Trends in Microbiology

CellPress

Review

A New Perspective on Microbes Formerly Known as Nitrite-Oxidizing Bacteria

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Nitrite-oxidizing bacteria (NOB) catalyze the second step of nitrification, nitrite oxidation to nitrate, which is an important process of the biogeochemical nitrogen cycle. NOB were traditionally perceived as physiologically restricted organisms and were less intensively studied than other nitrogen-cycling microorganisms. This picture is in contrast to new discoveries of an unexpected high diversity of mostly uncultured NOB and a great physiological versatility, which includes complex microbe–microbe interactions and lifestyles outside the nitrogen cycle. Most surprisingly, close relatives to NOB perform complete nitrification (ammonia oxidation to nitrate) and this finding will have far-reaching implications for nitrification research. We review recent work that has changed our perspective on NOB and provides a new basis for future studies on these enigmatic organisms.

Trends

Nitrite-oxidizing bacteria (NOB) are key players in the biogeochemical nitrogen cycle. They are a phylogenetically diverse guild with pronounced ecological niche specialization and they differ from each other in fundamental physiological and molecular traits.

NOB are involved in complex symbioses with ammonia-oxidizing and heterotrophic microorganisms. In a new type of interaction called reciprocal feeding, NOB recruit ammonia oxidizers to use urea or cyanate as energy source.

NOB are surprisingly versatile and can switch between nitrite oxidation and alternative metabolisms such as H_2 or formate oxidation. Thus, NOB may have diverse ecological functions within and beyond the nitrogen cycle.

The unexpected discovery of complete ammonia oxidizers ('comammox') in the NOB genus *Nitrospira* will have broad implications for future research on nitrification and the nitrogen cycle.

NOB: the 'Big Unknown' of the Nitrogen Cycle

Nitrogen is essential for all living organisms on Earth. In the biogeochemical nitrogen cycle, microorganisms mediate essential conversions of nitrogen compounds, such as N_2 fixation into organic molecules and the recycling of nitrogen from decaying biomass into the atmosphere. A key step of the nitrogen cycle is the sequential oxidation of ammonia via nitrite to nitrate, a process termed 'nitrification' (Figure 1). More than a century ago Sergei Winogradsky isolated the first chemolithoautotrophic bacteria that grew by nitrification, using ammonia or nitrite as their energy source and electron donor [1,2]. In the course of his monumental work, Winogradsky found the two nitrification steps to be catalyzed by distinct bacteria, **ammonia-oxidizing bacteria** (**AOB**; see Glossary) and NOB whose cooperation was needed to achieve complete nitrification. He also noticed that both groups were slow-growing organisms whose cultivation in the laboratory required patience [2]. In the following century, nitrification research made great progress by illuminating the biochemistry of nitrifiers from the genera *Nitrosomonas* (AOB) and *Nitrobacter* (NOB) (reviewed in [3]). This research was driven by scientific curiosity and the practical importance of nitrification. In agriculture, the conversion of fertilizer ammonium to nitrate leads to substantial nitrogen loss from soils. In contrast, nitrification is beneficial as a key step of biological wastewater treatment for eliminating excess nitrogen from sewage.

For decades, nitrification research has focused mainly on ammonia oxidizers. The reasons for this include (i) ammonia oxidation has been considered the rate-limiting nitrification step [4], (ii) research on ammonia oxidizers has received an impetus from the discovery of **ammonia-oxidizing archaea (AOA)** [5], and (iii) NOB have a reputation for being even more difficult to grow in the laboratory than AOA or AOB. Since nitrite does not accumulate in most ecosystems, the nitrite oxidation process receives less attention, although low-standing pools of a substrate do not allow conclusions on the importance of the consuming reactions. In addition, NOB were

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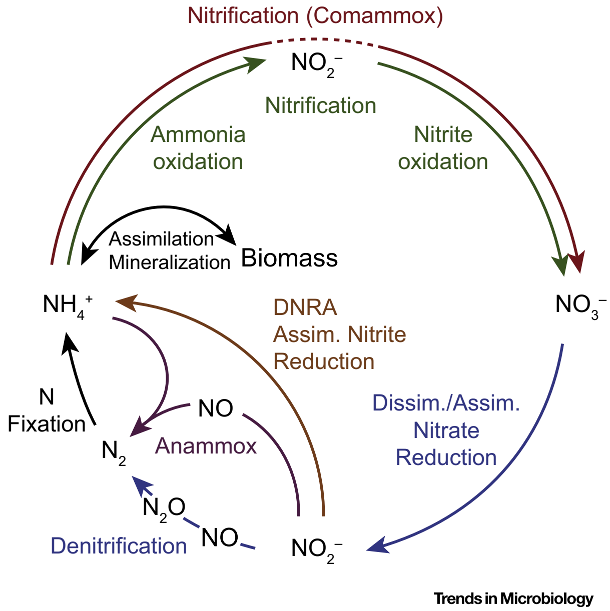

Figure 1. Nitrite, a Key Intermediate of the Biogeochemical Nitrogen Cycle. Schematic illustration of the key processes of the nitrogen cycle. Note that the fate of nitrite determines whether nitrogen remains fixed (as nitrite, nitrate, or ammonium) or is lost to the atmosphere (as NO, N_2O, or N_2). The stippled line for comammox indicates that nitrite is an intermediate but is oxidized to nitrate by the same organism. Abbreviations: Anammox, anaerobic ammonium oxidation; DNRA, dissimilatory nitrite reduction to ammonia; assim., assimilatory; dissim., dissimilatory.

perceived as obligate chemolithoautotrophs with a very limited physiological repertoire and thus little potential for the discovery of new physiologies. In consequence, progress in NOB-related research lagged behind the knowledge increase on other nitrogen-cycling microbes. However, the fate of nitrite determines whether fixed nitrogen remains in an ecosystem or is lost to the atmosphere (Figure 1). NOB counteract nitrogen loss by converting nitrite to nitrate, which is utilized as a nitrogen source by many microbes and plants and represents an impressive 88% of the fixed nitrogen in the oceans [6]. Hence, NOB have an important regulatory function in the nitrogen cycle. Furthermore, nitrite is toxic to eukaryotes [7] and inhibits bacterial growth [8]. NOB activity in **wastewater treatment plants (WWTPs)** tends to be unstable (in particular in industrial systems), and breakdowns of nitrite oxidation can cause tremendous ecological damage if nitrite from WWTPs leaks into natural waters. Considering the ecological importance of NOB and our limited knowledge on their biology, NOB are a 'big unknown' of the nitrogen cycle. Fortunately, this situation is now improving as metagenomics and other 'meta-omics' approaches [9,10], single-cell isotope labeling and genomic techniques [11,12], and refined cultivation methods [13,14] offer new opportunities to study NOB. This review provides an overview of recent insights and discoveries that have changed our perspective on these fascinating organisms. We focus on the chemolithoautotrophic NOB and skip the phototrophic NOB [15,16], which use light as an energy source and utilize nitrite only as electron donor for biosynthesis.

A Highly Diverse Functional Group

The known NOB belong to seven genera in four bacterial phyla (Figure 2), including a new candidate genus of uncultured marine NOB, '*Candidatus* Nitromaritima', which is related to *Nitrospina* and was proposed on the basis of comparative analyses of single-cell amplified genomes [17]. All NOB have Gram-negative cell envelopes except *Nitrolancea hollandica* which stains Gram-positive and forms thick cell wall layers [18]. The NOB lineages are unequally distributed in the environment (Figure 2). In particular, the Nitrospinae are the predominant

Glossary

Ammonia monooxygenase (AMO): the key enzyme of all known bacterial and archaeal ammonia oxidizers (including comammox), which oxidizes ammonia to hydroxylamine.

Ammonia-oxidizing archaea (AOA) and bacteria (AOB): mediate the first step of nitrification by oxidizing ammonia to nitrite.

Complete ammonia oxidizer (Comammox): an organism that is capable of performing complete nitrification on its own. Comammox was a hypothetical microbe until the recent discovery of completely nitrifying bacteria in the genus *Nitrospira*.

Extracellular polymeric substances (EPS): are secreted by microorganisms and are considered to be of key importance for the integrity and function of microbial biofilms and flocs.

Fluorescence *in situ* hybridization (FISH): FISH with rRNA-targeted probes is a widely used cultivation-independent method to detect and identify microorganisms in environmental samples.

Hydroxylamine dehydrogenase (HAO): a key enzyme of bacterial ammonia oxidizers (including comammox), which oxidizes hydroxylamine to nitrite. Formerly known as hydroxylamine oxidoreductase.

Nitrite oxidoreductase (NXR): the key enzyme of nitrite oxidizers (including comammox) that catalyzes nitrite oxidation to nitrate, but can also reduce nitrate to nitrite.

Nitrite-oxidizing bacteria (NOB): catalyze the second step of nitrification by oxidizing nitrite to nitrate.

Oxygen minimum zone (OMZ): oceanic regions where intense respiration of organic matter (exported from overlying productive waters) and a poor ventilation of water masses cause a depletion of dissolved oxygen.

Wastewater treatment plant (WWTP): modern WWTPs include a combination of the nitrification and denitrification processes to eliminate excess nitrogen from sewage.