Marcus A. Horn and Stefanie A. Hetz

# 4 Microbial nitrogen cycling in permafrost soils: implications for atmospheric chemistry

## 4.1 Introduction

Nitrogen (N) is the most abundant element in the atmosphere and a major constituent of the earth's crust. It is an essential macronutrient required by all organisms, a critical component of cellular compounds such as proteins and nucleic acids, and thus fundamental to the structures and biochemical processes that define life [1, 2]. Life takes an active part in the N-cycle; it is tightly coupled to transformations of reactive N compounds that include ammonia ($NH_3$), nitrous acid (HONO or $HNO_2$), nitrogen oxides ($NO_x$), and nitrous oxide ($N_2O$), all impacting atmospheric chemistry and climate change [3–6]. $NH_3$ is volatilized from soils, associated with the formation of particulate matter in the atmosphere, and contributes to the atmospheric deposition of reactive nitrogen [7]. Hydroxyl radicals ($OH^*$) represent a major oxidizing agent of the atmosphere, and reaction with $OH^*$ represents the most important methane ($CH_4$) sink in the troposphere [8, 9]. $NH_3$ might compete with $CH_4$ for $OH^*$, thus affecting $CH_4$ oxidation in the atmosphere [7]. Volatilized protonated soil nitrite ($NO_2^-$) gives rise to atmospheric HONO, a source of $OH^*$ in the atmosphere [10–12]. Nitric oxide (NO) reacts in the troposphere with ozone to nitrogen dioxide ($NO_2$) that is prone to photolysis. When water is available, nitrous acid (HONO) and, together with hydroxyl radicals, also nitric acid ($HNO_3$) can be formed [13]. $N_2O$ is one of the long-lived greenhouse gases, the third most important greenhouse gas on earth, and a major ozone depleting substance in the atmosphere [3, 4]. The global warming potential of $N_2O$ is 300-fold higher than that of carbon dioxide ($CO_2$) on a 100-year basis, and the atmospheric concentration of $N_2O$ increased from 270 ppb to 319 ppb from 1750 to 2005 [3, 14]. An increase of approximately 7% per decade is projected for $N_2O$ in the absence of mitigation efforts [15]. Long-lived greenhouse gases such as $N_2O$ are projected to become the dominant determinants of global mean temperature changes [3, 16]. 60–70% of the global annual emissions of $N_2O$ are soil derived [16–18]. Agricultural and pristine tropical soils and even drylands are recognized sources of $N_2O$, while the importance of arctic peatlands and permafrost-affected soils as sources of $N_2O$ is just emerging (e.g. [16, 19–22]) (Tab. 4.1). Permafrost-affected soils might even represent "hot-spots" for $N_2O$ emissions that emit $N_2O$ in the range of heavily fertilized agricultural soils in temperate zones [22] (Tab. 4.1). Available studies suggest that permafrost environments likewise represent sources of $NO_x$ and HONO, although considered of minor importance for global budgets [23] (Tab. 4.2). Thus, the terrestrial N-cycle in permafrost-affected soils has implications for atmospheric chemistry and climate change.

https://doi.org/10.1515/9783110497083-004

**Tab. 4.1:** $N_2O$ emission potentials and field measurements for representative sites in permafrost-affected regions.

| Laboratory-based emission potential | *In situ* emission [µg N $m^{-2}$ $h^{-1}$] | Soil moisture/ water content (mean) | pH | C/N ratio | Site descriptor | | Region | Coordinates | Reference |
|---|---|---|---|---|---|---|---|---|---|
| 0.63–1.54 µg N $g_{DW}^{-1}$ $h^{-1}$ [b] | 50.38–8.22 × $10^{2}$ [a,b,c] | 0.64 $dm^3$ $dm^{-3}$ VWC[d] | 4 | 21–24[d] | Cryoturbated peat circle | Tundra | Seida/Vorkuta, Russia | 67°03'N, 62°57'E | [42] |
| 1.46 × $10^{-4}$–5.83 × $10^{-4}$ µg N $g_{DW}^{-1}$ $h^{-1}$[b] | −0.56–0.28[b] | 74% SMC | 4.2–4.6 | 26–29 | Vegetated palsa peat | Peat | Northwestern Finnish Lapland | 69°49'13''N, 27°9'47''E | [214] |
| n.a. | 26.25 (mean)[b]<br>1.06–90.15[b] | 62–94% SMC | 3.2 | 22–23 | Bare peat | Tundra | Seida/Vorkuta, Russia | 67°03'N, 62°57'E | [24] |
| 5.30 µg N $m^{-2}$ $h^{-1}$ (mean)[b] | n.a. | 66–95% WFPS | 3.7–4.4 | 23–33 | Vegetated palsa mire | Peat | Finnish Lapland | 68°89'N, 21°05'EW | [36] |
| 74.51 µg N $m^{-2}$ $h^{-1}$ (mean)[b] | | 66–97% WFPS | 3.4–4.7 | 28–39 | Unvegetated palsa mire | | | | |
| n.a. | 16.44 (mean)[b]<br>3.18 (mean)[b] | 7–38% SMC | 4–5.6 | 30–40 | Vegetated peat<br>Cryoturbated, bare peat | Tundra | Seida/Vorkuta, Russia | 67°03'N, 62°55'E | [37] |
| n.a. | 7.72 (mean)[b]<br>3.21 (mean)[b] | 18.5–41.1% SMC<br>11.4–19.7% SMC | n.a. | n.a. | Trough<br>Interior | Ice-wedge polygon | Canadian High Arctic | 79°26'N, 90°46'W | [81] |

**Tab. 4.1** (continued)

| Laboratory-based emission potential | *In situ* emission [µg N $m^{-2}$ $h^{-1}$] | Soil moisture/ water content (mean) | pH | C/N ratio | Site descriptor | | Region | Coordinates | Reference |
|---|---|---|---|---|---|---|---|---|---|
| n.a. | 3.75 (mean)[b] | n.a. | 3.97 | n.a. | Bare palsa | Peat | Kevo, Finland | 69°48'N, 27°11'E | [173] |
| | −0.98 (mean)[b] | n.a. | 4.06 | n.a. | Vegetated palsa | | | | |
| | 18.79 (mean)[b] | 0.64 $dm^3$ $dm^{-3}$ VWC[d] | 3.86 | 23 | Bare peat | | Seida/Vorkuta, Russia | 67°03'N, 62°57'E | |
| | 1.34 (mean)[b] | 0.16 $dm^3$ $dm^{-3}$ VWC[d] | 3.75 | 59 | Vegetated peat | | | | |
| | 26.85 (mean)[b] | n.a. | 3.89 | n.a. | | | Taymyr, Russia | 74°00'N, 98°00'E | |
| | 4.41 (mean)[b] | n.a. | 3.93 | n.a. | Bare palsa Vegetated palsa | | | | |
| n.a. | 68.73–8.32 × $10^{2}$[a,b] | 14–78% WFPS | 3.3–4.8 | 22–78 | Peat and upland soil | Tundra | Seida/Vorkuta, Russia | 67°03'N, 62°57'E | [21] |
| | −15.91–4.35 × $10^{2}$[a,b] | 12–73% WFPS | 3.5–6.1 | 8–31 | Peat and upland soil | Palsa mire | Northern Finland | 69°35'N, 26°11'/12'E; 69°49'N, 27°10'E | |
| n.a. | −0.01–0.71[b] | 0.15–8.94 g $g^{-1}$ soil moisture | 6.7–7.5 | n.a. | Raised beach crest, lower foreslope, wet sedge meadow cryosols | Landscape zone | Canadian High Arctic | 75°40'N, 84°35'W | [167] |
| 1.40 × $10^{-4}$–6.72 × $10^{-5}$ µg N $g^{-1}$ $h^{-1}$ (mean)[b] | n.a. | 0.15–8.94 g $g^{-1}$ soil moisture | 6.7–7.5[c] | n.a. | Raised beach crest, lower foreslope, wet sedge meadow cryosols | Landscape zone | Canadian High Arctic | 75°40'N, 84°35'W | [168] |
| n.a. | 50.38–8.22 × $10^{2}$[a,b] | 0.15–0.65 $dm^3$ $dm^{-3}$ VWC | 3.1–4.9 | 21–94 | Peat, fen and upland soils | Tundra | Seida/Vorkuta, Russia | 67°03'N, 62°57'E | [22] |

**Tab. 4.1** (continued)

| Laboratory-based emission potential | *In situ* emission [μg N $m^{-2}$ $h^{-1}$] | Soil moisture/ water content (mean) | pH | C/N ratio | Site descriptor | | Region | Coordinates | Reference |
|---|---|---|---|---|---|---|---|---|---|
| n.a. | 58.33–124.62 (daily means)[b] | n.a. | n.a. | n.a. | Sedge, tussock tundra, open water, fresh water marsh | Landscape scale | Alaska | 69°48'N–70°24'N, 153°00'W–154°15'W | [359] |
| n.a. | 7.52–50.43[a,b] | n.a. | 4.8–5.2 | 12–20 | Swamp forests | n.a. | Northeast China | 122°06'–122°27'E, 53°17'–53°30'N | [360] |
| n.a. | −0.30–368.00[a,b] | 3–100% SMC[a] | 5.2–9.5 | 9–86 | Forrest and grassland | Taiga | Russia | 62°19'N, 129°30'E | [361] |
| n.a. | 0.69–82.20[b] | n.a. | 5.6 | n.a. | Swamp meadow | Alpine meadow | Tibetan Plateau | 37°28'N, 100°17'E | [38] |
| n.a. | −2.46–19.79[b] | 20–90% SMC | 4.5–5.8 | 14–17 | Dark brown forest soil | Forest | Northeast China | 53°17'–30'N, 1222°06'–27'E | [6] |
| n.a. | 2.63–4.81 (mean)[a,b] | 37.2–54.9% SMC | 4.2–6.7 | n.a. | Cryosols | Taiga | Canada | 66°22'N, 136°43'W 67°26'N, 133°45'W | [362] |
| n.a. | $8.18 \times 10^{2}$–$1.69 \times 10^{3}$[a,b] | 83.3–140.6% SMC | 5.4–8.0 | n.a. | Thermokarst soil | Thermokarst | Tibetan Plateau | 38.00°N, 100.91°E | [363] |
| n.a. | n.a. | 0.3–4.1 g $g^{-1}$ SMC | 5.5–6.6 | 13–20 | Cryosols (Gaspésie and Polygon) | Polar oasis | Canadian High Arctic (Truelove) | 75°33'N, 84°E 40'W | [364] |
| | −3.02 (mean)[b] | 0–6.7 g $g^{-1}$ SMC | 3.6–6.9 | 8–48 | Cryosols (Saguenay) | Tundra | Canadian Sub-Arctic (Churchill) | 58°45'N, 93°51'W | |
| | 16.13 (mean)[b] | 0–3.8 g $g^{-1}$ SMC | 3.2–4.6 | 19–40 | Cryosols (Dump, Buggy and Bear) | Esker | Canadian Low-Arctic (Daring Lake) | 64°E 50'N, 111° 38'W | |

**Tab. 4.1** (continued)

| Laboratory-based emission potential | *In situ* emission [µg N $m^{-2}$ $h^{-1}$] | Soil moisture/ water content (mean) | pH | C/N ratio | Site descriptor | | Region | Coordinates | Reference |
|---|---|---|---|---|---|---|---|---|---|
| n.a. | 0.57–8.79[b] | n.a. | 7.6–8.1 | n.a. | Alpine meadow | Alpine meadow | Tibetan Plateau | 43°43'43''N, 92°53'34''E | [365] |
| n.a. | −9.04–23.94[b] | n.a. | 4.1–5.1 | 15–25 | Shrub community | Peatland | Northeast China | 52°94'N, 122°86'E | [366] |
| 0.0–14 µg N $m^{-2}$ $h^{-1}$ | n.a. | 20–25 µg $g_{DW}^{-1}$ | n.a. | 22 | Dystric Regosol | Grassland | Eastern Finland | 63°09'N, 27°20'E | [367] |
| n.a. | 0.11 (mean)[b]<br>0.33 (max)[b] | n.a. | n.a. | n.a. | Ombrotrophic peatland (mosses, grasses, and dwarf scrub) | Tundra | Yukon Delta Wildlife Refuge, Alaska | 61°05.41'N, 162°00.92'W | [368] |
| n.a. | −2–0.6 | n.a. | 7.1 | 22 | Mosses and dwarf shrubs | Alpine heath | Abisko, North Sweden | 68°20'N, 20°51'E | [369]<br>[370] |
| <0.03 ng N $h^{-1}$ $g^{-1}$ | n.a. | n.a. | 5.5–6.4 | 7–20 | Forest tundra | Grawijka Creek catchment | Igarka (Russian Federation) | 67°29.9'N, 86°25.26'E | [371] |
| n.a. | <Detection limit<br><Detection limit<br><Detection limit | 200–400% of soil dry mass<br>400–700% of soil dry mass<br>100–200% of dry mass | 5.9<br>7.0<br>7.5 | 30<br>31<br>12 | Heath<br>Moss<br>Circle | Disturbance and vegetation gradient | Lake Torneträsk, Abisko, North Sweden | 68°19'N, 18°51'E | [372] |

**Tab. 4.1** (continued)

| Laboratory-based emission potential | *In situ* emission [μg N $m^{-2}$ $h^{-1}$] | Soil moisture/ water content (mean) | pH | C/N ratio | Site descriptor | | Region | Coordinates | Reference |
|---|---|---|---|---|---|---|---|---|---|
| n.a. | 0.036–8.57[b] | 292–716 g $H_2O$ $g_{soil}^{-1}$ (0–5 cm) | 5.0–6.7 (0–5 cm) | | Polygonized-peat plateau | Hudson bay area, plant community transect | Churchill, Manitoba, Canada | 58.73°N, 093.84°W | [373] |
| | 0.97–1.51[b] | 487–1040 g $H_2O$ $g_{soil}^{-1}$ (0–5 cm) | 5.9–7.3 (0–5 cm) | | Palsa fen | | | 58.64°N, 093.82°W | |
| | 0.11–0.40[b] | 389–1147 g $H_2O$ $g_{soil}^{-1}$ (0–5 cm) | 4.4–6.2 (0–5 cm) | | White Spruce/ Larch Forest | | | 58.66°N, 093.83°W | |

Please refer to the references provided for more details. Ranges were delineated from means by subtracting or adding standard deviations to provided means for upper and lower boundaries, respectively.

n.a. – not applicable, SMC – soil moisture content, WFPS – water-filled pore space, C/N – carbon to nitrogen ratio, VWC – volumetric water content.

[a] Data taken from Gao et al. 2019 [360].

[b] Data converted from original paper.

[c] Ma et al., 2007 [167].

[d] Repo et al., 2009 [22].

**Tab. 4.2:** Emission potentials, field measurements, and soil parameters for non-$N_2O$, N-cycle-derived trace gases with relevance for atmospheric chemistry.

| N-Gas | Laboratory-based emission potential | *In situ* emission range [µg N $m^{-2}$ $h^{-1}$] | Soil moisture/ water content (mean) | pH (mean) | C/N ratio (mean) | Site descriptor | | Region | Coordinates | Study |
|---|---|---|---|---|---|---|---|---|---|---|
| NO | 0.2–90.3 µg N $m^{-2}$ $h^{-1}$ | n.a. | 20–25 µg $g_{DW}^{-1}$ | n.a. | 22 | Dystric Regosol | Grassland | Eastern Finland | 63°09'N, 27°20'E | [367] |
| $NO_x$ | n.a. | 0.11 (mean)[a]; 0.33 (max)[a] | n.a. | n.a. | n.a. | Bog | Tundra | Alaska, North America | 61°05.41'N, 162°00.92'W | [24] [368] |
| HONO | 0–14 µg N $m^{-2}$ $h^{-1}$ | n.a. | 20–25 µg $g_{DW}^{-1}$ | n.a. | 22 | Dystric Regosol | Grassland | Eastern Finland | 63°09'N, 27°20'E | [367] |
| HONO | n.a. | –0.75–0.61[a] | 5.5 mg $kg^{-1}$ extractable water | 6.3 | n.a. | Snowpack | Snow and ice | European High Arctic, Svalbard | 78°55'N, 11°54'E | [374] |
| HONO | n.a. | –1.23–1.39[a] | n.a. | n.a. | n.a. | Snowpack | Snow and ice | Antarctica | 74°37'S, 163°56'E | [375] |

Data were compiled from literature and provided for representative sites in permafrost-affected regions.

n.a. – not available, C/N – carbon to nitrogen ratio, HONO – (abiotic) release of nitrous acid.

[a] Data converted from original paper.

Permafrost regions cover approximately 16–25% of the global soil surface area, include large peatland areas (up to 80% surface area in West Siberia), and are estimated to store 50% of the global below ground organic carbon representing potential electron donors for the generation of reactive N from less reactive N-species [25, 26]. High organic carbon content is correlated with high organic N content in northern peatlands [27], suggesting that permafrost soils are large N reservoirs. Indeed, a conservative estimate is that 67 Pg of N are stored in the upper 3 m [28]. Thus, permafrost nitrogen stocks are more than 500 times larger than the annual N loaded as fertilizer to soils globally [5, 29], with northern peatland soils alone storing approximately 10% of the global soil organic matter N (~8–15 Gt N) [30, 31]. Along these lines, estimates of global N stored in the upper 100 cm of soils worldwide are in the range of 140 Pg N [32]. Recent estimates of N stored in Yedoma permafrost alone are in the range of 40–60 Pg N, suggesting a massive underestimation of N stored in permafrost to date [33]. Only a small fraction of this N is currently bioavailable, which is in line with studies showing that N is the major limiting nutrient in Arctic soils [34, 35]. Anticipated permafrost thawing is expected to increase N mobilization and export of reactive N, thus alleviating N-limitation and fueling the N-cycle (Fig. 4.1). Thus, permafrost-affected soils have the potential to affect the global N-balance and accelerate global warming [21, 25]. Such an estimation was recently substantiated by *in situ* measurements showing

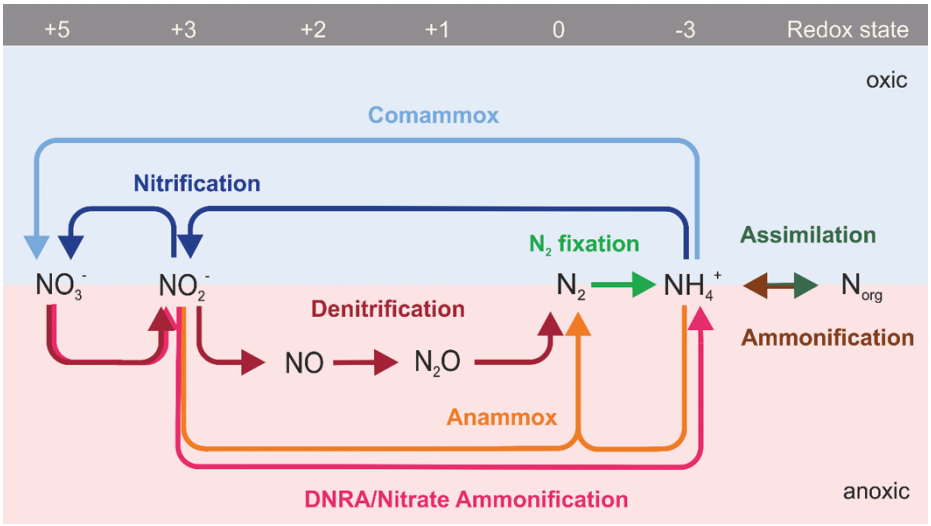


**Fig. 4.1:** The biological nitrogen cycle and microbial redox transformations. The oxidation state of N is given in white letters above the graph. Arrows indicate processes. Aerobic and anaerobic processes are indicated by a light blue and light red background, respectively. Reactions occurring under both oxic and anoxic conditions are presented at the interface. $NO_3^-$ – nitrate, $NO_2^-$ – nitrite, NO – nitric oxide, $N_2O$ – nitrous oxide, $N_2$ – dinitrogen gas, $NH_4^+$ – ammonium, $N_{org}$ – organic nitrogen, DNRA – dissimilatory nitrate reduction to ammonium or nitrate ammonification.

increased $N_2O$ emissions upon warming and permafrost thaw, highlighting the potential of N-cycle dependent impacts of permafrost soils for climate change [36–38].

Reaction rates of N-cycle processes and, thus, release of N-gases depend on the availability of reactive N. Input of reactive N occurs via atmospheric deposition, ground water infiltration, recycling of organic N by microbial mineralization, and biological nitrogen fixation (BNF). The latter two processes are most important for remote, pristine permafrost-affected soils with low atmospheric deposition. Under such boundary conditions, the availability of reactive N is primarily controlled by ammonification (mineralization of organic N) and N-fixation [39] (Fig. 4.1). The current understanding of the microbial redox N-cycle consists of five further N transformation processes that alter the oxidation state of nitrogen from –3 to +5 (Fig. 4.1): nitrification (including ammonium oxidation, nitrite oxidation, and comammox), nitrate dissimilation (including canonical denitrification, nitrifier-dependent and methane-oxidation-dependent denitrification, as well as nitrate ammonification), anammox, assimilation, and ammonification/mineralization. A huge versatility of N-dissimilating capabilities was found during the past decade of genomic data collection within single N-transforming microorganisms [2, 40]. In general, there are two main process categories: assimilation, i.e. the acquisition of N for incorporation into biomass, and dissimilation, i.e. processes that are associated with the conservation of energy in form of ATP [41]. It is common knowledge that essentially all living organisms (including plants) contribute to the assimilation of N into biomass. Thus, this book chapter will focus on BNF and dissimilatory processes of the N-cycle. Numerous strategies are available to identify key players of the N-cycle and associated process rates. “Metaomics” (i.e., metagenomics, -transcriptomics, -proteomics, and -metabolomics), traditional cultivation and isolation approaches, kinetic studies along with quantitative functional gene marker analyses, and the application of stable isotopes are some examples [42–44].

## 4.2 Microbial processes of the N-cycle

### 4.2.1 Biological nitrogen fixation (BNF) and associated organisms

Nitrogen fixation might be considered as the first process of the N-cycle and is the reaction of molecular dinitrogen ($N_2$) to ammonium ($NH_4^+$). Molecular dinitrogen ($N_2$) is the most prevalent N-compound in the atmosphere and chemically stable due to its high dissociation enthalpy of 945 kJ $mol^{-1}$ [45]. Thus, $N_2$ fixation is the most challenging reaction of the N-cycle in terms of activation energy. The only organisms capable of performing this energy demanding $N_2$ fixation reaction are diazotrophic prokaryotes of the Bacteria and Archaea [41]. Such BNF has been estimated to account for two-thirds of the annual global input of reactive N [39, 46]. BNF of 58 and 140 Tg N $yr^{-1}$ is estimated for terrestrial and marine systems, respectively. Atmospheric $N_2$ fixation associated with lightning is well known (e.g. [47]), although this naturally occurring

abiotic "N-fixation" is estimated to represent only 4 Tg N $yr^{-1}$ and thus makes small contributions relative to BNF [39, 48–52]. Hence, diazotrophic microorganisms play a key role in the terrestrial nitrogen cycle.

**Physiology and diversity of diazotrophs**

BNF is probably one of the oldest enzyme-catalyzed reactions that began to play a significant role when reactive inorganic nitrogen became scarce [53]. The ability to fix nitrogen is widely distributed in both bacteria and archaea, with these organisms having autotrophic, heterotrophic, chemolithotrophic, photo-heterotrophic, and methanogenic lifestyles [53, 54]. Microorganisms capable of BNF (diazotrophs) can either be free-living or symbiotic, e.g. associated with lichens and mosses [55, 56]. Diazotrophs depend on the oxygen sensitive nitrogenase metalloenzymes that catalyze the reduction of $N_2$ to ammonia ($NH_3$) with concomitant reduction of protons to molecular hydrogen [52, 57] (Equation 4.1).

$$N_2 + 8H^+ + 8e^- \xrightarrow{\text{NIF, VNF, ANF}} 2NH_3 + H_2 \tag{4.1}$$

NIF, VNF, and ANF represent molybdenum-, vanadium-, and iron-dependent nitrogenases, respectively.

The reaction requires a minimum of 16 ATP (adenosine triphosphate) per molecule $N_2$ and is one of the energetically most expensive processes in biology [58]. In the industrial conversion of $N_2$ to $NH_3$, i.e. the Haber-Bosch process, both high temperature and pressure are used in the presence of metal catalysts to combine $H_2$ and $N_2$ [57]. The enzyme complex consisting of nitrogenase and dinitrogenase reductase also utilizes metal catalysis for biological $N_2$ fixation. Nitrogenase accumulates electrons, which are donated from the dinitrogen reductase, and catalyzes the reduction of $N_2$ [57]. There are different classes of nitrogenases that differ in their catalytically active metal, catalytic rates, and efficiencies [59]. The metal content in the active site of these nitrogenases is either molybdenum (Mo), vanadium (V), or iron (Fe) [60, 61]. The molybdenum-nitrogenase is encoded by the *nif* regulon, the vanadium-nitrogenase by the *vnf* regulon, and the iron-only nitrogenase by the *anf* regulon [57]. The most efficient of the different nitrogenases in terms of specificity for $N_2$, energy requirement, and its reduction to $NH_3$ is the Nif, followed by Vnf and Anf [62, 63]. Despite these differences, the nitrogenase subtypes are structurally and phylogenetically related, with the Mo-dependent nitrogenase being the best studied and most prevalent nitrogenase [64, 65]. Its structural components are encoded by *nifHDK*, with the first dinitrogen-reductase encoding gene being commonly used as indicative of diazotrophs in molecular surveys [65]. The abundance and occurrence of the nitrogenase subtypes vary, with microorganisms harboring one or more of the different nitrogenases. *Azotobacter vinelandii*, a well-studied model organism,

contains all three types. To date, all isolated diazotrophs contain the *nif*-encoded molybdenum-dependent nitrogenase [57], which is also the most relevant nitrogenase catalyzing the majority of BNF. Nevertheless, in environments where Mo is scarce, the V- and Fe-nitrogenases are important alternatives for BNF [62]. Nitrogenases are also capable of reducing other triple- or double-bonded substrates, e.g. acetylene to ethylene, which in turn can be used to measure nitrogenase activity *in vivo* [57].

**Rates of BNF and associated diversity of diazotrophs in permafrost environments**

As in many other ecosystems, BNF is accepted to be the major source of nitrogen in terrestrial Arctic environments [66, 67] (Tab. 4.3), with the majority of BNF being carried out at the oxic soil-atmosphere interface by phototrophic cyanobacteria [68]. In contrast to temperate regions, BNF in the deeper soil profile of permafrost-affected environments by rhizospheric and free-living diazotrophic soil bacteria is thought to be of lesser importance [69–71]. Indeed, a metagenome study in the Nome Creek area, Alaska, demonstrated a decrease in relative abundance of BNF-associated genes from thawed upper to frozen lower layers [72]. In the High Arctic and subarctic, BNF by diazotrophs showed a strong moisture [68, 73, 74], temperature [75], and light [76] dependence. Such dependencies are common for both, the free-living [77] and symbiotic diazotrophs in the High Arctic [68]. In a metagenomic study with frozen permafrost soil cores from a black-spruce forest in Alaska, *nifH* represented a significant fraction of retrieved functional genes ($5 \times 10^5$–$3.5 \times 10^6$ per ng DNA) and decreased significantly after 7 days of thaw, demonstrating that diazotrophs are prevalent in permafrost and prone to respond to permafrost thaw [78]. Such a decrease in relative gene abundance in metagenomes might not necessarily correlate with a low transcriptional activity. Hultman et al. [79] showed that ratios of BNF-associated gene hits in metatranscriptomes vs. metagenomes were dramatically greater in the active layer than in the underlying permafrost, suggesting ongoing BNF at high rates in the active layer. Interestingly, most abundant hits of metaproteome data were indicative of putative diazotrophs like *Bradyrhizobium* and *Burkholderia*. The prevalence of diazotrophs in frozen permafrost soil and the viability of such permafrost diazotrophs are also shown by isolation techniques and the recovery of psychrophilic organisms from old permafrost (reviewed in [44]). In polygonal arctic tundra, *nifH* was abundant in metagenomes from high-, flat-, and low-centered polygons at two different depths representing organic and mineral soil [80]. Relative abundance data of *nifH* suggested the highest genetic potential for BNF in the organic soil from the low-centered polygons and a high BNF potential in the mineral soil of the flat-centered polygons. Such data demonstrate soil morphology-dependent differences in BNF potentials. A metatranscriptome study from the High Arctic of an $N_2O$-emitting site with 16S rRNA and *nifH* revealed distinct diazotrophic microbial communities from trough and polygon interior soils as well as a depth dependency [81]. *nifH* transcripts were primarily related to the classes Rhizobiales, Rhodobacterales, Desulfovibrionales,

and other Deltaproteobacteria related to Desulfobacterales or Desulfuromonadales, and Gallionelales [81]. 16S rRNA and *nifH* transcript sequences were related to the free-living diazotrophic genera *Azotobacter*, *Beijerinckia*, the cyanobacterial genus *Nostoc*, as well as symbiotic diazotrophs like *Frankia*, *Azorhizobium*, *Bradyrhizobium*, and *Rhizobium*, which further supports the presence of a rather diverse active microbial diazotrophic community in permafrost-affected soils [81]. Other studies with Arctic soil identified *nif* of Alphaproteobacteria as major diazotrophs as well as Betaproteobacteria and Cyanobacteria [66]. Cyanobacterial *Nostoc* spp. have been found in diverse Arctic environments and seem to be important diazotrophs that might live in associations with bryophytes [71, 73, 74, 82–85]. BNF by bryophytes varies between species; nevertheless, they account for a substantial part of reactive N input (2–58%) in certain Arctic ecosystems [71, 73, 83, 86–89]. Both biomass and high coverage of mosses in subarctic and arctic tundra support BNF that well exceeds N deposition [90, 91]. In a functional microarray study across an Antarctic latitudinal transect from the Falkland Islands to Coal Nunatak covering vegetated and unvegetated plots, *nifH* genes were among the five most abundant functional N-cycle-related genes in three out of eight sites [92]. *nifH* affiliated with Firmicutes (*Acetobacterium*) and many uncultured or unidentified bacteria, suggesting phylogenetic novelty. The abundance of *nifH* in Antarctic soils was supported by quantitative PCR (polymerase chain reaction) [93]. Such data demonstrate that the genetic BNF potentials of phylogenetically new and known diazotrophs are prevalent at Antarctic sites and widespread in permafrost-affected ecosystems. Rate measurements show that genetic potentials are expressed and provide an essential ecosystem function (Tab. 4.3).

A multiscale approach that estimated BNF during the growing season of different cyanobacterial associations and mapped the distribution of ecosystem types in a landscape study area estimated the nitrogen input via BNF of 0.68 kg N $ha^{-1}$ $yr^{-1}$ at a Low Arctic tundra region in Canada [73] (Tab. 4.3). Soils from the tropics, which are considered to be saturated with nitrogen [94, 95], show BNF rates within the range of 15–36 kg N $ha^{-1}$ $yr^{-1}$, which is similar to or higher than estimates for more temperate forests (7–27 kg N $ha^{-1}$ $yr^{-1}$) [96]. Thus, Arctic ecosystems generally show much lower BNF rates. Moisture and temperature as microclimatic controls were identified as key parameters of BNF and N input of arctic ecosystems, besides cyanobacterial associations and ecosystem type [73, 75]. Optimum temperatures for Arctic nitrogen-fixation are estimated to be around 10–30°C, depending on the site [55, 68, 75]. In the High Arctic, temperature optima below 15°C were observed, suggesting that there are certain permafrost ecosystems with cold adapted diazotrophs that might show lower BNF rates once the temperature exceeds such a temperature range [75]. Within these constraints, increased BNF as a result of climate change and global warming is a probable assumption. Thus, fueling the N-cycle with increasing amounts of reactive, readily available N for both microorganisms and plants is a likely scenario.

**Tab. 4.3:** Biological nitrogen fixation rates of various permafrost-affected sites as determined by laboratory incubations or in field studies.

| Laboratory process rates | *In situ* process rates [µg N $m^{-2}$ $h^{-1}$] | Soil moisture/ water content | pH | C/N ratio | Site descriptor | | Region | Coordinates | Reference |
|---|---|---|---|---|---|---|---|---|---|
| 0.17–25.0 µg N $g^{-1}$ $h^{-1}$[a,b] | n.a. | n.a. | n.a. | n.a. | Cyanobacteria from sedge meadow | Polar oasis | High Canadian Arctic | 75°33'N, 84°40'W | [84] |
| n.a. | 1040 (mean)[b,c] | n.a. | n.a. | 67.5–95.5 | *Sphagnum* bog | Tundra | Northwestern Sweden | 68°N, 18°E | [56] |
| n.a. | 2570 (mean)[b,c] | n.a. | n.a. | 23.2–28.3 | Lichen Heath | | | | |
| n.a. | 347 (mean)[b,c] | n.a. | n.a. | 14.5–24.6 | Cyanobacterial soil crust from polygon | | | | |
| n.a. | 2710 (mean)[b,c] | n.a. | n.a. | 23.7–32.5 | Grassland (legume *Astragalus alpinus*) | | | | |
| 132 µg N $m^{-2}$ $h^{-1}$ (mean)[b] | 267 (mean)[b] | n.a. | n.a. | n.a. | Hollow BSC | Tundra | Canadian Low Arctic | 64°52'N, 111°35'W | [73] |
| 168 µg N $m^{-2}$ $h^{-1}$ (mean)[b] | 395 (mean)[b] | n.a. | n.a. | n.a. | Hummock BSC | | | | |
| 396 µg N $m^{-2}$ $h^{-1}$ (mean)[b] | n.a. | n.a. | n.a. | n.a. | *Sphagnum* spp. *Stereocaulon paschale* | | | | |
| 749 µg N $m^{-2}$ $h^{-1}$ (mean)[b] | 3330 (mean)[b] | n.a. | n.a. | n.a. | | | | | |
| n.a. | 28.05–2100 (mean)[c] | 10–165% SMC[c] | 5–7 | 14.5–17.4 | Turbic cryosols | Heath | North Eastern Greenland | 74°30'N, 21°00'W | [75] |

**Tab. 4.3** (continued)

| Laboratory process rates | *In situ* process rates [μg N $m^{-2}$ $h^{-1}$] | Soil moisture/ water content | pH | C/N ratio | Site descriptor | | Region | Coordinates | Reference |
|---|---|---|---|---|---|---|---|---|---|
| n.a. | 140–393[b] | 5–90% SMC[c] | 4.8–6.5[d] | n.a. | Meadow, wet marsh, mosses, cyanobacterial colonies | Landscape transect | Spitzbergen, Svalbard | 78°47'N, 16°19'E | [75] |
| –20–243 μg N $m^{-2}$ $h^{-1}$[b] | n.a. | n.a. | n.a. | n.a. | Cyanolichens and bryophatey from birch forest, bog and fen | Subarctic Lowland | Abisko, North Sweden | Not provided | [90] |
| 5.95–99.9 μg N $m^{-2}$ $h^{-1}$ (mean)[b,f] | n.a. | 241–1046% SMC | 4.5–4.8[e] | 22.5–24.4[e] | Tussock and sedge tundra, heath | Tundra | Northern Alaska | 68°37'N, 149°18'W | [55] |
| <0.58 μg N $m^{-2}$ $h^{-1}$ (mean)[b] | n.a. | n.a. | n.a. | n.a. | Sediment | Fjord | Kongsfjorden, Svalbard, Norway | 78°59.43'N, 12°17.87'E | [307] |
| 11.67 μg N $m^{-2}$ $h^{-1}$ (mean)[b] | n.a. | n.a. | n.a. | n.a. | Sediment | Fjord | Smeerenburgfjorden, Svalbard, Norway | 79°42.01'N, 11°05.20'E | [307] |
| n.a. | 28.5 (lower estimate) | 200–400% of soil dry mass | 5.9 | 30 | Heath | Disturbance and vegetation gradient | Lake Torneträsk, Abisko, North Sweden | 68°19'N, 18°51'E | [372] |
| n.a. | 100 (lower estimate) | 400–700% of soil dry mass | 7.0 | 31 | Moss | | | | |
| n.a. | 126 (lower estimate) | 100–200% of soil dry mass | 7.5 | 12 | Circle | | | | |

**Tab. 4.3** (continued)

| Laboratory process rates | *In situ* process rates [µg N $m^{-2}$ $h^{-1}$] | Soil moisture/ water content | pH | C/N ratio | Site descriptor | | Region | Coordinates | Reference |
|---|---|---|---|---|---|---|---|---|---|
| n.a. | 11–48.7[b] | n.a. | n.a. | n.a. | Tundra | Alpine area | The Barrow, Alaska | 63°40'N, 146°5'W | [86] |
| n.a. | 308 (mean)[b] | n.a. | n.a. | n.a. | *P. scherberi* moss–*Nostoc* associations | Pine and spruce forests | Northern Sweden, Norway, Finland | 62–70°N, 13–20°E | [376] |
| 2210 µg N $m^{-2}$ $h^{-1}$ (mean)[a,b,c] | n.a. | n.a. | n.a. | n.a. | BSC | Polar desert | High Arctic, Truelove Lowland, Devon Island | 75°33'N, 84°24'W | [377] |
| 380 µg N $m^{-2}$ $h^{-1}$ (mean)[a,b,c] | n.a. | n.a. | n.a. | n.a. | Non-crusted mineral soil | | | | |
| 39–22,000 µg N $m^{-2}$ $h^{-1}$ (mean)[a,b,c] | n.a. | 13–850% of soil dry mass | n.a. | n.a. | Plant community transects | Rocky Point, Southwest meadow and lagoon | High Arctic, Truelove Lowland, Devon Island | 75°33'N, 84°40'W | [69] |

For more details, please refer to the references provided.

n.a. – not applicable, SMC – soil moisture content, C/N – carbon to nitrogen ratio, BSC – biological soil crust.

[a] Assuming a conversion factor of acetylene reduction to nitrogen fixation of 2.

[b] Data converted from original paper.

[c] Data extracted/estimated from figure.

[d] Vanderpuye et al., 2002 [378].

[e] Mercado-Díaz et al., 2014 [379].

[f] Assuming a conversion factor of acetylene reduction to nitrogen fixation of 5, according to previous results within the study.

### 4.2.2 Nitrification and associated organisms

The aerobic conversion of ammonia ($NH_3$) via nitrite ($NO_2^-$) to nitrate ($NO_3^-$) is primarily catalyzed by autotrophic Bacteria and Archaea [41] (classical view; theoretical oxidative half-cell reactions are given in Equations 4.2–4.4). Please note that molecular oxygen is needed for the conversion of $NH_3$.

$$NH_2 + H_2O \xrightarrow{AMO} NH_2OH + 2H^+ + 2e^- \quad (4.2)$$

$$NH_2OH + H_2O \xrightarrow{HAO} NO_2^- + 5H^+ + 4e^- \quad (4.3)$$

$$NO_2^- + H_2O \xrightarrow{NXR} NO_3^- + 2H^+ + 2e^- \quad (4.4)$$

Here, AMO is ammonia monooxygenase; HAO, hydroxylamine oxidioreductase; and NXR, nitrite oxidoreductase.

The process typically involves two distinct, interacting groups: the ammonia oxidizers that oxidize $NH_3$ to $NO_2^-$ and the nitrite oxidizers that oxidize $NO_2^-$ to $NO_3^-$ [97]. Recently, complete ammonia oxidizers (comammox) were discovered combining all nitrification half-cell reactions [98, 99] (Equations 4.2–4.4). Although the rate-limiting step of nitrification, the oxidation of $NH_3$, is performed by members of both Bacteria (AOB) and Archaea (AOA), their relative contributions to nitrification in natural environments remain tentative [100, 101]. However, recent evidence for a niche specialization of AOB and AOA suggests a dominance of AOB and AOA at high and low $NH_3$ availability, respectively [102]. A dominance of AOA is routinely observed in low pH environments, where $NH_3$ availability is generally low due to a pH-dependent shift in the equilibrium concentration of $NH_3$ relative to $NH_4^+$. Adaptions of AOB to low pH are currently not observed. Interestingly, yields of the greenhouse gas $N_2O$ are higher in AOB than AOA [102]. In contrast to AOA and AOB, the ecological niche of Comammox is less well defined. Growth in soils not amended with $NH_3$ rather than in those amended with $NH_3$ and high affinities for $NH_3$ suggest an oligotrophic life style [103, 104].

#### Physiology and diversity of ammonia oxidizers and comammox

All known aerobic ammonia-oxidizing bacteria and archaea use the enzyme ammonia monooxygenase (AMO), an integral membrane metalloenzyme, which catalyzes the oxidation of $NH_3$ to hydroxylamine ($NH_2OH$) [105]. AMO belongs, like the methanotrophic enzyme particulate methane monooxygenase (pMMO), to the family of copper containing membrane-bound monooxygenases [106]. A broad substrate range can be oxidized by AMO, including $CH_4$ [107], aromatic compounds [108], sulfides [109], and halogenated hydrocarbons [110, 111], suggesting that ammonia oxidizers are involved in many more transformation processes (although co-metabolically) than usually appreciated.

The classical view that the oxidation product of $NH_3$ by AMO, $NH_2OH$, is further oxidized to $NO_2^-$ via the multiheme enzyme hydroxylamine oxidoreductase (HAO) by ammonia oxidizing bacteria (AOB) is challenged to date [105, 112]. NO was believed to originate due to an abiotic side reaction of $NH_2OH$. However, recent evidence suggests that $NH_2OH$ is enzymatically oxidized to NO via HAO in the presence of oxygen [113] (Equation 4.5). NO is then further converted in an either biotic (enzymatically) and/or abiotic (NO reacting with $O_2$ in aqueous solutions at pH >7) reaction to $NO_2^-$ [113]. An enzyme acting as NO oxidoreductase is further suggested to be involved [112]. A possible candidate for this enzyme is the copper dependent NO-forming nitrite reductase NirK that can also catalyze NO oxidation to $NO_2^-$ [113, 114] (Equation 4.6). This implies that $NH_2OH$ and NO are both obligate intermediates of $NH_3$ oxidation by AOB.

$$2NH_2OH + \frac{3}{2}O_2 \xrightarrow{HAO} 2NO + 3H_2O \tag{4.5}$$

$$NO + 3H_2O \xrightarrow{NIR} NO_2^- + 2H^+ + e^- \tag{4.6}$$

Here, AMO is ammonia monooxygenase and NIR is nitrite reductase.

Within genomes obtained from either pure or enrichment cultures of AOA, homologues of HAO encoding genes were not detected [115–118]. Like for AOB, NO plays an important role in the ammonia oxidation pathway of AOA [119, 120] (postulated current view, Equations 4.7–4.9).

$$NH_3 + H_2O \xrightarrow{AMO} NH_2OH + 2H^+ + 2e^- \tag{4.7}$$

$$NH_2OH + NO + 2H_2O \xrightarrow{CytP460} 2NO_2^- + 7H^+ + 5e^- \tag{4.8}$$

$$NO_2^- + 2H^+ + e^- \xrightarrow{NIR} NO + 2H_2O \tag{4.9}$$

AMO indicates ammonia monooxygenase; CytP460, cytochrome P460; and NIR, nitrite reductase.

NO is an important intermediate and co-reactant for the oxidation of $NH_2OH$ to $NO_2^-$ in AOA, which is probably catalyzed by a novel copper enzyme capable of performing like the known heme-containing cytochrome P460 [121]. The NO that is required for this pathway is most likely generated through $NO_2^-$ reduction to NO by the nitrite reductase NirK, which is encoded in all genomes published for AOA [122–124]. In contrast to AOB that are capable of producing $N_2O$ enzymatically via nitrifier denitrification, the intermediates NO and $NH_2OH$ of archaeal ammonia oxidation might be released into the environment and then non-enzymatically converted to $N_2O$ under anoxic conditions [125].

AOB are represented by five proteobacterial (beta- and gammaproteobacterial) genera: *Nitrosomonas*, *Nitrosospira*, *Nitrosovibrio*, *Nitrosolobus*, and *Nitrosococcus* [126]. From these, *Nitrosospira* ssp. and *Nitrosomonas* ssp. inhabit soils, whereby

*Nitrosospira* ssp. often dominate soil populations [127, 128] and *Nitrosomonas* ssp. are common in soils with high N input, suggesting a niche differentiation within the AOB [129–131]. Nitrification and the associated fluxes of N compounds are strongly dependent on environmental conditions [132]. The inter-phylum niche differentiation between AOA and AOB communities is driven by pH and the availability of $NH_3$ (see above). Indeed, archaeal *amoA* genes have been detected in many acidic soils [133–137] and AOA often largely outnumber AOB (e.g. [136]). Some AOA seem to even prefer a low pH (<5.5) [138–140]. AOA are members of the phylum Thaumarchaeota and are ubiquitous as well as abundant in the environment [141–144]. The dogma that the oxidation of $NH_3$ to $NO_3^-$ requires two distinct groups of organisms, AOB and nitrite-oxidizing bacteria (NOB), has been refuted when complete ammonia oxidation to nitrate (comammox) was found to be catalyzed by a single organism, a member of the genus *Nitrospira* [99, 145]. Organisms capable of comammox are members of the phylum Nitrospirae, and organisms such as *Candidatus* "Nitrospira inopinata" appear to be well adapted to ammonia-limited environments and even outcompete many cultures of ammonia-oxidizing bacteria [103].

**Physiology and diversity of nitrite oxidizers**

The second step of nitrification, the oxidation of $NO_2^-$ to $NO_3^-$, is performed by autotrophic NOB, many of which are capable of a mixotrophic lifestyle, and catalyzed by the enzyme nitrite oxidoreductase (NXR). It is the main biochemical pathway that produces $NO_3^-$, and the aerobic nitrite oxidation is directly coupled to energy conservation [2]. By converting growth-inhibiting $NO_2^-$ to $NO_3^-$, NOB counteract $NO_2^-$ toxicity and provide $NO_3^-$ that is an essential N source for many plants. Thus, NOB have an important regulatory function in the nitrogen cycle [98, 146, 147]. The genes *nxrA/B* encoding the NXR are used as functional gene markers to detect and identify NOB in the environment [148, 149]. Known NOB belong to seven genera in four bacterial phyla, the Proteobacteria, Chloroflexi, Nitrospinae, and the Nitrospirae. With one exception (*Nitrolancea hollandica*), NOBs all have Gram-negative cell walls [145, 150]. NOBs have versatile metabolisms and can grow on substrates other than $NO_2^-$, such as the alternative electron donors formate or hydrogen [151].

**Physiology and diversity of heterotrophic nitrifiers**

Heterotrophic nitrification includes the oxidation of reduced forms of organic and inorganic nitrogen to more oxidized forms [152–154]. In contrast to autotrophic nitrification, the heterotrophic process is not coupled to energy conservation or cellular growth and the involved enzymes differ from those that catalyze autotrophic nitrification [155]. Heterotrophic nitrification has been found in chemoorganotrophic bacteria and fungi, where it seems to be linked to the re-oxidation of NAD(P)H under hypoxic conditions [156] and endogenous respiration [157], respectively. Model

organisms capable of heterotrophic nitrification host enzymes functionally similar to ammonia monooxygenase and hydroxylamine oxidases of autotrophic AOB or distinct enzymes that oxidize organic N to $NO_2^-$ [153]. Nitrite oxidation by heterotrophs is postulated to occur via a detoxification reaction by catalase. Example bacterial genera of heterotrophic nitrifiers include *Alcaligenes, Arthrobacter, Paracoccus*, and *Pseudomonas*. Most of them are also aerobic denitrifiers employing the strategy of co-respiring oxygen and $NO_3^-$ under hypoxic conditions [153, 158]. At low oxygen concentrations below 10 µM and a C/N ratio above 10, the heterotrophic nitrifier *Paracoccus pantotrophus* (formerly known as *Thiosphaera pantotropha*) outcompeted the autotrophic AOB *Nitrosomonas europaea*, suggesting that heterotrophic nitrification might dominate autotrophic nitrification under certain conditions in the environment [159]. Indeed, the importance of heterotrophic nitrification in terms of $NO_3^-$ production in acidic soils might exceed the one of autotrophic nitrification [160, 161], since the activity of autotrophic bacterial nitrification is impaired at low pH [162]. However, the cross-comparison of $N_2O$ emissions from different soils by heterotrophic nitrification could not be explained by the variability of pH between examined study sites, and the key factors controlling the process and its contribution to $N_2O$ emissions from soils are still to be elucidated [163]. In line with pure culture experiments, the availability of certain organic substrates with high C/N ratio may play an important role in the control of $N_2O$ emissions from soils by heterotrophic nitrification, since the gross heterotrophic nitrification rate increases linearly with the C/N ratio of examined soils [163].

**Rates of nitrification and associated nitrifier diversity in permafrost environments**

In Arctic soils, nitrification has frequently been documented (e.g. [164, 165]) (Tab. 4.4) and is reported to consume up to half of the N mineralized annually [166]. It has also been shown that nitrification contributes to the production of $N_2O$ in the Arctic and might represent the major source of $N_2O$ in certain Arctic soils [4, 18, 167, 168]. Both, AOA and AOB contribute to ammonia oxidation in permafrost soils. Ma et al. [167] studied lowland soils from Devon Island, Canadian High Arctic, and found that indigenous AOB communities have a high potential of releasing $N_2O$, contributing up to 86% of the $N_2O$ released from these environments [167]. Phylogenetic analysis of the soil community revealed sequences affiliating with known AOB from more temperate soil that are known to contribute to $N_2O$ emission [167]. AOB of the genera *Nitrosomonas*, *Nitrospira* (both belonging to the Betaproteobacteria), as well as *Nitrosococcus* (Gammaproteobacteria) were detected in permafrost-affected soil. In the same soils, the AOA *Nitrosopumilus* was detected [169]. Recently, AOA have been identified in Arctic, low pH permafrost-affected soils as numerically important [170, 171]. A combined study of phylogeny and ecophysiology at 10 permafrost-affected sites in Svalbard, Western Siberia, and Greenland (including shrub, tussock, and moss tundra as well as peat and forest)

showed a high β-diversity of Thaumarchaeota in Arctic soils, as well as a higher abundance of AOA compared to AOB in most samples. General niche partitioning of AOA clades in these soils followed soil moisture and nitrogen content [172]. AmoA encoding genes affiliating with clades A and B were widely distributed among the soils analyzed. The *Nitrososphaera* clade as well as clades C and D (distantly related to *Candidatus* Nitrosopumilus and *Candidatus* Nitrosoarchaeum) were less common. Interestingly, the data showed low intra-sample diversity of AOA, with a single phylotype dominating in each population [172]. In unvegetated (sub) arctic peat soil surfaces in Siberia and Finland, archaeal and bacterial *amoA* gene abundances were examined. Bacterial *amoA* were below the detection limit, and up to $1.3 \times 10^9$ archaeal *amoA* genes $g^{-1}$ dry soil were detected. Surprisingly, only two archaeal phylotypes associated with the order Nitrososphaerales (including *Candidatus* Nitrosocosmicus spp. and one clade without any cultured representative) were detected after sequencing, revealing a very low nitrifier diversity among these soils [173]. The gross nitrification rates in laboratory incubations in these soils reached up to 2.5 mg $NO_3$–N $kg^{-1}$ dry soil $h^{-1}$ [173]. Such soils showed high $N_2O$ emissions of up to 233 μg $N_2O$-N $m^{-2}$ $h^{-1}$ [37], which was similar to or even higher than managed peatlands in northern countries [174]. Interestingly, both soil $NO_3^-$ concentrations and the abundance of archaeal *amoA* genes were positively correlated with $N_2O$ emissions from the soils, linking high $N_2O$ emissions to AOA [173] and highlighting the importance of AOA as non-negligible direct or indirect source of $N_2O$ in Arctic permafrost soils. To verify these findings and to unravel the role of AOA in permafrost soils, further studies will be needed.

In pH neutral to mildly alkaline soils from Antarctica, AOA and AOB are routinely detected [93, 175]. Quantification of archaeal and bacterial *amoA* indicated that AOB outnumbered AOA [93]. The absence of detectable archaeal *amoA* supports this view [175]. AOB community changed due to altered organic soil carbon rather than increased temperatures when permafrost was subjected to experimental warming. Bacterial *amoA* were distantly related to *amoA* of *Nitrosospira* sp., In contrast to AOB, archaeal *amoA* decreased upon warming, suggesting a temperature sensitivity of AOA [93]. Antarctic desert soils (McMurdo Dry Valleys, pH 7–8) showed variable archaeal and bacterial *amoA* abundance from $<10^3$ to $5 \times 10^6$ genes per gram dry weight soil. AOB were more abundant than AOA at two of the four sites [176]. Harsher conditions (lower pH, higher electrical conductivity) favored AOA. The diversity of ammonia oxidizers was generally low; three and four phylotypes only distantly related to cultured organisms of AOB and AOA were detected, respectively. Most important AOB and AOA were related to Nitrosospiraceae, Nitrosomonadaceae, and Nitrosophaerales, respectively [176, 177]. Arctic cryosols (pH 6–7.3) of the Lena Delta hosted nitrifier communities accounting for 0.6–6.2% of 16S rRNA genes [178]. Most ammonia oxidizers affiliated with the genus *Nitrosospira*. The occurrence of AOA was restricted to low-organic C soils and represented by *Candidatus* Nitrososphaera, *Ca*. Nitrosopumilus, *Ca*. Nitrosocaldus, and *Ca*. Nitrosoarchaeum.

**Tab. 4.4:** Nitrification rates of various permafrost-affected sites as determined by laboratory incubations or in field studies.

| Laboratory process rates | *In situ* process rates [µg N $m^{-2}$ $h^{-1}$] (unless otherwise indicated) | Soil moisture/ water content | pH | C/N ratio | Site descriptor | | Region | Coordinates | Reference |
|---|---|---|---|---|---|---|---|---|---|
| −4.08–3.46 µg N $gdw^{-1}$ $h^{-1}$ (net)[a] | −3.96–0.13 µg N $gdw^{-1}$ $h^{-1}$[a] (net) | 17.1–907% SMC | 5.7–8.4 | 3.2–9.8 | Shrub, tussock and moss tundra, peat and fen | Tundra | Longyearben, Spitzbergen, Svalbard archipelago | 78°10'26''N, 16°1'29''E–78°56'33''N, 11°49'3''E | [172] |
| n.a. | 2.76 × $10^2$–3.35 × $10^2$ (mean)[b] | 326.7–853.6% SMC | 5.6[c] | n.a. | Sedge meadow hummock Willow herb hummocks | Coastal Lowland | High Canadian Arctic | 75°33'N, 84°40'W | [165] |
| n.a. | 1.49 × $10^3$–2.20 × $10^3$ (mean)[b] | 295.1–520.5% SMC | 5.9[c] | | | | | | |
| −4.48 × $10^{-4}$–0.04 µg N $g_{DW}^{-1}$ $h^{-1}$[b] | n.a. | 66.5–87.8% SMC | n.a. | 12–44 | Fen channels, sedge lawns, peat plateau, thermokarst | Peat | Manitoba, Canada | 58°48'N, 94°,09'W | [380] |
| n.a. | 13.70–45.66[a,b] | 65–553% SMC | 4.2–6.8 | 14.2–27.4 | Tussock and wet sedge tundra, heath, willow | Toposequence | Sagavanirktok River, Alaska | 68°46'40''N, 148°51'8''W | [166] |
| n.a. | 0.03–0.68 µg N $g_{DW}^{-1}$ $h^{-1}$ (AOB + AOA)[b] | n.a. | 6.0–7.3 | 12–37 | Polygon, dry river terrace, cliff, beach, floodplain | Tundra | Northeastern Siberia | 72°22'N, 126°28'E | [178] |
| n.a. | ≤0.02 µg N $g_{DW}^{-1}$ $h^{-1}$ (AOA)[b] | | | | | | | | |

**Tab. 4.4** (continued)

| Laboratory process rates | *In situ* process rates [µg N $m^{-2}$ $h^{-1}$] (unless otherwise indicated) | Soil moisture/ water content | pH | C/N ratio | Site descriptor | | Region | Coordinates | Reference |
|---|---|---|---|---|---|---|---|---|---|
| 1.5 × $10^{-3}$–0.02 µg N $gDW^{-1}$ $h^{-1b}$ | n.a. | 17.4–40.7% SMC | 7.5–7.9 | 11–18.9 | Moss, Regosolic cryosol | Tundra | Ny-Ålesund, Svalbard | 78°55'41–48"N, 11°51'25–46"E | [381] |
| 1.0 × $10^{-3}$–1.2 × $10^{-3}$ µg N $gDW^{-1}$ $h^{-1b}$ | n.a. | 15.4% SMC | 7.6 | 16.1 | Bare peat soil | | | | |
| 2.00 × $10^{2}$–4.60 × $10^{3}$ µg N $m^{-2}$ $h^{-1b}$ | n.a. | 2.6–29.6% SMC | 8.7–8.9 | 10.9–12.6 | Meadow and steppe | Alpine Grassland | Northern Tibet, China | 30°57'N, 88°42'E | [382] |
| n.a. | 5.64 × 105 µg N $g_{DS}^{-1}$ $h^{-1b}$ | 0.64 $dm^3$ $dm^{-3}$ VWC[d] | 3.86 | 23 | Bare peat | Peat | Seida/Vorkuta, Russia | 67°03'N, 62°57'E | [173] |
| n.a. | 1.60 × $10^{-3}$–0.21 µg N $g_{DS}^{-1}$ $h^{-1b}$ | 62–94% SMC | 3.2 | 22–23 | Bare peat | Tundra | Seida/Vorkuta, Russia | 67°03'N, 62°57'E | [24] |

For more details, please refer to the references provided.

n.a. – not applicable, SMC – soil moisture content, C/N – carbon to nitrogen ratio, DW – dry weight, AOB/A – ammonia oxidizing bacteria/archaea, DS – dry soil, VWC – volumetric water content.

[a] Data extracted/estimated from figure.

[b] Data converted from original paper.

[c] Bliss and Gold, 1994 [383].

[d] Repo et al., 2009 [22].

Thus, low pH (2.8–4.06), low temperatures and eventually low organic carbon content are crucial factors controlling specific archaeal ammonia oxidizing phylotypes and favor the dominance of AOA over AOB at permafrost-affected sites [168, 170–173].

A known cold-adapted NOB of the Betaproteobacteria, *Candidatus* "Nitrotoga arctica," was discovered from the active layer of permafrost-affected soil in the Siberian Arctic [179]. Results from experiments with a highly enriched *Candidatus* "Nitrotoga arctica" culture from permafrost soil revealed a generation time of 44 hours at 17°C and a $K_m$ value of 58 ± 28 µM for $NO_2^-$, indicating adaptation to low $NO_2^-$ concentrations [151]. Sanders et al. [178] identified the NOB *Nitrotoga* spp. via 16S rRNA Illumina amplicon sequencing in permafrost-affected soils in the Lena Delta, Northeastern Siberia. At one of the study sites, NOB associated with the genus *Nitrospira* dominated the nitrite oxidizer affiliated 16S rRNA gene sequences with up to 34% relative abundance. *Nitrobacter* spp. were likewise detected. Such a finding corroborates a metaproteome study where *Nitrobacter* genomes were among the 20 top scorers in accumulating peptides from frozen permafrost soil, the active layer, and a thermokarst bog [79]. In soils from a deglaciated valley in Peru that showed nitrification activity, sequences associated with *Candidatus* "Nitrotoga arctica" and *Nitrospira* sp. were also retrieved from 16S rRNA gene libraries [180]. Such findings indicate an important role of the NOB genera *Nitrospira* and *Nitrotoga* for $NO_2^-$ oxidation. Comammox are likewise common nitrifiers in the environment and host *amoA* as well as *nxr* genes, which is emphasized by metagenomic and PCR-based studies [145, 181, 182]. However, their occurrence and importance in permafrost systems are essentially unknown to date, necessitating further studies.

To distinguish between heterotrophic nitrification from organic N and autotrophic nitrification, inhibitors like acetylene or nitrapyrin are used, which both inhibit the oxidation of $NH_3$ by AMO [183, 184]. However, the application of stable isotopes is a more promising tool [185]. Due to the absence of established gene markers for heterotrophic nitrification, isolation and characterization of isolates are currently common strategies to identify heterotrophic nitrifiers. The gammaproteobacterial heterotrophic nitrifier *Pseudomonas* strain M19 has been isolated from a dry tundra meadow in the alpine permafrost of the Colorado Rocky Mountains and is capable of $NO_3^-$ production not only from organic nitrogen but also from $NH_4^+$ [186]. A study with three different cryosol sites from the Canadian High Arctic showed that heterotrophic ammonia oxidation potentials contributed to 29–47% of the total ammonia oxidizing potential measured in theses soils, with the highest potential of heterotrophic nitrification found at the site with the highest organic carbon and moisture content [170] (Tab. 4.4). The rates of heterotrophic ammonium oxidation ranged from 21 to 178 ng of $NO_2^-$-N g of dry soil$^{-1}$ h$^{-1}$, with the higher end of the rates being in the range of agricultural soils [187, 188]. Such studies suggest that heterotrophic nitrification should not be neglected in permafrost systems.

### 4.2.3 Dissimilatory nitrate reduction and associated organisms

When oxygen becomes limiting, alternative terminal electron acceptors are utilized by microbes [189]. Major anaerobic respiration pathways are dissimilatory nitrate reductions, including denitrification and dissimilatory nitrate reduction to ammonium (also called nitrate ammonification). Denitrification is a modular pathway comprising the sequential reduction of nitrate ($NO_3^-$) or nitrite ($NO_2^-$) via the intermediates NO and nitrous oxide ($N_2O$) to dinitrogen gas ($N_2$) [189]. Denitrification closes the nitrogen cycle by returning molecular $N_2$ to the atmosphere [190] (Equations 4.10–4.13). During nitrate ammonification, $NO_3^-$ is converted to $NH_4^+$ via $NO_2^-$ (Equations 4.15–4.16).

$$NO_3^- + 2H^+ + 2e^- \xrightarrow{\text{NAP, NAR}} NO_2^- + H_2O \quad (4.10)$$

$$NO_2^- + 2H^+ + e^- \xrightarrow{\text{NIR}} NO + H_2O \quad (4.11)$$

$$2NO + 2H^+ + 2e^- \xrightarrow{\text{NOR}} N_2O + H_2O \quad (4.12)$$

$$N_2O + 2H^+ + 2e^- \xrightarrow{\text{NOS}} N_2 + H_2O \quad (4.13)$$

Here, NAP indicates periplasmic nitrate reductase; NAR, membrane bound nitrate reductase; NIR, nitrite reductase; NOR, nitric oxide reductase; and NOS, nitrous oxide reductase

#### Physiology and diversity of dissimilatory nitrate reducers

*Canonical denitrifiers.* Most denitrifiers are facultative, heterotrophic anaerobes that consume sugars and/or organic acids but are generally not capable of growing by fermentation [191–193]. Autotrophic denitrifiers use reduced S-compounds, $H_2$, $NH_4^+$, $NO_2^-$, or $Fe^{2+}$ as electron donors. Complete denitrifiers convert $NO_3^-$ to $N_2$. However, the core reaction module of denitrifiers *sensu stricto* is the conversion of $NO_2^-$ to $N_2O$. Many denitrifiers miss some or all of the other reaction modules and associated genes, thus displaying different truncated forms of denitrification and either $N_2O$ or $N_2$ as end product [97, 194]. $N_2O$ is a strong greenhouse gas and an obligate intermediate or end product, depending on the organism. Denitrification can act as a source or sink for $N_2O$. Dynamic production and consumption processes at the soil/atmosphere interface result in varying $N_2O$ fluxes from the environment; denitrification is a major cause for nitrogen loss from many environments and useful for removing excess N from aquatic environments [191, 195]. Consequently, denitrification has been the focus of numerous studies due to its relevance for greenhouse gas metabolism and N-removal.

The ability to denitrify is widespread and can be found within more than 60 genera, including Bacteria, Archaea, and Eukarya, showing a high phylogenetic and functional variability [189, 196]. Genera belong primarily to the Alpha-, Beta-,

Gamma-, and Epsilonproteobacteria and the Firmicutes. Within the Eukarya, denitrification is mainly limited to fungi, even though it has also been reported to occur in some foraminifer species [197]. Many fungal species are capable of reducing $NO_3^-$ or $NO_2^-$ to $N_2O$ under suboxic conditions and generally lack nitrous oxide reductase [198–202]. The fungal nitrite reductase is a copper-dependent NirK-type enzyme; the NO reductase of fungi (P450nor), part of the cytochrome P450 superfamily, has been intensively studied, and sequence information on the genes encoding nitrite and NO-reductases is available from a variety of pure cultures and environments [89, 202–208]. Thus, $N_2O$ is the final product of fungal denitrification [200, 202]. Despite the fact that complete denitrification has not yet been found in fungi, they might still be able to produce $N_2$ via co-denitrification. Thereby, $NO_2^-$ is reduced to NO that can further react with organic N compounds [209]. Indeed, by a combination of biotic and abiotic processes, organisms lacking e.g. nitrite reductases are capable of complete denitrification [210].

Complete denitrification involves seven enzymes that catalyze four reductions during the process [189]. The first step of denitrification, common to denitrification and nitrate ammonification, is the reduction of $NO_3^-$ to $NO_2^-$. This step is catalyzed by Nar or Nap [189]. The catalytic α-subunit of the membrane-bound nitrate reductase Nar is encoded by *narG,* whereas the catalytic subunit of the periplasmic nitrate reductase Nap is encoded by *napA* [211]. Both genes are utilized as marker genes for nitrate reducers including denitrifiers in the environment (e.g. [212–215]). The key enzyme of denitrification is nitrite reductase, which further reduces $NO_2^-$ to NO, the first gaseous product of the process [189]. This step is catalyzed by either NirK or NirS, a Cu-containing or cytochrome $cd_1$ nitrite reductase, respectively [189]. The two enzymes have identical functions, despite differing in their structure and catalytic site [216]. Until recently, it was thought that one organism harbors either *nirK* or *nirS* genes [217]. The NO reductases cNor or qNor subsequently reduce NO to $N_2O$. The two proteins have different electron donor specificities; cNor is associated with cytochrome *c* or blue copper proteins and qNor derives electrons from the quinol pool [218]. qNor can also play a role in NO detoxification of non-denitrifying prokaryotes [218]. In *Bacillus azotoformans*, a third type of Nor was found, which is suggested to fulfill both bioenergetic and detoxifying functions [219]. The final step of denitrification is the reduction of $N_2O$ to $N_2$, catalyzed by the copper-containing nitrous oxide reductase Nos, the only known enzyme to be capable of this reaction [189, 216]. Nos is classified into two distinct groups: class I, prevalent in canonical denitrifiers, and class II, which also occurs among non-denitrifying $N_2O$ reducers [220]. While clade I Nos is associated primarily with Tat-dependent transport of the folded protein, class II Nos is primarily associated with Sec-dependent transport of the unfolded Nos precursor [221, 222].

Acidity, early growth phase, and high $NO_3^-$/organic carbon ratios stimulate release of $N_2O$ during denitrification [223–227]. Denitrifiers might lack nitrate reductases and/or $N_2O$ reductases and occupy diverse ecological niches [189, 191, 193, 228].

Denitrification rates and the product ratio of $N_2O$ to $N_2$ are regulated by the denitrifying community and *in situ* conditions (e.g. pH, temperature, C/N ratio, as well as the availability of substrates and electron acceptors [229–234]). Low pH (<5) impairs denitrification in certain systems and increases the product ratio of $N_2O$ to $N_2$ [235, 236]. The increased product ratio of $N_2O$ to $N_2$ is likely caused by post-transcriptional effects of low pH on $N_2O$ reductase assembly [237]. However, recently, acid-tolerant $N_2O$ reduction was identified in peatlands and pure cultures (e.g. [238–241]). The relative amount of $N_2O$ released from the environment depends also on the ratio of $N_2O$ to total N gases and reflects the relative abundance of the bacterial community capable of $N_2O$ reduction [190, 217, 242]. The natural terrestrial ecosystems where the highest known $N_2O$ emissions originate are in the tropics and have a high supply of mineral nitrogen and favorable soil moisture, both supporting conditions for $N_2O$ production [22, 243, 244]. Low $N_2O$ emissions, in contrast, have been reported from pristine terrestrial ecosystems in northern latitudes [243, 245]. It was thought that due to slow mineralization of organic matter under cold, humid conditions [246] and low atmospheric deposition of N [247], biological processes are generally N limited, which results in a competition for available nitrogen between vegetation and microorganisms [248], leading to low $N_2O$ emissions. Such a view is now changing for certain permafrost-affected systems as outlined below (see Section 4.2.3, "Rates of nitrate dissimilation and associated microbial diversity in permafrost environments" section; Tab. 4.1).

*Methane-dependent denitrification.* Known electron donors for denitrification include organic carbon and sulfur compounds as well as hydrogen [249]. Biochemically more challenging as a substrate than such compounds is methane ($CH_4$) that is subject to oxygen-, sulfate-, or $NO_3^-$/$NO_2^-$-dependent oxidation [250]. The first experimental evidence of $CH_4$-oxidation with $NO_3^-$/$NO_2^-$ via denitrification was obtained from a bioreactor operated with sludge [251]. The process was linked to bacteria of the candidate phylum NC10, which was also known from environmental genetic analyses [252]. Based on metagenomic data, one species of this clade, *Candidatus* "Methylomirabilis oxyfera", was described [253]. The bacterium has not yet been isolated but exists in enrichment cultures that can give insights into its physiology. $CH_4$ oxidation to $CO_2$ is coupled to the reduction of $NO_2^-$ to $N_2$ by *Ca*. "M. oxyfera" (Equation 4.14) [41].

$$3CH_4 + 8NO_2^- + 8H^+ \longrightarrow 3CO_2 + 4N_2 + 10H_2O \tag{4.14}$$

Members of the NC10 phylum have been identified from other environments, like waterlogged soils and freshwater sediments via 16S rRNA gene sequencing [252]. Even though experiments and resulting preliminary predictions based on these limited information do not suggest major $N_2$ production from $NO_2^-$-dependent $CH_4$ oxidation, further investigations are needed to reveal its importance in natural environments [41, 254].

*Denitrifying nitrifiers and aerobic denitrifiers.* Many of the classical "autotrophic" ammonia oxidizers are capable of denitrification under oxygen limiting conditions,

sometimes by using organic C-sources like pyruvate; such nitrifier-denitrification is often used by taxa within the autotrophic ammonia oxidizing *Nitrosospira* and was first described in pure cultures [255–259]. $NH_4^+$ is converted to $NO_2^-$ that is utilized as electron acceptor employing nitrite reductases when oxygen becomes limiting. $NO_2^-$ is then stepwise reduced to $N_2O$ via NO [260]. The stepwise reduction is controlled by enzymes that might not differ phylogenetically from nitrite (NIR) and nitrous oxide (NOR) reductases found in denitrifying organisms [261, 262]. Nitrifier denitrification can represent the main source of $N_2O$ released by nitrifiers in soils [185, 259, 263, 264]. Soils with moderately low pH and oxygen levels and high N content are thought to considerably contribute to $N_2O$ fluxes due to the occurrence of nitrifier denitrification [265, 266]. Proof of the process's actual occurrence in soil, despite its suggested importance as contributor to $N_2O$ emission from soils, still remains elusive [267, 268].

Interestingly, many heterotrophic nitrifiers are also capable of aerobic denitrification (see Section 4.2.3, "Physiology and diversity of dissimilatory nitrate reducers" section). Prokaryotes capable of aerobic denitrification are diverse and, among others, belong to the genera Proteobacteria, Firmicutes, and Actinobacteria. Aerobic denitrification proceeds via Nap that is expressed also under fully oxic conditions and *nirK/S* [153, 158]. Fungi as exemplified by the model organism *Fusarium oxysporum* are likewise aerobic denitrifiers. Fungal denitrification requires oxygen limited conditions, whereas nitrate ammonification (see Section 4.2.3, "Canonical denitrifiers" section) occurs only in the absence of oxygen [269, 270]. Fungal nitrate and nitrite reductases are structurally and functionally similar to the bacterial counterparts [205, 271]. The diversity of known fungal denitrifiers is currently increasing [201, 203, 206, 272]. All aerobic denitrification pathways, including nitrifier-denitrifiers, have in common that the end product is primarily $N_2O$.

*Nitrate ammonifiers (or dissimilatory nitrate reducers producing ammonium).* In contrast to denitrifiers that lead to N-loss, nitrate ammonifiers reduce $NO_3^-$ to $NH_4^+$ and thus retain fixed N in the system (Equations 4.15 and 4.16). Dissimilatory nitrate reduction to $NO_2^-$ or $NH_4^+$ is an anoxic process and is linked to ATP synthesis via electron transport and proton translocation [40, 273]. Energy yield during fermentation might likewise be increased via $NO_2^-$ reduction [208]. $N_2O$ is produced via the non-specific interaction of nitrate reductase with $NO_2^-$ [274, 275]. Two different sets of reductases are involved. The reduction of $NO_3^-$ is respiratory, while the reduction of $NO_2^-$ is also associated with fermentation [40, 276, 277], although both are dissimilatory processes [277]. During this fermentation process, $NO_2^-$ reduction regenerates oxidants like $NAD^+$, thus allowing for higher energy yields by substrate-level phosphorylation [278]. The production of $N_2O$ by nitrate-dissimilating bacteria is favored in high organic carbon systems like the rumen or the gastrointestinal tract of higher animals and, to a lesser extent, in organic-rich pH neutral soils [191, 273, 279, 280]. Oxidoreductases that catalyze the conversion of $NO_3^-$ to $NO_2^-$ and $NH_4^+$ in bacteria include nitrate reductases (encoded by *narGHI* and *napA*) and assimilatory

as well as dissimilatory nitrite reductases (encoded by *nirAB/nasB* and *nrfA*, respectively [40, 60, 281–283]). Except for some sulfate-reducing bacteria, which are only able to use $NO_2^-$ as a substrate, most organisms carrying out nitrate ammonification are likewise capable of $NO_3^-$ reduction [284, 285]. The key enzyme involved in nitrate ammonification that reduces $NO_2^-$ the whole way to $NH_4^+$ without the release of an intermediate is the cytochrome *c* Nrf and primers against this enzyme are used to detect *nrfA* genes in bacterial communities [276]. Dissimilatory nitrite reduction involving Nrf is favored over assimilatory nitrite reduction by NirB under low-nitrate conditions (i.e. $NO_3^-$ concentrations smaller than 2 mM), while the assimilatory nitrite reduction dominates when $NO_3^-$ exceeds 3 mM in *Escherichia coli* [283]. Similar findings were obtained for *Shewanella loihica*, a model organism that hosts denitrification and nitrate ammonification pathways [286, 287]. $NO_2^-/NO_3^-$ concentrations, pH, and C/N ratios were important for the regulation of differential electron flow toward denitrification and nitrate ammonification. Thus, N-oxide concentrations regulate the pathways for nitrate ammonification, and nitrate ammonifiers might compete for $NO_3^-$ with denitrifiers, with nitrate ammonification being favored when the electron acceptor $NO_3^-$ is the limiting factor [288, 289]. Nitrate reduction to ammonia is likewise catalyzed by fungi, predominantly under hypoxic conditions, and assimilatory enzymes like NiaD and NiiA are associated with this conversion [202]. Although dissimilatory nitrate reduction to $NO_2^-$ or $NH_4^+$ can occur in soil, the effect on the $N_2O$ emissions of soils appears negligible [279, 290]. However, provision of the intermediate $NO_2^-$ to denitrifiers could enhance $N_2O$ production by denitrification. Thus, dissimilatory nitrate reducers might indirectly contribute to $N_2O$ emissions in soils.

$$NO_3^- + 2H^+ + 2e^- \xrightarrow{\text{NAP, NAR}} NO_2^- + H_2O \tag{4.15}$$

$$NO_2^- + 8H^+ + 6e^- \xrightarrow{\text{NRF}} NH_4^- + 2H_2O \tag{4.16}$$

NAP represents periplasmic nitrate reductase; NAR, membrane bound nitrate reductase; and NRF, cytochrome c nitrite reductase.

Diverse bacteria are known to perform nitrate ammonification, which can be found within the Gamma-, Delta-, and Epsilonproteobacteria, Firmicutes, as well as within the Bacteroidetes [276, 291]. Obligate anaerobic (*Clostridium*), facultative aerobic (*Citrobacter, Enterobacter, Erwinia, Escherichia, Klebsiella*), and aerobic (*Bacillus, Pseudomonas*) lifestyles are well known for nitrate ammonifiers [191]. Already in 1938, the occurrence of nitrate ammonification in the common soil bacterium *Clostridium welchii* was shown by Woods [292]. Fungi like the anamorphic ascomycete *Fusarium oxysporum* are also be capable of nitrate ammonification. Depending on the availability of oxygen, *F. oxysporum* is likewise capable of denitrification (see Section 4.2.3, "Canonical denitrifiers" section above). Within the Deltaproteobacteria, many sulfate reducers are capable of nitrate ammonification when $NO_3^-$ is present [284, 285, 293].

Nitrate ammonification is mainly known from strongly reducing environments like sediments [249] but has also been found in other terrestrial and aquatic systems [273, 294, 295]. Nitrate ammonification has an important functional importance as it transfers $NO_3^-$ to a less mobile mineral form of N [191, 296]. Thus, the mobile $NO_3^-$-N that is prone to leach or to be denitrified to N-gases is retained in the ecosystem [191, 290, 296, 297]. Studies with $^{15}N$-labeled $NO_3^-$ in reducing sediments showed the simultaneous release of $NH_4^+$ and $N_2O$ via dissimilatory pathways, with $NH_4^+$ accounting typically for more than 90% of the released products [288]. Nitrate ammonification might act as a detoxification mechanism for $NO_2^-$, tolerating rather high concentrations of mM concentrations of $NO_2^-$ [298–300]. Nitrate ammonification is also a better electron sink than denitrification per mol of $NO_3^-$ (Equations 4.10–4.13, 4.15, and 4.16), allowing the organisms to efficiently regenerate their oxidants needed for survival, thus explaining the importance of nitrate ammonification in reduced environments.

**Rates of nitrate dissimilation and associated microbial diversity in permafrost environments**

Nitrate reduction and denitrification are important processes in permafrost-affected soils that emit the greenhouse gas $N_2O$, NO, and HONO (Tabs. 4.1 and 4.2). Recent studies showed that Arctic soils produce [301, 302] and release [21, 22] substantial amounts of $N_2O$. So-called cryoturbated peat circles in the discontinuous permafrost zone in the subarctic East European tundra emit $N_2O$ at exceptionally high rates throughout the growing season (1.9–31 mg $N_2O$ $m^{-2}$ $d^{-1}$) [22]. These peat circles, with an *in situ* pH around 4, lack vegetation and thus competition for nitrogen between plants and microorganisms. Coupled ammonification-nitrification reactions acting on old organic N-rich peat at the oxic/anoxic interface and an intermediate water content might explain high $NO_3^-$ concentration in peat circles, which is readily available for denitrification (Tab. 4.5), a main source of $N_2O$ under anoxic conditions [22, 42]. Studies with such peat circle and adjacent unturbated peat soil showed that *narG* abundance accounted for approximately 8% of the bacterial 16S rRNA genes in the cryoturbated peat circle soil [42]. *nirS* outnumbered *nirK* by up to three orders of magnitude, indicating a substantial role of *nirS*-type denitrifiers. The diversity of *nirS* was dominated by Alpha- and Betaproteobacteria, and the great majority of *nirK* sequences from both soil types affiliated with Alphaproteobacteria, although both *nirS* and *nirK* were only distantly related to *nirS* and *nirK* of cultured organisms [42]. Additionally, the *nirS* and *nirK* gene diversity of examined soils differed between sites. *nosZ* occurred at low frequencies in peat circles relative to *narG* and sequences were indicative of alphaproteobacterial *nosZ* (*Mesorhizobium* sp.). 60% of *nosZ* were only distantly related to *nosZ* of cultured microorganisms indicating a new, specific, and acid-tolerant denitrifier community capable of $N_2O$ reduction in these soils [42]. In addition to low pH, an electron donor limitation might favor $N_2O$ production in these peat soils, since $NO_3^-$ is not a limiting factor [42, 304]. In contrast,

unturbated vegetated peat soils from the same study site with the same acidic pH do not essentially emit $N_2O$ *in situ* [21, 22] (Tab. 4.1). Phylogenetic data show that denitrifier communities differ between bare cryoturbated and vegetated unturbated peat soils, correlate with denitrification potentials, and are likely accountable (along with AOA [173]; see Section 4.2.2, "Rates of nitrification and associated nitrifier diversity in permafrost environments" section) for contrasting $N_2O$ emissions between soils [21, 22 42]. New nitrate reducers were isolated, including two new *Caballeronia* strains hosting multiple nitrate reductases [303]. A huge quantitative imbalance between the genetic potential for dissimilatory nitrate relative to $N_2O$ reduction as indicated by *narG* outnumbering *nos*Z might contribute to the high $N_2O$ fluxes from peat circles.

Likewise, *narG* was abundant and accounted for 1–5% of bacterial 16S rRNA genes in a vegetated palsa peat (pH 4–5) in Finland [214], showing that a substantial amount of bacteria is capable of dissimilatory reduction of available $NO_3^-$ in these soils. However, denitrification potentials were low compared to those from peat circles. Most of the *narG* sequences were associated with *Actinosynema* sp. of the Actinobacteria, and Alphaproteobacteria related to *Oligotropha* sp. *nirK* were likewise indicative of Alphaproteobacteria (distantly related to *Methylobacterium* sp., *Mesorhizobium* sp., and uncultured taxa), although more diverse. The gene *nirS* was indicative of Beta- and Gammaproteobacteria affiliating primarily with uncultured taxa. The *nosZ* community as well mainly affiliated with alphaproteobacterial and betaproteobacterial *nosZ*, clustering with *Bradyrhizobium japonicum* and *Azospirillum lipoferum* [214]. The nitrate reducer community including denitrifiers was clearly different from that of the peat circles/unturbated vegetated peat, and $N_2O$ emissions from palsa peat were much lower, indicating that the nitrate reducer community including denitrifiers might be a key factor in terms of $N_2O$ metabolism in permafrost-affected peat soils [214].

Arctic thermokarst bog, the active layer and permafrost (pH 4.5–6) soil near Fairbanks, Alaska, showed generally a low expression of denitrification associated genes along with low denitrification potentials as indicated by metaproteomics, metatranscriptomics, and metagenomics [79]. Nar encoding genes were generally more abundant than genes encoding for all other denitrification associated reductases, and Nos encoding genes again were the least abundant. Metagenomics likewise indicated the presence of denitrification associated genes in black-spruce forest soil, suggesting a higher genetic potential for dissimilatory nitrate reduction to nitrite (*nar*) than nitrite reduction to N-gases (*nir*, *nor*) or ammonium (*nrf*) [72]. Low frequencies of *nos* suggested an even lower potential for $N_2O$ consumption than denitrification or nitrate ammonification (*nrf*). Similar findings were obtained for Arctic polygons by metagenomics and correlated with low $N_2O$ production potentials [80]. Almost complete metagenome assembled genomes affiliating with Actinobacteria, Bacteroidetes, and Verrucomicrobia had a high likelihood to host truncated denitrification pathways along with genes for short chain organic acid and alcohol catabolism, suggesting that such organisms are important players in Arctic polygons [80].

**Tab. 4.5:** Denitrification rates and one nitrate ammonification rate of various permafrost-affected sites as determined by laboratory incubations or in field studies.

| Laboratory process rates | *In situ* process rates [µg N $m^{-2}$ $h^{-1}$] | Soil moisture/ water content | pH | C/N ratio | Site descriptor | | Region | Coordinates | Study |
|---|---|---|---|---|---|---|---|---|---|
| 1.99–4.83 µg N $g_{DW}^{-1}$ $h^{-1}$ | 1.58 × $10^{2}$–2.58 × $10^{3a,b}$ | 0.64 $dm^3$ $dm^{-3}$ VWC[c] | 4 | 21–24[c] | Cryoturbated peat circle | Tundra | Seida/Vorkuta, Russia | 67°03’N, 62°57’E | [42] |
| 4.58 × $10^{-4}$–1.8 × $10^{-3}$ µg N $g_{DW}^{-1}$ $h^{-1}$ | –1.76–0.88[a] | 74% SMC | 4.2–4.6 | 26–29 | Vegetated palsa peat | Peat | Northwestern Finnish Lapland | 69°49’13’’N, 27°9’47’’E | [214] |
| n.a. | 0.70–1.06 (mean)[d] | 326.7–853.6% SMC | 5.6[e] | n.a. | Sedge meadow hummock | Coastal Lowland | High Canadian Arctic | 75°33’N, 84°40’W | [165] |
| n.a. | 3.07–4.13 (mean)[d] | 295.1–520.5% SMC | 5.9[e] | n.a. | Willow herb hummocks | | | | |
| n.a. | 0.09–0.18[d] | 0.12 g $g^{-1}$ soil moisture (mean) | 7.5 | n.a. | Raised beach crest cryosol | Landscape zone | Canadian High Arctic | 75°40’N, 84°35’W | [167] |
| n.a. | 0.25[d] | 0.66 g $g^{-1}$ soil moisture (mean) | 6.7 | | Lower foreslope cryosol | | | | |
| n.a. | –0.01–0.71[d] | 6.48 g $g^{-1}$ soil moisture (mean) | 6.9 | | Wet sedge meadow cryosol | | | | |
| 1.34 × $10^{-5}$–7.07 × $10^{-4}$ µg N $l^{-1}$ $h^{-1}$[d] | n.a. | n.a. | n.a. | 7.9–12.3 | Sediment | Coastal sediment | East and West Greenland | 69°17.2’N, 53°54.2’W–77°37.0’N, 07°38.6’W | [335] |
| 0–0.06 µg N $l^{-1}$ $h^{-1}$[d] | n.a. | n.a. | n.a. | n.a. | Sea ice cores | Fjord | Young Sound, Northeastern Greenland fjord | 74°18.59’N, 20°15.04’W | [337] |
| 0–0.20 µg N $l^{-1}$ $h^{-1}$[d] | n.a. | n.a. | n.a. | n.a. | Ice floats | Sea | Greenland Sea | 79°21.16’N, 11°08.20’W | |

**Tab. 4.5** (continued)

| Laboratory process rates | *In situ* process rates [μg N $m^{-2}$ $h^{-1}$] | Soil moisture/ water content | pH | C/N ratio | Site descriptor | | Region | Coordinates | Study |
|---|---|---|---|---|---|---|---|---|---|
| 21.59 μg N $m^{-2}$ $h^{-1}$ (mean)[d] | n.a. | n.a. | n.a. | n.a. | Sea ice cores | Bay | Franklin Bay, Canada | 70°02N, 126°18'W | [384] |
| 1.69 × $10^2$ μg N $m^{-2}$ $h^{-1}$ (mean)[d] | n.a. | n.a. | n.a. | n.a. | Sediment | Fjord | Smeerenburgfjorden, Svalbard, Norway | 79°42.01'N, 11°05.20'E | [307] |
| 19.84 μg N $m^{-2}$ $h^{-1}$ (mean)[d] | n.a. | n.a. | n.a. | n.a. | Sediment | Fjord | Kongsfjorden, Svalbard, Norway | 78°59.43'N, 12°17.87'E | [307] |
| 14.01–3.35 × $10^2$ μg N $m^{-2}$ $h^{-1}$[d] | n.a. | n.a. | n.a. | 8.2–9.6 | Sediment | Shoal | Hanna Shoal, Northwest Alaska | 71.3°–72.1°N, 158.3°–165.5°W | [306] |
| (Nitrate ammonification 0.00–9.94 μg N $m^{-2}$ $h^{-1}$[d]) | | | | | | | | | |

For more details, please refer to the references provided.

n.a. – not applicable, SMC – soil moisture content, C/N – carbon to nitrogen ratio, DW – dry weight, VWC – volumetric water content.

[a] Gao et al., 2019 [363].

[b] Ma et al., 2007 [167].

[c] Repo et al., 2009 [22].

[d] Data converted from original paper.

[e] Bliss and Gold, 1994 [383].

In the High Arctic of Canada (Axel Heiberg Island), $N_2O$ emissions were reported from high centered ice-wedge polygons. Quantification of gene markers, together with $N_2O$ gas flux data, suggests $N_2O$ production predominantly in the upper 5 cm of trough soil [81]. This study with Arctic mineral ice-wedge polygon cryosols of the Canadian High Arctic showed that *nirS* abundance as determined by quantitative PCR was significantly dependent on the sampled location, trough, or polygon interior soil, but not soil depth [81]. Diverse *nirS* genes were retrieved via targeted amplicon sequencing and clustered primarily with *nirS* genes of uncultured microorganisms. Diversity in terms of relative abundance of *nirS* derived operational taxonomic units was primarily determined by location and to a lesser extent by soil depth [81]. Bacteria at both locations as indicated by *nirS* and 16S rRNA were related to known denitrifying bacterial members of the genera *Thiobacillus*, *Denitrovibrio*, *Pseudomonas*, *Azospirillum*, and *Azorhizobium* and many uncultured organisms [81]. Dissimilar $N_2O$ emissions of trough or polygon interior were related to dissimilar *nirS* communities, suggesting that the topology impacts microbial communities and greenhouse gas emissions. Metatranscriptomics from sites in Svalbard showed a clear depth dependence of denitrifiers, with the number of transcripts associated with denitrification decreasing over depth [305].

Cloned *nosZ* genes retrieved from soils emitting $N_2O$ (−0.01–0.71 μg N $m^{-2}$ $h^{-1}$; Tab. 4.1) of the Canadian High Arctic, Devon Island, were affiliated with Alpha- and Betaproteobacteria and showed only minor similarities (≤83%) to known sequences in the database at that time and differed from denitrifier communities in temperate or Antarctic environments. Such *nosZ* clustered with *Achromobacter* spp., *Sinorhizobium* spp., and *Azospirillum* spp. [167]. The abundance of *nosZ* determined for different landforms was in the range of $10^5$ copies per gram soil, suggesting some potential for $N_2O$ consumption. Indeed, the contribution of denitrifiers to emitted $N_2O$ from examined soils ranged only from 3 to 18%, with the majority of $N_2O$ coming from AOB [167] (see Section 4.2.3, "Rates of nitrification and associated nitrifier diversity in permafrost environments" section).

Reported nitrate ammonification rates from sediment of five stations at Hanna Shoal, Alaska, measured via isotope pairing techniques revealed average nitrate ammonification rates of 0.23 ± 0.05 μmol N $m^{-2}$ $h^{-1}$, contributing only little to the overall sediment $NH_4^+$ turnover and thus suggesting that nitrate ammonification is not quantitatively important in these sediments [306] (Tab. 4.5). Another attempt to measure nitrate ammonification in Arctic fjord sediments of Svalbard by Gihring et al. was not successful [307]. Generally, few studies on nitrate ammonification in permafrost systems are available. Nevertheless, the genetic potential for nitrate ammonification was detected previously by metagenomics [80].

Collective data on nitrate dissimilating processes in permafrost-affected soils suggest that denitrification is more prominent than nitrate ammonification. A moderately diverse denitrifier community is inherent to many different Arctic environments, with novel not yet cultured microorganism. Denitrifier community and an imbalance

of the genetic potential for nitrate reduction and $N_2O$ consumption are microbial parameters contributing to large $N_2O$ emissions.

### 4.2.4 Anaerobic ammonia oxidation

Considering the classical view on the nitrogen cycle, oxygen was considered to be essential for $NH_4^+$ oxidation. Evidence for an anaerobic, oxygen-sensitive ammonia oxidation was first obtained from denitrifying bioreactors of wastewater treatment plants, 30 years after it was first proposed [308]. Isolation approaches have not yet been successful for anammox organisms, but highly purified enrichment cultures exist.

#### Physiology and diversity of Anaerobic Ammonia Oxidizers

The anaerobic ammonium oxidation (anammox) of $NH_4^+$ with $NO_2^-$ to $N_2$ gas via the intermediates NO and hydrazine ($N_2H_4$) is performed by bacteria of the order Planctomycetes [309] (Equations 4.17–4.20). Bacteria capable of anammox are found within five genera of the Planctomycetes: *Candidatus* Anammoxoglobus, *Candidatus* Brocadia, *Candidatus* Jettenia, *Candidatus* Kuenenia, and *Candidatus* Scalindua and classically considered to have an autotrophic lifestyle [309–312]. The energy from oxidation-reduction reactions of $NO_2^-$ and $NH_4^+$ is used to assimilate $CO_2$ and for growth [41]. However, there are anammox bacteria, which are capable of coupling of the oxidation of organic substances like propionate and $NH_4^+$ to the reduction of $NO_3^-$ to $NO_2^-$ [310, 313]. With doubling times of 10 to 20 days, anammox bacteria are generally extremely slow growers, even at 35°C and high substrate concentrations [309]. Only recently, doubling times were dramatically minimized to 3.3 days in bioreactors [314].

After $NO_2^-$ is reduced to NO, hydrazine synthase (HZS) converts NO together with $NH_4^+$ to hydrazine ($N_2H_4$). $NH_2OH$ might be formed as a side product. The enzyme hydrazine dehydrogenase (HDH) then further oxidizes $N_2H_4$ to $N_2$ [315] (Equations 4.17–20).

$$NO_2^- + 2H^+ + e^- \xrightarrow{\text{NIR}} NO + H_2O \tag{4.17}$$

$$NO + NH_4^+ + 2H^+ + 3e^- \xrightarrow{\text{HZS}} N_2H_4 + H_2O \quad (NH_2OH \text{ as intermediate}) \tag{4.18}$$

$$N_2H_4 \xrightarrow{\text{HDH}} N_2 + 4H^+ + 4e^- \tag{4.19}$$

$$NH_2OH \xrightarrow{\text{HAO–like HOX}} NO + 3H^+ + 3e^- \tag{4.20}$$

Here, NIR is nitrite reductase; HZS, hydrazine synthase; HDH, hydrazine dehydrogenase; and HAO-like HOX, HAO-like hydroxylamine oxidase.

The enzymes HZS and HDH are conserved in all known anammox genera [316]. An octaheme HAO that performs the reverse oxidation of $NH_2OH$ to NO, i.e. hydroxylamine oxidase (HOX), is also present in all anammox bacteria [113, 316, 317] (Equation 4.20). The microbial formation of NO is catalyzed by Cu-NIR or $cd_1$-NIR [315, 318, 319]. Whilst the *cd1*-NIR is encoded in the anammox species *Candidatus* "Kuenenia stuttgartiensis" and *Ca*. "Scalindua profunda" [320–322], the Cu-NIR is encoded in *Ca*. Jettenia spp. [323] and a yet unknown nitrite reductase is encoded in *Ca*. Brocadia spp. [324]. Recent studies suggest that the trait of nitrate reduction in anammox bacteria was acquired after the central catabolism of this pathway was established [315]. This is supported by the findings that the oxidation of $NH_4^+$ is coupled stoichiometrically to the reduction of NO, when $NO_2^-$ is absent; $NO_2^-$ is not required for growth under such conditions [315]. Interestingly, anammox bacteria do not detoxify NO to $N_2O$ under saturated NO conditions. Only 0.1% of consumed NO was converted to $N_2O$, which would suggest an efficient conversion of NO to $N_2$ [315]. Therefore, anammox bacteria have the potential to counteract the emission of the greenhouse gas $N_2O$ by scavenging the $N_2O$ precursor NO and to contribute to the control of both, NO and $N_2O$ emissions, in ecosystems.

The anammox process occurs in a unique specialized intracytoplasmic organelle, called the anammoxosome [309]. This organelle is membrane bound and comprises up to 60% of the cell volume [325]. The membrane of the compartment consists of another unique feature of anammox bacteria, the ladderane lipids, which form a dense barrier [326] that might protect the bacteria against the toxic intermediates $NH_2OH$ and $N_2H_4$ occurring during the anammox process [327]. The unique ladderanes have also been used as biomarkers for the presence of anammox bacteria [328].

Anammox bacteria are mainly found in marine environments [329] but also in freshwater systems [330]. The anaerobic anammox process depends on the presence of both oxidized and reduced inorganic nitrogen compounds; therefore, habitats with oxic/anoxic interfaces might be suitable for anammox bacteria [331]. Anammox activity was also shown in certain terrestrial environments, and detected anammox bacteria affiliated with the genera *Ca*. Brocadia and *Ca*. Kuenenia [331, 332]. In peat soil, the presence of anammox bacteria of the genera *Ca*. Brocadia and *Ca*. Jettenia was detected and shown to be strongly influenced by the slow release of organic matter like humic acids [333]. Thus, a contribution to N-cycling in peat of permafrost-affected systems is anticipated.

#### Rates of anaerobic ammonia oxidation and associated microbial diversity in permafrost environments

Indeed, anammox bacteria occur in permafrost systems, although low temperatures and low $NH_4^+$ as well as low $NO_2^-$ concentrations do not appear to be ideal for their growth [41]. Anammox bacteria were confirmed via PCR in alpine permafrost from Creux-du-Van, in the Swiss Jura, and 16S rRNA gene sequences retrieved were

affiliated with *Candidatus* Jettenia [331]. Sequences with >96% nucleotide identity to *Candidatus* Scalindua were detected in permafrost samples from Siberia [334]. Genetic potentials for anammox were complemented by process potentials in other systems (Tab. 4.6). In soil slurry experiments with Arctic coastal marine sediments from Greenland (–1.7 to 4.0°C), anammox accounted for up to 35% of total $N_2$ production, and anammox rates ranged from 1 to 92 µmol N $m^{-2}$ $d^{-1}$ [335] (Tab. 4.6). A strong correlation between location-specific anammox activity and bottom-water $NO_3^-$ concentrations was found, indicating that $NO_3^-$ concentrations in bottom-water might indirectly control anammox activity, which in turn are controlled by denitrifiers. Support for this conclusion came from a significant correlation between area-based anammox activity and denitrification activities [335]. Thus, anammox might contribute to a significant portion of $N_2$ production in cold marine sediments [335, 336]. In deeper layers of a multilayer sea ice floor from a Greenland fjord, the contribution of anammox to total $N_2$ production was up to 19%, but below detection limit in upper layers or annual sea ice [337]. This might be due to the more stable environment of deeper layers of multilayered sea ice, which the slow-growing anammox bacteria might favor [337, 338]. Diverse studies showed high amounts of organic and inorganic nitrogen stored in deep layers of permafrost [302, 339, 340], and anammox bacteria were shown to occur in ice and alpine permafrost. Thus, it is likely to find bacteria capable of anammox in deep permafrost, where environmental conditions are stable; however, future studies are needed to confirm this hypothesis [341].

### 4.2.5 Ammonification

Microbial mineralization of N containing organic compounds to their mineral constituents, including $CO_2$ and $NH_4^+$, is a key process determining the quantity of nitrogen that is available to the soil microbial community and plants from organic matter recycling. The conversion of organic nitrogen into inorganic nitrogen is known as ammonification [342] and releases $NH_4^+$-N [343, 344] (Equation 4.21, urea-mineralization as simplified example).

#### Physiology and diversity of ammonifiers

The ability to ammonify is widely distributed among heterotrophic microorganisms [345, 346], and several factors are known to influence ammonification. A major controlling factor of ammonification is temperature, as the process is primarily an enzymatic decomposition of organic nitrogen [342]. An increase of 10°C can lead to a doubling rate of ammonification [347]. Additionally, ammonification is influenced by pH, which was shown to be ideal between 6.5 and 8.5 [348]. Another well-known factor to influence ammonification is the C/N ratio. The greater the C/N ratio, the slower the process becomes. An explanation for this might be concurrent immobilization processes (i.e. assimilation) that outcompete or mask ammonification [349].

**Tab. 4.6:** Rates of anaerobic ammonia oxidation of various permafrost-affected sites as determined by laboratory incubations or in field studies.

| Laboratory process rates | C/N ratio | Soil type | Site descriptor | | Coordinates | Study |
|---|---|---|---|---|---|---|
| $2.33 \times 10^{-6}$ –$2.12 \times 10^{-4}$ µg N $l^{-1}$ $h^{-1}$[a] | 7.1–17.3 | Sediment | Coastal sediment | East and West Greenland | 69°17.2'N, 53°54.2'W–77°37.0'N, 07°38.6'W | [335] |
| 43.77 µg N $m^{-2}$ $h^{-1}$ (mean)[a] | n.a. | Sea ice cores | Bay | Franklin Bay, Canada | 70°02N, 126°18'W | [384] |
| 8.75 µg N $m^{-2}$ $h^{-1}$ (mean)[a] | n.a. | Sediment | Fjord | Smeerenburgfjorden, Svalbard, Norway | 79°42.01'N, 11°05.20'E | [307] |
| 5.84 µg N $m^{-2}$ $h^{-1}$ (mean)[a] | n.a. | Sediment | Fjord | Kongsfjorden, Svalbard, Norway | 78°59.43'N, 12°17.87'E | [307] |
| 0.84–5.74 µg N $m^{-2}$ $h^{-1}$[a] | 8.2–9.6 | Sediment | Shoal | Hanna Shoal, Northwest Alaska | 71.3°–72.1°N, 158.3°–165.5°W | [306] |

For more details, please refer to the references provided.

n.a. – not applicable, C/N – carbon to nitrogen ratio.

[a] Data converted from original paper.

$$CO(NH_2)_2 + H_2O \xrightarrow{URE} 2NH_3 + CO_2 \quad (4.21)$$

URE here indicates urease.

The largest pool of soil organic N is made up by amino acids [350]. Amino acids are catabolized by ammonification, which presumably includes several deamination reactions. Oxidative and reductive deaminations are possible options. Oxidative and reductive deamination are likely prominent in oxidized and reduced soil environments, respectively [342, 351]. The degradation of amino acids is intracellular, might rely on extracellular enzymes such as proteases, and is closely related to N mineralization in soil [352]. This is supported by high affinity kinetics ($K_M$ value ~ 50 µM), which reflect an intracellular microbial enzyme activity rather than an activity associated with the soil matrix [352]. In order to measure soil gross N mineralization by microorganisms, Alef and Kleiner proposed in 1986 [353] an arginine ammonification assay whereby $NH_4^+$ production and $O_2$ respiration are measured after arginine was added at saturating concentration. The simple-to-use arginine ammonification assay was compared to a $^{15}N$-$NH_4^+$ isotope dilution technique measuring gross N mineralization in different crop soils and showed reliable results in the examined soils, reflecting not only seasonal patterns but also short-term fluctuations of activity in the different soils [352].

**Ammonification associated diversity in permafrost environments**

Even at low temperatures, such as in the Arctic, high $NH_4^+$-N concentrations suggest high microbial activity, and $NH_4^+$-N concentrations may be used as indicators for organic matter mineralization in cold regions [354–356]. $NH_4^+$ and other inorganic N species were found in permafrost soils in a study across the Siberian Arctic with 11 soil profiles from different study sites, including peat and pure ice, demonstrating that these soils store significant amounts of inorganic nitrogen in the frozen ground. Thereby, higher amounts of inorganic N were detected in frozen parts of the permafrost soils relative to the active layer [339]. Other studies with permafrost-affected soils from Sweden and Greenland showed similar accumulations of $NH_4^+$ [302, 340]. Mineralization rates from two sites in the Canadian High Arctic, a sedge meadow and a willow-herb site, ranged from approximately 100 to 150 mg N $m^{-2}$ $d^{-1}$ during growing season and did not differ significantly between examined sites and depths [165] (Tab. 4.7). Results indicate that nitrogen mineralization is an important source of inorganic N in Arctic ecosystems [165]. Genes that were used as markers for ammonification from organic N (e.g. urease encoding *ureC;* Equation 4.21) were essentially as abundant as nitrate reduction associated genes in all soils analyzed, suggesting a widely distributed organic N-mineralization potential [80]. Studies on microbial diversity associated with ammonification is generally difficult, as most heterotrophic and also some autotrophic microbes possess the capabilities to ammonify, organo-N compounds are highly diverse, and genes involved in ammonification are not specific

**Tab. 4.7:** Mineralization (ammonificaton) rates of organic nitrogen for various permafrost-affected sites as determined by laboratory incubations or in field studies.

| Laboratory process rates | *In situ* process rates [µg N $m^{-2}$ $h^{-1}$] | Soil moisture/ water content | pH | C/N ratio | Site descriptor | | Region | Coordinates | Study |
|---|---|---|---|---|---|---|---|---|---|
| –0.11–0.14 µg N $g_{DW}^{-1}$ $h^{-1}$ | n.a. | 66.5–87.8% SMC | n.a. | 12–44 | Fen channels, sedge lawns, peat plateau, thermokarst | Peat | Manitoba, Canada | 58°48'N, 94°,09'W | [382] |
| n.a. | 4.24 × $10^3$–5.14 × $10^3$ (mean)[a]<br>5.70 × $10^3$–6.27 × $10^3$ (mean)[a] | 326.7–853.6% SMC<br>295.1–520.5% SMC | 5.6[b]<br>5.9[b] | n.a. | Sedge meadow hummock<br>Willow herb hummocks | Coastal Lowland | High Canadian Arctic | 75°33'N, 84°40'W | [165] |
| n.a. | 11.42–57.08[a] | 65–553% SMC | 4.2–6.8 | 14.2–27.4 | Tussock and wet sedge tundra, heath, willow | Toposequence | Sagavanirktok River, Alaska | 68°46'40''N, 148°51'8''W | [166] |
| 14.58–33.33 µg N $g^{-1}$ N $h^{-1}$ (mean)[c] | n.a. | n.a. | 5.2–6.3 | 13–19.1 | Heath tundra | Tundra | Eastern Greenland | 74°29'N, 20°32'W | [385] |
| 10.42–87.50 µg N $g^{-1}$ N $h^{-1}$ (mean)[c] | | | 4.1–6.1 | 11.7–29.1 | Shrub tundra | | North-Eastern Siberia | 68°45'N, 161°36'E | |
| 4.17–16.67 µg N $g^{-1}$ N $h^{-1}$ (mean)[c] | | | 5.1–5.9 | 13.9–24.6 | Tussock tundra | | North Eastern Siberia | 69°26'N, 161°44'E | |

For more details, please refer to the references provided.

n.a. – not applicable, SMC – soil moisture content, C/N – carbon to nitrogen ratio, DW – dry weight.

[a] Data converted from original paper.

[b] Bliss and Gold, 1994 [382].

[c] Data extracted/estimated from figure.

for the process. Thus, isolation and characterization of isolates are a suitable approach for studying ammonifiers. Isolates of oligotrophic soil microorganisms, like members of the genera *Mycobacterium* and *Streptomyces* (both Actinobacteria), from the upper layers of permafrost-affected soils have been tested positive for ammonification by plating soil dilutions on meat-peptone agar. Such strains are known to survive with low nutrient availability [357, 358]. Highest abundances of ammonifying bacteria in a cryosolic tundra soil were found in the lower horizons, between 30 and 40 cm [357]. This was explained by humic substances found in the lower soil horizons needed as organic substrates [357]. Knowledge on ammonifiers and ammonification in permafrost systems is highly limited.

## 4.3 Conclusions

The role of permafrost systems as important reservoirs of organic as well as inorganic nitrogen and as a source of nitrogenous greenhouse gases deserves attention. According to the International Panel of Climate Change, the air temperature in the Arctic and Antarctic might increase by 5–6°C, on average, by the end of this century [5]. This would result in big changes of such environments, including permafrost thaw. Studies showed that a substantial amount of inorganic N could be released upon permafrost thaw, and for now, the impact on the ecosystem cannot be foreseen [339]. However, permafrost soils are a large reservoir of hitherto undetected, new microbes; numerous microbial key players of permafrost soils and their ecophysiology are currently unknown. Thus, it is inevitable that such knowledge gaps are closed. A deeper understanding of microbial potentials and communities associated with the N-cycle, their regulation and ecophysiology as well as systematic field studies is urgently needed to better quantify the effects of future permafrost development. Interdisciplinary collaboration spanning many different scales, from single organism to landscapes, will support such an endeavor.

## References

[1] Francis CA, Beman JM, Kuypers MMM. New processes and players in the nitrogen cycle: the microbial ecology of anaerobic and archaeal ammonia oxidation. *ISME J*. 2007;1:19–27.
[2] Kuypers MMM, Marchant HK, Kartal B. The microbial nitrogen-cycling network. *Nat Rev Microbiol*. 2018;16(5):263–76.
[3] Forster P, Ramaswamy V, Artaxo P, et al. Changes in atmospheric constituents and in radiative forcing. In: Solomon S, Qin D, Manning M, et al., editors. Climate change 2007: the physical science basis. Contribution of Working Group I to the fourth assessment report of the Intergovernmental Panel on Climate Change. Cambridge: Cambridge University Press; 2007. p. 129–234.
[4] Ravishankara AR, Daniel JS, Portmann RW. Nitrous oxide ($N_2O$): The dominant ozone-depleting substance emitted in the 21st century. *Science*. 2009;326(5949):123–5.

[5] Stocker T. F, Qin D, Plattner G-K, et al. IPCC 2018. Climate change 2013: the physical science basis. Contribution of Working Group I to the fifth assessment report of the Intergovernmental Panel on Climate Change. 2018. Available from: https://www.ipcc.ch/report/ar5/wg1/
[6] Wu D, Horn MA, Behrendt T, et al. Soil HONO emissions at high moisture content are driven by microbial nitrate reduction to nitrite: tackling the HONO puzzle. *ISME J*. 2019;13:1688–99.
[7] Behera SN, Sharma M, Aneja VP, Balasubramanian R. Ammonia in the atmosphere: a review on emission sources, atmospheric chemistry and deposition on terrestrial bodies. *Environ Sci Pollut Res*. 2013;20(11):8092–131.
[8] Kirschke S, Bousquet P, Ciais P, et al. Three decades of global methane sources and sinks. *Nat Geosci*. 2013;6(10):813–23.
[9] Gligorovski S, Strekowski R, Barbati S, Vione D. Environmental implications of hydroxyl radicals (•OH). *Chem Rev*. 2015;115(24):13051–92.
[10] Su H, Cheng Y, Oswald R, et al. Soil nitrite as a source of atmospheric HONO and OH radicals. *Science*. 2011;333(6049):1616–8.
[11] Kulmala M, Petäjä T. Soil nitrites influence atmospheric chemistry. *Science*. 2011;333(6049):1586–7.
[12] Kleffmann J, Gavriloaiei T, Hofzumahaus A, et al. Daytime formation of nitrous acid: A major source of OH radicals in a forest. *Geophys Res Lett*. 2005;32(5):L05818.
[13] Atkinson R. Atmospheric chemistry of VOCs and NOx. *Atmos Environ*. 2000;34(12):2063–101.
[14] Spahni R, Chappellaz J, Stocker TF, et al. Atmospheric methane and nitrous oxide of the late pleistocene from Antarctic ice cores. *Science*. 2005;310(5752):1317–21.
[15] Montzka SA, Dlugokencky EJ, Butler JH. Non-$CO_2$ greenhouse gases and climate change. *Nature*. 2011;476(7358):43–50.
[16] Denman KL, Brasseur G, Chidthaisong A, et al. Couplings between changes in the climate system and biogeochemistry. In: Solomon S, Qin D, Manning M, et al., editors. Climate change 2007: the physical science basis. Contribution of Working Group I to the fourth assessment report of the Intergovernmental Panel on Climate Change. Cambridge: Cambridge University Press; 2007. p. 499–587.
[17] Mosier A, Kroeze C, Nevison C, Oenema O, Seitzinger S, van Cleemput O. Closing the global $N_2O$ budget: Nitrous oxide emissions through the agricultural nitrogen cycle – OECD/IPCC/IEA phase II development of IPCC guidelines for national greenhouse gas inventory methodology. *Nutr Cycl Agroecosystems*. 1998;52(2–3):225–48.
[18] Conrad R. Soil microorganisms as controllers of atmospheric trace gases ($H_2$, CO, $CH_4$, OCS, $N_2O$, and NO). *Microbiol Rev*. 1996;60(4):609–40.
[19] Behrendt T, Agam N, Horn MA. Microbial nitric oxide, nitrous oxide, and nitrous acid emissions from drylands. In: D'Odorico P, Porporato A, Wilkinson Runyan C, editors. Dryland ecohydrology. Cham: Springer International Publishing; 2019. p. 335–65.
[20] Christensen TR. Climate science: patchy peat. *Nat Geosci*. 2009;2(3):163–4.
[21] Marushchak ME, Pitkämäki A, Koponen H, et al. Hot spots for nitrous oxide emissions found in different types of permafrost peatlands. *Glob Chang Biol*. 2011;17(8):2601–14.
[22] Repo ME, Susiluoto S, Lind SE, et al. Large $N_2O$ emissions from cryoturbated peat soil in tundra. *Nat Geosci*. 2009;2(3):189–92.
[23] Steinkamp J, Lawrence MG. Improvement and evaluation of simulated global biogenic soil NO emissions in an AC-GCM. *Atmos Chem Phys*. 2011;11(12):6063–82.
[24] Gil J, Pérez T, Boering K, Martikainen PJ, Biasi C. Mechanisms responsible for high $N_2O$ emissions from subarctic permafrost peatlands studied via stable isotope techniques. *Global Biogeochem Cycles*. 2017;31(1):172–89.
[25] Anisimov OA. Potential feedback of thawing permafrost to the global climate system through methane emission. *Environ Res Lett*. 2007;2(4).

[26] Tarnocai C, Canadell JG, Schuur EAG, Kuhry P, Mazhitova G, Zimov S. Soil organic carbon pools in the northern circumpolar permafrost region. *Global Biogeochem Cycles*. 2009;23.
[27] Post WM, Pastor J, Zinke PJ, Stangenberger AG. Global patterns of soil-nitrogen storage. *Nature*. 1985;317(6038):613–6.
[28] Harden JW, Koven CD, Ping CL, et al. Field information links permafrost carbon to physical vulnerabilities of thawing. *Geophys Res Lett*. 2012;39(15):1–6.
[29] Bouwman L, Goldewijk KK, Van Der Hoek KW, et al. Exploring global changes in nitrogen and phosphorus cycles in agriculture induced by livestock production over the 1900–2050 period. *Proc Natl Acad Sci*. 2013;110(52):20882–7.
[30] Limpens J, Berendse F, Blodau C, et al. Peatlands and the carbon cycle: From local processes to global implications – a synthesis. *Biogeosciences*. 2008;5(5):1475–91.
[31] Loisel J, Yu Z, Beilman DW, et al. A database and synthesis of northern peatland soil properties and Holocene carbon and nitrogen accumulation. *Holocene*. 2014;24(9):1028–42.
[32] Batjes N. Total carbon and nitrogen in the soils of the world. *Eur J Soil Sci*. 1996;47:151–63.
[33] Strauss J, Abbott B, Beermann F, et al. The nitrogen stock of the ice-rich yedoma domain. In: Deline P, Bodin X, Ravanel L, editors. 5th European Conference on Permafrost. Chamonix Mont-Blanc, France: Laboratoire EDYTEM, CNRS, Université Savoie Mont-Blanc; 2018. p. 784–5.
[34] Nordin A, Schmidt IK, Shaver GR. Nitrogen uptake by arctic soil microbes and plants in relation to soil nitrogen supply. *Ecology*. 2004;85(4):955–62.
[35] Shaver GR, Chapin III FS. Response to fertilization by various plant growth forms in an Alaskan tundra: nutrient accumulation and growth. *Ecology*. 1980;61(3):662–75.
[36] Voigt C, Marushchak ME, Lamprecht RE, et al. Increased nitrous oxide emissions from Arctic peatlands after permafrost thaw. *Proc Natl Acad Sci*. 2017;114(24):6238–43.
[37] Voigt C, Lamprecht RE, Marushchak ME, et al. Warming of subarctic tundra increases emissions of all three important greenhouse gases – carbon dioxide, methane, and nitrous oxide. *Glob Chang Biol*. 2017;23(8):3121–38.
[38] Yang G, Peng Y, Marushchak ME, et al. Magnitude and pathways of increased nitrous oxide emissions from uplands following permafrost thaw. *Environ Sci Technol*. 2018;52(16):9162–9.
[39] Canfield DE, Glazer AN, Falkowski PG. The evolution and future of Earth's nitrogen cycle. *Science*. 2010;330(6001):192–6.
[40] Simon J. Enzymology and bioenergetics of respiratory nitrite ammonification. *FEMS Microbiol Rev*. 2002;26(3):285–309.
[41] Thamdrup B. New pathways and processes in the global nitrogen cycle. *Annu Rev Ecol Evol Syst*. 2012;43(1):407–28.
[42] Palmer K, Biasi C, Horn MA. Contrasting denitrifier communities relate to contrasting $N_2O$ emission patterns from acidic peat soils in arctic tundra. *ISME J*. 2012;6(5):1058–77.
[43] Mackelprang R, Saleska SR, Jacobsen CS, Jansson JK, Taş N. Permafrost meta-omics and climate change. *Annu Rev Earth Planet Sci*. 2016;44(1):439–62.
[44] Jansson JK, Taş N. The microbial ecology of permafrost. *Nat Rev Microbiol*. 2014;12(6): 414–25.
[45] Kalescky R, Kraka E, Cremer D. Identification of the strongest bonds in chemistry. *J Phys Chem A*. 2013;117(36):8981–95.
[46] Vitousek PM, Howarth RW. Nitrogen limitation on land and in the sea: how can it occur? *Biogeochemistry*. 1991;13:87.115.
[47] Tai Y-L, Dempsey BA. Nitrite reduction with hydrous ferric oxide and Fe(II): stoichiometry, rate, and mechanism. *Water Res*. 2009;43(2):546–52.
[48] Canfield DE, Kristensen E, Thamdrup B. The nitrogen cycle. In: Canfield DE, Kristensen E, Thamdrup B, editors. Advances in marine biology. Cambridge, MA, USA: Academic Press; 2005. p. 205–67.

[49] Gruber N, Galloway JN. An Earth-system perspective of the global nitrogen cycle. *Nature*. 2008;451(7176):293–6.
[50] Samarkin VA, Madigan MT, Bowles MW, et al. Abiotic nitrous oxide emission from the hypersaline Don Juan Pond in Antarctica. *Nat Geosci*. 2010;3(5):341–4.
[51] Schumann U, Huntrieser H. The global lightning-induced nitrogen oxides source. *Atmos Chem Phys*. 2007;7(14):3823–907.
[52] Vitousek PM, Menge DNL, Reed SC, Cleveland CC. Biological nitrogen fixation: rates, patterns and ecological controls in terrestrial ecosystems. *Philos Trans R Soc Lond B Biol Sci*. 2013;368(1621):20130119.
[53] Raymond J, Siefert JL, Staples CR, Blankenship RE. The natural history of nitrogen fixation. *Mol Biol Evol*. 2004;21(3):541–54.
[54] Reed SC, Cleveland CC, Townsend AR. Functional ecology of free-living nitrogen fixation: a contemporary perspective. *Annu Rev Ecol Evol Syst*. 2011;42(1):489–512.
[55] Hobara S, McCalley C, Koba K, et al. Nitrogen fixation in surface soils and vegetation in an Arctic tundra watershed: A key source of atmospheric nitrogen. *Arctic Antarct Alp Res*. 2006;38(3):363–72.
[56] Rousk K, Sorensen PL, Michelsen A. Nitrogen transfer from four nitrogen-fixer associations to plants and soils. *Ecosystems*. 2016;19(8):1491–504.
[57] Ludden PW. Nitrogenase complex. In: Encyclopedia of life sciences. Chichester, UK: John Wiley & Sons, Ltd; 2001.
[58] Simpson F, Burris R. A nitrogen pressure of 50 atmospheres does not prevent evolution of hydrogen by nitrogenase. *Science*. 1984;224(4653):1095–7.
[59] Eady RR. Structure–function relationships of alternative nitrogenases. *Chem Rev*. 1996;96(7):3013–30.
[60] Einsle O. Nitrogenase MoFe-protein at 1.16 Å resolution: a central ligand in the FeMo-cofactor. *Science*. 2002;297(5587):1696–700.
[61] Spatzal T, Aksoyoglu M, Zhang L, et al. Evidence for interstitial carbon in nitrogenase FeMo cofactor. *Science*. 2011;334(6058):940.
[62] Joerger RD, Bishop PE, Evans HJ. Bacterial alternative nitrogen fixation systems. *CRC Crit Rev Microbiol*. 1988;16(1):1–14.
[63] Miller RW, Eady RR. Molybdenum and vanadium nitrogenases of *Azotobacter chroococcum*. Low temperature favours $N_2$ reduction by vanadium nitrogenase. *Biochem J*. 1988;256(2):429–32.
[64] Hartmann LS, Barnum SR. Inferring the evolutionary history of Mo-dependent nitrogen fixation from phylogenetic studies of *nifK* and *nifDK*. *J Mol Evol*. 2010;71(1):70–85.
[65] Seefeldt LC, Hoffman BM, Dean DR. Mechanism of Mo-dependent nitrogenase. *Annu Rev Biochem*. 2009;78(1):701–22.
[66] Deslippe JR, Egger KN, Henry GHR. Impacts of warming and fertilization on nitrogen-fixing microbial communities in the Canadian High Arctic. *FEMS Microbiol Ecol*. 2005;53(1):41–50.
[67] Knorr K-H, Horn MA, Borken W. Significant nonsymbiotic nitrogen fixation in Patagonian ombrotrophic bogs. *Glob Chang Biol*. 2015;21(6):2357–65.
[68] Stewart KJ, Grogan P, Coxson DS, Siciliano SD. Topography as a key factor driving atmospheric nitrogen exchanges in Arctic terrestrial ecosystems. *Soil Biol Biochem*. 2014;70(3):96–112.
[69] Chapin DM, Bliss LC, Bledsoe LJ. Environmental regulation of nitrogen fixation in a High Arctic lowland ecosystem. *Can J Bot*. 1991;69(12):2744–55.
[70] Jordan DC, McNicol PJ, Marshall MR. Biological nitrogen fixation in the terrestrial environment of a High Arctic ecosystem (Truelove Lowland, Devon Island, N.W.T.). *Can J Microbiol*. 1978;24(6):643–9.
[71] Solheim B, Endal A, Vigstad H. Nitrogen fixation in Arctic vegetation and soils from Svalbard, Norway. *Polar Biol*. 1996;16(1):35–40.

[72] Taş N, Prestat E, McFarland JW, et al. Impact of fire on active layer and permafrost microbial communities and metagenomes in an upland Alaskan boreal forest. *ISME J*. 2014;8(9):1904–19.
[73] Stewart KJ, Coxson D, Grogan P. Nitrogen inputs by associative cyanobacteria across a Low Arctic tundra landscape. *Arctic Antarct Alp Res*. 2011;43(2):267–78.
[74] Zielke M, Solheim B, Spjelkavik S, Olsen RA. Nitrogen fixation in the High Arctic: role of vegetation and environmental conditions. *Arctic Antarct Alp Res*. 2005;37(3):372–8.
[75] Rousk K, Sorensen PL, Michelsen A. What drives biological nitrogen fixation in High Arctic tundra: moisture or temperature? *Ecosphere*. 2018;9(2):e02117.
[76] Sorensen PL, Lett S, Michelsen A. Moss-specific changes in nitrogen fixation following two decades of warming, shading, and fertilizer addition. *Plant Ecol*. 2012;213(4):695–706.
[77] Belnap J. Factors influencing nitrogen fixation and nitrogen release in biological soil crusts. In: Belnap J, Lange OL, editors. Biological soil crust: structure, function, and management. Berlin, Heidelberg, Germany: Springer; 2001. p. 241–61.
[78] Mackelprang R, Waldrop MP, DeAngelis KM, et al. Metagenomic analysis of a permafrost microbial community reveals a rapid response to thaw. *Nature*. 2011;480(7377):368–71.
[79] Hultman J, Waldrop MP, Mackelprang R, et al. Multi-omics of permafrost, active layer and thermokarst bog soil microbiomes. *Nature*. 2015;521(7551):208–12.
[80] Taş N, Prestat E, Wang S, et al. Landscape topography structures the soil microbiome in Arctic polygonal tundra. *Nat Commun*. 2018;9(1):777.
[81] Altshuler I, Ronholm J, Layton A, Onstott TC, W. Greer C, Whyte LG. Denitrifiers, nitrogen-fixing bacteria and $N_2O$ soil gas flux in High Arctic ice-wedge polygon cryosols. *FEMS Microbiol Ecol*. 2019;95(5):1–12.
[82] Davey A, Marchant HJ. Seasonal variation in nitrogen fixation by *Nostoc commune* vaucher at the Vestfold Hills, Antarctica. *Phycologia*. 1983;22(4):377–85.
[83] Henry GHR, Svoboda J. Dinitrogen fixation (acetylene reduction) in High Arctic sedge meadow communities. *Arct Alp Res*. 1986;18(2):181–7.
[84] Lennihan R, Chapin DM, Dickson LG. Nitrogen fixation and photosynthesis in High Arctic forms of *Nostoc commune*. *Can J Bot*. 1994;72(7):940–5.
[85] Chapin DM, Bledsoe CS. Nitrogen fixation in Arctic plant communities. In: Chapin III RS, Jefferies RL, Reynolds JF, Shaver GR, Svoboda J, editors. Arctic ecosystems in a changing climate: an ecophysiological perspective. San Diego: Academic Press; 1992. p. 301–19.
[86] Alexander V, Schell DM. Seasonal and spatial variation of nitrogen fixation in the Barrow, Alaska, Tundra. *Arct Alp Res*. 1973;5(2):77.
[87] Granhall U, Lid-Torsvik V. Nitrogen fixation by bacteria and free-living blue-green algae in tundra areas – Fennoscandian tundra ecosystems: part 1 plants and microorganisms. In: Wielgolaski FE, editor. Berlin, Heidelberg: Springer Berlin Heidelberg; 1975. p. 305–15.
[88] Stewart KJ, Lamb EG, Coxson DS, Siciliano SD. Bryophyte-cyanobacterial associations as a key factor in $N_2$-fixation across the Canadian Arctic. *Plant Soil*. 2011;344(1–2):335–46.
[89] Usuda K, Torizsuka N, Matsuo Y, Kim D-H, Shoun H. Denitrification by the fungus *Cylindrocarpon tonkinese*: anaerobic cell growth and two isozyme forms of cytochrome P-450nor. *Appl Environ Microbiol*. 1995;61(3):883–9.
[90] Gavazov KS, Soudzilovskaia NA, van Logtestijn RSP, Braster M, Cornelissen JHC. Isotopic analysis of cyanobacterial nitrogen fixation associated with Subarctic lichen and bryophyte species. *Plant Soil*. 2010;333(1–2):507–17.
[91] Michelsen A, Rinnan R, Jonasson S. Two decades of experimental manipulations of heaths and forest understory in the Subarctic. *Ambio*. 2012;41(S3):218–30.
[92] Yergeau E, Kang S, He Z, Zhou J, Kowalchuk GA. Functional microarray analysis of nitrogen and carbon cycling genes across an Antarctic latitudinal transect. *ISME J*. 2007;1(2):163–79.

[93] Jung J, Yeom J, Kim J, et al. Change in gene abundance in the nitrogen biogeochemical cycle with temperature and nitrogen addition in Antarctic soils. *Res Microbiol*. 2011;162(10):1018–26.
[94] Cleveland CC, Townsend AR, Taylor P, et al. Relationships among net primary productivity, nutrients and climate in tropical rain forest: a pan-tropical analysis. *Ecol Lett*. 2011;14(9): 939–47.
[95] Hedin LO, Brookshire ENJ, Menge DNL, Barron AR. The nitrogen paradox in tropical forest ecosystems. *Annu Rev Ecol Evol Syst*. 2009;40(1):613–35.
[96] Cleveland CC, Townsend AR, Schimel DS, et al. Global patterns of terrestrial biological nitrogen ($N_2$) fixation in natural ecosystems. *Global Biogeochem Cycles*. 1999;13(2):623–45.
[97] Stein LY, Klotz MG. The nitrogen cycle. *Curr Biol*. 2016;26(3):R94–8.
[98] Daims H, Lücker S, Wagner M. A new perspective on microbes formerly known as nitrite-oxidizing bacteria. *Trends Microbiol*. 2016;24(9):699–712.
[99] van Kessel MAHJ, Speth DR, Albertsen M, et al. Complete nitrification by a single microorganism. *Nature*. 2015;528(7583):555–9.
[100] Prosser JI, Nicol GW. Relative contributions of archaea and bacteria to aerobic ammonia oxidation in the environment. *Environ Microbiol*. 2008;10(11):2931–41.
[101] Schleper C, Nicol GW. Ammonia-oxidising archaea – physiology, ecology and evolution. In: Poole RK, editor. Advances in microbial physiology. Cambridge, MA, USA: Academic Press; 2010. p. 1–41.
[102] Prosser JI, Hink L, Gubry-Rangin C, Nicol GW. Nitrous oxide production by ammonia oxidizers: physiological diversity, niche differentiation and potential mitigation strategies. *Glob Chang Biol*. 2020;26(1):103–18.
[103] Kits KD, Sedlacek CJ, Lebedeva EV, et al. Kinetic analysis of a complete nitrifier reveals an oligotrophic lifestyle. *Nature*. 2017;549(7671):269–72.
[104] Wang Z, Cao Y, Zhu-Barker X, et al. Comammox *Nitrospira* clade B contributes to nitrification in soil. *Soil Biol Biochem*. 2019;135.
[105] Hooper AB, Vannelli T, Bergmann DJ, Arciero DM. Enzymology of the oxidation of ammonia to nitrite by bacteria. *Antonie van Leeuwenhoek*. 1997;71(1–2):59–67.
[106] Tavormina PL, Orphan VJ, Kalyuzhnaya MG, Jetten MSM, Klotz MG. A novel family of functional operons encoding methane/ammonia monooxygenase-related proteins in gammaproteobacterial methanotrophs. *Environ Microbiol Rep*. 2011;3(1):91–100.
[107] Hyman MR, Murton IB, Arp DJ. Interaction of ammonia monooxygenase from *Nitrosomonas europaea* with alkanes, alkenes, and alkynes. *Appl Environ Microbiol*. 1988;54(12):3187–90.
[108] Keener WK, Arp DJ. Transformations of aromatic compounds by *Nitrosomonas europaea*. *Appl Environ Microbiol*. 1994;60(6):1914–20.
[109] Arp D, Sayavedra-Soto L, Hommes N. Molecular biology and biochemistry of ammonia oxidation by *Nitrosomonas europaea*. *Arch Microbiol*. 2002;178(4):250–5.
[110] Arciero D, Vannelli T, Logan M, Hopper AB. Degradation of trichloroethylene by the ammonia-oxidizing bacterium *Nitrosomonas europaea*. *Biochem Biophys Res Commun*. 1989;159(2):640–3.
[111] Vannelli T, Logan M, Arciero DM, Hooper AB. Degradation of halogenated aliphatic compounds by the ammonia- oxidizing bacterium *Nitrosomonas europaea*. *Appl Environ Microbiol*. 1990;56(4):1169–71.
[112] Lancaster KM, Caranto JD, Majer SH, Smith MA. Alternative bioenergy: Updates to and challenges in nitrification metalloenzymology. *Joule*. 2018;2(3):421–41.
[113] Caranto JD, Lancaster KM. Nitric oxide is an obligate bacterial nitrification intermediate produced by hydroxylamine oxidoreductase. *Proc Natl Acad Sci*. 2017;114(31):8217–22.
[114] Wijma HJ, Canters GW, de Vries S, Verbeet MP. Bidirectional catalysis by copper-containing nitrite reductase. *Biochemistry*. 2004;43(32):10467–74.

[115] Kim BK, Jung M-Y, Yu DS, et al. Genome sequence of an ammonia-oxidizing soil archaeon, '*Candidatus* Nitrosoarchaeum koreensis' MY1. *J Bacteriol*. 2011;193(19):5539–40.
[116] Spang A, Poehlein A, Offre P, et al. The genome of the ammonia-oxidizing *Candidatus* Nitrososphaera gargensis: insights into metabolic versatility and environmental adaptations. *Environ Microbiol*. 2012;14(12):3122–45.
[117] Tourna M, Stieglmeier M, Spang A, et al. *Nitrososphaera viennensis*, an ammonia oxidizing archaeon from soil. *Proc Natl Acad Sci*. 2011;108(20):8420–5.
[118] Walker CB, de la Torre JR, Klotz MG, et al. *Nitrosopumilus maritimus* genome reveals unique mechanisms for nitrification and autotrophy in globally distributed marine crenarchaea. *Proc Natl Acad Sci*. 2010;107(19):8818–23.
[119] Martens-Habbena W, Berube PM, Urakawa H, de la Torre JR, Stahl DA. Ammonia oxidation kinetics determine niche separation of nitrifying archaea and bacteria. *Nature*. 2009;461(7266):976–9.
[120] Shen T, Stieglmeier M, Dai J, Urich T, Schleper C. Responses of the terrestrial ammonia-oxidizing archaeon *Ca*. Nitrososphaera viennensis and the ammonia-oxidizing bacterium *Nitrosospira multiformis* to nitrification inhibitors. *FEMS Microbiol Lett*. 2013;344(2):121–9.
[121] Kozlowski JA, Stieglmeier M, Schleper C, Klotz MG, Stein LY. Pathways and key intermediates required for obligate aerobic ammonia-dependent chemolithotrophy in bacteria and Thaumarchaeota. *ISME J*. 2016;10(8):1836–45.
[122] Bartossek R, Nicol GW, Lanzen A, Klenk H, Schleper C. Homologues of nitrite reductases in ammonia-oxidizing archaea: diversity and genomic context. *Environ Microbiol*. 2010;12(4):1075–88.
[123] Bartossek R, Spang A, Weidler G, Lanzen A, Schleper C. Metagenomic analysis of ammonia-oxidizing archaea affiliated with the soil group. *Front Microbiol*. 2012;3:208.
[124] Stieglmeier M, Mooshammer M, Kitzler B, et al. Aerobic nitrous oxide production through N-nitrosating hybrid formation in ammonia-oxidizing archaea. *ISME J*. 2014;8(5):1135–46.
[125] Zhu-Barker X, Cavazos AR, Ostrom NE, Horwath WR, Glass JB. The importance of abiotic reactions for nitrous oxide production. *Biogeochemistry*. 2015;126(3):251–67.
[126] Fiencke C, Spieck E, Bock E. Nitrifying bacteria. In: Werner D, Newton WE, editors. Nitrogen fixation in agriculture, forestry, ecology, and the environment. Berlin/Heidelberg: Springer-Verlag; 2005. p. 255–76.
[127] Kowalchuk GA, Stephen JR, De Boer W, Prosser JI, Embley TM, Woldendorp JW. Analysis of ammonia-oxidizing bacteria of the beta subdivision of the class Proteobacteria in coastal sand dunes by denaturing gradient gel electrophoresis and. *Appl Environ Microbiol*. 1997;63(4):1489–97.
[128] Okano Y, Hristova KR, Leutenegger CM, et al. Application of real-time PCR to study effects of ammonium on population size of ammonia-oxidizing bacteria in soil. *Appl Environ Microbiol*. 2004;70(2):1008–16.
[129] Briones AM, Okabe S, Umemiya Y, Ramsing N-B, Reichardt W, Okuyama H. Influence of different cultivars on populations of ammonia-oxidizing bacteria in the root environment of rice. *Appl Environ Microbiol*. 2002;68(6):3067–75.
[130] Hastings RC, Ceccherini MT, Miclaus N, Saunders JR, Bazzicalupo M, McCarthy AJ. Direct molecular biological analysis of ammonia oxidising bacteria populations in cultivated soil plots treated with swine manure. *FEMS Microbiol Ecol*. 2006;23(1):45–54.
[131] Oved T, Shaviv A, Goldrath T, Mandelbaum RT, Minz D. Influence of effluent irrigation on community composition and function of ammonia-oxidizing bacteria in soil. *Appl Environ Microbiol*. 2001;67(8):3426–33.
[132] Booth MS, Stark JM, Rastetter E. Controls on nitrogen cycling in terrestrial ecosystems: A synthetic analysis of literature data. *Ecol Monogr*. 2005;75(2):139–57.

[133] Boyle-Yarwood SA, Bottomley PJ, Myrold DD. Community composition of ammonia-oxidizing bacteria and archaea in soils under stands of red alder and Douglas fir in Oregon. *Environ Microbiol*. 2008;10(11):2956–65.
[134] Chen X, Zhu Y, Xia Y, Shen J, He J. Ammonia-oxidizing archaea: important players in paddy rhizosphere soil? *Environ Microbiol*. 2008;10(8):1978–87.
[135] Isobe K, Koba K, Suwa Y, et al. High abundance of ammonia-oxidizing archaea in acidified subtropical forest soils in southern China after long-term N deposition. *FEMS Microbiol Ecol*. 2012;80(1):193–203.
[136] Leininger S, Urich T, Schloter M, et al. Archaea predominate among ammonia-oxidizing prokaryotes in soils. *Nature*. 2006;442(7104):806–9.
[137] Stopnisek N, Gubry-Rangin C, Hofferle S, Nicol GW, Mandic-Mulec I, Prosser JI. Thaumarchaeal ammonia oxidation in an acidic forest peat soil is not influenced by ammonium amendment. *Appl Environ Microbiol*. 2010;76(22):7626–34.
[138] Gubry-Rangin C, Nicol GW, Prosser JI. Archaea rather than bacteria control nitrification in two agricultural acidic soils. *FEMS Microbiol Ecol*. 2010;74(3):566–74.
[139] Nicol GW, Leininger S, Schleper C, Prosser JI. The influence of soil pH on the diversity, abundance and transcriptional activity of ammonia oxidizing archaea and bacteria. *Environ Microbiol*. 2008;10(11):2966–78.
[140] Yao H, Campbell CD, Chapman SJ, Freitag TE, Nicol GW, Singh BK. Multi-factorial drivers of ammonia oxidizer communities: evidence from a national soil survey. *Environ Microbiol*. 2013;15(9):2545–56.
[141] Stahl DA, de la Torre JR. Physiology and diversity of ammonia-oxidizing archaea. *Annu Rev Microbiol*. 2012;66(1):83–101.
[142] Brochier-Armanet C, Boussau B, Gribaldo S, Forterre P. Mesophilic crenarchaeota: proposal for a third archaeal phylum, the Thaumarchaeota. *Nat Rev Microbiol*. 2008;6(3):245–52.
[143] De La Torre JR, Walker CB, Ingalls AE, Könneke M, Stahl DA. Cultivation of a thermophilic ammonia oxidizing archaeon synthesizing crenarchaeol. *Environ Microbiol*. 2008;10(3): 810–8.
[144] Lehtovirta-Morley LE, Stoecker K, Vilcinskas A, Prosser JI, Nicol GW. Cultivation of an obligate acidophilic ammonia oxidizer from a nitrifying acid soil. *Proc Natl Acad Sci*. 2011;108(38):15892–7.
[145] Daims H, Lebedeva VE, Pjevac P, et al. Complete nitrification by *Nitrospira* bacteria. *Nature*. 2015;528(7583):504–9.
[146] Castellani AG, Niven, Jr. CF. Factors affecting the bacteriostatic action of sodium nitrite. *Appl Microbiol*. 1955;3(3):154–9.
[147] Lewis, JR WM, Morris DP. Toxicity of nitrite to fish: a review. *Trans Am Fish Soc*. 1986;115(2):183–95.
[148] Pester M, Maixner F, Berry D, et al. *NxrB* encoding the beta subunit of nitrite oxidoreductase as functional and phylogenetic marker for nitrite-oxidizing *Nitrospira*. *Environ Microbiol*. 2014;16(10):3055–71.
[149] Poly F, Wertz S, Brothier E, Degrange V. First exploration of *Nitrobacter* diversity in soils by a PCR cloning-sequencing approach targeting functional gene *nxrA*. *FEMS Microbiol Ecol*. 2008;63(1):132–40.
[150] Sorokin DY, Lücker S, Vejmelkova D, et al. Nitrification expanded: discovery, physiology and genomics of a nitrite-oxidizing bacterium from the phylum Chloroflexi. *ISME J*. 2012;6(12):2245–56.
[151] Nowka B, Daims H, Spieck E. Comparison of oxidation kinetics of nitrite-oxidizing bacteria: Nitrite availability as a key factor in niche differentiation. *Appl Environ Microbiol*. 2015;81(2):745–53.

[152] Verstraete W, Focht DD. Biochemical ecology of nitrification and denitrification. *Adv Microbiol Ecol*. 1977;1:135–214.
[153] Stein L. Heterotrophic nitrification and nitrifier denitrification. In: Ward BB, Arp DJ, Klotz MG, editors. Nitrification. Washington, D.C., USA: ASM Press; 2011;95–114.
[154] Prosser JI. Chapter 15 – the ecology of nitrifying bacteria. In: Bothe H, Ferguson SJ, Newton WE, editors. Biology of the nitrogen cycle. Amsterdam: Elsevier; 2007. p. 223–43.
[155] Braker G, Conrad R. Diversity, structure, and size of $N_2O$-producing microbial communities in soils – what matters for their functioning? In: Laskin IL, Sariaslani S, Gadd GM, editors. Advances in applied microbiology. Cambridge, MA, USA: Academic Press; 2011. p. 33–70.
[156] Robertson LA, Kuenen JG. Combined heterotrophic nitrification and aerobic denitrification in *Thiosphaera pantotropha* and other bacteria. *Antonie Van Leeuwenhoek*. 1990;57(3):139–52.
[157] Van Gool AP, Schmidt EL. Nitrification in relation to growth in *Aspergillus flavus*. *Soil Biol Biochem*. 1973;5(2):259–65.
[158] Ji B, Yang K, Zhu L, et al. Aerobic denitrification: a review of important advances of the last 30 years. *Biotechnol Bioprocess Eng*. 2015;20(4):643–51.
[159] van Niel EWJ, Arts PAM, Wesselink BJ, Robertson LA, Kuenen JG. Competition between heterotrophic and autotrophic nitrifiers for ammonia in chemostat cultures. *FEMS Microbiol Ecol*. 1993;11(2):109–18.
[160] Stange CF, Spott O, Arriaga H, Menéndez S, Estavillo JM, Merino P. Use of the inverse abundance approach to identify the sources of NO and $N_2O$ release from Spanish forest soils under oxic and hypoxic conditions. *Soil Biol Biochem*. 2013;57:451–8.
[161] Zhang J, Cai Z, Zhu T. $N_2O$ production pathways in the subtropical acid forest soils in China. *Environ Res*. 2011;111(5):643–9.
[162] Weber DF, Gainey PL. Relative sensitivity of nitrifying organisms to hydrogen ions in soils and in solutions. *Soil Sci*. 1962;94(3):138–45.
[163] Zhang J, Müller C, Cai Z. Heterotrophic nitrification of organic N and its contribution to nitrous oxide emissions in soils. *Soil Biol Biochem*. 2015;84:199–209.
[164] Binkley D, Stottlemyer R, Suarez F, Cortina J. Soil nitrogen availability in some Arctic ecosystems in northwest Alaska: Responses to temperature and moisture. *Ecoscience*. 1994;1(1):64–70.
[165] Chapin DM. Nitrogen mineralization, nitrification, and denitrification in a High Arctic lowland ecosystem, Devon Island, N.W.T., Canada. *Arct Alp Res*. 1996;28(1):85.
[166] Giblin AE, Nadelhoffer KJ, Shaver GR, Laundre JA, McKerrow AJ. Biogeochemical diversity along a riverside toposequence in Arctic Alaska. *Ecol Monogr*. 1991;61(4):415–35.
[167] Ma WK, Schautz A, Fishback LAE, Bedard-Haughn A, Farrell RE, Siciliano SD. Assessing the potential of ammonia-oxidizing bacteria to produce nitrous oxide in soils of a High Arctic lowland ecosystem on Devon Island, Canada. *Soil Biol Biochem*. 2007;39(8): 2001–13.
[168] Siciliano SD, Ma WK, Ferguson S, Farrell RE. Nitrifier dominance of Arctic soil nitrous oxide emissions arises due to fungal competition with denitrifiers for nitrate. *Soil Biol Biochem*. 2009;41(6):1104–10.
[169] Yergeau E, Hogues H, Whyte LG, Greer CW. The functional potential of High Arctic permafrost revealed by metagenomic sequencing, qPCR and microarray analyses. *ISME J*. 2010;4(9):1206–14.
[170] Banerjee S, Siciliano SD. Factors driving potential ammonia oxidation in Canadian Arctic ecosystems: does spatial scale matter? Appl Environ Microbiol. 2012;78(2):346–53.
[171] Lamb EG, Han S, Lanoil BD, et al. A High Arctic soil ecosystem resists long-term environmental manipulations. *Glob Chang Biol*. 2011;17(10):3187–94.
[172] Alves RJE, Wanek W, Zappe A, et al. Nitrification rates in Arctic soils are associated with functionally distinct populations of ammonia-oxidizing archaea. *ISME J*. 2013;7(8):1620–31.

[173] Siljanen HMPP, Alves RJEE, Ronkainen Jussi G, et al. Archaeal nitrification is a key driver of high nitrous oxide emissions from arctic peatlands. *Soil Biol Biochem*. 2019;53(3):107539.
[174] Maljanen M, Sigurdsson BD, Guðmundsson J, Óskarsson H, Huttunen JT, Martikainen PJ. Greenhouse gas balances of managed peatlands in the Nordic countries – present knowledge and gaps. *Biogeosciences*. 2010;7(9):2711–38.
[175] Barnard S, Van Goethem MW, de Scally SZ, et al. Increased temperatures alter viable microbial biomass, ammonia oxidizing bacteria and extracellular enzymatic activities in Antarctic soils. *FEMS Microbiol Ecol*. 2020;96(5): fiaa065.
[176] Magalhães CM, Machado A, Frank-Fahle B, Lee CK, Cary SC. The ecological dichotomy of ammonia-oxidizing archaea and bacteria in the hyper-arid soils of the Antarctic Dry Valleys. *Front Microbiol*. 2014;5:515.
[177] Monteiro M, Baptista MS, Séneca J, et al. Understanding the response of nitrifying communities to disturbance in the McMurdo Dry Valleys, Antarctica. *Microorganisms*. 2020;8(3):404.
[178] Sanders T, Fiencke C, Hüpeden J, Pfeiffer EM, Spieck E. Cold adapted *Nitrosospira* sp.: a potential crucial contributor of ammonia oxidation in cryosols of permafrost-affected landscapes in Northeast Siberia. *Microorganisms*. 2019;7(12):699.
[179] Alawi M, Lipski A, Sanders T, Pfeiffer EM, Spieck E. Cultivation of a novel cold-adapted nitrite oxidizing betaproteobacterium from the Siberian Arctic. *ISME J*. 2007;1(3):256–64.
[180] Schmidt SK, Nemergut DR, Miller AE, Freeman KR, King AJ, Seimon A. Microbial activity and diversity during extreme freeze–thaw cycles in periglacial soils, 5400 m elevation, Cordillera Vilcanota, Perú. *Extremophiles*. 2009;13(5):807–16.
[181] Wang B, Zhao J, Guo Z, Ma J, Xu H, Jia Z. Differential contributions of ammonia oxidizers and nitrite oxidizers to nitrification in four paddy soils. *ISME J*. 2015;9(5):1062–75.
[182] Pjevac P, Schauberger C, Poghosyan L, et al. *AmoA*-targeted polymerase chain reaction primers for the specific detection and quantification of comammox *Nitrospira* in the environment. *Front Microbiol*. 2017;8:1508.
[183] De Boer W, Kowalchuk GA. Nitrification in acid soils: micro-organisms and mechanisms. *Soil Biol Biochem*. 2001;33(7–8):853–66.
[184] Hynes RK, Knowles R. Inhibition by acetylene of ammonia oxidation in *Nitrosomonas europaea*. *FEMS Microbiol Lett*. 1978;4(6):319–21.
[185] Wrage-Mönning N, Horn MA, Well R, Müller C, Velthof G, Oenema O. The role of nitrifier denitrification in the production of nitrous oxide revisited. *Soil Biol Biochem*. 2018;123: A3–16.
[186] Nemergut DR, Schmidt SK. Disruption of *narH*, *narJ*, and *moaE* inhibits heterotrophic nitrification in *Pseudomonas* strain M19. *Appl Environ Microbiol*. 2002;68(12):6462–5.
[187] He J, Shen J, Zhang L, et al. Quantitative analyses of the abundance and composition of ammonia-oxidizing bacteria and ammonia-oxidizing archaea of a Chinese upland red soil under long-term fertilization practices. *Environ Microbiol*. 2007;9(9):2364–74.
[188] Li X, Xiao Y, Ren W, Liu Z, Shi J, Quan Z. Abundance and composition of ammonia-oxidizing bacteria and archaea in different types of soil in the Yangtze River estuary. *J Zhejiang Univ Sci B*. 2012;13(10):769–82.
[189] Zumft WG. Cell biology and molecular basis of denitrification. *Microbiol Mol Biol Rev*. 1997;61(4):533–616.
[190] Philippot L, Hallin S, Börjesson G, Baggs EM. Biochemical cycling in the rhizosphere having an impact on global change. *Plant Soil*. 2009;321(1–2):61–81.
[191] Tiedje JM. Ecology of denitrification and dissimilatory nitrate reduction to ammonium – environmental microbiology of anaerobes. *Environ Microbiol Anaerobes*. 1988;717: 179–244.
[192] Zumft WG. The denitrifying prokaryotes. In: Balows A, Trüper HG, Dworkin M, Harder W, Schleifer K-H, editors. The prokaryotes. New York: Springer Verlag; 1992. p. 554–82.

[193] Shapleigh JP. The denitrifying prokaryotes. In: Dworkin M, Falkow S, Rosenberg E, Schleifer K-H, Stackebrandt E, editors. The prokaryotes. New York: Springer; 2006. p. 769–92.
[194] Cofman Anderson I, Levine JS. Relative rates of nitric oxide and nitrous oxide production by nitrifiers, denitrifiers, and nitrate respirers. *Appl Environ Microbiol*. 1986;51(5):938–45.
[195] Conrad R. Compensation concentration as critical variable for regulating the flux of trace gases between soil and atmosphere. *Biogeochemistry*. 1994;27(3):155–70.
[196] Philippot L, Hallin S, Schloter M. Ecology of denitrifying prokaryotes in agricultural soil. In: Sparks DL, editor. Advances in agronomy. Cambridge, MA, USA: Academic Press; 2007. p. 249–305.
[197] Kraft B, Strous M, Tegetmeyer HE. Microbial nitrate respiration – genes, enzymes and environmental distribution. *J Biotechnol*. 2011;155(1):104–17.
[198] Chen H, Mothapo N V, Shi W. The significant contribution of fungi to soil $N_2O$ production across diverse ecosystems. *Appl Soil Ecol*. 2014;73:70–7.
[199] Lavrent'ev R, Zaitsev S, Sudnitsyn I, Kurakov A. Nitrous oxide production by fungi in soils under different moisture levels. *Moscow Univ Soil Sci Bull*. 2008;63:178–83.
[200] Morozkina E V, Kurakov A V. Dissimilatory nitrate reduction in fungi under conditions of hypoxia and anoxia: a review. *Appl Biochem Microbiol*. 2007;43(5):544–9.
[201] Mothapo N, Chen H, Cubeta MA, Grossman JM, Fuller F, Shi W. Phylogenetic, taxonomic and functional diversity of fungal denitrifiers and associated $N_2O$ production efficacy. *Soil Biol Biochem*. 2015;83:160–75.
[202] Takaya N. Response to hypoxia, reduction of electron acceptors, and subsequent survival by filamentous fungi. *Biosci Biotechnol Biochem*. 2009;73(1):1–8.
[203] Higgins SA, Welsh A, Orellana LH, et al. Detection and diversity of fungal nitric oxide reductase genes *( p450nor)* in agricultural soils. *Appl Environ Microbiol*. 2016;82(10):2919–28.
[204] Kim S-W, Fushinobu S, Zhou S, Wakagi T, Shoun H. Eukaryotic *nirK* genes encoding copper-containing nitrite reductase: originating from the protomitochondrion? *Appl Environ Microbiol*. 2009;75(9):2652–8.
[205] Kobayashi M, Shoun H. The copper-containing dissimilatory nitrite reductase involved in the denitrifying system of the fungus *Fusarium oxysporum*. *J Biol Chem*. 1995;270(8):4146–51.
[206] Maeda K, Spor A, Edel-Hermann V, et al. $N_2O$ production, a widespread trait in fungi. *Sci Rep*. 2015;5(1):9697.
[207] Shoun H, Kim D-H, Uchiyama H, Sugiyama J. Denitrification by fungi. *FEMS Microbiol Lett*. 1992;94:277–82.
[208] Zhou Z, Takaya N, Shoun H. Multi-energy metabolic mechanisms of the fungus *Fusarium oxysporum* in low oxygen environments. *Biosci Biotechnol Biochem*. 2010;74(12):2431–7.
[209] Spott O, Russow R, Stange CF. Formation of hybrid $N_2O$ and hybrid $N_2$ due to codenitrification: First review of a barely considered process of microbially mediated N-nitrosation. *Soil Biol Biochem*. 2011;43(10):1995–2011.
[210] Onley JR, Ahsan S, Sanford RA, Loffler FE. Denitrification by *Anaeromyxobacter dehalogenans*, a common soil bacterium lacking nitrite reductase senes (*nirS/nirK*). *Appl Environ Microbiol*. 2018;84(4):e01985–17
[211] Blaud A, Lerch TZ, Phoenix GK, Osborn AM. Arctic soil microbial diversity in a changing world. *Res Microbiol*. 2015;166(10):796–813.
[212] Bru D, Sarr A, Philippot L. Relative abundances of proteobacterial membrane-bound and periplasmic nitrate reductases in selected environments. *Appl Environ Microbiol*. 2007;73(18):5971–4.
[213] Levy-Booth DJ, Prescott CE, Grayston SJ. Microbial functional genes involved in nitrogen fixation, nitrification and denitrification in forest ecosystems. *Soil Biol Biochem*. 2014;75: 11–25.

[214] Palmer K, Horn MA. Actinobacterial nitrate reducers and proteobacterial denitrifiers are abundant in $N_2O$-metabolizing palsa peat. *Appl Environ Microbiol*. 2012;78(16):5584–96.
[215] Papaspyrou S, Smith CJ, Dong LF, Whitby C, Dumbrell AJ, Nedwell DB. Nitrate reduction functional genes and nitrate reduction potentials persist in deeper estuarine sediments. Why? *PLoS One*. 2014;9(4):e94111.
[216] Jones CM, Stres B, Rosenquist M, Hallin S. Phylogenetic analysis of nitrite, nitric oxide, and nitrous oxide respiratory enzymes reveal a complex evolutionary history for denitrification. *Mol Biol Evol*. 2008;25(9):1955–66.
[217] Graf DRH, Jones CM, Hallin S. Intergenomic comparisons highlight modularity of the denitrification pathway and underpin the importance of community structure for $N_2O$ emissions. *PLoS One*. 2014;9(12):e114118.
[218] Zumft WG. Nitric oxide reductases of prokaryotes with emphasis on the respiratory, heme-copper oxidase type. *J Inorg Biochem*. 2005;99(1):194–215.
[219] Suharti, Strampraad MJF, Schröder I, de Vries S. A novel copper A containing menaquinol NO reductase from *Bacillus azotoformans*. *Biochemistry*. 2001;40(8):2632–9.
[220] Sanford RA, Wagner DD, Wu Q, et al. Unexpected nondenitrifier nitrous oxide reductase gene diversity and abundance in soils. *Proc Natl Acad Sci*. 2012;109(48):19709–14.
[221] Hallin S, Philippot L, Löffler F, Sanford R, Jones C. Genomics and ecology of novel $N_2O$-reducing microorganisms. *Trends Microbiol*. 2018;26:43–55.
[222] Natale P, Brüser T, Driessen AJM. Sec- and Tat-mediated protein secretion across the bacterial cytoplasmic membrane – distinct translocases and mechanisms. *Biochim Biophys Acta Biomembr*. 2008;1778(9):1735–56.
[223] Baggs EM, Smales CL, Bateman EJ. Changing pH shifts the microbial source as well as the magnitude of $N_2O$ emission from soil. *Biol Fertil Soils*. 2010;46(8):793–805.
[224] Baumann B, Snozzi M, Zehnder AJB, van der Meer JR. Dynamics of denitrification activity of *Paracoccus denitrificans* in continuous culture during aerobic-anaerobic changes. *J Bacteriol*. 1996;178(15):4367–74.
[225] Stevens RJ, Laughlin RJ, Malone JP. Soil pH affects the processes reducing nitrate to nitrous oxide and dinitrogen. *Soil Biol Biochem*. 1998;30:1119–26.
[226] Thomsen JK, Geest R, Cox RP. Mass spectrometric studies of the effect of pH on the accumulation of intermediates in denitrification by *Paracoccus denitrificans*. *Appl Environ Microbiol*. 1994;60:536–41.
[227] van Breemen N, Feijtel TCJ. Soil processes and properties involved in the production of greenhouse gases, with special relevance to soil taxonomic systems. In: Bouwman AF, editor. Soils and the greenhouse effect. Chichester: John Wiley & Sons; 1990. p. 195–223.
[228] Philippot L, Andert J, Jones CM, Bru D, Hallin S. Importance of denitrifiers lacking the genes encoding the nitrous oxide reductase for $N_2O$ emissions from soil. *Glob Chang Biol*. 2011;17(3):1497–504.
[229] Bergaust L, Mao Y, Bakken LR, Frostegård A. Denitrification response patterns during the transition to anoxic respiration and posttranscriptional effects of suboptimal pH on nitrogen oxide reductase in *Paracoccus denitrificans*. *Appl Environ Microbiol*. 2010;76(19):6387–96.
[230] Dorsch P, Holtan-Hartwig L, Bakken LR. Low temperature control of soil denitrifying communities: kinetics of $N_2O$ production and reduction. *Soil Biol Biochem*. 2002;34(11):1797–806.
[231] Enwall K, Philippot L, Hallin S. Activity and composition of the denitrifying bacterial community respond differently to long-term fertilization. *Appl Environ Microbiol*. 2005;71(12):8335–43.
[232] Holtan-Hartwig L, Dorsch P, Bakken LR. Comparison of denitrifying communities in organic soils: kinetics of $NO_3^-$ and $N_2O$ reduction. *Soil Biol Biochem*. 2000;32(6):833–43.

[233] Philippot L, Bru D, Ramette A, et al. Determinants of the distribution of nitrogen-cycling microbial communities at the landscape scale. *Isme J*. 2011;5(3):532–42.
[234] van Cleemput O. Subsoils: chemo-and biological denitrification, $N_2O$ and $N_2$ emissions. *Nutr Cycl Agroecosystems*. 1998;52(2):187–94.
[235] Cuhel J, Simek M, Laughlin RJ, et al. Insights into the effect of soil pH on $N_2O$ and $N_2$ emissions and denitrifier community size and activity. *Appl Environ Microbiol*. 2010;76(6):1870–8.
[236] Simek M, Jisova L, Hopkins DW. What is the so-called optimum pH for denitrification in soil? *Soil Biol Biochem*. 2002;34(9):1227–34.
[237] Liu BB, Mørkved PT, Frostegård Å, et al. Denitrification gene pools, transcription and kinetics of NO, $N_2O$ and $N_2$ production as affected by soil pH. *Fems Microbiol Ecol*. 2010;72(3):407–17.
[238] Kostka JE, Green SJ, Prakash O, et al. Denitrifying bacteria isolated from terrestrial subsurface sediments exposed to mixed-waste contamination. *Appl Environ Microbiol*. 2010;76(10):3244–54.
[239] Lycus P, Lovise Bøthun K, Bergaust L, Peele Shapleigh J, Reier Bakken L, Frostegård Å. Phenotypic and genotypic richness of denitrifiers revealed by a novel isolation strategy. *ISME J*. 2017;11(10):2219–32.
[240] Palmer K, Drake HL, Horn MA. Association of novel and highly diverse acid-tolerant denitrifiers with $N_2O$ fluxes of an acidic fen. *Appl Environ Microbiol*. 2010;76(4):1125–34.
[241] Kolb S, Horn M. Microbial $CH_4$ and $N_2O$ consumption in acidic wetlands. *Front Microbiol*. 2012;3:78.
[242] Hallin S, Welsh A, Stenstrom J, et al. Soil functional operating range linked to microbial biodiversity and community composition using denitrifiers as model guild. *PLoS One*. 2012;7(12): e51962.
[243] Potter CS, Matson PA, Vitousek PM, Davidson EA. Process modeling of controls on nitrogen trace gas emissions from soils worldwide. *J Geophys Res Atmos*. 1996;101(D1):1361–77.
[244] Werner C, Butterbach-Bahl K, Haas E, Hickler T, Kiese R. A global inventory of $N_2O$ emissions from tropical rainforest soils using a detailed biogeochemical model. *Global Biogeochem Cycles*. 2007;21(3):GB3010.
[245] Martikainen PJ, Nykänen H, Crill P, Silvola J. Effect of a lowered water table on nitrous oxide fluxes from northern peatlands. *Nature*. 1993;366(6450):51–3.
[246] Shaver GR, Billings WD, Chapin, FS, et al. Global change and the carbon balance of Arctic ecosystems. *Bioscience*. 1992;42(6):433–41.
[247] Dentener F, Drevet J, Lamarque JF, et al. Nitrogen and sulfur deposition on regional and global scales: a multimodel evaluation. *Global Biogeochem Cycles*. 2006;20(4).
[248] Jonasson S, Michelsen A, Schmidt IK. Coupling of nutrient cycling and carbon dynamics in the Arctic, integration of soil microbial and plant processes. *Appl Soil Ecol*. 1999; 11(2–3):135–46.
[249] Thamdrup B, Dalsgaard T. Nitrogen cycling in sediments. In: Kirchman DL, editor. Microbial ecology of the oceans. New York, USA: John Wiley & Sons, Inc.; 2008. p. 527–68.
[250] Thauer RK, Shima S. Methane as fuel for anaerobic microorganisms. *Ann N Y Acad Sci*. 2008;1125(1):158–70.
[251] Islas-Lima S, Thalasso F, Gómez-Hernandez J. Evidence of anoxic methane oxidation coupled to denitrification. *Water Res*. 2004;38(1):13–6.
[252] Ettwig KF, van Alen T, van de Pas-Schoonen KT, Jetten MSM, Strous M. Enrichment and molecular detection of denitrifying methanotrophic bacteria of the NC10 phylum. *Appl Environ Microbiol*. 2009;75(11):3656–62.
[253] Ettwig KF, Butler MK, Le Paslier D, et al. Nitrite-driven anaerobic methane oxidation by oxygenic bacteria. *Nature*. 2010;464(7288):543–8.

[254] Luesken FA, Sánchez J, van Alen TA, et al. Simultaneous nitrite-dependent anaerobic methane and ammonium oxidation processes. *Appl Environ Microbiol*. 2011;77(19):6802–7.
[255] Hooper AB. A nitrite-reducing enzyme from *Nitrosomonas europaea*. Preliminary characterization with hydroxylamine as electron donor. *Biochim Biophys Acta*. 1968;162(1):49–65.
[256] Ritchie GAF, Nicholas DJD. Identification of the sources of nitrous oxide produced by oxidative and reductive processes in *Nitrosomonas europaea*. *Biochem J*. 1972;126(5):1181–91.
[257] Shaw LJ, Nicol GW, Smith Z, Fear J, Prosser JI, Baggs EM. *Nitrosospira* spp. can produce nitrous oxide via a nitrifier denitrification pathway. *Environ Microbiol*. 2006;8(2):214–22.
[258] Zart D, Bock E. High rate of aerobic nitrification and denitrification by *Nitrosomonas eutropha* grown in a fermentor with complete biomass retention in the presence of gaseous $NO_2$ or NO. *Arch Microbiol*. 1998;169:282–6.
[259] Beyer S, Gilch S, Meyer O, Schmidt I. Transcription of genes coding for metabolic key functions in *Nitrosomonas europaea* during aerobic and anaerobic growth. *J Mol Microbiol Biotechnol*. 2008;16:187–97.
[260] Colliver BB, Stephenson T. Production of nitrogen oxide and dinitrogen oxide by autotrophic nitrifiers. *Biotechnol Adv*. 2000;18(3):219–32.
[261] Casciotti KL, Ward BB. Dissimilatory nitrite reductase genes from autotrophic ammonia-oxidizing bacteria. *Appl Environ Microbiol*. 2001;67(5):2213–21.
[262] Garbeva P, Baggs EM, Prosser JI. Phylogeny of nitrite reductase (*nirK*) and nitric oxide reductase (*norB*) genes from *Nitrosospira* species isolated from soil. *FEMS Microbiol Lett*. 2007;266(1):83–9.
[263] Anderson IC, Poth M, Homstead J, Burdige D. A comparison of NO and $N_2O$ production by the autotrophic nitrifier *Nitrosomonas europea* and the heterotrophic nitrifier *Alcaligenes faecalis*. *Appl Environ Microbiol*. 1993;59(11):3525–33.
[264] Bollmann A, Conrad R. Influence of $O_2$ availability on NO and $N_2O$ release by nitrification and denitrification in soils. *Glob Chang Biol*. 1998;4:387–96.
[265] Gharahi Ghehi N, Werner C, Cizungu Ntaboba L, et al. Spatial variations of nitrogen trace gas emissions from tropical mountain forests in Nyungwe, Rwanda. *Biogeosciences*. 2012;9(4):1451–63.
[266] Wrage N, Velthof GL, Laanbroek HL, Oenema O. Pitfalls in measuring nitrous oxide production by nitrifiers. In: Hatch DJ, Chadwick DR, Jarvis SC, Roker JA, editors. Controlling nitrogen flows and losses. The Netherlands: Wageningen Academic Publishers; 2004. p. 260–7.
[267] Webster EA, Hopkins DW. Contributions from different microbial processes to $N_2O$ emission from soil under different moisture regimes. *Biol Fertil Soils*. 1996;22(4):331–5.
[268] Van Groenigen JW, Huygens D, Boeckx P, et al. The soil N cycle: new insights and key challenges. *Soil*. 2015;1(1):235–56.
[269] Zhou Z, Takaya N, Nakamura A, Yamaguchi M, Takeo K, Shoun H. Ammonia fermentation, a novel anoxic metabolism of nitrate by fungi. *J Biol Chem*. 2002;277(3):1892–6.
[270] Zhou Z, Takaya N, Sakairi MAC, Shoun H. Oxygen requirement for denitrification by the fungus *Fusarium oxysporum*. *Arch Microbiol*. 2001;175(1):19–25.
[271] Uchimura H, Enjoji H, Seki T, Taguchi A, Tsakaya N, Shoun H. Nitrate reductase-formate dehydrogenase couple involved in the fungal denitrification by *Fusarium oxysporum*. *J Biochem*. 2002;131(4):579–86.
[272] Novinscak A, Goyer C, Zebarth BJ, Burton DL, Chantigny MH, Filion M. Novel *P450nor* gene detection assay used to characterize the prevalence and diversity of soil fungal denitrifiers. *Appl Environ Microbiol*. 2016;82(15):4560–9.
[273] Rütting T, Boeckx P, Müller C, Klemedtsson L. Assessment of the importance of dissimilatory nitrate reduction to ammonium for the terrestrial nitrogen cycle. *Biogeosciences*. 2011;8(7):1779–91.

[274] Smith MS, Zimmermann K. Nitrous oxide production by nondenitrifying soil nitrate reducers. *Soil Sci Soc Am J*. 1981;45:865–71.
[275] Philippot L, Hojberg O. Dissimilatory nitrate reductases in bacteria. *Biochim Biophys Acta-Gene Struct Expr*. 1999;1446(1–2):1–23.
[276] Mohan SB, Schmid M, Jetten M, Cole J. Detection and widespread distribution of the *nrfA* gene encoding nitrite reduction to ammonia, a short circuit in the biological nitrogen cycle that competes with denitrification. *Fems Microbiol Ecol*. 2004;49(3):433–43.
[277] Moreno-Vivián C, Ferguson SJ. Definition and distinction between assimilatory, dissimilatory and respiratory pathways. *Mol Microbiol*. 1998;29(2):664–6.
[278] Bonin P. Anaerobic nitrate reduction to ammonium in two strains isolated from coastal marine sediment: a dissimilatory pathway. *FEMS Microbiol Ecol*. 1996;19(1):27–38.
[279] Kaspar HF, Tiedje JM. Dissimilatory reduction of nitrate and nitrite to ammonium: Nitrous oxide production and effect of acetylene. *Appl Environ Microbiol*. 1981;41:705–9.
[280] Strohm TO, Griffin B, Zumft WG, Schink B. Growth yields in bacterial denitrification and nitrate ammonification. *Appl Environ Microbiol*. 2007;73(5):1420–4.
[281] 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*. 1999;181(21):6573–84.
[282] Richardson DJ, Berks BC, Russell DA, Spiro S, Taylor CJ. Functional, biochemical and genetic diversity of prokaryotic nitrate reductases. *Cell Mol Life Sci*. 2001;58(2):165–78.
[283] Wang H, Gunsalus RP. The *nrfA* and *nirB* nitrite reductase operons in *Escherichia coli* are expressed differently in response to nitrate than to nitrite. *J Bacteriol*. 2000;182(20):5813–22.
[284] Dannenberg S, Kroder M, Dilling W, Cypionka H. Oxidation of $H_2$, organic compounds and inorganic sulfur compounds coupled to reduction of $O_2$ or nitrate by sulfate-reducing bacteria. *Arch Microbiol*. 1992;158(2):93–9.
[285] Mitchell GJ, Jones JG, Cole JA. Distribution and regulation of nitrate and nitrite reduction by *Desulfovibrio* and *Desulfotomaculum* species. *Arch Microbiol*. 1986;144(1):35–40.
[286] Yoon S, Cruz-Garcia C, Sanford R, Ritalahti KM, Loffler FE. Denitrification versus respiratory ammonification: environmental controls of two competing dissimilatory $NO_3^-/NO_2^-$ reduction pathways in *Shewanella loihica* strain PV-4. *ISME J*. 2015;9(5):1093–104.
[287] Yoon S, Sanford RA, Loffler FE. Nitrite control over dissimilatory nitrate/nitrite reduction pathways in *Shewanella loihica* strain PV-4. *Appl Environ Microbiol*. 2015;81(10):3510–7.
[288] Bleakley BH, Tiedje JM. Nitrous oxide production by organisms other than nitrifiers or denitrifiers. *Appl Environ Microbiol*. 1982;44(6):1342–8.
[289] Fazzolari É, Nicolardot B, Germon JC. Simultaneous effects of increasing levels of glucose and oxygen partial pressures on denitrification and dissimilatory nitrate reduction to ammonium in repacked soil cores. *Eur J Soil Biol*. 1998;34(1):47–52.
[290] Silver WL, Herman DJ, Firestone MK. Dissimilatory nitrate reduction to ammonium in upland tropical forest soils. *Ecology*. 2001;82(9):2410–6.
[291] Smith CJ, Nedwell DB, Dong LF, Osborn AM. Diversity and abundance of nitrate reductase genes (*narG* and *napA*), nitrite reductase genes (*nirS* and *nrfA*), and their transcripts in estuarine sediments. *Appl Environ Microbiol*. 2007;73(11):3612–22.
[292] Woods DD. The reduction of nitrate to ammonia by *Clostridium welchii*. *Biochem J*. 1938;32(11):2000–12.
[293] Marietou A, Griffiths L, Cole J. Preferential reduction of the thermodynamically less favorable electron acceptor, sulfate, by a nitrate-reducing strain of the sulfate-reducing bacterium *Desulfovibrio desulfuricans* 27774. *J Bacteriol*. 2009;191(3):882–9.
[294] Dong LF, Sobey MN, Smith CJ, et al. Dissimilatory reduction of nitrate to ammonium, not denitrification or anammox, dominates benthic nitrate reduction in tropical estuaries. *Limnol Oceanogr*. 2011;56(1):279–91.

[295] Lam P, Lavik G, Jensen MM, et al. Revising the nitrogen cycle in the Peruvian oxygen minimum zone. *Proc Natl Acad Sci*. 2009;106(12):4752–7.
[296] Buresh RJ, Patrick WH. Nitrate reduction to ammonium in anaerobic soil. *Soil Sci Soc Am J*. 1978;42(6):913.
[297] Huygens D, Boeckx P, Templer P, et al. Mechanisms for retention of bioavailable nitrogen in volcanic rainforest soils. *Nat Geosci*. 2008;1(8):543–8.
[298] Kaspar HF. Nitrite reduction to nitrous oxide by propionibacteria: detoxication mechanism. *Arch Microbiol*. 1982;133(2):126–30.
[299] Stevens RJ, Laughlin RJ. Measurement of nitrous oxide and di-nitrogen emissions from agricultural soils. *Nutr Cycl Agroecosystems*. 1998;52:131–9.
[300] Sun Y, De Vos P, Heylen K. Nitrous oxide emission by the non-denitrifying, nitrate ammonifier *Bacillus licheniformis*. *BMC Genomics*. 2016;17(1):68.
[301] Abbott BW, Jones JB. Permafrost collapse alters soil carbon stocks, respiration, $CH_4$, and $N_2O$ in upland tundra. *Glob Chang Biol*. 2015;21(12):4570–87.
[302] Elberling B, Christiansen HH, Hansen BU. High nitrous oxide production from thawing permafrost. *Nat Geosci*. 2010;3(5):332–5.
[303] Hetz SA, Poehlein A, Horn MA. Whole-genome sequences of two new caballeronia strains isolated from cryoturbated peat circles of the permafrost-affected eastern european tundra. *Microbiol Resour Announc*. 2020;9(31):e00731–20.
[304] Schalk-Otte S, Seviour RJ, Kuenen JG, Jetten MSM. Nitrous oxide ($N_2O$) production by *Alcaligenes faecalis* during feast and famine regimes. *Water Res*. 2000;34(7):2080–8.
[305] Tveit AT, Urich T, Svenning MM. Metatranscriptomic analysis of Arctic peat soil microbiota. *Appl Environ Microbiol*. 2014;80(18):5761–72.
[306] McTigue ND, Gardner WS, Dunton KH, Hardison AK. Biotic and abiotic controls on co-occurring nitrogen cycling processes in shallow Arctic shelf sediments. *Nat Commun*. 2016;7(1):13145.
[307] Gihring TM, Lavik G, Kuypers MMM, Kostka JE. Direct determination of nitrogen cycling rates and pathways in Arctic fjord sediments (Svalbard, Norway). *Limnol Oceanogr*. 2010;55(2): 740–52.
[308] Mulder A. Anaerobic ammonium oxidation discovered in a denitrifying fluidized bed reactor. *FEMS Microbiol Ecol*. 1995;16(3):177–83.
[309] Strous M, Fuerst JA, Kramer EHM, et al. Missing lithotroph identified as new planctomycete. *Nature*. 1999;400(6743):446–9.
[310] Kartal B, Rattray J, van Niftrik LA, et al. *Candidatus* 'Anammoxoglobus propionicus': a new propionate oxidizing species of anaerobic ammonium oxidizing bacteria. *Syst Appl Microbiol*. 2007;30(1):39–49.
[311] Quan Z-X, Rhee S-K, Zuo J-E, et al. Diversity of ammonium-oxidizing bacteria in a granular sludge anaerobic ammonium-oxidizing (anammox) reactor. *Environ Microbiol*. 2008;10(11):3130–9.
[312] Schmid M, Walsh K, Webb R, et al. *Candidatus* 'Scalindua brodae', sp. nov., *Candidatus* 'Scalindua wagneri', sp. nov., two new species of anaerobic ammonium oxidizing bacteria. *Syst Appl Microbiol*. 2003;26(4):529–38.
[313] Guven D, Dapena A, Kartal B, et al. Propionate oxidation by and methanol inhibition of anaerobic ammonium-oxidizing bacteria. *Appl Environ Microbiol*. 2005;71(2):1066–71.
[314] Lotti T, Kleerebezem R, van Loosdrecht MCM. Effect of temperature change on anammox activity. *Biotechnol Bioeng*. 2015;112(1):98–103.
[315] Hu Z, Wessels HJCT, van Alen T, Jetten MSM, Kartal B. Nitric oxide-dependent anaerobic ammonium oxidation. *Nat Commun*. 2019;10(1):1244.
[316] Maalcke WJ, Dietl A, Marritt SJ, et al. Structural basis of biological NO generation by octaheme oxidoreductases. *J Biol Chem*. 2014;289(3):1228–42.

[317] Kartal B, Keltjens JT. Anammox biochemistry: a tale of heme *c* proteins. *Trends Biochem Sci*. 2016;41(12):998–1011.
[318] Payne WJ. Reduction of nitrogenous oxides by microorganisms. *Bacteriol Rev*. 1973;37(4): 409–52.
[319] Simon J, Klotz MG. Diversity and evolution of bioenergetic systems involved in microbial nitrogen compound transformations. *Biochim Biophys Acta Bioenerg*. 2013;1827(2):114–35.
[320] Kartal B, Maalcke WJ, de Almeida NM, et al. Molecular mechanism of anaerobic ammonium oxidation. *Nature*. 2011;479(7371):127–30.
[321] Strous M, Pelletier E, Mangenot S, et al. Deciphering the evolution and metabolism of an anammox bacterium from a community genome. *Nature*. 2006;440(7085):790–4.
[322] van de Vossenberg J, Woebken D, Maalcke WJ, et al. The metagenome of the marine anammox bacterium '*Candidatus* Scalindua profunda' illustrates the versatility of this globally important nitrogen cycle bacterium. *Environ Microbiol*. 2013;15(5):1275–89.
[323] Hu Z, Speth DR, Francoijs K-J, Quan Z-X, Jetten MSM. Metagenome analysis of a complex community reveals the metabolic blueprint of anammox bacterium '*Candidatu*s Jettenia asiatica.' *Front Microbiol*. 2012;3:366.
[324] Kartal B, Tan NCG, Van de Biezen E, Kampschreur MJ, van Loosdrecht MCM, Jetten MSM. Effect of nitric oxide on anammox bacteria. *Appl Environ Microbiol*. 2010;76(18):6304–6.
[325] van Niftrik L, Geerts WJC, van Donselaar EG, et al. Combined structural and chemical analysis of the anammoxosome: a membrane-bounded intracytoplasmic compartment in anammox bacteria. *J Struct Biol*. 2008;161(3):401–10.
[326] Sinninghe Damsté JS, Strous M, Rijpstra WIC, et al. Linearly concatenated cyclobutane lipids form a dense bacterial membrane. *Nature*. 2002;419(6908):708–12.
[327] Jetten MSM, Sliekers O, Kuypers M, et al. Anaerobic ammonium oxidation by marine and freshwater planctomycete-like bacteria. *Appl Microbiol Biotechnol*. 2003;63(2):107–14.
[328] Kuypers MMM, Sliekers AO, Lavik G, et al. Anaerobic ammonium oxidation by anammox bacteria in the Black Sea. *Nature*. 2003;422(6932):608–11.
[329] Dalsgaard T, Thamdrup B, Canfield DE. Anaerobic ammonium oxidation (anammox) in the marine environment. *Res Microbiol*. 2005;156(4):457–64.
[330] Moore TA, Xing Y, Lazenby B, et al. Prevalence of anaerobic ammonium-oxidizing bacteria in contaminated groundwater. *Environ Sci Technol*. 2011;45(17):7217–25.
[331] Humbert S, Tarnawski S, Fromin N, Mallet M-P, Aragno M, Zopfi J. Molecular detection of anammox bacteria in terrestrial ecosystems: Distribution and diversity. *ISME J*. 2010;4(3):450–4.
[332] Zhu G, Wang S, Wang Y, et al. Anaerobic ammonia oxidation in a fertilized paddy soil. *ISME J*. 2011;5(12):1905–12.
[333] Hu B, Rush D, van der Biezen E, et al. New anaerobic, ammonium-oxidizing community enriched from peat soil. *Appl Environ Microbiol*. 2011;77(3):966–71.
[334] Penton CR, Devol AH, Tiedje JM. Molecular evidence for the broad distribution of anaerobic ammonium-oxidizing bacteria in freshwater and marine sediments. *Appl Environ Microbiol*. 2006;72(10):6829.
[335] Rysgaard S, Glud RN, Risgaard-Petersen N, Dalsgaard T. Denitrification and anammox activity in Arctic marine sediments. *Limnol Oceanogr*. 2004;49(5):1493–502.
[336] Thamdrup B, Dalsgaard T. Production of $N_2$ through anaerobic ammonium oxidation coupled to nitrate reduction in marine sediments. *Appl Environ Microbiol*. 2002;68(3):1312–8.
[337] Rysgaard S, Glud RN. Anaerobic $N_2$ production in Arctic sea ice. *Limnol Oceanogr*. 2004;49(1):86–94.
[338] Jetten MS.M, Wagner M, Fuerst J, van Loosdrecht MCM, Kuenen G, Strous M. Microbiology and application of the anaerobic ammonium oxidation ('anammox') process. *Curr Opin Biotechnol*. 2001;12(3):283–8.

[339] Beermann F, Langer M, Wetterich S, et al. Permafrost thaw and liberation of inorganic nitrogen in Eastern Siberia. *Permafr Periglac Process*. 2017;28(4):605–18.
[340] Keuper F, Bodegom PM, Dorrepaal E, et al. A frozen feast: thawing permafrost increases plant-available nitrogen in subarctic peatlands. *Glob Chang Biol*. 2012;18(6):1998–2007.
[341] Penton CR. Anaerobic ammonium oxidation (anammox). In: Margesin R, editor. Permafrost soils. Berlin, Heidelberg: Springer Berlin Heidelberg; 2009. p. 149–58.
[342] Savant NK, De Datta SK. Nitrogen transformations in wetland rice soils. In: Brady NC, editor. Advances in Agronomy (Volume 35). Cambridge, MA, USA: Academic Press; 1982. p. 241–302.
[343] Anand R, Germon J-C, Groffman PM, et al. Nitrogen transformations. In: Huang PM, Li Y, Sumner ME, editors. Handbook of soil sciences: properties and processes. Boca Raton, FL, USA: CRC Press; 1982. p. 873–925.
[344] Prosser JI. Autotrophic nitrification in bacteria. *Adv Microb Physiol*. 1990;30:125–81.
[345] Bengtsson G, Torstensson L. Soil biological variables in environmental hazard assessment. In: National Swedish Environmental Protection Board, Solna, Sweden. 1988. p. 60.
[346] Somerville L, Greaves MP, Domsch KH, et al. Recommended laboratory tests for assessing the side-effects of pesticides on the soil microflora. In: Proc. 3rd Int. Workshop, Cambridge, 1987. p. 29.
[347] Kadlec RH, Knight RI, Vymazal J, Brix H, Cooper P, Haberl R. Constructed wetlands for pollution control: processes, performance, design and operation. London, UK: IWA Publishing; 2000.
[348] Patrick WH, Wyatt R. Soil nitrogen loss as a result of alternate submergence and drying. *Soil Sci Soc Am J*. 1964;28(5):647.
[349] Kawaguchi K, Kyuma K. Paddy soils in tropical Asia, their material nature and fertility. Honolulu, HI, USA: University of Hawaii Press; 1977.
[350] Stevenson FJ. Organic forms of soil nitrogen. In: Nitrogen in agricultural soils, Agronomy Monographs Volume 22. American Society of Agronomy, Crop Science Society of America, Soil Science Society of America; 1982. p. 67–122.
[351] Rose AH. Chemical microbiology. 3rd ed. London, UK: Butterworth-Heinemann; 1976.
[352] Bonde T, Nielsen T, Miller M, Sørensen J. Arginine ammonification assay as a rapid index of gross N mineralization in agricultural soils. *Biol Fertil Soils*. 2001;34(3):179–84.
[353] Alef K, Kleiner D. Arginine ammonification, a simple method to estimate microbial activity potentials in soils. *Soil Biol Biochem*. 1986;18(2):233–5.
[354] Atkin OK. Reassessing the nitrogen relations of Arctic plants: a mini-review. *Plant Cell Environ*. 2006;19:695–704.
[355] Kawahigashi M, Kaiser K, Kalbitz K, Rodionov A, Guggenberger G. Dissolved organic matter in small streams along a gradient from discontinuous to continuous permafrost. *Glob Chang Biol*. 2004;10(9):1576–86.
[356] Tanski G, Lantuit H, Ruttor S, et al. Transformation of terrestrial organic matter along thermokarst-affected permafrost coasts in the Arctic. *Sci Total Environ*. 2017;581–582: 434–47.
[357] Ivanova TI, Kuz'mina NP, Isaev AP. A microbiological characterization of the permafrost soil of Tit-Ary Island (Yakutia). *Contemp Probl Ecol*. 2012;5(6):589–96.
[358] Shi T, Reeves RH, Gilichinsky DA, Friedmann EI. Characterization of viable bacteria from Siberian permafrost by 16S rDNA sequencing. *Microb Ecol*. 1997;33(3):169–79.
[359] Wilkerson J, Dobosy R, Sayres DS, et al. Permafrost nitrous oxide emissions observed on a landscape scale using the airborne eddy-covariance method. *Atmos Chem Phys*. 2019;19(7):4257–68.
[360] Gao W, Yao Y, Liang H, et al. Emissions of nitrous oxide from continuous permafrost region in the Daxing'an Mountains, Northeast China. *Atmos Environ*. 2019;198:34–45.

[361] Takakai F, Desyatkin AR, Lopez CML, Fedorov AN, Desyatkin R, Hatano R. $CH_4$ and $N_2O$ emissions from a forest-alas ecosystem in the permafrost taiga forest region, eastern Siberia, Russia. *J Geophys Res Biogeosciences*. 2008;113(G2).
[362] Köster E, Köster K, Berninger F, Aaltonen H, Zhou X, Pumpanen J. Carbon dioxide, methane and nitrous oxide fluxes from a fire chronosequence in subarctic boreal forests of Canada. *Sci Total Environ*. 2017;601–602:895–905.
[363] Mu CC, Abbott BW, Zhao Q, et al. Permafrost collapse shifts alpine tundra to a carbon source but reduces $N_2O$ and $CH_4$ release on the northern Qinghai-Tibetan Plateau. *Geophys Res Lett*. 2017;44(17):8945–52.
[364] Paré MC, Bedard-Haughn A. Landscape-scale N mineralization and greenhouse gas emissions in Canadian Cryosols. *Geoderma*.2012;189–190:469–79.
[365] Chen X, Wang G, Zhang T, et al. Effects of warming and nitrogen fertilization on GHG flux in the permafrost region of an alpine meadow. *Atmos Environ*. 2017;157:111–24.
[366] Cui Q, Song C, Wang X, Shi F, Yu X, Tan W. Effects of warming on $N_2O$ fluxes in a boreal peatland of Permafrost region, Northeast China. *Sci Total Environ*. 2018;616–617:427–34.
[367] Bhattarai HR, Virkajärvi P, Yli-Pirilä P, Maljanen M. Emissions of atmospherically important nitrous acid (HONO) gas from northern grassland soil increases in the presence of nitrite ($NO_2$-). *Agric Ecosyst Environ*. 2018;256:194–9.
[368] Bakwin PS, Wofsy SC, Fan S-M, Fitzjarrald DR. Measurements of NOx and NOy concentrations and fluxes over Arctic tundra. *J Geophys Res*. 1992;97(D15):16545.
[369] Christensen TR, Michelsen A, Jonasson S. Exchange of $CH_4$ and $N_2O$ in a subarctic heath soil: effects of inorganic N and P and amino acid addition. *Soil Biol Biochem*. 1999;31(4):637–41.
[370] Jonasson S, Michelsen A. Nutrient cycling in Subarctic and Arctic ecosystems, with special reference to the Abisko and Tornetrask region. *Plant Ecol Subarct Swedish Lapl*. 1996;(45): 45–52.
[371] Rodionow A, Flessa H, Kazansky O, Guggenberger G. Organic matter composition and potential trace gas production of permafrost soils in the forest tundra in northern Siberia. *Geoderma*. 2006;135:49–62.
[372] Sorensen PL, Jonasson S, Michelsen A. Nitrogen fixation, denitrification, and ecosystem nitrogen pools in relation to vegetation development in the subarctic. *Arct Antarct Alp Res*. 2006;38(2):263–72.
[373] Churchill, JA. Spatial variations of soil methane and nitrous oxide emissions in subarctic environments of Churchill, Manitoba. University of Manitoba, Winnipeg Manitoba (Thesis). 2007.
[374] Spataro F, Ianniello A, Salvatori R, Nardino M, Esposito G, Montagnoli M. Sources of atmospheric nitrous acid (HONO) in the European High Arctic. *Rend Lincei*. 2017;28(1):25–33.
[375] Beine HJ, Amoroso A, Dominé F, et al. Surprisingly small HONO emissions from snow surfaces at Browning Pass, Antarctica. *Atmos Chem Phys*. 2006;6(9):2569–80.
[376] DeLuca TH, Zackrisson O, Nilsson M-C, Sellstedt A. Quantifying nitrogen-fixation in feather moss carpets of boreal forests. *Nature*. 2002;419(6910):917–20.
[377] Dickson LG. Constraints to nitrogen fixation by cryptogamic crusts in a polar desert ecosystem, Devon Island, N.W.T., Canada. *Arct Antarct Alp Res*. 2000;32(1):40–5.
[378] Vanderpuye AW, Elvebakk A, Nilsen L. Plant communities along environmental gradients of high-arctic mires in Sassendalen, Svalbard. *J Veg Sci*. 2002;13(6):875–84.
[379] Mercado-Díaz JA, Gould WA, González G. Soil nutrients, landscape age, and Sphagno-Eriophoretum vaginati; plant communities in Arctic moist-acidic tundra landscapes. *Open J Soil Sci*. 2014;04(11):375–87.
[380] Morison MQ, Macrae ML, Petrone RM, Fishback L. Climate-induced changes in nutrient transformations across landscape units in a thermokarst subarctic peatland. *Arct Antarct Alp Res*. 2018;50(1):e1519366.

[381] Hayashi K, Shimomura Y, Morimoto S, Uchida M, Nakatsubo T, Hayatsu M. Characteristics of ammonia oxidation potentials and ammonia oxidizers in mineral soil under *Salix polaris*–moss vegetation in Ny-Ålesund, Svalbard. *Polar Biol*. 2016;39(4):725–41.
[382] Lu X, Yan Y, Fan J, Wang X. Gross Nitrification and denitrification in alpine grassland ecosystems on the Tibetan Plateau. *Arct Antarct Alp Res*. 2012;44(2):188–96.
[383] Bliss LC, Gold WG. The patterning of plant communities and edaphic factors along a high arctic coastline: implications for succession. *Can J Bot*.1994;72(8):1095–107.
[384] Rysgaard S, Glud RN, Sejr MK, Blicher ME, Stahl HJ. Denitrification activity and oxygen dynamics in Arctic sea ice. *Polar Biol*. 2008;31(5):527–37.
[385] Wild B, Schnecker J, Bárta J, et al. Nitrogen dynamics in Turbic Cryosols from Siberia and Greenland. *Soil Biol Biochem*. 2013;67:85–93.