

# Microbial roles in the terrestrial and aquatic nitrogen cycle—implications in climate change

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## Abstract

Nitrogen, as an essential component for living organisms, is the primary limiting nutrient on Earth. The availability and effective utilization of nitrogenous compounds for metabolic and other essential biochemical reactions are dependent on the myriad and phylogenetically diverse microbial communities. The microorganisms harmoniously interact and participate in every reaction of the nitrogen cycle to continuously transform nitrogen into its various bio-available forms. Research on the nitrogen cycle continues to disclose that there are many reactions that remain unknown. In this review, we summarize the recent discoveries that have contributed to advancing our understanding of the microbial involvement in reactions of the nitrogen cycle in soil and aquatic systems that influence climate change. Additionally, the mini-review highlights, which anthropogenic activities cause disturbances in the nitrogen cycle and proposes how beneficial microbes may be harnessed to replenish nitrogen in agricultural ecosystems.

**Keywords:** terrestrial nitrogen cycle, marine nitrogen cycle, nitrification, denitrification

## Introduction

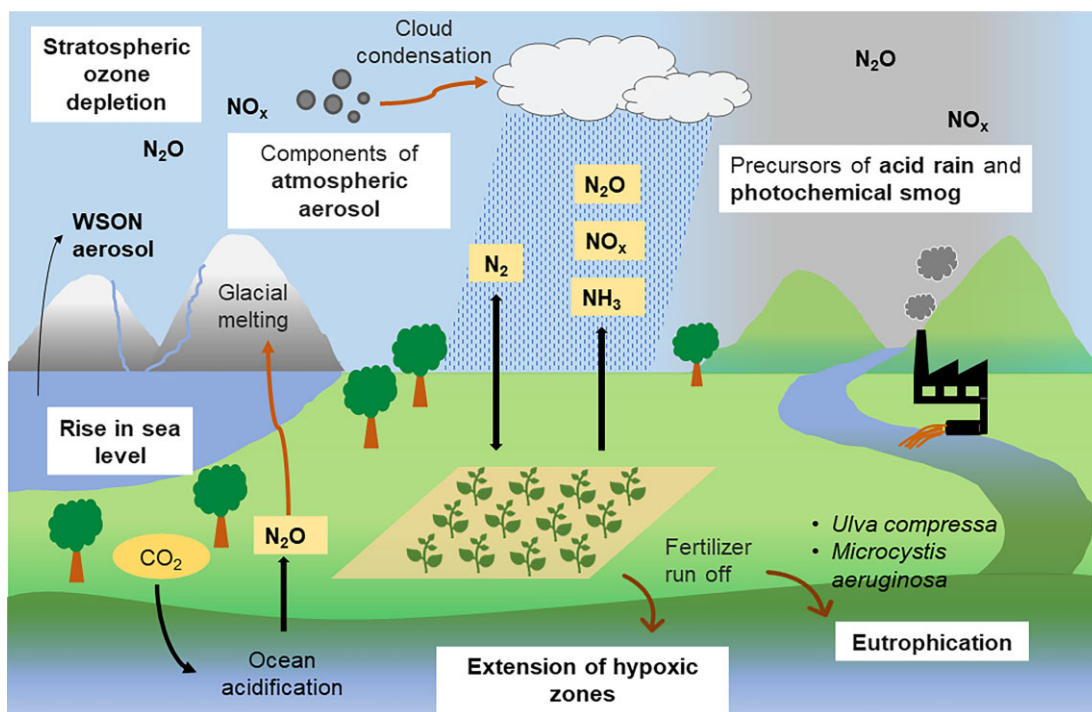
As a vital requirement for all life forms, nitrogen (N) accounts for 78% of the Earth's atmosphere, comprising of  $\sim 4 \times 10^9$  Tg N (Johnson and Goldblatt 2015). Globally, N in the first 100 cm of soil has been estimated to be 133–140 Pg (Batjes 2014) while marine water systems account for  $6.6 \times 10^5$  Tg N (Capone et al. 2008). Nitrogen exists as dinitrogen ( $N_2$ ) and its oxides ( $NO_x$ ) in the atmosphere, while in soil and aquatic environments, ammonia ( $NH_3$ ) and nitrates ( $NO_3^-$ ) predominate. Atmospheric nitrogen predominantly exists in its molecular form, which cannot be directly assimilated by living beings due to the energy required to break the bonds between its atoms. Hence, nature utilizes a series of biochemical reactions to transform atmospheric, marine, and soil nitrogen into its various chemical forms, employing microbial action. As depicted in Fig. 1, the nitrogen cycle not only influences climate change through the release of greenhouse gases such as nitric oxide (NO) and nitrous oxide ( $N_2O$ ) but also through anthropogenic activities, including excess chemical fertiliser, which offset critical N concentrations in soil, water, and air. We have also discussed the primary nitrogen transformations that occur in terrestrial and marine systems, respectively, along with the latest research findings on the pathways involved (Figs 2 and 3). Additionally, we discuss that the interactions of nitrogen and its forms with other elements of the ecosystem influence the global nitrogen cycle and need more future exploration (Fig. 4).

## Recent findings in the terrestrial nitrogen cycle

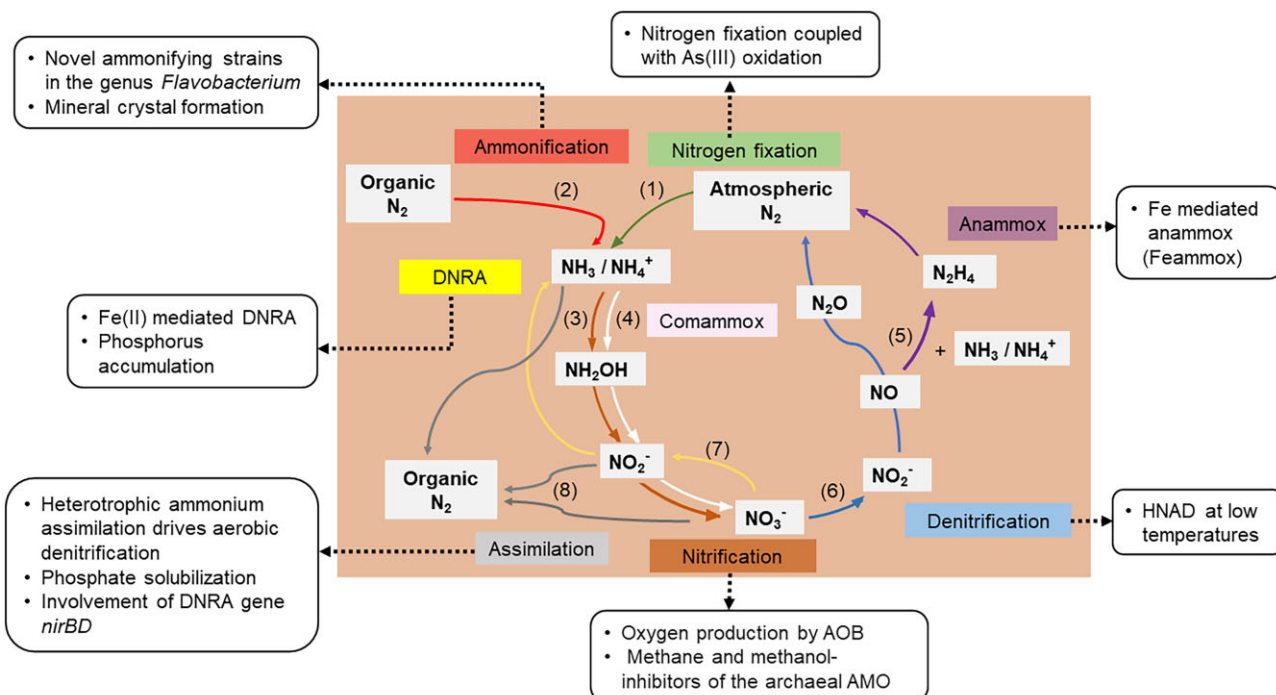
Biological nitrogen fixation (BNF) in plants is initiated (Fig. 2, Step 1) by diazotroph-encoding nitrogenases. Recently, novel diazotrophs, *Geomonas* sp., *Kosakonia sacchari*, and *Paraburkholderia guartelaensis*, performing nitrogen fixation in terrestrial environments (Giri 2019, Paulitsch et al. 2019, Liu et al. 2022), have been

discovered. While root nodules of leguminous plants employ Rhizobia to fix nitrogen, non-nodulating legumes utilize endophytic associative nitrogen-fixing bacteria such as *Caulobacter segnis* and *C. crescentus* under nitrogen deficient conditions (Thamizhseran and Shendye 2022). While chemolithotrophic diazotrophy by nitrogen fixers such as *Azospirillum* sp. employs sulphur, nitrogen fixation fuelled by arsenic (III) oxidation has been reported in *Serratia* sp. This is a novel biogeochemical process shedding light on alternate electron donors for BNF other than reduced sulphur or organic matter (Li et al. 2022b, Kwak and Shin 2016). Further, nutrient addition experiments have disclosed that regulation of nitrogen fixation by phosphorus (P) increases diazotroph diversity and hence the rate of nitrogen fixation (Wang et al. 2022b), whereas long-term nitrogen fertilization dramatically suppresses N-fixing diazotrophs (Fan et al. 2019).

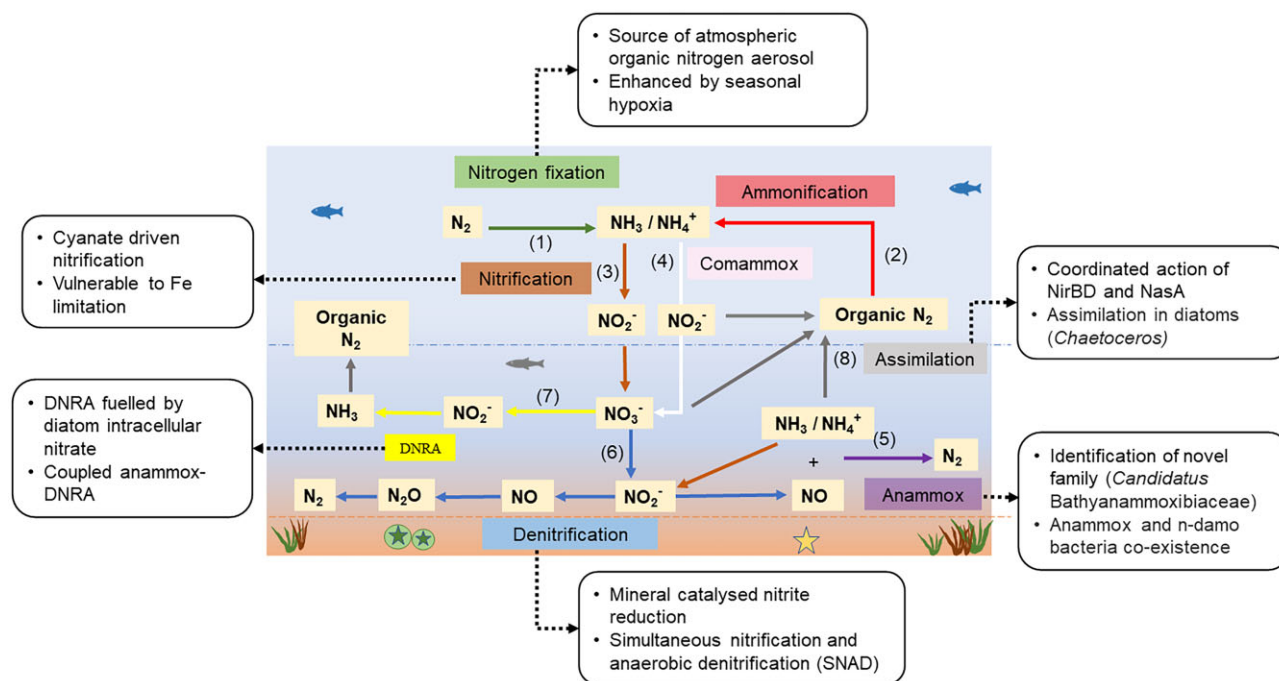
Nitrogenases participating in BNF are efficient microbial enzymes, among which molybdenum nitrogenases are best elucidated. Recently, vanadium (V) and iron-only (Fe) nitrogenases discovered in free-living soil bacteria, cyanobacteria, and yeasts are beginning to be investigated (Bellenger et al. 2020, López-Torrejón et al. 2021). Interestingly, Mo, V, and Fe nitrogenases may follow similar catalytic mechanisms since their cofactors and protein scaffolds display similar structural stoichiometry. X-ray crystallographic studies have provided detailed structures of the nitrogenases with their constituent metallo-cofactors, including recent crystal structures of ligand-bound forms of nitrogenases (Einsle and Rees 2020). Significant progress has been made in the characterization of the enzyme's active site structure employing nuclear resonance spectroscopy (Van Stappen et al. 2023). Because nitrogenase gets inactivated by oxygen, microorganisms have evolved protective cellular strategies in aerobic environments to overcome enzyme inactivation. Under a reduced



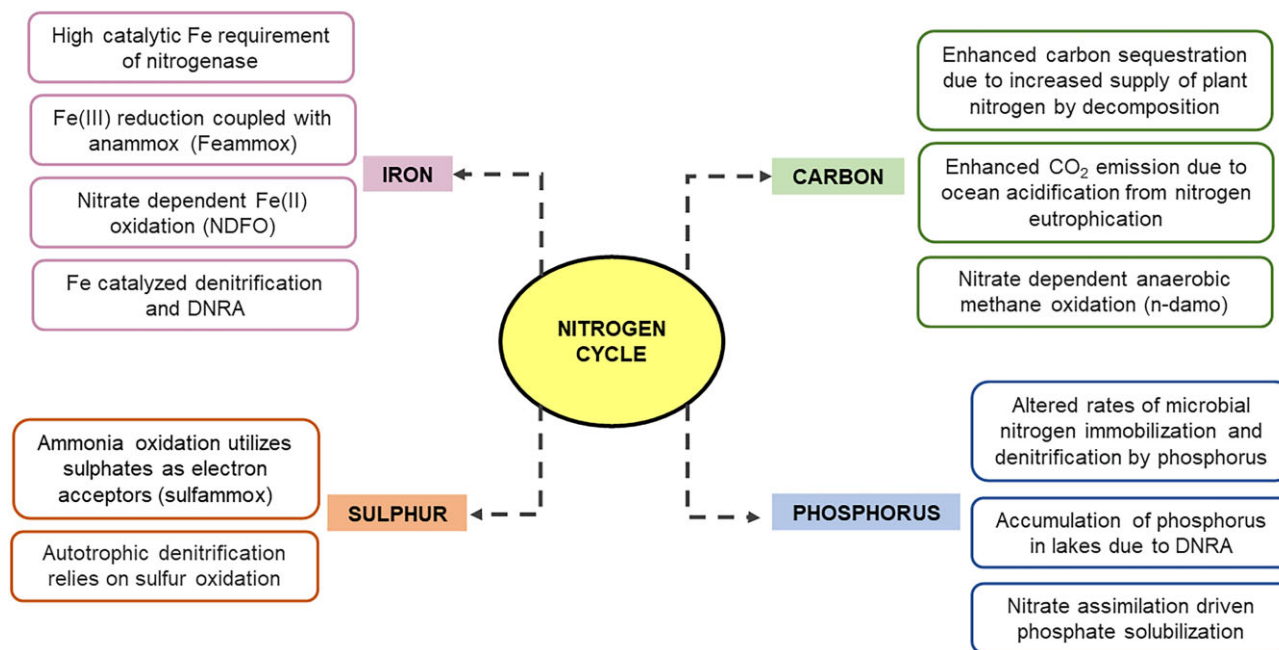
**Figure 1.** Cartoon displaying how the reactive nitrogen species including nitrogen oxides (NO<sub>x</sub>), ammonia (NH<sub>3</sub>), and nitrous oxide (N<sub>2</sub>O) in atmospheric, terrestrial, and marine ecosystems participate in climate change. The water-soluble organic nitrogen and inorganic forms of nitrogen are components of aerosols, which have roles in glacial melting which consequently rises sea levels. Aerosols influence atmospheric chemistry and hydrological cycle by functioning as cloud condensation nuclei (CCN). Reactive nitrogen oxides may be precursors of photochemical smog and also acid rain. Loss prone nitrogen exits agricultural lands to aquatic bodies causing pollution through eutrophication, creating hypoxic zones and ocean acidification.



**Figure 2.** Microbial roles in the transformation of nitrogen along with its compounds in terrestrial systems has been depicted. The numbers 1–8 represent the sequential steps involved in nitrogen cycle. The text in squares indicate novel findings related to the pathways. Data for the figures have been sourced from references of Huang et al. (2020), Zhao et al. (2020), Kraft et al. (2022), Krämer et al. (2022), Li et al. (2022a, 2022b), Ma et al. (2022), Tan et al. (2022), Watanabe et al. (2022), Wu et al. (2022), Yuan et al. (2022), Garcia-Sanchez et al. (2023), and Oudova-Rivera et al. (2023).



**Figure 3.** Microbial involvement in transformation of nitrogen and its compounds in marine systems has been diagrammatically depicted. The numbers 1–8 indicate various steps involved in nitrogen cycle. Text in squares indicate novel findings related to the pathways. The figure has been drawn using information from Li et al. (2021a), Morando and Capone (2018), Buessecker et al. (2022), Hu et al. (2022), Mao et al. (2022), Mduityana et al. (2022), Stief et al. (2022), Valiente et al. (2022), Zhao et al. (2022), Zhou et al. (2022), Dobashi et al. (2023), and Yao et al. (2023).



**Figure 4.** Cartoon representation of link between nitrogen and iron, carbon, sulphur, and phosphorus in terrestrial and marine ecosystems.

oxygen concentration of 20%, overexpression of *nafU* was observed in *Azotobacter vinelandii*, which subsequently led to the promotion of nitrogenase activity by the NafU protein (Takimoto et al. 2022). Over-expressing *nafU* in *Escherichia coli* resulted in a marked enhancement of its nitrogenase function under aerobic conditions.

Novel bacterial strains capable of ammonification (Fig. 2, Step 2) were isolated from aquatic systems and characterized, show-

ing a close relation to the *Flavobacterium* genus and leading to the proposal of their names as *Flavobacterium ammonificans* sp. nov. and *F. ammoniigenes* sp. nov. (Watanabe et al. 2022). *Brevibacillus laterosporus* could induce mineral crystal formation through ammonification, highlighting the significance of recognizing this process as biologically induced mineralization (BIM) (Zhao et al. 2020). While there have been numerous recent studies that have extensively examined bacterial ammonification in diverse

systems, including treatment plants, their detailed discussion is beyond the scope of this review.

Nitrification (Fig. 2, Step 3) is performed by ammonia-oxidizing bacteria (AOB: Nitrosomonas, Nitrosospira, and Nitrosococcus) and ammonia-oxidizing archaea (AOA: Nitrososphaera and Nitrosotalea) in synergy with nitrite-oxidizing bacteria (Nitrobacter, Nitrospina, Nitrococcus, and Nitrospira; Pajares and Bohannan 2016). The identification of comammox, the complete oxidation of ammonia into nitrate by a single organism, Nitrospira (Fig. 2, Step 4), disproves the notion that the oxidation of ammonia and nitrite require two distinct groups of microorganisms (van Kessel et al. 2015). One model highlights  $\text{NH}_3$  oxidation in the formation of nitrite ( $\text{NO}_2^-$ ) via a single obligate intermediate, hydroxylamine ( $\text{NH}_2\text{OH}$ ), enzymatically catalyzed by ammonia monooxygenase (AMO) and hydroxylamine oxidoreductase (HAO). However, other studies demonstrate that nitric oxide functions as an additional obligate intermediate to be oxidized to nitrite (Caranto and Lancaster 2017). All AOB that oxidizes ammonia to nitrate encodes AMO and HAO. However, AOA may not harbour HAO; hence, the archaeal enzyme responsible for hydroxylamine oxidation remains undiscovered (Kuypers et al. 2018). The elucidation of structural similarity between archaeal AMO and particulate methane monooxygenase (PPMO) involved in methane oxidation and the finding that methane and methanol act as competitive inhibitors of archaeal AMO lay the foundation for future studies on the metabolism of one-carbon (C1) compounds in AOA (Oudova-Rivera et al. 2023). The discovery of an oxygen-producing pathway by AOA highlights the role of AOA under anoxic conditions, with interesting implications for nitrogen cycling in oxygen-depleted environments (Kraft et al. 2022).

Anammox (Fig. 2, Step 5), reported by Humbert et al. (2010), performed by anammox bacterial genera (*Candidatus Scalindua*, *Ca. Brocadia*, *Ca. Jettenia*, and *Ca. Kuenenia*) in both mangrove and rice fields (Padhy et al. 2022) may have applications in agricultural settings. The new deep-branching bacteria *Ca. Bathyanammoxi* within the order *Ca. Brocadiales*, possessing genetic potential for anammox metabolism, have been reported in terrestrial and marine environments (Zhao et al. 2022). Identification of such diverse bacteria furnishes a vital link for biogeochemical cycles and climate change. Recently, a novel pathway for ammonia oxidation, the microbial anammox coupled to Fe (III) reduction, namely ferric ammonium oxidation (Feammox), has been discovered in terrestrial and aquatic ecosystems (Tan et al. 2022). Possibly, feammox pathways might offer novel solutions for effective wastewater management.

*Micrococcus denitrificans*, *Thiobacillus denitrificans* including species of *Pseudomonas* and *Serratia*, involved in conversion of nitrate to nitrogen (Fig. 2, Step 6) are active participants in denitrification. Recently, *Acinetobacter calcoaceticus* TY1 displaying dual heterotrophic nitrification and aerobic denitrification abilities at low temperatures has been reported, which could have potential in wastewater management (Wu et al. 2022). Functional genes contributing to denitrification include nitrate reductase (Nar), nitrite reductase (Nir), nitric oxide reductase (Nor), and nitrous oxide reductase (Nos). Interestingly, most denitrifiers harbour either copper-containing NirK or cytochrome  $\text{cd}_1$ -containing NirS nitrite reductases encoded by nirK and nirS, respectively; however, *Bradyrhizobium nitroreducens*, isolated from a rice field, harbours both genes (Jang et al. 2018). Recently, Nir protein identified from *Chloroflexi* (Schwartz et al. 2022) was found to display a chimeric arrangement, hinting that an ancestral horizontal transfer and a fusion event may have generated this. Identification of novel variants of the nirS and eNOR genes reveals that the denitrification

enzymes are diverse and perform important structural functions that warrant thorough investigation.

DNRA (respiratory ammonification, Fig. 2, Step 7), performed by heterotrophic and anaerobic chemolithoautotrophs, transforms nitrate (loss-prone) into a soil-retainable form of ammonium, thus alleviating N loss by competing with denitrification and anammox. Majorly, *Clostridium*, *Bacillus*, and *Enterobacter* perform DNRA in soils. Recently, Fe (II)-dependent nitrate reducers affiliated with betaproteobacteria (Li et al. 2022a) have been found to participate in DNRA. Noteworthy is that DNRA may assume high importance in soil amendment regimes in agricultural settings to prevent the loss of nitrogen from the soil. Nitrate reductase coded by the *nrfA* gene is found in different groups of DNRA performing bacteria, including Proteobacteria, Planctomycetes, Bacteroides, and Firmicutes (Pandey et al. 2020). Interestingly, the DNRA and denitrification pathways coexist, as observed in experimental studies involving *Shewanella loihica* strain PV-4, capable of performing the dual processes (Yoon et al. 2015). This study revealed that under  $\text{NO}_3^-/\text{NO}_2^-$  limitations and at high C/N ratios, transcription levels of denitrification genes (*nirK* and *nosZ*) decreased, leading to the predominance of the respiratory ammonification pathway, whereas acetate limitations at low C/N ratios increased *nirK* and *nosZ* transcription, leading to the predominance of denitrification. Although DNRA was previously believed to be a strictly anaerobic process, several studies have demonstrated that it occurs under aerobic conditions (Minick et al. 2016, Yang et al. 2017). The chemolithoautotrophic metabolism of DNRA bacteria facilitates the release of phosphorus in terrestrial water habitats, influencing eutrophication. Hence, this forms an important link between the phosphorus and nitrogen cycles (Yuan et al. 2022, Fig. 4).

Studies on the heterotrophic assimilation of ammonium (Fig. 2, Step 8) in the *Rhodococcus erythropolis* strain reveal its direct influence on denitrification under aerobic conditions (Ma et al. 2022). Ammonium increases reactions involved in the reduction of nitrate and nitrite and facilitates the transformation of total nitrogen. The assimilation of  $\text{NO}_3^-$  (Fig. 2, Step 8) by bacteria such as *Bacillus* and *Paenibacillus* drives the production of organic acids, which, in turn, showed a close association with rock phosphate solubilization (Garcia-Sanchez et al. 2023, Fig. 4). In organisms such as *Ps. putida* strain Y-9, the gene *nirBD*, a functional gene of DNRA, is also associated with the assimilation of  $\text{NO}_3^-$  (Huang et al. 2020). Interestingly, the genomic arrangement of *nirBD* in the strain Y-9 is similar to the arrangement of genes responsible for the assimilation of  $\text{NO}_3^-$  in *Ps. aeruginosa* (Jiang and Jiao 2016).

## Marine nitrogen cycle and recent trends

Marine ecosystems harbour metabolically versatile functional microbial communities performing complex biochemical transformations that drive the nitrogen cycle (Fig. 3), affecting atmosphere-ocean carbon flux and thus global climate change. Symbiotic associations form a predominant component of ocean diazotrophic communities contributing to nitrogen fixation (Fig. 3, Step 1). Examples include the heterocystous cyanobacterium *Richelia intracellularis* and certain oligotrophic diatoms such as *Rhizosolenia* and *Hemiaulus* (Caputo et al. 2019) and associations between a rhopalodiacean diatom and a diazotrophic non-photosynthetic cyanobacterium (*Pierella Karlusich* et al. 2021). Another form of symbiosis occurs with nitrogen-fixing microorganisms in the unicellular cyanobacterium 'Ca. Atelocyanobacterium thalassa' (UCYN-A), which lives in symbiosis with small unicellular haptophyte algae such as *Braarudosphaera bigelowii* (Martínez-Pérez et al. 2016). Nitrogen fixation in benthic

environments is performed by seagrass meadow and coral reef ecosystems. A specific association between the tropical seagrass *Posidonia oceanica* and *Gammaproteobacterium* (*Ca. Celerinatantimonas neptuna*) reveals that bacteria promote the survival and adaptation of seagrass under nitrogen-limiting conditions (Mohr et al. 2021). In seasonally hypoxic coastal zones, the synergy between iron-reducing bacteria and sulphate-reducing bacteria contributes significantly to the active nitrogen concentration in eutrophic sediments (Yao et al. 2021). Recent studies suggest that reactive nitrogen (dissolved organic nitrogen and ammonium) exuded by nitrogen-fixing microorganisms in surface sea water, contributes to the formation of water soluble organic nitrogen (WSON) hinting at direct but unidentified roles of microbes in nitrogen aerosols from marine habitats (Dobashi et al. 2023, Fig. 1).

Recent discoveries of microorganisms that catalyze nitrification in marine environments, (Fig. 3, Step 3) include nitrite-oxidizing bacteria belonging to the phyla Nitrospinota and Nitrospirata (Park et al. 2020). Advancements in culture and culture-free techniques have significantly broadened the phylogenetic diversity of nitrite-oxidizing bacteria, incorporating novel lineages such as *Ca. Nitrotoga* and *Ca. Nitromaritima* (Ngugi et al. 2016, Kitzinger et al. 2018). Comammox bacteria (*Nitrospira* sp.) (Fig. 3, Step 4) found in marine wetlands and coastal waters are distributed in estuaries with high runoff but lower salinity (Sun et al. 2020). Abundantly present cyanases, in nitrite-oxidisers and phytoplankton, encoded by *cynS*, catalyze the conversion of cyanate to ammonium and carbon dioxide and participate in organic N-utilization and the formation of  $\text{NO}_3^-$  (Mao et al. 2022). Nitrite-oxidoreductase (Nxr), an iron-metalloenzyme harboured by nitrite-oxidizing bacteria, exists as the most abundant (60 billion molecules per litre) microbial protein in the mesopelagic zone (Saito et al. 2020). In addition to oxidation of nitrite from sinking organic matter, it has moonlighting roles in biochemical reactions involving nitrogen and redox-sensitive metals. *Candidatus Nitrotoga fabula* has a unique nitrite oxidoreductase, enabling it to tolerate high nitrite and nitrate concentrations by utilizing electron donors, namely  $\text{H}_2$  and sulphite (Kitzinger et al. 2018). Studies reveal that the abundance of nitrite oxidoreductases may increase with prolonged deoxygenation in the oceans, leading to a mesopelagic demand for iron and potential changes to marine biogeochemical cycles (Saito et al. 2020).

$\text{N}_2$  production in offshore marine sediments, accounting to 67% (Thamdrup and Dalsgaard 2002), is contributed by anammox (Fig. 3, Step 5), and so far, six *Candidatus* genera (*Brocadia*, *Kuenenia*, *Jettenia*, *Scalindua*, *Anammoxoglobus*, and *Anammoximicrobium*) have been identified as anammox bacteria (Zhang and Okabe 2020). Recent studies further indicate that bacteria can directly couple nitric oxide reduction to ammonia oxidation for nitrate production (Hu et al. 2019, Babbín et al. 2020). Evidence suggests that the hadal trenches (>6 kilometres deep) are hotspots for the anammox process due to the high deposition of reactive organic matter acting as a source of electron donors for anammox and denitrification (Thamdrup et al. 2021). The co-existence and co-abundance of anammox bacteria and n-Damo (nitrite-dependent anaerobic methane oxidation) bacteria illuminate their biochemical importance in the marine biogeochemical cycles (Zhou et al. 2022).

Historically, marine heterotrophic denitrification (Fig. 3, Step 6) known to be performed by proteobacteria and archaea has now been demonstrated even by fungi, including *Acremonium* sp. and *Aspergillus versicolor* (Gao et al. 2020). Importantly, chemodenitrifiers including *Thiobacillus denitrificans* and *Thiomicrospira denitrifi-*

*cans* generate  $\text{N}_2$  from hydrogen sulphide ( $\text{H}_2\text{S}$ ) (Devol 2015), implicating this important reaction in atmospheric chemistry.

DNRA (Fig. 3, Step 7) is exhibited by marine Proteobacteria, Firmicutes, Verrucomicrobia, Planctomycetes, Acidobacteria, and Chloroflexi (Welsh et al. 2014). Recently, intracellular nitrate-storing diatoms have emerged as significant contributors to benthic nitrogen cycling, harbouring DNRA activity under anoxic conditions (Stief et al. 2022). Anoxia in saline environments promotes nitrate reduction via coupled DNRA and anammox, contributing to a reduction in atmospheric  $\text{N}_2\text{O}$  emissions (Valiente et al. 2022).

Stable isotope probing methods have been employed to investigate specific functional and phylogenetic groups involved in assimilation (Fig. 3, Step 8), revealing that the assimilation of  $\text{NO}_3^-$  and urea is predominantly carried out by organisms closely related to the eukaryotic diatom *Chaetoceros*, while  $\text{NH}_4^+$  assimilation is primarily dominated by prokaryotic members of Flavobacteriaceae and Rhodobacteraceae (Morando and Capone 2018). Furthermore, taxonomic and comparative genomic analyses conducted in heterotrophic proteobacterial lineages, including *Methylophaga*, *Marinobacterium*, *Pseudomonas*, and *Acinetobacter*, have provided evidence that the assimilatory nitrate reduction (ANR) process in estuarine ecosystems involves the coordinated action of nitrite reductase (NirBD) and assimilatory nitrate reductase (NasA) (Hu et al. 2022).

## Impact of climate change on nitrogen cycling

Various climate change events directly influence nitrogen transformations and dynamics. Increased drought decreases the rates of denitrification and mineralization, causing nitrogen accumulation (Van Metre et al. 2016). Natural forest wildfires positively influence soil organic carbon and nitrogen pools but negatively affect N mineralization. Wildfires in the Arctic tundra retain higher soil nitrogen due to the increased incorporation of nitrogen into microbial biomass (Xu et al. 2022). Further, N losses from forest ecosystems occur through leaching and a decline in microbial activity (Cobo-Díaz et al. 2015), which pave the way for the reestablishment of beneficial microbes.

Experiments demonstrate that soil freeze-thaw events further supplement the soil's labile C, leading to the enhancement of catabolic (N mineralization and nitrification) and anabolic (N assimilation leading to an increase in abundance) activities of the microbial communities during winter (Isobe et al. 2022). Decreases in soil moisture may exacerbate N limitation and water deficits, thereby suppressing soil enzyme activity. During limitation of N, alpine meadow ecosystems display enhanced cycling of nitrogen to restrict loss of nitrogenous forms (mainly nitrate), resulting in increased  $\text{N}_2\text{O}$  emissions (Zhang et al. 2022a). Such studies are important to understand the effect of climate change on soil microbial populations that directly influence nitrogen cycling in different habitats.

Alterations in precipitation may influence  $\text{N}_2\text{O}$  flux in alpine meadow ecosystems, leading to N loss in the future (Zhang et al. 2022a). Further, high intensity rainstorms flush 14 times more N from soils than undisturbed areas (Gustine et al. 2022). Waterlogging alters the proportion of  $\text{NH}_4^+$  and  $\text{NO}_3^-$  in the soil, which affects N accumulation in plant tissues (Gu et al. 2019). Prolonged precipitation and flooding increase leaching of nitrate, increase N mineralization, and translocate mobile  $\text{NO}_3^-$  in the upper root zone (Murphy et al. 2021). Nitrogen-containing soil organic matter undergoes microbially mediated decomposition, releasing  $\text{NH}_4^+$  to the surrounding groundwater (Liang et al. 2022), exuberating

anthropogenic and natural eutrophication of aquatic bodies with higher nitrogen flux (Sinha et al. 2017).

Increased carbon dioxide (CO<sub>2</sub>) directly stimulates nitrogen-fixing cyanobacteria, inhibits nitrification by archaea and bacteria (Hutchins et al. 2019) and indirectly (oxygen depletion) influences marine ecosystems (Voss et al. 2013). A meta-analysis (Wannicke et al. 2018) demonstrated that ocean acidification enhanced diazotrophic nitrogen fixation by 29% ± 4% and decreased nitrification by 29% ± 10%. The declining ocean pH significantly increases N<sub>2</sub>O production by nitrifiers. If pH continues to decline at the same rate, N<sub>2</sub>O production in the Subarctic North Pacific is predicted to increase up to 491% by the end of this century (Breider et al. 2019). Surface warming and ocean stratification could reduce nutrient supplies to the euphotic zone (Kwiatkowski et al. 2020), causing diminished primary production, decreased nitrification rates, and associated N<sub>2</sub>O releases. Incubation experiments demonstrate that warming inhibits ammonia oxidation by ocean AOA communities, although additional ammonia inputs from atmospheric deposition may aid in thermal adaptation (Zheng et al. 2020). Although oxygen-depleted zones represent ideal environments for nitrogen fixers as reduced O<sub>2</sub> levels benefit the O<sub>2</sub>-sensitive nitrogenase enzyme, expanding oxygen-deficient zones (ODZs), as a result of climate change, have become major sites of natural nitrous oxide (N<sub>2</sub>O) production and emissions. While denitrification is responsible for the highest N<sub>2</sub>O production below the oxic–anoxic surface in marine systems, ammonia oxidation accounts for the production of N<sub>2</sub>O at the upper oxic levels (Frey et al. 2020).

### Exploring the links between nitrogen and other biogeochemical cycles

The interactions of nitrogen with other elements in the ecosystem (Fig. 4) influence their overall stocks in the ecosystem. Here, we have discussed the interactions of nitrogen with other elements that influence the global nitrogen cycle.

The incorporation of carbon is repressed when decomposition is affected by climate change. Warm temperatures favour decomposition, leading to increased net primary production (NPP) and C sequestration (Shaver et al. 2000). Nitrogen-induced NPP accounts for 13% of the global forest NPP and dominantly affects carbon sinks in forests (Du and de Vries 2018). In marine systems, excess nitrogen stimulates the production of C-rich algal biomass and ocean acidification, resulting in C loss through increased organic C mineralization and the release of CO<sub>2</sub> (Nakayama 2022).

Various links between microbial sulphur and nitrogen pathways have been attempted to decipher the interconnected pathways (Gurung and Mattoo 2021). Thiosulfate, an electron donor in the denitrification reaction, is found in *Thiobacillus*, *Lentimicrobium*, *Sulfurovum*, and *Hydrogenophaga*. Elemental sulphur stimulates denitrification and DNRA in *Thiobacillus* (with NrfA and NirB) and *Nocardioideis* (with only NirB) by acting as an electron donor (Li et al. 2022). A novel link between nitrogen and sulphur cycles with respect to sulphate-dependent ammonium oxidation (Sulfammox) involving coupling ammonia oxidation with sulphate reduction under anaerobic conditions has been unravelled in natural environments (Liu et al. 2021). Assessing the rate of microbial sulphate reduction in designed wetlands employing extended <sup>34</sup>S-stable isotope analysis and mass balance where sulphur oxidation co-occurs with pathways of S conversion provides intriguing evidence with links to sulfammox (Liu et al. 2021). *Neisseria* and *Treponema* catalyze key nitrogen and sulphur transformations such

as sulphur reduction and anammox in mangroves (Meng et al. 2022).

Nitrogenase (molybdenum nitrogenase), along with its alternative forms, namely vanadium and iron only nitrogenase (under molybdenum limiting conditions), utilize iron for nitrogen fixation. The nitrogenase complex is composed of two 38 kDa Fe proteins, each containing one 4Fe-4S cluster, and a ~220 kDa dimeric MoFe protein, which contains 30 Fe atoms. Therefore, the high demand for iron for nitrogenase synthesis suggests that nitrogen fixation may be limited by Fe availability (Whittaker et al. 2011). Reactions coupling iron and nitrogen, such as Fe (III) reduction coupled to anammox (Feammox) and nitrate-dependent Fe (II) oxidation (NDFO), have been detected in tropical forests and riparian zones, resulting in significant N utilization (Ding et al. 2017, Yang et al. 2021). *Geobacter*, *Shewanella*, *Proteobacteria*, *Actinobacteria*, and *Acidimicrobiaceae* actively participate in the Feammox process (Xia et al. 2022).

As depicted in Fig 4, phosphorus influences nitrogen cycling. As a key element, phosphorus, alters microbial nitrogen immobilization in soils, thereby influencing denitrification and nitrous oxide emissions. Nitrogen fixation and mineralization, stimulated by phosphorus additions, integrate further nitrogen in the nitrifying or denitrifying processes, expediting increased N<sub>2</sub>O emissions (Mori et al. 2017). Links between nitrogen and phosphorus cycles are further revealed when phosphorus deprived ecosystems display reductions in N<sub>2</sub>O emissions due to a decline in nitrate availability and affecting denitrification (Wang et al. 2022a). Synergistic reactions between phosphate solubilizing bacteria (*Paenibacillus* sp.) and diazotrophs (*Pa. beijingensis*) have demonstrated improvement in total N in soil, doubling the expression of the nifH gene compared to a single bacteria inoculation, implicating its importance in agriculture (Li et al. 2020). Assessing the responses of nitrogen in relation to other elements, including phosphorus, sulphur, and carbon, would be of vital importance (Fig. 4) in climate smart agricultural techniques (Gurung and Mattoo 2021).

### Anthropogenic disturbances in the global nitrogen cycle

Agricultural fertilization and fossil fuel combustion contribute to ~150 Tg y<sup>-1</sup> of reactive nitrogen to soil ecosystems. Application of chemical fertilisers affects the abundance of microbial communities and nitrogen cycling genes (Lin et al. 2021, Mattoo et al. 2021). Paddy fields, when supplemented with synthetic fertilisers, generate ~60% of N<sub>2</sub>O (Timilsina et al. 2020), perhaps due to alterations in microbial communities causing imbalances in nitrogen cycling and nutrient content. Application of agricultural chemicals including urea (540 kg<sup>-1</sup> h<sup>-1</sup> y<sup>-1</sup> dm), potassium (180 kg<sup>-1</sup> h<sup>-1</sup> y<sup>-1</sup> dm), phosphate (540 kg<sup>-1</sup> h<sup>-1</sup> y<sup>-1</sup> dm), di-ammonium phosphate (DAP) (180 kg<sup>-1</sup> h<sup>-1</sup> y<sup>-1</sup> dm), gypsum (63 kg<sup>-1</sup> h<sup>-1</sup> y<sup>-1</sup> dm), and boric acid (63 kg<sup>-1</sup> h<sup>-1</sup> y<sup>-1</sup> dm) during rice cultivation decreases soil nitrate, ammonia, soil organic carbon (SOC), and total N and C compositions (Rahman et al. 2020). Additionally, fertiliser runoff leads to eutrophication, causing hypoxia (Fig. 1) and limiting nitrification, which, in turn, restricts denitrification in aquatic bodies (Yao et al. 2021).

Anthropogenic practises involving excessive soil tillage result in the loss of soil N due to substrate exposure (Xiao et al. 2019). Furthermore, increased tillage decreases soil nitrate concentrations due to nitrate leaching resulting from alterations in soil structure and water movement (Li et al. 2021b). Microplastic accumulation restricts the expression of key functional genes encoding the enzymes of the nitrogen cycle (Seeley et al. 2020). Polyvinyl

chloride (PVC) microplastics from fertiliser application, wastewater irrigation, and plastic mulching manifest in an increase in bacterial (*Amycolatopsis*, *Sinomonas*, *Nocardia*, *Bradyrhizobium*, and *Burkholderia*) and fungal (*Exophiala* and *Cladophialophora*) populations and a decrease in archaea (*Ca. Nitrosocosmicus*), leading to increased  $\text{NH}_4^+$  and decreased  $\text{NO}_3^-$  content in soil (Zhu et al. 2022).

Long-term exposure of soils to pharmaceutical compounds, namely tylosin, chlortetracycline, and sulfamethazine, through farming practises such as the addition of manures has been found to affect nitrogen fixation by altering the composition of *Bradyrhizobium* populations thriving in nodules of leguminous plants (Revellin et al. 2018). Nitrification reactions display a higher sensitivity to pharmaceutical chemicals than natural mineralization or ammonia volatilization processes (Pashaei et al. 2022). Hence, circumventing disturbing anthropogenic activities, especially in agricultural settings, could minimize increased greenhouse gas emissions while mitigating additional influences of climate change.

### Supplementing nitrogen through the inclusion of beneficial nitrogen-fixing bacteria

Because excess application of nitrogen fertiliser results in contamination of ecosystems, alternative approaches attained by introducing microorganisms for soil nitrogen management have been recommended (Gurung and Mattoo 2021, Mattoo and Gowda 2022). For example, microbial consortia comprising *Rhizobium phaseoli*, *Sinorhizobium americanum*, and *Azospirillum brasilense* promote nitrogen fixation in maize (Gómez-Godínez et al. 2019). *Nostoc*, *Calothrix*, *Anabaena*, and *Wollea* fulfil nitrogen requirements for rice cultivation (Iniesta-Pallarés et al. 2021). When the nitrogen-fixing strain, *Rhizobium mayense*, isolated from the groundnut rhizosphere, was inoculated into green gram and finger millet fields, it enhanced crop growth and chlorophyll content by influencing high nitrogenase activity and hence served as a potential biofertiliser (Shameem M et al. 2023). Inoculating legumes with endosymbionts leads to an increase in nitrogen uptake from the soil, resulting in better plant yields. Nitrogen fixation in soya beans is enhanced by the application of *Bradyrhizobium* at the time of sowing (Pedrozo et al. 2018). Additionally, improved nitrogen fixation and nodulation have been observed in pigeon pea when inoculated with *Bradyrhizobium* (Soni et al. 2021). Fungal inoculations into legumes enhance nitrogen fixation through improved root functions. For example, inoculation of the white rot fungus *Ceriporia lacerata* HG2011 into *Vicia faba* produced more lateral roots with large surfaces, which provided more attachment sites for rhizobia in their nodules, thereby boosting nitrogen fixation (Yin et al. 2022). The application of microbe harbouring legumes (intercropping) is an effective approach towards meeting the nitrogen requirements of plants as they improve the abundance of diazotrophs, thereby boosting BNF.

Conservation agricultural techniques, including raised beds and conservation tillage, benefit N-cycling bacterial communities, manifesting in enhanced crop productivity (Singh et al. 2021). Field management involving crop residue retention exhibits better aggregation, improved soil moisture, and increased organic carbon matter due to enhanced microbial denitrification activity. Biochar, as a soil amendment, has shown positive effects in inhibiting soil nitrogen loss and improving the nitrogen fixation capacity of soils (Zhang et al. 2022b). Combining biochar and nitrogen-fixing microorganisms could be a key strategy for improving nitrogen utilization efficiency in plants by supporting enzyme activities associated with nitrogen cycling (Gou et al. 2023).

Microbial inoculants offer promise in not only restricting synthetic nitrogen fertilisers, which are not always required, but also serve as a valuable source of BNF.

### Conclusions

Microorganisms participating in the nitrogen cycle are diverse, myriad, and functionally versatile, warranting thorough future investigations. Microbial reactions contribute to nitrogen generation, greenhouse gas emissions and consumption in soil, eutrophication, and the removal of nitrogenous compounds from aquatic systems. Microbial webs in nature are complex and link nitrogen to other vital elements such as phosphorus, sulphur, etc. Identification of the contributing factors and substrates involving nitrogen transformation is necessary and requires insight and deeper analysis of microbial abundance and evolution. From past research, we learn that microorganisms in the nitrogen cycle may possess moonlighting roles, and future research should be oriented towards interdisciplinary, cutting-edge techniques to unravel the mysterious nitrogen cycle for applied research in mitigating climate change.

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