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Review

Diversity and ecology of NrfA-dependent ammonifying microorganisms

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Nitrate ammonifiers are a taxonomically diverse group of microorganisms that reduce nitrate to ammonium, which is released, and thereby contribute to the retention of nitrogen in ecosystems. Despite their importance for understanding the fate of nitrate, they remain a largely overlooked group in the nitrogen cycle. Here, we present the latest advances on free-living microorganisms using NrfA to reduce nitrite during ammonification. We describe their diversity and ecology in terrestrial and aquatic environments, as well as the environmental factors influencing the competition for nitrate with denitrifiers that reduce nitrate to gaseous nitrogen species, including the greenhouse gas nitrous oxide (N_2O). We further review the capacity of ammonifiers for other redox reactions, showing that they likely play multiple roles in the cycling of elements.

Highlights

Non-fermentative nitrite reduction to ammonium, resulting in the release of ammonium in the environment, can be performed by a variety of enzymes found among bacteria and archaea.

NrfA-dependent ammonifiers are phylogenetically diverse and are present in terrestrial and aquatic environments, and for the latter especially in sediments.

NrfA-dependent ammonifiers often carry genes involved in denitrification and redox reactions with sulfur or iron compounds, suggesting that they play a role in multiple biogeochemical cycles.

In most aquatic and terrestrial environments, denitrifiers dominate over nitrate ammonifiers, and denitrification is the dominating process of nitrate reduction. However, nitrate ammonification rates can be significant under certain redox conditions.

Nitrate ammonifiers support nitrogen retention in ecosystems

Nitrogen is an essential nutrient for all living organisms and the element most often limiting primary production in ecosystems. The bioavailability of nitrogen, and in which form nitrogen is present, is controlled by a number of oxidation and reduction reactions (Figure 1) mediated by microorganisms, predominantly bacteria and archaea. These transformations drive input, recycling, retention, and loss of gaseous and water-soluble nitrogen from ecosystems. The **dissimilatory** (see Glossary) reduction of nitrate to ammonium is known as nitrate ammonification or dissimilatory nitrate reduction to ammonium (DNRA). The organisms performing this process are among the least studied in the nitrogen cycle [1] despite being very diverse and present in many different ecosystems (e.g., [2,3]). Nitrate ammonifiers couple the oxidation of various electron donors, most often organic carbon compounds, to the reduction of nitrate under anoxic conditions in a two-step reaction. First, nitrate is reduced to nitrite, which is subsequently reduced to ammonium, and this second step is what defines nitrate ammonification [4,5].

The reduction of nitrate to ammonium creates a short-circuit in the nitrogen cycle [6] and results in nitrogen retention (Figure 1). This can be beneficial in nitrogen-limited ecosystems or agricultural soils where it is desirable to improve nitrogen use efficiency [7] but is negative in eutrophicated systems [8] and wastewater treatment supporting nitrogen removal [1]. However, nitrate can also be reduced via **denitrification**, resulting in gaseous nitrogen losses from ecosystems (Figure 1). Denitrification is the major global nitrogen sink, returning nitrogen as dinitrogen gas to the atmosphere. Nevertheless, during denitrification a substantial amount of nitrate is reduced to N_2O, which exhibits a global warming potential approximately 300 times higher than that of carbon dioxide (CO_2) and is now the primary cause of ozone layer depletion [9]. The N_2O concentration in the atmosphere is increasing at an accelerating rate, of which microbial activity in soils, oceans, inland and coastal waters, as well as agriculture and forestry contribute nearly 90% to global N_2O emissions [10], with denitrification as the main source [11]. Thus, the predominant

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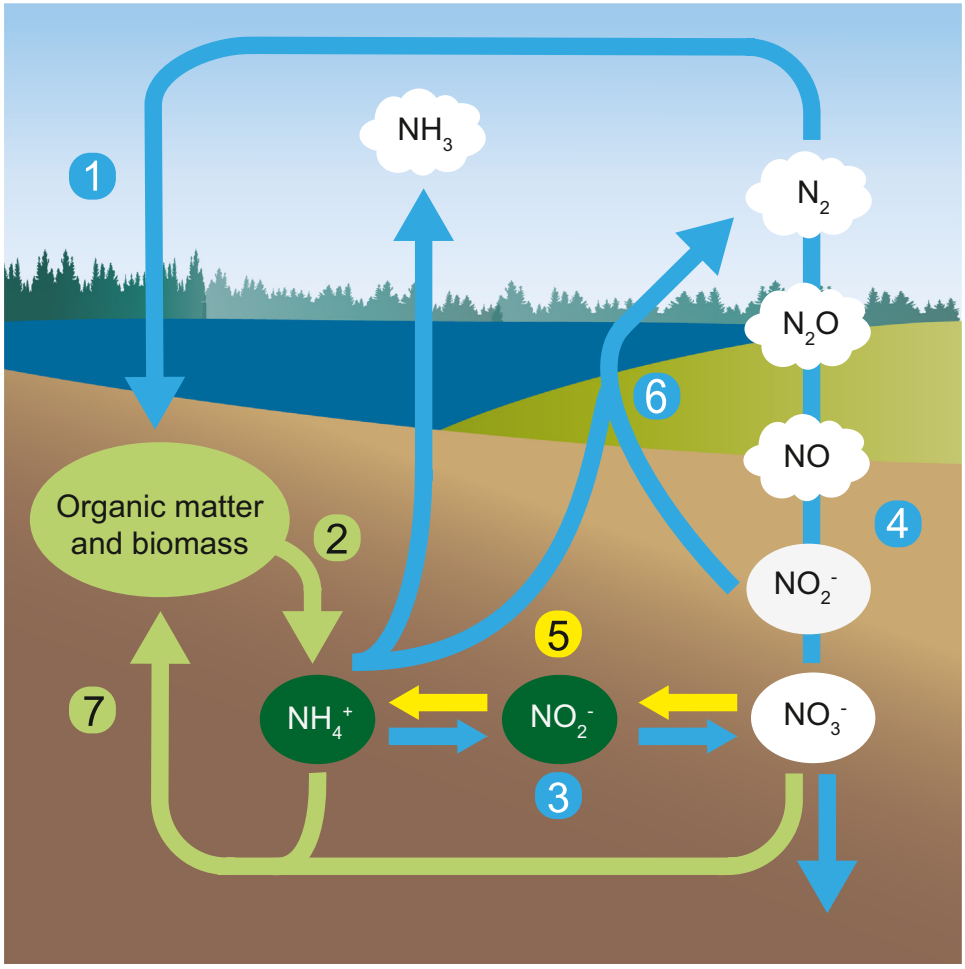

Figure 1. Schematic illustration of the inorganic nitrogen cycle. The major nitrogen transformation pathways are indicated by numbers: (1) di-nitrogen fixation, (2) nitrogen mineralization, (3) nitrification, (4) denitrification, (5) nitrate ammonification, (6) anaerobic ammonium oxidation (anammox), and (7) assimilation of ammonium and nitrate. Products and a selection of intermediates are shown by their chemical names. Nitrogen is lost from ecosystems through leaching of nitrate and gaseous nitrogen losses. Adapted from [7] (copyright license: CC BY 4.0).

nitrate reduction pathway has major consequences for ecosystem primary production, eutrophication, and climate change.

In this review, we synthesize the current understanding of the diversity and ecology of free-living ammonifiers in aquatic and terrestrial environments. This includes a summary of the enzymes catalyzing nitrate and nitrite reduction to ammonium, with more details on the latter being the key step in nitrate ammonification. We specifically focus on the ecology of NrfA-dependent ammonifiers because the NrfA nitrite reductase is a periplasmic protein known to release ammonium in the environment and its gene is the most widely used marker to study ammonifiers, while knowledge on the phylogenetic and environmental distribution of other nitrite-ammonifying enzymes is limited.

Enzymes involved in nitrate ammonification

The reduction of nitrate to nitrite, the first step in nitrate ammonification, is catalyzed by either of the two molybdenum-containing nitrate reductases Nap or Nar [12]. Nar is membrane-bound

Glossary

Assimilation (biology): the process by which organisms absorb and convert food substrates or nutrients that are incorporated into the cells.
Biogeochemical cycles: the movement and transformation of elements and compounds between living and non-living forms on Earth and its atmosphere.
Comammox: complete ammonia oxidation, a microbial process in which ammonia is oxidized to nitrate within a single organism.
Denitrification: an anaerobic respiratory pathway consisting of the sequential reduction of soluble nitrate (NO_3^-) or nitrite (NO_2^-) to the gaseous products nitric oxide (NO), nitrous oxide (N_2O), and di-nitrogen gas (N_2). The entire pathway can be achieved by a single organism or shared across several organisms specialized in one or several steps (see Figure 1 in the main text).
Dissimilatory: metabolic processes where compounds or elements are reduced or oxidized to remove or provide electrons to obtain energy without their incorporation into the cell (see assimilation). Both nitrate ammonification in fermentative bacteria (using e.g., NirB) and respiratory nitrate ammonification (using e.g., NrfA) are dissimilatory processes.
Facultative (in microbiology): an organism capable of living under different conditions and not restricted to one specific condition by switching between different metabolic pathways.
Fermentative bacteria: bacteria able to obtain energy by converting organic molecules into energy under anoxic conditions in a process in which the substrate is partly oxidized and partly reduced, and ATP is produced by substrate-level phosphorylation.
Homologs (genes): homologous genes have a common ancestor and share sequence similarities.
Metagenome-assembled genomes (MAGs): microbial genomes obtained by assembling metagenomic sequences. MAGs reflect the average genetic repertoire of co-occurring and closely related subpopulations present in a metagenome, contrary to genomes of cultivated isolates which represent single genetic variants.
Metagenomics: a cultivation-independent and sequencing-based method in which the collective genome of a community (i.e., the metagenome) is obtained.

and releases protons in the **periplasm**, directly contributing to proton motive force generation. By contrast, Nap is periplasmic and is not involved in proton translocation across the membrane. Nap and Nar are present either alone or in combination in organisms, and their production is regulated in response to oxygen and nitrate/nitrite [13]. However, these enzymes are not unique to nitrate ammonification and are present in other pathways, like denitrification.

Two families of **metalloenzymes** are known to catalyze the six-electron reduction of nitrite to ammonium. Siroheme-containing nitrite reductases (e.g., NirA, NirB) using ferredoxin or NAD(P)H as electron donor are found in archaea, bacteria, fungi, protists, and plants, where they are involved in ammonium **assimilation** and nitrite detoxification [14–18]. In **fermentative bacteria**, NirB can also be used to regenerate the NAD^+ pools for glycolysis during anaerobic growth, resulting in the generation of one extra ATP molecule by substrate-level phosphorylation for each molecule of acetate generated [19]. The role of NirB in assimilatory nitrite ammonification and in fermentative bacteria will not be discussed further in this review. The five multiheme cytochrome *c* (MHC) nitrite reductases that have been characterized so far [pentaheme NrfA (Figure 2A and Box 1) and those with eight heme-binding sites: octaheme nitrite reductase (ONR), octaheme tetrathionate reductase, epsilonproteobacterial hydroxylamine oxidoreductase, and Ih octaheme cytochrome *c*) are exclusively found in archaea and bacteria [20–25]. Because these enzymes are located in the periplasm, ammonium can be released into the environment and not directly assimilated. However, the degree to which these enzymes are functionally redundant is not known. The MHC nitrite reductases belong to a family of **redox** enzymes that play key roles in the **biogeochemical cycles** of nitrogen, sulfur, and iron, which has stimulated attempts to resolve their origin and evolutionary history. Challenging the idea that the main evolutionary mechanism was the progressive fusion of redox modules towards more complexity [26,27], a recent study incorporating newly identified MHC reductases has argued for an octaheme ancestor with nitrite reduction activity and subsequent grafting and pruning events of redox modules to explain the diversity of extant MHCs [28]. Yet, relatively little information is available on the octaheme nitrite reductases, which are constrained to certain taxa and, in the case of ONR, display nearly negligible abundance in terrestrial environments [29]. By contrast, NrfA-dependent bacteria are phylogenetically widespread and likely contribute to the recycling of nitrogen at the global scale [29]. In addition to the ammonium-forming nitrite reductases, there are nitric oxide-forming nitrite reductases, most known for performing a key step in denitrification. There are two evolutionarily distinct nitric oxide-forming nitrite reductases, the heme-coordinating cytochrome cd_1 NirS and the multicopper-oxidase NirK [30], and they compete with NrfA for nitrite.

Physiological roles of NrfA

NrfA is best known for its role in **respiratory** nitrate ammonification under oxygen-limited conditions, where it contributes to the proton motive force by regenerating the menaquinone pool that is necessary for the oxidation of dihydrogen, sulfide, or nonfermentable organic molecules (e.g., acetate, formate, lactate) [31–33] (Figure 2B and Box 1). The proton motive force is generated by menaquinone turnover during electron transport from the electron donor to nitrite through a redox loop [13,34].

Like other MHC nitrite reductases, NrfA is a promiscuous enzyme with a broad substrate spectrum. Specific activities for other compounds are, however, always much lower than for nitrite and vary among different organisms [35,36]. NrfA contributes to oxidative and nitrosative stress defense mechanisms by mediating the reduction of hydrogen peroxide to water, hydroxylamine to ammonium, and nitric oxide to N_2O or ammonium [37]. NrfA is also able to reduce N_2O, possibly with dinitrogen as reaction product, but with very low specific activity [35]. Finally, NrfA can perform the six-electron reduction of sulfite to sulfide and thus connects the nitrogen and sulfur

Metalloenzymes: enzymes that use a metal cation (e.g., Fe^{2+}) as a cofactor in their active site. The metal cation is essential for the structural stability and/or catalytic activity of the enzyme.
Monophyletic clade: a group of genes, proteins, or genomes in a phylogeny that includes their most recent common ancestor and all the descendants of that most recent common ancestor.
Periplasm: the space between the outer and inner membranes of Gram-negative bacteria or between the cell wall and plasma membrane in Gram-positive bacteria.
Phylogeny: the inferred evolutionary relationship of a set of genes, proteins, or genomes based on the similarities and differences in their characteristics.
Redox: the short form for reduction–oxidation, which is a reaction causing a change in the oxidation state of a substrate. Electrons are released during oxidation (increase in the oxidation state of the substrate) while they are gained during reduction (decrease in the oxidation state of the substrate).
Respiratory: refers to a metabolic process by which substrates are oxidized together with the reduction of an external electron acceptor, and converted into energy via the generation of a proton motive force and where ATP is produced by oxidative phosphorylation.

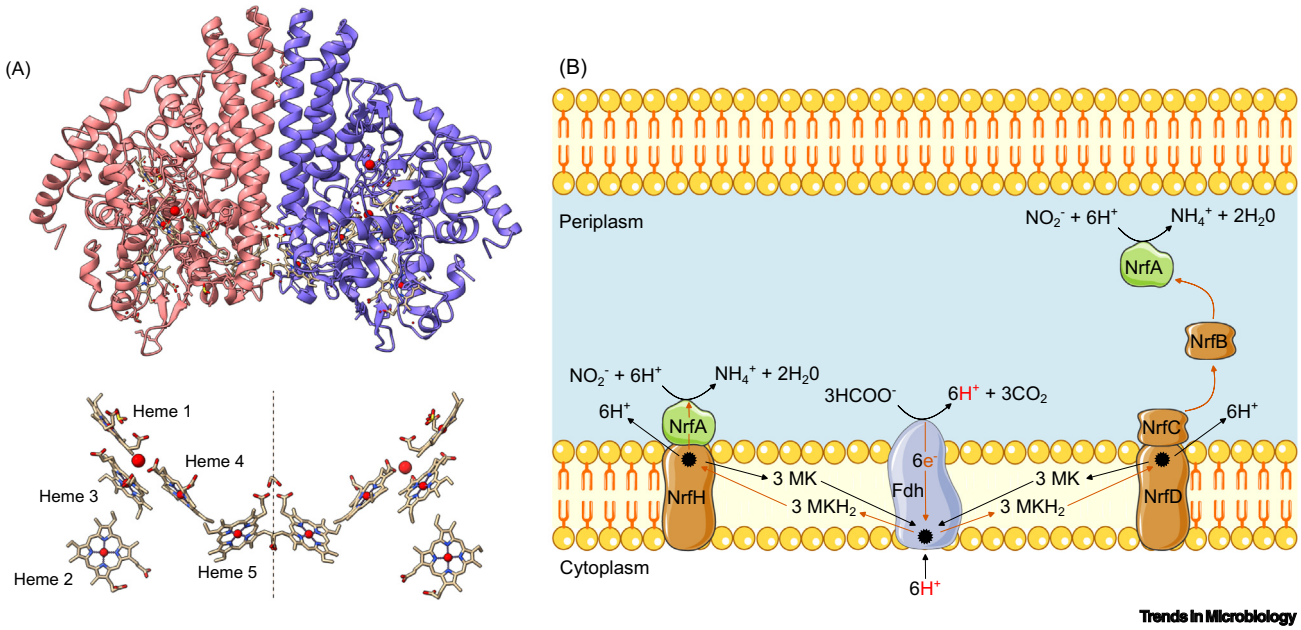

Figure 2. Structure of NrfA and schematics of the enzyme complexes involved in the electron transport chain from formate to nitrite. (A) Upper panel: overall structure of the NrfA homodimer from *Escherichia coli* [Protein Data Bank (PDB)-ID 3L1T]. Front view with the dimer axis oriented vertically, the five heme groups in each monomer (stick representation), the calcium ions (large red circles) and the iron atoms (small red circles). Monomers are shown in pink and purple, respectively. Lower panel: heme groups in stick representation in the same orientation as in the upper panel. Hemes in the left monomer are numbered according to their attachment to the protein chain. The active site is located at heme 1. Small red circles represent the central iron atoms and large red circles depict calcium ions. The two hemes 5 interact across the dimer interface (indicated by a vertical line). Generated using the protein structure visualization program ChimeraX [100]. (B) Electron transport from formate to nitrite in respiratory nitrite ammonification, with either NrfH (left) or NrfBCD (right) as electron carriers, as characterized in *Wolinella succinogenes* and *E. coli*, respectively [4]. The subunits of the formate dehydrogenase (Fdh) are not shown. Protons colored in red are involved in the electrogenic oxidation of formate by menaquinone (MK), thus generating a proton motive force by a redox loop mechanism. Substrates and products of the redox reactions are written in their neutral forms for simplicity. Protein and membrane icons from Bioicons' open-source illustrations (https://bioicons.com/).

cycles [38]. However, sulfite does not induce NrfA formation, the conformation of NrfA is optimized for a negatively charged substrate and a positively charged product, and the rates of sulfite reduction are orders of magnitude lower than those of nitrite. This indicates that sulfite reduction is not the primary function of NrfA but could rather be a way to prevent inhibition of the enzyme's active site, as sulfite is a competitive inhibitor of nitrite [39]. A similar mechanism is at play in sulfate-reducing bacteria, where nitrite is a competitive inhibitor of sulfate for the sulfate reductase, and NrfA is used to limit nitrite entry into the cells [40].

Diversity of NrfA-dependent ammonifiers

A recent analysis of more than one million microbial genomes of isolates and **metagenome-assembled genomes** has shown that **homologs** of the gene *nrfA*, which codes for NrfA, are predominantly present in single copy and in a wider range of bacterial phyla than previously thought as well as in a few archaea (Figure 3) [29]. Sequencing of additional genomes is needed to assess whether the comparatively smaller number of archaeal genomes carrying *nrfA* is characteristic of this domain or simply reflects the current under-representation of archaea in genome databases (<1%). Among bacteria, nitrite ammonifiers are found in most phyla, and the dominating phyla represented in the NrfA **phylogeny** are Bacteroidota, Bacillota, Desulfobacterota, Pseudomonadota, Actinomycetota, and Chloroflexota in descending order. Further, Cys-X-X-Cys-His-NrfA sequences form a highly diverse and **monophyletic clade** nested within the larger Cys-X-X-Cys-Lys-NrfA clade, supporting a later emergence of the Cys-X-X-Cys-His variant [28]. NrfA-dependent ammonification activity has been experimentally demonstrated for only a handful

Box 1. Structure of NrfA and regulatory mechanisms

NrfA is a homodimer (~120 kDa) containing five *c* hemes per monomer and performs the reduction of nitrite to ammonium without releasing intermediates (see Figure 2A in the main text). Seven crystal structures are available to date, four obtained from Pseudomonadota and three from Desulfobacterota (former Delta-proteobacteria) [83–89] (see Figure 3 in the main text). NrfA has separate channels for nitrite entry and ammonium release, with matching electrostatic surface potentials to facilitate the flux (positive for nitrite, negative for ammonium). The catalytic site is located in the first heme-biding motif, of which two variants, Cys-X-X-Cys-His and Cys-X-X-Cys-Lys, exist. The other four heme-biding motifs have a Cys-X-X-Cys-His structure and act as a 'wire' to transfer the electrons received from the electron carrier to the catalytic center. NrfA proteins from different bacterial taxa display structural specificities, including electron storage and distribution properties and the presence/absence of a calcium ion (Ca^{2+}) near the active site [89,90]. The most common electron carrier is NrfH, a tetraheme cytochrome of the NapC/NirT family [91], whereas NrfB, a pentaheme cytochrome c unrelated to NrfH, and CymA, also belonging to the NapC/NirT family, are restricted to Gammaproteobacteria [92,93]. Only the NrfA sequences predicted to have NrfB as electron carrier contain the seven-residue insertion near the heme 2 binding motif that is the putative docking site for NrfB [86].

The regulation of *nrfA* has been investigated in a few model organisms belonging to Pseudomonadota, Bacillota, and Desulfobacterota. These studies have shown that *nrfA* expression under respiratory ammonification is under the control of complex nitrate/nitrite, carbon, and oxygen responsive systems including NarQP/NarXL, cyclic-AMP receptor proteins, and FNR, respectively [94–96], whereas transcriptional regulators of the NsrR and Nss families [97,98], as well as NrfR two-component systems [99], respond to nitric oxide and nitrite under nitrosative stress. However, whether members of the environmentally abundant CXXCH clade use these regulatory systems has yet to be confirmed.

of strains (Figure 3, and Table S1 in the supplemental information online) and our knowledge of the diversity of microorganisms performing this process relies mainly on gene function predictions based on sequence similarity with characterized NrfA proteins.

NrfA-dependent ammonifiers are functionally diverse and can be **facultative** anaerobes or obligate anaerobes. Some are involved in redox reactions with sulfur or iron compounds (e.g., [41,42]) and include members of Campylobacterota (e.g., *Sulfurospirillum deleyianum*, *Wolinella succinogenes*), Desulfurobacterota (e.g., *Desulfovibrio* and *Geobacter* spp.), and Pseudomonadota (e.g., *Shewanella* spp.), as well as thermophilic sulfate reducers (classes Thermodesulfovibrionia in Nitrospirota and Archaeoglobi in Halobacteriota). The *nrfA* gene is also present in the genomes of bacterial (Methylomirabilota) and archaeal (*Methanoperedens* and *Methanimicrococcus* spp.) methane oxidizers. In the latter, nitrite ammonification is coupled to methane oxidation [43]. Relevant to the global circulation of nitrogen is the coexistence of *nrfA* with genes involved in other nitrogen transformations within the same genome. For example, *nrfA* has been detected in some **comammox** genomes [44] and in four of the six known genera of bacteria capable of anaerobic ammonium oxidation (anammox; *Candidatus* Brocadiae, Jettenia, Loosdretchtia, and Scalindua) [29,45]. Likewise, 45% of the ca. 1200 genomes included in the most recent NrfA phylogeny [29] carry at least one denitrification gene, and among those, 50% had the genetic potential for N_2O production whereas only 38% had a *nosZ* gene indicating capacity for N_2O reduction, though this varies both between and within phyla. The N_2O production capacity was mainly linked to nitric oxide detoxification via a nitric oxide reductase rather than denitrification. Nevertheless, N_2O can also originate from reduction of nitric oxide by the hybrid cluster protein (Hcp) [46], and its expression is induced by nitric oxide [47]. Thus, there are several mechanisms apart from N_2O production by NrfA in ammonifiers that can explain observations of N_2O production from soil isolates of nitrate-ammonifying bacteria [48]. Overall, it can be concluded that nitrite ammonifiers represent a diverse group of microorganisms with the potential to be important players not only in the nitrogen cycle, but also in the cycling of organic matter, methane, and elements such as sulfur, iron, and hydrogen.

Ecology of NrfA-dependent ammonifiers

NrfA-ammonifiers have until fairly recently received little attention in environmental studies and comprehensive molecular approaches to study these organisms developed later than for other functional groups in the nitrogen cycle [3,49]. These methods have been used to quantify the

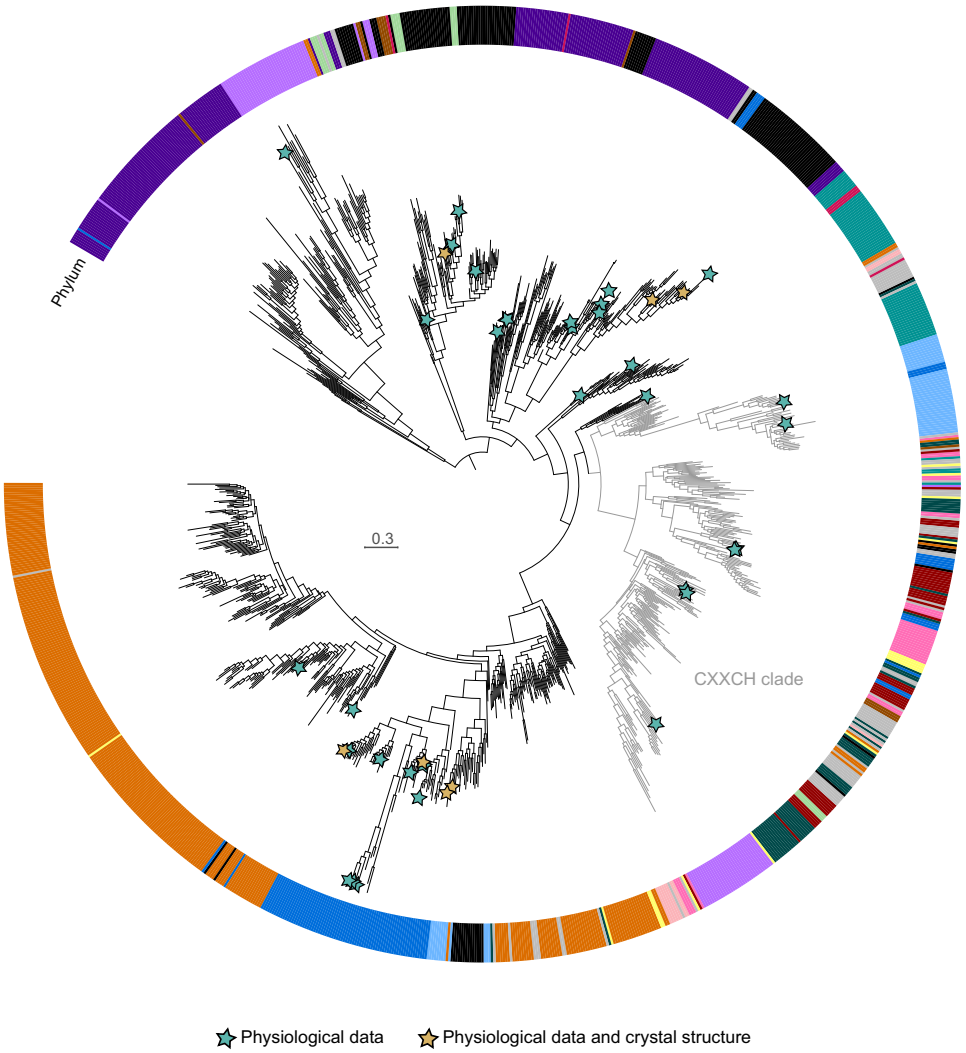

Figure 3. Maximum likelihood phylogeny of 1150 full-length NrfA sequences from 1121 genome assemblies inferred from the alignment of 350 amino acid positions. Taxonomic classification at the phylum level is indicated by the color in the outer ring and is based on the Genome Taxonomy DataBase. Taxa with <10 representative sequences are shown as 'Other' (gray color in the outer ring). Branches in gray correspond to sequences harboring the Cys-X-X-Cys-His variant in the first heme-biding site. Blue stars indicate bacterial isolates demonstrated to reduce nitrate or nitrite to ammonium and yellow stars those for which the crystal structure of NrfA is available. References describing the physiological activity of organisms (indicated by a star on the tree) are given in Table S1 in the supplemental information online. The scale bar denotes the amino acid exchange rate (WAG+R10). The outgroup is not shown. Based on data reported in [29] (copyright license: CC BY 4.0).

abundance and expression of *nrfA* genes in aquatic and terrestrial ecosystems (e.g., [50–53]), often showing a significant correlation with ammonification rates [51,52]. To increase our understanding of the diversity of nitrate ammonifiers and their ecology, **metagenomic** approaches [29,54,55] and sequencing of the *nrfA* gene [51,56] have been used, but community studies of nitrate ammonifiers are still rare.

Ecology of NrfA-dependent ammonifiers in aquatic ecosystems

In aquatic ecosystems, the oxic–anoxic interface in sediments of coastal waters and freshwater environments are hotspots for nitrate ammonification [56–58]. Estuaries are especially well studied and here the abundance of nitrate ammonifiers have been shown to increase with organic content, and the community composition changes in relation to salinity [51]. In sediments, nitrate ammonification is often linked to oxidation or availability of ferrous iron (Fe^{2+}) [55,59]. However, a direct link between iron oxidation and nitrate reduction to ammonium has not yet been shown [60]. In such reduced environments rich in organic matter, *Geobacter* spp. are considered to be important community members that couple nitrate ammonification to the decomposition of organic matter [61,62] and nitrate ammonifiers may represent a relevant source of nitrogen in nitrogen-limited rice paddies [63]. In oceanic oxygen deficient zones, *nrfA* reads in metagenomes as well as *nrfA* expression combined with stable-isotope experiments indicate that nitrate ammonifiers could play a role by supplying ammonium to anammox bacteria, but their importance and the proportion of nitrite reduced via ammonification remains unclear [50,64,65].

Ecology of NrfA-dependent ammonifiers in terrestrial ecosystems

In contrast to sediments, nitrate ammonifiers have been less studied in soils but an unexpectedly high frequency of bacteria carrying nitrate ammonification genes in soil metagenomes was first reported by Nelson *et al.* [54]. A more recent study of global soils shows that *nrfA* genes are found in metagenomes of all major terrestrial biomes and are especially abundant in cropland soils, rhizosphere, and tropical and subtropical broadleaf forests [29]. However, the diversity was highest in tundra soils and different forest biomes. The majority of the *nrfA* reads from these global soils and, to a lesser degree, from aquatic environments, are found in the taxonomically highly diverse Cys-X-X-Cys-His clade in the NrfA phylogeny (Figure 4); a clade not well represented among isolated and genome-sequenced ammonifiers. Compared with the rest of the NrfA phylogeny, genomes within this clade show similar genetic potential for N_2O production but lower genetic potential for N_2O reduction. Since nitrate ammonification recycles fixed nitrogen, there is an interest to support the activity of nitrate ammonifiers over denitrifiers in agricultural soils as a mean to conserve nitrogen and minimize negative environmental impact (e.g., [52]). However, there are several challenges and knowledge gaps that need to be filled before this could be a feasible option [7]. For example, although there is a negative correlation between nitrate ammonification rates and N_2O emissions in unfertilized soils at the global scale [66], the idea that enhancing ammonification activity is a viable strategy to decrease greenhouse gas emissions in agroecosystems ([67] and references therein) needs to be revisited as fertilization may weaken the microbial controls on nitrogen transformations [68] and many nitrate ammonifiers can produce N_2O [29]. Further, there is limited information on the relative importance of nitrogen mineralization and nitrate ammonification for delivering ammonium in crop production systems as well if factors promoting nitrate ammonifiers over denitrifiers can be translated into management practices [7].

Competition between NrfA-dependent ammonifiers and denitrifiers

Respiratory NrfA-dependent ammonifiers and denitrifiers compete for the use of nitrate as electron acceptor under oxygen-limited conditions, and the majority of both groups use carbon compounds as electron donors. For a long time, it has been considered that an electron donor

excess, in relation to electron acceptor levels, that is, the organic carbon to nitrate ratio, as well as more reduced conditions, promote the activity of nitrate ammonifiers relative to denitrifiers [2,69], because of the additional electrons donated to nitrite during ammonification compared with denitrification [70]. This suggests a shift towards ammonification when the electron acceptor becomes limiting compared with the electron donor, but recent reports show that regulatory controls are more complex. Studies on bacterial species and enrichment cultures having pathways for both respiratory ammonification and denitrification show that type, ratio, and concentrations of resources (i.e., nitrate, nitrite, and organic carbon) affect the fate of nitrate but with inconsistent pathway selection in different organisms [71–73]. Additional work with enrichment cultures and isolates indicate that the carbon source, rather than the amount, determines the end-product of nitrate respiration, although there was no general conclusion about carbon types [33].

Several recent studies have also investigated the environmental controls of the competition between denitrifiers and ammonifiers at the community level as well as the relative abundance of the two groups. An analysis of 227 soil metagenomes and the corresponding metadata shows that the partitioning between denitrifiers and ammonifiers is mediated by soil nitrate rather than amount of carbon, which only seems to play a role in soils with low carbon content [29]. The same study reports that denitrification genes are more abundant than *nrfA* across 1861 global

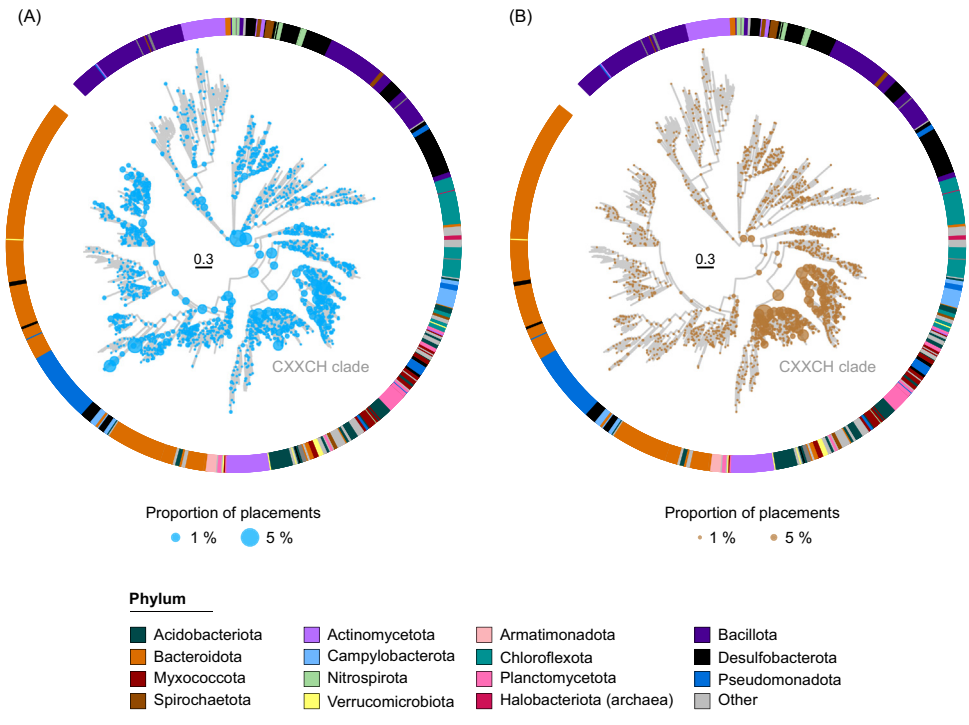

Figure 4. Phylogenetic placement of the metagenomic *nrfA* sequence fragments across biomes. Phylogenetic placement of the metagenomic *nrfA* sequence fragments on the NrfA tree across (A) aquatic and (B) terrestrial biomes. Taxonomic classification at the phylum level is indicated by the color in the outer ring and is based on the Genome Taxonomy DataBase. Panel B is based on data reported in [29] (copyright license: CC BY 4.0) and placements in panel A were obtained by using the same approach, with accession numbers for the aquatic metagenomes (including sediment, freshwater and marine metagenomes) provided in Table S2 in the supplemental information online. The size of the dots indicates the proportion of placements within aquatic and terrestrial biomes. The scale bar denotes the amino acid exchange rate (WAG+R10). The outgroup is not shown.

soil and rhizosphere metagenomes, and the only exceptions were some tundra and tropical and subtropical dry broadleaf forest soils. Approaches based on qPCR targeting *nrfA* and denitrification genes also show that denitrifiers are more abundant than nitrate ammonifiers in soils, although it is mainly agricultural soils and grasslands that have been investigated [52,74]. Altogether, this suggests that denitrification is the dominant nitrate reduction pathway in soils.

The effects of oxic/anoxic conditions and redox have mainly been addressed in freshwater and coastal sediments, wetlands, and paddy soils (e.g., [59,75]). Although denitrification is usually the dominating nitrate sink in sediments, higher nitrate ammonification than denitrification rates have frequently been observed in hypoxic sediments with high organic carbon-to-nitrate ratios and in iron- and sulfur-rich sediments [59,76]. This agrees with the greater abundance of *nrfA* relative to denitrification genes in constantly anoxic sediments compared with those subjected to fluctuating anoxic-oxic conditions [77]. For soils, there is mixed evidence as to whether denitrifiers and ammonifiers are favored under different redox potentials [78,79], maybe because redox potential in soils fluctuates both in the short term (e.g., due to root respiration and rain fall) and seasonally (e.g., due to spring thaw and flooding). Redox conditions also control the oxidation state of iron and availability of sulfide. The relative importance of nitrate ammonification for nitrate reduction in a periodically hypoxic estuary has been shown to increase from <1% under hypoxic to about 18% under oxic conditions in the presence of Fe^{2+}, suggesting that availability of Fe^{2+}, which binds to sulfide under anoxic conditions, can control the fate of nitrate [80]. Nevertheless, both denitrification and nitrate ammonification were recently shown to be stimulated by nitrate and iron in the absence of external organic carbon [60]. Sulfide can be used as electron donor by NrfA in some taxa [32] but inconsistent effects of sulfide levels on the relative importance of nitrate ammonification versus denitrification have been reported in the literature [76,81], suggesting that nitrate ammonifier community composition likely plays a central role.

Concluding remarks and future perspectives

Recent advances in research on nitrate ammonification have revealed that NrfA-dependent ammonifiers are phylogenetically diverse and contribute to nitrogen retention in terrestrial and aquatic environments. However, they are more versatile than previously thought as they often carry genes involved in transformation of other nitrogen species as well as other elements, calling for a reassessment of their roles in biogeochemical cycles beyond nitrate reduction to ammonium. Yet, current knowledge on the physiology of NrfA-dependent ammonifiers and the biochemistry and regulation of enzymes involved in the nitrate ammonification pathway is based on a few model organisms with limited relevance in aquatic and terrestrial environments, particularly within the abundant and elusive Cys-X-X-Cys-His clade. Furthermore, microorganisms can use a variety of other enzymes, both cytoplasmic and periplasmic, to reduce nitrite to ammonium but their phylogenetic distribution and their relevance for nitrogen retention in the environment are largely unknown. Future progress on the diversity and ecology of nitrate ammonifiers, as well as on the factors controlling the competition with denitrifiers (see Outstanding questions), will be crucial to improve our ability to predict biogeochemical fluxes in the environment. This could also help design agricultural practices that result in retention of nitrogen in soils as a way to reduce the environmental footprint of the global food system [82].

Outstanding questions

How redundant are the different nitrite-ammonifying enzymes? The degree to which the five known MHC nitrite reductases that catalyze nitrite reduction to ammonium are redundant is not known and neither is the proportion of organisms that use nitrite ammonifying enzymes for respiration *vs*. detoxification.

What characterizes the diverse Cys-X-X-Cys-His clade in the NrfA phylogeny? Phenotypic and genotypic characterization of members of the Cys-X-X-Cys-His clade is especially important since most environmental NrfA-dependent ammonifiers are found within this clade.

What is the occurrence of MHC nitrite reductases beyond NrfA in the environment, and does it differ across biomes? Recent work shows that NrfA-dependent outnumbers ONR-dependent ammonifiers in terrestrial ecosystems, but we lack knowledge about the aquatic environments and the prevalence of other MHC nitrite reductases. Further, the relative importance of respiratory and fermentative ammonifiers needs attention.

What is the frequency of coexistence between *nrfA* and other nitrogen-transformation genes in microbial genomes across taxa and different biomes? Our understanding of nitrate ammonifiers in the environment is limited, and knowledge on the co-occurrence patterns between *nrfA* and other nitrogen cycling genes in environmental microorganisms is crucial to understand the role of nitrate ammonifiers in nitrogen cycling.

How do environmental conditions govern the distribution and competition between nitrate ammonifiers and denitrifiers, as well as ammonifiers with different repertoires of denitrification genes? This is crucial for a mechanistic understanding of what drives nitrogen retention and loss, and N_2O emission in different ecosystems.

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Declaration of interests

No interests are declared.

Supplemental information

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References

1. Kuypers, M.M.M. *et al.* (2018) The microbial nitrogen-cycling network. *Nat. Rev. Microbiol.* 16, 263–276
2. Rütting, T. *et al.* (2011) Assessment of the importance of dissimilatory nitrate reduction to ammonium for the terrestrial nitrogen cycle. *Biogeosciences* 8, 1779–1791
3. Welsh, A. *et al.* (2014) Refined NrfA phylogeny improves PCR-based *nrfA* gene detection. *Appl. Environ. Microbiol.* 80, 2110–2119
4. Simon, J. (2002) Enzymology and bioenergetics of respiratory nitrite ammonification. *FEMS Microbiol. Rev.* 26, 285–309
5. Kroneck, P.M.H. (2022) Nature's nitrite-to-ammonia expressway, with no stop at dinitrogen. *J. Biol. Inorg. Chem.* 27, 1–21
6. Cole, J.A. and Brown, C.M. (1980) Nitrate reduction to ammonia by fermentative bacteria: a short circuit of the bacterial nitrogen cycle. *FEMS Microbiol. Lett.* 7, 65–72
7. Hallin, S. and Saghaï, A. (2023) Can nitrate-reducing ammonifiers increase nitrogen retention in soil and support ammonium-based cropping systems? *J. Sustain. Agric. Environ.* 2, 541–545
8. Song, G. *et al.* (2021) Response of benthic nitrogen cycling to estuarine hypoxia. *Limnol. Oceanogr.* 66, 652–666
9. Ravishankara, R. *et al.* (2009) Nitrous oxide (N_2O): the dominant ozone-depleting substance emitted in the 21st century. *Science* 326, 123–125
10. Tian, H. *et al.* (2020) A comprehensive quantification of global nitrous oxide sources and sinks. *Nature* 586, 248–256
11. Thompson, R.L. *et al.* (2019) Acceleration of global N_2O emissions seen from two decades of atmospheric inversion. *Nat. Clim. Chang.* 9, 993–998
12. Moreno-Vivián, C. *et al.* (1999) Prokaryotic nitrate reduction: molecular properties and functional distinction among bacterial nitrate reductases. *J. Bacteriol.* 181, 6573–6584
13. Simon, J. and Klotz, M.G. (2013) Diversity and evolution of bioenergetic systems involved in microbial nitrogen compound transformations. *Biochim. Biophys. Acta Bioenerg.* 1827, 114–135
14. Suzuki, I. *et al.* (1995) A novel nitrite reductase gene from the cyanobacterium *Plectonema boryanum*. *J. Bacteriol.* 177, 6137–6143
15. Zhou, Z. *et al.* (2002) Ammonia fermentation, a novel anoxic metabolism of nitrate by fungi. *J. Biol. Chem.* 277, 1892–1896
16. Bonete, M.J. *et al.* (2008) Nitrogen metabolism in haloarchaea. *Saline Syst.* 4, 9
17. Kamp, A. *et al.* (2015) Nitrate storage and dissimilatory nitrate reduction by eukaryotic microbes. *Front. Microbiol.* 6, 1492
18. Fenn, S. *et al.* (2021) NirA is an alternative nitrite reductase from *Pseudomonas aeruginosa* with potential as an antivirulence target. *mBio* 12, e00207-21
19. Cole, J.A. (1978) The rapid accumulation of large quantities of ammonia during nitrite reduction by *Escherichia coli*. *FEMS Microbiol. Lett.* 4, 327–329
20. Fujita, T. and Sato, R. (1966) Studies on soluble cytochromes in Enterobacteriaceae: IV. Possible involvement of cytochrome c-552 in anaerobic nitrite metabolism. *J. Biochem.* 60, 691–700
21. Mowat, C.G. *et al.* (2004) Octaheme tetrathionate reductase is a respiratory enzyme with novel heme ligation. *Nat. Struct. Mol. Biol.* 11, 1023–1024
22. Tikhonova, T.V. *et al.* (2012) Octaheme nitrite reductases: structure and properties. *Biochemistry* 77, 1129–1138
23. Parey, K. *et al.* (2016) In meso crystal structure of a novel membrane-associated octaheme cytochrome c from the Crenarchaeon *Ignicoccus hospitalis*. *FEBS J.* 283, 3807–3820
24. Haase, D. *et al.* (2017) Epsilonproteobacterial hydroxylamine oxidoreductase (εHao): characterization of a 'missing link' in the multihaem cytochrome c family. *Mol. Microbiol.* 105, 127–138
25. Sorokin, D.Y. *et al.* (2023) *Trichlorobacter ammonificans*, a dedicated acetate-dependent ammonifier with a novel module for dissimilatory nitrate reduction to ammonia. *ISME J.* 17, 1639–1648
26. Klotz, M.G. *et al.* (2008) Evolution of an octahaem cytochrome c protein family that is key to aerobic and anaerobic ammonia oxidation by bacteria. *Environ. Microbiol.* 10, 3150–3163
27. Costa, N.L. *et al.* (2019) How thermophilic gram-positive organisms perform extracellular electron transfer: characterization of the cell surface terminal reductase OcwA. *mBio* 10, e01210-19
28. Soares, R. *et al.* (2022) A new paradigm of multiheme cytochrome evolution by grafting and pruning protein modules. *Mol. Biol. Evol.* 39, msac139
29. Saghaï, A. *et al.* (2023) Phyloecology of nitrate ammonifiers and their importance relative to denitrifiers in global terrestrial biomes. *Nat. Commun.* 14, 8249
30. Zumft, W.G. (1997) Cell biology and molecular basis of denitrification. *Microbiol. Mol. Biol. Rev.* 61, 533–616
31. Seitz, H.J. and Cypionka, H. (1986) Chemolithotrophic growth of *Desulfovibrio desulfuricans* with hydrogen coupled to ammonification of nitrate or nitrite. *Arch. Microbiol.* 146, 63–67
32. Eisenmann, E. *et al.* (1995) Lithotrophic growth of *Sulfurospirillum deleyianum* with sulfide as electron donor coupled to respiratory reduction of nitrate to ammonia. *Arch. Microbiol.* 164, 180–185
33. Carlson, H.K. *et al.* (2020) Selective carbon sources influence the end products of microbial nitrate respiration. *ISME J.* 14, 2034–2045
34. Simon, J. *et al.* (2008) The organisation of proton motive and non-proton motive redox loops in prokaryotic respiratory systems. *Biochim. Biophys. Acta Bioenerg.* 1777, 1480–1490
35. Stach, P. *et al.* (2000) Bacterial cytochrome *c* nitrite reductase: new structural and functional aspects. *J. Inorg. Biochem.* 79, 381–385
36. Clarke, T.A. *et al.* (2006) Comparison of the structural and kinetic properties of the cytochrome *c* nitrite reductases from *Escherichia coli*, *Wolinella succinogenes*, *Sulfurospirillum deleyianum* and *Desulfovibrio desulfuricans*. *Biochem. Soc. Trans.* 34, 143–145
37. Simon, J. *et al.* (2011) Physiological function and catalytic versatility of bacterial multihaem cytochromes *c* involved in nitrogen and sulfur cycling. *Biochem. Soc. Trans.* 39, 1864–1870
38. Lukat, P. *et al.* (2008) Binding and reduction of sulfite by cytochrome *c* nitrite reductase. *Biochemistry* 47, 2080–2086
39. Kemp, G.L. *et al.* (2010) Kinetic and thermodynamic resolution of the interactions between sulfite and the pentahaem cytochrome NrfA from *Escherichia coli*. *Biochem. J.* 431, 73–80
40. Haveman, S.A. *et al.* (2004) Physiological and gene expression analysis of inhibition of *Desulfovibrio vulgaris* Hildenborough by nitrite. *J. Bacteriol.* 186, 7944–7950
41. Myers, C.R. and Nealson, K.H. (1990) Respiration-linked proton translocation coupled to anaerobic reduction of manganese(IV) and iron(III) in *Shewanella putrefaciens* MR-1. *J. Bacteriol.* 172, 6232–6238
42. Greene, E.A. *et al.* (2003) Nitrite reductase activity of sulphate-reducing bacteria prevents their inhibition by nitrate-reducing, sulphide-oxidizing bacteria. *Environ. Microbiol.* 5, 607–617
43. Ettwig, K.F. *et al.* (2016) Archaea catalyze iron-dependent anaerobic oxidation of methane. *Proc. Natl. Acad. Sci.* 113, 12792–12796
44. Koch, H. *et al.* (2019) Complete nitrification: insights into the ecophysiology of comammox *Nitrospira*. *Appl. Microbiol. Biotechnol.* 103, 177–189
45. Yang, Y. *et al.* (2022) Discovery of a new genus of anaerobic ammonium oxidizing bacteria with a mechanism for oxygen tolerance. *Water Res.* 226, 119165
46. Hagen, W.R. (2022) Structure and function of the hybrid cluster protein. *Coord. Chem. Rev.* 457, 214405
47. Wang, J. *et al.* (2016) The roles of the hybrid cluster protein, Hcp and its reductase, Hcr, in high affinity nitric oxide reduction that protects anaerobic cultures of *Escherichia coli* against nitrosative stress. *Mol. Microbiol.* 100, 877–892

48. Heo, H. *et al.* (2020) Involvement of NO_3^- in ecophysiological regulation of dissimilatory nitrate/nitrite reduction to ammonium (DNRA) is implied by physiological characterization of soil DNRA bacteria isolated via a colorimetric screening method. *Appl. Environ. Microbiol.* 86, e01054-20
49. Cannon, J. *et al.* (2019) Optimization of PCR primers to detect phylogenetically diverse *nrfA* genes associated with nitrite ammonification. *J. Microbiol. Methods* 160, 49–59
50. Lam, P. *et al.* (2009) Revising the nitrogen cycle in the Peruvian oxygen minimum zone. *Proc. Natl. Acad. Sci.* 106, 4752–4757
51. Song, B. *et al.* (2014) Linking DNRA community structure and activity in a shallow lagoonal estuarine system. *Front. Microbiol.* 5, 460
52. Putz, M. *et al.* (2018) Relative abundance of denitrifying and DNRA bacteria and their activity determine nitrogen retention or loss in agricultural soil. *Soil Biol. Biochem.* 123, 97–104
53. Lai, T.V. *et al.* (2021) Dissimilatory nitrate reduction to ammonium increased with rising temperature. *Biol. Fertil. Soils* 57, 363–372
54. Nelson, M.B. *et al.* (2016) Global biogeography of microbial nitrogen-cycling traits in soil. *Proc. Natl. Acad. Sci. USA* 113, 8033–8040
55. Jäntti, H. *et al.* (2022) The role of organic matter and microbial community controlling nitrate reduction under elevated ferrous iron concentrations in boreal lake sediments. *Hydrobiologia* 849, 2145–2160
56. Pang, Y. and Ji, G. (2019) Biotic factors drive distinct DNRA potential rates and contributions in typical Chinese shallow lake sediments. *Environ. Pollut.* 254, 112903
57. Giblin, A. *et al.* (2013) The importance of dissimilatory nitrate reduction to ammonium (DNRA) in the nitrogen cycle of coastal ecosystems. *Oceanography* 26, 124–131
58. Bourceau, O.M. *et al.* (2023) Simultaneous sulfate and nitrate reduction in coastal sediments. *ISME Commun.* 3, 17
59. Kessler, A.J. *et al.* (2018) Biogeochemical controls on the relative importance of denitrification and dissimilatory nitrate reduction to ammonium in estuaries. *Glob. Biogeochem. Cycles* 32, 1045–1057
60. Li, S. *et al.* (2022) Denitrification and dissimilatory nitrate reduction to ammonia in long-term lake sediment microcosms with iron(II). *Sci. Total Environ.* 807, 150835
61. Zhao, Y. *et al.* (2020) Survey of dissimilatory nitrate reduction to ammonium microbial community at national wetland of Shanghai, China. *Chemosphere* 250, 126195
62. Li, X. *et al.* (2022) Role of organic/sulfide ratios on competition of DNRA and denitrification in a co-driven sequencing biofilm batch reactor. *Environ. Sci. Pollut. Res.* 29, 18793–18804
63. Pandey, A. *et al.* (2021) Dissimilatory nitrate ammonification and N_2 fixation helps maintain nitrogen nutrition in resource-limited rice paddies. *Biol. Fertil. Soils* 57, 107–115
64. Babbin, A.R. *et al.* (2017) Multiple metabolisms constrain the anaerobic nitrite budget in the Eastern Tropical South Pacific. *Glob. Biogeochem. Cycles* 31, 258–271
65. Fuchsman, C.A. *et al.* (2017) Niche partitioning of the N cycling microbial community of an offshore oxygen deficient zone. *Front. Microbiol.* 8, 2384
66. Cheng, Y. *et al.* (2022) Global patterns and drivers of soil dissimilatory nitrate reduction to ammonium. *Environ. Sci. Technol.* 56, 3791–3800
67. Yoon, S. *et al.* (2019) Ecological and physiological implications of nitrogen oxide reduction pathways on greenhouse gas emissions in agroecosystems. *FEMS Microbiol. Ecol.* 95, fiz066
68. Jones, C.M. *et al.* (2022) Reactive nitrogen restructures and weakens microbial controls of soil N_2O emissions. *Commun. Biol.* 5, 273
69. Tiedje, J.M. *et al.* (1982) Denitrification: ecological niches, competition and survival. *Antonie Van Leeuwenhoek* 48, 569–583
70. Kraft, B. *et al.* (2014) The environmental controls that govern the end product of bacterial nitrate respiration. *Science* 345, 676–679
71. Yoon, S. *et al.* (2015) Nitrite control over dissimilatory nitrate/nitrite reduction pathways in *Shewanella loihica* strain PV-4. *Appl. Environ. Microbiol.* 81, 3510–3517
72. Vuono, D.C. *et al.* (2019) Resource concentration modulates the fate of dissimilated nitrogen in a dual-pathway actinobacterium. *Front. Microbiol.* 10, 3389
73. Stremińska, M.A. *et al.* (2012) Nitrous oxide production in soil isolates of nitrate-ammonifying bacteria. *Environ. Microbiol. Rep.* 4, 66–71
74. Tatti, E. *et al.* (2017) Over-winter dynamics of soil bacterial denitrifiers and nitrite ammonifiers influenced by crop residues with different carbon to nitrogen ratios. *Appl. Soil Ecol.* 110, 53–64
75. Pandey, A. *et al.* (2019) Dissimilatory nitrate reduction to ammonium dominates nitrate reduction in long-term low nitrogen fertilized rice paddies. *Soil Biol. Biochem.* 131, 149–156
76. Murphy, A.E. *et al.* (2020) Sulphide addition favours respiratory ammonification (DNRA) over complete denitrification and alters the active microbial community in salt marsh sediments. *Environ. Microbiol.* 22, 2124–2139
77. Wittorf, L. *et al.* (2016) Habitat partitioning of marine benthic denitrifier communities in response to oxygen availability. *Environ. Microbiol. Rep.* 8, 486–492
78. Pett-Ridge, J. *et al.* (2006) Redox fluctuations frame microbial community Impacts on N-cycling rates in a humid tropical forest soil. *Biogeochemistry* 81, 95–110
79. Friedl, J. *et al.* (2018) Dissimilatory nitrate reduction to ammonium (DNRA), not denitrification dominates nitrate reduction in subtropical pasture soils upon rewetting. *Soil Biol. Biochem.* 125, 340–349
80. Roberts, K.L. *et al.* (2014) Increased rates of dissimilatory nitrate reduction to ammonium (DNRA) under oxic conditions in a periodically hypoxic estuary. *Geochim. Cosmochim. Acta* 133, 313–324
81. Cojean, A.N.Y. *et al.* (2020) Controls of H_2S, Fe^{2+}, and Mn^{2+} on microbial NO_3^--reducing processes in sediments of an eutrophic lake. *Front. Microbiol.* 11, 1158
82. Clark, M.A. *et al.* (2020) Global food system emissions could preclude achieving the 1.5° and 2°C climate change targets. *Science* 370, 705–708
83. Einsle, O. *et al.* (1999) Structure of cytochrome *c* nitrite reductase. *Nature* 400, 476–480
84. Einsle, O. *et al.* (2000) Cytochrome *c* nitrite reductase from *Wolinella succinogenes*. *J. Biol. Chem.* 275, 39608–39616
85. Pereira, I.A.C. *et al.* (2000) Characterization of a heme *c* nitrite reductase from a non-ammonifying microorganism, *Desulfovibrio vulgaris* Hildenborough. *Biochim. Biophys. Acta Protein Struct. Mol. Enzymol.* 1481, 119–130
86. Bamford, V.A. *et al.* (2002) Structure and spectroscopy of the periplasmic cytochrome *c* nitrite reductase from *Escherichia coli*. *Biochemistry* 41, 2921–2931
87. Cunha, C.A. *et al.* (2003) Cytochrome *c* nitrite reductase from *Desulfovibrio desulfuricans* ATCC 27774. *J. Biol. Chem.* 278, 17455–17465
88. Youngblut, M. *et al.* (2012) Laue crystal structure of *Shewanella oneidensis* cytochrome *c* nitrite reductase from a high-yield expression system. *JBIC J. Biol. Inorg. Chem.* 17, 647–662
89. Campeciño, J. *et al.* (2020) Cytochrome *c* nitrite reductase from the bacterium *Geobacter lovleyi* represents a new NrfA subclass. *J. Biol. Chem.* 295, 11455–11465
90. Sosa Alfaro, V. *et al.* (2021) Elucidating electron storage and distribution within the pentaheme scaffold of cytochrome *c* nitrite reductase (NrfA). *Biochemistry* 60, 1853–1867
91. Simon, J. *et al.* (2000) A NapC/NirT-type cytochrome c (NrfH) is the mediator between the quinone pool and the cytochrome c nitrite reductase of *Wolinella succinogenes*. *Mol. Microbiol.* 35, 686–696
92. Schwalb, C. *et al.* (2003) The tetraheme cytochrome CymA is required for anaerobic respiration with dimethyl sulfoxide and nitrite in *Shewanella oneidensis*. *Biochemistry* 42, 9491–9497
93. Clarke, T.A. *et al.* (2007) The crystal structure of the pentahaem c-type cytochrome NrfB and characterization of its solution-state interaction with the pentahaem nitrite reductase NrfA. *Biochem. J.* 406, 19–30
94. Darwin, A.J. *et al.* (1997) Differential regulation by the homologous response regulators NarL and NarP of *Escherichia coli* K-12 depends on DNA binding site arrangement. *Mol. Microbiol.* 25, 583–595

95. Mania, D. *et al.* (2016) Regulation of nitrogen metabolism in the nitrate-ammonifying soil bacterium *Bacillus vireti* and evidence for its ability to grow using N_2O as electron acceptor. *Environ. Microbiol.* 18, 2937–2950
96. Liu, S. *et al.* (2021) Dissimilatory nitrate reduction to ammonium (DNRA) and denitrification pathways are leveraged by cyclic AMP receptor protein (CRP) paralogues based on electron donor/acceptor limitation in *Shewanella loihica* PV-4. *Appl. Environ. Microbiol.* 87, e01964-20
97. Filenko, N. *et al.* (2007) The NsrR regulon of *Escherichia coli* K-12 includes genes encoding the hybrid cluster protein and the periplasmic, respiratory nitrite reductase. *J. Bacteriol.* 189, 4410–4417
98. Kern, M. and Simon, J. (2016) Three transcription regulators of the Nss family mediate the adaptive response induced by nitrate, nitric oxide or nitrous oxide in *Wolinella succinogenes*. *Environ. Microbiol.* 18, 2899–2912
99. Rajeev, L. *et al.* (2015) Regulation of nitrite stress response in *Desulfovibrio vulgaris* Hildenborough, a model sulfate-reducing bacterium. *J. Bacteriol.* 197, 3400–3408
100. Pettersen, E.F. *et al.* (2021) UCSF ChimeraX: structure visualization for researchers, educators, and developers. *Protein Sci.* 30, 70–82