# **Supporting Information**

## Brunner et al. 10.1073/pnas.1310488110

#### 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

1.3 
$$NO_2^- + 1 NH_4^+ \rightarrow 1 N_2 + 0.3 NO_3^- + 2 H_2O_1$$
 [S1]

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<sub>2</sub> (Fig. S1):

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

and

$$\frac{d}{dt}NO_3^{-}(t) = -x \cdot \frac{d}{dt}NO_2^{-}(t).$$
 [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**):

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

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

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

Integration of Eq. S3 and Eq. S5 yields

$$NO_3^{-}(t) = -x \cdot NO_2^{-}(t) + const$$
 [S6]

and

$$NH_4^+(t) = (1-x) \cdot NO_2^-(t) + const,$$
 [S7]

plots were larger than values from NO<sub>3</sub><sup>-</sup> vs. NO<sub>2</sub><sup>-</sup> 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<sub>2</sub><sup>-</sup> and NH<sub>4</sub><sup>+</sup> consumption (anamnox stoichiometry) of  $1.32 \pm 0.05$ , which is consistent with Eq. S1.

**Ammonium Isotope Fractionation.** Ammonium isotope fractionation followed closed-system Rayleigh isotope fractionation systematics. Thus, the ammonium isotope fractionation  $\epsilon^{15}N_{NH_4^+} \rightarrow N_2$  could be determined from a  $R^{15}N_{NH_4^+}$  vs.  $ln(f_{NH_4^+})$  plot (Fig. S3), according to

$$-\ln\left(R^{15}N_{NH_{4}^{+}}\right) = \epsilon^{15}N_{NH_{4}^{+}\to N_{2}}\cdot\ln\left(f_{NH_{4}^{+}}\right),$$
 [S8]

where

$$\ln\left(\mathbf{R}^{15}\mathbf{N}_{\mathrm{NH}_{4}^{+}}\right) = \ln\left(\frac{\delta^{15}\mathbf{N}_{\mathrm{NH}_{4}^{+}}(t) + 1,000\%o}{\delta^{15}\mathbf{N}_{\mathrm{NH}_{4}^{+}}(t_{0}) + 1,000\%o}\right)$$
[**S9**]

and

$$\ln(f_{\rm NH_4^+}) = \ln\left(\frac{\rm NH_4^+(t)}{\rm NH_4^+(t_0)}\right).$$
 [S10]

Data obtained during sampling campaign 1 (C1) (Exps.  $C_{1_a}$  and  $C_{1_b}$ ) overlap in the  $-\ln(R^{15}N_{NH_4^+})$  vs.  $\ln(f_{NH_4^+})$  plot, allowing us to combine the two datasets to determine  $\epsilon^{15}N_{NH_4^+\to N_2}$  (29.1  $\pm$  0.7‰, Fig. S3*A*), yielding an excellent match between observed data and modeled trend (Fig. S3*B*). The  $\epsilon^{15}N_{NH_4^+\to N_2}$  (23.5  $\pm$  0.6‰) for experiment  $C_{3_b}$  is smaller (Fig. S3*C*) but also yields an excellent match between observed data and modeled trend (Fig. 1*B* and Fig. S3*D*; for discussion, see main text).

<sup>15</sup>N Labeling Experiments: Numerical Model. To derive an iterative numerical model of the changes in the size of <sup>14</sup>N and <sup>15</sup>N pools, we transformed the mass and isotope mass balances into functions with discrete time steps ( $\Delta t$ ), with discrete flux sizes for total nitrite consumption (<sup>14</sup>NO<sub>2</sub><sup>-</sup> and <sup>15</sup>NO<sub>2</sub><sup>-</sup>), and accordingly for nitrate and N<sub>2</sub> production.

The <sup>14</sup>N nitrite mass balance becomes

$${}^{14}\text{NO}_2^{-}(t+\Delta t) = {}^{14}\text{NO}_2^{-}(t) - f_{\text{NO}_2^{-}\text{cons}} \cdot \left( (1+r_{ex} \cdot x) \cdot \frac{{}^{14}\text{NO}_2^{-}(t)}{{}^{14}\text{NO}_2^{-}(t) + {}^{15}\text{NO}_2^{-}(t)} - r_{ex} \cdot x \cdot \frac{{}^{14}\text{NO}_3^{-}(t)}{{}^{14}\text{NO}_3^{-}(t) + {}^{15}\text{NO}_3^{-}(t)} \right).$$
[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<sub>4</sub><sup>+</sup> vs. NO<sub>2</sub><sup>-</sup>

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  $(x \cdot f_{NO_2^-}cons)$ . The total flux is divided into a flux of <sup>15</sup>N and <sup>14</sup>N isotopes, according to the relative abundance of those two isotopes in the respective pool. Similarly, the <sup>15</sup>N nitrite mass balance is described as

$${}^{15}\mathrm{NO}_{2}^{-}(t+\Delta t) = {}^{15}\mathrm{NO}_{2}^{-}(t) - f_{\mathrm{NO}_{2}^{-}\text{-cons}} \cdot \left( (1+r_{ex} \cdot x) \cdot \frac{{}^{15}\mathrm{NO}_{2}^{-}(t)}{{}^{14}\mathrm{NO}_{2}^{-}(t)} + {}^{15}\mathrm{NO}_{2}^{-}(t)} - r_{ex} \cdot x \cdot \frac{{}^{15}\mathrm{NO}_{3}^{-}(t)}{{}^{14}\mathrm{NO}_{3}^{-}(t)} + {}^{15}\mathrm{NO}_{3}^{-}(t)} \right).$$
[S12]

Accordingly, the <sup>14</sup>N and <sup>15</sup>N nitrate mass balance is described as

$${}^{14}\text{NO}_{3}^{-}(t+\Delta t) = {}^{14}\text{NO}_{3}^{-}(t) + x \cdot f_{\text{NO}_{2}^{-}\text{cons}} \cdot \left( (1+r_{ex}) \cdot \frac{{}^{14}\text{NO}_{2}^{-}(t)}{{}^{14}\text{NO}_{2}^{-}(t)} - r_{ex} \cdot \frac{{}^{14}\text{NO}_{3}^{-}(t)}{{}^{14}\text{NO}_{3}^{-}(t) + {}^{15}\text{NO}_{3}^{-}(t)} \right)$$
[S13]

and

$${}^{15}\text{NO}_{3}^{-}(t+\Delta t) = {}^{15}\text{NO}_{3}^{-}(t) + x \cdot f_{\text{NO}_{2}^{-}\text{-cons}} \cdot \left( (1+r_{ex}) \frac{{}^{15}\text{NO}_{2}^{-}(t)}{{}^{14}\text{NO}_{2}^{-}(t)} + {}^{15}\text{NO}_{2}^{-}(t)} - r_{ex} \cdot \frac{{}^{15}\text{NO}_{3}^{-}(t)}{{}^{14}\text{NO}_{3}^{-}(t) + {}^{15}\text{NO}_{3}^{-}(t)} \right).$$
[S14]

The <sup>14</sup>N and <sup>15</sup>N of N in N<sub>2</sub> that is derived from nitrite are calculated as

$${}^{14}N_2(t+\Delta t) = {}^{14}N_2(t) + (1-x) \cdot f_{NO_2^--cons} \cdot \frac{{}^{14}NO_2^-(t)}{{}^{14}NO_2^-(t) + {}^{15}NO_2^-(t)}$$
[S15]

and

$${}^{15}N_2(t+\Delta t) = {}^{15}N_2(t) + (1-x) \cdot f_{NO_2^- - cons} \cdot \frac{{}^{15}NO_2^{-}(t)}{{}^{14}NO_2^{-}(t) + {}^{15}NO_2^{-}(t)}.$$
[S16]

Using this iterative modeling approach, we obtained good fits of modeled <sup>15</sup>N and <sup>14</sup>N trends to experimental data with x = 0.24 and  $r_{ex} = 25$  and 35 for experiment C<sub>3</sub>\_d and C<sub>3</sub>\_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<sub>x</sub> 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 ( $\Delta t$ ), with discrete flux sizes. Further, we simplified the model by using  $\delta$  values instead of isotope ratios. The nitrite mass balance becomes

$$NO_2^{-}(t + \Delta t) = NO_2^{-}(t) - f_{NO_2^{-} consumption}$$
  
= NO<sub>2</sub><sup>-</sup>(t)-(1-x) · f<sub>NO\_2^{-} consumption [S17]  
-x · f\_{NO\_2^{-} consumption}.</sub>

The nitrite isotope mass balance becomes

$$\delta \operatorname{NO}_{2}^{-}(t + \Delta t) \cdot \operatorname{NO}_{2}^{-}(t + \Delta t) = \delta \operatorname{NO}_{2}^{-}(t) \cdot \operatorname{NO}_{2}^{-}(t)$$

$$-f_{\operatorname{NO}_{2}^{-}\_cons} \cdot (1 - x) \cdot (\delta \operatorname{NO}_{2}^{-}(t) - \varepsilon_{\operatorname{NO}_{2}^{-} \to \operatorname{NO}_{2}^{-}})$$

$$-f_{\operatorname{NO}_{2}^{-}\_cons} \cdot x \cdot (\delta \operatorname{NO}_{2}^{-}(t) - \varepsilon_{\operatorname{NO}_{2}^{-} \to \operatorname{NO}_{3}^{-}})$$

$$-f_{\operatorname{NO}_{2}^{-}\_cons} \cdot r_{ex} \cdot x \cdot (\delta \operatorname{NO}_{2}^{-}(t) - \varepsilon_{\operatorname{NO}_{2}^{-} \to \operatorname{NO}_{3}^{-}})$$

$$+f_{\operatorname{NO}_{2}^{-}\_cons} \cdot r_{ex} \cdot x \cdot \delta \operatorname{NO}_{3}^{-}(t).$$
[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

$$\delta \operatorname{NO}_{2}^{-}(t+\Delta t) = \frac{\delta \operatorname{NO}_{2}^{-}(t) \cdot \operatorname{NO}_{2}^{-}(t)}{\operatorname{NO}_{2}^{-}(t+\Delta t)} - \frac{f_{\operatorname{NO}_{2}^{-}cons}}{\operatorname{NO}_{2}^{-}(t+\Delta t)} \cdot \begin{pmatrix} \delta \operatorname{NO}_{2}^{-}(t) \cdot (1+r_{ex} \cdot x) - (1-x) \cdot \varepsilon_{\operatorname{NO}_{2}^{-} \to \operatorname{NO}_{2}^{-} \to \operatorname{NO}_{3}^{-}} \\ -r_{ex} \cdot x \cdot (\varepsilon_{\operatorname{NO}_{2}^{-} \to \operatorname{NO}_{3}^{-}} + \delta \operatorname{NO}_{3}^{-}(t)) \end{pmatrix}.$$
[S21]

The nitrate mass balance becomes

 $NO_{3}^{-}(t + \Delta t) = NO_{3}^{-}(t) + x \cdot f_{NO_{2}^{-} - consumption}.$  [S18]

The mass balance for nitrogen in  $N_{\rm 2}$  that was derived from nitrite becomes

$$N_2(t + \Delta t) = N_2(t) + (1 - x) \cdot f_{NO_2^- \_consumption}.$$
 [S19]

Analogously, the nitrate isotope mass balance becomes

$$\delta \operatorname{NO}_{3}^{-}(t + \Delta t) \cdot \operatorname{NO}_{3}^{-}(t + \Delta t) = \delta \operatorname{NO}_{3}^{-}(t) \cdot \operatorname{NO}_{3}^{-}(t) + f_{\operatorname{NO}_{2}^{-}-cons} \cdot x \cdot \left(\delta \operatorname{NO}_{2}^{-}(t) - \varepsilon_{\operatorname{NO}_{2}^{-} \to \operatorname{NO}_{3}^{-}}\right) + f_{\operatorname{NO}_{2}^{-}-cons} \cdot r_{ex} \cdot x \cdot \left(\delta \operatorname{NO}_{2}^{-}(t) - \varepsilon_{\operatorname{NO}_{2}^{-} \to \operatorname{NO}_{3}^{-}}\right) - f_{\operatorname{NO}_{2}^{-}-cons} \cdot r_{ex} \cdot x \cdot \delta \operatorname{NO}_{3}^{-}(t).$$
[S22]

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

$$\delta \mathbf{N}_{2}(t + \Delta t) \cdot \mathbf{N}_{2}(t + \Delta t) = \delta \mathbf{N}_{2}(t) \cdot \mathbf{N}_{2}(t) + f_{\mathbf{NO}_{2}^{-}cons} \cdot (1 - x)$$
$$\cdot \left(\delta \mathbf{NO}_{2}^{-}(t) - \varepsilon_{\mathbf{NO}_{2}^{-} \rightarrow \mathbf{N}_{2}}\right).$$
[S23]

Using this iterative modeling approach, we obtained good fits for modeled  $\delta^{15}N$  trends to experimental data with x = 0.24,  $\epsilon_{NO2^- \rightarrow NO3^-} = -30\%o$ , and  $\epsilon_{NO2^- \rightarrow NO3^-} = -60\%o$ . For C2 and C3,  $\epsilon_{NO2^- \rightarrow N_2}$  was kept constant at +15%o, whereas for C1  $\epsilon_{NO2^- \rightarrow N_2}$ was gradually changed from +15%o to -10%o (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 C<sub>2</sub> a (Fig. S5F) to a fairly long duration of exchange, however, with strongly reduced exchange rates, for C<sub>3</sub> 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 C<sub>3</sub> c and C<sub>3</sub> 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<sub>2</sub>, associated with a kinetic isotope fractionation ( $\varepsilon_{NO_2^- \rightarrow N_2}$ ) and a nitrogen flux from nitrite to nitrate, associated with another kinetic isotope fractionation ( $\varepsilon_{NO_2^- \rightarrow N_2}$ ):

$$\frac{d}{dt} \left( \mathrm{NO}_{2}^{-}(t) \cdot \delta \mathrm{NO}_{2}^{-}(t) \right) = (1 - x) \cdot \frac{d}{dt} \mathrm{NO}_{2}^{-}(t) \cdot \left( \delta \mathrm{NO}_{2}^{-}(t) - \varepsilon_{\mathrm{NO}_{2}^{-} \rightarrow \mathrm{N}_{2}} \right)$$
$$+ x \cdot \frac{d}{dt} \mathrm{NO}_{2}^{-}(t) \cdot \left( \delta \mathrm{NO}_{2}^{-}(t) - \varepsilon_{\mathrm{NO}_{2}^{-} \rightarrow \mathrm{NO}_{3}^{-}} \right).$$
[S24]

Similarly, the nitrate isotope mass balance is written as

$$\frac{d}{dt} (\mathrm{NO}_3^{-}(t) \cdot \delta \mathrm{NO}_3^{-}(t)) = -x \cdot \frac{d}{dt} \mathrm{NO}_2^{-}(t) \cdot (\delta \mathrm{NO}_2^{-}(t) - \varepsilon_{\mathrm{NO}_2^{-} \to \mathrm{NO}_3^{-}}).$$
[S25]

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

$$\frac{d}{dt}(\mathrm{NO}_{2}^{-}(t)\cdot\delta\mathrm{NO}_{2}^{-}(t)) = \frac{d}{dt}\mathrm{NO}_{2}^{-}(t)\cdot\delta\mathrm{NO}_{2}^{-}(t) + \mathrm{NO}_{2}^{-}(t)\cdot\frac{d}{dt}\delta\mathrm{NO}_{2}^{-}(t)$$

$$= \left((1-x)\cdot\frac{d}{dt}\mathrm{NO}_{2}^{-}(t) + x\cdot\frac{d}{dt}\mathrm{NO}_{2}^{-}(t)\right)$$

$$\cdot\delta\mathrm{NO}_{2}^{-}(t) + \mathrm{NO}_{2}^{-}(t)\cdot\frac{d}{dt}\delta\mathrm{NO}_{2}^{-}(t)$$

$$= (1-x)\cdot\frac{d}{dt}\mathrm{NO}_{2}^{-}(t)\cdot(\delta\mathrm{NO}_{2}^{-}(t) - \varepsilon_{\mathrm{NO}_{2}^{-}\to\mathrm{NO}_{2}})$$

$$+ x\cdot\frac{d}{dt}\mathrm{NO}_{2}^{-}(t)\cdot(\delta\mathrm{NO}_{2}^{-}(t) - \varepsilon_{\mathrm{NO}_{2}^{-}\to\mathrm{NO}_{3}^{-}}),$$
[S26]

which can be simplified to

$$NO_{2}^{-}(t) \cdot \frac{d}{dt} \delta NO_{2}^{-}(t) = (1-x) \cdot \frac{d}{dt} NO_{2}^{-}(t) \cdot -\varepsilon_{NO_{2}^{-} \to N_{2}} + x \cdot \frac{d}{dt} NO_{2}^{-}(t) \cdot -\varepsilon_{NO_{2}^{-} \to NO_{3}^{-}}.$$
 [S27]

Eq. S27 is rearranged:

$$\frac{d}{dt}\delta \mathrm{NO}_{2}^{-}(t) = -\left((1-x)\cdot\varepsilon_{\mathrm{NO}_{2}^{-}\to\mathrm{N}_{2}} + x\cdot\varepsilon_{\mathrm{NO}_{2}^{-}\to\mathrm{NO}_{3}^{-}}\right)\cdot\frac{\frac{d}{dt}\mathrm{NO}_{2}^{-}(t)}{\mathrm{NO}_{2}^{-}(t)}.$$
[S28]

Integration yields

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

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

$$f_{\text{NO}_2^-}(t) = \frac{\text{NO}_2^-(t)}{\text{NO}_2^-(t_0)},$$
 [S30]

and defining

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

results in

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

Under the assumption that the initial amount of nitrate and N<sub>2</sub> is very small (NO<sub>3</sub><sup>-</sup>(t) ~ N<sub>2</sub>(t) ~ 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<sub>2</sub> (N<sub>2</sub>(t)) and the isotope composition of N<sub>2</sub> ( $\delta$ N<sub>2</sub>(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

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

and

$$N_2(t) = (1-x) \cdot (NO_2^{-}(t_0) - NO_2^{-}(t)).$$
 [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

$$NO_{3}^{-}(t) = x \cdot \left( NO_{2}^{-}(t_{0}) - f_{NO_{2}^{-}}(t) \cdot NO_{2}^{-}(t_{0}) \right)$$
$$= x \cdot NO_{2}^{-}(t_{0}) \cdot \left( 1 - f_{NO_{2}^{-}}(t) \right)$$
[S36]

and

$$N_{2}(t) = (1 - x) \cdot \left( NO_{2}^{-}(t_{0}) - f_{NO_{2}^{-}}(t) \cdot NO_{2}^{-}(t_{0}) \right)$$
$$= (1 - x) \cdot NO_{2}^{-}(t_{0}) \cdot \left( 1 - f_{NO_{2}^{-}}(t) \right).$$
[837]

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

$$NO_{2}^{-}(t_{0}) \cdot \delta NO_{2}^{-}(t_{0}) - NO_{2}^{-}(t) \cdot \delta NO_{2}^{-}(t)$$
  
= NO<sub>3</sub><sup>-</sup>(t) \cdot \delta NO<sub>3</sub><sup>-</sup>(t) + N<sub>2</sub>(t) \cdot \delta N<sub>2</sub>(t). [S38]

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

$$NO_{2}^{-}(t_{0}) \cdot \delta NO_{2}^{-}(t_{0}) - NO_{2}^{-}(t) \cdot \delta NO_{2}^{-}(t) = x \cdot NO_{2}^{-}(t_{0})$$
  
 
$$\cdot (1 - f_{NO_{2}^{-}}(t)) \cdot \delta NO_{3}^{-}(t) + (1 - x) \cdot NO_{2}^{-}(t_{0})$$
 [S39]  
 
$$\cdot (1 - f_{NO_{2}^{-}}(t)) \cdot \delta N_{2}(t).$$

This equation is further simplified by dividing with  $NO_2^{-}(t_0)$ 

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

Substitution of  $\delta NO_2^{-}(t)$  with Eq. **S32** results in

$$\delta NO_{2}^{-}(t_{0}) - f_{NO_{2}^{-}}(t) \cdot \left(\delta NO_{2}^{-}(t_{0}) - E \cdot \ln f_{NO_{2}^{-}}(t)\right)$$
  
=  $x \cdot \left(1 - f_{NO_{2}^{-}}(t)\right) \cdot \delta NO_{3}^{-}(t) + (1 - x) \cdot \left(1 - f_{NO_{2}^{-}}(t)\right) \cdot \delta N_{2}(t).$   
[S41]

Eq. S41 can be simplified to

$$\delta \text{NO}_{2}^{-}(t_{0}) \cdot \left(1 - f_{\text{NO}_{2}^{-}}(t)\right) + f_{\text{NO}_{2}^{-}}(t) \cdot E \cdot \ln f_{\text{NO}_{2}^{-}}(t)$$
  
=  $x \cdot \left(1 - f_{\text{NO}_{2}^{-}}(t)\right) \cdot \delta \text{NO}_{3}^{-}(t) + (1 - x) \cdot \left(1 - f_{\text{NO}_{2}^{-}}(t)\right) \cdot \delta \text{N}_{2}(t)$   
[S42]

and finally to

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$$\delta NO_2^{-}(t_0) + \frac{f_{NO_2^{-}}(t) \cdot \ln f_{NO_2^{-}}(t)}{1 - f_{NO_2^{-}}(t)} \cdot E = x \cdot \delta NO_3^{-}(t) + (1 - x) \cdot \delta N_2(t).$$
[S43]

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

$$\frac{d}{dt} \operatorname{NO}_{3}^{-}(t) \cdot \delta \operatorname{NO}_{3}^{-}(t) + \operatorname{NO}_{3}^{-}(t) \cdot \frac{d}{dt} \delta \operatorname{NO}_{3}^{-}(t)$$
$$= -x \cdot \frac{d}{dt} \operatorname{NO}_{2}^{-}(t) \cdot \left( \delta \operatorname{NO}_{2}^{-}(t) - \varepsilon_{\operatorname{NO}_{2}^{-} \to \operatorname{NO}_{3}^{-}} \right), \qquad [S44]$$

$$\frac{d}{dt}\delta NO_{3}^{-}(t)_{ansatz} = \frac{d}{dt} NO_{2}^{-}(t) \cdot \left( \left( \ln \frac{NO_{2}^{-}(t)}{NO_{2}^{-}(t_{0})} + 1 \right) \cdot \left( NO_{2}^{-}(t_{0}) - NO_{2}^{-}(t) \right)^{-1} + \left( \left( NO_{2}^{-}(t) \cdot \ln \frac{NO_{2}^{-}(t)}{NO_{2}^{-}(t_{0})} \right) \cdot \left( NO_{2}^{-}(t_{0}) - NO_{2}^{-}(t) \right)^{-2} \right) \cdot E$$
[S46]

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

$$-x \cdot \frac{d}{dt} \operatorname{NO}_{2}^{-}(t) \cdot \delta \operatorname{NO}_{3}^{-}(t) + x \cdot \left(\operatorname{NO}_{2}^{-}(t_{0}) - \operatorname{NO}_{2}^{-}(t)\right) \cdot \frac{d}{dt} \delta \operatorname{NO}_{3}^{-}(t)$$
$$= -x \cdot \frac{d}{dt} \operatorname{NO}_{2}^{-}(t) \cdot \left(\delta \operatorname{NO}_{2}^{-}(t) - \varepsilon_{\operatorname{NO}_{2}^{-} \to \operatorname{NO}_{3}^{-}}\right).$$
[S47]

Eq. S47 can be divided by -x,

$$\frac{d}{dt} \operatorname{NO}_{2}^{-}(t) \cdot \delta \operatorname{NO}_{3}^{-}(t) - \left(\operatorname{NO}_{2}^{-}(t_{0}) - \operatorname{NO}_{2}^{-}(t)\right) \cdot \frac{d}{dt} \delta \operatorname{NO}_{3}^{-}(t)$$

$$= \frac{d}{dt} \operatorname{NO}_{2}^{-}(t) \cdot \left(\delta \operatorname{NO}_{2}^{-}(t) - \varepsilon_{\operatorname{NO}_{2}^{-} \to \operatorname{NO}_{3}^{-}}\right),$$
[S48]

and be rearranged as

$$-\left(\mathrm{NO}_{2}^{-}(t_{0}) - \mathrm{NO}_{2}^{-}(t)\right) \cdot \frac{d}{dt} \delta \mathrm{NO}_{3}^{-}(t)$$
$$= \frac{d}{dt} \mathrm{NO}_{2}^{-}(t) \cdot \left(\delta \mathrm{NO}_{2}^{-}(t) - \delta \mathrm{NO}_{3}^{-}(t) - \varepsilon_{\mathrm{NO}_{2}^{-} \to \mathrm{NO}_{3}^{-}}\right).$$
[S49]

In Eq. **S49**, the derivative after the time of  $\delta_{NO_3^-}$  is substituted with the derivative after the time of the ansatz for  $\delta_{NO_3^-}$  (Eq. **S46**), the function for  $\delta_{NO_2^-}$  is substituted with Eq. **S32**, and the function of  $\delta_{NO_3^-}$  is substituted with the ansatz for  $\delta_{NO_3^-}$ , (Eq. **S55**):

$$-\left(\mathrm{NO}_{2}^{-}(t_{0})-\mathrm{NO}_{2}^{-}(t)\right)\cdot\frac{d}{dt}\mathrm{NO}_{2}^{-}(t)\cdot\left(\left(\mathrm{ln}\frac{\mathrm{NO}_{2}^{-}(t)}{\mathrm{NO}_{2}^{-}(t_{0})}+1\right)\cdot\left(\mathrm{NO}_{2}^{-}(t_{0})-\mathrm{NO}_{2}^{-}(t)\right)^{-1}+\right)\cdot E\left(\mathrm{NO}_{2}^{-}(t)\cdot\mathrm{ln}\frac{\mathrm{NO}_{2}^{-}(t)}{\mathrm{NO}_{2}^{-}(t_{0})}\right)\cdot\left(\mathrm{NO}_{2}^{-}(t_{0})-\mathrm{NO}_{2}^{-}(t)\right)^{-2}\right)\cdot E\left(\mathrm{NO}_{2}^{-}(t)\cdot\mathrm{ln}\frac{\mathrm{NO}_{2}^{-}(t_{0})}{\mathrm{NO}_{2}^{-}(t_{0})}\right)\cdot\left(\mathrm{NO}_{2}^{-}(t_{0})-\mathrm{NO}_{2}^{-}(t)\right)^{-2}\right)\cdot E\left(\mathrm{S50}\right)$$
$$=\frac{d}{dt}\mathrm{NO}_{2}^{-}(t)\cdot\left(\left(\delta\mathrm{NO}_{2}^{-}(t_{0})-E\cdot\mathrm{ln}f_{\mathrm{NO}_{2}^{-}}(t)\right)-\left(\delta\mathrm{NO}_{2}^{-}(t_{0})+\left(\frac{f_{\mathrm{NO}_{2}^{-}}(t)\cdot\mathrm{ln}f_{\mathrm{NO}_{2}^{-}}(t)}{1-f_{\mathrm{NO}_{2}^{-}}(t)}+k_{1}\right)\cdot E+k_{2}\right)-\varepsilon_{\mathrm{NO}_{2}^{-}\to\mathrm{NO}_{3}^{-}}\right).$$

which is in the form of

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

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

With Eq. S30, Eq. S50 simplifies to

$$k_1 \cdot E + k_2 = +1 \cdot E - \varepsilon_{NO_2^- \to NO_3^-}.$$
 [S51]

From Eq. S51, we can determine the values for the constants used in the ansatz for the function for  $\delta_{NO_3^-}$  (Eq. S45):

$$k_1 = 1 \text{ and } k_2 = -\varepsilon_{NO_2^- \to NO_3^-}.$$
 [S52]

Thus, Eq. S45 becomes

$$\delta \text{NO}_{3}^{-}(t) = \delta \text{NO}_{2}^{-}(t_{0}) + \left(\frac{f_{\text{NO}_{2}^{-}}(t) \cdot \ln f_{\text{NO}_{2}^{-}}(t)}{1 - f_{\text{NO}_{2}^{-}}(t)} + 1\right) \cdot E - \varepsilon_{\text{NO}_{2}^{-} \to \text{NO}_{3}^{-}}.$$
[S53]

Eqs. **S32** and **S53** can be used to graphically determine *E* and  $\varepsilon_{NO_2} \rightarrow NO_3^-$ :

$$\delta NO_2^{-}(t) - \delta NO_2^{-}(t_0) = E \cdot - \ln f_{NO_2^{-}}(t)$$
 [S54]

$$\delta NO_{3}^{-}(t) - \delta NO_{2}^{-}(t_{0}) = \left(\frac{f_{NO_{2}^{-}}(t) \cdot \ln f_{NO_{2}^{-}}(t)}{1 - f_{NO_{2}^{-}}(t)} + 1\right) \cdot E - \varepsilon_{NO_{2}^{-} \to NO_{3}^{-}}.$$
[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  $-\varepsilon_{NO_2^- \to NO_3^-}$ . To determine  $\varepsilon_{NO_2^- \to N_2}$  Eq. **S31** has to be rearranged:

$$E = ((1-x) \cdot \varepsilon_{\mathrm{NO}_{2}^{-} \to \mathrm{N}_{2}} + x \cdot \varepsilon_{\mathrm{NO}_{2}^{-} \to \mathrm{NO}_{3}^{-}}) \Rightarrow \varepsilon_{\mathrm{NO}_{2}^{-} \to \mathrm{N}_{2}} = \frac{E - x \cdot \varepsilon_{\mathrm{NO}_{2}^{-} \to \mathrm{NO}_{3}^{-}}}{1-x}$$
[S56]

This demonstrates that, to determine a value for  $\varepsilon_{NO_2^- \to N_2}$ , the relative proportion of the nitrogen flux from nitrite to nitrate to the nitrogen flux from nitrite to N<sub>2</sub>, i.e., the parameter *x*, must be known.

Using this analytical approach and an estimate for x (0.24  $\pm$  0.04), we obtained estimates  $\varepsilon_{NO_2^- \to NO_3^-}$  and  $\varepsilon_{NO_2^- \to N_2}$  (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<sub>2</sub><sup>-</sup> (beginning of the experiment) and the concentration of NO<sub>2</sub><sup>-</sup> (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  $\varepsilon_{NO_2^- \to NO_3^-}$  and  $\varepsilon_{NO_2^- \to N_2}$ , and the SD for the averaged values serves as an error estimate ( $\varepsilon_{NO_2^- \to NO_3^-} = -31.1 \pm 3.9\%$ , Table S3). In the case of  $\varepsilon_{NO_2^- \to NO_3^-}$  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.,  $\varepsilon_{NO_2^- \to N_2} = 16.0 \pm 4.5\%$  (Table S3).

Equilibrium N Isotope Fractionation Between Nitrite and Nitrate. For an estimate for the equilibrium isotope fractionation we took the maximum offset between  $\delta^{15}N_{NO_3^-}$  and  $\delta^{15}N_{NO_2^-}$  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  $\delta^{15}N_{NO_3^-}$  and  $\delta^{15}N_{NO_2^-}$  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  $\delta^{15}N_{NO_2^-}$  and an overestimate of  $\delta^{15}N_{NO_3^-}$ . Thus, we attribute a value of  $-60.5 \pm 1.0\%$  to  $\epsilon_{NO_3^-}$ .

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 <sup>15</sup>N isotope exchange, whereas the chemical control shows that there is no abiotic <sup>15</sup>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<sub>2</sub> 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  $\varepsilon_{NO2^- \rightarrow N2}$  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. S1.** Model for N fluxes and isotope fractionation during anammox. Anammox converts ammonium and nitrite to nitrate and dinitrogen according to the approximate stoichiometry,  $1.3 \text{ NO}_2^- + 1 \text{ NH}_4^+ \rightarrow 1 \text{ N}_2 + 0.3 \text{ NO}_3^- + 2 \text{ H}_2\text{O}$ . The depicted N pools, fluxes (*f*), isotope effects ( $\varepsilon$ ), and parameters  $r_{ex}$  (for N exchange between nitrate and nitrite) and *x* (for anammox stoichiometry) have been used for numerical models and for isotope fractionation calculations. The overall N isotope effect for nitrite consumption is designated to the parameter *E*, which is composed of the isotope effects for nitrite conversion to dinitrogen and to nitrate (i.e.,  $E = (1 - x) \cdot \varepsilon_{NO_2^- \rightarrow NO_2^-} + x \cdot \varepsilon_{NO_2^- \rightarrow NO_3^-}$ ). According to the approximate anammox stoichiometry,  $x \sim 0.23$  (measured  $x \sim 0.24 \pm 0.04$ ; Table S1). Note that the pools N<sub>2</sub>, <sup>14</sup>N<sub>2</sub>, and <sup>15</sup>N<sub>2</sub> and the isotope composition  $\delta N_2$  refer to nitrogen derived from the nitrite pool and do not refer to nitrogen derived from the ammonium pool (shaded area).



**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.



**Fig. S3.** Ammonium N isotope fractionation. The ammonium isotope fractionation by anammox follows a typical closed-system Rayleigh trend. (*A*) determination of  $\epsilon^{15}N_{NH_4^+ \to N_2}$  from the combined datasets C<sub>1</sub>\_a and C<sub>1</sub>\_b. (*B*) Match between model isotope trend and data from C<sub>1</sub>\_b. (*C*) Determination of  $\epsilon^{15}N_{NH_4^+ \to N_2}$  from experiment C<sub>3</sub>\_b. (*D*) Match between model isotope trend and data from C<sub>3</sub>\_b. (*D*) Match between model isotope trend and data from C<sub>3</sub>\_b. The *x*-axis label f<sub>NH4^+</sub> refers to the fraction of remaining NH<sub>4</sub><sup>+</sup>.



**Fig. 54.** <sup>15</sup>N labeling experiments. (A) Experiment  $C_3\_d$ : ~1.5 mM <sup>15</sup>N-NO<sub>2</sub><sup>-</sup> was directly added to a culture (containing ~0.3 mM NO<sub>2</sub><sup>-</sup> and ~10 mM NO<sub>3</sub><sup>-</sup> with natural abundance isotope composition). The strong initial increase in <sup>15</sup>N-NO<sub>3</sub><sup>-</sup> and <sup>14</sup>N-NO<sub>2</sub><sup>-</sup> and the strong initial decrease in <sup>15</sup>N-NO<sub>2</sub><sup>-</sup> are evidence for rapid isotope exchange between NO<sub>2</sub><sup>-</sup> and NO<sub>3</sub><sup>-</sup>. (*B* and *C*) Experiment  $C_3\_c$ : ~1.5 mM <sup>15</sup>N-NO<sub>3</sub><sup>-</sup> was directly added to a culture (containing ~1.5 mM NO<sub>2</sub><sup>-</sup> and ~10 mM NO<sub>3</sub><sup>-</sup> with natural abundance isotope composition). The rapid initial increase in <sup>15</sup>N-NO<sub>2</sub><sup>-</sup> followed by gradual increase in <sup>29</sup>N<sub>2</sub>, is consistent with an initial rapid isotope exchange between NO<sub>2</sub><sup>-</sup> and NO<sub>3</sub><sup>-</sup> (*B*, note different concentration scale). The modeled rapid change in <sup>15</sup>N-NO<sub>3</sub><sup>-</sup> at an initial stage of the experiment (shaded area in *C*) is not captured by actual measurements. There is a good match between modeled concentration trends (x = 0.24) and data for a scenario where the initial N isotope exchange flux and NO<sub>2</sub><sup>-</sup> consumption of 6–8). The *x*-axis label  $f_{NO_2}^{-}$  refers to the fraction of remaining NO<sub>2</sub><sup>-</sup>. Symbols represent data, and lines are modeled trends.



**Fig. S5.** Concentration and  $\delta^{15}$ N trends. N isotope exchange between nitrite and nitrate appears to occur throughout C1 (*B* and *D*), whereas the variable  ${}^{15}$ N enrichment in the nitrate pool at an initial stage of C2 and C3 (*F*, *H*, *J*, and *L*) indicates a variable degree of N isotope exchange between nitrite and nitrate at an initial stage (shaded areas). Potentially, isotope exchange decreases gradually over time (modeled and illustrated as a series of exchange modes that decrease in intensity for C<sub>3</sub> a in *J*). Modeling allows estimation of exchange fluxes (Table S2) and of isotope fractionations. (*A* and *B*) C<sub>1</sub> a; (*C* and *D*) C<sub>1</sub> b; (*E* and *F*) C<sub>2</sub> a; (*G* and *H*) C<sub>2</sub> b; (*I* and *J*) C<sub>3</sub> b. The *x*-axis label f<sub>NO2</sub><sup>-</sup> refers to the fraction of remaining NO<sub>2</sub><sup>-</sup>. Symbols represent data, lines are modeled trends, and concentration and isotope composition of N<sub>2</sub> was calculated from NO<sub>x</sub>.



**Fig. S6.** NO<sub>x</sub> isotope effects. Shown is graphical determination of  $\varepsilon_{NO_2^- \to N_2}$  and  $\varepsilon_{NO_2^- \to NO_3^-}$  from experimental data, according to Rayleigh-type relationships between  $\delta^{15}N_{NO_2^-}$  and  $f_{NO_2^-}$  (*A*, *C*, *E*, and *G*) and between  $\delta^{15}N_{NO_3^-}$  and  $f_{NO_2^-}$  (*B*, *D*, *F*, and *H*), where  $f_{NO_2^-}$  refers to the fraction of remaining NO<sub>2</sub><sup>-</sup> (*SI Text*). (*A* and *B*) C<sub>2</sub>\_a; (*C* and *D*) C<sub>2</sub>\_b; (*E* and *F*) C<sub>3</sub>\_a; (*G* and *H*) C<sub>3</sub>\_b.

### Table S1. Results from C1–C3: Anammox stoichiometry

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Campaign	Experiment	x from $NH_4^+$ vs. $NO_2^-$	Estimated error, SE	x from $NO_3^-$ vs. $NO_2^-$	Estimated error, SE	Average x
C1	C <sub>1</sub> _a	0.24	0.02	0.17	0.01	0.20
C1	C <sub>1</sub> _b	0.33	0.02	0.15	0.01	0.24
C2	C <sub>2</sub> _a	0.39	0.04	0.21	0.02	0.30
C2	C <sub>2</sub> _b	0.48	0.02	0.21	0.01	Not used
C3	C <sub>3</sub> _a	0.3	0.15	0.18	0	0.24
C3	C₃_b	0.27	0.04	0.19	0	0.23
						Average: 0.24 $\pm$ 0.04, 1 SD

Table S2. Results from C1–C3: Modeled isotope exchange between  $NO_2^-$  and  $NO_3^-$ 

Campaign	Experiment	Ratio between exchange flux and net $NO_3^-$ production	Ratio between exchange flux and $NO_2^-$ consumption	From $f_{NO_2}$ -	To f <sub>NO₂</sub> -
C1	C <sub>1_</sub> a	20	4.80	1	0
C1	C <sub>1</sub> _b	15	3.60	1	0
C2	C <sub>2</sub> _a	No exchange	No exchange	_	_
C2	C2_b	0.17	0.04	1	0.993
C3	C <sub>3</sub> _a	3.00	0.72	1	0.993
		0.67	0.16	0.993	0.962
		0.33	0.08	0.962	0.844
		0.03	0.01	0.844	0.800
C3	C <sub>3</sub> _b	2.67	0.64	1	0.995
C3	C <sub>3</sub> _c	35	8.40	1	0.951
C3	C <sub>3</sub> _d	25	6.00	1	0.943

## Table S3. Results from C1–C3: Estimates for $\epsilon^{15}N_{NO_2^- \rightarrow NO_3^-}$ and $\epsilon^{15}N_{NO_2^- \rightarrow N_2}$

Campaign	Experiment	ε <sup>15</sup> N <sub>NO2</sub> -→ <sub>NO3</sub> - from intercept (Fig. S6), ‰	Estimated error (SE), ‰	$\epsilon^{15}N_{NO_2^- \rightarrow N_2}$ from $\delta^{15}N_{NO_2^-}$ (Fig. S6), ‰	Estimated error (SE), ‰	$\epsilon^{15}N_{NO_2^-\rightarrow N_2}$ from $\delta^{15}N_{NO_3^-}$ (Fig. S6), ‰	Estimated error (SE), ‰
C2	C <sub>2</sub> _a	-26.7	1.1	15.6	1.7	18.6	2.4
C2	C2_b	-35.0	4.2	18.9	2.5	12.2	7.9
C3	C <sub>3</sub> _a	-33.7	0.5	18.5	2.1	14.5	2.2
C3	C <sub>3</sub> _b	-28.8	1.1	16.6	2.2	13.4	2.8
Average: –31.1 ± 3.9,		1 ± 3.9, 1 SD	Average: 17.4	4 ± 1.5, 1 SD	Average: 14.7 $\pm$	2.8, 1 SD	
$x=0.24\pm0.2$	.04	-		-		-	
Estimate $\epsilon^{15} N_{NO_2^- \to NO_3^-} = -31.1 \pm 3.9\%$		- 31.1 ± 3.9‰	Average: 16.0, Range 11.9–18.9 $\epsilon^{15}N_{NO_2} \rightarrow N_2 = 16.0 \pm 4.5\%$				

Active exchange

### Table S4. Isotope exchange between $NO_3^-$ and $NO_2^-$ dependent on sampling procedures

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Identifier	% <sup>15</sup> N exchange (no. replicates)	Description*				
C <sub>4</sub> _a: chemical control	0 (7)	<sup>15</sup> N nitrate label added to medium in ice bath.				
C4_b: intact cells, HgCl killed	5 (5)	Sample containing anammox biomass and medium added to medium that contains <sup>15</sup> N nitrate label and 100 μL saturated (7%) HgCl <sub>2</sub> solution at room temperature and left standing for 1 h, after which sample was frozen.				
C <sub>4</sub> _c: intact cells	26 (5)	Sample containing anammox biomass and medium added to medium that contains <sup>15</sup> N nitrate label at room temperature and left standing for 1 h, after which sample was frozen.				
C <sub>4</sub> _d: filtered, cold	16 (6)	Sample containing anammox biomass and medium filtered through syringe filter (0.2 μm) into ice-bath cooled medium that contains <sup>15</sup> N nitrate label. Sample was frozen immediately after filtering.				
C4_e: filtered, room temperature	28 (6)	Sample containing anammox biomass and medium filtered through syringe filter (0.2 $\mu$ m) into medium that contains <sup>15</sup> N nitrate label at room temperature and left standing for 1 h, after which sample was frozen.				
C4_f: French press, cold	34 (5)	Sample containing anammox biomass and medium treated with French press and filtered (0.2 μm) into ice-bath cooled medium that contains <sup>15</sup> N nitrate label. Sample was frozen immediately after filtering.				
C <sub>4</sub> _g: French press, room temperature	44 (5)	Sample containing anammox biomass and medium treated with French press and filtered through syringe filter (0.2 $\mu$ m) into medium that contains <sup>15</sup> N nitrate label at room temperature and left standing for 1 h, after which sample was frozen.				

\*Experimental conditions, chemical control: Medium contained  $\sim$ 1 mM nitrite, with label addition of  $\sim$ 1 mM <sup>15</sup>N nitrate. All other experiments: Medium contained  $\sim$ 2.8 mM nitrate,  $\sim$ 1 mM nitrite, with label addition of  $\sim$ 1 mM <sup>15</sup>N nitrate.