

LETTERS

Ammonia oxidation kinetics determine niche separation of nitrifying Archaea and Bacteria

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The discovery of ammonia oxidation by mesophilic and thermophilic Crenarchaeota and the widespread distribution of these organisms in marine and terrestrial environments indicated an important role for them in the global nitrogen cycle^{1–7}. However, very little is known about their physiology or their contribution to nitrification⁸. Here we report oligotrophic ammonia oxidation kinetics and cellular characteristics of the mesophilic crenarchaeon ‘*Candidatus Nitrosopumilus maritimus*’ strain SCM1. Unlike characterized ammonia-oxidizing bacteria, SCM1 is adapted to life under extreme nutrient limitation, sustaining high specific oxidation rates at ammonium concentrations found in open oceans. Its half-saturation constant ($K_m = 133$ nM total ammonium) and substrate threshold (≤ 10 nM) closely resemble kinetics of *in situ* nitrification in marine systems^{9,10} and directly link ammonia-oxidizing Archaea to oligotrophic nitrification. The remarkably high specific affinity for reduced nitrogen (68,700 l per g cells per h) of SCM1 suggests that *Nitrosopumilus*-like ammonia-oxidizing Archaea could successfully compete with heterotrophic bacterioplankton and phytoplankton. Together these findings support the hypothesis that nitrification is more prevalent in the marine nitrogen cycle than accounted for in current biogeochemical models¹¹.

Aerobic ammonia oxidation is the first, rate-limiting step of nitrification, a two-step process catalysed by ammonia-oxidizing and nitrite-oxidizing microorganisms. It is the only oxidative biological process linking reduced and oxidized pools of inorganic nitrogen in nature^{12,13}. This key process in the global nitrogen cycle was thought to be restricted to ammonia-oxidizing bacteria (AOB), two narrow clades of Beta- and Gammaproteobacteria^{8,13}. These slow-growing, autotrophic bacteria use ammonia oxidation as their sole source of energy. The growth rates of AOB are thus directly linked to the availability of ammonium and the kinetics of its oxidation^{13,14}. *In situ* rate measurements in natural marine and terrestrial environments, however, indicate that nitrification occurs almost ubiquitously, even in the most oligotrophic environments with ammonium levels significantly below the growth threshold of AOB^{9–11,13–15}, raising the question whether these bacteria could represent the dominant ammonia-oxidizer assemblage in oligotrophic environments^{9,10,13,14}.

The demonstration of autotrophic ammonia oxidation by mesophilic and thermophilic Crenarchaeota^{3–6} and the widespread distribution of putative archaeal ammonia monooxygenase (*amo*) genes in marine and terrestrial environments^{1–4,6,8} changed the perspective on nitrification. The isolation of ammonia-oxidizing Archaea (AOA) strains affiliated with Crenarchaeota clades constituting up to 39% of microbial plankton in meso- and bathypelagic oceans suggested that AOA could potentially play an important part in nitrification^{8,16–18}. However, AOA would need to compete with AOB, organotrophic bacterioplankton and perhaps even phytoplankton for ammonium^{11,12,19}. To test this hypothesis, we determined the ammonia

oxidation stoichiometry and kinetics in the only isolated AOA strain, ‘*Candidatus Nitrosopumilus maritimus*’ strain SCM1. The results demonstrate that ammonia oxidation by AOA could indeed sustain significant growth rates and standing stocks of these Crenarchaeota, even in nutrient-depleted natural marine and terrestrial environments.

In ammonium-limited batch cultures, SCM1 grew exponentially with high rates and depleted ammonium below the detection limit of 10 nM ($n = 4$; Fig. 1). This ammonium concentration approaches values measured in oligotrophic open ocean water^{11,16} and is more than 100-fold lower than the minimum concentration required for growth (> 1 μ M near neutral pH) of cultivated AOB^{13,15}. SCM1 attained maximum growth rates (0.027 h⁻¹; doubling time = 26 h) and activities (51.9 μ mol ammonium per mg protein per h) comparable to AOB strains (range 30–80 μ mol ammonium per mg protein per h)¹³ (Fig. 1, Supplementary Note 1). Thus, SCM1 seems particularly well adapted to growth at ammonium levels prevailing in nutrient-limited open oceans.

We observed that growth of SCM1 was significantly impaired by agitation, compared to static batch cultures, which precluded continuous culture experiments (Supplementary Note 1). To gain insight into the ammonia oxidation kinetics of SCM1, we therefore independently determined ammonium and oxygen uptake with ammonium-depleted (< 10 nM) cells from batch cultures (Fig. 2). Ammonium-depleted, early-stationary phase cells consumed very low amounts of oxygen (0.51 μ M O₂ h⁻¹ = 0.67 μ mol O₂ per mg protein per h, s.d. 0.29, $n = 10$). Oxygen uptake increased more than 50-fold to maximum rates of 27.51 μ M O₂ h⁻¹ (36.29 μ mol O₂ per mg protein per h, s.d. 3.35, $n = 10$; ≈ 24.2 μ mol ammonium per mg

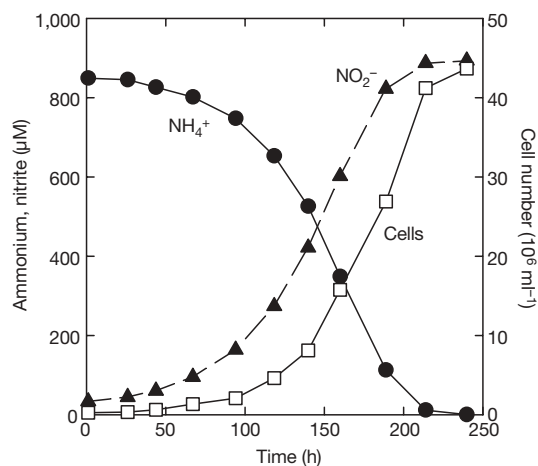


Figure 1 | Growth of SCM1 in ammonium-limited artificial sea water batch culture. Cultures entered stationary phase after ammonium was depleted below the detection limit of 10 nM.

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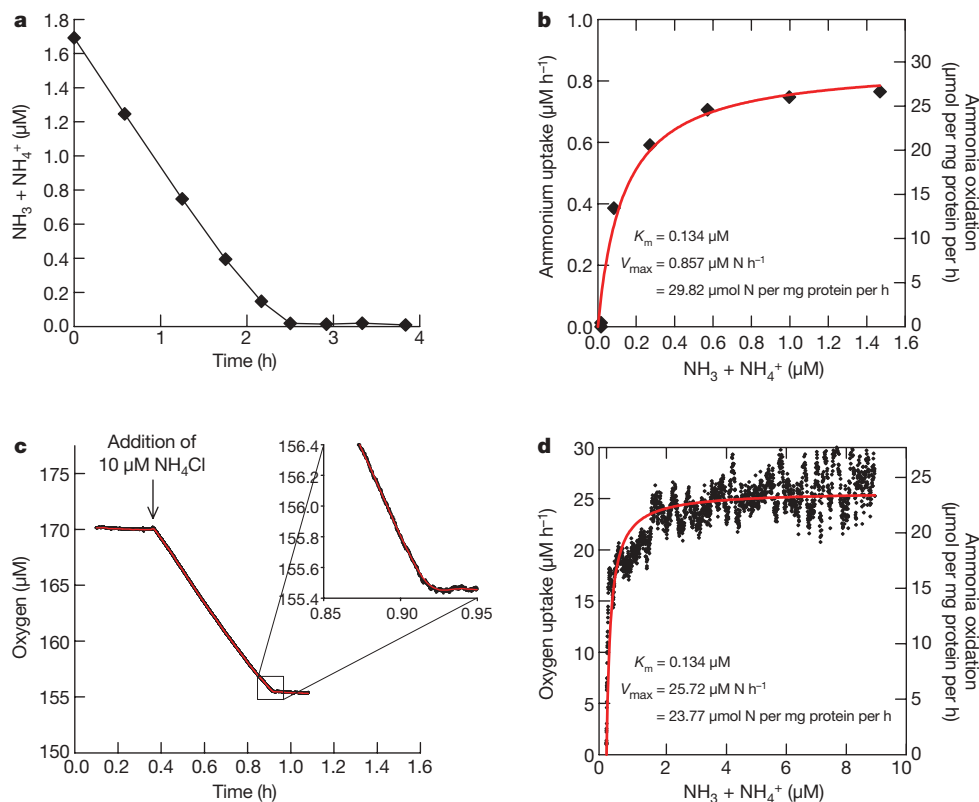


Figure 2 | Ammonia oxidation kinetics of SCM1. **a**, Ammonium uptake of early-stationary SCM1 cells on transfer to fresh medium containing 1.7 μM ammonium. **b**, Michaelis–Menten plot of ammonium uptake. **c**, Ammonium-dependent oxygen uptake of early-stationary phase SCM1 cells. Suspensions of cells were equilibrated for ~ 20 min before the

protein per h) within a few minutes of ammonium addition. Ammonium and oxygen were consumed with an AOB-like stoichiometry of 1:1.52 (s.d. 0.06, $n = 10$). Notably, late-exponential and early-stationary phase SCM1 cells both exhibited maximum rates of ammonium and oxygen uptake at as low as 2 μM ammonium (Fig. 2b, d and Supplementary Fig. 1b).

Ammonia oxidation by SCM1 followed Michaelis–Menten-type kinetics (Fig. 2b, d and Supplementary Fig. 1b). The mean apparent half-saturation constants (K_m) for ammonium uptake (0.132 μM $\text{NH}_3 + \text{NH}_4^+$, range 0.005, $n = 2$, equivalent to ~ 3 nM NH_3) and oxygen uptake (0.133 μM $\text{NH}_3 + \text{NH}_4^+$, s.d. 0.038, $n = 13$) were indistinguishable. Supplementation with as little as 0.2 μM ammonium chloride triggered more than 50% of maximum oxygen uptake. In contrast, identical experiments with *Nitrosomonas europaea* and *Nitrosococcus oceanus* cells showed no stimulation of activity by 0.2 μM ammonium. Their K_m values, determined by the same methods used to characterize SCM1, were in a typical range previously reported (Supplementary Note 2). Identical K_m values and activities of SCM1 were observed in up to 5 cycles of substrate addition and depletion, up to 2 mM ammonium, and even with cells from late-exponential cultures which had not experienced ammonium concentrations below 250 μM for more than 50 generations of exponential growth (Supplementary Fig. 1b). More frequent ammonium additions, as well as high ammonium concentrations (≥ 2 mM), caused a decline of activity (data not shown, Supplementary Note 3). Together these data suggest that SCM1 has a constitutively low apparent half-saturation constant for ammonium.

Previous studies have indicated that *amo* gene copy numbers of AOA significantly outnumber those of AOB in oligotrophic ocean gyres and unfertilized natural soils, suggesting that substrate availability may account for the abundance patterns of AOA and AOB^{7,8,16,20}. A comparison of the apparent K_m value for SCM1 to

experiment was started by the addition of 10 μM ammonium chloride. **d**, Michaelis–Menten plot of ammonium-dependent oxygen uptake. Uptake rates were calculated from smoothed data (red line in **c**). For calculation of kinetic parameters, a Michaelis–Menten equation was fitted to the data. See Methods for experimental details and calculations.

in situ measurements strongly supports this hypothesis (Fig. 3a). The K_m of SCM1 is by far the lowest half-saturation constant determined of any ammonia-oxidizing microorganism to date; it is very similar to *in situ* nitrification measurements made in oligotrophic, ammonium-depleted oceanic provinces^{9,10} and is even lower than in natural unfertilized soils²¹ (Fig. 3a). Assuming Michaelis–Menten-type saturation kinetics, an apparent K_m of ammonia oxidation of $\sim 0.15 \mu\text{M}$ ammonium was estimated in the upper oxic Cariaco basin⁹ (Fig. 3a), and it was found that in the primary nitrite maximum off the coast of California, ammonia oxidation was saturated at 0.1 μM ammonium¹⁰. Similarly, the K_m values for nitrification in uncultivated soil areas were significantly lower than the K_m of known AOB²¹. To account for these *in situ* nitrification measurements, organisms with *Nitrosopumilus*-like kinetics must vastly outnumber organisms typified by AOB. In contrast, a kinetic response consistent with the activity of known AOB becomes prevalent only in coastal environments and soils with higher nutrient levels^{21,22} (Fig. 3a). Nonetheless, even in nutrient-poor marine environments, organic-matter-rich particles provide niches for nitrifiers with probably significantly different kinetic properties^{23,24}—for example, members of the *Nitrosomonas eutropha* lineage²⁴. However, significant ammonium is lost to the surrounding water during particle-associated mineralization²³, and these AOB lineages occur in very low numbers in oligotrophic open ocean gyres^{7,23}. Although it remains unknown if as-yet uncultured AOB lineages (for example, members of the marine *Nitrospira* cluster 1 (ref. 25) or novel, unidentified organisms) participate in oligotrophic nitrification, these data suggest that *Nitrosopumilus*-related AOA indeed are predominantly responsible for the observed nitrification in oligotrophic environments.

Using the specific affinity^{26,27} (a^0) for ammonium as a basis for comparison, the kinetic characteristics of SCM1 further suggest that *Nitrosopumilus*-related AOA might successfully compete with other

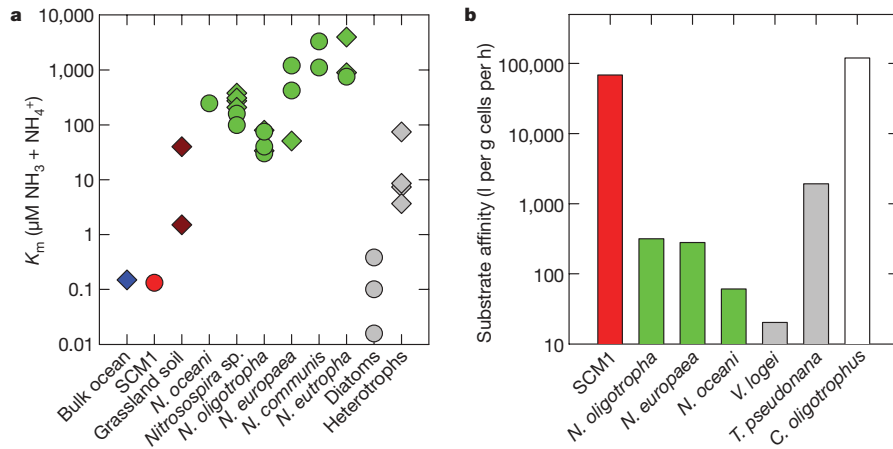


Figure 3 | High-affinity ammonia oxidation by AOA dominates in oligotrophic environments. **a**, Apparent K_m of SCM1 (red), the AOB strains *Nitrosococcus oceani*, *Nitrosospira* spp. cluster 0, 2 and 3, *Nitrosomonas oligotropha*, *Nitrosomonas europaea*, *Nitrosomonas communis*, *Nitrosomonas eutropha* (green), *in situ* nitrification in ocean water (blue) and soils (brown), as well as the lowest K_m for ammonium assimilation of diatoms and heterotrophic bacteria (grey). K_m values are given for activity

measurements (circles) and growth (diamonds). **b**, Specific affinity (a^0) of SCM1 (red), bacterial ammonia oxidizers (green), as well as the highest values for ammonium-assimilating diatoms, and heterotrophic bacteria (grey). The highest reported specific affinity of an organotrophic organism (*Cycloclasticus oligotrophus*, open bar) for its carbon substrate is shown for comparison. See Methods for calculations and references.

marine phototrophs and chemotrophs for ammonium, the preferred inorganic nitrogen source for assimilation²⁸ (Fig. 3b). The specific affinity of SCM1 (68,700 l per g wet weight per h) is among the highest affinities reported for microbial substrates (Fig. 3b). It exceeds that of AOB by more than 200-fold and is still 30-fold higher than of the oligotrophic diatom, *Thalassiosira pseudonana*²⁹. Although limited data are available for the specific ammonium affinity of marine heterotrophs, it is unlikely that ammonium-assimilating organisms possess higher specific ammonium affinities than *Nitrosopumilus*. The highest specific affinity reported for an oligotrophic heterotroph for its carbon and energy source (120,000 l per g wet weight per h, *Cycloclasticus oligotrophus*^{26,27}) is only twofold higher than the ammonium affinity of SCM1 (Fig. 3b). If ammonium served as the sole source of nitrogen (C:N ratio of 5:1, 50% of carbon substrate assimilated into biomass)²⁷ and the K_m for ammonium was comparable to the carbon source, the specific ammonium affinity of *C. oligotrophus*-like heterotrophs would still be >5 times lower (~12,000 l per g wet weight per h) than that of SCM1. The highest reported ammonium affinities of marine heterotrophs (for example, marine *Vibrio* strains) are in fact significantly lower (Fig. 3b). Hence, we predict that, in direct nutrient-limited competition, the ammonium turnover per unit biomass of *Nitrosopumilus*-like AOA would be at least 5 times higher than of oligotrophic heterotrophs, and more than 30 times higher than of the most oligotrophic diatoms known so far. These data suggest that *Nitrosopumilus*-like AOA not only out-compete AOB under limiting ammonium concentrations, but may well compete effectively with heterotrophs and phytoplankton, especially in deeper water layers.

A previously unrecognized competition for ammonium by AOA is entirely consistent with a recent synopsis of global oceanic nitrification rates which suggested that up to 50% of the nitrite and nitrate assimilated by phytoplankton is generated from ammonium by nitrification near the water surface, rather than transported from deeper water layers¹¹. Our results strongly indicate that *Nitrosopumilus*-like AOA supply the oxidized nitrogen, offering an ecophysiological explanation for the conspicuously high nitrification rates in nutrient-poor oceanic regions^{9,10,14,22} as well as the high nitrite/nitrate assimilation rates of heterotrophs and phototrophs²⁸. Although the true maintenance energy demand of SCM1 cells remains to be determined, the low endogenous respiration rates of SCM1 (1.8% of maximum metabolic activity) may be suggestive of a small maintenance requirement. The growth threshold concentration of SCM1 could thus be as low as 10–20 nM ammonium, suggesting a strong

competition by *Nitrosopumilus*-like AOA even in nutrient-poor meso- and bathypelagic waters.

Despite the isolation of strain SCM1 from the gravel of a marine aquarium and not directly from natural sea water, the kinetic and cellular characteristics of strain SCM1 presented here are unequivocal evidence for the existence of oligotrophic ammonia oxidizers among the Crenarchaeota and their ability to compete for ammonium as energy source in nutrient-deprived oligotrophic oceans. Notably, similar growth rates, cell yields or low residual ammonium concentrations have also been reported in the thermophilic enrichment cultures '*Candidatus Nitrosocaldus yellowstonii*' and '*Candidatus Nitrososphaera gargensis*'^{4,6}. Moreover, it has been reported⁶ that even CO₂ fixation in '*Candidatus Nitrososphaera gargensis*' was inhibited by ammonium concentrations similar to those that inhibited SCM1, supporting a widespread distribution of oligotrophic ammonia oxidation within the Crenarchaeota.

Substrate availability is unlikely to be the only factor determining the abundance and distribution of AOA and AOB and the ecological niches of these organisms^{7,8,16,20}. For example, if AOA may indeed compete with phototrophs and thus be responsible for the detected nitrification rates in euphotic surface water^{10,11}, specialized ecotypes or AOA in general should be considerably more light tolerant than previously investigated AOB strains¹³. As recognized AOA now span considerable diversity within the Crenarchaeota^{1,2,8}, we also anticipate that significant physiological diversity will ultimately be revealed among its members. For example, analysis of the SCM1 genome sequence revealed several putative ABC-type transporter systems for organic carbon acquisition (C. B. Walker *et al.*, unpublished results), consistent with previous findings of organic matter assimilation by marine Crenarchaeota *in situ*^{18,30}. If the marine Crenarchaeota also harbour lineages of obligate organotrophs as suggested^{16,18,30}, these organisms seem to be competitive only in permanently nutrient-deprived deep ocean provinces with ammonium concentrations significantly below 10 nM. Although the genome sequence has revealed two Amt-type ammonium transporters (C. B. Walker *et al.*, unpublished results) that could have unusually high affinity for ammonia, the mechanistic basis for the unprecedented capacity for ammonium acquisition and the biochemical pathway for its oxidation remain unknown. Nonetheless, this novel metabolism probably rendered mesophilic ammonia-oxidizing Crenarchaeota one of the dominant microbial clades in the ocean, with fundamental impact on the global biogeochemical cycles of nitrogen and carbon^{1,4,8}.

METHODS SUMMARY

All experiments with 'Candidatus Nitrosopumilus maritimus' strain SCM1 were carried out in HEPES-buffered synthetic Crenarchaeota medium (SCM, 32‰ salinity, pH 7.5, 1 mM NH₄Cl). *Nitrosomonas europaea* strain ATCC 19718 was grown in basal mineral medium (pH 7.8, 0.0003% phenol red, 10 mM NH₄Cl). *Nitrosococcus oceani* strain ATCC 19707 was cultured in HEPES-buffered SCM medium (10 mM NH₄Cl). All strains were maintained at 30 °C in the dark. Nitrite and ammonium were determined spectrophotometrically and fluorometrically, respectively. Cells were counted by epi-fluorescence microscopy after SybrGreen I staining. Protein was quantified using the Nano Orange kit (Invitrogen). Oxygen uptake was monitored using Clark-type oxygen microsensors in 2-ml respiration chambers in a modified micro-respirometry system (Unisense AS) at 30 °C in a thermostatted water bath. Ammonium uptake activity was examined in static 5-l batches inoculated with early-stationary phase cells. Kinetic constants were obtained by fitting a Michaelis–Menten equation to oxygen and ammonium uptake rates using the equation:

$$V = (V_{\max} \times [S]) \times (K_m + [S])^{-1}$$

Here V is velocity, V_{\max} is maximum velocity ($\mu\text{M h}^{-1}$), K_m is half-saturation constant for ammonium oxidation (μM), and $[S]$ is concentration of ammonium (μM). Specific affinities were calculated from kinetic constants of SCM1 and literature data of AOB, diatoms and heterotrophic bacteria using the equation:

$$a^0 = V_{\max} \times K_m^{-1}$$

Here a^0 is specific affinity (l per g wet cells per h), V_{\max} is maximum velocity (g substrate per g wet cells per h) and K_m is half-saturation constant (in g substrate)²⁷.

Full Methods and any associated references are available in the online version of the paper at www.nature.com/nature.

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Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

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METHODS

Strain cultivation and maintenance. Cultures of 'Candidatus Nitrosopumilus maritimus' strain SCM1 were maintained and all physiological experiments were carried out in synthetic Crenarchaeota medium (SCM) as described earlier³ with the following modifications: all necessary glassware used for media preparation or cultivation was solely used for this purpose and carefully acid washed (1% HCl) and rinsed with MilliQ water (>18.2 MΩ resistance) before use. The basal artificial sea water was autoclaved, cooled to room temperature, and supplemented with the following sterile stock solutions (per litre): 10 ml HEPES (1 M HEPES, 0.6 M NaOH, pH 7.8), 2 ml sodium bicarbonate (1 M), 5 ml KH₂PO₄ (0.4 g l⁻¹), 1 ml FeNaEDTA (7.5 mM), 1 ml modified non-chelated trace element solution. The trace element solution contained (per litre) 8 ml conc. HCl (~12.5 M), 30 mg H₃BO₃, 100 mg MnCl₂·4H₂O, 190 mg CoCl₂·6H₂O, 24 mg NiCl₂·6H₂O, 2 mg CuCl₂·2H₂O, 144 mg ZnSO₄·7H₂O, 36 mg Na₂MoO₄·2H₂O. The medium was finally supplemented with 0.5 to 1 ml of NH₄Cl (1 M). The final pH of this medium at 30 °C was ~7.5. Strain SCM1 did not grow if directly transferred from solely bicarbonate-buffered medium³ to HEPES-buffered medium. However, no difference in growth rates between both media was observed if the HEPES concentration was incrementally increased to 10 mM during exponential growth over three consecutive transfers. Cultures were subsequently maintained in HEPES-buffered SCM medium at 25 °C or 30 °C in the dark and transferred (0.1 to 1% inoculum size) to fresh medium when ~2/3 of the ammonium was oxidized. Shaking and stirring were avoided. Purity of the culture was monitored by phase contrast microscopy and by quantitative real-time PCR (qPCR) of bacterial 16S rRNA genes and *amoA* genes (see below). Contaminations were neither detected by quantitative PCR, nor by phase contrast microscopy of 10 μl samples of 200-fold concentrated suspensions of late exponential SCM1 cultures.

Nitrosomonas europaea strain ATCC 19718 was grown in liquid medium supplemented with phenol red as pH indicator (0.0003% final concentration) as described elsewhere³¹. *Nitrosococcus oceanii* strain ATCC 19707 was grown in HEPES-buffered SCM medium as described above containing 10 mM NH₄Cl. Both strains were cultured at 30 °C on a rotary shaker (150 r.p.m.).

qPCR assays were carried out on a LightCycler System (Roche Applied Science) using the LightCycler FastStart DNA Master SYBR Green I kit (Roche) with the following reaction chemistry: 1 μl of LightCycler FastStart DNA master mix, 3.5 mM MgCl₂, 0.5 μM each primer and 0.01–1 ng of DNA template in a final volume of 10 μl. Bacterial 16S rRNA gene abundance was quantified using the GM3 and EUB338 primer set³ with the following real-time PCR amplification protocol: initial denaturation at 95 °C for 5 min; followed by 55 cycles of 95 °C for 10 s, 55 °C for 10 s, and 72 °C for 20 s, and a melting curve analysis (60 °C to 95 °C) with a heating rate of 0.1 °C s⁻¹. Bacterial *amoA* genes were quantified using the same reaction chemistry with the bacterial *amoA* primer set³² and the following PCR conditions: initial denaturation 95 °C for 5 min; 55 cycles of 95 °C for 7 s, 55 °C for 20 s and 72 °C for 20 s and a detection step at 78 °C for 7 s, followed by a melting curve analysis (65 °C to 95 °C) with a heating rate of 0.1 °C s⁻¹. The standard curves for both bacterial 16S rRNA genes and *amoA* genes were generated using *N. europaea* genomic DNA in a dilution series of 10¹–10⁶ copies per reaction. Data were analysed with the second derivative maximum method using the Light Cycler Software (ver. 3.5.3).

Nutrient measurements, cell counts, and protein quantification. Nitrite was determined spectrophotometrically³³. Ammonium was determined by fluorescence measurement after *o*-phthalaldehyde derivatization in a Trilogy Laboratory fluorometer (Turner Designs) or a fluorescence microplate reader (Tecan Inc.)³⁴. Detection limits were 5–10 nM, and 100 nM, respectively. Cell counts were performed on the day of sampling by epi-fluorescence microscopy after SybrGreen I staining as described elsewhere³⁵. Protein content of mid- and late-exponential cultures was quantified using the Nano Orange kit (Invitrogen) according to manufacturer's instructions after cells were collected by centrifugation in Centricon YM-100 units (Amicon Inc.) and rinsed with MilliQ water to remove salt.

Activity measurements. Oxygen uptake was measured in a micro-respiration system (Unisense AS) equipped with 2-ml glass micro-respiration chambers, glass-coated stir bars, Clark-type OX-MR oxygen microsensors, PA 2000 picoammeter, and MicOx 2.6 data acquisition software. Oxygen microsensors were polarized continuously for >7 days before use. Oxygen uptake of the employed sensors was insignificant (below 1 nM d⁻¹)³⁶ and 90% response time was <10 s (Supplementary Fig. 3a). To reduce electronic noise and interference from other laboratory and computer equipment, all electrical components were powered by a stabilized power source and the data transmission from AD-converter to PC computer was shielded using an USB port optical isolator (Black Box). All measurements were done in a re-circulated water bath at 30 °C (ΔT < 0.1 °C) and 100 r.p.m. stirring. Initial kinetic measurements with cells from late-exponential cultures of *N. europaea* and *N. oceanii* collected by

centrifugation or filtration as described previously^{37,38} were performed to test the set-up (Supplementary Note 2, Supplementary Fig. 2). Cells of SCM1 lost more than 90% of activity through a similar centrifugation or filtration treatment. Activity measurements with late-exponential or early-stationary phase cells were therefore carried out as follows: ammonia oxidation and nitrite production of SCM1 cultures was monitored daily. Depletion of substrate was predictable within two to four hours. Aliquots of 20–30 ml were removed from cultures either within a few hours before (late-exponential) or up to 12 h after ammonium depletion (early-stationary phase) and immediately transferred to pre-warmed 40-ml glass vials in a 30 °C water bath. Sub-samples were then filled into 2-ml micro-respiration vessels with several volumes of overflow, carefully sealed with glass lids, and immediately immersed in the water bath. Oxygen uptake was monitored continuously after an initial equilibration of at least 10 min. Ammonium or nitrite was added as necessary from concentrated stock solutions in basal SCM salts by means of a Hamilton syringe.

Ammonium uptake activity was determined in static 5-l batches of fresh, pre-warmed SCM medium with given ammonium concentrations at 30 °C inoculated with early-stationary phase cells. Sub-samples (80 ml) were withdrawn and immediately assayed for residual ammonium concentration as described above. **Calculation of kinetic constants and specific affinities.** Kinetic characteristics of SCM1 were estimated from multiple individual oxygen traces. High-frequency noise was removed using the 'Smooth 2D data' function implemented in Sigma Plot 8.0 (SPSS Inc.). Ammonium concentrations were calculated from oxygen uptake according to the ratio of ammonia oxidation to oxygen uptake of 1:1.5. Michaelis–Menten plots of oxygen uptake and ammonium uptake rates by SCM1 versus total ammonium concentration were then obtained by fitting a Michaelis–Menten kinetic to the data. Kinetic data of AOB, diatoms, organotrophic microorganisms, soil and ocean water were compiled from refs 9, 13, 21, 26, 27, 29 and 37–50. If only NH₄⁺ or NH₃ concentrations were given by the authors, the corresponding values were calculated based on given salinity, temperature and pH and the respective stoichiometric dissociation constants of NH₃ and NH₄⁺ given in ref. 51. For the estimation of specific affinities (*a*⁰; ref. 27), we calculated growth rates, μ (h⁻¹), metabolic coefficients, *q* (g substrate per g wet cells per h), and cell yields, *Y* (g wet cells per g substrate), as described^{26,27,40}. If necessary, the following conversion factors were used: 3 g wet weight per g dry weight, 5.7 g wet weight per g protein (ref. 27), and 0.55 g carbon per g dry weight (refs 52, 53).

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