The microbial nitrogen-cycling network

Marcel M. M. Kuypers, Hannah K. Marchant and Boran Kartal*

Abstract | Nitrogen is an essential component of all living organisms and the main nutrient limiting life on our planet. By far, the largest inventory of freely accessible nitrogen is atmospheric dinitrogen, but most organisms rely on more bioavailable forms of nitrogen, such as ammonium and nitrate, for growth. The availability of these substrates depends on diverse nitrogen-transforming reactions that are carried out by complex networks of metabolically versatile microorganisms. In this Review, we summarize our current understanding of the microbial nitrogen-cycling network, including novel processes, their underlying biochemical pathways, the involved microorganisms, their environmental importance and industrial applications.

Nitrogen is an essential element for all living organisms and is required for the biosynthesis of key cellular components, such as proteins and nucleic acids. Atmospheric dinitrogen gas is the largest inventory of freely accessible nitrogen, and it is biologically available to highly diverse but rare nitrogen-fixing bacteria and archaea. Other organisms must rely on more reactive forms of nitrogen for growth, such as ammonium and nitrate. This bioavailable nitrogen is rare in many environments, and the availability of this growth-limiting nutrient is controlled primarily by microbial reactions that alter the oxidation state of nitrogen.

Human activity has had a profound effect on the amount of bioavailable nitrogen, mainly owing to the high input of industrial nitrogen-based fertilizers[1]. Food production for about 50% of the human population currently relies on industrial fertilizers[2]. This fertilizer use and legume cultivation have nearly doubled the nitrogen input to terrestrial and marine ecosystems[1]. To predict the consequences of this input, there is a pressing need to understand the basic mechanisms that underlie microbial nitrogen transformations.

Microorganisms can transform nitrogen compounds as reactive and toxic as nitric oxide or as inert and harmless as dinitrogen gas. Microbial transformations of nitrogen are often depicted as a cycle consisting of six distinct processes that proceed in an orderly fashion. This view of the nitrogen cycle implies that a molecule of dinitrogen gas is first fixed to ammonia, which is assimilated into organic nitrogen (that is, biomass). The degradation of organic nitrogen, ammonification, releases a molecule of ammonia, which is subsequently oxidized to nitrate through nitrification ($NH_4^+ \rightarrow NO_2^- \rightarrow NO_3^-$) and eventually converted back to a molecule of dinitrogen gas through denitrification ($NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2$) or anaerobic ammonium oxidation (anammox; $NO_2^- + NH_4^+ \rightarrow N_2$). In reality, there is not one balanced nitrogen cycle. Instead, the six distinct processes are associated with nitrogen fluxes of vastly different magnitude (BOX 1).

Nitrogen-transforming microorganisms are generally classified according to one of the six processes they are involved in: nitrifiers carry out nitrification; denitrifiers, denitrification; nitrogen-fixers, nitrogen fixation; and so on. However, genomic data collected during the past decade challenge this classification, as they have revealed tremendous metabolic versatility within nitrogen-transforming microorganisms. We now know that diverse microorganisms can fix dinitrogen gas and denitrify simultaneously[3,4], and organisms classified as nitrite oxidizers can also grow on formate, hydrogen and sulfide[5,6]. Thus, owing to their metabolic versatility, it has become nearly impossible to objectively classify nitrogen-transforming microorganisms according to the six classical processes (BOX 1). We will use process names, such as denitrification and nitrification, but refrain from classifying organisms accordingly. This Review focuses on the redox reactions that convert nitrogen compounds, biochemical pathways, and the responsible enzymes (FIG. 1) and microorganisms.

Based on our current understanding, microorganisms can convert nitrogen compounds spanning redox states from −3 to +5 using 14 discrete redox reactions (FIG. 1). There is no change in redox state in the interconversion of organic nitrogen to ammonia. Nitrogen-converting enzymes are often found in very diverse microorganisms (see below) and many of these enzymes have only recently been identified. In the past decade, four new reactions were discovered: hydroxylamine oxidation to nitric oxide[7,8] (FIG. 1; reaction 7), nitric oxide dismutation to dinitrogen gas and oxygen[9] (reaction 9), hydrazine synthesis[10] (reaction 13) and hydrazine oxidation to dinitrogen gas[10] (reaction 12). In addition, many new metabolic capabilities were

Max Planck Institute for Marine Microbiology, Bremen, Germany.
**e-mail: mkuypers@mpi-bremen.de*

doi:10.1038/nrmicro.2018.9
Published online 5 Feb 2018

discovered, such as phototrophic nitrite oxidation[11] and complete ammonia oxidation (comammox) to nitrate[12,13], and novel microorganisms were identified, such as ammonia-oxidizing archaea[14], denitrifying eukaryotic foraminifera[15] and symbiotic heterotrophic nitrogen-fixing cyanobacteria[16].

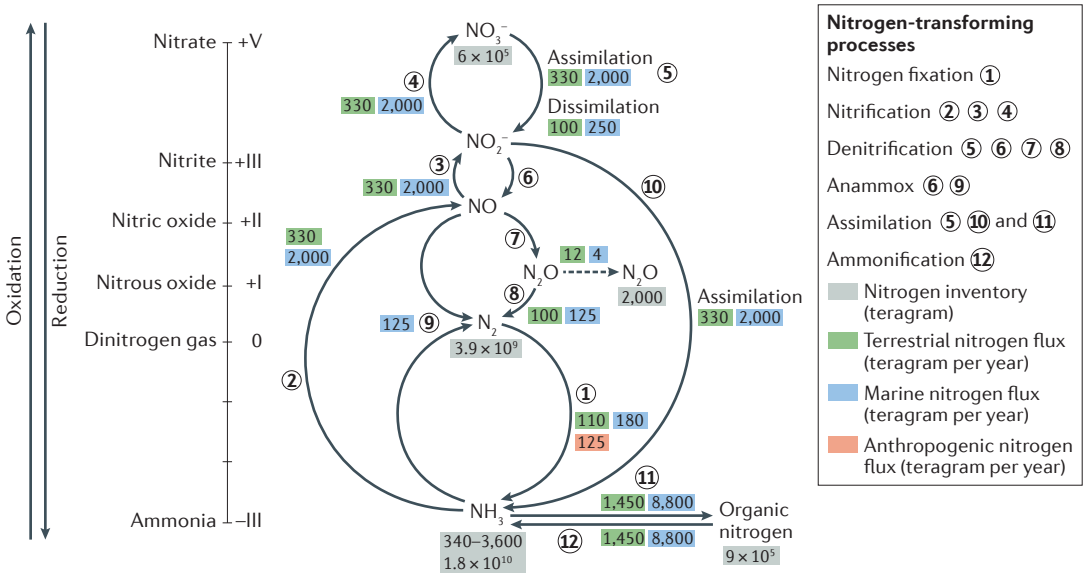
In this Review, we present these new findings in the context of our current understanding of microbial transformations of nitrogen. We describe microbial nitrogen-transforming reactions and microorganisms and their physiological and environmental function, and we also present reactions that are likely to exist but have

Box 1 | **Biogeochemical nitrogen cycling: global inventories, processes and fluxes**

The largest global nitrogen inventory, with 1.8×10^{10} Tg nitrogen, is ammonia bound in rocks and sediments[139] (nitrogen inventories in grey boxes; see the figure). Although this bound ammonia becomes available upon erosion, it has a minor role in annual biogeochemical nitrogen cycling. Whereas the terrestrial inventory of freely accessible ammonia is unknown[140], the marine inventory[70,139] is estimated to be between 340 and 3,600 Tg nitrogen. The largest freely accessible global nitrogen inventory is dinitrogen gas with 3.9×10^9 Tg nitrogen followed by organic nitrogen, nitrate and nitrous oxide inventories[70,139]. Global nitrite and nitric oxide inventories are negligible.

Biogeochemical nitrogen cycling between these inventories is often attributed to the following six distinct nitrogen-transforming processes: assimilation, ammonification, nitrification, denitrification, anaerobic ammonium oxidation (anammox) and nitrogen fixation (see the figure). We estimated the annual nitrogen fluxes for a number of these processes from the available literature[129,141–143] and by using simple assumptions (see below). In the figure, the fluxes between major nitrogen species are shown in Tg nitrogen per year, in green, blue and red boxes referring to terrestrial, marine and anthropogenic nitrogen fluxes, respectively. The best-defined fluxes involve nitrogen loss and fixation because they have been the focus of many studies[129,141,143]. These fluxes are comparatively small (see the figure) but regulate the availability of bioavailable nitrogen, which largely controls the removal of atmospheric carbon dioxide through the biological carbon pump[129]. Current estimates suggest that biological nitrogen fixation (~300 Tg nitrogen yr^{-1}) combined with industrial nitrogen fixation (~125 Tg nitrogen yr^{-1})[129,143] exceeds the production of dinitrogen gas by anammox and denitrification (~350 Tg nitrogen yr^{-1})[129,141]. Not all nitrous oxide produced from nitric oxide reduction is further reduced to dinitrogen gas. The resulting nitrous oxide release from the marine and terrestrial environments is 4 and 12 Tg nitrogen yr^{-1}, respectively[129]. Although the nitrous oxide flux is small compared to the other nitrogen fluxes, it has a profound effect on the environment because nitrous oxide is the main ozone-depleting agent and a powerful greenhouse gas[92].

As shown in the figure, the nitrogen-transforming processes have vastly different fluxes and do not form one balanced nitrogen cycle, as often depicted in papers and textbooks. The largest nitrogen fluxes are associated with the interconversion of ammonia and organic nitrogen. In the marine environment alone, the fluxes associated with ammonification and ammonium assimilation are an order of magnitude larger (~8,800 Tg nitrogen yr^{-1})[142] than marine nitrogen loss and gain combined (~400 Tg nitrogen yr^{-1})[141]. Another substantial nitrogen flux is associated with the oxidation of ammonia to nitrate via nitrite (that is, nitrification). Marine nitrification is associated with a flux of ~2,000 Tg nitrogen per year, which explains why marine ammonia-oxidizing archaea are among the most abundant microorganisms even though ammonia concentrations are low in the ocean. Nitrate assimilation-related fluxes are in the same order of magnitude. Marine phytoplankton account for 2,000 Tg nitrate reduced per year[142]. Compared to this, the fluxes associated with dissimilatory nitrate reduction to ammonium are most likely smaller. Although there are no available estimates for the terrestrial environment, assimilation-related fluxes are likely six times smaller owing to the lower nitrogen requirement of land plants, which require about 1 molecule of nitrogen for every 40 carbon molecules fixed[144], compared to 1 molecule of nitrogen per 6.6 molecules of carbon fixed by marine algae. Assuming steady-state conditions (when gain of a nitrogen compound equals its loss), we estimated the terrestrial nitrification and ammonification fluxes by dividing the marine fluxes by six.



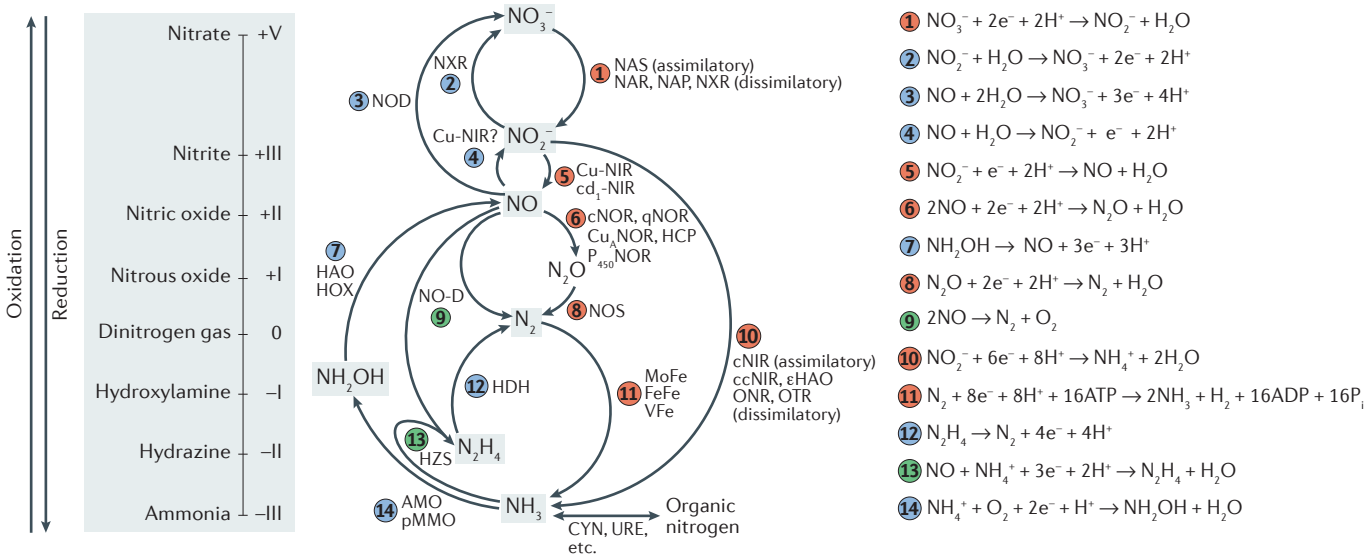


Fig. 1 | **Microbial transformations of nitrogen compounds.** Microorganisms carry enzymes that perform 14 redox reactions involving 8 key inorganic nitrogen species of different oxidation states (enzyme-bound intermediates and their redox states are not shown). The interconversion of ammonia and organic nitrogen does not involve a change in the redox state of the nitrogen atom. The reactions involve reduction (red), oxidation (blue) and disproportionation and comproportionation (green). The following enzymes perform the nitrogen transformations: assimilatory nitrate reductase (NAS, *nasA* and *nirA*); membrane-bound (NAR, *narGH*) and periplasmic (NAP, *napA*) dissimilatory nitrate reductases; nitrite oxidoreductase (NXR, *nxrAB*); nitric oxide oxidase (NOD, *hmp*); haem-containing (cd_1-NIR, *nirS*) and copper-containing (Cu-NIR, *nirK*) nitrite reductases; cytochrome *c*-dependent (cNOR, *cnorB*), quinol-dependent (qNOR, *norZ*) and copper-containing quinol-dependent nitric oxide reductases (Cu_ANOR); NADH-dependent cytochrome P_{450} nitric oxide reductase (P_{450}NOR, *p450nor*); flavo-diiron nitric oxide reductase (NORvw, *norVW*); hybrid cluster protein (HCP, *hcp*); hydroxylamine oxidoreductase (HAO, *hao*); hydroxylamine oxidase (HOX; *hox*); nitrous oxide reductase (NOS, *nosZ*); nitric oxide dismutase (NO-D, *norZ*); assimilatory nitrite reductase (cNIR; *nasB* and *nirB*); dissimilatory periplasmic cytochrome *c* nitrite reductase (ccNIR, *nrfAH*); ε-hydroxylamine oxidoreductase (εHAO; *haoA*); octahaem nitrite reductase (ONR); octahaem tetrathionate reductase (OTR); molybdenum-iron (MoFe, *nifHDK*), iron-iron (FeFe, *anfHGDK*) and vanadium-iron (VFe, *vnfHGDK*) nitrogenases; hydrazine dehydrogenase (HDH, *hdh*); hydrazine synthase (HZS, *hzsCBA*); ammonia monooxygenase (AMO, *amoCAB*); particulate methane monooxygenase (pMMO, *pmoCAB*); cyanase (CYN, *cynS*); and urease (URE, *ureABC*).

not yet been discovered. Furthermore, we discuss the complex network of interactions between nitrogen-transforming microorganisms and its impact on global biogeochemical nitrogen cycling.

Nitrogen-transforming reactions

Nitrogen fixation. Atmospheric dinitrogen gas is the largest reservoir of freely accessible nitrogen, but it is biologically available only to microorganisms that carry the nitrogenase metalloenzyme and thus can fix dinitrogen into ammonia. Nitrogenase is widespread in bacteria and archaea, and provides them with a competitive advantage in environments that are depleted of bioavailable nitrogen. There are three different types of nitrogenases — iron-iron (FeFe), vanadium-iron (VFe) and molybdenum-iron (MoFe) nitrogenases[17]. They have similar sequences and structural and functional properties but vary in their metal cofactor. All nitrogenases are composed of two components (FIG. 2a). *anfDGK*, *vnfDGK* or *nifDK* encode the catalytic component of nitrogenases that have iron, vanadium or molybdenum in the active centre, respectively[17,18]. In addition, *anfH*, *vnfH* or *nifH* encode iron-containing electron transfer proteins (known as nitrogenase reductase or iron protein). *NifH* is used as a gene marker for the detection of nitrogen-fixing microorganisms in the environment[18]. The soil bacterium *Azotobacter vinelandii* encodes all three types of nitrogenases, whereas other microorganisms, such as the marine nitrogen-fixers *Trichodesmium* spp., have only MoFe nitrogenase[18]. Whereas vanadium is seldom limiting, molybdenum and iron are rare in the terrestrial and marine environment, respectively, and can therefore limit nitrogen fixation in these ecosystems[19].

During nitrogen fixation, an electron carrier such as ferredoxin first reduces the iron protein, which subsequently reduces the catalytic component. This requires the iron and catalytic proteins to dissociate and reassociate[20]. Per molecule of nitrogen fixed, 16 molecules of ATP are consumed[20]. Additional bioenergetic costs arise from the production of powerful reductants, such as ferredoxin, and the protection of the oxygen-labile nitrogenase[21]. Because oxygen exposure deactivates nitrogenases, oxygenic phototrophs, such as *Trichodesmium* spp., *Crocosphaera watsonii* and *Nodularia* spp., often separate nitrogen fixation from photosynthesis, either spatially (for example, in heterocysts, which are specialized nitrogen-fixing cells), or temporally[22]. Even non-photosynthetic organisms living in oxic environments require mechanisms, such as increased oxygen respiration, detoxification via

Reductants
The electron-donating compounds in a redox reaction.

Oxygenic phototrophs
Organisms that obtain energy from light and use water as the electron donor, forming molecular oxygen and sugar as products.

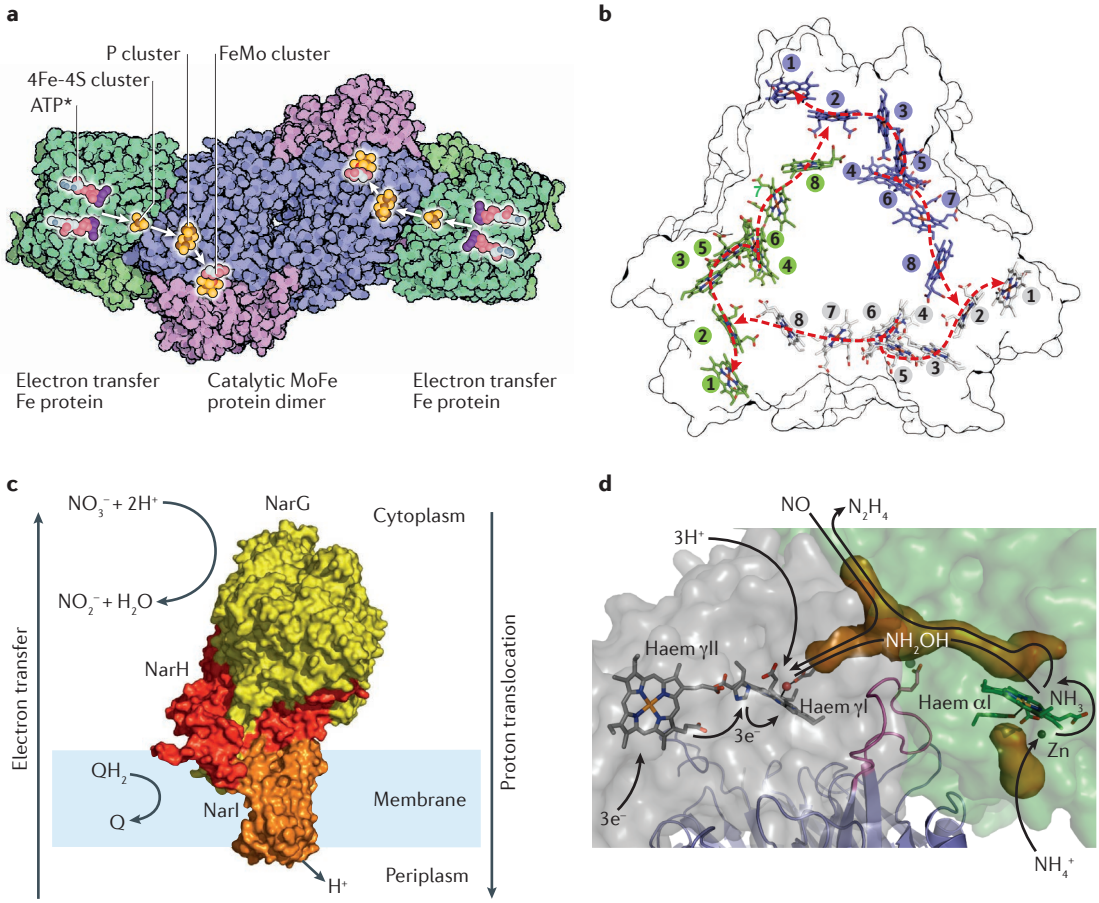


Fig. 2 | **Enzymes catalysing four key nitrogen-cycling reactions. a** | The molybdenum-iron (MoFe) nitrogenase enzyme contains the electron transfer protein (green; encoded by *nifH*) and the α-subunit (magenta; encoded by *nifD*) and β-subunit (purple; encoded by *nifK*) of the catalytic enzyme. *nifH* is used to detect nitrogen-fixing microorganisms in the environment. The iron-sulfur clusters mediate electron transfer to the catalytic centre. The association and dissociation of the electron transfer and catalytic proteins require the input of ATP. **b** | In the anaerobic ammonium-oxidizing bacterium *Kuenenia stuttgartiensis*, electrons flow through the haems of the octahaem hydroxylamine oxidase (red arrows). Haems belonging to different monomers are depicted in green, blue and grey. Haem 4 is the catalytic centre. **c** | In the membrane-bound bacterial nitrate reductase (NAR), the catalytic dimer is encoded by *narG* and *narH*, whereas the membrane anchor is encoded by *narI*. *narG* is used to detect denitrifying microorganisms in the environment. Nitrate reduction to nitrite occurs in the cytoplasm, and protons are translocated into the periplasm. Thereby, NAR contributes to the proton motive force. **d** | In *K. stuttgartiensis*, *hzsA*, *hzsB* and *hzsC* encode hydrazine synthase. The former two genes are used to detect anaerobic ammonium-oxidizing bacteria in the environment. This enzyme is proposed to perform a two-step reaction. It starts in the γ-subunit (grey) with the reduction of nitric oxide to hydroxylamine, which is transported through the substrate channel (brown) to the α-subunit (green). The α-subunit comproportionates hydroxylamine with ammonia into hydrazine. Both reactions are catalysed by cytochrome *c*-type haem proteins. *ADP-ammonium fluoride; ATP analogue. Part **a** is adapted with permission from David Goodsell, doi:10.2210/rcsb_pdb/mom_2002_2 (February 2002). Part **b** was originally published in the *J. Biol Chem*. Maalcke, W. J. et al. Structural basis of biological NO generation by octaheme oxidoreductases. 2014; **289**: 1228–1242. © the American Society for Biochemistry and Molecular Biology (REF. 8). Part **c** is adapted with permission from REF. 154, Elsevier. Part **d** is adapted with permission from REF. 115, Macmillan Publishers Limited.

superoxide dismutase and conformational changes of nitrogenase, to protect their nitrogenase from oxygen[23]. The existence of a completely different, oxygen-insensitive pathway of nitrogen fixation using an unusual nitrogenase was recently refuted[24].

Although no nitrogen-fixing eukaryotes have been found, many nitrogen-fixing microorganisms live in symbioses with eukaryotes. The unicellular cyanobacterium '*Candidatus* Atelocyanobacterium thalassa' (UCYN-A), which lives in symbiosis with small unicellular haptophyte algae such as *Braarudosphaera bigelowii*, is one of the most widespread nitrogen-fixing microorganisms and has a key role in marine nitrogen fixation[16,25]. Symbiotic nitrogen-fixing microorganisms are also part of the gut microbiota of animals such as termites and can be found in special bacteriocytes in bivalves[26,27]. Moreover, nitrogen-fixing members of the Rhizobiales order live in special root nodules of crop legumes, such as alfalfa, beans, peas and soy, which provide 20% of food protein worldwide[28].

Bacteriocytes
Special cells in animals that contain endosymbiotic bacteria.

Ammonia oxidation to hydroxylamine. All known aerobic ammonia-oxidizing bacteria and archaea activate ammonia by oxidizing it to hydroxylamine using ammonia monooxygenase (AMO)[29]. Most ammonia-oxidizing bacteria belong to the Betaproteobacteria and Gammaproteobacteria classes, and are chemolithoautotrophs that oxidize ammonia to nitrite[30]. They can be found in nearly all environments, including fertilized soils[31] and wastewater treatment plants. Archaea belonging to the Thaumarchaeota phylum, such as *Nitrosopumilus maritimus*, can also grow chemolithoautotrophically by oxidizing ammonia to nitrite[14]. Their discovery resolved the long-standing mystery of the apparently rare ammonia oxidizers in the oceans[32,33]. Thaumarchaeota are more abundant than bacteria in some sandy and silty clay soils[31,34]. Furthermore, the isolation of the acidophilic ammonia-oxidizing archaeon '*Candidatus* Nitrosotalea devanaterra' overturned the common assumption that chemolithoautotrophic ammonia oxidation could not occur at low pH because of low ammonia availability[35]. Many ammonia oxidizers, such as *Nitrosospira* sp. and *Nitrososphaera viennensis*, can also degrade organic nitrogen compounds, for example, by hydrolysing urea with ureases, to produce additional ammonia[36,37]. The archaeon *Nitrososphaera gargensis* can also produce ammonia by hydrolysing cyanate with a cyanase[38].

Recently, the ability to oxidize ammonia has also been found in members of the genus *Nitrospira*, which were previously assumed to only be capable of nitrite oxidation[12,13]. The discovery of these bacteria that oxidize ammonia to nitrate (complete ammonia oxidation (comammox)), refuted the dogma that the oxidation of ammonia and nitrite requires two distinct groups of microorganisms. The bacteria that perform the comammox process, such as '*Candidatus* Nitrospira inopinata', appear well adapted to ammonia-limited environments and can outcompete most cultured ammonia-oxidizing microorganisms for ammonia[39]. The transient accumulation of nitrite in comammox cultures grown on ammonia indicates that they more efficiently oxidize ammonia than nitrite[12,13,39]. We hypothesize that bacteria that perform the comammox process oxidize ammonia to nitrate under ammonia-limited conditions and perform partial ammonia oxidation to nitrite under oxygen-limited conditions.

AMO is closely related to methane monooxygenase (MMO), which is found in methanotrophs such as gammaproteobacteria[40] and candidate phylum NC10 (REF. 9). MMO can also oxidize ammonia to hydroxylamine, although very inefficiently[41] (FIG. 1). Similarly, AMO can also oxidize methane but less efficiently than MMO[30]. Intriguingly, *amo* sequences of bacteria that perform the comammox process were detected in the environment (for example, in groundwater) already before their discovery but were wrongly assigned as particulate MMO (*pmo*) genes of the filamentous methane-oxidizing *Crenothrix polyspora*[42]. Recent resequencing of *C. polyspora* and other *Crenothrix* species revealed that they actually contain typical gammaproteobacterial *pmo* and not *amo*[43].

Hydroxylamine oxidation to nitric oxide and further to nitrite. Aerobic oxidation of ammonia to hydroxylamine is an endergonic reaction. Therefore, all aerobic ammonia oxidizers conserve energy by further oxidizing hydroxylamine. It was believed that aerobic ammonia-oxidizing bacteria oxidize hydroxylamine to nitrite using octahaem hydroxylamine oxidoreductase (HAO). Recently, it was shown that the product of HAO is not nitrite but nitric oxide, which is further oxidized to nitrite by an unknown enzyme[7]. Although the enzyme catalysing the latter reaction has not been conclusively identified, copper-containing nitrite reductase (Cu-NIR) working in reverse has been suggested to catalyse it[7]. All ammonia-oxidizing bacteria, including the newly discovered *Nitrospira* spp., which can oxidize ammonia all the way to nitrate, contain AMO and HAO[12,13]. By contrast, known ammonia-oxidizing archaea do not encode HAO, and the archaeal enzyme responsible for hydroxylamine oxidation remains unknown[44,45].

HAO belongs to a family of octahaem proteins (FIG. 2b) found in diverse microorganisms[44,46]. The genomes of anaerobic ammonium-oxidizing bacteria encode approximately ten HAO-like proteins[46], and one of these also oxidizes hydroxylamine to nitric oxide[8]. In anaerobic ammonium-oxidizing bacteria, this hydroxylamine oxidase (HOX) recycles hydroxylamine, which leaks from hydrazine synthase (HZS; see below).

Methane-oxidizing bacteria also produce hydroxylamine as a result of their unspecific ammonia oxidation activity[41] (see above), and diverse methanotrophs in the Proteobacteria, Verrucomicrobia and NC10 phyla (for example, '*Candidatus* Methylomirabilis oxyfera') encode HAO-like proteins that likely oxidize hydroxylamine to nitric oxide, which is further oxidized to nitrite or reduced to nitrous oxide[8,47,48]. Currently, it is unknown whether this reaction directly contributes to energy conservation in methane-oxidizing bacteria.

Nitrite oxidation to nitrate. Nitrite oxidation is the main biochemical pathway that produces nitrate and is catalysed by nitrite oxidoreductase (NXR). NXR is encoded by aerobic nitrite-oxidizing bacteria (members of the Alphaproteobacteria, Betaproteobacteria, Gammaproteobacteria, Chloroflexi, Nitrospinae and Nitrospirae phyla)[6], anoxygenic phototrophs (for example, *Thiocapsa* sp. KS1 and *Rhodopseudomonas* sp. LQ17)[11,49] and anaerobic ammonium-oxidizing bacteria[50]. Whereas aerobic nitrite-oxidizing bacteria directly couple nitrite oxidation by NXR to energy conservation, anaerobic nitrite-oxidizing bacteria do not. *Thiocapsa* sp. KS1 and *Rhodopseudomonas* sp. LQ17 can oxidize nitrite anaerobically by coupling it directly to phototrophy[11,49]. Further, anaerobic ammonium-oxidizing bacteria might couple anaerobic nitrite oxidation to carbon fixation[51].

Nitrite-oxidizing bacteria are metabolically versatile and can grow on substrates other than nitrite[6]. Indeed, the comammox *Nitrospira* species oxidizes ammonia to nitrate[12,13]. *Nitrospira moscoviensis* grows aerobically on hydrogen and anaerobically on organic acids while respiring nitrate[52,53]. Nitrate reduction

Thaumarchaeota
The phylum that contains the ammonia-oxidizing archaea.

Acidophilic
The propensity of organisms to grow in acidic environments (pH < 6).

Methanotrophs
Organisms that oxidize methane to conserve energy.

NC10
A candidate bacterial phylum, named after the Nullarbor Caves in Australia, that contains '*Candidatus* Methylomirabilis oxyfera', which is the first organism discovered that performs methane oxidation coupled to oxygenic denitrification.

Endergonic
A reaction that requires energy input.

Verrucomicrobia
A bacterial phylum with only a few described species, some of which appear to be important in the methane cycle.

Anoxygenic phototrophs
These microorganisms obtain energy from light and use compounds such as hydrogen sulfide instead of water as an electron donor and thus do not produce molecular oxygen.

Eutrophication
An increased input of nutrients that leads to excessive growth of algae or cyanobacteria.

Proton motive force
Proton dislocation creates a difference of charge and pH between two sides of a cell membrane and thereby generates an electrochemical potential, which is used for energy conservation.

Anaerobic sludge digesters
Bioreactors in which excess microbial biomass (sludge) produced during wastewater treatment is anaerobically converted to carbon dioxide, methane, ammonium and reduced sulfur compounds.

in these nitrite-oxidizing bacteria is also catalysed by NXR, which is related to bacterial and archaeal nitrate reductases[54].

The concerted activity of nitrite and ammonia-oxidizing microorganisms in agricultural soils converts nitrogen-based fertilizers to nitrate and has a key role in the loss of fertilizers to river and groundwaters, leading to the eutrophication of rivers, lakes and coastal waters. The same two processes are also used in wastewater treatment plants as the first step of conventional nitrogen removal (BOX 2). In marine environments, nitrite-oxidizing bacteria generate nitrate, which is the dominant form of biologically available nitrogen in the ocean, and contribute to carbon fixation[55].

Nitrate reduction to nitrite. Nitrate reduction to nitrite is used for respiration, known as dissimilatory nitrate reduction, and for nitrogen assimilation into biomass. Dissimilatory nitrate reduction to nitrite can be carried out by microorganisms from all three domains of life. These microorganisms occur in all anoxic environments in which nitrate is present, including soils[56], oxygen minimum zones[57], marine sediments[58] and the human gastrointestinal tract[59]. The reaction is catalysed by either a membrane-bound nitrate reductase (NAR) or periplasmic nitrate reductase (NAP)[60]. Many organisms, including the model organism *Paracoccus denitrificans*, contain both NAP and NAR[60]. NAR reduces nitrate in the cytoplasm and releases protons into the periplasm (FIG. 2c) and thereby directly contributes to energy conservation through the proton motive force. By contrast, NAP reduces nitrate to nitrite in the periplasm and thus does not translocate protons that could contribute to the proton motive force[60].

Dissimilatory nitrate reduction to nitrite is not merely the first step in denitrification. Some microorganisms

Box 2 | Nitrogen removal by microorganisms in wastewater treatment

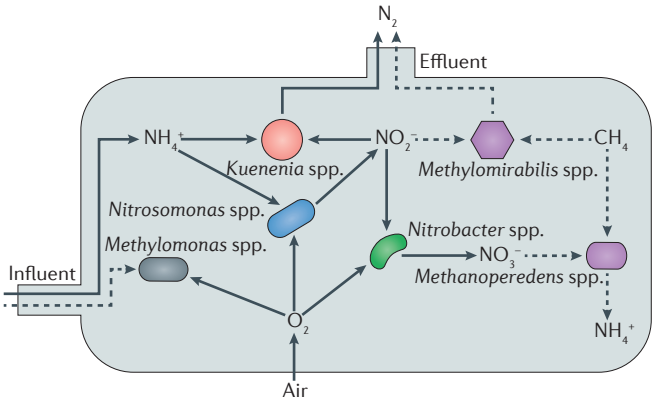
Since the industrial revolution, agriculture, burning of fossil fuels and domestic and industrial wastewater production have been the major drivers of nitrogen pollution, which severely affects life on Earth[141,142]. Nitrogen has been recognized as an important pollutant in wastewater only in the past 40 years, when it became clear that excess nitrogen leads to eutrophication and fish mortality owing to the toxic effects of ammonia. Consequently, nitrogen-removing systems were added to many wastewater treatment plants, which were originally used to remove organic carbon. Nevertheless, most conventional wastewater treatment plants do not remove nitrogen.

In contrast to most natural ecosystems in which precious nitrogen is recycled and retained, nitrogen-removing treatment plants are designed to convert ammonium to dinitrogen gas, which is lost to the atmosphere. In these treatment plants, organic carbon is removed first. This results in organic carbon-poor and ammonium-rich wastewater, which is fed into a nitrogen-removal system. Conventional systems rely on nitrification ($NH_4^+ \rightarrow NO_2^- \rightarrow NO_3^-$) to oxidize ammonium to nitrate, which is subsequently reduced to dinitrogen gas by denitrification ($NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2$). Nitrification requires extensive aeration to create conditions that are suitable for ammonium oxidation to nitrate (2 molecules of O_2 are needed per molecule of ammonium). Subsequently, external organic carbon (often methanol) is added to induce heterotrophic denitrification, which reduces nitrate to dinitrogen. Hence, conventional nitrogen removal is costly, as well as energy and resource intensive, and also produces nitrous oxide, which contributes to global warming. To alleviate these problems, different reactor configurations have been implemented to minimize external carbon addition and aeration. For example, in some systems, part of the raw wastewater, which is rich in organic carbon, is fed directly to the denitrification step or, in others, intermittent aeration is used to promote nitrification and denitrification in a single tank[145].

In the past decade, anaerobic ammonium oxidation (anammox) emerged as an alternative process for nitrogen removal. In compact bioreactors, aerobic ammonia-oxidizing bacteria, such as *Nitrosomonas europea*, convert half of the available ammonia to nitrite under oxygen limitation, which is termed 'partial nitritation'. This is followed by the conversion of nitrite with the remaining ammonium to dinitrogen gas by bacteria performing the anammox process, such as '*Candidatus* Kuenenia stuttgartiensis' (solid arrows, see the figure)[146]. In these partial nitritation-anammox systems, nitrate production by aerobic nitrite oxidizers such as *Nitrospira* spp. or *Nitrobacter* spp. is undesired as it decreases the efficiency of nitrogen removal. Oxygen-limited partial nitritation-anammox reactors have lower aeration requirements than conventional nitrogen-removal systems, do not require the addition of organic carbon and produce less nitrous oxide. Currently, partial nitritation-anammox systems are increasingly used for ammonium-rich wastewaters[146,147], such as effluents from anaerobic sludge digesters. Implementation of these systems in full-scale municipal wastewater treatment, which has much lower ammonium concentrations, could pave the way to more sustainable sewage treatment[146].

Some of the recently discovered nitrogen-cycling microorganisms could also be applied in wastewater treatment. Archaea that oxidize ammonia to nitrite and bacteria that oxidize ammonia to nitrate (in the complete ammonia oxidation (comammox process)) have been detected in nitrogen-removing wastewater treatment plants[133,148], but their role in these systems is unclear. In oxygen-limited nitrogen-removal systems, such as partial nitritation-anammox bioreactors, bacteria performing comammox[12,13,39] will most likely act as conventional ammonia oxidizers that produce nitrite. Exciting new possibilities for wastewater treatment are offered by the newly discovered nitrite-dependent and nitrate-dependent anaerobic methane-oxidizing microorganisms[66,149]. A bioreactor that combines anaerobic methanotrophs, such as '*Candidatus* Methylomirabilis' spp. and '*Candidatus* Methanoperedens' spp., with microorganisms that perform the anammox process could simultaneously remove ammonium, nitrate and methane (dashed arrows; see the figure). Such co-cultures have already been established under laboratory conditions; however, a full-scale wastewater treatment system has not been implemented[66,149]. In these systems, aerobic methane oxidizers such as *Methylomonas* spp. would also contribute to methane removal.

Fundamental physiological and biochemical research into nitrogen-cycling microorganisms and their application have always progressed hand in hand — newly discovered microorganisms led to more efficient and sustainable treatment systems and vice versa. It is apparent that this trend will continue to help safeguard the environment for future generations.



such as the giant sulfur-oxidizing *Beggiatoa* sp.[61], which is widespread in freshwater and marine sediments, reduce nitrate via nitrite to ammonium, and many microorganisms, such as some members of the ubiquitous marine clade SAR11 (REF. 62), only reduce nitrate to nitrite (FIG. 1). Nitrate reduction is a major source of nitrite for other nitrogen-cycling processes, including aerobic nitrite oxidation and anammox[62–64]. Dissimilatory nitrate reduction can be coupled to the oxidation of electron donors such as organic matter[65], methane[66,67] (for example, in '*Candidatus* Methanoperedens' spp.), sulfur compounds (for example, in *Thiobacillus denitrificans*)[68], hydrogen (for example, in *Cupriavidus necator*, also known as *Alcaligenes eutrophus*) or iron (for example, in *Ferroglobus placidus*)[69].

Nitrate is a major nitrogen source for eukaryotes, bacteria and archaea that contain assimilatory nitrate reductases (NAS)[60]. Considering that nitrate supports at least 20% of marine algal growth[70], nitrate assimilation likely exceeds the magnitude of most other redox driven nitrogen-cycle processes in the ocean (BOX 1). NAS, together with assimilatory nitrite reductases (see below), produces ammonia, which is incorporated into biomass[60]. Because NAS is located in the cytoplasm, nitrate assimilation requires nitrate transport into the cell by ATP-dependent transporters[60]. Due to this energy requirement, NAS expression is repressed in ammonia-replete environments, such as fertilized soils[60].

Bacterial and archaeal NAS, together with NAP, NAR and NXR, belong to the dimethylsulfoxide reductase family, whereas eukaryotic assimilatory nitrate reductases belong to the sulfite oxidase family[71]. This suggests multiple origins of nitrate reductases. The distinction between assimilatory and dissimilatory nitrate reduction pathways is not absolute. In principle, nitrite produced by assimilatory nitrate reduction could be reduced further in the respiratory chain. Conversely, *Mycobacterium tuberculosis* has been shown to use the NAR complex for nitrate assimilation[72].

Nitrite reduction to ammonium. Nitrite reduction to ammonium is used for both dissimilatory and assimilatory purposes. Dissimilatory nitrite reduction to ammonium is carried out by most bacterial lineages, the thermophilic *Pyrolobus fumarii*[73], which is a member of the Crenarcheota, methane-oxidizing archaea[67], diatoms[74] and fungi[75]. This reaction is catalysed by the periplasmic cytochrome *c* nitrite reductase (ccNIR) encoded by *nrfAH*, the octahaem nitrite reductase (ONR)[76] or the octahaem tetrathionate reductase (OTR)[77]. It is unclear whether the latter two enzymes are used for respiration or detoxification of nitrite or hydroxylamine. Reduction of nitrite to ammonium involves the formation of hydroxylamine as an intermediate, which remains bound to the enzyme until it is reduced to ammonium[78].

Interestingly, the anaerobic ammonium-oxidizing bacterium *K. stuttgartiensis* can reduce nitrite to ammonium but lacks known ammonium-producing nitrite reductases. It is hypothesized that nitrite reduction to ammonium instead might be accomplished by an HAO-like protein[46]. Recently, an HAO encoded by an Epsilonproteobacteria (εHAO), such as *Campylobacter fetus* and *Nautilia profundicola*, was shown to reduce nitrite and hydroxylamine to ammonium, although with poor efficiency[79].

Dissimilatory nitrite reduction to ammonium is the key reaction in the so-called dissimilatory nitrate reduction to ammonium (DNRA) process[80]. Microorganisms can grow using DNRA by coupling it to the oxidation of electron donors, such as organic matter, ferrous iron, hydrogen, sulfide and methane[67,81–83]. Little is known about the environmental importance of DNRA[84,85]; however, in marine and lake sediments, DNRA appears to be favoured over denitrification when there is an excess of electron donor relative to nitrate[58].

Assimilatory nitrite reductases produce ammonium and are as widespread as NAS, and both types of enzymes are often encoded on the same *nas* operon[54]. The formation of primary nitrite maxima in the ocean has been attributed to the release of nitrite owing to an uncoupling of assimilatory nitrate and nitrite reduction in phytoplankton[86]. The physiological reasons for this uncoupling are still unclear.

Nitrite reduction to nitric oxide. Many microorganisms have the ability to reduce nitrite to nitric oxide, such as Proteobacteria, anaerobic ammonium-oxidizing bacteria and Bacteroidetes[54]. These microorganisms are found in many environments in which nitrate is available and oxygen concentrations are low, such as soils[56], oxygen minimum zones[57] and marine sediments[58]. This reaction can be catalysed by two unrelated enzymes: a haem-containing cd_1 nitrite reductase (cd_1-NIR; encoded by *nirS*) or a Cu-containing nitrite reductase (Cu-NIR; encoded by *nirK*), which are widespread among bacteria and archaea[87]. Both enzymes are located in the periplasm and do not contribute directly to energy conservation[54,65]. These two enzymes also occur together in a single microorganism, for example, in *Rhodothermus marinus*[87].

Commonly, *nirS* and *nirK* are used in environmental studies as gene markers for denitrifiers; however, these genes are present in many other microorganisms, including anaerobic ammonium-oxidizing bacteria, nitrite and methane-oxidizing bacteria, and ammonia-oxidizing bacteria and archaea[88]. Apart from Cu-NIR and cd_1-NIR, other nitrite-reducing enzymes might exist; for example, some anaerobic ammonium-oxidizing bacteria contain neither of the genes but can reduce nitrite to nitric oxide[89]. To carry out this reaction, these bacteria might use an HAO-like octahaem oxidoreductase[46].

Nitric oxide reduction to nitrous oxide or dinitrogen gas. Nitric oxide is a signalling molecule, a toxin[90] and an intermediate of the denitrification, nitrification and anammox processes. Additionally, bacteria that perform oxygenic denitrification dismutate two molecules of nitric oxide to one molecule of dinitrogen gas and one molecule of oxygen[9]. Therefore, microorganisms capable of nitric oxide reduction can be found in a wide range of environments, including wastewater treatment plants[46], agricultural soils[56,91], marine sediments[58] and

Primary nitrite maxima
The peak in nitrite concentrations at the base of the euphotic zone.

marine oxygen minimum zones[57]. Microbial nitric oxide reduction (FIG. 1) is the main source of nitrous oxide, a powerful greenhouse gas (310 times more potent than carbon dioxide) and the dominant ozone-depleting agent[92]. Nitrous oxide-producing nitric oxide reductases (NOR) are used for detoxification or respiration of nitric oxide and belong to a diverse group of enzymes ranging from flavoproteins to haem copper oxidases, which are widespread throughout the tree of life. Flavo-diiron proteins, such as flavorubredoxin nitric oxide reductase (NORvw), are used to detoxify nitric oxide, for example, by the sulfate-reducing bacterium *Desulfovibrio gigas*[93,94]. Other NOR-type enzymes are the NADH-dependent cytochrome P_{450}NOR found in the mitochondria of fungi, such as *Fusarium oxysporum*[95], and the hybrid cluster protein HCP recently discovered in *Escherichia coli*[96].

The haem copper oxidase family contains terminal oxidases, the cytochrome *c*-dependent cNOR, quinol-dependent qNOR and the copper-containing Cu_ANOR, which all have a role in nitric oxide respiration[97–99]. Nitrous oxide is an intermediate of denitrification, and NOR is present in microorganisms such as *P. denitrificans* and *Pseudomonas stutzeri*[65]. Nitrous oxide can also be the end product of denitrification in some microorganisms, such as *Pseudomonas chlororaphis*[65]. Ammonia-oxidizing bacteria can produce nitrous oxide in a process termed nitrifier–denitrification, in which NOR is used to reduce nitric oxide formed upon nitrite reduction[30]. In cultures of ammonia-oxidizing bacteria and bacteria capable of carrying out the comammox process, nitrous oxide can also be formed through abiotic reactions of the extracellular intermediates hydroxylamine and nitric oxide[100]. Additionally, ammonia-oxidizing bacteria can produce nitrous oxide through the NOR-catalysed reduction of nitric oxide, which is produced during hydroxylamine oxidation[7,30]. Similar to ammonia-oxidizing bacteria, methanotrophic bacteria produce nitrous oxide through the NOR-catalysed reduction of nitric oxide formed upon hydroxylamine oxidation (see above) and nitrite reduction[47,48]. By contrast, nitrous oxide production in ammonia-oxidizing archaea might exclusively involve the abiotic reactions of the intermediates nitric oxide and hydroxylamine[45].

The use of nitrogen-based fertilizers has drastically increased nitrous oxide emissions[101]. Due to the concerted activity of nitrogen-transforming microorganisms, 3–5% of the nitrogen used as agricultural fertilizer is converted into nitrous oxide[102,103]. Nitrogen-based fertilizers are increasingly used to grow crops for biofuel production, which represents a potential replacement for fossil fuels. Herein lies a dilemma — the more fertilizer is used to produce biofuels, the more nitrous oxide emissions increase. Therefore, the fertilizer use for biofuel production counteracts the reduction in greenhouse gas emissions that is achieved by reducing the use of fossil fuels[103].

Nitric oxide dismutation to dinitrogen and oxygen gas (FIG. 1) is a recently discovered nitrogen-transforming reaction[104]. Microorganisms such as '*Ca.* Methylomirabilis oxyfera' found in anoxic systems rich in methane and nitrate (for example, in eutrophied lakes and wetlands) use this reaction to produce their own molecular oxygen from nitrite[9]. This enables '*Ca.* Methylomirabilis oxyfera' to live in anoxic environments and to use the aerobic methane oxidation pathway[9]. The dismutation reaction might involve an unusual qNOR, tentatively called nitric oxide dismutase (NO-D)[9]. Nitric oxide dismutation might be more widespread than previously thought, as similar unusual qNOR sequences are present in other phyla, such as Gammaproteobacteria (for example, strain HdN1) and Bacteroidetes (for example, *Muricauda ruestringensis*)[104].

Nitrous oxide reduction to nitrogen gas. Microbial nitrous oxide reduction to nitrogen gas is the main sink of this powerful greenhouse gas. The only known enzyme that catalyses this reaction is nitrous oxide reductase (NOS), which, owing to its location in the periplasm, does not directly contribute to energy conservation through the proton motive force[105]. Diverse bacteria, including members of the Proteobacteria, Bacteroidetes and Chlorobi phyla, and archaea from Crenarchaeota and Halobacteria[106] utilize NOS. The discovery of a slightly different NOS-encoding gene in *Wolinella succinogenes*[107] revealed an overlooked diversity of NOS sequences in soils[108]. Intriguingly, organisms encoding this NOS variant often have no other nitrogen-oxide reductases[87,91,109]. Some eukaryotes, the Foraminifera and Gromiida, also reduce nitrous oxide, but their enzymatic machinery is unknown[15,110].

For a long time, it was believed that NOS was more sensitive to oxygen, pH and sulfide than other nitrogen-oxide reductases[105]. Based on that apparent sensitivity, environmental emissions of nitrous oxide were fully attributed to inhibition of NOS in organisms that reduce nitrate all the way to nitrogen, the so-called complete denitrifiers. Additionally, interactions of so-called incomplete denitrifiers, which are microorganisms that only perform, for example, nitrite reduction to nitrous oxide or nitrous oxide reduction to dinitrogen gas, and their niche differentiation might cause imbalances between nitrous oxide production and consumption in many environments, such as soils and marine environments[91,109,111].

Hydrazine synthesis and hydrazine oxidation to dinitrogen gas. Until recently, it was generally believed that ammonium could be activated only with molecular oxygen and that bioavailable nitrogen could be lost only as dinitrogen gas through denitrification[112]. The discovery of anaerobic ammonium oxidation (anammox) to dinitrogen gas with nitrite as the terminal electron acceptor overturned both of these dogmas[51,113,114]. HZS is the only known enzyme that can activate ammonium anaerobically[89], and it is found exclusively in anaerobic ammonium-oxidizing bacteria that belong to five genera in the phylum Planctomycetes[89,115,116]. HZS is also the only enzyme known to form an N–N bond from two discrete N-compounds, producing hydrazine as a free intermediate in a two-step reaction[10,115]. The

Nitric oxide dismutation
Two molecules of nitric oxide are disproportionated into one molecule of molecular oxygen and one molecule of dinitrogen gas.

hypothetical mechanism of hydrazine synthesis starts with nitric oxide reduction to hydroxylamine (FIG. 2d), which subsequently undergoes comproportionation together with ammonium into hydrazine, one of the most potent reductants in nature[10,115]. During this reaction, hydroxylamine is transferred from one active site to the next (FIG. 2d), which might result in hydroxylamine loss from HZS. Two of the genes encoding HZS, *hzsA* and *hzsB*, are used as genetic markers for anaerobic ammonium-oxidizing bacteria in the environment[117,118].

Hydrazine is oxidized to dinitrogen gas by hydrazine dehydrogenase (HDH)[10,119]. Based on amino acid sequences, this enzyme is related to HOX and HAO; however, it is inhibited by hydroxylamine and can oxidize only hydrazine[119]. Hydrazine oxidation occurs in a unique membrane-bound structure called the anammoxosome and is most likely directly associated with energy conservation[46,120,121]. Intriguingly, all catabolic enzymes of anaerobic ammonium-oxidizing bacteria (HDH, HZS, NIR, HOX and NXR) are located exclusively in the anammoxosome[122].

HDH is responsible for the release of a substantial amount of dinitrogen to the atmosphere[119]. In the past decade, it became clear that the anammox process is a major nitrogen sink in the ocean[123–125], and it could also have an important role in terrestrial ecosystems[126].

Microbial nitrogen-transforming networks

There is an astonishing diversity of microorganisms that transform nitrogen, and each of these microorganisms has discrete physiological requirements for optimal growth. As growth conditions in nature are highly variable and seldom optimal, nitrogen turnover by individual microorganisms is bound to be inefficient. However, nitrogen transformations in the environment are carried out by microbial communities that recycle nitrogen more efficiently than single microorganisms. Consequently, very little bioavailable nitrogen escapes to the atmosphere, and the small amount lost as dinitrogen gas is balanced by nitrogen fixation (BOX 1). This apparent nitrogen homeostasis not only characterizes the global biosphere; it also characterizes many ecosystems, such as forest soils and ocean gyres. The microbial communities required to efficiently recycle nitrogen in these ecosystems retain nitrogen-transforming reactions even when the species composition changes in response to environmental perturbations. The nitrogen-transforming reactions are linked by microorganisms that form complex networks in both natural and man-made ecosystems (FIG. 3).

The ocean gyres, the world's largest ecosystems, are nearly nitrogen-balanced owing to extensive nitrogen recycling (FIG. 3a). Here, the main nitrogen-transforming processes are nitrogen assimilation by cyanobacteria, such as *Prochlorococcus marinus*[70], ammonification by mesozooplankton[127] and heterotrophic bacteria, such as '*Candidatus* Pelagibacter ubique' (REF. 128) and nitrification by *Nitrosopumilus* spp. and *Nitrospina* spp. (BOX 1; FIG. 3a). Nitrogen fixation by microorganisms, such as *Trichodesmium* spp. and '*Ca.* Atelocyanobacterium' spp., is a rather minor nitrogen-transforming process in the gyres[70]. Yet, owing to the sheer extent of the area in which nitrogen fixation occurs, it is the main supply of new bioavailable nitrogen to the ocean.

In contrast to the ocean gyres, oxygen minimum zone waters cover less than 1% of the open ocean area but might account for 30–50% of oceanic nitrogen loss[57,70,125] (BOX 1). Here, anaerobic microorganisms such as '*Candidatus* Scalindua' spp. co-occur with aerobic organisms such as *Nitrosopumilus* spp. and *Nitrospina* spp.[57]. The microbial nitrogen-transforming network in open-ocean oxygen minimum zones is complex[57], with all the known nitrogen-converting processes occurring alongside each other (FIG. 3b).

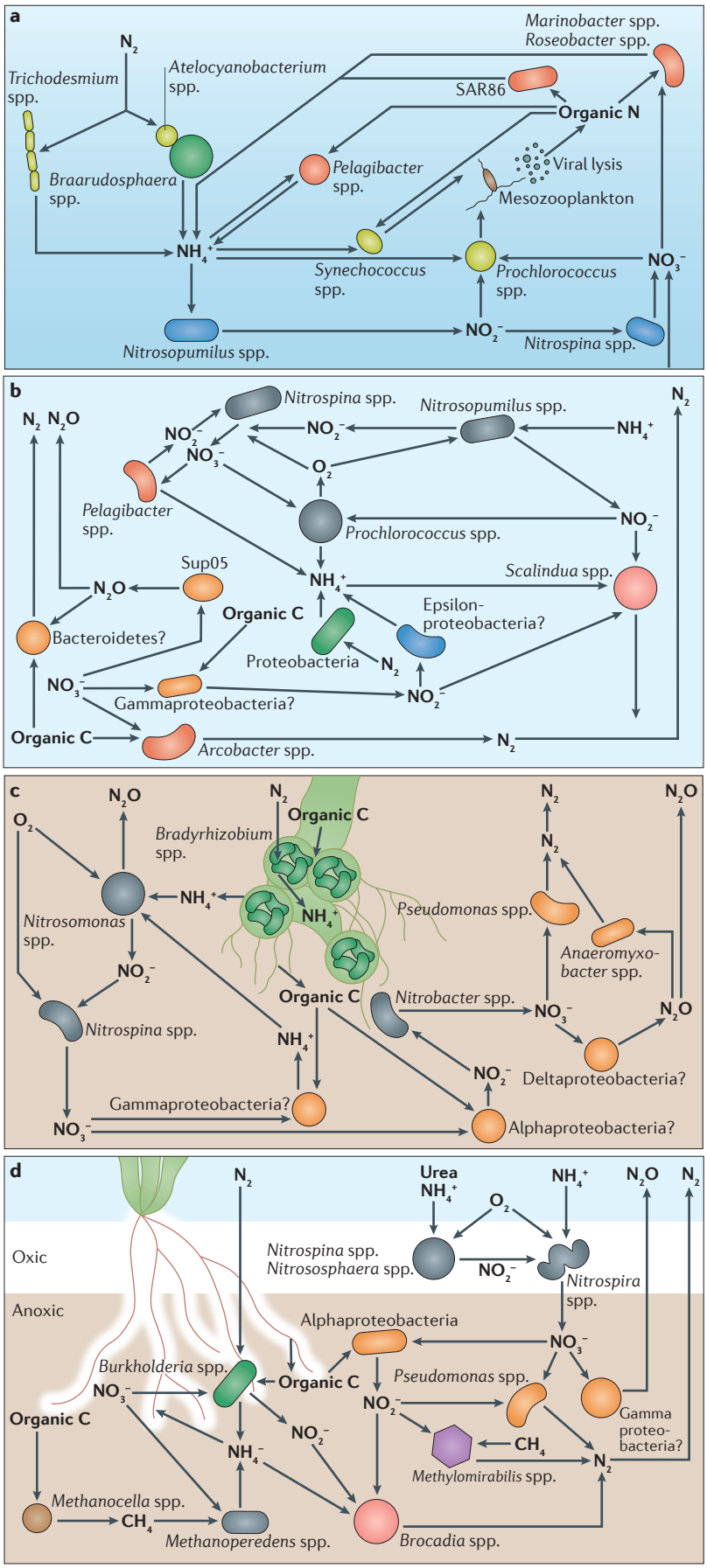
Similar to oxygen minimum zone waters, nitrogen-removing wastewater treatment plants are characterized by imbalanced nitrogen transformations. These man-made systems are designed to convert ammonium to dinitrogen gas, which is lost to the atmosphere (BOX 2).

Agricultural fields are among the largest man-made ecosystems, and their microbial nitrogen-transforming networks have been strongly affected by the anthropogenic input of nitrogen. The cultivation of legumes that form symbioses with nitrogen-fixing microorganisms have substantially increased the nitrogen input into the environment[2,129]. Nitrogen-fixing microorganisms, such as *Bradyrhizobium* spp., often live in specialized root nodules and provide ammonium to the legumes (FIG. 3c). Ammonium that leaks out into the surrounding soil fuels other microbial nitrogen transformations, such as aerobic ammonia oxidation. In rice paddy fields, the use of industrial fertilizers has resulted in intense nitrification and increased nitrogen loss[126]. Recent studies reveal that these systems have highly complex nitrogen-transforming networks, which include nitrite-reducing ('*Ca.* Methylomirabilis' spp.) and nitrate-reducing ('*Ca.* Methanoperedens' spp.) methanotrophs[130] (FIG. 3d).

In these ecosystems, some nitrogen-transforming microorganisms, such as anaerobic ammonium-oxidizing bacteria, can perform multiple redox reactions (reactions 1, 2, 5, 7, 10, 12 and 13; FIG. 1). Still, processes such as nitrification and denitrification are performed by a complex network of specialists in a modular fashion (FIG. 3). Such modularity, which is a general feature of nitrogen-transforming microbial networks, results in cooperative and competitive interactions (examples in FIG. 3). A cooperative interaction exists between *Nitrosopumilus* spp. and *Nitrospina* spp. that together oxidize ammonia to nitrate (FIGS. 3a,b). In most environments, nitrification is carried out by diverse assemblages of ammonia-oxidizing and nitrite-oxidizing microorganisms, which also compete for ammonia and nitrite, respectively. Substrate competition also exists between microorganisms with very different metabolisms, such as *Nitrospira* spp., '*Ca.* Methylomirabilis' spp., '*Candidatus* Brocadia' spp., '*Ca.* Methanoperedens' spp. and *Pseudomonas* spp., which all compete for nitrite (FIG. 3d). Microbial interactions can also be simultaneously cooperative and competitive: *Nitrosopumilus* spp. produce nitrite for '*Ca* Scalindua' spp., but both also compete for ammonia (FIG. 3b).

Comproportionation
A chemical reaction in which two reactants containing the same element with a different oxidation state react to create a product with a single oxidation state.

Anammoxosome
A bacterial organelle found in anaerobic ammonium oxidizing (anammox) bacteria that is the only known prokaryotic membrane-bound structure that is equally divided into daughter cells upon cell division.



The factors that control these interactions are poorly understood. Sometimes, a single physiological characteristic is used to explain the dominance of certain nitrogen-transforming microorganisms in the environment. For example, the abundance of ammonia-oxidizing archaea relative to bacteria in ammonia-depleted environments was attributed to the superior ammonia affinity of the archaea[31,131,132]. Recently, however, it was shown that the terrestrial bacterium '*Ca.* Nitrospira inopinata', which performs the comammox process, has a higher ammonia affinity than all cultured terrestrial ammonia-oxidizing archaea[39]. Yet, the microorganisms that perform the comammox process do not dominate all the ammonia-depleted terrestrial environments[133]. The success of nitrogen-transforming microorganisms also depends on other factors, such as the use of alternative substrates and cellular energy requirements. Such variables might have general roles in shaping nitrogen-transforming microbial networks.

Fig. 3 | **Potential nitrogen-transforming microbial networks in different ecosystems. a** | The open ocean gyres are vast nutrient-limited regions in which nitrogen is extensively recycled. In the sunlit surface waters, cyanobacteria mainly assimilate ammonium and/or organic nitrogen compounds for growth. Viral lysis and grazing by mesozooplankton release organic nitrogen (for example, urea), which is subsequently mineralized back to ammonium by heterotrophic bacteria. Nitrogen-fixing bacteria provide additional ammonium. In deeper waters, ammonium is oxidized to nitrate. Some of this nitrate diffuses up into the surface waters and is assimilated by phytoplankton. **b** | Marine oxygen minimum zones are found on the eastern boundaries of oceans, where wind-driven upwelling of nutrient rich waters stimulates primary productivity in the surface waters. The subsequent aerobic mineralization of sinking organic matters depletes oxygen in the underlying waters. Aerobic nitrifying communities that are well adapted to low oxygen conditions perform ammonia oxidation to nitrite and nitrate. The oxygen minimum zones are major regions of nitrogen loss owing to the activity of anaerobic ammonium-oxidizing bacteria and to a lesser extent denitrification. Complex communities of microorganisms are involved in the denitrification process. **c** | Among the largest man-made ecosystems are agricultural fields that are used for crop production. Legumes are common crops and an important source of protein. They influence the microbial community in the surrounding soil by releasing organic carbon and live in symbiosis with nitrogen-fixing microorganisms, such as *Bradyrhizobium* spp. Ammonium that leaks out into the surrounding soil can fuel aerobic ammonia and nitrite oxidation. Subsequent diffusion of nitrate to anoxic zones in soil fuels nitrogen-transforming processes, such as dissimilatory nitrate reduction to ammonium, nitrous oxide and dinitrogen gas. **d** | Rice paddies are flooded agricultural fields, which are fertilized with nitrogen-containing compounds such as urea to grow rice[155]. Urea hydrolysis and nitrogen fixation generate ammonia, which is oxidized to nitrate in oxic soils surrounding the rice-plant roots. Subsequent diffusion of nitrate to the underlying anoxic soil fuels processes such as denitrification, anaerobic ammonium oxidation (anammox) and the oxidation of methane produced by methanogenesis.

Exergonic
A reaction that results in the release of free energy.

Disproportionation
A chemical reaction in which a reactant is split into two species containing the same element with different oxidation states, one more oxidized and the other more reduced than the reactant.

Box 3 | **Undiscovered biochemical reactions**

Numerous new microbial nitrogen-transforming reactions and pathways have been discovered in the past decade. Based on thermodynamic considerations, further exergonic reactions exist that could be exploited by microorganisms (reactions 15–26). Whereas some reactions could be catalysed by known enzymes, others would require hitherto unknown biochemistry (reactions 15–19, 25 and 26). For example, nitrate-dependent ammonium oxidation (reaction 15) cannot proceed through the known anaerobic ammonium oxidation pathway because ammonia first needs to be oxidized to the intermediate hydroxylamine or a similar oxygen-containing species[46].

Reaction 15 $5NH_4^+ + 3NO_3^- \rightarrow 4N_2 + 9H_2O + 2H^+$ ($\Delta G^{0'} = -297$ kJ mol^{-1} NH_4^+)

Similarly, novel biochemical pathways for ammonia activation would be necessary for iron-dependent and manganese-dependent ammonium oxidation (reactions 16–19).

Reaction 16 $NH_4^+ + 6Fe^{3+} + 2H_2O \rightarrow NO_2^- + 6Fe^{2+} + 8H^+$ ($\Delta G^{0'} = -247$ kJ mol^{-1} NH_4^+)

Reaction 17 $2NH_4^+ + 6Fe^{3+} \rightarrow N_2 + 6Fe^{2+} + 8H^+$ ($\Delta G^{0'} = -303$ kJ mol^{-1} NH_4^+)

Reaction 18 $NH_4^+ + 3MnO_2 + 4H^+ \rightarrow NO_2^- + 3Mn^{2+} + 4H_2O$ ($\Delta G^{0'} = -60$ kJ mol^{-1} NH_4^+)

Reaction 19 $2NH_4^+ + 3MnO_2 + 4H^+ \rightarrow N_2 + 3Mn^{2+} + 6H_2O$ ($\Delta G^{0'} = -249$ kJ mol^{-1} NH_4^+)

Conversely, several disproportionation reactions (reactions 20–24) could be carried out by known microorganisms using the existing biochemical machinery. Anaerobic ammonium-oxidizing bacteria could perform nitrite (reaction 20) and nitric oxide (reaction 21) disproportionation[46].

Reaction 20 $5NO_2^- + 2H^+ \rightarrow N_2 + 3NO_3^- + 2H_2O$ ($\Delta G^{0'} = -60$ kJ mol^{-1} NO_2^-)

Reaction 21 $10NO + 2H_2O \rightarrow 3N_2 + 4NO_3^- + 4H^+$ ($\Delta G^{0'} = -100$ kJ mol^{-1} NO)

Similarly, disproportionation of nitrite into nitrous oxide and nitrate (reaction 22) and nitric oxide into nitrite and nitrous oxide (reaction 23) or nitrate and nitrous oxide (reaction 24) could theoretically be carried out by aerobic nitrite-oxidizing bacteria.

Reaction 22 $4NO_2^- + 2H^+ \rightarrow N_2O + 2NO_3^- + H_2O$ ($\Delta G^{0'} = -32$ kJ mol^{-1} NO_2^-)

Reaction 23 $4NO + H_2O \rightarrow 2NO_2^- + N_2O + 2H^+$ ($\Delta G^{0'} = -40$ kJ mol^{-1} NO)

Reaction 24 $8NO + H_2O \rightarrow 2NO_3^- + 3N_2O + 2H^+$ ($\Delta G^{0'} = -56$ kJ mol^{-1} NO)

These microorganisms could use nitrite oxidoreductase to oxidize nitrite to nitrate and nitrite reductases present in *Nitrobacter* spp., *Nitrococcus* spp., *Nitrospira* spp. and *Nitrospina* spp. could reduce nitrite to nitric oxide[6]. Nitric oxide oxidation has been observed in *Nitrobacter* spp.[150, 151], but it is unclear whether this reaction is biotic or abiotic, and the responsible enzyme remains unknown. Nitric oxide oxidation to nitrite could also be catalysed by Cu-containing nitrite reductases (encoded by *nirK*), which are known to be bidirectional[152]. The remaining reaction, reduction of nitric oxide to nitrous oxide, can be carried out by terminal oxidases, which are evolutionarily related to nitric oxide reductases[153].

Nitrous oxide, a potent greenhouse gas, is reduced to dinitrogen gas in the absence of oxygen, whereas it is assumed to be biologically stable under oxic conditions. Intriguingly, aerobic nitrous oxide oxidation to either nitrite or nitrate is thermodynamically feasible (reactions 25, 26), but this reaction would also require a new biochemical pathway.

Reaction 25 $N_2O + O_2 + H_2O \rightarrow 2NO_2^- + 2H^+$ ($\Delta G^{0'} = -21$ kJ/mol N_2O)

Reaction 26 $N_2O + 2O_2 + H_2O \rightarrow 2NO_3^- + 2H^+$ ($\Delta G^{0'} = -89$ kJ/mol N_2O)

The only way to identify microorganisms that catalyse these undiscovered reactions is to grow them under controlled laboratory conditions. It is clear that the physiology and biochemistry of nitrogen-transforming microorganisms will remain fertile fields of research for years to come.

Concluding remarks

Identifying the factors that shape nitrogen-transforming networks will require greater insight into the physiology of the involved microorganisms and a deeper understanding of their ecology and evolution. Only a fraction of all microorganisms has been cultivated, and the uncultivated majority likely contains undiscovered metabolic pathways (BOX 3). Cultivation, followed by painstaking biochemical, physiological and genomic characterization, has already changed our perspective of key nitrogen-cycle processes. Aerobic nitrite-oxidizing bacteria and anaerobic ammonium-oxidizing bacteria have a hitherto unexpected metabolic versatility that renders their classification as mere aerobic nitrite oxidizers or anaerobic ammonia oxidizers inadequate. Many aerobic nitrite oxidizers might grow as hydrogen oxidizers, ammonia oxidizers or nitrate reducers in the environment[6]. Anaerobic ammonium-oxidizing bacteria can also use short-chain fatty acids, methylamines and Fe(II) as electron donors[46,134], and they can use nitrate, Mn(IV) and Fe(III) as electron acceptors[46,135,136].

Conversely, there is a growing realization that complete denitrification by single microorganisms is the exception rather than the rule, with many microorganisms being specialists that perform only one or a few nitrogen oxide reduction reactions[3,91,137]. Specialized nitrogen oxide reducers often lack known genes enabling them to reduce nitrate all the way to nitrogen[87,138]. These specialist nitrogen oxide reducers are often described as incomplete denitrifiers, which is comparable to describing ammonia oxidizers such as *Nitrosomonas* spp. as incomplete nitrifiers.

Undoubtedly, it will become increasingly difficult to classify organisms according to the classical six nitrogen-cycling processes, leaving it up to the eye of the beholder to define the function of an organism. If we can learn one thing from the past few decades of research, it is that microorganisms do not conform to boundaries. They will do whatever is necessary in the perpetual struggle to survive.

1. Galloway, J. N. et al. Transformation of the nitrogen cycle: recent trends, questions, and potential solutions. *Science* **320**, 889–892 (2008). **A comprehensive overview of the human impact on biogeochemical nitrogen cycling.**
2. Erisman, J. W., Sutton, M. A., Galloway, J., Klimont, Z. & Winiwarter, W. How a century of ammonia synthesis changed the world. *Nat. Geosci.* **1**, 636–639 (2008).
3. Stein, L. Y. & Klotz, M. G. The nitrogen cycle. *Curr. Biol.* **26**, R94–R98 (2016).
4. Yan, Y. et al. Nitrogen fixation island and rhizosphere competence traits in the genome of root-associated Pseudomonas stutzeri A1501. *Proc. Natl Acad. Sci. USA* **105**, 7564–7569 (2008).
5. Füssel, J. et al. Adaptability as the key to success for the ubiquitous marine nitrite oxidizer Nitrococcus. *Sci. Adv.* **3**, e1700807 (2017).
6. Daims, H., Lücker, S. & Wagner, M. A. New perspective on microbes formerly known as nitrite-oxidizing bacteria. *Trends Microbiol.* **24**, 699–712 (2016).
7. Caranto, J. D. & Lancaster, K. M. Nitric oxide is an obligate bacterial nitrification intermediate produced by hydroxylamine oxidoreductase. *Proc. Natl Acad. Sci. USA* **114**, 8217–8222 (2017).
8. Maalcke, W. J. et al. Structural basis of biological NO generation by octaheme oxidoreductases. *J. Biol. Chem.* **289**, 1228–1242 (2014).
9. Ettwig, K. F. et al. Nitrite-driven anaerobic methane oxidation by oxygenic bacteria. *Nature* **464**, 543–548 (2010). **The discovery of oxygenic denitrification.**
10. Kartal, B. et al. Molecular mechanism of anaerobic ammonium oxidation. *Nature* **479**, 127–130 (2011). **Isolation and characterization of the unique enzyme that produces free hydrazine.**
11. Griffin, B. M., Schott, J. & Schink, B. Nitrite, an electron donor for anoxygenic photosynthesis. *Science* **316**, 1870–1870 (2007). **The discovery of phototrophic nitrite oxidation.**
12. Daims, H. et al. Complete nitrification by Nitrospira bacteria. *Nature* **528**, 504–509 (2015).
13. van Kessel, M. A. et al. Complete nitrification by a single microorganism. *Nature* **528**, 555–559 (2015). **Together with reference 12, this article reports the discovery of complete nitrification by a single microorganism.**
14. Konneke, M. et al. Isolation of an autotrophic ammonia-oxidizing marine archaeon. *Nature* **437**, 543–546 (2005). **The discovery of ammonia-oxidizing archaea.**
15. Risgaard-Petersen, N. et al. Evidence for complete denitrification in a benthic foraminifer. *Nature* **443**, 93–96 (2006).
16. Thompson, A. W. et al. Unicellular cyanobacterium symbiotic with a single-celled eukaryotic alga. *Science* **337**, 1546–1550 (2012). **Shows that the ubiquitous unicellular cyanobacterium UCYN-A forms a nitrogen-fixing symbiosis with an algae.**
17. Eady, R. R. Structure-function relationships of alternative nitrogenases. *Chem. Rev.* **96**, 3013–3030 (1996).
18. Zehr, J. P., Jenkins, B. D., Short, S. M. & Steward, G. F. Nitrogenase gene diversity and microbial community structure: a cross-system comparison. *Environ. Microbiol.* **5**, 539–554 (2003).
19. Vitousek, P. M. & Howarth, R. W. Nitrogen limitation on land and in the sea: How can it occur? *Biogeochemistry* **13**, 87–115 (1991).
20. Bothe, H., Schmitz, O., Yates, M. G. & Newton, W. E. Nitrogen Fixation and Hydrogen Metabolism in Cyanobacteria. *Microbiol. Mol. Biol. Rev.* **74**, 529–551 (2010).
21. Robson, R. L. & Postgate, J. R. Oxygen and hydrogen in biological nitrogen fixation. *Annu. Rev. Microbiol.* **34**, 183–207 (1980).
22. Berman-Frank, I., Lundgren, P. & Falkowski, P. Nitrogen fixation and photosynthetic oxygen evolution in cyanobacteria. *Res. Microbiol.* **154**, 157–164 (2003).
23. Inomura, K., Bragg, J. & Follows, M. J. A quantitative analysis of the direct and indirect costs of nitrogen fixation: a model based on Azotobacter vinelandii. *ISME J.* **11**, 166–175 (2017).
24. MacKellar, D. et al. Streptomyces thermoautotrophicus does not fix nitrogen. *Sci. Rep.* **6**, 20086 (2016).
25. Martinez-Perez, C. et al. The small unicellular diazotrophic symbiont, UCYN-A, is a key player in the marine nitrogen cycle. *Nat. Microbiol.* **1**, 16163 (2016).
26. Brune, A. Symbiotic digestion of lignocellulose in termite guts. *Nat. Rev. Microbiol.* **12**, 168–180 (2014).
27. Lechene, C. P., Luyten, Y., McMahon, G. & Distel, D. L. Quantitative imaging of nitrogen fixation by individual bacteria within animal cells. *Science* **317**, 1563–1566 (2007).
28. Burris, R. H. & Roberts, G. Biological nitrogen fixation. *Annu. Rev. Nutr.* **13**, 317–335 (1993).
29. Hooper, A. B., Vannelli, T., Bergmann, D. J. & Arciero, D. M. Enzymology of the oxidation of ammonia to nitrite by bacteria. *Antonie Leeuwenhoek* **71**, 59–67 (1997).
30. Arp, D. J. & Stein, L. Y. Metabolism of inorganic N compounds by ammonia-oxidizing bacteria. *Crit. Rev. Biochem. Mol. Biol.* **38**, 471–495 (2003).
31. Prosser, J. I. & Nicol, G. W. Relative contributions of archaea and bacteria to aerobic ammonia oxidation in the environment. *Environ. Microbiol.* **10**, 2931–2941 (2008).
32. Wuchter, C. et al. Archaeal nitrification in the ocean. *Proc. Natl Acad. Sci. USA* **103**, 12317–12322 (2006).
33. Francis, C. A., Roberts, K. J., Beman, J. M., Santoro, A. E. & Oakley, B. B. Ubiquity and diversity of ammonia-oxidizing archaea in water columns and sediments of the ocean. *Proc. Natl Acad. Sci. USA* **102**, 14683–14688 (2005). **Shows that ammonia-oxidizing archaea are ubiquitous in the oceans.**
34. Leininger, S. et al. Archaea predominate among ammonia-oxidizing prokaryotes in soils. *Nature* **442**, 806–809 (2006). **Shows that archaea are major ammonia oxidizers in soils.**
35. Lehtovirta-Morley, L. E., Stoecker, K., Vilcinskas, A., Prosser, J. I. & Nicol, G. W. Cultivation of an obligate acidophilic ammonia oxidizer from a nitrifying acid soil. *Proc. Natl Acad. Sci. USA* **108**, 15892–15897 (2011). **The discovery of an acidophilic ammonia oxidizer.**
36. Tourna, M. et al. Nitrososphaera viennensis, an ammonia oxidizing archaeon from soil. *Proc. Natl Acad. Sci. USA* **108**, 8420–8425 (2011).
37. Burton, S. A. & Prosser, J. I. Autotrophic ammonia oxidation at low pH through urea hydrolysis. *Appl. Environ. Microbiol.* **67**, 2952–2957 (2001).
38. Palatinszky, M. et al. Cyanate as an energy source for nitrifiers. *Nature* **524**, 105–108 (2015).
39. Kits, K. D. et al. Kinetic analysis of a complete nitrifier reveals an oligotrophic lifestyle. *Nature* **549**, 269–272 (2017).
40. Hakemian, A. S. & Rosenzweig, A. C. The biochemistry of methane oxidation. *Annu. Rev. Biochem.* **76**, 223–241 (2007).
41. Stein, L. Y. & Klotz, M. G. Nitrifying and denitrifying pathways of methanotrophic bacteria. *Biochem. Soc. Trans.* **39**, 1826–1831 (2011).
42. Stoecker, K. et al. Cohn's Crenothrix is a filamentous methane oxidizer with an unusual methane monooxygenase. *Proc. Natl Acad. Sci. USA* **103**, 2363–2367 (2006).
43. Oswald, K. et al. Crenothrix are major methane consumers in stratified lakes. *ISME J.* **11**, 2124–2140 (2017).
44. Simon, J. & Klotz, M. G. Diversity and evolution of bioenergetic systems involved in microbial nitrogen compound transformations. *Biochim. Biophys. Acta* **1827**, 114–135 (2013). **An extensive overview of enzymes involved in microbial nitrogen transformations.**
45. Kozlowski, J. A., Stieglmeier, M., Schleper, C., Klotz, M. G. & Stein, L. Y. Pathways and key intermediates required for obligate aerobic ammonia-dependent chemolithotrophy in bacteria and Thaumarchaeota. *ISME J.* **10**, 1836–1845 (2016).
46. Kartal, B. et al. How to make a living from anaerobic ammonium oxidation. *FEMS Microbiol. Rev.* **37**, 428–461 (2013).
47. Ren, T., Roy, R. & Knowles, R. Production and consumption of nitric oxide by three methanotrophic bacteria. *Appl. Environ. Microbiol.* **66**, 3891–3897 (2000).
48. Nyerges, G. & Stein, L. Y. Ammonia cometabolism and product inhibition vary considerably among species of methanotrophic bacteria. *FEMS Microbiol. Lett.* **297**, 131–136 (2009).
49. Schott, J., Griffin, B. M. & Schink, B. Anaerobic phototrophic nitrite oxidation by Thiocapsa sp. strain KS1 and Rhodopseudomonas sp. strain LQ17. *Microbiology* **156**, 2428–2437 (2010).
50. Strous, M. et al. Deciphering the evolution and metabolism of an anammox bacterium from a community genome. *Nature* **440**, 790–794 (2006).
51. Strous, M. et al. Missing lithotroph identified as new planctomycete. *Nature* **400**, 446–449 (1999). **The discovery of anaerobic ammonium-oxidizing bacteria.**
52. Koch, H. et al. Growth of nitrite-oxidizing bacteria by aerobic hydrogen oxidation. *Science* **345**, 1052–1054 (2014).
53. Koch, H. et al. Expanded metabolic versatility of ubiquitous nitrite-oxidizing bacteria from the genus Nitrospira. *Proc. Natl Acad. Sci. USA* **112**, 11371–11376 (2015).
54. Maia, L. B. & Moura, J. J. How biology handles nitrite. *Chem. Rev.* **114**, 5273–5357 (2014).
55. Pachiadaki, M. G. et al. Major role of nitrite-oxidizing bacteria in dark ocean carbon fixation. *Science* **358**, 1046–1051 (2017).
56. Philippot, L., Hallin, S. & Schloter, M. Ecology of denitrifying prokaryotes in agricultural soil. *Adv. Agronomy* **96**, 249–305 (2007).
57. Lam, P. & Kuypers, M. M. Microbial nitrogen-cycling processes in oxygen minimum zones. *Annu. Rev. Mar. Sci.* **3**, 317–345 (2011).
58. Kraft, B. et al. The environmental controls that govern the end product of bacterial nitrate respiration. *Science* **345**, 676–679 (2014).
59. Lundberg, J. O., Weitzberg, E. & Gladwin, M. T. The nitrate-nitrite-nitric oxide pathway in physiology and therapeutics. *Nat. Rev. Drug Discov.* **7**, 156–167 (2008).
60. Moreno-Vivian, C., Cabello, P., Martinez-Luque, M., Blasco, R. & Castillo, F. Prokaryotic nitrate reduction: molecular properties and functional distinction among bacterial nitrate reductases. *J. Bacteriol.* **181**, 6573–6584 (1999).
61. Preisler, A. et al. Biological and chemical sulfide oxidation in a Beggiatoa inhabited marine sediment. *ISME J.* **1**, 341–353 (2007).
62. Tsementzi, D. et al. SAR11 bacteria linked to ocean anoxia and nitrogen loss. *Nature* **536**, 179–183 (2016).
63. Lam, P. et al. Revising the nitrogen cycle in the Peruvian oxygen minimum zone. *Proc. Natl Acad. Sci. USA* **106**, 4752–4757 (2009).
64. Bristow, L. A. et al. N2 production rates limited by nitrite availability in the Bay of Bengal oxygen minimum zone. *Nat. Geosci.* **10**, 24–29 (2017).
65. Zumft, W. G. Cell biology and molecular basis of denitrification. *Microbiol. Mol. Biol. Rev.* **61**, 533–616 (1997).
66. Haroon, M. F. et al. Anaerobic oxidation of methane coupled to nitrate reduction in a novel archaeal lineage. *Nature* **500**, 567–570 (2013). **The discovery of nitrate-reducing methanotrophic archaea.**
67. Ettwig, K. F. et al. Archaea catalyze iron-dependent anaerobic oxidation of methane. *Proc. Natl Acad. Sci. USA* **113**, 12792–12796 (2016).
68. Cardoso, R. B. et al. Sulfide oxidation under chemolithoautotrophic denitrifying conditions. *Biotechnol. Bioengineer.* **95**, 1148–1157 (2006).
69. Weber, K. A., Achenbach, L. A. & Coates, J. D. Microorganisms pumping iron: anaerobic microbial iron oxidation and reduction. *Nat. Rev. Microbiol.* **4**, 752–764 (2006).
70. Gruber, N. The marine nitrogen cycle: overview and challenges. *Nitrogen Marine Environ.* **2**, 1–50 (2008).
71. Stolz, J. F. & Basu, P. Evolution of nitrate reductase: molecular and structural variations on a common function. *Chembiochem* **3**, 198–206 (2002).
72. Malm, S. et al. The roles of the nitrate reductase NarGHJI, the nitrite reductase NirBD and the response regulator GlnR in nitrate assimilation of *Mycobacterium tuberculosis*. *Microbiology* **155**, 1332–1339 (2009).
73. Blöchl, E. et al. Pyrolobus fumarii, gen. and sp. nov., represents a novel group of archaea, extending the upper temperature limit for life to 113 C. *Extremophiles* **1**, 14–21 (1997).
74. Kamp, A., de Beer, D., Nitsch, J. L., Lavik, G. & Stief, P. Diatoms respire nitrate to survive dark and anoxic conditions. *Proc. Natl Acad. Sci. USA* **108**, 5649–5654 (2011).
75. Zhou, Z. et al. Ammonia fermentation, a novel anoxic metabolism of nitrate by fungi. *J. Biol. Chem.* **277**, 1892–1896 (2002).

76. Tikhonova, T. V. et al. Molecular and catalytic properties of a novel cytochrome *c* nitrite reductase from nitrate-reducing haloalkaliphilic sulfur-oxidizing bacterium Thioalkalivibrio nitratireducens. *Biochim. Biophys. Acta* **1764**, 715–723 (2006).
77. Atkinson, S. J., Mowat, C. G., Reid, G. A. & Chapman, S. K. An octaheme c-type cytochrome from Shewanella oneidensis can reduce nitrite and hydroxylamine. *FEBS Lett.* **581**, 3805–3808 (2007).
78. Einsle, O. et al. Structure of cytochrome *c* nitrite reductase. *Nature* **400**, 476–480 (1999).
79. Haase, D., Hermann, B., Einsle, O. & Simon, J. Epsilonproteobacterial hydroxylamine oxidoreductase (εHao): characterization of a 'missing link' in the multihaem cytochrome *c* family. *Mol. Microbiol.* **105**, 127–138 (2017).
80. Tiedje, J. M. in in *Biology of Anaerobic Microorganisms* (ed. Zehnder, A. J. B.) 179–244 (Wiley, New York, NY, USA, 1988).
81. Brunet, R. & Garcia-Gil, L. Sulfide-induced dissimilatory nitrate reduction to ammonia in anaerobic freshwater sediments. *FEMS Microbiol. Ecol.* **21**, 131–138 (1996).
82. Seitz, H.-J. & Cypionka, H. Chemolithotrophic growth of Desulfovibrio desulfuricans with hydrogen coupled to ammonification of nitrate or nitrite. *Arch. Microbiol.* **146**, 63–67 (1986).
83. Robertson, E. K., Roberts, K. L., Burdorf, L. D. W., Cook, P. & Thamdrup, B. Dissimilatory nitrate reduction to ammonium coupled to Fe(II) oxidation in sediments of a periodically hypoxic estuary. *Limnol. Oceanogr.* **61**, 365–381 (2016).
84. Rütting, T., Boeckx, P., Müller, C. & Klemedtsson, L. Assessment of the importance of dissimilatory nitrate reduction to ammonium for the terrestrial nitrogen cycle. *Biogeosciences* **8**, 1779–1791 (2011).
85. Burgin, A. J. & Hamilton, S. K. Have we overemphasized the role of denitrification in aquatic ecosystems? A review of nitrate removal pathways. *Front. Ecol. Environ.* **5**, 89–96 (2007).
86. Lomas, M. W. & Lipschultz, F. Forming the primary nitrite maximum: nitrifiers or phytoplankton? *Limnol. Oceanogr.* **51**, 2453–2467 (2006).
87. Graf, D. R., Jones, C. M. & Hallin, S. Intergenomic comparisons highlight modularity of the denitrification pathway and underpin the importance of community structure for N_2O emissions. *PloS ONE* **9**, e114118 (2014).
88. Bartossek, R., Nicol, G. W., Lanzen, A., Klenk, H. P. & Schleper, C. Homologues of nitrite reductases in ammonia-oxidizing archaea: diversity and genomic context. *Environ. Microbiol.* **12**, 1075–1088 (2010).
89. Kartal, B. & Keltjens, J. T. Anammox biochemistry: a tale of heme c proteins. *Trends Biochem. Sci.* **41**, 998–1011 (2016).
90. Fang, F. C. Antimicrobial reactive oxygen and nitrogen species: concepts and controversies. *Nat. Rev. Microbiol.* **2**, 820–832 (2004).
91. Hallin, S., Philippot, L., Löffler, F. E., Sanford, R. A. & Jones, C. M. Genomics and ecology of novel N_2O-reducing microorganisms. *Trends Microbiol.* **26**, 43–55 (2018).
92. Ravishankara, A. R., Daniel, J. S. & Portmann, R. W. Nitrous Oxide (N_2O): the dominant ozone-depleting substance emitted in the 21st century. *Science* **326**, 123–125 (2009).
93. Saraiva, L. M., Vicente, J. B. & Teixeira, M. The role of the flavodiiron proteins in microbial nitric oxide detoxification. *Adv. Microb. Physiol.* **49**, 77–129 (2004).
94. Rodrigues, R. et al. Desulfovibrio gigas flavodiiron protein affords protection against nitrosative stress in vivo. *J. Bacteriol.* **188**, 2745–2751 (2006).
95. Shoun, H., Fushinobu, S., Jiang, L., Kim, S.-W. & Wakagi, T. Fungal denitrification and nitric oxide reductase cytochrome P450nor. *Phil. Trans. R. Soc. B Biol Sci.* **367**, 1186–1194 (2012).
96. Wang, J. et al. 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 (2016).
97. Hino, T. et al. Structural basis of biological N_2O generation by bacterial nitric oxide reductase. *Science* **330**, 1666–1670 (2010).
98. Matsumoto, Y. et al. Crystal structure of quinol-dependent nitric oxide reductase from Geobacillus stearothermophilus. *Nat. Struct. Mol. Biol.* **19**, 238–245 (2012).
99. Al-Attar, S. & de Vries, S. An electrogenic nitric oxide reductase. *FEBS Lett.* **589**, 2050–2057 (2015).
100. Liu, S. et al. Abiotic conversion of extracellular NH2OH contributes to N2O emission during ammonia oxidation. *Environ. Sci. Technol.* **51**, 13122–13132 (2017).
101. Stocker, T. F. et al. *IPCC, 2013: Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change* (Cambridge Univ. Press, 2013).
102. Davidson, E. A. The contribution of manure and fertilizer nitrogen to atmospheric nitrous oxide since 1860. *Nat. Geosci.* **2**, 659–662 (2009).
103. Crutzen, P., Mosier, A., Smith, K. & Winiwarter, W. N_2O release from agro-biofuel production negates global warming reduction by replacing fossil fuels. *Atmos. Chem. Phys.* **8**, 389–395 (2008).
104. Ettwig, K. F. et al. Bacterial oxygen production in the dark. *Front. Microbiol.* **3**, 273 (2012).
105. Zumft, W. G. & Kroneck, P. M. Respiratory transformation of nitrous oxide (N2O) to dinitrogen by Bacteria and Archaea. *Adv. Microb. Physiol.* **52**, 107–227 (2006).
106. Cabello, P., Roldan, M. D. & Moreno-Vivian, C. Nitrate reduction and the nitrogen cycle in archaea. *Microbiology* **150**, 3527–3546 (2004).
107. Simon, J., Einsle, O., Kroneck, P. M. & Zumft, W. G. The unprecedented nos gene cluster of Wolinella succinogenes encodes a novel respiratory electron transfer pathway to cytochrome *c* nitrous oxide reductase. *FEBS Lett.* **569**, 7–12 (2004).
108. Sanford, R. A. et al. Unexpected nondenitrifier nitrous oxide reductase gene diversity and abundance in soils. *Proc. Natl Acad. Sci. USA* **109**, 19709–19714 (2012).
109. Jones, C. M. et al. Recently identified microbial guild mediates soil N2O sink capacity. *Nat. Climate Change* **4**, 801–805 (2014).
110. Piña-Ochoa, E. et al. Widespread occurrence of nitrate storage and denitrification among Foraminifera and Gromiida. *Proc. Natl Acad. Sci. USA* **107**, 1148–1153 (2010).
111. Philippot, L., Andert, J., Jones, C. M., Bru, D. & Hallin, S. Importance of denitrifiers lacking the genes encoding the nitrous oxide reductase for N2O emissions from soil. *Global Change Biol.* **17**, 1497–1504 (2011).
112. Codispoti, L. & Christensen, J. Nitrification, denitrification and nitrous oxide cycling in the eastern tropical South Pacific Ocean. *Mar. Chem.* **16**, 277–300 (1985).
113. Van de Graaf, A. A., de Bruijn, P., Robertson, L. A., Jetten, M. S. & Kuenen, J. G. Autotrophic growth of anaerobic ammonium-oxidizing micro-organisms in a fluidized bed reactor. *Microbiology* **142**, 2187–2196 (1996).
114. Mulder, A., Vandegraaf, A. A., Robertson, L. A. & Kuenen, J. G. Anaerobic ammonium oxidation discovered in a denitrifying fluidized bed reactor. *FEMS Microbiol. Ecol.* **16**, 177–183 (1995).
The discovery of anaerobic ammonium oxidation.
115. Dietl, A. et al. The inner workings of the hydrazine synthase multiprotein complex. *Nature* **527**, 394–397 (2015).
116. Jetten, M. S. M., den Camp, H. J. M. O., Gijs Kuenen, J. & Strous, M. in *Bergey's Manual of Systematics of Archaea and Bacteria* (ed. Whitman, W. B.) https://doi.org/10.1002/9781118960608.fbm00160 (John Wiley & Sons, Ltd, 2015).
117. Harhangi, H. R. et al. Hydrazine synthase, a unique phylomarker with which to study the presence and biodiversity of anammox bacteria. *Appl. Environ. Microbiol.* **78**, 752–758 (2012).
118. Wang, Y. et al. Co-occurrence and distribution of nitrite-dependent anaerobic ammonium and methane-oxidizing bacteria in a paddy soil. *Fems Microbiol. Lett.* **336**, 79–88 (2012).
119. Maalcke, W. J. et al. Characterization of anammox hydrazine dehydrogenase, a key N2-producing enzyme in the global nitrogen cycle. *J. Biol. Chem.* **291**, 17077–17092 (2016).
120. Neumann, S. et al. Isolation and characterization of a prokaryotic cell organelle from the anammox bacterium Kuenenia stuttgartiensis. *Mol. Microbiol.* **94**, 794–802 (2014).
121. de Almeida, N. M. et al. Membrane-bound electron transport systems of an anammox bacterium: a complexome analysis. *Biochim. Biophys. Acta* **1857**, 1694–1704 (2016).
122. de Almeida, N. M. et al. Immunogold localization of key metabolic enzymes in the anammoxosome and on the tubule-like structures of Kuenenia stuttgartiensis. *J. Bacteriol.* **197**, 2432–2441 (2015).
123. Kuypers, M. M. et al. Anaerobic ammonium oxidation by anammox bacteria in the Black Sea. *Nature* **422**, 608–611 (2003).
The first detection of anaerobic ammonium-oxidizing bacteria in the environment.
124. Kuypers, M. M. M. et al. Massive nitrogen loss from the Benguela upwelling system through anaerobic ammonium oxidation. *Proc. Natl Acad. Sci. USA* **102**, 6478–6483 (2005).
125. Devol, A. H. Nitrogen cycle: Solution to a marine mystery. *Nature* **422**, 575–576 (2003).
126. Zhu, G. et al. Anaerobic ammonia oxidation in a fertilized paddy soil. *ISME J.* **5**, 1905–1912 (2011).
127. Bronk, D. A. & Steinberg, D. K. in *Nitrogen in the in Marine Environment* 2nd edn 385–467 (Academic Press, San Diego, 2008).
128. Sunagawa, S. et al. Structure and function of the global ocean microbiome. *Science* **348**, 1261359 (2015).
129. Gruber, N. & Galloway, J. N. An Earth-system perspective of the global nitrogen cycle. *Nature* **451**, 293–296 (2008).
130. Vaksmaa, A. et al. Enrichment of anaerobic nitrate-dependent methanotrophic 'Candidatus Methanoperedens nitroreducens' archaea from an Italian paddy field soil. *Appl. Microbiol. Biotechnol.* **101**, 7075–7084 (2017).
131. Martens-Habbena, W., Berube, P. M., Urakawa, H., de la Torre, J. R. & Stahl, D. A. Ammonia oxidation kinetics determine niche separation of nitrifying Archaea and Bacteria. *Nature* **461**, 976–979 (2009).
132. Schleper, C. & Nicol, G. W. Ammonia-oxidising archaea — physiology, ecology and evolution. *Adv. Microb. Physiol.* **57**, 41 (2010).
133. Pjevac, P. et al. AmoA-targeted polymerase chain reaction primers for the specific detection and quantification of comammox Nitrospira in the environment. *Front. Microbiol.* **8**, 1508 (2017).
134. Oshiki, M. et al. Nitrate-dependent ferrous iron oxidation by anaerobic ammonium oxidation (anammox) bacteria. *Appl. Environ. Microbiol.* **79**, 4087–4093 (2013).
135. Ferousi, C. et al. Iron assimilation and utilization in anaerobic ammonium oxidizing bacteria. *Curr. Opin. Chem. Biol.* **37**, 129–136 (2017).
136. Jensen, M. M. et al. Intensive nitrogen loss over the Omani Shelf due to anammox coupled with dissimilatory nitrite reduction to ammonium. *ISME J.* **5**, 1660–1670 (2011).
137. Jones, C. M., Graf, D. R. H., Bru, D., Philippot, L. & Hallin, S. The unaccounted yet abundant nitrous oxide-reducing microbial community: a potential nitrous oxide sink. *ISME J.* **7**, 417–426 (2013).
138. Heylen, K. & Keltjens, J. Redundancy and modularity in membrane-associated dissimilatory nitrate reduction in Bacillus. *Front. Microbiol.* **3**, 371 (2012).
139. Canfield, D. E., Thamdrup, B. & Kristensen, E. *Aquatic Geomicrobiology* (Elsevier Academic Press, 2005).
140. Sutton, M. A. et al. *The European Nitrogen Assessment: Sources, Effects and Policy Perspectives* (Cambridge Univ. Press, 2011).
141. Canfield, D. E., Glazer, A. N. & Falkowski, P. G. The evolution and future of Earth's nitrogen cycle. *Science* **330**, 192–196 (2010).
142. Duce, R. A. et al. Impacts of atmospheric anthropogenic nitrogen on the open ocean. *Science* **320**, 893–897 (2008).
143. Grosskopf, T. et al. Doubling of marine dinitrogen-fixation rates based on direct measurements. *Nature* **488**, 361–364 (2012).
144. McGroddy, M. E., Daufresne, T. & Hedin, L. O. Scaling of C:N:P stoichiometry in forests worldwide: implications of terrestrial redfield-type ratios. *Ecology* **85**, 2390–2401 (2004).
145. Rittmann, B. E. & McCarty, P. L. *Environmental Biotechnology: Principles and Applications* (Tata McGraw-Hill Education, 2012).
146. Kartal, B., Kuenen, J.v. & Van Loosdrecht, M. Sewage treatment with anammox. *Science* **328**, 702–703 (2010).
147. Lackner, S. et al. Full-scale partial nitritation/anammox experiences — an application survey. *Water Res.* **55**, 292–303 (2014).
148. Park, H.-D., Wells, G. F., Bae, H., Criddle, C. S. & Francis, C. A. Occurrence of ammonia-oxidizing archaea in wastewater treatment plant bioreactors. *Appl. Environ. Microbiol.* **72**, 5643–5647 (2006).

149. Luesken, F. A. et al. Simultaneous nitrite-dependent anaerobic methane and ammonium oxidation processes. *Appl. Environ. Microbiol.* **77**, 6802–6807 (2011).
150. Starkenburg, S. R., Arp, D. J. & Bottomley, P. J. Expression of a putative nitrite reductase and the reversible inhibition of nitrite-dependent respiration by nitric oxide in Nitrobacter winogradskyi Nb-255. *Environ. Microbiol.* **10**, 3036–3042 (2008).
151. Freitag, A. & Bock, E. Energy conservation in Nitrobacter. *FEMS Microbiol. Lett.* **66**, 157–162 (1990).
152. Wijma, H. J., Canters, G. W., de Vries, S. & Verbeet, M. P. Bidirectional catalysis by copper-containing nitrite reductase. *Biochemistry* **43**, 10467–10474 (2004).
153. Sousa, F. L. et al. The superfamily of heme–copper oxygen reductases: types and evolutionary considerations. *Biochim. Biophys. Acta* **1817**, 629–637 (2012).
154. Rothery, R. A., Workun, G. J. & Weiner, J. H. The prokaryotic complex iron–sulfur molybdoenzyme family. *Biochim. Biophys. Acta* **1778**, 1897–1929 (2008).
155. Ishii, S., Ikeda, S., Minamisawa, K. & Senoo, K. Nitrogen cycling in rice paddy environments: past achievements and future challenges. *Microbes Environ.* **26**, 282–292 (2011).

Acknowledgements
The authors thank W. Mohr and J. Milucka (Max Planck Institute for Marine Microbiology, Bremen, Germany) for discussions. This work was supported by the Max Planck Society (MPG) and the European Research Council Grant 640422 to B.K.

Competing interests
The authors declare no competing financial interests.

Author contributions
M.M.M.K., H.K.M. and B.K. researched data for the article, made substantial contributions to discussions of the content, wrote the article and reviewed and edited the manuscript before submission.

Publisher's note
Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Reviewer information
Nature Reviews Microbiology thanks Holger Daims, Sarah Hallin and the other anonymous reviewer(s) for their contribution to the peer review of this work.