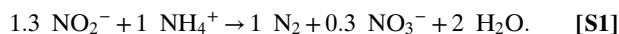


Supporting Information

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SI Text

Anaerobic Ammonium Oxidation Stoichiometry: Mass Balances. As a net reaction, anaerobic ammonium oxidation (anammox) converts nitrite and ammonium to dinitrogen and nitrate, according to an approximate stoichiometry of



We designate the parameter x to be the relative proportion of the nitrogen flux from nitrite to nitrate to the nitrogen flux from nitrite to N_2 (Fig. S1):

$$\frac{d}{dt} \text{NO}_2^-(t) = (1-x) \cdot \frac{d}{dt} \text{NO}_2^-(t) + x \cdot \frac{d}{dt} \text{NO}_2^-(t) \quad [\text{S2}]$$

and

$$\frac{d}{dt} \text{NO}_3^-(t) = -x \cdot \frac{d}{dt} \text{NO}_2^-(t). \quad [\text{S3}]$$

The approximate anammox stoichiometry (0.3 times increase in nitrate concentration equals the 1.3 times decrease in nitrite concentration) follows (Eq. S1, combined with Eq. S3):

$$x = \frac{0.3}{1.3} \approx 0.23. \quad [\text{S4}]$$

The relationship between change in nitrite and ammonium concentrations is described by

$$-(1-x) \cdot \frac{d}{dt} \text{NO}_2^-(t) = -\frac{d}{dt} \text{NH}_4^+(t). \quad [\text{S5}]$$

Integration of Eq. S3 and Eq. S5 yields

$$\text{NO}_3^-(t) = -x \cdot \text{NO}_2^-(t) + \text{const} \quad [\text{S6}]$$

and

$$\text{NH}_4^+(t) = (1-x) \cdot \text{NO}_2^-(t) + \text{const}, \quad [\text{S7}]$$

plots were larger than values from NO_3^- vs. NO_2^- plots. The estimate for $x = 0.24 \pm 0.04$ was calculated as the average of the determined values, whereby one outlying dataset was omitted (Table S1). This value for x corresponds to a ratio between NO_2^- and NH_4^+ consumption (anammox stoichiometry) of 1.32 ± 0.05 , which is consistent with Eq. S1.

Ammonium Isotope Fractionation. Ammonium isotope fractionation followed closed-system Rayleigh isotope fractionation systematics. Thus, the ammonium isotope fractionation $\epsilon^{15}\text{N}_{\text{NH}_4^+ \rightarrow \text{N}_2}$ could be determined from a $R^{15}\text{N}_{\text{NH}_4^+}$ vs. $\ln(f_{\text{NH}_4^+})$ plot (Fig. S3), according to

$$-\ln(R^{15}\text{N}_{\text{NH}_4^+}) = \epsilon^{15}\text{N}_{\text{NH}_4^+ \rightarrow \text{N}_2} \cdot \ln(f_{\text{NH}_4^+}), \quad [\text{S8}]$$

where

$$\ln(R^{15}\text{N}_{\text{NH}_4^+}) = \ln\left(\frac{\delta^{15}\text{N}_{\text{NH}_4^+}(t) + 1,000\text{‰}}{\delta^{15}\text{N}_{\text{NH}_4^+}(t_0) + 1,000\text{‰}}\right) \quad [\text{S9}]$$

and

$$\ln(f_{\text{NH}_4^+}) = \ln\left(\frac{\text{NH}_4^+(t)}{\text{NH}_4^+(t_0)}\right). \quad [\text{S10}]$$

Data obtained during sampling campaign 1 (C1) (Exps. C1_a and C1_b) overlap in the $-\ln(R^{15}\text{N}_{\text{NH}_4^+})$ vs. $\ln(f_{\text{NH}_4^+})$ plot, allowing us to combine the two datasets to determine $\epsilon^{15}\text{N}_{\text{NH}_4^+ \rightarrow \text{N}_2}$ ($29.1 \pm 0.7\text{‰}$, Fig. S3A), yielding an excellent match between observed data and modeled trend (Fig. S3B). The $\epsilon^{15}\text{N}_{\text{NH}_4^+ \rightarrow \text{N}_2}$ ($23.5 \pm 0.6\text{‰}$) for experiment C3_b is smaller (Fig. S3C) but also yields an excellent match between observed data and modeled trend (Fig. 1B and Fig. S3D; for discussion, see main text).

^{15}N Labeling Experiments: Numerical Model. To derive an iterative numerical model of the changes in the size of ^{14}N and ^{15}N pools, we transformed the mass and isotope mass balances into functions with discrete time steps (Δt), with discrete flux sizes for total nitrite consumption ($^{14}\text{NO}_2^-$ and $^{15}\text{NO}_2^-$), and accordingly for nitrate and N_2 production.

The ^{14}N nitrite mass balance becomes

$$^{14}\text{NO}_2^-(t + \Delta t) = ^{14}\text{NO}_2^-(t) - f_{\text{NO}_2^- \text{cons}} \cdot \left((1 + r_{\text{ex}} \cdot x) \cdot \frac{^{14}\text{NO}_2^-(t)}{^{14}\text{NO}_2^-(t) + ^{15}\text{NO}_2^-(t)} - r_{\text{ex}} \cdot x \cdot \frac{^{14}\text{NO}_3^-(t)}{^{14}\text{NO}_3^-(t) + ^{15}\text{NO}_3^-(t)} \right). \quad [\text{S11}]$$

with const being an integration constant. The value of x can be determined from the slope of the regression line in a nitrate vs. nitrite concentration plot, whereas $(1-x)$ can be determined from the slope of the regression line in an ammonium vs. nitrite concentration plot (Fig. S2). Values for x based on NH_4^+ vs. NO_2^-

The parameter r_{ex} is a measure of the isotope exchange flux between nitrate and nitrite relative to the oxidation flux of nitrite to nitrate ($x \cdot f_{\text{NO}_2^- \text{cons}}$). The total flux is divided into a flux of ^{15}N and ^{14}N isotopes, according to the relative abundance of those two isotopes in the respective pool.

Similarly, the ^{15}N nitrite mass balance is described as

$$^{15}\text{NO}_2^-(t + \Delta t) = ^{15}\text{NO}_2^-(t) - f_{\text{NO}_2^- \text{ consumption}} \cdot \left((1 + r_{ex} \cdot x) \cdot \frac{^{15}\text{NO}_2^-(t)}{^{14}\text{NO}_2^-(t) + ^{15}\text{NO}_2^-(t)} - r_{ex} \cdot x \cdot \frac{^{15}\text{NO}_3^-(t)}{^{14}\text{NO}_3^-(t) + ^{15}\text{NO}_3^-(t)} \right). \quad [\text{S12}]$$

Accordingly, the ^{14}N and ^{15}N nitrate mass balance is described as

$$^{14}\text{NO}_3^-(t + \Delta t) = ^{14}\text{NO}_3^-(t) + x \cdot f_{\text{NO}_2^- \text{ consumption}} \cdot \left((1 + r_{ex}) \cdot \frac{^{14}\text{NO}_2^-(t)}{^{14}\text{NO}_2^-(t) + ^{15}\text{NO}_2^-(t)} - r_{ex} \cdot \frac{^{14}\text{NO}_3^-(t)}{^{14}\text{NO}_3^-(t) + ^{15}\text{NO}_3^-(t)} \right) \quad [\text{S13}]$$

and

$$^{15}\text{NO}_3^-(t + \Delta t) = ^{15}\text{NO}_3^-(t) + x \cdot f_{\text{NO}_2^- \text{ consumption}} \cdot \left((1 + r_{ex}) \cdot \frac{^{15}\text{NO}_2^-(t)}{^{14}\text{NO}_2^-(t) + ^{15}\text{NO}_2^-(t)} - r_{ex} \cdot \frac{^{15}\text{NO}_3^-(t)}{^{14}\text{NO}_3^-(t) + ^{15}\text{NO}_3^-(t)} \right). \quad [\text{S14}]$$

The ^{14}N and ^{15}N of N in N_2 that is derived from nitrite are calculated as

$$^{14}\text{N}_2(t + \Delta t) = ^{14}\text{N}_2(t) + (1 - x) \cdot f_{\text{NO}_2^- \text{ consumption}} \cdot \frac{^{14}\text{NO}_2^-(t)}{^{14}\text{NO}_2^-(t) + ^{15}\text{NO}_2^-(t)} \quad [\text{S15}]$$

and

$$^{15}\text{N}_2(t + \Delta t) = ^{15}\text{N}_2(t) + (1 - x) \cdot f_{\text{NO}_2^- \text{ consumption}} \cdot \frac{^{15}\text{NO}_2^-(t)}{^{14}\text{NO}_2^-(t) + ^{15}\text{NO}_2^-(t)}. \quad [\text{S16}]$$

Using this iterative modeling approach, we obtained good fits of modeled ^{15}N and ^{14}N trends to experimental data with $x = 0.24$ and $r_{ex} = 25$ and 35 for experiment $\text{C}_3\text{-d}$ and $\text{C}_3\text{-c}$, respectively, which corresponds to a ratio between the nitrate–nitrite N exchange flux and the total nitrite consumption of 6–8 (Fig. S4).

NO_x Isotope Fractionation: Numerical Model. For the iterative numerical model of natural abundance isotope trends, we transformed the mass and isotope mass balances into functions with discrete time steps (Δt), with discrete flux sizes. Further, we simplified the model by using δ values instead of isotope ratios. The nitrite mass balance becomes

$$\begin{aligned} \text{NO}_2^-(t + \Delta t) &= \text{NO}_2^-(t) - f_{\text{NO}_2^- \text{ consumption}} \\ &= \text{NO}_2^-(t) - (1 - x) \cdot f_{\text{NO}_2^- \text{ consumption}} \\ &\quad - x \cdot f_{\text{NO}_2^- \text{ consumption}}. \end{aligned} \quad [\text{S17}]$$

The nitrite isotope mass balance becomes

$$\begin{aligned} \delta\text{NO}_2^-(t + \Delta t) \cdot \text{NO}_2^-(t + \Delta t) &= \delta\text{NO}_2^-(t) \cdot \text{NO}_2^-(t) \\ &\quad - f_{\text{NO}_2^- \text{ consumption}} \cdot (1 - x) \cdot (\delta\text{NO}_2^-(t) - \epsilon_{\text{NO}_2^- \rightarrow \text{N}_2}) \\ &\quad - f_{\text{NO}_2^- \text{ consumption}} \cdot x \cdot (\delta\text{NO}_2^-(t) - \epsilon_{\text{NO}_2^- \rightarrow \text{NO}_3^-}) \\ &\quad - f_{\text{NO}_2^- \text{ consumption}} \cdot r_{ex} \cdot x \cdot (\delta\text{NO}_2^-(t) - \epsilon_{\text{NO}_2^- \leftrightarrow \text{NO}_3^-}) \\ &\quad + f_{\text{NO}_2^- \text{ consumption}} \cdot r_{ex} \cdot x \cdot \delta\text{NO}_3^-(t). \end{aligned} \quad [\text{S20}]$$

This equation includes the isotope fractionation during removal of nitrogen from nitrite to the N_2 pool, the isotope fractionation during the removal of nitrogen from the nitrite pool to nitrate, and the equilibrium isotope fractionation during the isotope exchange between nitrite and nitrate.

Eq. S20 can be simplified to

$$\delta\text{NO}_2^-(t + \Delta t) = \frac{\delta\text{NO}_2^-(t) \cdot \text{NO}_2^-(t)}{\text{NO}_2^-(t + \Delta t)} - \frac{f_{\text{NO}_2^- \text{ cons}}}{\text{NO}_2^-(t + \Delta t)} \cdot \left(\frac{\delta\text{NO}_2^-(t) \cdot (1 + r_{ex} \cdot x) - (1 - x) \cdot \epsilon_{\text{NO}_2^- \rightarrow \text{N}_2} - x \cdot \epsilon_{\text{NO}_2^- \rightarrow \text{NO}_3^-}}{-r_{ex} \cdot x \cdot (\epsilon_{\text{NO}_2^- \leftrightarrow \text{NO}_3^-} + \delta\text{NO}_3^-(t))} \right). \quad [\text{S21}]$$

The nitrate mass balance becomes

$$\text{NO}_3^-(t + \Delta t) = \text{NO}_3^-(t) + x \cdot f_{\text{NO}_2^- \text{ consumption}}. \quad [\text{S18}]$$

The mass balance for nitrogen in N_2 that was derived from nitrite becomes

$$\text{N}_2(t + \Delta t) = \text{N}_2(t) + (1 - x) \cdot f_{\text{NO}_2^- \text{ consumption}}. \quad [\text{S19}]$$

Analogously, the nitrate isotope mass balance becomes

$$\begin{aligned} \delta\text{NO}_3^-(t + \Delta t) \cdot \text{NO}_3^-(t + \Delta t) &= \delta\text{NO}_3^-(t) \cdot \text{NO}_3^-(t) \\ &\quad + f_{\text{NO}_2^- \text{ cons}} \cdot x \cdot (\delta\text{NO}_2^-(t) - \epsilon_{\text{NO}_2^- \rightarrow \text{NO}_3^-}) \\ &\quad + f_{\text{NO}_2^- \text{ cons}} \cdot r_{ex} \cdot x \cdot (\delta\text{NO}_2^-(t) - \epsilon_{\text{NO}_2^- \leftrightarrow \text{NO}_3^-}) \\ &\quad - f_{\text{NO}_2^- \text{ cons}} \cdot r_{ex} \cdot x \cdot \delta\text{NO}_3^-(t). \end{aligned} \quad [\text{S22}]$$

The nitrogen isotope mass balance for nitrogen in N_2 derived from nitrite becomes

$$\delta N_2(t + \Delta t) \cdot N_2(t + \Delta t) = \delta N_2(t) \cdot N_2(t) + f_{NO_2^- \rightarrow N_2} \cdot (1 - x) \cdot (\delta NO_2^-(t) - \epsilon_{NO_2^- \rightarrow N_2}). \quad [S23]$$

Using this iterative modeling approach, we obtained good fits for modeled $\delta^{15}N$ trends to experimental data with $x = 0.24$, $\epsilon_{NO_2^- \rightarrow NO_3^-} = -30\%$, and $\epsilon_{NO_2^- \rightarrow NO_3^-} = -60\%$. For C2 and C3, $\epsilon_{NO_2^- \rightarrow N_2}$ was kept constant at $+15\%$, whereas for C1 $\epsilon_{NO_2^- \rightarrow N_2}$ was gradually changed from $+15\%$ to -10% (Fig. S5). Exchange rates were adjusted to obtain good matches between model output and experimental data (Table S2). The best-fit exchange rates vary considerably between different experiments, from no exchange in C2_a (Fig. S5F) to a fairly long duration of exchange, however, with strongly reduced exchange rates, for C3_a (Fig. S5J). For the determination of exchange rates, the isotope labeling approach is likely more robust because changes are more strongly expressed, which may explain the higher rates observed for C3_c and C3_d.

NO_x Isotope Fractionation: Analytical Solution for a Scenario with Unidirectional Fluxes (i.e., No Isotope Exchange Between Nitrite and Nitrate). We can describe the nitrite isotope mass balance by a nitrogen flux from nitrite to N_2 , associated with a kinetic isotope fractionation ($\epsilon_{NO_2^- \rightarrow N_2}$) and a nitrogen flux from nitrite to nitrate, associated with another kinetic isotope fractionation ($\epsilon_{NO_2^- \rightarrow NO_3^-}$):

$$\frac{d}{dt}(NO_2^-(t) \cdot \delta NO_2^-(t)) = (1-x) \cdot \frac{d}{dt}NO_2^-(t) \cdot (\delta NO_2^-(t) - \epsilon_{NO_2^- \rightarrow N_2}) + x \cdot \frac{d}{dt}NO_2^-(t) \cdot (\delta NO_2^-(t) - \epsilon_{NO_2^- \rightarrow NO_3^-}). \quad [S24]$$

Similarly, the nitrate isotope mass balance is written as

$$\frac{d}{dt}(NO_3^-(t) \cdot \delta NO_3^-(t)) = -x \cdot \frac{d}{dt}NO_2^-(t) \cdot (\delta NO_2^-(t) - \epsilon_{NO_2^- \rightarrow NO_3^-}). \quad [S25]$$

Using product rule for Eq. S25 and substituting the derivative after the time of the nitrite concentration with Eq. S2 results in

$$\begin{aligned} \frac{d}{dt}(NO_2^-(t) \cdot \delta NO_2^-(t)) &= \frac{d}{dt}NO_2^-(t) \cdot \delta NO_2^-(t) + NO_2^-(t) \cdot \frac{d}{dt}\delta NO_2^-(t) \\ &= \left((1-x) \cdot \frac{d}{dt}NO_2^-(t) + x \cdot \frac{d}{dt}NO_2^-(t) \right) \\ &\quad \cdot \delta NO_2^-(t) + NO_2^-(t) \cdot \frac{d}{dt}\delta NO_2^-(t) \\ &= (1-x) \cdot \frac{d}{dt}NO_2^-(t) \cdot (\delta NO_2^-(t) - \epsilon_{NO_2^- \rightarrow N_2}) \\ &\quad + x \cdot \frac{d}{dt}NO_2^-(t) \cdot (\delta NO_2^-(t) - \epsilon_{NO_2^- \rightarrow NO_3^-}), \end{aligned} \quad [S26]$$

which can be simplified to

$$NO_2^-(t) \cdot \frac{d}{dt}\delta NO_2^-(t) = (1-x) \cdot \frac{d}{dt}NO_2^-(t) \cdot -\epsilon_{NO_2^- \rightarrow N_2} + x \cdot \frac{d}{dt}NO_2^-(t) \cdot -\epsilon_{NO_2^- \rightarrow NO_3^-}. \quad [S27]$$

Eq. S27 is rearranged:

$$\frac{d}{dt}\delta NO_2^-(t) = -((1-x) \cdot \epsilon_{NO_2^- \rightarrow N_2} + x \cdot \epsilon_{NO_2^- \rightarrow NO_3^-}) \cdot \frac{\frac{d}{dt}NO_2^-(t)}{NO_2^-(t)}. \quad [S28]$$

Integration yields

$$\delta NO_2^-(t) = \delta NO_2^-(t_0) - ((1-x) \cdot \epsilon_{NO_2^- \rightarrow N_2} + x \cdot \epsilon_{NO_2^- \rightarrow NO_3^-}) \cdot \ln \frac{NO_2^-(t)}{NO_2^-(t_0)}. \quad [S29]$$

Defining $f_{NO_2^-}(t)$ as the fraction of remaining nitrite,

$$f_{NO_2^-}(t) = \frac{NO_2^-(t)}{NO_2^-(t_0)}, \quad [S30]$$

and defining

$$E = ((1-x) \cdot \epsilon_{NO_2^- \rightarrow N_2} + x \cdot \epsilon_{NO_2^- \rightarrow NO_3^-}) \quad [S31]$$

results in

$$\delta NO_2^-(t) = \delta NO_2^-(t_0) - E \cdot \ln f_{NO_2^-}(t). \quad [S32]$$

Under the assumption that the initial amount of nitrate and N_2 is very small ($NO_3^-(t) \sim N_2(t) \sim 0$), we can use an isotope mass balance for calculating the time-dependent evolution of the N isotope composition of nitrate. In the calculations presented below, the amount of N_2 ($N_2(t)$) and the isotope composition of N_2 ($\delta N_2(t)$) solely refer to nitrogen derived from the nitrite pool (i.e., not the sum of nitrogen from the nitrite and the ammonium pool). The amount of nitrate and N_2 can be calculated as

$$NO_3^-(t) = x \cdot (NO_2^-(t_0) - NO_2^-(t)) \quad [S33]$$

and

$$N_2(t) = (1-x) \cdot (NO_2^-(t_0) - NO_2^-(t)). \quad [S34]$$

Using the relationship

$$f_{NO_2^-}(t) = \frac{NO_2^-(t)}{NO_2^-(t_0)} \Rightarrow NO_2^-(t) = f_{NO_2^-}(t) \cdot NO_2^-(t_0), \quad [S35]$$

Eqs. S33 and S34 become

$$\begin{aligned} NO_3^-(t) &= x \cdot (NO_2^-(t_0) - f_{NO_2^-}(t) \cdot NO_2^-(t_0)) \\ &= x \cdot NO_2^-(t_0) \cdot (1 - f_{NO_2^-}(t)) \end{aligned} \quad [S36]$$

and

$$\begin{aligned} N_2(t) &= (1-x) \cdot (NO_2^-(t_0) - f_{NO_2^-}(t) \cdot NO_2^-(t_0)) \\ &= (1-x) \cdot NO_2^-(t_0) \cdot (1 - f_{NO_2^-}(t)). \end{aligned} \quad [S37]$$

The isotope mass balance of all nitrogen species derived from nitrite is written as follows:

$$\begin{aligned} &NO_2^-(t_0) \cdot \delta NO_2^-(t_0) - NO_2^-(t) \cdot \delta NO_2^-(t) \\ &= NO_3^-(t) \cdot \delta NO_3^-(t) + N_2(t) \cdot \delta N_2(t). \end{aligned} \quad [S38]$$

Using Eqs. S36 and S37, Eq. S38 is rewritten as

$$\begin{aligned} \text{NO}_2^-(t_0) \cdot \delta\text{NO}_2^-(t_0) - \text{NO}_2^-(t) \cdot \delta\text{NO}_2^-(t) &= x \cdot \text{NO}_2^-(t_0) \\ &\cdot \left(1 - f_{\text{NO}_2^-}(t)\right) \cdot \delta\text{NO}_3^-(t) + (1-x) \cdot \text{NO}_2^-(t_0) \\ &\cdot \left(1 - f_{\text{NO}_2^-}(t)\right) \cdot \delta\text{N}_2(t). \end{aligned} \quad \text{[S39]}$$

This equation is further simplified by dividing with $\text{NO}_2^-(t_0)$

$$\begin{aligned} \delta\text{NO}_2^-(t_0) - f_{\text{NO}_2^-}(t) \cdot \delta\text{NO}_2^-(t) &= x \cdot \left(1 - f_{\text{NO}_2^-}(t)\right) \\ &\cdot \delta\text{NO}_3^-(t) + (1-x) \cdot \left(1 - f_{\text{NO}_2^-}(t)\right) \cdot \delta\text{N}_2(t). \end{aligned} \quad \text{[S40]}$$

Substitution of $\delta\text{NO}_2^-(t)$ with Eq. S32 results in

$$\begin{aligned} \delta\text{NO}_2^-(t_0) - f_{\text{NO}_2^-}(t) \cdot \left(\delta\text{NO}_2^-(t_0) - E \cdot \ln f_{\text{NO}_2^-}(t)\right) \\ = x \cdot \left(1 - f_{\text{NO}_2^-}(t)\right) \cdot \delta\text{NO}_3^-(t) + (1-x) \cdot \left(1 - f_{\text{NO}_2^-}(t)\right) \cdot \delta\text{N}_2(t). \end{aligned} \quad \text{[S41]}$$

Eq. S41 can be simplified to

$$\begin{aligned} \delta\text{NO}_2^-(t_0) \cdot \left(1 - f_{\text{NO}_2^-}(t)\right) + f_{\text{NO}_2^-}(t) \cdot E \cdot \ln f_{\text{NO}_2^-}(t) \\ = x \cdot \left(1 - f_{\text{NO}_2^-}(t)\right) \cdot \delta\text{NO}_3^-(t) + (1-x) \cdot \left(1 - f_{\text{NO}_2^-}(t)\right) \cdot \delta\text{N}_2(t) \end{aligned} \quad \text{[S42]}$$

and finally to

$$\delta\text{NO}_2^-(t_0) + \frac{f_{\text{NO}_2^-}(t) \cdot \ln f_{\text{NO}_2^-}(t)}{1 - f_{\text{NO}_2^-}(t)} \cdot E = x \cdot \delta\text{NO}_3^-(t) + (1-x) \cdot \delta\text{N}_2(t). \quad \text{[S43]}$$

Eq. S43 provides an ansatz for integrating the isotope mass balance for nitrate (Eq. S25),

$$\begin{aligned} \frac{d}{dt} \text{NO}_3^-(t) \cdot \delta\text{NO}_3^-(t) + \text{NO}_3^-(t) \cdot \frac{d}{dt} \delta\text{NO}_3^-(t) \\ = -x \cdot \frac{d}{dt} \text{NO}_2^-(t) \cdot \left(\delta\text{NO}_2^-(t) - \varepsilon_{\text{NO}_2^- \rightarrow \text{NO}_3^-}\right), \end{aligned} \quad \text{[S44]}$$

$$\begin{aligned} -(\text{NO}_2^-(t_0) - \text{NO}_2^-(t)) \cdot \frac{d}{dt} \text{NO}_2^-(t) \cdot \left(\left(\ln \frac{\text{NO}_2^-(t)}{\text{NO}_2^-(t_0)} + 1 \right) \cdot (\text{NO}_2^-(t_0) - \text{NO}_2^-(t))^{-1} + \right. \\ \left. \left(\text{NO}_2^-(t) \cdot \ln \frac{\text{NO}_2^-(t)}{\text{NO}_2^-(t_0)} \right) \cdot (\text{NO}_2^-(t_0) - \text{NO}_2^-(t))^{-2} \right) \cdot E \\ = \frac{d}{dt} \text{NO}_2^-(t) \cdot \left(\left(\delta\text{NO}_2^-(t_0) - E \cdot \ln f_{\text{NO}_2^-}(t) \right) - \left(\delta\text{NO}_2^-(t) + \left(\frac{f_{\text{NO}_2^-}(t) \cdot \ln f_{\text{NO}_2^-}(t)}{1 - f_{\text{NO}_2^-}(t)} + k_1 \right) \cdot E + k_2 \right) - \varepsilon_{\text{NO}_2^- \rightarrow \text{NO}_3^-} \right). \end{aligned} \quad \text{[S50]}$$

which is in the form of

$$\delta\text{NO}_3^-(t)_{\text{ansatz}} = \delta\text{NO}_2^-(t_0) + \left(\frac{f_{\text{NO}_2^-}(t) \cdot \ln f_{\text{NO}_2^-}(t)}{1 - f_{\text{NO}_2^-}(t)} + k_1 \right) \cdot E + k_2. \quad \text{[S45]}$$

The constants k_1 and k_2 can be determined by calculating the derivative of the ansatz:

$$\begin{aligned} \frac{d}{dt} \delta\text{NO}_3^-(t)_{\text{ansatz}} \\ = \frac{d}{dt} \text{NO}_2^-(t) \cdot \left(\left(\ln \frac{\text{NO}_2^-(t)}{\text{NO}_2^-(t_0)} + 1 \right) \cdot (\text{NO}_2^-(t_0) - \text{NO}_2^-(t))^{-1} + \right. \\ \left. \left(\text{NO}_2^-(t) \cdot \ln \frac{\text{NO}_2^-(t)}{\text{NO}_2^-(t_0)} \right) \cdot (\text{NO}_2^-(t_0) - \text{NO}_2^-(t))^{-2} \right) \cdot E. \end{aligned} \quad \text{[S46]}$$

Using Eqs. S3 and S33, Eq. S44 can now be rewritten as

$$\begin{aligned} -x \cdot \frac{d}{dt} \text{NO}_2^-(t) \cdot \delta\text{NO}_3^-(t) + x \cdot (\text{NO}_2^-(t_0) - \text{NO}_2^-(t)) \cdot \frac{d}{dt} \delta\text{NO}_3^-(t) \\ = -x \cdot \frac{d}{dt} \text{NO}_2^-(t) \cdot \left(\delta\text{NO}_2^-(t) - \varepsilon_{\text{NO}_2^- \rightarrow \text{NO}_3^-}\right). \end{aligned} \quad \text{[S47]}$$

Eq. S47 can be divided by $-x$,

$$\begin{aligned} \frac{d}{dt} \text{NO}_2^-(t) \cdot \delta\text{NO}_3^-(t) - (\text{NO}_2^-(t_0) - \text{NO}_2^-(t)) \cdot \frac{d}{dt} \delta\text{NO}_3^-(t) \\ = \frac{d}{dt} \text{NO}_2^-(t) \cdot \left(\delta\text{NO}_2^-(t) - \varepsilon_{\text{NO}_2^- \rightarrow \text{NO}_3^-}\right), \end{aligned} \quad \text{[S48]}$$

and be rearranged as

$$\begin{aligned} -(\text{NO}_2^-(t_0) - \text{NO}_2^-(t)) \cdot \frac{d}{dt} \delta\text{NO}_3^-(t) \\ = \frac{d}{dt} \text{NO}_2^-(t) \cdot \left(\delta\text{NO}_2^-(t) - \delta\text{NO}_3^-(t) - \varepsilon_{\text{NO}_2^- \rightarrow \text{NO}_3^-}\right). \end{aligned} \quad \text{[S49]}$$

In Eq. S49, the derivative after the time of δNO_3^- is substituted with the derivative after the time of the ansatz for δNO_3^- (Eq. S46), the function for δNO_2^- is substituted with Eq. S32, and the function of δNO_3^- is substituted with the ansatz for δNO_3^- , (Eq. S55):

With Eq. S30, Eq. S50 simplifies to

$$k_1 \cdot E + k_2 = +1 \cdot E - \varepsilon_{\text{NO}_2^- \rightarrow \text{NO}_3^-}. \quad \text{[S51]}$$

From Eq. S51, we can determine the values for the constants used in the ansatz for the function for δNO_3^- (Eq. S45):

$$k_1 = 1 \text{ and } k_2 = -\varepsilon_{\text{NO}_2^- \rightarrow \text{NO}_3^-}. \quad \text{[S52]}$$

Thus, Eq. S45 becomes

$$\delta\text{NO}_3^-(t) = \delta\text{NO}_2^-(t_0) + \left(\frac{f_{\text{NO}_2^-(t)} \cdot \ln f_{\text{NO}_2^-(t)}}{1 - f_{\text{NO}_2^-(t)}} + 1 \right) \cdot E - \varepsilon_{\text{NO}_2^- \rightarrow \text{NO}_3^-} \quad [\text{S53}]$$

Eqs. S32 and S53 can be used to graphically determine E and $\varepsilon_{\text{NO}_2^- \rightarrow \text{NO}_3^-}$:

$$\delta\text{NO}_2^-(t) - \delta\text{NO}_2^-(t_0) = E \cdot -\ln f_{\text{NO}_2^-(t)} \quad [\text{S54}]$$

$$\delta\text{NO}_3^-(t) - \delta\text{NO}_2^-(t_0) = \left(\frac{f_{\text{NO}_2^-(t)} \cdot \ln f_{\text{NO}_2^-(t)}}{1 - f_{\text{NO}_2^-(t)}} + 1 \right) \cdot E - \varepsilon_{\text{NO}_2^- \rightarrow \text{NO}_3^-} \quad [\text{S55}]$$

The value of E corresponds to the slope of the regression line for data plotted according to Eqs. S54 and S55, whereas the intercept of the regression line of data plotted according to Eq. S55 corresponds to $-\varepsilon_{\text{NO}_2^- \rightarrow \text{NO}_3^-}$. To determine $\varepsilon_{\text{NO}_2^- \rightarrow \text{N}_2}$ Eq. S31 has to be rearranged:

$$E = \left((1-x) \cdot \varepsilon_{\text{NO}_2^- \rightarrow \text{N}_2} + x \cdot \varepsilon_{\text{NO}_2^- \rightarrow \text{NO}_3^-} \right) \Rightarrow \varepsilon_{\text{NO}_2^- \rightarrow \text{N}_2} = \frac{E - x \cdot \varepsilon_{\text{NO}_2^- \rightarrow \text{NO}_3^-}}{1-x} \quad [\text{S56}]$$

This demonstrates that, to determine a value for $\varepsilon_{\text{NO}_2^- \rightarrow \text{N}_2}$, the relative proportion of the nitrogen flux from nitrite to nitrate to the nitrogen flux from nitrite to N_2 , i.e., the parameter x , must be known.

Using this analytical approach and an estimate for x (0.24 ± 0.04), we obtained estimates $\varepsilon_{\text{NO}_2^- \rightarrow \text{NO}_3^-}$ and $\varepsilon_{\text{NO}_2^- \rightarrow \text{N}_2}$ (Fig. S6 and Table S3). To avoid potential errors in the graphical determination of regressions, we selected data that can be considered to be robust, i.e., where the concentration of NO_2^- (beginning of the experiment) and the concentration of NO_3^- (toward the end of the experiment) are large. Still, the uncertainty for these estimates is fairly large. The average of the obtained values can be used as an estimate for $\varepsilon_{\text{NO}_2^- \rightarrow \text{NO}_3^-}$ and $\varepsilon_{\text{NO}_2^- \rightarrow \text{N}_2}$, and the SD for the averaged values serves as an error estimate ($\varepsilon_{\text{NO}_2^- \rightarrow \text{NO}_3^-} = -31.1 \pm 3.9\%$, Table S3). In the case of $\varepsilon_{\text{NO}_2^- \rightarrow \text{NO}_3^-}$ there are two independent estimates ($14.7 \pm 2.8\%$ and $17.4 \pm 1.5\%$, Table S3). Here, we take as an estimate the average between the two values and use as a conservative error estimate the range covered by the values; i.e., $\varepsilon_{\text{NO}_2^- \rightarrow \text{N}_2} = 16.0 \pm 4.5\%$ (Table S3).

Equilibrium N Isotope Fractionation Between Nitrite and Nitrate. For an estimate for the equilibrium isotope fractionation we took the maximum offset between $\delta^{15}\text{N}_{\text{NO}_3^-}$ and $\delta^{15}\text{N}_{\text{NO}_2^-}$ from the experiments of C1 because the true equilibrium isotope fractionation can be only equal to or larger than this offset. As an error estimate for this value, we considered that there may be an analytical and procedural error of 0.5% for both $\delta^{15}\text{N}_{\text{NO}_3^-}$ and $\delta^{15}\text{N}_{\text{NO}_2^-}$ measurements. A conservative error estimate of the addition of the two errors, as the maximum value in a data series, may have resulted from the combination of an underestimate of $\delta^{15}\text{N}_{\text{NO}_2^-}$ and an overestimate of $\delta^{15}\text{N}_{\text{NO}_3^-}$. Thus, we attribute a value of $-60.5 \pm 1.0\%$ to $\varepsilon_{\text{NO}_2^- \leftrightarrow \text{NO}_3^-}$.

Factors Influencing the Observed Isotope Exchange Between Nitrate and Nitrite. Even an immediate killing of anammox cells with mercury (which also inhibits enzyme activity) does not fully impede ^{15}N isotope exchange, whereas the chemical control shows that there is no abiotic ^{15}N isotope exchange between nitrate and nitrite (Table S4). Our experiments show that N isotope exchange between nitrate and nitrite proceeds after the filtration of cells and treatment with a French press, because the exchange is higher than in the experiment where the cells were poisoned with mercury. This demonstrates that anammox enzymes that mediate N isotope exchange between nitrite and nitrate remain functional. Obviously, sample treatment plays a role; i.e., higher temperatures (e.g., room temperature instead of ice bath) enhance isotope exchange. However, the fact that no N isotope exchange between nitrate and nitrite was observed for the major parts of C2 and C3 shows that our sampling procedure (identical to “filtered cold”, Table S4) does not cause N isotope exchange when the harvested biomass is actively performing the anammox reaction.

The fact that we observe N isotope exchange at the initiation of sampling campaign points and that cell lysis does not impede this exchange indicates that anammox can release enzymes catalyzing N isotope exchange between nitrite and nitrate when exposed to environmental stress, such as the exposure to O_2 before the experiments. In light of these findings, it is likely that during C1 N isotope exchange between nitrite and nitrate occurred throughout the experiment and continued during sample preparation. The latter may have partially overprinted the original N isotope signature of nitrite and nitrate, which could explain why, for C1, we obtain good fits between model trends only for a scenario where $\varepsilon_{\text{NO}_2^- \rightarrow \text{N}_2}$ gradually changes (Fig. S5 B and D). Nevertheless, the large isotope offset between nitrite and nitrate in the initial stages of C2/C3 and throughout C1 cannot be explained by the exchange observed in the death controls.

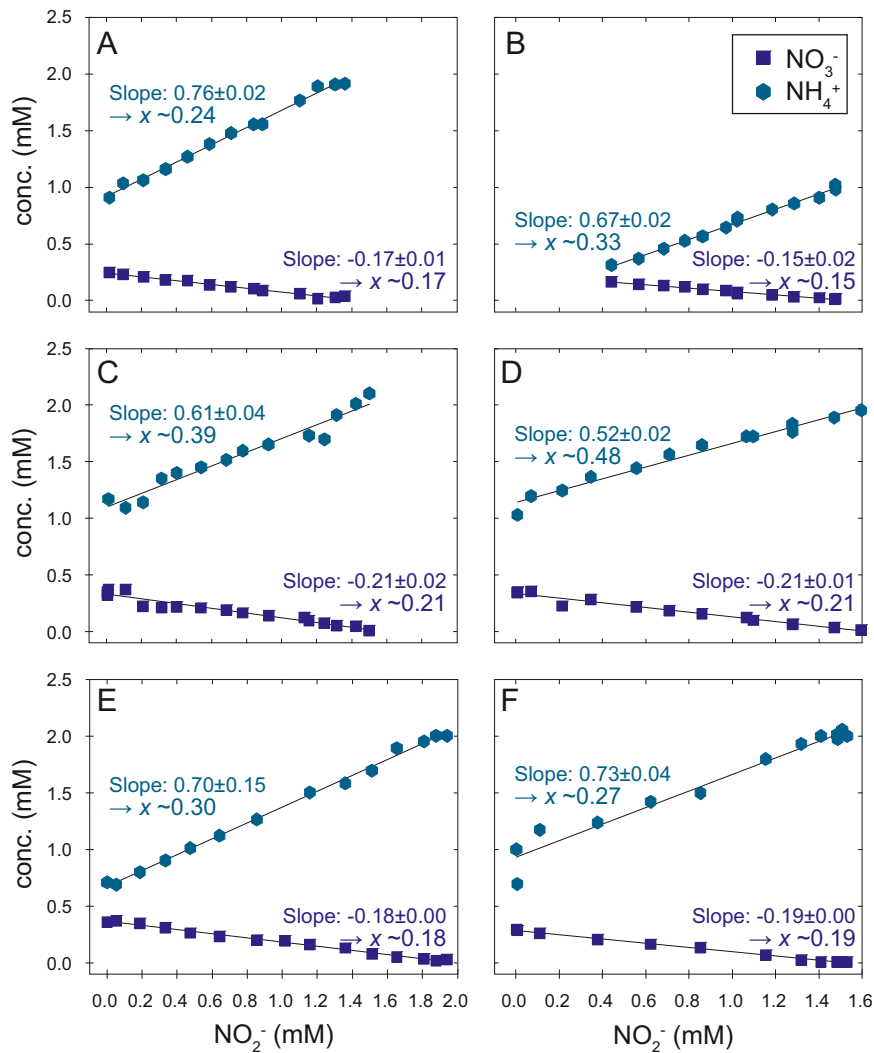


Fig. S2. Determination of anammox stoichiometry (x). The value for x can be determined from the relationship between NH_4^+ and NO_2^- consumption (positive slopes), as well as from the relationship between NO_3^- production and NO_2^- consumption (negative slopes; *SI Text*). The values found for x based on NH_4^+ vs. NO_2^- plots always exceed the values found for x based on NO_3^- vs. NO_2^- plots. (A) C_{1_a}; (B) C_{1_b}; (C) C_{2_a}; (D) C_{2_b}; (E) C_{3_a}; (F) C_{3_b}. For a compilation of the determined values for x , see Table S1.

