# Expanded metabolic versatility of ubiquitous nitrite-oxidizing bacteria from the genus *Nitrospira*

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***Nitrospira* are a diverse group of nitrite-oxidizing bacteria and among the environmentally most widespread nitrifiers. However, they remain scarcely studied and mostly uncultured. Based on genomic and experimental data from *Nitrospira moscoviensis* representing the ubiquitous *Nitrospira* lineage II, we identified ecophysiological traits that contribute to the ecological success of *Nitrospira*. Unexpectedly, *N. moscoviensis* possesses genes coding for a urease and cleaves urea to ammonia and $CO_2$. Ureolysis was not observed yet in nitrite oxidizers and enables *N. moscoviensis* to supply ammonia oxidizers lacking urease with ammonia from urea, which is fully nitrified by this consortium through reciprocal feeding. The presence of highly similar urease genes in *Nitrospira lenta* from activated sludge, in metagenomes from soils and freshwater habitats, and of other ureases in marine nitrite oxidizers, suggests a wide distribution of this extended interaction between ammonia and nitrite oxidizers, which enables nitrite-oxidizing bacteria to indirectly use urea as a source of energy. A soluble formate dehydrogenase lends additional ecophysiological flexibility and allows *N. moscoviensis* to use formate, with or without concomitant nitrite oxidation, using oxygen, nitrate, or both compounds as terminal electron acceptors. Compared with *Nitrospira defluvii* from lineage I, *N. moscoviensis* shares the *Nitrospira* core metabolism but shows substantial genomic dissimilarity including genes for adaptations to elevated oxygen concentrations. Reciprocal feeding and metabolic versatility, including the participation in different nitrogen cycling processes, likely are key factors for the niche partitioning, the ubiquity, and the high diversity of *Nitrospira* in natural and engineered ecosystems.**

*Nitrospira* | nitrification | genome | urease | formate

Nitrification, a key aerobic process of the biogeochemical nitrogen (N) cycle, is catalyzed by two guilds of chemolithoautotrophic microorganisms. The ammonia-oxidizing microorganisms (bacteria and archaea; AOM) oxidize ammonia to nitrite, which is subsequently oxidized to nitrate by nitrite-oxidizing bacteria (NOB). Nitrification links aerobic and anaerobic pathways of the N cycle by providing nitrate or nitrite as electron acceptors for dissimilatory nitrate reduction, denitrification, respiratory ammonification, and anaerobic ammonium oxidation (1, 2). The end product of nitrification, nitrate, is an important source of nitrogen for assimilation by many microorganisms and plants. Moreover, nitrification is a key step of biological wastewater treatment but also contributes to N losses from fertilized agricultural soils (3). Being a two-step process that involves two functional groups, nitrification is a prime example of a tight metabolic interaction between free-living microorganisms.

Current insights into the ecology of chemolithoautotrophic NOB suggest that two of the six known NOB genera are restricted to marine ecosystems (*Nitrospina* and *Nitrococcus*) (4). The recently identified *Nitrolancea* was enriched from activated sludge from a wastewater treatment plant (WWTP) (5), whereas *Nitrotoga* occur in soils and WWTPs (6, 7). *Nitrobacter* are generally common in terrestrial and limnic habitats. Analyses of *Nitrobacter* genome sequences provided first insights into the genomic makeup of NOB and revealed a greater metabolic flexibility than anticipated earlier, which included the mixotrophic utilization of various organic substrates (8, 9). However, *Nitrobacter* require high nitrite concentrations (10, 11), and molecular surveys indicated that *Nitrobacter* are not the primary NOB in ecosystems with low ambient nitrite levels such as unfertilized soils (12), freshwater habitats (13), and most WWTPs (14).

Among all known NOB, the genus *Nitrospira* appears to be most widespread in different habitat types and is phylogenetically most diverse. *Nitrospira* are well adapted to low nitrite concentrations (10, 11) and form at least six phylogenetic lineages (15, 16) that are globally distributed in soils (17, 18), the oceans (19), freshwater habitats (20), hot springs (16), and many other oxic habitats (15). In addition, *Nitrospira* members are the key NOB in most WWTPs (14, 15). *Nitrospira* are notoriously difficult to culture under laboratory conditions and, hence, despite their ecological and biotechnological importance, little is known about their ecophysiology. Interestingly, not all members of this genus are restricted to nitrite as their sole source of energy and reductant. Some *Nitrospira* from marine ecosystems or activated sludge can use simple organic substrates, such as pyruvate,

## Significance

**Nitrification, the sequential aerobic oxidation of ammonia via nitrite to nitrate, is a key process of the biogeochemical nitrogen cycle and catalyzed by two aerobic microbial guilds (nitrifiers): ammonia oxidizers and nitrite-oxidizing bacteria (NOB). NOB are generally considered as metabolically restricted and dependent on ammonia oxidizers. Here, we report that, surprisingly, key NOB of many ecosystems (*Nitrospira*) convert urea, an important ammonia source in nature, to ammonia and $CO_2$. Thus, *Nitrospira* supply urease-negative ammonia oxidizers with ammonia and receive nitrite produced by ammonia oxidation in return, leading to a reciprocal feeding interaction of nitrifiers. Moreover, *Nitrospira* couple formate oxidation with nitrate reduction to remain active in anoxia. Accordingly, *Nitrospira* are unexpectedly flexible and contribute to nitrogen cycling beyond nitrite oxidation.**

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formate, and glycerol, for carbon assimilation and probably also as energy sources in addition to $CO_2$ and nitrite (mixotrophy) (15, 19, 21, 22). *Nitrospira moscoviensis* even grows by aerobic hydrogen oxidation as an alternative lifestyle outside the N cycle (23). Furthermore, this organism can reduce nitrate with $H_2$ as an electron donor, but under these conditions, growth was not detected (24).

So far, only one study analyzed a fully sequenced *Nitrospira* genome, which was obtained from *N. defluvii* (25). This *Nitrospira* lineage I member had been enriched from a WWTP (26). Here, we analyzed the genome of *N. moscovienis* representing *Nitrospira* lineage II, which is the *Nitrospira* clade most widely distributed in both natural and engineered ecosystems (15). This newly sequenced *Nitrospira* genome revealed surprising metabolic features that were experimentally confirmed. These findings change the current perception on the interdependence of nitrifiers and demonstrate an unexpected ecophysiological versatility of *Nitrospira* with contributions to N-cycling processes other than nitrite oxidation.

## Results and Discussion

**Hydrolysis of Urea.** The complete genome of *N. moscoviensis* comprises 4.59 Mb with 4,863 predicted coding sequences (CDS) (Table S1). Among the most striking features identified in the genome were a functional hydrogenase for nitrite-independent growth (23) and a gene cluster for the utilization of urea. This locus codes for the urea-binding subunit of a urea ABC transporter (NITMOv2_1251), all three subunits of a putative nickel-binding urease (UreABC; NITMOv2_1253, NITMOv2_1255, and NITMOv2_1260), and the accessory proteins UreF, UreG, UreD required for urease maturation (27) (Fig. S1*A*, Dataset S1, and *SI Results and Discussion*). Urease (EC 3.5.1.5) catalyzes the ATP-independent hydrolysis of urea resulting in ammonia and carbamate, which spontaneously decomposes into a second molecule of ammonia and bicarbonate (27). In the context of nitrification, urease is an important enzyme that enables some ammonia-oxidizing bacteria and archaea to obtain both ammonia and $CO_2$ from urea. Thus, these urease-positive AOM can use urea as a source of energy, N, and carbon (28). In contrast, the presence of urease-encoding genes in a nitrite-oxidizing bacterium was unexpected because NOB would not be able to use ammonia as energy source and urea degradation by NOB had never been observed. To confirm the ureolytic activity, a pure culture of *N. moscoviensis* was incubated in liquid mineral medium that contained 1 mM urea. Indeed, within a few hours of incubation, a strong increase of the free ammonium concentration was observed in the culture supernatant, which was independent from the concomitant presence of nitrite and did not occur in the control experiments without *N. moscoviensis* biomass (Fig. 1). This result suggests that after uptake into the *Nitrospira* cells by an ABC transporter or passive diffusion (*SI Results and Discussion*), urea was cleaved by the cytoplasmic urease (Fig. S1*B*). Ammonia then diffused through the cell membrane into the medium or ammonium was exported by AmtB transporters, which are encoded by three *amtB* genes in the close vicinity of the urease genes (Fig. S1*A*). Consistent with the lack of urease genes in the genome of *N. defluvii*, no ureolytic activity was observed during incubation of this *Nitrospira* strain in medium containing urea (Fig. S2*A*).

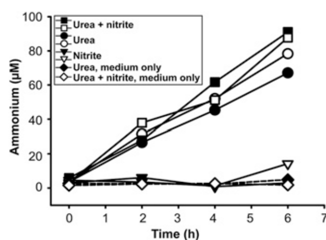


**Fig. 1.** Ureolytic activity of a *N. moscoviensis* pure culture during incubations with urea or both urea and nitrite. Supply of urea led to the accumulation of ammonium in the culture supernatant. Ammonium formation was not detected in the absence of urea (nitrite-only incubations) and in the control experiments without addition of biomass. The results of two biological replicates are shown for each incubation experiment.

**Ureolytic Activity of NOB Drives Full Nitrification.** Urea is an important dissolved organic N compound in marine (coastal and open water) and freshwater ecosystems, where it is produced by heterotrophic bacteria and also released by phytoplankton, microfauna, and macrofauna (29). Because commercially synthesized urea plays a major role as plant fertilizer, urea is globally widespread in agricultural soils. Fertilization also contributes to increased urea levels in coastal waters due to riverine input. In addition, a large portion of the N in municipal wastewater occurs as urea. Despite the environmental abundance of urea, not all AOM possess urease (28), and urease-negative AOM are thought to thrive mainly in habitats where free ammonia ($NH_3$) levels are relatively high, such as eutrophic waters and neutral or alkaline soils. They appear to be outcompeted by urease-positive AOM in acid soils where free $NH_3$ is scarce but urea is a source of $NH_3$ (28).

AOM and NOB are generally considered to be mutualistic symbionts because AOM produce nitrite, which is required as substrate and also detoxified by NOB (30). Accordingly, nitrifiers often tightly coaggregate in flocs and biofilms (14), and the abundances of AOA and *Nitrospira* in soils have been found to correlate (17). This classical scheme dictates that NOB strongly depend on AOM to initiate nitrification. The presence of urease and ureolytic activity in *N. moscoviensis* changes this picture and opens an interesting perspective on the interactions between NOB and AOM. Ureolytic NOB could actually feed urease-negative AOM by cleaving urea and releasing ammonia. The AOM would subsequently oxidize the ammonia to nitrite, providing the NOB with their actual source of energy (Fig. 2*A*). The ammonia and $CO_2$ obtained from urea would also be N and carbon sources for assimilation by both partners. Interestingly, the cleavage of urea by NOB represents the initial step that fuels nitrification by such NOB–AOM consortia.

To test whether this "reciprocal feeding" NOB–AOM interaction actually occurs, we coincubated a pure culture of *N. moscoviensis* with a pure culture of the urease-negative ammonia-oxidizing bacterium *Nitrosomonas europaea*, whose sequenced genome lacks any genes for urea utilization (31) and which did not show ureolytic activity in a control experiment (Fig. S2*B*). The only substrates provided to this coincubation were 1 mM urea as well as $O_2$ and $CO_2$ from air. During 7 d of incubation, nitrate accumulated in the culture supernatant, whereas the concentrations of ammonium and nitrite did not increase (Fig. 2*B*). The only explanation for this result is full nitrification by reciprocal feeding (Fig. 2*A*). Full nitrification was also observed in a second coincubation experiment with an initial urea concentration of only 50 μM, which is closer to the micromolar levels of urea found in natural habitats (29) (Fig. S2*C*).

Intriguingly, genes for urea utilization occur also in NOB other than *N. moscoviensis*. The recently sequenced draft genome of *Nitrospira lenta*, a strain which also belongs to *Nitrospira* lineage II (32), encodes a urea ABC transporter and the urease at a genomic locus that is mostly syntenic with the homologous region in *N. moscoviensis* (Fig. S1*A* and Dataset S1). According to the phylogeny of the urease alpha subunit UreC, these two *Nitrospira* ureases are closely related and clearly distinct from the ureases of all known AOM (Fig. S3). Two *Nitrospira*-like UreC sequences were also found in an activated sludge metagenome from a municipal WWTP (Aalborg West, Denmark). They were linked to scaffolds that encoded several other proteins with highest sequence similarities to homologs in *N. defluvii*, strongly suggesting a *Nitrospira* origin. Together with the UreC sequences

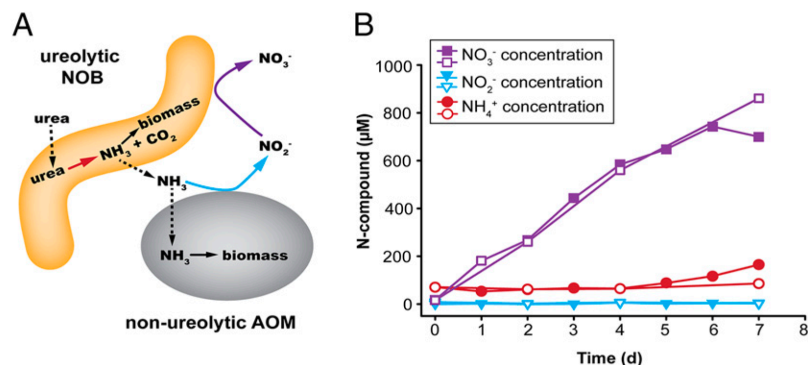


**Fig. 2.** Full nitrification by *N. moscoviensis* and urease-negative AOM through reciprocal feeding. (*A*) Schematic illustration of the proposed reciprocal feeding interaction between ureolytic NOB such as *N. moscoviensis* (yellow) and urease-negative AOM such as *N. europaea* (gray). Solid arrows represent conversions of substrates; dashed arrows the uptake or release of substrates. (*B*) Concentrations of ammonium, nitrite, and nitrate in a coincubation of *N. moscoviensis* and urease-negative *N. europaea* during 7 d of incubation with urea as the sole source of energy and nitrogen. The results of two biological replicates are shown for all incubations.

from *N. moscoviensis* and *N. lenta*, they form a distinct monophyletic clade (Fig. S3). A screening of publicly available metagenomes from various environments retrieved additional UreC sequences that fall into this *Nitrospira* UreC clade and share high amino acid identity of 85.3–96.3% with UreC from *N. moscoviensis*, *N. lenta*, and the sequences from the Danish WWTP. These sequences from soil and freshwater metagenomes (Fig. S3) suggest that *Nitrospira* with urease occur widespread in different terrestrial and aquatic habitats where urea is available (e.g., due to fertilization). Hence, it is tempting to speculate that reciprocal feeding of *Nitrospira* and AOM could be a common phenomenon, whose contribution to the total nitrification in different ecosystems remains to be determined.

Single-cell genomes from uncultured *Nitrospina*-like NOB (accession nos. PRJNA199992 and PRJNA199055) also contain complete sets of urease genes. The genus *Nitrospina* (4) represents the major known group of marine NOB, and the capability of using urea as N source and for reciprocal feeding with AOM could be beneficial in oceanic habitats, where the availability of urea can be higher than that of ammonium (33). Because the genome of the type strain *Nitrospina gracilis* (34) lacks urease, this feature is not homogeneously distributed in this genus, as it is not among *Nitrospira* (see above). The ureases of *Nitrospira* and *Nitrospina* are phylogenetically not closely related. The *Nitrospina* UreC sequences fall into a lineage of ureases from *Proteobacteria* (Fig. S3), indicating that the *ureC* gene was subject to lateral gene transfer. All three sequenced *Nitrobacter* genomes lack urease but encode putative urea carboxylases and allophanate hydrolases, which together might cleave urea by an ATP-dependent mechanism (9). However, these candidate proteins in *Nitrobacter* are shorter than their functionally characterized homologs in other microorganisms. Similar short variations occur also in the genomes of *N. defluvii* and *N. europaea*, both of which are unable to use urea (Fig. S2 *A* and *B*), suggesting that these enzymes play a different and yet unknown functional role.

Nitrification by reciprocal feeding may influence the population structure of nitrifiers. AOM lacking urease could survive by tightly interacting with ureolytic NOB if urea is the main source of ammonia. This mechanism could influence the local distribution of urease-negative AOM in microniches shared with ureolytic NOB within soils and biofilms, and it might contribute to a high nitrifier diversity by sustaining populations of urease-negative AOM in otherwise unfavorable habitats. Additionally, it demonstrates that NOB can take critical roles within AOM–NOB consortia beyond nitrite oxidation and shows that the interplay between nitrifiers can be surprisingly complex. Reciprocal feeding interactions represent a compartmentalization of functions, which balances the metabolic and genomic costs among the partners (35) and might thus make a nitrifier consortium bioenergetically more efficient. Additional aspects of complemental AOM–NOB symbioses might include the interspecies transfer of Fe-loaded siderophores and of various organic N and C compounds and cofactors (36). Such complex interactions of nitrifiers may need a tight regulation, which could be achieved by cell-cell communication with diffusible signal molecules (36, 37).

**Use of Formate with $O_2$ or Nitrate as Electron Acceptor.** *Nitrospira* can reach high abundances in deep layers of nitrifying biofilms where low dissolved oxygen concentrations prevail (38, 39). To elucidate whether *Nitrospira* may survive in such niches by other metabolic activities than aerobic nitrite oxidation, we incubated *N. moscoviensis* cells under anoxic conditions in the presence of formate as energy source and electron donor and nitrate as terminal electron acceptor. Nitrate was chosen because its reduction by *N. moscoviensis* to nitrite had already been observed with $H_2$ as electron donor (24). Formate was added because the genomes of *N. moscoviensis* (Dataset S1) and *N. defluvii* (25) encode a $NAD^+$-dependent soluble formate dehydrogenase and a transporter from the formate/nitrite transporter family (NITMOv2_3821, NITMOv2_3822, NITMOv2_3823, NITMOv2_3825) (Fig. S1*B*). Formate is a common product of fermenting organisms, which may occur in the spatial proximity of *Nitrospira* in hypoxic or anoxic habitats.

Indeed, the provided nitrate was reduced to nitrite upon addition of 1 mM formate, which was completely consumed during the incubations (Fig. 3*A*). When formate was provided in excess, its consumption stopped after all nitrate had been reduced to nitrite (Fig. S4*A*). These results demonstrate the utilization of nitrate instead of oxygen as terminal electron acceptor. The observed nitrate reduction was most likely catalyzed by nitrite oxidoreductase (NXR) (*SI Results and Discussion*) operating in the reverse direction because no other enzyme for dissimilatory nitrate reduction was found in the genome of *N. moscoviensis*. All supplied nitrate was nearly stoichiometrically reduced to nitrite (Fig. 3*A* and Fig. S4*A*). *N. moscoviensis* encodes four copper-containing dissimilatory nitrite reductases (NirK), which were apparently not strongly active during these incubations. Because no nitrite was formed in medium containing only nitrate but no formate (Fig. 3*A*), we can exclude the possibility that *N. moscoviensis* reduced nitrate with electrons derived from intracellular storage compounds. No nitrate reduction was observed with some other simple organic substrates tested (Fig. S4*B* and *SI Results and Discussion*).

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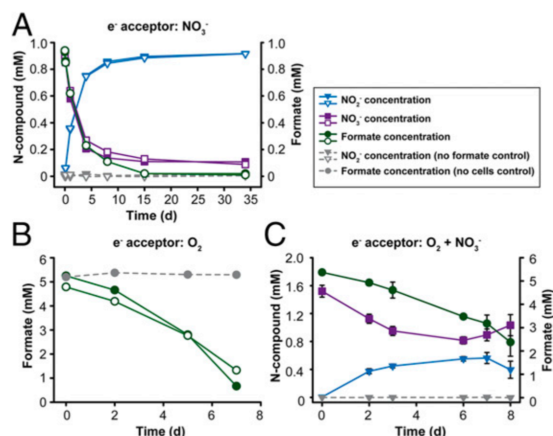


**Fig. 3.** Formate utilization by a pure culture of *N. moscoviensis*. (*A*) Anaerobic consumption of formate with nitrate as terminal electron acceptor. Nitrate was nearly stoichiometrically reduced to nitrite. No nitrite formation from nitrate was observed in the control experiment without formate. The results of two biological replicates are shown for all incubations. (*B*) Aerobic use of formate with $O_2$ as terminal electron acceptor. For the incubations with *N. moscoviensis* cells, the results of two biological replicates are shown. Please note that the control experiment with formate but without cells, which confirms the chemical stability of formate, was performed for these incubation conditions only. (*C*) Aerobic use of formate with both $O_2$ and nitrate as terminal electron acceptors. The oxidation of the formed nitrite became detectable on the seventh day of incubation. Data points show the means of biological replicates ($n$ = 3). Error bars represent SD and are not shown if smaller than symbols. An extended incubation experiment (23 d) confirming the concomitant utilization of formate and nitrite is shown in Fig. S5 *D–F*.

Additional experiments showed that *N. moscoviensis* also used formate as sole substrate with $O_2$ as terminal electron acceptor (Fig. 3*B* and Fig. S5*A*) and grew under these conditions, although growth on formate was weaker than growth on nitrite with $O_2$ as electron acceptor (Fig. S5 *B* and *C*). When formate and both electron acceptors nitrate and $O_2$ were supplied, the formate concentration decreased by 1.9 mM, whereas the nitrate concentration decreased only by 0.7 mM during the first 6 d of incubation (Fig. 3*C*). An explanation could be that the electrons derived from formate were mainly channeled to $O_2$ or used for $CO_2$ fixation. In addition, concurrent reoxidation of the produced nitrite (with $O_2$) may replenish the nitrate pool and, thus, reduce the net consumption of nitrate. Indeed, net nitrite oxidation became detectable after 6 d while formate was still being consumed (Fig. 3*C*). These data suggested that *N. moscoviensis* was simultaneously performing three different metabolic reactions: the oxidation of formate with nitrate and $O_2$ as electron acceptors and the oxidation of nitrite with $O_2$ as electron acceptor. This striking physiological flexibility was further investigated in additional experiments. In an extended oxic incubation, formate and nitrate were supplied initially and nitrite was formed by nitrate reduction. The subsequent net decrease of nitrite and increase of nitrate, together with ongoing formate consumption, confirmed that formate and nitrite were used concomitantly as electron donors (Fig. S5 *D–F*). Net nitrite consumption was slower than in the absence of formate (Fig. S5 *E* and *G*). The utilization of $O_2$ as electron acceptor with formate as electron donor was monitored by microrespirometry (Fig. S6). In these experiments, the rate of $O_2$ consumption decreased in the presence of nitrate as an alternative electron acceptor. This decrease of the $O_2$ consumption rate and the concurrent formation of nitrite from nitrate confirmed that *N. moscoviensis* was using both electron acceptors simultaneously (Fig. S6).

The aerobic use of formate by *N. moscoviensis* is consistent with the utilization of $^{14}C$-labeled formate by uncultured *Nitrospira* in oxic-activated sludge, which was recently observed by fluorescence in situ hybridization and microautoradiography (21), and the growth on formate of *Nitrospira japonica* isolated from activated sludge (40). Formate is aerobically metabolized also by *Nitrobacter* (41, 42) and *Nitrolancea hollandica* (5). However, the anaerobic use of an organic compound by NOB, as shown here for a member of the widely distributed lineage II *Nitrospira* (Fig. 3*A*), has only been reported for *Nitrobacter* so far (43, 44). The simultaneous oxidation of an organic substrate and nitrite, together with the parallel use of nitrate and oxygen as electron acceptors, demonstrate a high degree of metabolic versatility that was not anticipated for NOB.

**Comparative Genomics Reveals Putative Features for Niche Partitioning of *Nitrospira*.** The genome of *N. moscoviensis* is slightly larger than the previously sequenced genome of *N. defluvii* (25). Key features of both genomes are summarized in Table S1. Only 56% of all *N. moscoviensis* CDS have homologs in *N. defluvii*, and 40–44% of the CDS in each genome code for functions not found in the respective other *Nitrospira* strain (Table S1). This genomic dissimilarity reflects the affiliation to different *Nitrospira* lineages and the relatively low 16S rRNA sequence identity of 94.2% between *N. moscoviensis* and *N. defluvii*. Nevertheless, the two *Nitrospira* strains share a highly conserved core metabolism for their chemolithoautotrophic nitrite-oxidizing lifestyle (Fig. S7 and *SI Results and Discussion*).

A possibly important, outstanding feature of *N. moscoviensis* is related to the assimilation of N from nitrite. All cultured *Nitrospira* (including *N. moscoviensis*) grow on nitrite as the sole N source for assimilation. In *N. defluvii* and *N. lenta*, nitrite reduction to ammonia is most likely catalyzed by a ferredoxin-dependent nitrite reductase, whose gene (*nirA*) is located in the vicinity of various other genes for N acquisition and assimilation (Fig. S1*A* and Dataset S1). Surprisingly, *N. moscoviensis* does not possess *nirA* (Fig. S1*A*) or any other known pathway to assimilate N from nitrite. An interesting candidate for this function is an octaheme cytochrome *c* (OCC) encoded in the same genomic region where the other two *Nitrospira* genomes contain *nirA* (Fig. S1*A* and *SI Results and Discussion*). Various forms of OCCs have been linked to assimilatory or dissimilatory nitrite reduction in other organisms (2, 45). Intriguingly, *N. moscoviensis* might link nitrite reduction to ammonia by the OCC with energy conservation by proton translocation across the cell membrane (*SI Results and Discussion*). This energy gain could partly compensate for the costs of the reverse electron transport, which is needed to provide reductants for assimilatory pathways if nitrite is the sole electron donor. This feature could be an adaptation to highly oligotrophic environments and would distinguish *N. moscoviensis* from *N. defluvii* and *N. lenta*, which use canonical NirA (Fig. S1*A*).

Consistent with the capability of *N. moscoviensis* to grow by aerobic $H_2$ oxidation (23), its genome encodes a cytoplasmic group 2a uptake hydrogenase and several proteins that could be involved in electron transfer from the hydrogenase to quinone (23) (NITMOv2_1637 to NITMOv2_1657). These genes are missing in *N. defluvii*.

Because nitrite oxidation is an aerobic process, *Nitrospira* must be able to cope with reactive oxygen species (ROS). Interestingly, *N. defluvii* lacks any genes of superoxide dismutase (SOD), superoxide reductase, and catalase (25), which are widely distributed key enzymes for the defense against ROS. In stark contrast, the genome of *N. moscoviensis* encodes a canonical SOD and a catalase (Fig. S1*B*, Dataset S1, and *SI Results and Discussion*). In addition, *N. moscoviensis* possesses the putative alternative ROS defense mechanisms as predicted for *N. defluvii* (25) (*SI Results and Discussion*). The larger repertoire for ROS defense of *N. moscoviensis* should confer ecological advantages

at elevated oxygen levels. Consistently, the *Nitrospira* community composition shifted from lineage I (related to *N. defluvii*) to lineage II *Nitrospira* (related to *N. moscoviensis*) in nitrifying chemostats after an increase in the dissolved oxygen concentration (46).

## Conclusions

The genome of *N. moscoviensis* revealed a previously unknown type of interaction between NOB and AOM, which could contribute to the ecological success of *Nitrospira*. Reciprocal feeding with AOM enables *Nitrospira* possessing urease to indirectly use urea as an energy source, independently from the presence and ureolytic activity of urease-positive AOM. This interaction could facilitate the colonization by *Nitrospira* of habitats where urea is available and reduce the competition for free nitrite with other NOB, denitrifiers, and anaerobic ammonium oxidizers. Hence, reciprocal feeding should contribute to a high nitrification performance in WWTPs but could partly be responsible for N losses from soils fertilized with urea.

The ecophysiological flexibility, which results from the aerobic and anaerobic use of formate (this study) or $H_2$ (23, 24), may enable *Nitrospira* to survive periods of nitrite or oxygen deprivation. These metabolisms uncouple the growth of NOB from the nitrification process and could contribute to the unexpected higher abundances of NOB compared with AOM observed in nitrifying activated sludge, biofilm, and freshwater sediment (10, 20, 47, 48). Taken together, *Nitrospira* play diverse functional roles and even participate in N-cycle processes other than nitrification. For example, nitrate-reducing *Nitrospira* may provide nitrite as substrate for denitrification or anaerobic ammonium oxidation (anammox) in natural ecosystems and bioreactors. The pronounced genomic differences between *N. moscoviensis* and *N. defluvii*, which include urease, ROS defense, and a large number of yet-uncharacterized genes (Table S1), suggest a high degree of functional versatility as basis of ecological niche partitioning within the ubiquitous genus *Nitrospira*. This versatility likely explains the surprisingly high diversity of *Nitrospira* that coexist in natural soils (17) and activated sludge (21). Unraveling this complexity will be crucial to deepen our microbiological understanding of nitrification and other N-cycle processes, to optimize wastewater treatment strategies, and to improve the efficiency of N fertilization in agriculture.

## Materials and Methods

**Genome Sequencing and Annotation.** DNA was isolated from a pure culture of *N. moscoviensis* (24) as described (34). Paired-end and mate-pair sequencing libraries were prepared and sequenced on an Illumina MiSeq DNA sequencer (*SI Materials and Methods*). The trimmed reads were assembled by using the CLC genomics workbench v. 6.5.1. The genome annotation platform MicroScope (49) was used for automated prediction and annotation of coding sequences. See *SI Materials and Methods* for further details.

**Metagenomic Screening for *Nitrospira*-like *UreC* Sequences and Phylogenetic Analyses.** In total, 3,217 publicly available metagenomes in the IMG database (50) and an additional metagenome form the Aalborg WWTP (MG-RAST ID: 4611649.3) were screened for the presence of *Nitrospira*-like *UreC* sequences (*SI Materials and Methods*). Phylogeny of the metagenomic sequences and UreC proteins of AOB and NOB was reconstructed by using Bali-phy (51) (*SI Materials and Methods*).

**Physiological Experiments.** *N. moscoviensis*, *N. defluvii*, and *N. europaea* were incubated separately in medium with urea as described in *SI Materials and Methods*. Urea as sole N and energy source was added during coincubations to mixtures of *N. moscoviensis* and *N. europaea*. *N. moscoviensis* was incubated in medium with formate, or formate plus nitrate, under oxic or anoxic conditions. The conversion of substrates was monitored by chemical analyses of aliquots of the medium. See *SI Materials and Methods* for detailed descriptions.

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