis, W. (2010). Burrowing deeper into benthic nitrogen cycling: The impact of bioturbation on nitrogen fixation coupled to sulfate reduction. *Marine Ecology Progress Series*, *409*, 1–15. https://doi.org/10.3354/meps08639

Bombar, D., Paerl, R. W., & Riemann, L. (2016). Marine non-cyanobacterial diazotrophs: Moving beyond molecular detection. *Trends in Microbiology*, *24*(11), 916–927. https://doi.org/10.1016/j.tim.2016.07.002

Brown, S. M., & Jenkins, B. D. (2014). Profiling gene expression to distinguish the likely active diazotrophs from a sea of genetic potential in marine sediments. *Environmental Microbiology*, *16*(10), 3128–3142. https://doi.org/10.1111/1462-2920.12403

Cai, W. J., & Sayles, F. L. (1996). Oxygen penetration depths and fluxes in marine sediments. *Marine Chemistry*, *52*(2), 123–131. https://doi.org/10.1016/0304-4203(95)00081-x

Capone, D. G. (1983). *Benthic nitrogen fixation* (Vol. 105). Academic Press.

Capone, D. G., & Carpenter, E. J. (1982a). Nitrogen fixation in the marine environment. *Science*, *217*(4565), 1140–1142. https://doi.org/10.1126/science.217.4565.1140

Capone, D. G., & Carpenter, E. J. (1982b). Perfusion method for assaying microbial activities in sediments: Applicability to studies of N2 fixation by C_2H_2 reduction. *Applied and Environmental Microbiology*, *43*(6), 1400–1405. https://doi.org/10.1128/aem.43.6.1400-1405.1982

Cardini, U., Bartoli, M., Lücker, S., Mooshammer, M., Polzin, J., Lee, R. W., et al. (2019). Chemosymbiotic bivalves contribute to the nitrogen budget of seagrass ecosystems. *The ISME Journal*, *13*(12), 3131–3134. https://doi.org/10.1038/s41396-019-0486-9

Carpenter, E. J., & Culliney, J. L. (1975). Nitrogen fixation in marine shipworms. *Science*, *187*(4176), 551–552. https://doi.org/10.1126/science.187.4176.551

Chakraborty, S., Andersen, K. H., Visser, A. W., Inomura, K., Follows, M. J., & Riemann, L. (2021). Quantifying nitrogen fixation by heterotrophic bacteria in sinking marine particles. *Nature Communications*, *12*(1), 4085. https://doi.org/10.1038/s41467-021-23875-6

Codispoti, L. A. (2007). An oceanic fixed nitrogen sink exceeding 400 Tg N a^{-1} vs the concept of homeostasis in the fixed-nitrogen inventory. *Biogeosciences*, *4*(2), 233–253. https://doi.org/10.5194/bg-4-233-2007

Codispoti, L. A., Brandes, J., Christensen, J. P., Devol, A. H., Naqvi, S. W. A., Paerl, H., & Yoshinari, T. (2001). The oceanic fixed nitrogen and nitrous oxide budgets: Moving targets as we enter the anthropocene? *Scientia Marina*, *65*(S2), 85–105. https://doi.org/10.3989/scimar.2001.65s285

Dekas, A. E., Poretsky, R. S., & Orphan, V. J. (2009). Deep-sea archaea fix and share nitrogen in methane-consuming microbial consortia. *Science*, *326*(5951), 422–426. https://doi.org/10.1126/science.1178223

Desai, M. S., Assig, K., & Dattagupta, S. (2013). Nitrogen fixation in distinct microbial niches within a chemoautotrophy-driven cave ecosystem. *The ISME Journal*, *7*(12), 2411–2423. https://doi.org/10.1038/ismej.2013.126

Dicker, H. J., & Smith, D. W. (1980). Physiological ecology of acetylene reduction (nitrogen fixation) in a Delaware salt marsh. *Microbial Ecology*, *6*(2), 161–171. https://doi.org/10.1007/bf02010555

Dutkiewicz, S., Ward, B. A., Monteiro, F., & Follows, M. J. (2012). Interconnection of nitrogen fixers and iron in the Pacific Ocean: Theory and numerical simulations. *Global Biogeochemical Cycles*, *26*(1), GB1012. https://doi.org/10.1029/2011gb004039

Fulweiler, R. W., Brown, S. M., Nixon, S. W., & Jenkins, B. D. (2013). Evidence and a conceptual model for the co-occurrence of nitrogen fixation and denitrification in heterotrophic marine sediments. *Marine Ecology Progress Series*, *482*, 57–68. https://doi.org/10.3354/meps10240

Fulweiler, R. W., & Heiss, E. M. (2014). (Nearly) A decade of directly measured sediment N_2 fluxes: What can Narragansett Bay tell us about the global ocean nitrogen budget? *Oceanography*, *27*(1), 184–195. https://doi.org/10.5670/oceanog.2014.22

Fulweiler, R. W., Nixon, S. W., Buckley, B. A., & Granger, S. L. (2007). Reversal of the net dinitrogen gas flux in coastal marine sediments. *Nature*, *448*(7150), 180–182. https://doi.org/10.1038/nature05963

Gandy, E. L., & Yoch, D. C. (1988). Relationship between nitrogen-fixing sulfate reducers and fermenters in salt marsh sediments and roots of *Spartina alterniflora*. *Applied and Environmental Microbiology*, *54*(8), 2031–2036. https://doi.org/10.1128/aem.54.8.2031-2036.1988

Garcia, A. K., McShea, H., Kolaczkowski, B., & Kaçar, B. (2020). Reconstructing the evolutionary history of nitrogenases: Evidence for ancestral molybdenum-cofactor utilization. *Geobiology*, *18*(3), 394–411. https://doi.org/10.1111/gbi.12381

Gattuso, J. P., Gentili, B., Duarte, C. M., Kleypas, J. A., Middelburg, J. J., & Antoine, D. (2006). Light availability in the coastal ocean: Impact on the distribution of benthic photosynthetic organisms and their contribution to primary production. *Biogeosciences*, *3*(4), 489–513. https://doi.org/10.5194/bg-3-489-2006

Gauthier, F., Neufeld, J. D., Driscoll, B. T., & Archibald, F. S. (2000). Coliform bacteria and nitrogen fixation in pulp and paper mill effluent treatment systems. *Applied and Environmental Microbiology*, *66*(12), 5155–5160. https://doi.org/10.1128/aem.66.12.5155-5160.2000

Gier, J., Sommer, S., Löscher, C. R., Dale, A. W., Schmitz, R. A., & Treude, T. (2016). Nitrogen fixation in sediments along a depth transect through the Peruvian oxygen minimum zone. *Biogeosciences*, *13*(14), 4065–4080. https://doi.org/10.5194/bg-13-4065-2016

Gruber, N. (2008). The marine nitrogen cycle: Overview and challenges. *Nitrogen in the Marine Environment*, *2*, 1–50.

Hou, L., Wang, R., Yin, G., Liu, M., & Zheng, Y. (2018). Nitrogen fixation in the intertidal sediments of the Yangtze Estuary: Occurrence and environmental implications. *Journal of Geophysical Research: Biogeosciences*, *123*(3), 936–944. https://doi.org/10.1002/2018jg004418

Howarth, R. W., Marino, R., & Cole, J. J. (1988). Nitrogen fixation in freshwater, estuarine, and marine ecosystems. 2. Biogeochemical controls. *Limnology & Oceanography*, *33*(4), 688–701. https://doi.org/10.4319/lo.1988.33.4_part_2.0688

Huang, W. S., Wang, L. T., Chen, J. S., Chen, Y. T., Wei, S. T. S., Chiang, Y. R., et al. (2021). *Vibrio nitrifigilis* sp. nov., a marine nitrogen-fixing bacterium isolated from the lagoon sediment of an islet inside an atoll. *Antonie van Leeuwenhoek*, *114*(7), 933–945. https://doi.org/10.1007/s10482-021-01567-x

Ikuta, T., Takaki, Y., Nagai, Y., Shimamura, S., Tsuda, M., Kawagucci, S., et al. (2016). Heterogeneous composition of key metabolic gene clusters in a vent mussel symbiont population. *The ISME Journal*, *10*(4), 990–1001. https://doi.org/10.1038/ismej.2015.176

Inomura, K., Bragg, J., & Follows, M. J. (2017). A quantitative analysis of the direct and indirect costs of nitrogen fixation: A model based on *Azotobacter vinelandii*. *The ISME Journal*, *11*(1), 166–175. https://doi.org/10.1038/ismej.2016.97

Jabir, T., Vipindas, P. V., Jesmi, Y., Valliyodan, S., Parambath, P. M., Singh, A., & Abdulla, M. H. (2020). Nutrient stoichiometry (N:P) controls nitrogen fixation and distribution of diazotrophs in a tropical eutrophic estuary. *Marine Pollution Bulletin*, *151*, 110799. https://doi.org/10.1016/j.marpolbul.2019.110799

Jickells, T. D., Buitenhuis, E., Altieri, K., Baker, A. R., Capone, D., Duce, R. A., et al. (2017). A reevaluation of the magnitude and impacts of anthropogenic atmospheric nitrogen inputs on the ocean. *Global Biogeochemical Cycles*, *31*(2), 289–305. https://doi.org/10.1002/2016gb005586

Joshi, H. M., & Tabita, F. R. (1996). A global two component signal transduction system that integrates the control of photosynthesis, carbon dioxide assimilation, and nitrogen fixation. *Proceedings of the National Academy of Sciences*, *93*(25), 14515–14520. https://doi.org/10.1073/pnas.93.25.14515

Kapili, B. J., Barnett, S. E., Buckley, D. H., & Dekas, A. E. (2020). Evidence for phylogenetically and catabolically diverse active diazotrophs in deep-sea sediment. *The ISME Journal*, *14*(4), 971–983. https://doi.org/10.1038/s41396-019-0584-8

Knapp, A. N. (2012). The sensitivity of marine N2 fixation to dissolved inorganic nitrogen. *Frontiers in Microbiology*, *3*, 374. https://doi.org/10.3389/fmicb.2012.00374

Lechene, C. P., Luyten, Y., McMahon, G., & Distel, D. L. (2007). Quantitative imaging of nitrogen fixation by individual bacteria within animal cells. *Science*, *317*(5844), 1563–1566. https://doi.org/10.1126/science.1145557

Liau, P., Kim, C., Saxton, M. A., & Malkin, S. Y. (2022). Microbial succession in a marine sediment: Inferring interspecific microbial interactions with marine cable bacteria. *Environmental Microbiology*, *24*(12), 6348–6364. https://doi.org/10.1111/1462-2920.16230

Liesirova, T., Aarenstrup-Launbjerg, T., Hallstrøm, S., Bittner, M. J., Riemann, L., & Voss, M. (2023). Nitrogen-fixing sulfate reducing bacteria in shallow coastal sediments under simulated resuspension. *Estuarine, Coastal and Shelf Science*, *280*, 108165. https://doi.org/10.1016/j.ecss.2022.108165

Madigan, M. T., Martinko, J. M., Dunlap, P. V., & Clark, D. P. (2008). Brock biology of microorganisms 12th edn. *International Microbiology*, *11*, 65–73.

Marcarelli, A. M., Fulweiler, R. W., & Scott, J. T. (2022). Nitrogen fixation: A poorly understood process along the freshwater-marine continuum. *Limnology and Oceanography Letters*, *7*(1), 1–10. https://doi.org/10.1002/lol2.10220

Marzocchi, U., Bonaglia, S., Zaiko, A., Quero, G. M., Vybernaite-Lubiene, I., Politi, T., et al. (2021). Zebra mussel Holobionts fix and recycle nitrogen in lagoon sediments. *Frontiers in Microbiology*, *11*, 610269. https://doi.org/10.3389/fmicb.2020.610269

McRose, D. L., Zhang, X., Kraepiel, A. M., & Morel, F. M. (2017). Diversity and activity of alternative nitrogenases in sequenced genomes and coastal environments. *Frontiers in Microbiology*, *8*, 267. https://doi.org/10.3389/fmicb.2017.00267

Metcalfe, K. S., Murali, R., Mullin, S. W., Connon, S. A., & Orphan, V. J. (2021). Experimentally-validated correlation analysis reveals new anaerobic methane oxidation partnerships with consortium-level heterogeneity in diazotrophy. *The ISME Journal*, *15*(2), 377–396. https://doi.org/10.1038/s41396-020-00757-1

Mohr, W., Lehnen, N., Ahmerkamp, S., Marchant, H. K., Graf, J. S., Tschitschko, B., et al. (2021). Terrestrial-type nitrogen-fixing symbiosis between seagrass and a marine bacterium. *Nature*, *600*(7887), 105–109. https://doi.org/10.1038/s41586-021-04063-4

Newell, S. E., McCarthy, M. J., Gardner, W. S., & Fulweiler, R. W. (2016). Sediment nitrogen fixation: A call for re-evaluating coastal N budgets. *Estuaries and Coasts*, *39*(6), 1626–1638. https://doi.org/10.1007/s12237-016-0116-y

Newell, S. E., Pritchard, K. R., Foster, S. Q., & Fulweiler, R. W. (2016). Molecular evidence for sediment nitrogen fixation in a temperate New England estuary. *PeerJ*, *4*, e1615. https://doi.org/10.7717/peerj.1615

Paredes, G. F., Viehboeck, T., Lee, R., Palatinszky, M., Mausz, M. A., Reipert, S., et al. (2021). Anaerobic sulfur oxidation underlies adaptation of a chemosynthetic symbiont to oxic-anoxic interfaces. *mSystems*, *6*(3), 10–1128. https://doi.org/10.1128/msystems.01186-20

Perez, C. A., Carmona, M. R., & Armesto, J. J. (2010). 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*, *35*(2), 148–156. https://doi.org/10.1111/j.1442-9993.2009.02020.x

Pernthaler, A., Dekas, A. E., Brown, C. T., Goffredi, S. K., Embaye, T., & Orphan, V. J. (2008). Diverse syntrophic partnerships from deep-sea methane vents revealed by direct cell capture and metagenomics. *Proceedings of the National Academy of Sciences*, *105*(19), 7052–7057. https://doi.org/10.1073/pnas.0711303105

Petersen, J. M., & Yuen, B. (2021). The symbiotic "all-rounders": Partnerships between marine animals and chemosynthetic nitrogen-fixing bacteria. *Applied and Environmental Microbiology*, *87*(5), e02129-20. https://doi.org/10.1128/aem.02129-20

Postgate, J. R., & Kent, H. M. (1984). Depression of nitrogen fixation in *Desulfovibrio gigas* and its stability to ammonia or oxygen stress in vivo. *Microbiology*, *130*(11), 2825–2831. https://doi.org/10.1099/00221287-130-11-2825

Raut, Y., & Capone, D. G. (2021). Macroalgal detrital systems: An overlooked ecological niche for heterotrophic nitrogen fixation. *Environmental Microbiology*, *23*(8), 4372–4388. https://doi.org/10.1111/1462-2920.15622

Sayavedra, L., Li, T., Bueno Batista, M., Seah, B. K., Booth, C., Zhai, Q., et al. (2021). *Desulfovibrio diazotrophicus* sp. nov., a sulfate-reducing bacterium from the human gut capable of nitrogen fixation. *Environmental Microbiology*, *23*(6), 3164–3181. https://doi.org/10.1111/1462-2920.15538

Schink, B. (2002). Synergistic interactions in the microbial world. *Antonie van Leeuwenhoek*, *81*(1/4), 257–261. https://doi.org/10.1023/a:1020579004534

Soetaert, K., & Middelburg, J. J. (2009). Modeling eutrophication and oligotrophication of shallow-water marine systems: The importance of sediments under stratified and well-mixed conditions. In *Eutrophication in coastal ecosystems: Towards better understanding and management strategies selected papers from the second international symposium on research and management of eutrophication in coastal ecosystems, 20–23 June 2006, Nyborg, Denmark* (pp. 239–254). Springer.

Sohm, J. A., Webb, E. A., & Capone, D. G. (2011). Emerging patterns of marine nitrogen fixation. *Nature Reviews Microbiology*, *9*(7), 499–508. https://doi.org/10.1038/nrmicro2594

Staal, M., Rabouille, S., & Stal, L. J. (2007). On the role of oxygen for nitrogen fixation in the marine cyanobacterium *Trichodesmium* sp. *Environmental Microbiology*, *9*(3), 727–736. https://doi.org/10.1111/j.1462-2920.2006.01195.x

Stal, L. J. (2015). Nitrogen fixation in cyanobacteria. In *eLS* (pp. 1–9). https://doi.org/10.1002/9780470015902.a0021159.pub2

Streicher, S. L., & Valentine, R. C. (1973). Comparative biochemistry of nitrogen fixation. *Annual Review of Biochemistry*, *42*(1), 279–302. https://doi.org/10.1146/annurev.bi.42.070173.001431

Welsh, D. T. (2000). Nitrogen fixation in seagrass meadows: Regulation, plant–bacteria interactions and significance to primary productivity. *Ecology Letters*, *3*(1), 58–71. https://doi.org/10.1046/j.1461-0248.2000.00111.x

Yoch, D. C., & Whiting, G. J. (1986). Evidence for NH_4^+ switch-off regulation of nitrogenase activity by bacteria in salt marsh sediments and roots of the grass *Spartina alterniflora*. *Applied and Environmental Microbiology*, *51*(1), 143–149. https://doi.org/10.1128/aem.51.1.143-149.1986

Zehr, J. P., & Capone, D. G. (2020). Changing perspectives in marine nitrogen fixation. *Science*, *368*(6492), eaay9514. https://doi.org/10.1126/science.aay9514

Zhang, X., McRose, D. L., Darnajoux, R., Bellenger, J. P., Morel, F. M., & Kraepiel, A. M. (2016). Alternative nitrogenase activity in the environment and nitrogen cycle implications. *Biogeochemistry*, *127*(2–3), 189–198. https://doi.org/10.1007/s10533-016-0188-6

Zhang, X., Sigman, D. M., Morel, F. M., & Kraepiel, A. M. (2014). Nitrogen isotope fractionation by alternative nitrogenases and past ocean anoxia. *Proceedings of the National Academy of Sciences*, *111*(13), 4782–4787. https://doi.org/10.1073/pnas.1402976111

Zilius, M., Bonaglia, S., Broman, E., Chiozzini, V. G., Samuiloviene, A., Nascimento, F. J., et al. (2020). N_2 fixation dominates nitrogen cycling in a mangrove fiddler crab holobiont. *Scientific Reports*, *10*(1), 13966. https://doi.org/10.1038/s41598-020-70834-0