Cyanate as an energy source for nitrifiers

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Ammonia- and nitrite-oxidizing microorganisms are collectively responsible for the aerobic oxidation of ammonia via nitrite to nitrate and have essential roles in the global biogeochemical nitrogen cycle. The physiology of nitrifiers has been intensively studied, and urea and ammonia are the only recognized energy sources that promote the aerobic growth of ammonia-oxidizing bacteria and archaea. Here we report the aerobic growth of a pure culture of the ammonia-oxidizing thaumarchaeote *Nitrososphaera gargensis* using cyanate as the sole source of energy and reductant; to our knowledge, the first organism known to do so. Cyanate, a potentially important source of reduced nitrogen in aquatic and terrestrial ecosystems, is converted to ammonium and carbon dioxide in *Nitrososphaera gargensis* by a cyanase enzyme that is induced as a result of cyanate addition. Within the cyanate gene family, this cyanase is a member of a distinct clade also containing cyanases of nitrite-oxidizing bacteria of the genus *Nitrospira*. We demonstrate by co-culture experiments that these nitrite oxidizers supply cyanate-lacking ammonia oxidizers with ammonium from cyanate, which is fully nitrified by this microbial consortium through reciprocal feeding. By screening a comprehensive set of more than 3,000 publicly available metagenomes from environmental samples, we reveal that cyanase-encoding genes clustering with the cyanases of these nitrifiers are widespread in the environment. Our results demonstrate an unexpected metabolic versatility of nitrifying microorganisms, and suggest a previously unrecognized importance of cyanate in cycling of nitrogen compounds in the environment.

Cyanate is a small molecule containing carbon, nitrogen, and oxygen atoms. It is formed spontaneously within cells from urea and carbamoyl phosphate, but also occurs in the environment where it may be produced from the chemical/physicochemical decomposition of urea or cyanide. Until recently, environmental cyanate concentrations were difficult to obtain, as the available analytical methods were inadequate for sub-micromolar detection. Furthermore, cyanate is not chemically stable and decomposes relatively slowly to ammonium and carbon dioxide (CO2). The decomposition rate is linearly related to the concentration of cyanate and thus the compound is reasonably stable at low concentrations (Extended Data Fig. 1). A more sensitive chromatographic method for the detection of cyanate in aquatic samples was recently developed and revealed nanomolar-range cyanate concentrations in seawater. These cyanate levels are in the same order of magnitude as ammonia concentrations typically found in oligotrophic marine environments. Consistently, cyanate has been postulated to serve as a nitrogen source for the growth of certain marine cyanobacteria under nitrogen limitation. For assimilation of cyanate, these phototrophic bacteria convert it to ammonium and CO2 with the enzyme cyanase (also known as cyanate lyase and cyanate hydratase). Cyanases are also found in a variety of other bacteria and archaea, where they have been reported to play a role in nitrogen assimilation or detoxification as cyanate chemically modifies proteins through carbamylation. However, to our knowledge, no microorganism has been described that can grow using cyanate as a source of energy and reductant.

Nitrifying microorganisms are generally considered to be highly specialized chemolithoautotrophs that oxidize either ammonia or nitrite to generate energy and reductant for growth, and use CO2 as a carbon source. Over the past few decades, however, this perception has been challenged by several studies. For example, it was reported that uncultured thaumarchaeota closely related to the ammonia oxidizer *Nitrosophaera gargensis* thrive in wastewater treatment plants using unknown sources of energy and reductant other than ammonium or urea and that nitrite oxidizers of the genus *Nitrospira* can derive energy for growth by aerobic hydrogen oxidation. Furthermore, the growth of some thaumarchaeotal ammonia oxidizers is stimulated by the addition of organic compounds, while others may be obligate mixotrophs. However, aerobic growth of ammonia-oxidizing microorganisms has thus far only been demonstrated in the presence of urea or ammonium.

Recently, we sequenced the genome of *N. gargensis* enriched from a thermal spring sample. Unexpectedly, a gene encoding a putative cyanase was detected close to the gene of a putative cyanate/nitrite/formate transporter. In contrast, all other sequenced genomes of archaeal or bacterial ammonia oxidizers, including its closest relative *Nitrosophaera viennensis*, do not contain a cyanase-encoding gene. As *N. gargensis* shares most central metabolic pathways with other thaumarchaeotes, it is very unlikely that it requires cyanase for detoxification of internally produced cyanate. We therefore hypothesized that *N. gargensis* may instead use cyanate as a source of energy and reductant for growth. Prior to testing our hypothesis, we obtained a pure culture of *N. gargensis* by repeated serial dilutions over a period of 16 months (see Supplementary Information). The pure culture of *N. gargensis* grew well in the presence of 2 mM ammonium and growth was not inhibited by addition of 0.5 mM cyanate. After a short period of growth in the presence of both ammonium and cyanate, the biomass of *N. gargensis* was transferred to a medium in which cyanate was the sole source of energy, reductant and nitrogen. In this medium, *N. gargensis* stoichiometrically converted cyanate via ammonium to nitrite (Fig. 1a), and cyanate degradation was the rate-limiting step of the overall process (Extended Data Fig. 1). A much slower conversion of cyanate to ammonium, reflecting chemical decay, was observed in control experiments with equal amounts of dead biomass of *N. gargensis* (Fig. 1b). Notably, growth of *N. gargensis* in the medium containing only cyanate as an energy source was demonstrated by total protein measurements (Fig. 1c) and by a quantitative polymerase chain reaction (qPCR) assay targeting its 16S rRNA gene (Extended Data Fig. 2). During growth on 0.5 mM cyanate, *N. gargensis* showed, according to total protein measurements, a mean generation time of 136.3 h (±11.4 (s.d.)), which is slightly higher than the mean generation time observed during growth on 0.5 mM ammonium, which was determined to be 113.4 h (±6.1). This difference might reflect the toxicity of cyanate, despite the presence of a cyanase, or the additional energy demand for the synthesis of cyanate during growth. Proteomic analyses revealed that on first exposure of *N. gargensis* to 0.5 mM cyanate for 48 h, cyanate was the most strongly induced protein...
Figure 1 | *N. gargensis* grows on cyanate. a, Concentration changes of cyanate, ammonium, and nitrite during the growth of *N. gargensis* in a mineral medium containing 0.5 mM cyanate as the sole source of energy and reductant. Arrows indicate additions of 0.5 mM cyanate. b, Control experiment with an identical amount of dead biomass of *N. gargensis*. Nitrite was added at different time points, as indicated by the arrows, to mimic the conditions in the experiment with living biomass. All experiments shown in panels a and b were performed in four replicates and the chemical measurements were done in three technical replicates (averaged). Data points are mean values of four biological replicates, error bars show s.d. c, Total protein concentration of *N. gargensis* during growth on cyanate. For comparison, the respective protein concentration of *N. gargensis* after growth in medium with 0.5 mM ammonium is presented. Protein concentration increased 4.99-fold during growth on ammonium and 3.81-fold during growth on cyanate over 263 h. Significance was calculated by paired t-test, *p < 0.05 compared to 0 h. Columns show mean values of four biological replicates; error bars show s.d. Biomass increase was independently confirmed by qPCR (Extended Data Fig. 2).
These findings show that this novel cyanase gene family is widespread in the environment. Most of these cyanases were located on very small contigs, preventing an independent phylogenetic classification of the organisms carrying these genes. The metagenomic cyanase fragments most closely related to *N. gargensis* (47–55% amino acid similarity) were retrieved from three different peat and cultured organisms\textsuperscript{19}. We searched a collection of 3,000 metagenomic data sets available from the Integrated Microbial Genomics (IMG) system\textsuperscript{20} and identified 225 additional metagenomic cyanase genes (fragments) that are related to the cyanases of these known nitrifiers (Fig. 3). These findings show that this novel cyanase gene family is by fluorescence in situ hybridization. c, d. Concentration changes of cyanate, ammonium, nitrite, and nitrate during the growth of the cyanase-negative ammonia-oxidizing bacterium *N. nitrosa* and the cyanase-positive nitrite oxidizer *N. moscoviensis* in a mineral medium containing 1 mM cyanate (c) or 1 mM cyanate and 1 mM ammonium (d). Error bars show s.d. of three technical replicates. For each experiment, three biological replicates were performed (one replicate is displayed in panels c and d, all replicates including mass balances are shown in Extended Data Fig. 8). Note that *N. nitrosa* did not grow equally well in all replicates.
permafrost soils in Alaska, while the sequences most closely affiliated with Nitrospira cyanases (67–80% amino acid similarity) were mostly found in temperate forest and agricultural soil from lower latitudes as well as in lakes, freshwater sediment and groundwater, matching the known distribution of Nitrospira in a broad range of different ecosystems26 (Fig. 3b).

Our findings show that an archaeal ammonia oxidizer can grow on cyanate, utilizing it as the sole source of energy, reductant, and nitrogen. Furthermore, nitrite oxidizers of the genus Nitrospira (and probably all nitrite oxidizers) can convert cyanate to ammonium and are capable of fully nitrifying it through a newly discovered type of reciprocal feeding with cyanase-negative ammonia oxidizers. This metabolic capability potentially provides them with a selective advantage in environments where cyanate is present, in particular if ammonium concentrations are low, and thus may be an important facet of the ecology of nitrifiers. Cyanate forms spontaneously by isomerization of urea in aqueous solution. The high concentration of urea in many environments where cyanate is present, in particular if ammonium is present, is due to processes such as urea fertilization in agriculture and the high concentration of urea in many aquatic environments.

Online Content Methods, along with any additional Extended Data display items and Source Data, are available in the online version of the paper; references unique to these sections appear only in the online paper.

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METHODS

Purification and standard cultivation of *Nitrososphaera gargensis*. A pure culture of the ammonia-oxidizing archaeon *Nitrososphaera gargensis* was obtained through a series of antibiotics treatments (50 mg l\(^{-1}\) kanamycin; 50 mg l\(^{-1}\) penicillin-G; 100 mg l\(^{-1}\) streptomycin; 100 mg l\(^{-1}\) carbenicillin; 50 mg l\(^{-1}\) ampicillin; 20 mg l\(^{-1}\) erythromycin; 20 mg l\(^{-1}\) doxycyclin) and repeated serial dilutions in the ammonia-oxidizer medium described below. Purity of the culture was confirmed by phase contrast microscopy and by using a specific catalysed reported deposition–fluorescence in situ hybridization (CARD–FISH) assay, as well as by PCR targeting the 16S rRNA gene, using various universal eubacterial and archaeal primer combinations (27F 5′-AGAGTTTGATCCTGGCTCAG-3′; Arch21F 5′-TTCCTGGTTAGCCYGCGGA-3′; 907F 5′-AAACTCAGAAGATTGACCG-3′; 909F 5′-CGCTC WATTCCMTTTGAGT-3′; 1390F 5′-GAGCGGGCGGTGTCAAC-3′; 1492F 5′-GGYTACCTTGTTACCGTT-3′) on DNA extracted by three different DNA isolation methods (bead beating with phenolchloroform extraction; MoBio UltraClean Soil DNA kit; FastDNA SPIN Kit for Soil). Any PCR product obtained was cloned and sequenced, retrieving only *N. gargensis* 16S rRNA gene sequences. In addition, no growth was observed if the *N. gargensis* culture was inoculated into various rich media such as lysogeny broth, nutrient agar and tryptic soy agar. Subsequently, *N. gargensis* was grown at 46 °C in a modified ammonia-oxidizing archaea (AOA) medium \(^{30}\) containing (per litre): 50 mg KH\(_2\)PO\(_4\); 75 mg KCl; 50 mg MgSO\(_4\) \(_7\)H\(_2\)O; 584 mg NaCl; 4 g CaCO\(_3\); (mostly undisolved, acting as a solid buffering system and growth surface); 1 ml of specific trace element solution (AOA-TES); and 1 ml of selenium-wolfram solution (SWS) \(^{31}\). The composition of TES and SWS is described below. Both solutions were added to the autoclaved medium by sterile filtration using 0.2 μm pore-size cellulose acetate filters (Thermo Scientific). The pH of the medium was around 8.4 after autoclaving and was kept around 8.2 during growth of *N. gargensis* by the CaCO\(_3\) buffering system. AOA-TES contained (per litre): 34.4 mg MnSO\(_4\) \(_7\)H\(_2\)O; 50 mg H\(_2\)BO\(_3\); 70 mg ZnCl\(_2\); 72.6 mg Na\(_2\)MoO\(_4\) \(_2\)H\(_2\)O; 20 mg CuCl\(_2\) \(_2\)H\(_2\)O; 24 mg NiCl\(_2\) \(_2\)H\(_2\)O; 80 mg CoCl\(_2\) \(_2\)H\(_2\)O; 1 g FeSO\(_4\) \(_7\)H\(_2\)O. All salts except the FeSO\(_4\) \(_7\)H\(_2\)O were dissolved in 997.5 ml Milli-Q water and 2.5 ml of 37% (smoking) HCl was added before dissolving the FeSO\(_4\) \(_7\)H\(_2\)O salt. After autoclaving, 1 mM (final concentration) of filter-sterilized NaNO\(_2\) (if not stated otherwise) was added to the medium. All cultures were grown in the dark without shaking at 37 °C. If all nitrite was consumed, it was re-added to a final concentration of 1 mM.

Cyanate degradation by *N. moscoviensis*. Nitrite-oxidizing cultures of *N. moscoviensis* were supplied with 0.5 mM (final concentration) KOCN and incubated for 48 h at 37 °C to induce the expression of cyanase. Biomass was harvested (8,500 rpm for 15 min at room temperature) and washed twice with fresh NOB medium without nitrite. Cells were then transferred into 50 ml NOB medium, which either contained 1 mM NaNO\(_2\) or 1 mM KOCN. Biomass concentrations were inferred from total protein concentrations, which were 27.6 ± 3.9 μg ml\(^{-1}\) as measured by the Pierce BCA Protein Assay Kit (Thermo Scientific). Abiotic experiments were performed by adding 1 mM KOCN to the NOB medium in the absence of nitrite. Dead biomass controls were performed by treating similar amounts of *N. moscoviensis* biomass fixed with paraformaldehyde (4%) as described above. The dead biomass was incubated in nitrite-free NOB medium containing 1 mM KOCN. All incubations were amended by filter-sterilized 1.5 mM NaHCO\(_3\) (final concentration). All incubations were performed in 250 ml Schott bottles closed by rubber stoppers without shaking at 37 °C in the dark for 96 h. All experiments were performed in triplicate.

In order to evaluate the effect of increasing cyanate concentrations on nitrite oxidation by *N. moscoviensis*, biomass was harvested (9,300 rpm for 15 min at room temperature) and washed twice with fresh NOB medium without nitrite. Cells were then transferred into 100 ml NOB medium. Incubations were performed with 1 mM NaNO\(_2\), 0 mM, 1 mM, 2 mM, 3 mM, 4 mM, or 5 mM of KOCN. As an abiotic control, medium containing 5 mM KOCN and 1 mM NaNO\(_2\) was incubated without addition of biomass. All incubations were performed in 250 ml Schott bottles closed by rubber stoppers without shaking at 37 °C in the dark for 60 h. All experiments were performed in duplicate.

Response of *Nitrososphaera nitrosa Nm90* to cyanate. The ammonia-oxidizing bacterium *N. nitrosa* Nm90 (strain collection of the University of Hamburg, Germany) was grown in AOA medium amended by filter-sterilized (1.5 mM) NaHCO\(_3\) (final concentration). The ammonia-oxidizing cultures of *N. moscoviensis* were supplied with 0.5 mM (final concentration) KOCN and incubated for 48 h to induce the expression of cyanase. Biomass was harvested (8,500 rpm for 15 min at room temperature) and washed twice with fresh AOA medium without ammonium. Cells were inoculated into 25 ml batches of AOA medium containing either 1 mM KOCN alone, 1 mM NH\(_4\)Cl and 1 mM KOCN, or 10 mM NH\(_4\)Cl and 1 mM KOCN. Cultures were inoculated in 50 ml CELLSTAR plastic suspension culture flasks (Greiner Bio-One) at 37 °C in the dark and shaken at 150 rpm.

Co-culture experiments with *N. nitrosa Nm90 and *N. moscoviensis*. Nitrite-oxidizing cultures of *N. moscoviensis* were supplied with 0.5 mM (final concentration) KOCN and incubated for 48 h to induce the expression of cyanase. Biomass was harvested (8,500 rpm for 15 min at room temperature) and washed twice with fresh AOA medium without nitrite. *N. nitrosa* Nm90 was grown in AOA medium supplied with 10 mM NH\(_4\)Cl. Biomass was harvested (8,500 rpm for 15 min at room temperature) and washed twice with fresh AOA medium without ammonium. Biomass concentrations were measured separately for *N. moscoviensis* and *N. nitrosa* Nm90, inferred from total protein concentrations which were 446.5 μg ml\(^{-1}\) and 164 μg ml\(^{-1}\) respectively in 50 ml final volumes for each culture-stock, measured by the Pierce BCA Protein Assay kit (Thermo Scientific). All biomass were combined and diluted up to 1 litre serving as a master-mix, which was aliquoted to 100 ml batches for the experimental setups resulting in a starting protein concentration 20 times less than the separate stocks measured. Subsequently, either 1 mM KOCN, 1 mM KOCN and 1 mM NH\(_4\)Cl or only 1 mM NH\(_4\)Cl was added to the experiments (final concentrations). In addition, abiotic experiments were performed by adding either 1 mM KOCN or 1 mM KOCN and 1 mM NH\(_4\)Cl to 100 mM AOA medium. All incubations were amended by filter-sterilized 1.5 mM NaHCO\(_3\) (final concentration). All experiments were done in 250 ml Schott bottles closed by rubber stoppers, incubated without shaking at 37 °C in the dark for 168 h. All experiments were performed in triplicate.

Chemical analysis. Nitrite levels were measured by photometry with the sulfanilamide–N-(1-naphthyl)ethylenediamine dihydrochloride (NED) reagent \(^{32}\). Ammonium levels were measured photometrically as described previously \(^{33}\). Cyanate was measured fluorometrically after derivatization with 2-aminobenzoic acid to quinazoline-2,4-dione \(^{34}\), with the modification using fluorescence readout (excitation, 312 nm; emission, 370 nm). All photometric and fluorometric reads were performed with an Infinite 200 Pro spectrophotometer (Tecan Group AG).

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qPCR quantification of N. garragenis. A qPCR assay was developed using the newly designed N. garragenis 16S rRNA gene-specific primers NG1052 5′-TATGG GCTAACCTCTGGTTC-3′ and NG1436 5′-ACCTGTGAAGACTCTC-3′. The qPCR reactions were run with three technical replicates in a Bio-Rad C1000-
CFX96 Real-Time PCR system, using the Bio-Rad iQ SYBR Green Supermix kit (Bio-Rad).

Fluorescence in situ hybridization. Prior to FISH, calcium carbonate-containing formaldehyde-fixed samples were treated with 0.1 M HCl for 3 min. After the calcium carbonate dissolved, the cells were centrifuged (3 min, 10,000g) and the supernatant discarded. The pellet was resuspended in 50 µL EtOH and PBS (50-50) and the cell suspension was spotted on slides. The FISH procedure was performed according to the standard protocol with 16S rRNA-targeted probes Ntspa712 (specific for the phylum Nitrospiraprotobacteria) and Nso1225 (specific for β-proteobacterial ammonia-oxidizing bacteria). Images were acquired with a Leica SP8 confocal laser scanning microscope (Leica).

Total protein quantification. Protein concentrations were measured using the Pierce BCA protein assay kit (Thermo Scientific).

Replication of physiological experiments. The number of replications are detailed in the subsections for each specific experiment, and were mostly determined by the amount of biomass available for the different nitrifier cultures. In all experiments, a minimum of three biological replications were used, with the exception of one auxiliary experiment: decelerating effect of increasing cyanate concentrations on nitrite oxidation by N. moscoviensis (Extended Data Fig. 6). No statistical methods were used to predetermine sample size.

Proteomic analysis. Concentrated N. garragenis biomass was inoculated in 140 ml modified AOA medium (amended with 1 mM ammonium final concentration, no cyanate) in three replicates. Prior to incubation for 24 h, 40-ml samples were taken for proteomic analysis (time point 1) and the remaining cultures were amended with 0.5 mM KOCN and 0.1 mM NH4Cl (final concentrations) and further incubated. Cultures were regularly fed afterwards with 0.5 mM KOCN (final concentration), keeping the concentration between 0.1 mM and 0.6 mM based on residual KOCN determination of ammonium. Cultures were regularly fed afterwards with 0.5 mM KOCN (final concentration), and three singleton sequences that aligned poorly were discarded. Cyanase from N. garragenis and N. moscoviensis were added into the data set. Alignment and phylogeny for the set of 99 representative cyanase genes was calculated using BAli-Phy with an initial alignment randomization and the number of iterations in each run set to 1,100, with a burnin of 600. Posterior tree pools from three independent runs were combined to assess bipartition support. The 225 environmental cyanase sequences identified in a Nitrosobacter/Nitrospira clade were clustered into 61 representative sequences using USEARCH at 99% minimum identity. Alignment and phylogenetic reconstruction for these representative sequences and ten broadly sampled outgroup cyanases was carried out in BALi-Phy (randomized alignment sets, iterations set to 1,100, burnin of 600). Posterior tree pools from four independent runs were combined to generate an 80% posterior probability (PP) consensus tree to assess bipartition support.
Extended Data Figure 1 | Biotic and abiotic cyanate degradation kinetics.
a. Degradation of 500 μM cyanate and utilization of ammonium by *N. gargensis* modelled as two consecutive first order reactions (cyanate–ammonium–nitrite). Measured data are shown as dots and error bars (mean ± s.e.m.) and model predictions with estimated rate parameters are shown as solid lines. Estimated rate constants were $k_{\text{cyanate-ammonium}} = 4.872 \times 10^{-4}$ min$^{-1}$ and $k_{\text{ammonium-nitrite}} = 1.064 \times 10^{-2}$ min$^{-1}$. The abiotic hydrolysis of 500 μM cyanate in this medium was measured to be much slower than enzymatic degradation ($k_{\text{cyanate-hydrolysis}} = 8.71 \times 10^{-5}$ min$^{-1}$). b. The abiotic degradation of low (100 nM; left) and high (500 μM; right) concentrations of isocyanic acid/cyanate across a range of temperatures and pH. Degradation was modelled using a well-established model of three first-order reactions: (1) hydronium-ion-catalysed hydrolysis of isocyanic acid ($k_1 = e^{25.97} \times e^{-27201.29/T}$); (2) direct hydrolysis of isocyanic acid ($k_2 = e^{72.30} \times e^{-21646.66/T}$); and (3) direct hydrolysis of cyanate ($k_3 = e^{22.23} \times e^{-8725/T}$). The log-transformed degradation rates are shown (as min$^{-1}$). The conditions that were used to test cyanate degradation by *N. gargensis* are marked with a cross.
Extended Data Figure 2 | *Nitrososphaera gargensis* grows on cyanate. 16S rRNA gene copy numbers of *N. gargensis* as determined by qPCR at three different time points during the experiment shown in Fig. 1. For comparison, the respective gene copy numbers after growth in medium with 0.5 mM ammonium are displayed. The gene copy numbers increased 6.49-fold during growth on ammonium and 4.98-fold during growth on cyanate over 263 h. Columns show means, error bars show s.d. of four biological replicates. Significance was calculated by a paired $t$-test.
Extended Data Figure 3 | Cyanase increase upon exposure of *N. gargensis* to cyanate. Fold-increase and -decrease of the 35 most affected proteins after 48 h exposure of *N. gargensis* to 0.5 mM cyanate (in comparison to t = 0 of *N. gargensis* biomass that had not been exposed to cyanate). Experiments were performed in three biological replicates. Proteins with a significant difference in expression are colour coded. Significance of difference was calculated by a one-sample *t*-test on log-fold induction, with the Benjamini–Hochberg false discovery rate set to 0.05 (*P* value cutoff 0.00878). For the proteomic analyses, 10 μg protein and 500 ng peptide lysate per sample was used. Protein abundances within a sample were normalized by dividing the peak area for a given protein by the median peak area for all detected proteins. Note that during growth on cyanate, *N. gargensis* experiences much lower concentrations of ammonium than during growth on ammonium in batch culture, which probably influences the expression patterns of some of the listed proteins.
Extended Data Figure 4 | Conversion of 0.05 mM cyanate by N. gargensis.

a, Concentration changes of cyanate, ammonium, and nitrite caused by N. gargensis in a mineral medium containing 0.05 mM cyanate as the only source of energy and reductant. b, Abiotic control experiment. All experiments were performed in four biological replicates and the chemical measurements were done in three technical replicates (averaged). Data points are mean values of four biological replicates, error bars show s.d.
Extended Data Figure 5 | *Nitrospira moscoviensis* has a functional cyanase. Concentration changes of cyanate and ammonium during incubation of *N. moscoviensis* (27.6 ± 3.9 μg ml⁻¹ protein) in a mineral medium containing cyanate, but no nitrite. Results from a control experiment with identical amounts of dead biomass of *N. moscoviensis* are also displayed. All experiments were performed in triplicate and the chemical measurements from each replicate were done three replicates. Data points are mean values, error bars show s.d. Asterisks indicate statistical significance between *N. moscoviensis* and dead biomass, *P < 0.05, ***P < 0.001. Significance was assessed by two-way analysis of variance (ANOVA) including Tukey’s honest significant difference (HSD) test.
Extended Data Figure 6 | Decelerating effect of increased cyanate concentrations on nitrite oxidation by *N. moscoviensis*. Biomass was incubated for 60 h in medium containing 1 mM nitrite and cyanate concentrations ranging from 0 mM to 5 mM, and nitrite oxidation was monitored. Incubations were performed in duplicates.
Extended Data Figure 7 | *Nitrosomonas nitrosa* Nm90 has no cyanase activity and is not inhibited by 1 mM cyanate. Concentration of nitrite during incubation of *N. nitrosa* in a mineral medium containing: 1 mM ammonium (filled circles); 1 mM cyanate (filled squares); 1 mM cyanate and 1 mM ammonium (open circles); 10 mM ammonium (filled triangles); and 1 mM cyanate and 10 mM ammonium (open triangles). All experiments were performed in three biological replicates, data points are mean values, error bars show s.d.
1 mM cyanate

1 mM cyanate + 1 mM ammonium

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Extended Data Figure 8 | Reciprocal feeding of ammonia and nitrite oxidizers during cyanate conversion. As activities differed between biological replicates (as often observed for nitrifying strains that are very sensitive to rubber stoppers, contaminants on glass material, etc.), data are displayed for each replicate individually. Concentrations of cyanate, ammonium, nitrite, and nitrate are displayed as bar (left) and line charts (right) during the growth of the cyanase-negative ammonium-oxidizing bacterium *Nitrosomonas nitrosa* Nm90 and the cyanase-positive nitrite oxidizer *N. moscoviensis* in a mineral medium containing 1 mM cyanate (a–c) or 1 mM cyanate and 1 mM ammonium (d–f). Data points are mean values, error bars show s.d. of three technical replicates.
Extended Data Figure 9 | Abiotic controls for the reciprocal-feeding experiment. See also Fig. 2 and Extended Data Fig. 8. Concentration changes of cyanate, ammonium, nitrite, and nitrate during 168 h of incubation under similar conditions to the biotic experiments: mineral medium containing 1 mM cyanate (left) or 1 mM cyanate and 1 mM ammonium (right). The rate of cyanate decay is much slower than in biotic setups (see Extended Data Fig. 8). Cyanate decay in the presence of ammonium led to formation of a product other than ammonium (possibly carbamylate). No nitrite or nitrate was formed abiotically. Data points are mean values, error bars show s.d. of three technical replicates.
Extended Data Table 1 | Presence of cyanase, nitrite/nitrate transporters and enzymes related to urea metabolism in ammonia- and nitrite-oxidizing microorganisms with fully a sequenced genome.

| Organism                                      | Cyanase | Nitrite/nitrate transporter | Urea metabolism |
|-----------------------------------------------|---------|-----------------------------|-----------------|
|                                               |         | FNT Family  | NNP Family | ABC transporter | § | || | $ || | $ |
| Ammonia oxidizing archaea                     |         |             |            |                |  |  |  |  |  |  |
| Nitrososphaera gargensis                     | +       | +            | -          | +              | + | + | + | + |
| Nitrososphaera viennensis EN76               | -       | -            | -          | +              | + | + | + | + |
| Ca. Nitrososphaera evergladensis SR1         | -       | -            | -          | +              | + | + | + | + |
| Nitrosopumilus maritimus SCM1                | -       | -            | -          | +              | - | + | - | - |
| Nitrosopumilus sp. AR                        | -       | -            | -          | +              | + | + | + | + |
| Nitrosopumilus sp. SJ                        | -       | -            | -          | +              | - | + | - | - |
| Ca. Nitrosopumilus koreensis AR1             | -       | -            | -          | +              | - | + | - | - |
| Ca. Nitrosopumilus salaria BD31              | -       | -            | -          | +              | - | + | - | - |
| Ca. Nitrosopumilus sp. AR2                   | -       | -            | -          | +              | + | + | + | + |
| Ca. Nitrosoarchaeum koreensis MY1            | -       | -            | -          | +              | - | + | - | - |
| Ca. Nitrosoarchaeum limnia BG20              | -       | -            | -          | +              | - | + | - | - |
| Ca. Nitrosoarchaeum limnia SFB1              | -       | -            | -          | +              | - | + | - | - |
| Ca. Nitrosotenuis uzonensis N4               | -       | -            | -          | +              | - | + | - | - |
| Cenarchaeum symbiosum A                      | -       | -            | -          | +              | + | + | + | + |
| Ammonia oxidizing bacteria                   |         |             |            |                |  |  |  |  |  |  |
| Nitrosococcus halophilus No4                 | -       | +            | -          | -              | - | - | - | - |
| Nitrosococcus oceani AFC27                  | -       | +            | -          | -              | + | + | + | + |
| Nitrosococcus oceani ATCC 19707              | -       | +            | -          | -              | + | + | + | + |
| Nitrosococcus watsonii C-113                 | -       | +            | -          | -              | + | + | + | + |
| Nitrosomonas europaea ATCC 19718             | -       | +            | -          | +              | - | + | - | - |
| Nitrosomonas utropha C91                     | -       | +            | -          | -              | + | + | - | - |
| Nitrosomonas sp. AL212                      | -       | +            | -          | -              | + | + | - | - |
| Nitrosomonas sp. Is79A3                      | -       | +            | -          | -              | + | + | - | - |
| Nitrospira multiformis ATCC 25196            | -       | +            | -          | -              | + | + | - | - |
| Nitrite oxidizing bacteria                   |         |             |            |                |  |  |  |  |  |  |
| Nitrospira moscovicensis                     | +       | +            | +          | +              | + | + | + | + |
| Ca. Nitrospira defluii                      | +       | +            | +          | -              | - | + | - | - |
| Nitrobacter sp. Nb-311A                      | +       | +            | +          | -              | - | + | - | - |
| Nitrobacter winogradskyi Nb-255              | +       | +            | +          | -              | - | + | - | - |
| Nitrobacter hamburgensis X14                 | +       | +            | +          | -              | - | + | - | - |
| Nitrooccus mobilis Nb-231                    | +       | +            | +          | -              | - | + | - | - |
| Nitrodevancea hollandica                     | +       | +            | +          | -              | + | + | - | - |
| Nitrospina gracilis                         | +       | +            | -          | -              | - | + | - | - |

*Formate-nitrite transporter family; encoded by focA/nvC, has been postulated to transport cyanate
†Nitrate/nitrite transporter family; encoded by nark, might also transport cyanate due to its chemical similarity to nitrite.
‡ABC transporter of nitrate/sulfate/bicarbonate: this transporter family has been shown to transport cyanate as well.
§Urea transporter: ABC transporter, or urea channel.
||Urea produced internally by the enzymes agmatine amidinohydrolase or arginine amidinohydrolase.
||Urea consumed internally by the enzymes urease or urea carboxylase and allophanate hydrolase.