Calculations for the Expanded Rayleigh model

To apply the Expanded Rayleigh model, additional values ($^{15}N^{bulk}$ and τ) must be calculated from experimental values. If necessary, δ values ($\delta^{15}N^{bulk}$, $\delta^{15}N^\alpha$, and $\delta^{15}N^\beta$) must be converted to the corresponding R values (Eq. (6)). $^{14}N^{bulk}$ and $^{15}N^{bulk}$ can then be calculated using N^{bulk} and R_{bulk} ($N^{bulk} = 2*[mol\ N_2O]$).

$$^{14}N^{bulk} = \frac{N^{bulk}}{1+R_{bulk}} \tag{S1}$$

$$^{15}N^{bulk} = R_{bulk} * {}^{14}N^{bulk} \tag{S2}$$

Equations S1 and S2 are derived from the definition of R ($R = {}^{15}N/{}^{14}N$, Eq. (5)) and the fact that $^{15}N^{bulk} + {}^{14}N^{bulk} = N^{bulk}$. Similar equations can be used to calculate $^{14}N^\alpha$ and $^{15}N^\alpha$:

$$^{14}N^\alpha = \frac{N^\alpha}{1+R_\alpha} = \frac{0.5*N^{bulk}}{1+R_\alpha} \tag{S3}$$

$$^{15}N^\alpha = R_\alpha * {}^{14}N^\alpha \tag{S4}$$

The values of $^{14}N^\alpha$ and $^{14}N^{bulk}$ can then be used to calculate τ for every step of the reaction ($\tau = {}^{14}N^\alpha/{}^{14}N^{bulk}$, Eq. (24)).

Standard error calculations for the Expanded Rayleigh model

To calculate the standard error for KIE $^{15}N^\alpha$ or KIE $^{15}N^\beta$, the standard errors for ρ, τ, and α_{N-bulk} need to be determined (see Table S15). The standard error for ρ (se_ρ) was extracted from nonlinear model 1 or nonlinear model 2 (Baty et al., 2015). For τ, the standard error of the mean (se_τ) was calculated in R using the std.error function (Lemon, 2006). The standard error for α_{N-bulk} ($se_{\alpha N-bulk}$) can be determined by propagating the standard error for ϵ_{N-bulk} ($se_{\epsilon N-bulk}$):

$$\alpha_{N-bulk} = \frac{\epsilon_{N-bulk}}{1000} + 1 \tag{S5}$$

$$se_{\alpha N-bulk} = \frac{d\alpha_{N-bulk}}{d\epsilon_{N-bulk}} * se_{\epsilon N-bulk} \tag{S6}$$

Equation (S6) simplifies to Eq. (S7),

$$se_{\alpha N-bulk} = \frac{se_{\epsilon N-bulk}}{1000} \tag{S7}$$

where $se_{\epsilon N-bulk}$ is the standard error of the slope of a linear regression plot of $\delta^{15}N^{bulk}$ against $[-flnf/(1-f)]$.

The standard error for $\alpha_{N-\alpha}$ is calculated by combining the standard errors for ρ, τ, and α_{N-bulk} (*e.g.*, error propagation for Eq. (21)):

$$se_{\alpha N-\alpha} = \sqrt{(\frac{\partial \alpha_{N-\alpha}}{\partial \rho} * se_\rho)^2 + (\frac{\partial \alpha_{N-\alpha}}{\partial \tau} * se_\tau)^2 + (\frac{\partial \alpha_{N-\alpha}}{\partial \alpha_{N-bulk}} * se_{\alpha N-bulk})^2} \tag{S8}$$

Replacing the partial derivatives in Eq. (S8) yields Eq. (S9)

$$se_{\alpha N-\alpha} = \sqrt{(\frac{\alpha_{N-bulk}}{\tau} * se_\rho)^2 + (-\frac{\rho*\alpha_{N-bulk}}{\tau^2} * se_\tau)^2 + (\frac{\rho}{\tau} * se_{\alpha N-bulk})^2} \tag{S9}$$

Similarly, the standard error for $\alpha_{N-\beta}$ is calculated by performing error propagation for Eq. (22):

$$se_{\alpha N-\beta} = \sqrt{(\frac{\partial \alpha_{N-\beta}}{\partial \rho} * se_\rho)^2 + (\frac{\partial \alpha_{N-\beta}}{\partial \tau} * se_\tau)^2 + (\frac{\partial \alpha_{N-\beta}}{\partial \alpha_{N-bulk}} * se_{\alpha N-bulk})^2} \tag{S10}$$

Replacing the partial derivatives in Eq. (S10) produces Eq. (S11):

$$se_{\alpha N-\beta} = \sqrt{(-\frac{\alpha_{N-bulk}}{1-\tau} * se_\rho)^2 + (\frac{(1-\rho)*\alpha_{N-bulk}}{(1-\tau)^2} * se_\tau)^2 + (\frac{1-\rho}{1-\tau} * se_{\alpha N-bulk})^2} \tag{S11}$$

The standard error values for $\alpha_{N-\alpha}$ and $\alpha_{N-\beta}$ can then be converted to the corresponding errors for ε (Eq. (S12)) or KIE (Eq. (S13)):

$$se_\varepsilon = \frac{d\varepsilon}{d\alpha} * se_\alpha = 1000 * se_\alpha \tag{S12}$$

$$se_{KIE} = \left|\frac{dKIE}{d\alpha} * se_\alpha\right| = \left|-\frac{1}{\alpha^2} * se_\alpha\right| \tag{S13}$$

Calculation of $\delta^{15}N^\alpha$, $\delta^{15}N^\beta$, and ρ when $\varepsilon_{N-\alpha}$ or $\varepsilon_{N-\beta}$ is equal to 0 (*e.g.*, Simulated Datasets 1 and 5)

For simulated Dataset 1, $\varepsilon_{N-\alpha}$ was set to 0 (no isotope effect at N^α); for simulated Dataset 5, $\varepsilon_{N-\beta}$ was set to 0 (no isotope effect at N^β). Here we show the additional calculations required to determine $\delta^{15}N^\alpha$, $\delta^{15}N^\beta$, and ρ for Datasets 1 and 5.

At or near natural abundance of ^{15}N, $^{14}N^\alpha \approx {}^{14}N^\beta$ and $\frac{1}{2}(\varepsilon_{N-\alpha} + \varepsilon_{N-\beta})$ is equal to ε_{N-bulk}. Therefore, when $\varepsilon_{N-\alpha} = 0$ and ε_{N-bulk} is set at -20‰, $\varepsilon_{N-\beta}$ must equal -40‰ (and vice versa). Conversion of these ε values to the corresponding values of α (Eq. (1)) yields values of 1.0000, 0.9600, and 0.9800 for $\alpha_{N-\alpha}$, $\alpha_{N-\beta}$, and α_{N-bulk}, respectively. Because the values of $\alpha_{N-\alpha}$ and $\alpha_{N-\beta}$ are known in this scenario, the values of ρ/τ and $(1-\rho)/(1-\tau)$ can be calculated using Eq. (S14) and Eq. (S15) (derived from Eq. (21-22)). For simplicity, ρ/τ is designated as *A*, and $(1-\rho)/(1-\tau)$ is designated as *B*:

$$A = \frac{\rho}{\tau} = \frac{\alpha_{N-\alpha}}{\alpha_{N-bulk}} \tag{S14}$$

$$B = \frac{1-\rho}{1-\tau} = \frac{\alpha_{N-\beta}}{\alpha_{N-bulk}} \tag{S15}$$

Additionally, as long as the average of $\alpha_{N-\alpha}$ and $\alpha_{N-\beta}$ is approximately equal to α_{N-bulk}, the sum of *A* and *B* must be equal to 2:

$$A + B = 2 \tag{S16}$$

For Dataset 1, *B* was calculated using Eq. (S15), and *A* was calculated using Eq. (S16). For Dataset 5, *A* was calculated using Eq. (S14), and *B* was calculated using Eq. (S16). R_α and R_β were then calculated using Eq. (S17) and Eq. (S18),

$$R_\alpha = A * R_{bulk} = \frac{\rho}{\tau} * R_{bulk} \tag{S17}$$

$$R_\beta = B * R_{bulk} = \frac{1-\rho}{1-\tau} * R_{bulk} \tag{S18}$$

and the R values were converted to the corresponding δ values (Eq. (6)).

To determine ρ for Datasets 1 and 5, ρ was calculated for f = 0.7-0.3 using Eq. (23) ($\rho = {}^{15}N^\alpha/{}^{15}N^{bulk}$). Values of $^{15}N^\alpha$ and $^{15}N^{bulk}$ were calculated using Eq. (S4) and Eq. (S2), respectively. For Dataset 1, the average value of $\rho \pm$ standard deviation was $0.5102 \pm 9 \times 10^{-8}$. For Dataset 5, the average value of $\rho \pm$ standard deviation was $0.4898 \pm 8 \times 10^{-8}$. Thus, the variation in calculated ρ values was minimal.

Estimation of experimental error for N^s

To simulate experimental error in simulated Datasets 1-5, randomly generated numbers were added to N^s, $\delta^{15}N^{bulk}$, and $\delta^{15}N^\alpha$. These simulated error values were randomly generated from a skew-normal distribution where the mean was set to 0 and the standard deviation was set to the estimated value of measurement error. For N_2O synthesis reactions, we assumed that values of N^s (*i.e.*, moles of substrate) are back-calculated from N_2O concentrations, where the main source of error is volume measurements. Measurement error estimates were calculated for a hypothetical experiment where 1 ± 0.01 mL of headspace from a reaction bottle with N_2O was transferred to a sample bottle, followed by removal of 1 ± 0.01 mL of headspace from the sample bottle for quantification. The experimental error for N^s (se_{Ns}) can be approximated by propagating the error for two gas volume measurements (Eq. (S19)):

$$se_{N^s} = N_s * \sqrt{\left(\frac{error_{v1}}{v_1}\right)^2 + \left(\frac{error_{v2}}{v_2}\right)^2} = 0.014 * N_s \tag{S19}$$

where v_1 and v_2 represent volume measurements 1 and 2, respectively. Thus, the error for N^s is estimated to be 1.4% of N^s. To err on the side of caution, we set the standard deviation of the distribution for our simulated error values to 1.5% of N^{s0}.

Calculation of f, $\delta^{15}N^\alpha$, and $\delta^{15}N^\beta$ values for previously published experimental data

To apply the Expanded Rayleigh model (and the standard Rayleigh model) to the isotopic data published by Sutka and colleagues (Sutka et al., 2006), values of f, $\delta^{15}N^\alpha$, and $\delta^{15}N^\beta$ had to be calculated (Table S14). To calculate f, N^{bulk} (in terms of µmol) was calculated using the reported N_2O concentrations and a headspace volume ($v_{headspace}$) of 0.0227 L (Sutka et al., 2006):

$$N^{bulk} = 2 * c_{N_2O} * v_{headspace} \tag{S20}$$

N^s (moles of unconsumed substrate) was calculated using Eq. (S21) ($N^{s0} = 3$ µmol),

$$N^s = N^{s0} - N^{bulk} \tag{S21},$$

and f was calculated using N^s and N^{s0}:

$$f = \frac{N^s}{N^{s0}} \tag{S22}.$$

The f values for *Methylosinus trichosporium* replicate B ranged from 0.8-0.4 (see Table S14).

By combining Eq. (15) and Eq. (32) to make Eq. (S23), values of $\delta^{15}N^\alpha$ can be calculated using the reported values of $\delta^{15}N^{bulk}$ and site preference (SP):

$$\delta^{15}N^\alpha = \delta^{15}N^{bulk} + \frac{SP}{2} \tag{S23}$$

Values of $\delta^{15}N^\beta$ can then be calculated using Eq. (24) (a rearrangement of Eq. (32)):

$$\delta^{15}N^\beta = \delta^{15}N^\alpha - SP \tag{S24}$$

Table S1. Absolute relative error of $\varepsilon_{N\text{-}bulk}$ and KIE values introduced by Mariotti's approximation of the Rayleigh equation at various $\delta^{15}N^{s0}$ values[a]

	Actual				Estimate				Absolute relative difference[b]			
$\delta^{15}N^{s0}$ (‰)	$\varepsilon_{N\text{-}bulk}$ (‰)	KIE $^{15}N^{bulk}$ [c]	KIE $^{15}N^{\alpha}$ [c]	KIE $^{15}N^{\beta}$ [c]	$\varepsilon_{N\text{-}bulk}$ [d] (‰)	KIE $^{15}N^{bulk}$ [e]	KIE $^{15}N^{\alpha}$ [e]	KIE $^{15}N^{\beta}$ [e]	$\varepsilon_{N\text{-}bulk}$	KIE $^{15}N^{bulk}$	KIE $^{15}N^{\alpha}$	KIE $^{15}N^{\beta}$
-100	-20	1.0204	1.0103	1.0307	-17.81	1.0181	1.0080	1.0285	0.11	0.0022	0.0022	0.0022
-90	-20	1.0204	1.0103	1.0307	-18.01	1.0183	1.0082	1.0287	0.10	0.0020	0.0020	0.0020
-80	-20	1.0204	1.0103	1.0307	-18.21	1.0185	1.0084	1.0289	0.09	0.0018	0.0018	0.0018
-70	-20	1.0204	1.0103	1.0307	-18.41	1.0188	1.0086	1.0291	0.08	0.0016	0.0016	0.0016
-60	-20	1.0204	1.0103	1.0307	-18.60	1.0190	1.0088	1.0293	0.07	0.0014	0.0014	0.0014
-50	-20	1.0204	1.0103	1.0307	-18.80	1.0192	1.0090	1.0295	0.06	0.0012	0.0012	0.0012
-40	-20	1.0204	1.0103	1.0308	-19.00	1.0194	1.0092	1.0297	0.05	0.0010	0.0010	0.0010
-30	-20	1.0204	1.0103	1.0308	-19.20	1.0196	1.0094	1.0299	0.04	0.0008	0.0008	0.0008
-20	-20	1.0204	1.0103	1.0308	-19.40	1.0198	1.0096	1.0301	0.03	0.0006	0.0006	0.0006
-10	-20	1.0204	1.0103	1.0308	-19.59	1.0200	1.0099	1.0303	0.02	0.0004	0.0004	0.0004
0	-20	1.0204	1.0103	1.0308	-19.79	1.0202	1.0101	1.0305	0.01	0.0002	0.0002	0.0002
10	-20	1.0204	1.0103	1.0308	-19.99	1.0204	1.0103	1.0307	5.0E-04	1.0E-05	1.0E-05	1.0E-05
20	-20	1.0204	1.0103	1.0308	-20.19	1.0206	1.0105	1.0310	0.01	0.0002	0.0002	0.0002
30	-20	1.0204	1.0103	1.0308	-20.39	1.0208	1.0107	1.0312	0.02	0.0004	0.0004	0.0004
40	-20	1.0204	1.0103	1.0308	-20.58	1.0210	1.0109	1.0314	0.03	0.0006	0.0006	0.0006
50	-20	1.0204	1.0103	1.0308	-20.78	1.0212	1.0111	1.0316	0.04	0.0008	0.0008	0.0008
60	-20	1.0204	1.0103	1.0308	-20.98	1.0214	1.0113	1.0318	0.05	0.0010	0.0010	0.0010
70	-20	1.0204	1.0103	1.0308	-21.18	1.0216	1.0115	1.0320	0.06	0.0012	0.0012	0.0012
80	-20	1.0204	1.0103	1.0308	-21.38	1.0218	1.0117	1.0322	0.07	0.0014	0.0014	0.0014
90	-20	1.0204	1.0103	1.0308	-21.57	1.0220	1.0119	1.0324	0.08	0.0016	0.0016	0.0016
100	-20	1.0204	1.0103	1.0308	-21.77	1.0223	1.0121	1.0326	0.09	0.0018	0.0018	0.0018

[a] Simulated $\delta^{15}N^s$, $\delta^{15}N^{bulk}$, $\delta^{15}N^\alpha$, and $\delta^{15}N^\beta$ values were calculated without Mariotti's approximation (Mariotti et al., 1981) that $\ln[(\delta^{15}N^s/1000 +1)/(\delta^{15}N^{s0}/1000 +1)] \approx \delta^{15}N^s - \delta^{15}N^{s0}$. (This approximation is only valid when $\delta^{15}N^s/1000$ and $\delta^{15}N^{s0}/1000$ are small relative to 1.) $\varepsilon_{N\text{-}bulk}$ was set at -20‰ and ρ was set at 0.5050 (normal KIE $^{15}N^\alpha$, normal KIE $^{15}N^\beta$). The average value of τ for each simulation was 0.49998.

[b] Absolute relative difference (Eq. (31)) is the absolute value of the difference between the estimated value and actual value divided by the actual value (|(estimate – actual)/actual|).

[c] Actual KIE values were calculated with Expanded Rayleigh model 1 using f values between 0.7-0.2. $\alpha_{N\text{-}bulk}$ was determined without the $\ln(1+u) = u$ approximation by linear regression of $\ln(\delta^{15}N^s/1000 +1)$ against $\ln(f)$; $\varepsilon_{N\text{-}bulk}$ is the slope in this model (Hayes, 2004). (The variable u represents $\delta^{15}N^s$ or $\delta^{15}N^{s0}$.) The value of ρ was determined via nonlinear regression (model 1) Eq. (29), and τ was determined by averaging $^{14}N^\alpha/^{14}N^{bulk}$ for every step of the reaction Eq. (24). The results for Expanded Rayleigh model 2 (standard Rayleigh model combined with nonlinear model 2, data not shown) are essentially identical to the results from Expanded Rayleigh model 1.

[d] Estimated $\varepsilon_{N\text{-}bulk}$ values were determined using Eq. (2), Mariotti's approximation of the Rayleigh equation (*i.e.*, via linear regression of $\delta^{15}N^{bulk}$ against [-flnf/(1-f)]) using f values between 0.7-0.2. $\varepsilon_{N\text{-}bulk}$ is the slope in this model.

[e] Estimated KIE values for Expanded Rayleigh model 1 were calculated using Mariotti's approximation of the standard Rayleigh equation to determine $\alpha_{N\text{-}bulk}$ (calculated from estimated $\varepsilon_{N\text{-}bulk}$, see note d and Eq. (2)). Values for ρ and τ were calculated as described in note b above; these values were used to determine KIE $^{15}N^\alpha$ and KIE $^{15}N^\beta$.

Table S2. Absolute relative error of $\varepsilon_{\text{N-bulk}}$ and KIE values introduced by varying $\varepsilon_{\text{N-bulk}}$ values[a]

$\delta^{15}N^{s0}$ (‰)	$\varepsilon_{\text{N-bulk}}$ (‰)	KIE $^{15}N^{bulk}$ [c]	KIE $^{15}N^{\alpha}$ [c]	KIE $^{15}N^{\beta}$ [c]	$\varepsilon_{\text{N-bulk}}$ [d] (‰)	KIE $^{15}N^{bulk}$ [e]	KIE $^{15}N^{\alpha}$ [e]	KIE $^{15}N^{\beta}$ [e]	$\varepsilon_{\text{N-bulk}}$	KIE $^{15}N^{bulk}$	KIE $^{15}N^{\alpha}$	KIE $^{15}N^{\beta}$
		Actual				**Estimate**				**Absolute relative difference[b]**		
0	-100	1.1111	1.1001	1.1224	-94.52	1.1044	1.0934	1.1156	0.05	0.0061	0.0061	0.0061
0	-90	1.0989	1.0880	1.1100	-85.59	1.0936	1.0827	1.1047	0.05	0.0048	0.0048	0.0048
0	-80	1.0870	1.0762	1.0980	-76.54	1.0829	1.0721	1.0939	0.04	0.0037	0.0037	0.0037
0	-70	1.0753	1.0646	1.0862	-67.37	1.0722	1.0616	1.0831	0.04	0.0028	0.0028	0.0028
0	-60	1.0638	1.0533	1.0746	-58.08	1.0617	1.0511	1.0724	0.03	0.0020	0.0020	0.0020
0	-50	1.0526	1.0422	1.0633	-48.67	1.0512	1.0407	1.0618	0.03	0.0014	0.0014	0.0014
0	-40	1.0417	1.0313	1.0522	-39.16	1.0408	1.0304	1.0513	0.02	0.0009	0.0009	0.0009
0	-30	1.0309	1.0207	1.0414	-29.53	1.0304	1.0202	1.0409	0.02	0.0005	0.0005	0.0005
0	-20	1.0204	1.0103	1.0308	-19.79	1.0202	1.0101	1.0305	0.01	0.0002	0.0002	0.0002
0	-10	1.0101	1.0001	1.0203	-9.95	1.0100	1.0000	1.0203	0.01	0.0001	0.0001	0.0001
0	10	0.9901	0.9803	1.0001	10.05	0.9900	0.9802	1.0001	0.01	0.0001	0.0001	0.0001
0	20	0.9804	0.9706	0.9903	20.20	0.9802	0.9705	0.9901	0.01	0.0002	0.0002	0.0002
0	30	0.9709	0.9612	0.9807	30.45	0.9704	0.9608	0.9803	0.02	0.0004	0.0004	0.0004
0	40	0.9615	0.9520	0.9713	40.80	0.9608	0.9513	0.9705	0.02	0.0008	0.0008	0.0008
0	50	0.9524	0.9429	0.9620	51.24	0.9513	0.9418	0.9609	0.02	0.0012	0.0012	0.0012
0	60	0.9434	0.9340	0.9530	61.77	0.9418	0.9325	0.9514	0.03	0.0017	0.0017	0.0017
0	70	0.9346	0.9253	0.9441	72.40	0.9325	0.9232	0.9419	0.03	0.0022	0.0022	0.0022
0	80	0.9259	0.9167	0.9353	83.11	0.9233	0.9141	0.9326	0.04	0.0029	0.0029	0.0029
0	90	0.9174	0.9083	0.9267	93.91	0.9141	0.9051	0.9234	0.04	0.0036	0.0036	0.0036
0	100	0.9091	0.9001	0.9183	104.80	0.9051	0.8961	0.9143	0.05	0.0043	0.0043	0.0043

[a] Simulated $\delta^{15}N^{s}$, $\delta^{15}N^{bulk}$, $\delta^{15}N^{\alpha}$, and $\delta^{15}N^{\beta}$ values were calculated without Mariotti's approximation (Mariotti et al., 1981) that $\ln[(\delta^{15}N^{s}/1000 +1)/(\delta^{15}N^{s0}/1000 +1)] \approx \delta^{15}N^{s} - \delta^{15}N^{s0}$. (This approximation is only valid when $\delta^{15}N^{s}/1000$ and $\delta^{15}N^{s0}/1000$ are small relative to 1.) $\delta^{15}N^{s0}$ was set at 0‰ and ρ was set at 0.5050 (normal KIE $^{15}N^{\alpha}$, normal KIE $^{15}N^{\beta}$). The average value of τ for each simulation was 0.49998.

[b] Absolute relative difference (Eq. (31)) is the absolute value of the difference between the estimated value and actual value divided by the actual value (|(estimate – actual)/actual|).

[c] Actual KIE values were calculated with Expanded Rayleigh model 1 using f values between 0.7-0.2. $\alpha_{N\text{-bulk}}$ was determined without the $\ln(1+u) = u$ approximation by linear regression of $\ln(\delta^{15}N^s/1000 +1)$ against $\ln(f)$; $\varepsilon_{N\text{-bulk}}$ is the slope in this model (Hayes, 2004). (The variable u represents $\delta^{15}N^s$ or $\delta^{15}N^{s0}$.) The value of ρ was determined via nonlinear regression (model 1) Eq. (29), and τ was determined by averaging $^{14}N^\alpha/^{14}N^{bulk}$ for every step of the reaction Eq. (24). The results for Expanded Rayleigh model 2 (standard Rayleigh model combined with nonlinear model 2, data not shown) are essentially identical to the results from Expanded Rayleigh model 1.

[d] Estimated $\varepsilon_{N\text{-bulk}}$ values were determined using Eq. (2), Mariotti's approximation of the Rayleigh equation (*i.e.*, via linear regression of $\delta^{15}N^{bulk}$ against $[-flnf/(1-f)]$) using f values between 0.7-0.2. $\varepsilon_{N\text{-bulk}}$ is the slope in this model.

[e] Estimated KIE values for Expanded Rayleigh model 1 were calculated using Mariotti's approximation of the standard Rayleigh equation to determine $\alpha_{N\text{-bulk}}$ (calculated from estimated $\varepsilon_{N\text{-bulk}}$, see note d and Eq. (2)). Values for ρ and τ were calculated as described in note b above; these values were used to determine KIE $^{15}N^\alpha$ and KIE $^{15}N^\beta$.

Table S3. Testing the impact of ρ and τ values on the Expanded Rayleigh model

ρ	τ [a]	KIE ^{15}N$^\alpha$ [b]	KIE ^{15}N$^\beta$ [b]	δ^{15}N^{bulk} − 0.5(δ^{15}N$^\alpha$ + δ^{15}N$^\beta$) (‰) [c, d]
0.4700	0.50011 ± 3.5E-07	1.0858	0.9624	-0.013 ± 8.4E-05
0.4800	0.50007 ± 2.3E-07	1.0631	0.9810	-0.006 ± 3.7E-05
0.4900	0.50004 ± 1.2E-07	1.0413	1.0003	-0.001 ± 9.3E-06
0.5000	0.50000 ± 5.0E-17	1.0204	1.0204	0.000 ± 6.8E-14
0.5100	0.49996 ± 1.2E-07	1.0003	1.0413	-0.001 ± 9.3E-06
0.5200	0.49993 ± 2.3E-07	0.9810	1.0631	-0.006 ± 3.7E-05
0.5300	0.49989 ± 3.5E-07	0.9624	1.0858	-0.013 ± 8.4E-05

[a] Average τ ± standard deviation. τ was calculated for each value of f (f = 0.2-0.7) using Eq. (24).

[b] KIE values were calculated using the input values of ε$_{N-bulk}$ and ρ and average τ.

[c] Average difference ± standard deviation of δ^{15}N^{bulk} and the average of δ^{15}N$^\alpha$ and δ^{15}N$^\beta$. Equation 2 was used to calculate δ^{15}N^{bulk} values where ε$_{N-bulk}$ is set at -20‰ and δ^{15}N^{s0} is set at 0‰. Equations 27-28 were used to calculate δ^{15}N$^\alpha$ and δ^{15}N$^\beta$ values using the specified value of ρ.

[d] All of the average differences shown here are less than the analytical error values typical for δ values (0.5-0.7‰).(Yang et al., 2014) Only very extreme ρ values (*e.g.*, ρ < 0.32 or ρ > 0.68) violate the assumption that δ^{15}N^{bulk} is equal to the average of δ^{15}N$^\alpha$ and δ^{15}N$^\beta$.

Table S4. Dataset 1: Simulated no-error values for N₂O synthesis in a closed system (no isotope effect for N^α, normal isotope effect for N^β).

$\delta^{15}N$ values for three replicates with evenly-spaced f values between 0.7 and 0.3 were simulated for a hypothetical reaction where $\varepsilon_{N\text{-bulk}}$ = -20‰, $\delta^{15}N^{s0}$ = 0‰, and $\varepsilon_{N\text{-}\alpha}$ = 0‰. $\delta^{15}N^s$ values were calculated using Eq. (18), and $\delta^{15}N^{bulk}$ values were calculated using Eq. (2). $\delta^{15}N^\alpha$ and $\delta^{15}N^\beta$ values were calculated using Eq. (S17) or Eq. (S18) and Eq. (6). Accumulated N^{bulk} and N^α values (in nmol) were calculated for a hypothetical reaction where N^{s0} = 10,000 nmol. (See SI section "Calculations for the Expanded Rayleigh model" for details).

Replicate	f	$\delta^{15}N^s$	$\delta^{15}N^{bulk}$	$\delta^{15}N^\alpha$	$\delta^{15}N^\beta$	N^{bulk}	$^{14}N^{bulk}$	$^{15}N^{bulk}$	$^{14}N^\alpha$	$^{15}N^\alpha$
1	0.7	7.1	-16.6	3.4	-36.7	3000	2989.2	10.8	1494.5	5.5
1	0.6	10.2	-15.3	4.8	-35.4	4000	3985.6	14.4	1992.7	7.3
1	0.5	13.9	-13.9	6.3	-34.0	5000	4982.0	18.0	2490.8	9.2
1	0.4	18.3	-12.2	7.9	-32.4	6000	5978.4	21.6	2989.0	11.0
1	0.3	24.1	-10.3	9.9	-30.5	7000	6974.7	25.3	3487.1	12.9
2	0.7	7.1	-16.6	3.4	-36.7	3000	2989.2	10.8	1494.5	5.5
2	0.6	10.2	-15.3	4.8	-35.4	4000	3985.6	14.4	1992.7	7.3
2	0.5	13.9	-13.9	6.3	-34.0	5000	4982.0	18.0	2490.8	9.2
2	0.4	18.3	-12.2	7.9	-32.4	6000	5978.4	21.6	2989.0	11.0
2	0.3	24.1	-10.3	9.9	-30.5	7000	6974.7	25.3	3487.1	12.9
3	0.7	7.1	-16.6	3.4	-36.7	3000	2989.2	10.8	1494.5	5.5
3	0.6	10.2	-15.3	4.8	-35.4	4000	3985.6	14.4	1992.7	7.3
3	0.5	13.9	-13.9	6.3	-34.0	5000	4982.0	18.0	2490.8	9.2
3	0.4	18.3	-12.2	7.9	-32.4	6000	5978.4	21.6	2989.0	11.0
3	0.3	24.1	-10.3	9.9	-30.5	7000	6974.7	25.3	3487.1	12.9

Table S5. Dataset 2: Simulated no-error values for N₂O synthesis in a closed system (normal isotope effect for N^α, normal isotope effect for N^β).

$\delta^{15}N$ values for three replicates with evenly-spaced f values between 0.7 and 0.3 were simulated for a hypothetical reaction where $\varepsilon_{\text{N-bulk}}$ = -20‰, $\delta^{15}N^{s0}$ = 0‰, and ρ = 0.5050. $\delta^{15}N^s$ values were calculated using Eq. (18), and $\delta^{15}N^{bulk}$ values were calculated using Eq. (2). $\delta^{15}N^\alpha$ and $\delta^{15}N^\beta$ values were calculated using Eq. (27) or Eq. (28). Accumulated N^{bulk} and N^α values (in nmol) were calculated for a hypothetical reaction where N^{s0} = 10,000 nmol. (See SI section "Calculations for the Expanded Rayleigh model" for details).

Replicate	f	$\delta^{15}N^s$	$\delta^{15}N^{bulk}$	$\delta^{15}N^\alpha$	$\delta^{15}N^\beta$	N^{bulk}	$^{14}N^{bulk}$	$^{15}N^{bulk}$	$^{14}N^\alpha$	$^{15}N^\alpha$
1	0.7	7.1	-16.6	-6.8	-26.5	3000	2989.2	10.8	1494.6	5.4
1	0.6	10.2	-15.3	-5.4	-25.2	4000	3985.6	14.4	1992.7	7.3
1	0.5	13.9	-13.9	-4.0	-23.8	5000	4982.0	18.0	2490.9	9.1
1	0.4	18.3	-12.2	-2.3	-22.1	6000	5978.4	21.6	2989.1	10.9
1	0.3	24.1	-10.3	-0.4	-20.3	7000	6974.7	25.3	3487.2	12.8
2	0.7	7.1	-16.6	-6.8	-26.5	3000	2989.2	10.8	1494.6	5.4
2	0.6	10.2	-15.3	-5.4	-25.2	4000	3985.6	14.4	1992.7	7.3
2	0.5	13.9	-13.9	-4.0	-23.8	5000	4982.0	18.0	2490.9	9.1
2	0.4	18.3	-12.2	-2.3	-22.1	6000	5978.4	21.6	2989.1	10.9
2	0.3	24.1	-10.3	-0.4	-20.3	7000	6974.7	25.3	3487.2	12.8
3	0.7	7.1	-16.6	-6.8	-26.5	3000	2989.2	10.8	1494.6	5.4
3	0.6	10.2	-15.3	-5.4	-25.2	4000	3985.6	14.4	1992.7	7.3
3	0.5	13.9	-13.9	-4.0	-23.8	5000	4982.0	18.0	2490.9	9.1
3	0.4	18.3	-12.2	-2.3	-22.1	6000	5978.4	21.6	2989.1	10.9
3	0.3	24.1	-10.3	-0.4	-20.3	7000	6974.7	25.3	3487.2	12.8

Table S6. Dataset 3: Simulated no-error values for N₂O synthesis in a closed system (inverse isotope effect for N^α, normal isotope effect for N^β).

$\delta^{15}N$ values for three replicates with evenly-spaced f values between 0.7 and 0.3 were simulated for a hypothetical reaction where $\varepsilon_{\text{N-bulk}}$ = -20‰, $\delta^{15}N^{s0}$ = 0‰, and ρ = 0.5200. $\delta^{15}N^s$ values were calculated using Eq. (18), and $\delta^{15}N^{\text{bulk}}$ values were calculated using Eq. (2). $\delta^{15}N^\alpha$ and $\delta^{15}N^\beta$ values were calculated using Eq. (27) or Eq. (28). Accumulated N^{bulk} and N^α values (in nmol) were calculated for a hypothetical reaction where N^{s0} = 10,000 nmol. (See SI section "Calculations for the Expanded Rayleigh model" for details).

Replicate	f	$\delta^{15}N^s$	$\delta^{15}N^{\text{bulk}}$	$\delta^{15}N^\alpha$	$\delta^{15}N^\beta$	N^{bulk}	$^{14}N^{\text{bulk}}$	$^{15}N^{\text{bulk}}$	$^{14}N^\alpha$	$^{15}N^\alpha$
1	0.7	7.1	-16.6	22.8	-56.1	3000	2989.2	10.8	1494.4	5.6
1	0.6	10.2	-15.3	24.2	-54.8	4000	3985.6	14.4	1992.5	7.5
1	0.5	13.9	-13.9	25.7	-53.4	5000	4982.0	18.0	2490.6	9.4
1	0.4	18.3	-12.2	27.4	-51.9	6000	5978.4	21.6	2988.8	11.2
1	0.3	24.1	-10.3	29.4	-50.0	7000	6974.7	25.3	3486.9	13.1
2	0.7	7.1	-16.6	22.8	-56.1	3000	2989.2	10.8	1494.4	5.6
2	0.6	10.2	-15.3	24.2	-54.8	4000	3985.6	14.4	1992.5	7.5
2	0.5	13.9	-13.9	25.7	-53.4	5000	4982.0	18.0	2490.6	9.4
2	0.4	18.3	-12.2	27.4	-51.9	6000	5978.4	21.6	2988.8	11.2
2	0.3	24.1	-10.3	29.4	-50.0	7000	6974.7	25.3	3486.9	13.1
3	0.7	7.1	-16.6	22.8	-56.1	3000	2989.2	10.8	1494.4	5.6
3	0.6	10.2	-15.3	24.2	-54.8	4000	3985.6	14.4	1992.5	7.5
3	0.5	13.9	-13.9	25.7	-53.4	5000	4982.0	18.0	2490.6	9.4
3	0.4	18.3	-12.2	27.4	-51.9	6000	5978.4	21.6	2988.8	11.2
3	0.3	24.1	-10.3	29.4	-50.0	7000	6974.7	25.3	3486.9	13.1

Table S7. Dataset 4: Simulated no-error values for N₂O synthesis in a closed system (inverse isotope effect for N^α, inverse isotope effect for N^β).

$\delta^{15}N$ values for three replicates with evenly-spaced f values between 0.7 and 0.3 were simulated for a hypothetical reaction where $\varepsilon_{N\text{-bulk}} = +20‰$, $\delta^{15}N^{s0} = 0‰$, and $\rho = 0.5050$. $\delta^{15}N^s$ values were calculated using Eq. (18), and $\delta^{15}N^{bulk}$ values were calculated using Eq. (2). $\delta^{15}N^\alpha$ and $\delta^{15}N^\beta$ values were calculated using Eq. (27) or Eq. (28). Accumulated N^{bulk} and N^α values (in nmol) were calculated for a hypothetical reaction where $N^{s0} = 10,000$ nmol. (See SI section "Calculations for the Expanded Rayleigh model" for details).

Replicate	f	$\delta^{15}N^s$	$\delta^{15}N^{bulk}$	$\delta^{15}N^\alpha$	$\delta^{15}N^\beta$	N^{bulk}	$^{14}N^{bulk}$	$^{15}N^{bulk}$	$^{14}N^\alpha$	$^{15}N^\alpha$
1	0.7	-7.1	16.6	26.8	6.4	3000	2988.9	11.1	1494.4	5.6
1	0.6	-10.2	15.3	25.5	5.1	4000	3985.2	14.8	1992.5	7.5
1	0.5	-13.9	13.9	24.0	3.7	5000	4981.5	18.5	2490.7	9.3
1	0.4	-18.3	12.2	22.4	2.1	6000	5977.8	22.2	2988.8	11.2
1	0.3	-24.1	10.3	20.5	0.2	7000	6974.2	25.8	3487.0	13.0
2	0.7	-7.1	16.6	26.8	6.4	3000	2988.9	11.1	1494.4	5.6
2	0.6	-10.2	15.3	25.5	5.1	4000	3985.2	14.8	1992.5	7.5
2	0.5	-13.9	13.9	24.0	3.7	5000	4981.5	18.5	2490.7	9.3
2	0.4	-18.3	12.2	22.4	2.1	6000	5977.8	22.2	2988.8	11.2
2	0.3	-24.1	10.3	20.5	0.2	7000	6974.2	25.8	3487.0	13.0
3	0.7	-7.1	16.6	26.8	6.4	3000	2988.9	11.1	1494.4	5.6
3	0.6	-10.2	15.3	25.5	5.1	4000	3985.2	14.8	1992.5	7.5
3	0.5	-13.9	13.9	24.0	3.7	5000	4981.5	18.5	2490.7	9.3
3	0.4	-18.3	12.2	22.4	2.1	6000	5977.8	22.2	2988.8	11.2
3	0.3	-24.1	10.3	20.5	0.2	7000	6974.2	25.8	3487.0	13.0

Table S8. Dataset 5: Simulated no-error values for N₂O synthesis in a closed system (normal isotope effect for N^α, no isotope effect for N^β).

$\delta^{15}N$ values for three replicates with evenly-spaced f values between 0.7 and 0.3 were simulated for a hypothetical reaction where $\varepsilon_{N\text{-bulk}}$ = -20‰, $\delta^{15}N^{s0}$ = 0‰, and $\varepsilon_{N\text{-}\beta}$ = 0‰. $\delta^{15}N^s$ values were calculated using Eq. (18), and $\delta^{15}N^{bulk}$ values were calculated using Eq. (2). $\delta^{15}N^\alpha$ and $\delta^{15}N^\beta$ values were calculated using Eq. (S17) or Eq. (S18) and Eq. (6). Accumulated N^{bulk} and N^α values (in nmol) were calculated for a hypothetical reaction where N^{s0} = 10,000 nmol. (See SI section "Calculations for the Expanded Rayleigh model" for details).

Replicate	f	$\delta^{15}N^s$	$\delta^{15}N^{bulk}$	$\delta^{15}N^\alpha$	$\delta^{15}N^\beta$	N^{bulk}	$^{14}N^{bulk}$	$^{15}N^{bulk}$	$^{14}N^\alpha$	$^{15}N^\alpha$
1	0.7	7.1	-16.6	-36.7	3.4	3000	2989.2	10.8	1494.7	5.3
1	0.6	10.2	-15.3	-35.4	4.8	4000	3985.6	14.4	1993.0	7.0
1	0.5	13.9	-13.9	-34.0	6.3	5000	4982.0	18.0	2491.2	8.8
1	0.4	18.3	-12.2	-32.4	7.9	6000	5978.4	21.6	2989.4	10.6
1	0.3	24.1	-10.3	-30.5	9.9	7000	6974.7	25.3	3487.6	12.4
2	0.7	7.1	-16.6	-36.7	3.4	3000	2989.2	10.8	1494.7	5.3
2	0.6	10.2	-15.3	-35.4	4.8	4000	3985.6	14.4	1993.0	7.0
2	0.5	13.9	-13.9	-34.0	6.3	5000	4982.0	18.0	2491.2	8.8
2	0.4	18.3	-12.2	-32.4	7.9	6000	5978.4	21.6	2989.4	10.6
2	0.3	24.1	-10.3	-30.5	9.9	7000	6974.7	25.3	3487.6	12.4
3	0.7	7.1	-16.6	-36.7	3.4	3000	2989.2	10.8	1494.7	5.3
3	0.6	10.2	-15.3	-35.4	4.8	4000	3985.6	14.4	1993.0	7.0
3	0.5	13.9	-13.9	-34.0	6.3	5000	4982.0	18.0	2491.2	8.8
3	0.4	18.3	-12.2	-32.4	7.9	6000	5978.4	21.6	2989.4	10.6
3	0.3	24.1	-10.3	-30.5	9.9	7000	6974.7	25.3	3487.6	12.4

Table S9. Precision and accuracy of values calculated with the Expanded Rayleigh model using simulated datasets derived from Dataset 1 (no isotope effect for N^α, normal isotope effect for N^β).

Model	Level of error[a]	Skewness [a]	R^{2} [b, c]	ρ [b]	τ [b]	$\varepsilon_{N\text{-}bulk}$ [b] (‰)	KIE $^{15}N^{bulk}$ [b]	KIE $^{15}N^{\alpha}$ [b]	KIE $^{15}N^{\beta}$ [b]
Actual	None	No	NA	0.5102 ± 9e-08 [d]	0.49996 ± 9e-08 [d]	-20 ± NA	1.0204 ± NA	1 ± NA	1.0417 ± NA
Expanded Rayleigh 1	Low	No	0.95 ± 0.02	0.5102 ± 1e-04	0.49996 ± 4.1e-07	-19.78 ± 1.27	1.0202 ± 0.0013	0.9998 ± 0.0013	1.0414 ± 0.0014
		Left	0.94 ± 0.02	0.5102 ± 1e-04	0.49996 ± 4e-07	-19.79 ± 1.28	1.0202 ± 0.0013	0.9998 ± 0.0013	1.0414 ± 0.0014
		Right	0.94 ± 0.02	0.5102 ± 1e-04	0.49996 ± 4e-07	-19.83 ± 1.26	1.0202 ± 0.0013	0.9998 ± 0.0013	1.0415 ± 0.0014
	Medium	No	0.75 ± 0.09	0.5102 ± 3e-04	0.49996 ± 9.9e-07	-19.18 ± 2.95	1.0196 ± 0.0031	0.9992 ± 0.003	1.0408 ± 0.0032
		Left	0.74 ± 0.1	0.5101 ± 3e-04	0.49996 ± 1.01e-06	-19.32 ± 3.05	1.0197 ± 0.0032	0.9994 ± 0.0032	1.0409 ± 0.0033
		Right	0.74 ± 0.1	0.5102 ± 3e-04	0.49996 ± 1e-06	-19.35 ± 3.01	1.0197 ± 0.0031	0.9993 ± 0.0031	1.041 ± 0.0033
	High	No	0.44 ± 0.17	0.5102 ± 6e-04	0.49996 ± 2.05e-06	-18.24 ± 5.75	1.0186 ± 0.006	0.9982 ± 0.006	1.0398 ± 0.0061
		Left	0.43 ± 0.17	0.5101 ± 6e-04	0.49996 ± 1.99e-06	-18.57 ± 5.61	1.019 ± 0.0058	0.9987 ± 0.0058	1.0401 ± 0.0061
		Right	0.43 ± 0.17	0.5102 ± 6e-04	0.49996 ± 2.04e-06	-18.36 ± 5.68	1.0187 ± 0.0059	0.9983 ± 0.0059	1.04 ± 0.0062
Expanded Rayleigh 2	Low	No	0.95 ± 0.02	0.5102 ± 1e-04	0.49996 ± 4.1e-07	-19.78 ± 1.27	1.0202 ± 0.0013	0.9998 ± 0.0013	1.0414 ± 0.0014
		Left	0.94 ± 0.02	0.5102 ± 1e-04	0.49996 ± 4e-07	-19.79 ± 1.28	1.0202 ± 0.0013	0.9998 ± 0.0013	1.0414 ± 0.0014
		Right	0.94 ± 0.02	0.5102 ± 1e-04	0.49996 ± 4e-07	-19.83 ± 1.26	1.0202 ± 0.0013	0.9998 ± 0.0013	1.0415 ± 0.0014
	Medium	No	0.75 ± 0.09	0.5102 ± 3e-04	0.49996 ± 9.9e-07	-19.18 ± 2.95	1.0196 ± 0.0031	0.9992 ± 0.003	1.0408 ± 0.0032
		Left	0.74 ± 0.1	0.5102 ± 3e-04	0.49996 ± 1.01e-06	-19.32 ± 3.05	1.0197 ± 0.0032	0.9994 ± 0.0032	1.0409 ± 0.0033
		Right	0.74 ± 0.1	0.5102 ± 3e-04	0.49996 ± 1e-06	-19.35 ± 3.01	1.0197 ± 0.0031	0.9993 ± 0.0031	1.041 ± 0.0033
	High	No	0.44 ± 0.17	0.5102 ± 6e-04	0.49996 ± 2.05e-06	-18.24 ± 5.75	1.0186 ± 0.006	0.9982 ± 0.006	1.0398 ± 0.0061
		Left	0.43 ± 0.17	0.5101 ± 6e-04	0.49996 ± 1.99e-06	-18.57 ± 5.61	1.019 ± 0.0058	0.9987 ± 0.0058	1.0401 ± 0.0061
		Right	0.43 ± 0.17	0.5102 ± 6e-04	0.49996 ± 2.04e-06	-18.36 ± 5.68	1.0187 ± 0.0059	0.9983 ± 0.0059	1.04 ± 0.0062

ᵃ See Table 2 for details.

ᵇ Average value ± standard deviation calculated from 1000 R^2 values generated from 1000 simulated datasets.

ᶜ Average R^2 value for linear regression of $\delta^{15}N^{bulk}$ against [-flnf/(1-f)] (using standard Rayleigh equation) calculated from 1000 R^2 values generated from 1000 simulated datasets.

ᵈ Average value ± standard deviation for the ρ or τ values from the no-error dataset. τ was calculated for each value of f using Eq. (24). ρ was calculated for each value of f using Eq. (23); see SI section "Calculation of $\delta^{15}N^{\alpha}$, $\delta^{15}N^{\beta}$, and ρ when $\varepsilon_{N-\alpha}$ or $\varepsilon_{N-\beta}$ is equal to 0 *(e.g.*, Simulated Datasets 1 and 5)" for details.

Table S10. Precision and accuracy of values calculated with the Expanded Rayleigh model using simulated datasets derived from Dataset 2 (normal isotope effects for N^α and N^β).

Model	Level of error[a]	Skewness [a]	R^{2} [b,c]	ρ [b]	τ [b]	$\varepsilon_{N\text{-bulk}}$ [b] (‰)	KIE $^{15}N^{bulk}$ [b]	KIE $^{15}N^{\alpha}$ [b]	KIE $^{15}N^{\beta}$ [b]
Actual	None	No	NA	0.505 ± NA	0.49998 ± 4e-08 [d]	-20 ± NA	1.0204 ± NA	1.0103 ± NA	1.0308 ± NA
Expanded Rayleigh 1	Low	No	0.95 ± 0.02	0.505 ± 1e-04	0.49998 ± 4.1e-07	-19.78 ± 1.27	1.0202 ± 0.0013	1.01 ± 0.0014	1.0305 ± 0.0013
		Left	0.94 ± 0.02	0.505 ± 1e-04	0.49998 ± 4e-07	-19.79 ± 1.28	1.0202 ± 0.0013	1.0101 ± 0.0013	1.0305 ± 0.0014
		Right	0.94 ± 0.02	0.505 ± 1e-04	0.49998 ± 4e-07	-19.83 ± 1.26	1.0202 ± 0.0013	1.0101 ± 0.0013	1.0306 ± 0.0013
	Medium	No	0.75 ± 0.09	0.505 ± 3e-04	0.49998 ± 9.9e-07	-19.18 ± 2.95	1.0196 ± 0.0031	1.0094 ± 0.0031	1.0299 ± 0.0032
		Left	0.74 ± 0.1	0.505 ± 3e-04	0.49998 ± 1.01e-06	-19.32 ± 3.05	1.0197 ± 0.0032	1.0096 ± 0.0032	1.03 ± 0.0033
		Right	0.74 ± 0.1	0.505 ± 3e-04	0.49998 ± 1e-06	-19.35 ± 3.01	1.0197 ± 0.0031	1.0096 ± 0.0032	1.0301 ± 0.0032
	High	No	0.44 ± 0.17	0.505 ± 6e-04	0.49998 ± 2.05e-06	-18.24 ± 5.75	1.0186 ± 0.006	1.0085 ± 0.0061	1.0289 ± 0.006
		Left	0.43 ± 0.17	0.5049 ± 6e-04	0.49998 ± 1.99e-06	-18.57 ± 5.61	1.019 ± 0.0058	1.0089 ± 0.0059	1.0292 ± 0.006
		Right	0.43 ± 0.17	0.505 ± 6e-04	0.49998 ± 2.04e-06	-18.36 ± 5.68	1.0187 ± 0.0059	1.0086 ± 0.0059	1.0291 ± 0.0061
Expanded Rayleigh 2	Low	No	0.95 ± 0.02	0.505 ± 1e-04	0.49998 ± 4.1e-07	-19.78 ± 1.27	1.0202 ± 0.0013	1.01 ± 0.0014	1.0305 ± 0.0013
		Left	0.94 ± 0.02	0.505 ± 1e-04	0.49998 ± 4e-07	-19.79 ± 1.28	1.0202 ± 0.0013	1.0101 ± 0.0013	1.0305 ± 0.0014
		Right	0.94 ± 0.02	0.505 ± 1e-04	0.49998 ± 4e-07	-19.83 ± 1.26	1.0202 ± 0.0013	1.0101 ± 0.0013	1.0306 ± 0.0013
	Medium	No	0.75 ± 0.09	0.505 ± 3e-04	0.49998 ± 9.9e-07	-19.18 ± 2.95	1.0196 ± 0.0031	1.0094 ± 0.0031	1.0299 ± 0.0032
		Left	0.74 ± 0.1	0.505 ± 3e-04	0.49998 ± 1.01e-06	-19.32 ± 3.05	1.0197 ± 0.0032	1.0096 ± 0.0032	1.03 ± 0.0033
		Right	0.74 ± 0.1	0.505 ± 3e-04	0.49998 ± 1e-06	-19.35 ± 3.01	1.0197 ± 0.0031	1.0096 ± 0.0032	1.0301 ± 0.0032
	High	No	0.44 ± 0.17	0.505 ± 6e-04	0.49998 ± 2.05e-06	-18.24 ± 5.75	1.0186 ± 0.006	1.0085 ± 0.0061	1.0289 ± 0.006
		Left	0.43 ± 0.17	0.5049 ± 6e-04	0.49998 ± 1.99e-06	-18.57 ± 5.61	1.019 ± 0.0058	1.0089 ± 0.0059	1.0292 ± 0.006
		Right	0.43 ± 0.17	0.505 ± 6e-04	0.49998 ± 2.04e-06	-18.36 ± 5.68	1.0187 ± 0.0059	1.0086 ± 0.0059	1.0291 ± 0.0061

[a] See Table 2 for details.

[b] Average value ± standard deviation calculated from 1000 R^2 values generated from 1000 simulated datasets.

[c] Average R^2 value for linear regression of $\delta^{15}N^{bulk}$ against [-flnf/(1-f)] (using standard Rayleigh equation) calculated from 1000 R^2 values generated from 1000 simulated datasets.

[d] Standard deviation for the τ values from the no-error dataset. τ was calculated for each value of f using Eq. (24).

Table S11. Precision and accuracy of values calculated with the Expanded Rayleigh model using simulated datasets derived from Dataset 3 (inverse isotope effect for N^α, normal isotope effect for N^β).

Model	Level of error[a]	Skewness[a]	R^2 [b,c]	ρ [b]	τ [b]	$\varepsilon_{N\text{-bulk}}$ [b] (‰)	KIE $^{15}N^{bulk}$ [b]	KIE $^{15}N^{\alpha}$ [b]	KIE $^{15}N^{\beta}$ [b]
Actual	None	No	NA	$0.52 \pm$ NA	$0.49993 \pm 1.7e\text{-}07$ [d]	$-20 \pm$ NA	$1.0204 \pm$ NA	$0.981 \pm$ NA	$1.0631 \pm$ NA
Expanded Rayleigh 1	Low	No	0.95 ± 0.02	$0.52 \pm 1e\text{-}04$	$0.49993 \pm 4.1e\text{-}07$	-19.78 ± 1.27	1.0202 ± 0.0013	0.9808 ± 0.0013	1.0628 ± 0.0014
		Left	0.94 ± 0.02	$0.52 \pm 1e\text{-}04$	$0.49993 \pm 4e\text{-}07$	-19.79 ± 1.28	1.0202 ± 0.0013	0.9808 ± 0.0013	1.0628 ± 0.0014
		Right	0.94 ± 0.02	$0.52 \pm 1e\text{-}04$	$0.49993 \pm 4e\text{-}07$	-19.83 ± 1.26	1.0202 ± 0.0013	0.9808 ± 0.0013	1.0629 ± 0.0014
	Medium	No	0.75 ± 0.09	$0.52 \pm 3e\text{-}04$	$0.49993 \pm 9.9e\text{-}07$	-19.18 ± 2.95	1.0196 ± 0.0031	0.9802 ± 0.003	1.0622 ± 0.0033
		Left	0.74 ± 0.1	$0.52 \pm 3e\text{-}04$	$0.49993 \pm 1.01e\text{-}06$	-19.32 ± 3.05	1.0197 ± 0.0032	0.9804 ± 0.0031	1.0623 ± 0.0034
		Right	0.74 ± 0.1	$0.52 \pm 3e\text{-}04$	$0.49993 \pm 1e\text{-}06$	-19.35 ± 3.01	1.0197 ± 0.0031	0.9804 ± 0.0031	1.0624 ± 0.0033
	High	No	0.44 ± 0.17	$0.52 \pm 6e\text{-}04$	$0.49993 \pm 2.05e\text{-}06$	-18.24 ± 5.75	1.0186 ± 0.006	0.9793 ± 0.0059	1.0612 ± 0.0062
		Left	0.43 ± 0.17	$0.5199 \pm 6e\text{-}04$	$0.49993 \pm 1.99e\text{-}06$	-18.57 ± 5.61	1.019 ± 0.0058	0.9797 ± 0.0057	1.0615 ± 0.0062
		Right	0.43 ± 0.17	$0.52 \pm 6e\text{-}04$	$0.49993 \pm 2.04e\text{-}06$	-18.36 ± 5.68	1.0187 ± 0.0059	0.9794 ± 0.0057	1.0614 ± 0.0063
Expanded Rayleigh 2	Low	No	0.95 ± 0.02	$0.52 \pm 1e\text{-}04$	$0.49993 \pm 4.1e\text{-}07$	-19.78 ± 1.27	1.0202 ± 0.0013	0.9808 ± 0.0013	1.0629 ± 0.0014
		Left	0.94 ± 0.02	$0.52 \pm 1e\text{-}04$	$0.49993 \pm 4e\text{-}07$	-19.79 ± 1.28	1.0202 ± 0.0013	0.9808 ± 0.0013	1.0629 ± 0.0014
		Right	0.94 ± 0.02	$0.52 \pm 1e\text{-}04$	$0.49993 \pm 4e\text{-}07$	-19.83 ± 1.26	1.0202 ± 0.0013	0.9808 ± 0.0013	1.0629 ± 0.0014
	Medium	No	0.75 ± 0.09	$0.52 \pm 3e\text{-}04$	$0.49993 \pm 9.9e\text{-}07$	-19.18 ± 2.95	1.0196 ± 0.0031	0.9802 ± 0.003	1.0622 ± 0.0033
		Left	0.74 ± 0.1	$0.52 \pm 3e\text{-}04$	$0.49993 \pm 1.01e\text{-}06$	-19.32 ± 3.05	1.0197 ± 0.0032	0.9804 ± 0.0031	1.0623 ± 0.0034
		Right	0.74 ± 0.1	$0.52 \pm 3e\text{-}04$	$0.49993 \pm 1e\text{-}06$	-19.35 ± 3.01	1.0197 ± 0.0031	0.9804 ± 0.0031	1.0624 ± 0.0033
	High	No	0.44 ± 0.17	$0.52 \pm 6e\text{-}04$	$0.49993 \pm 2.05e\text{-}06$	-18.24 ± 5.75	1.0186 ± 0.006	0.9793 ± 0.0059	1.0612 ± 0.0062
		Left	0.43 ± 0.17	$0.52 \pm 6e\text{-}04$	$0.49993 \pm 1.99e\text{-}06$	-18.57 ± 5.61	1.019 ± 0.0058	0.9797 ± 0.0057	1.0615 ± 0.0062
		Right	0.43 ± 0.17	$0.52 \pm 6e\text{-}04$	$0.49993 \pm 2.04e\text{-}06$	-18.36 ± 5.68	1.0187 ± 0.0059	0.9794 ± 0.0057	1.0614 ± 0.0063

[a] See Table 2 for details.

[b] Average value ± standard deviation calculated from 1000 R^2 values generated from 1000 simulated datasets.

[c] Average R^2 value for linear regression of $\delta^{15}N^{bulk}$ against [-flnf/(1-f)] (using standard Rayleigh equation) calculated from 1000 R^2 values generated from 1000 simulated datasets.

[d] Standard deviation for the τ values from the no-error dataset. τ was calculated for each value of f using Eq. (24).

Table S12. Precision and accuracy of values calculated with the Expanded Rayleigh model using simulated datasets derived from Dataset 4 (inverse isotope effects for N^α and N^β).

Model	Level of error[a]	Skewness [a]	R^{2} [b, c]	ρ [b]	τ [b]	$\varepsilon_{N\text{-bulk}}$ [b] (‰)	KIE $^{15}N^{bulk}$ [b]	KIE $^{15}N^{\alpha}$ [b]	KIE $^{15}N^{\beta}$ [b]
Actual	None	No	NA	0.505 ± NA	0.49998 ± 4e-08 [d]	20 ± NA	0.9804 ± NA	0.9706 ± NA	0.9903 ± NA
Expanded Rayleigh 1	Low	No	0.95 ± 0.02	0.505 ± 1e-04	0.49998 ± 4e-07	19.83 ± 1.27	0.9806 ± 0.0012	0.9708 ± 0.0012	0.9905 ± 0.0012
		Left	0.94 ± 0.02	0.505 ± 1e-04	0.49998 ± 4.1e-07	19.8 ± 1.3	0.9806 ± 0.0013	0.9708 ± 0.0012	0.9905 ± 0.0013
		Right	0.94 ± 0.02	0.505 ± 1e-04	0.49998 ± 4.1e-07	19.83 ± 1.31	0.9806 ± 0.0013	0.9708 ± 0.0013	0.9905 ± 0.0013
	Medium	No	0.75 ± 0.09	0.505 ± 3e-04	0.49998 ± 9.9e-07	19.31 ± 2.94	0.9811 ± 0.0028	0.9713 ± 0.0028	0.991 ± 0.0029
		Left	0.74 ± 0.1	0.505 ± 3e-04	0.49998 ± 1.01e-06	19.22 ± 2.98	0.9811 ± 0.0029	0.9714 ± 0.0029	0.9911 ± 0.003
		Right	0.74 ± 0.1	0.505 ± 3e-04	0.49998 ± 1.01e-06	19.42 ± 3	0.981 ± 0.0029	0.9712 ± 0.0029	0.9909 ± 0.003
	High	No	0.45 ± 0.16	0.505 ± 5e-04	0.49998 ± 2e-06	18.59 ± 5.71	0.9818 ± 0.0055	0.972 ± 0.0056	0.9917 ± 0.0056
		Left	0.43 ± 0.17	0.505 ± 5e-04	0.49998 ± 2.02e-06	18.3 ± 5.59	0.9821 ± 0.0054	0.9724 ± 0.0054	0.9919 ± 0.0056
		Right	0.43 ± 0.16	0.505 ± 6e-04	0.49998 ± 2.05e-06	18.69 ± 5.72	0.9817 ± 0.0055	0.9719 ± 0.0055	0.9917 ± 0.0057
Expanded Rayleigh 2	Low	No	0.95 ± 0.02	0.505 ± 1e-04	0.49998 ± 4e-07	19.83 ± 1.27	0.9806 ± 0.0012	0.9708 ± 0.0012	0.9905 ± 0.0012
		Left	0.94 ± 0.02	0.505 ± 1e-04	0.49998 ± 4.1e-07	19.8 ± 1.3	0.9806 ± 0.0013	0.9708 ± 0.0012	0.9905 ± 0.0013
		Right	0.94 ± 0.02	0.505 ± 1e-04	0.49998 ± 4.1e-07	19.83 ± 1.31	0.9806 ± 0.0013	0.9708 ± 0.0013	0.9905 ± 0.0013
	Medium	No	0.75 ± 0.09	0.505 ± 3e-04	0.49998 ± 9.9e-07	19.31 ± 2.94	0.9811 ± 0.0028	0.9713 ± 0.0028	0.991 ± 0.0029
		Left	0.74 ± 0.1	0.505 ± 3e-04	0.49998 ± 1.01e-06	19.22 ± 2.98	0.9811 ± 0.0029	0.9714 ± 0.0029	0.9911 ± 0.003
		Right	0.74 ± 0.1	0.505 ± 3e-04	0.49998 ± 1.01e-06	19.42 ± 3	0.981 ± 0.0029	0.9712 ± 0.0029	0.9909 ± 0.003
	High	No	0.45 ± 0.16	0.505 ± 5e-04	0.49998 ± 2e-06	18.59 ± 5.71	0.9818 ± 0.0055	0.972 ± 0.0056	0.9917 ± 0.0056
		Left	0.43 ± 0.17	0.505 ± 5e-04	0.49998 ± 2.02e-06	18.3 ± 5.59	0.9821 ± 0.0054	0.9724 ± 0.0054	0.9919 ± 0.0056
		Right	0.43 ± 0.16	0.505 ± 6e-04	0.49998 ± 2.05e-06	18.69 ± 5.72	0.9817 ± 0.0055	0.9719 ± 0.0055	0.9917 ± 0.0057

[a] See Table 2 for details.

[b] Average value $\pm$ standard deviation calculated from 1000 R^2 values generated from 1000 simulated datasets.

[c] Average R^2 value for linear regression of $\delta^{15}N^{bulk}$ against [-flnf/(1-f)] (using standard Rayleigh equation) calculated from 1000 R^2 values generated from 1000 simulated datasets.

[d] Standard deviation for the τ values from the no-error dataset. τ was calculated for each value of f using Eq. (24).

Table S13. Precision and accuracy of values calculated with the Expanded Rayleigh model using simulated datasets derived from Dataset 5 (normal isotope effects for N^α, no isotope effect for N^β).

Model	Level of error[a]	Skewness[a]	R^{2}[b,c]	ρ[b]	τ[b]	$\varepsilon_{N\text{-bulk}}$[b] (‰)	KIE $^{15}N^{bulk}$[b]	KIE $^{15}N^{\alpha}$[b]	KIE $^{15}N^{\beta}$[b]
Actual	None	No	NA	0.4898 ± 8e-08[d]	0.50004 ± 9e-08[d]	-20 ± NA	1.0204 ± NA	1.0417 ± NA	1 ± NA
Expanded Rayleigh 1	Low	No	0.95 ± 0.02	0.4898 ± 1e-04	0.50004 ± 4.1e-07	-19.78 ± 1.27	1.0202 ± 0.0013	1.0414 ± 0.0014	0.9998 ± 0.0013
		Left	0.94 ± 0.02	0.4898 ± 1e-04	0.50004 ± 4e-07	-19.79 ± 1.28	1.0202 ± 0.0013	1.0415 ± 0.0014	0.9998 ± 0.0013
		Right	0.94 ± 0.02	0.4898 ± 1e-04	0.50004 ± 4e-07	-19.83 ± 1.26	1.0202 ± 0.0013	1.0415 ± 0.0014	0.9998 ± 0.0013
	Medium	No	0.75 ± 0.09	0.4898 ± 3e-04	0.50004 ± 9.9e-07	-19.18 ± 2.95	1.0196 ± 0.0031	1.0408 ± 0.0032	0.9992 ± 0.0031
		Left	0.74 ± 0.1	0.4898 ± 3e-04	0.50004 ± 1.01e-06	-19.32 ± 3.05	1.0197 ± 0.0032	1.041 ± 0.0033	0.9993 ± 0.0032
		Right	0.74 ± 0.1	0.4898 ± 3e-04	0.50004 ± 1e-06	-19.35 ± 3.01	1.0197 ± 0.0031	1.041 ± 0.0033	0.9994 ± 0.0031
	High	No	0.44 ± 0.17	0.4898 ± 6e-04	0.50004 ± 2.05e-06	-18.24 ± 5.75	1.0186 ± 0.006	1.0398 ± 0.0063	0.9982 ± 0.0059
		Left	0.43 ± 0.17	0.4898 ± 5e-04	0.50004 ± 1.99e-06	-18.57 ± 5.61	1.019 ± 0.0058	1.0403 ± 0.006	0.9985 ± 0.0058
		Right	0.43 ± 0.17	0.4899 ± 6e-04	0.50004 ± 2.04e-06	-18.36 ± 5.68	1.0187 ± 0.0059	1.0399 ± 0.0061	0.9984 ± 0.0059
Expanded Rayleigh 2	Low	No	0.95 ± 0.02	0.4898 ± 1e-04	0.50004 ± 4.1e-07	-19.78 ± 1.27	1.0202 ± 0.0013	1.0414 ± 0.0014	0.9998 ± 0.0013
		Left	0.94 ± 0.02	0.4898 ± 1e-04	0.50004 ± 4e-07	-19.79 ± 1.28	1.0202 ± 0.0013	1.0415 ± 0.0014	0.9998 ± 0.0013
		Right	0.94 ± 0.02	0.4898 ± 1e-04	0.50004 ± 4e-07	-19.83 ± 1.26	1.0202 ± 0.0013	1.0415 ± 0.0014	0.9998 ± 0.0013
	Medium	No	0.75 ± 0.09	0.4898 ± 3e-04	0.50004 ± 9.9e-07	-19.18 ± 2.95	1.0196 ± 0.0031	1.0408 ± 0.0032	0.9992 ± 0.0031
		Left	0.74 ± 0.1	0.4898 ± 3e-04	0.50004 ± 1.01e-06	-19.32 ± 3.05	1.0197 ± 0.0032	1.041 ± 0.0033	0.9993 ± 0.0032
		Right	0.74 ± 0.1	0.4898 ± 3e-04	0.50004 ± 1e-06	-19.35 ± 3.01	1.0197 ± 0.0031	1.041 ± 0.0033	0.9994 ± 0.0031
	High	No	0.44 ± 0.17	0.4898 ± 6e-04	0.50004 ± 2.05e-06	-18.24 ± 5.75	1.0186 ± 0.006	1.0398 ± 0.0063	0.9982 ± 0.0059
		Left	0.43 ± 0.17	0.4898 ± 5e-04	0.50004 ± 1.99e-06	-18.57 ± 5.61	1.019 ± 0.0058	1.0403 ± 0.006	0.9985 ± 0.0058
		Right	0.43 ± 0.17	0.4899 ± 6e-04	0.50004 ± 2.04e-06	-18.36 ± 5.68	1.0187 ± 0.0059	1.0399 ± 0.0061	0.9984 ± 0.0059

ᵇ Average value ± standard deviation calculated from 1000 R^2 values generated from 1000 simulated datasets.

ᶜ Average R^2 value for linear regression of $\delta^{15}N^{bulk}$ against [-flnf/(1-f)] (using standard Rayleigh equation) calculated from 1000 R^2 values generated from 1000 simulated datasets.

ᵈ Average value ± standard deviation for the ρ or τ values from the no-error dataset. τ was calculated for each value of f using Eq. (24). ρ was calculated for each value of f using Eq. (23); see SI section "Calculation of $\delta^{15}N^{\alpha}$, $\delta^{15}N^{\beta}$, and ρ when $\varepsilon_{N-\alpha}$ or $\varepsilon_{N-\beta}$ is equal to 0 *(e.g.*, Simulated Datasets 1 and 5)" for details.

Table S14. N$_2$O concentrations and $\delta^{15}N^{bulk}$ values (reported) and values of $\delta^{15}N^{\alpha}$, $\delta^{15}N^{\beta}$ (back-calculated) for N$_2$O production from NH$_2$OH by *M. trichosporium* replicate B [isotopic data previously published (Sutka et al., 2006)].

Previously reported by Sutka et al., 2006				*Back-calculated*		
Time (min.)	[N$_2$O] (μM)	$\delta^{15}N^{bulk}$ (‰)	SP (‰)	f	$\delta^{15}N^{\alpha}$ (‰)	$\delta^{15}N^{\beta}$ (‰)
95	12.7	5.2	34.9	0.81	22.7	-12.3
155	19.5	5.0	33.3	0.70	21.7	-11.7
195	23.3	4.7	35.1	0.65	22.3	-12.9
290	30.8	4.2	35.0	0.53	21.7	-13.3
330	30.9	4.2	35.0	0.53	21.7	-13.3
450	38	3.9	34.4	0.42	21.1	-13.3

Table S15. Comparison of values calculated for the standard and Expanded Rayleigh models for N$_2$O production from NH$_2$OH by an axenic culture of *M. trichosporium* (*Methylocystis* sp.). Values were calculated using isotopic data previously published for *M. trichosporium* replicate B (Sutka et al., 2006).

Model	R^{2} [a]	$\delta^{15}N^{s0}$ measured [b]	$\delta^{15}N^{s0}$ calculated [a, c]	ρ [c]	τ [c]	$\varepsilon_{N\text{-bulk}}$ [c] (‰)	KIE $^{15}N^{bulk}$ [c]	KIE $^{15}N^{\alpha}$ [c]	KIE $^{15}N^{\beta}$ [c]
Standard [d]	0.98	-2.3	0.5 ± 0.3	NA	NA	5.32 ± 0.4	$0.9947 \pm 4\text{e-}04$	0.9952 ± 0.0015	0.9942 ± 0.0021
Expanded 1 [e]	0.98	-2.3	0.5 ± 0.3	$0.5086 \pm 1\text{e-}04$	$0.49997 \pm 2.6\text{e-}07$	5.32 ± 0.4	$0.9947 \pm 4\text{e-}04$	$0.9779 \pm 4\text{e-}04$	$1.0121 \pm 4\text{e-}04$
Expanded 2 [e]	0.98	-2.3	0.5 ± 0.3	$0.5086 \pm 1\text{e-}04$	$0.49997 \pm 2.6\text{e-}07$	5.32 ± 0.4	$0.9947 \pm 4\text{e-}04$	$0.9779 \pm 4\text{e-}04$	$1.0122 \pm 4\text{e-}04$

[a] Value from linear regression of $\delta^{15}N^{bulk}$ against [-flnf/(1-f)] (using standard Rayleigh equation, Eq. (2)). (The calculated value of $\delta^{15}N^{s0}$ is the intercept of Eq. (2)).

[b] Experimentally measured $\delta^{15}N$ value for NH$_2$OH (Sutka et al., 2006)

[c] Calculated value ± standard error.

[d] KIE values were calculated from $\varepsilon_{N\text{-bulk}}$, $\varepsilon_{N\text{-}\alpha}$, or $\varepsilon_{N\text{-}\beta}$ values obtained via linear regression of $\delta^{15}N^{bulk}$, $\delta^{15}N^{\alpha}$, or $\delta^{15}N^{\beta}$ against [-flnf/(1-f)]).

[e] For the Expanded Rayleigh model, $\alpha_{N\text{-bulk}}$ was determined with the standard Rayleigh approach, ρ was determined via nonlinear regression (nonlinear model 1 or 2, Eq. (29) or Eq. (30)), and τ was determined by averaging $^{14}N^{\alpha}/^{14}N^{bulk}$ for every step of the reaction. Then $\alpha_{N\text{-}\alpha}$ and $\alpha_{N\text{-}\beta}$ were calculated with Eq. (21) or Eq. (22) and converted to KIE values using Eq. (10).

Table S16. N$_2$O concentrations and product δ values ($\delta^{15}N^{bulk}$, $\delta^{15}N^{\alpha}$, and $\delta^{15}N^{\beta}$) reported for N$_2$O production from NO by purified *Histoplasma capsulatum* (fungal) P450 NOR [isotopic data previously published (Yang et al., 2014)].

Replicate	Time (min.)	[N$_2$O] (nmol/mL)	f	$\delta^{15}N^{bulk}$	$\delta^{15}N^{\alpha}$	$\delta^{15}N^{\beta}$	SP
A	5	12.24	0.87	-37.68	-30.08	-45.26	15.18
A	8	17.07	0.81	-37.89	-29.18	-46.57	17.39
A	11	19.16	0.79	-37.51	-29.5	-45.49	16
A	14	22.82	0.75	-37.23	-29.5	-44.93	15.44
A	17	24.71	0.73	-37.92	-28.08	-47.69	19.61
A	20	28.74	0.68	-38.43	-28.52	-48.25	19.73
A	23	27.32	0.70	-38.55	-28.4	-48.72	20.32
A	26	27.87	0.69	-38.98	-28.05	-49.92	21.87
A	32	35.02	0.62	-37.92	-27.09	-48.76	21.68
A	38	37.23	0.59	-39.04	-27.23	-50.85	23.62
A	51	43.73	0.52	-39.05	-27.08	-51.02	23.95
A	61	43.9	0.52	-39.43	-26.81	-52.07	25.26
A	71	46.38	0.49	-39.37	-27	-51.75	24.75
B	11	18.13	0.80	-38.68	-31.03	-46.32	15.29
B	14	17.4	0.81	-38.85	-29.37	-48.25	18.88
B	17	21.99	0.76	-38.76	-29.04	-48.41	19.37
B	20	26.36	0.71	-39.45	-30.01	-48.82	18.81
B	26	30.16	0.67	-40.14	-30.24	-49.74	19.5
B	29	32.78	0.64	-39.75	-29.51	-49.76	20.25
B	31	35.95	0.60	-40.22	-28.88	-51.46	22.57
B	34	36.22	0.60	-40.22	-29.4	-50.93	21.53
B	38	36.38	0.60	-40.33	-29	-51.56	22.56
B	41	39.27	0.57	-40.42	-28.64	-52.22	23.58
B	51	43.62	0.52	-40.82	-28.42	-53.38	24.96
B	61	45.26	0.50	-40.75	-28.11	-53.62	25.51
B	71	47.92	0.47	-41.05	-28.02	-54.39	26.36
C	11	20.33	0.78	-37.7	-30.22	-45.16	14.93

C	14	20.69	0.77	-38.5	-29.12	-47.8	18.67
C	17	25.06	0.72	-38.89	-28.34	-49.36	21.01
C	20	29.43	0.68	-38.85	-29.22	-48.4	19.19
C	23	27.55	0.70	-39.56	-27.56	-51.68	24.12
C	26	31.08	0.66	-39.33	-27.5	-51.28	23.78
C	29	32.21	0.65	-39.73	-27.18	-52.39	25.21
C	32	35.06	0.61	-39.43	-26.74	-52.24	25.5
C	35	36.1	0.60	-39.88	-26.4	-53.48	27.08
C	38	37.5	0.59	-39.78	-27.26	-52.42	25.16
C	41	38.41	0.58	-40.13	-27.29	-53.1	25.81
C	51	44.06	0.52	-40.16	-26.82	-53.63	26.81
C	71	52.38	0.42	-40.17	-25.79	-54.67	28.88

Table S17. Comparison of values calculated for the standard and Expanded Rayleigh models for N₂O production from NO by purified *Histoplasma capsulatum* (fungal) P450 NOR [calculated using previously published isotopic data (Yang et al., 2014)].

Model	Replicate	Extent of reaction [f value(s)]		R^2 [a]	$\delta^{15}N^{s0}$ calculated [a] (‰)	ρ	τ	$\varepsilon_{N\text{-bulk}}$ [a] (‰)	KIE $^{15}N^{\text{bulk}}$ [a]	KIE $^{15}N^{\alpha}$	KIE $^{15}N^{\beta}$
Standard [b]	A	all	0.49-0.87	0.68	-44.72	NA	NA	7.81	0.9922	1.0134	0.9718
	B	all	0.47-0.81	0.94	-48.32	NA	NA	10.63	0.9895	1.0094	0.9690
	C	all	0.42-0.78	0.69	-46.39	NA	NA	8.87	0.9912	1.0154	0.9673
	Avg ± SD	all	NA	NA	NA	NA	NA	9.1 ± 1.42 [c]	0.9910 ± 0.0014 [c]	1.0127 ± 0.003 [c]	0.9694 ± 0.0022 [c]
Expanded [d]	A	early	0.81	0.68	-44.72	0.5045	0.49998	7.81	0.9922	0.9833	1.0013
	A	early	0.79	0.68	-44.72	0.5041	0.49999	7.81	0.9922	0.9841	1.0006
	B	early	0.80	0.94	-48.32	0.5040	0.49999	10.63	0.9895	0.9817	0.9974
	B	early	0.81	0.94	-48.32	0.5049	0.49998	10.63	0.9895	0.9798	0.9993
	C	early	0.78	0.69	-46.39	0.5039	0.49999	8.87	0.9912	0.9836	0.9990
	C	early	0.77	0.69	-46.39	0.5049	0.49998	8.87	0.9912	0.9816	1.0010
	Avg ± SD	early	NA	NA	NA	0.5044 ± 5e-04 [e]	0.49998 ± 1.58e-06 [e]	NA	NA	0.9823 ± 0.0016 [e]	0.9998 ± 0.0015 [e]
Expanded [d]	A	late	0.52	0.68	-44.72	0.5062	0.49998	7.81	0.9922	0.98	1.0048
	A	late	0.52	0.68	-44.72	0.5065	0.49998	7.81	0.9922	0.9794	1.0055
	A	late	0.49	0.68	-44.72	0.5064	0.49998	7.81	0.9922	0.9796	1.0052
	B	late	0.52	0.94	-48.32	0.5064	0.49998	10.63	0.9895	0.9769	1.0024
	B	late	0.50	0.94	-48.32	0.5066	0.49998	10.63	0.9895	0.9766	1.0027
	B	late	0.47	0.94	-48.32	0.5068	0.49998	10.63	0.9895	0.9762	1.0031
	C	late	0.52	0.69	-46.39	0.5069	0.49998	8.87	0.9912	0.9776	1.0052
	Avg ± SD	late	NA	NA	NA	0.5066 ± 2e-04 [e]	0.49998 ± 8.2e-07 [e]	NA	NA	0.9781 ± 0.0016 [e]	1.0041 ± 0.0013 [e]

[a] Values were calculated by analyzing each replicate separately (13 observations/replicate) via linear regression of $\delta^{15}N^{\text{bulk}}$ against [-flnf/(1-f)] (using standard Rayleigh equation, Eq. (2)). (The calculated value of $\delta^{15}N^{s0}$ is the intercept of Eq. (2) when y = $\delta^{15}N^{\text{bulk}}$). For each individual observation listed for the Expanded Rayleigh model, the corresponding standard Rayleigh value from the appropriate replicate is listed.

[b] KIE values were calculated from $\varepsilon_{\text{N-bulk}}$, $\varepsilon_{\text{N-}\alpha}$, or $\varepsilon_{\text{N-}\beta}$ values obtained via linear regression of $\delta^{15}N^{\text{bulk}}$, $\delta^{15}N^{\alpha}$, or $\delta^{15}N^{\beta}$ against [-flnf/(1-f)]). The standard Rayleigh model values presented here differ slightly from the previously published values (Yang et al., 2014) due to our exclusion of the earliest observation(s) from each replicate (*i.e.*, observations with the highest values of f were excluded).

[c] Average value ± standard deviation calculated for values from three separate replicates (13 observations/replicate) using the standard Rayleigh model.

[d] For the Expanded Rayleigh model, bulk values ($\alpha_{\text{N-bulk}}$, $\varepsilon_{\text{N-bulk}}$, and KIE $^{15}N^{\text{bulk}}$) were determined by analyzing each replicate separately (13 observations/replicate) with the standard Rayleigh approach. Values of ρ and τ were calculated for each observation using Eq. (23) ($\rho = {}^{15}N^{\alpha}/{}^{15}N^{\text{bulk}}$) and Eq. (24) ($\tau = {}^{14}N^{\alpha}/{}^{14}N^{\text{bulk}}$). Then $\alpha_{\text{N-}\alpha}$ and $\alpha_{\text{N-}\beta}$ were calculated for each individual observation with Eq. (21) or Eq. (22) and converted to KIE values using Eq. (10).

[e] Average value ± standard deviation calculated for values from for six (early) or seven (late) individual observations using the Expanded Rayleigh model. Individual early-reaction or late-reaction values from all three replicates were pooled and averaged.
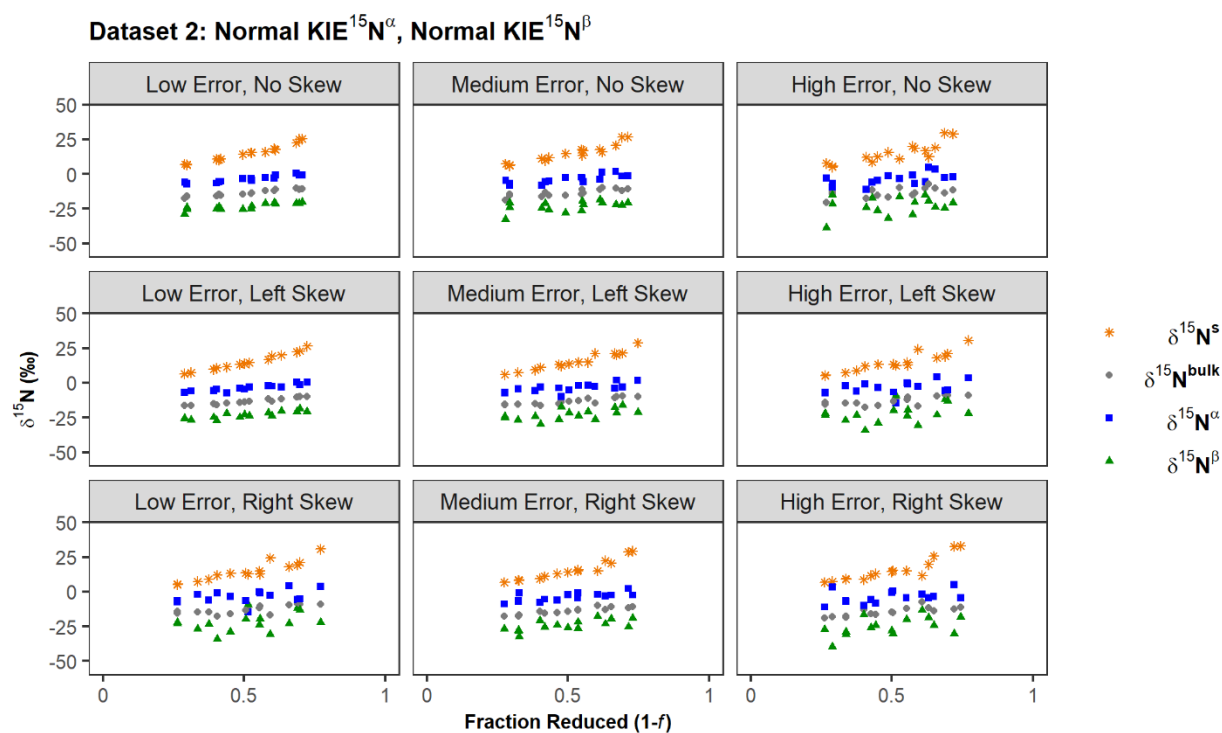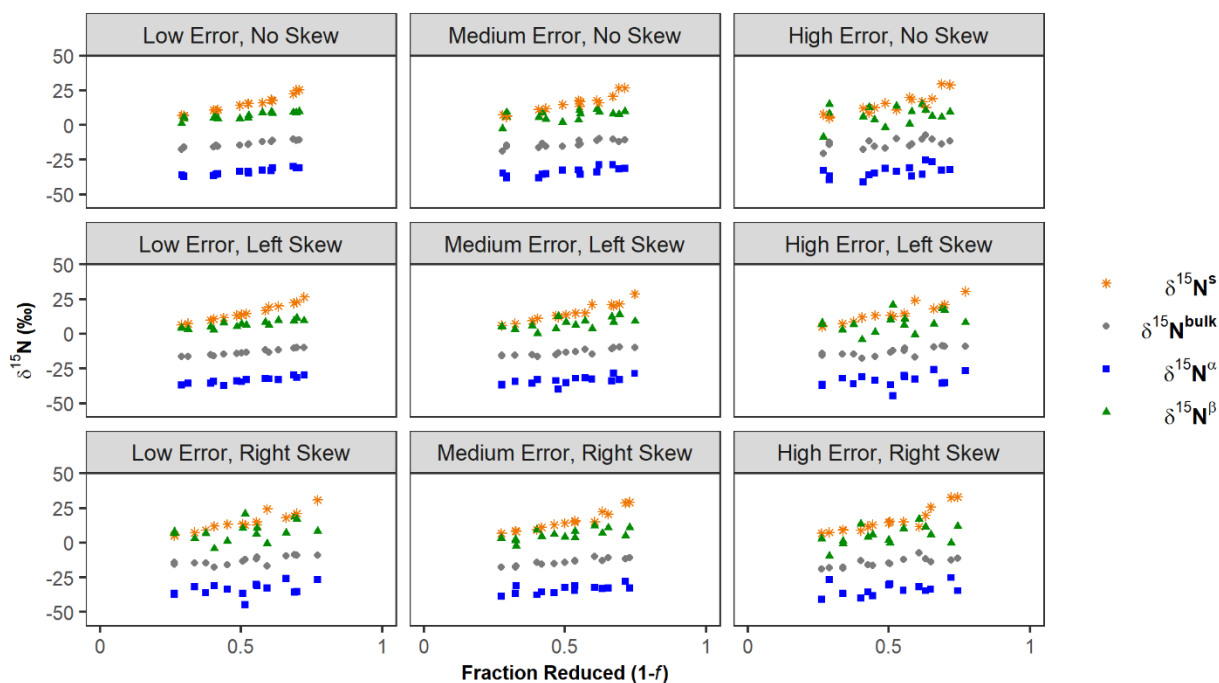
Figure S1. Example datasets derived from Dataset 2 with varying levels of error and types of skewness.

Each panel shows a single dataset (representative of 1000 simulated datasets) consisting of three replicates with five timepoints each. All graphs were derived from Dataset 2 ($\delta^{15}N^{s0}$ = 0‰, $\varepsilon_{N\text{-bulk}}$ = -20‰, KIE $^{15}N^{\alpha}$ = 1.0103, and KIE $^{15}N^{\beta}$ = 1.0308). Each level of error and type of skewness is described in Table 2.
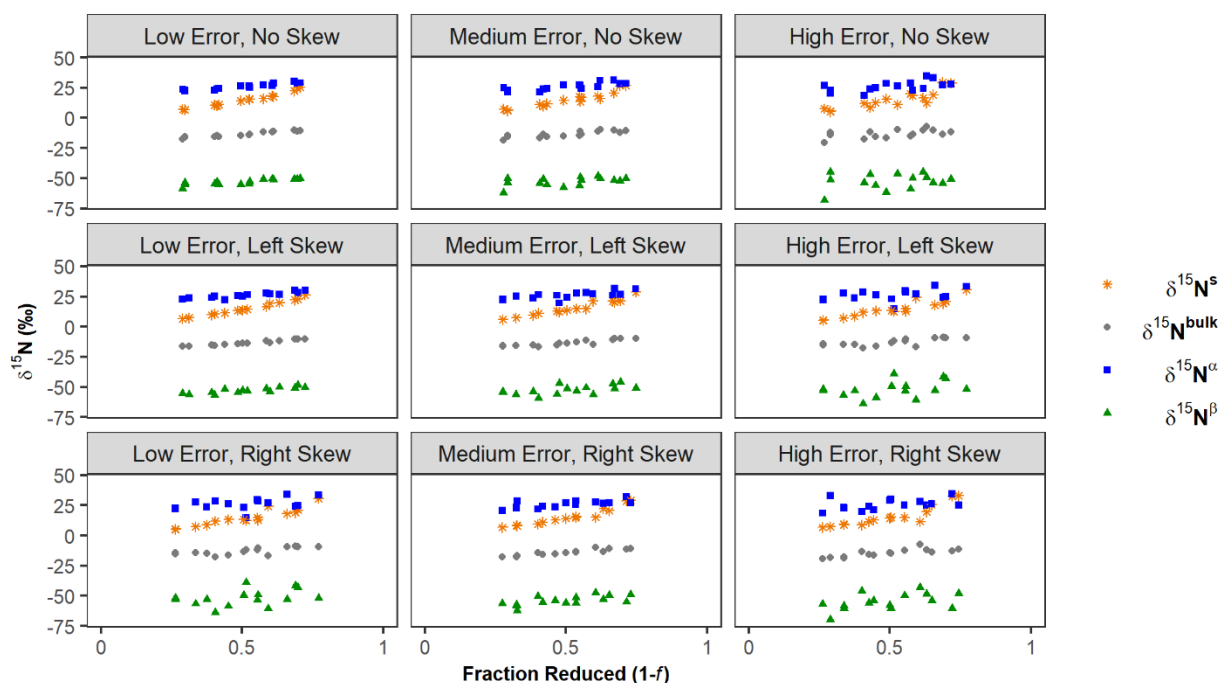
Figure S2. Example datasets derived from Dataset 3 with varying levels of error and types of skewness.

Each panel shows a single dataset (representative of 1000 simulated datasets) consisting of three replicates with five timepoints each. All graphs were derived from Dataset 3 ($\delta^{15}N^{s0}$ = 0‰, $\varepsilon_{N\text{-bulk}}$ = -20‰, KIE $^{15}N^{\alpha}$ = 0.9810, and KIE $^{15}N^{\beta}$ = 1.0631). Each level of error and type of skewness is described in Table 2.
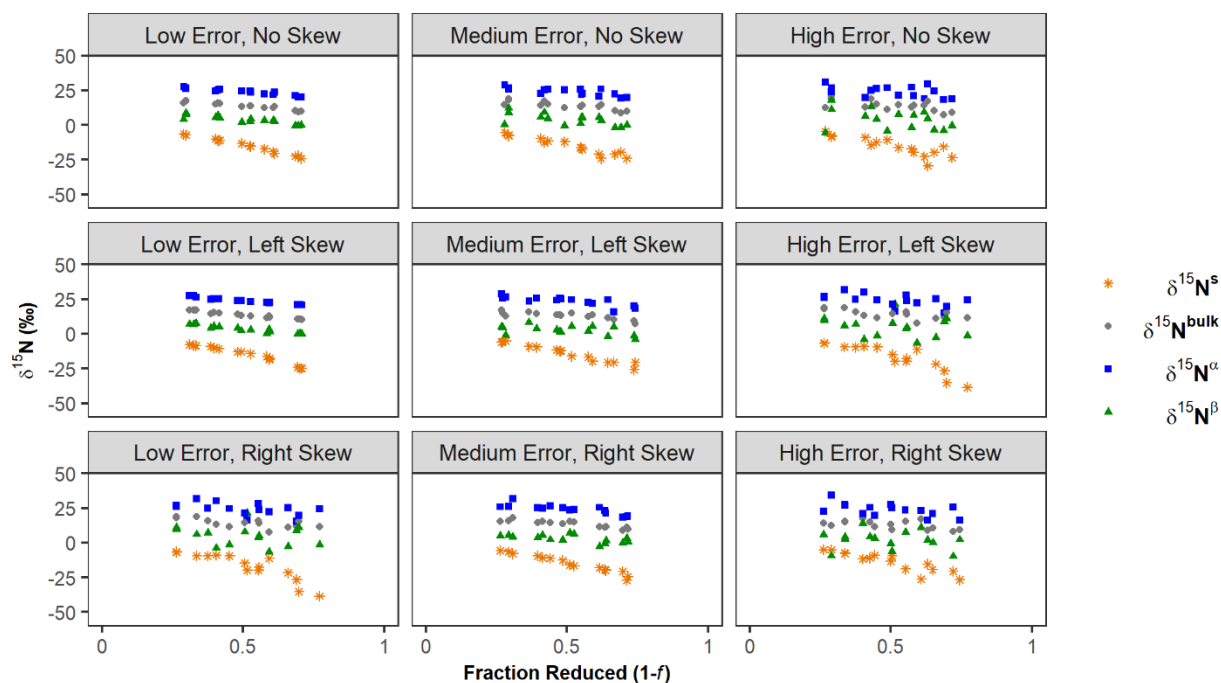
Figure S3. Example datasets derived from Dataset 4 with varying levels of error and types of skewness.

Each panel shows a single dataset (representative of 1000 simulated datasets) consisting of three replicates with five timepoints each. All graphs were derived from Dataset 4 ($\delta^{15}N^{s0}$ = 0‰, $\varepsilon_{\text{N-bulk}}$ = 20‰, KIE $^{15}N^{\alpha}$ = 0.9706, and KIE $^{15}N^{\beta}$ = 0.9903). Each level of error and type of skewness is described in Table 2.

Figure S4. Example datasets derived from Dataset 5 with varying levels of error and types of skewness.

Each panel shows a single dataset (representative of 1000 simulated datasets) consisting of three replicates with five timepoints each. All graphs were derived from Dataset 5 ($\delta^{15}N^{s0} = 0‰$, $\varepsilon_{N\text{-bulk}} = -20‰$, KIE $^{15}N^{\alpha} = 1.0417$, and KIE $^{15}N^{\beta} = 1.0000$). Each level of error and type of skewness is described in Table 2.
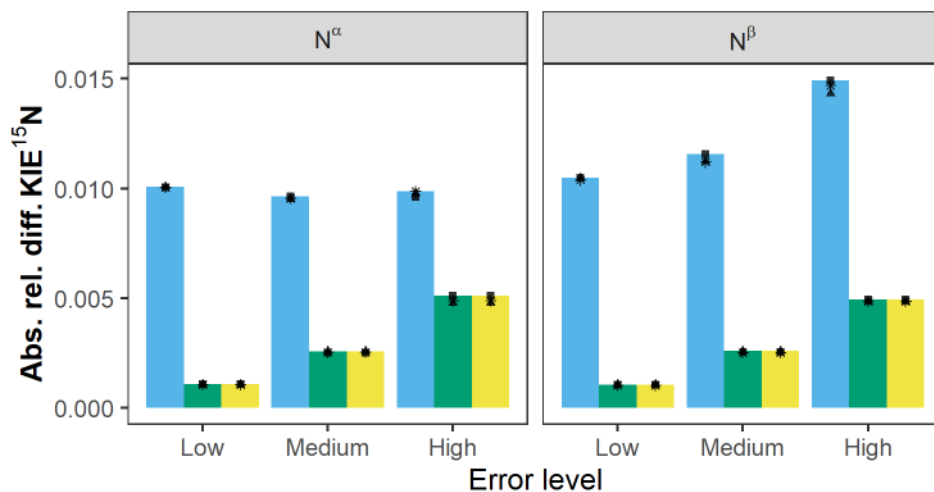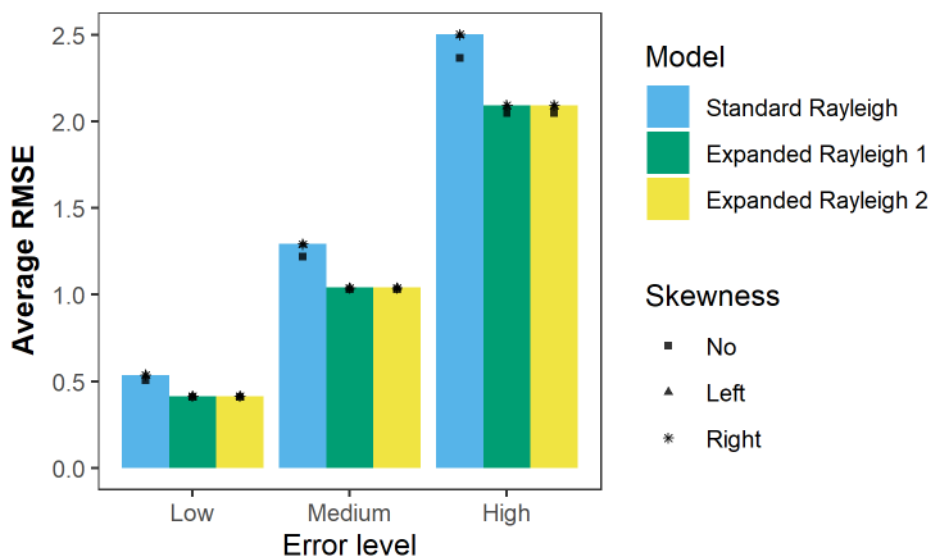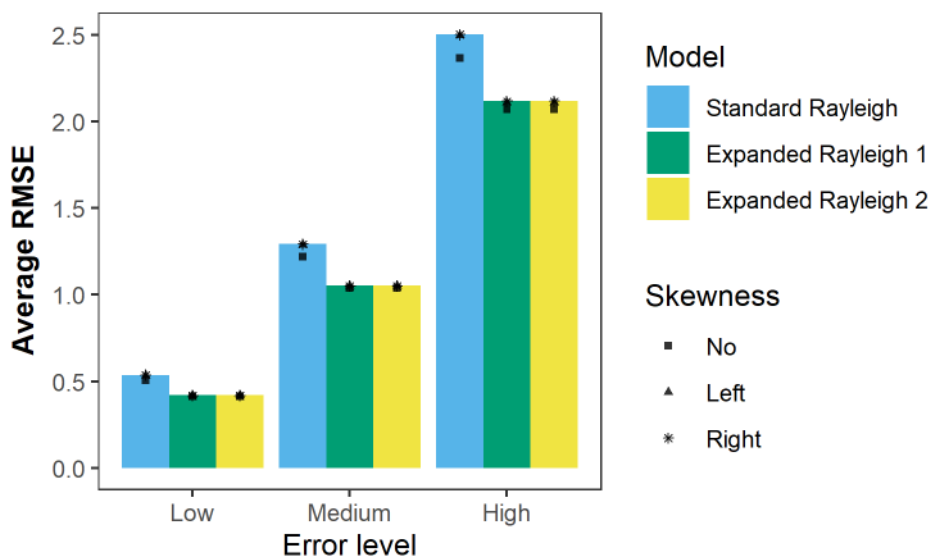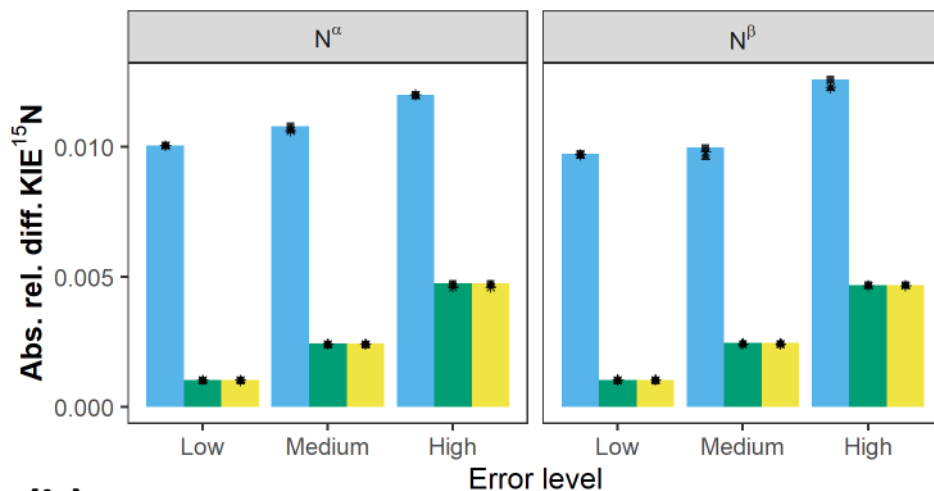
Figure S5. Dataset 2: Comparison of the accuracy and goodness of fit of the standard Rayleigh model and Expanded Rayleigh models 1 and 2.

A. Comparison of the accuracy of KIE $^{15}N^\alpha$ and KIE $^{15}N^\beta$ values. For each model, the absolute relative difference for KIE $^{15}N^\alpha$ and KIE $^{15}N^\beta$ values (average for 1000 simulated datasets derived from Dataset 2) are shown. Actual values for Dataset 2: $\delta^{15}N^{s0}$ = 0‰, $\varepsilon_{N\text{-bulk}}$ = -20‰, KIE $^{15}N^\alpha$ = 1.0103, and KIE $^{15}N^\beta$ = 1.0308. Absolute relative difference (Eq. (31)) is the absolute value of the difference between the estimated value and actual value divided by the actual value (|(estimate – actual)/actual|). B. Comparison of the average RMSE values for each set of 1000 simulated datasets derived from Dataset 2. Both the standard Rayleigh model and the Expanded Rayleigh model (1 and 2) use $\delta^{15}N^{bulk}$ as the

dependent variable, so average RMSE values can be compared directly. Lower RMSE values indicate a better goodness of fit. At each error level (low, medium, and high), the absolute relative difference value (A) or average RMSE (B) is depicted with a symbol that represents skewness type as shown in the legend. Note that in most cases these symbols overlap.

(a) Dataset 3: Inverse KIE $^{15}N^{\alpha}$, Normal KIE $^{15}N^{\beta}$
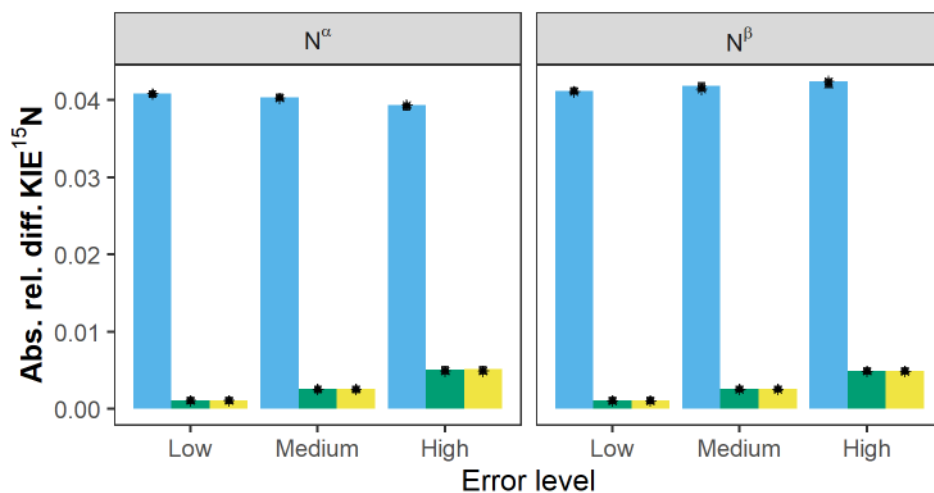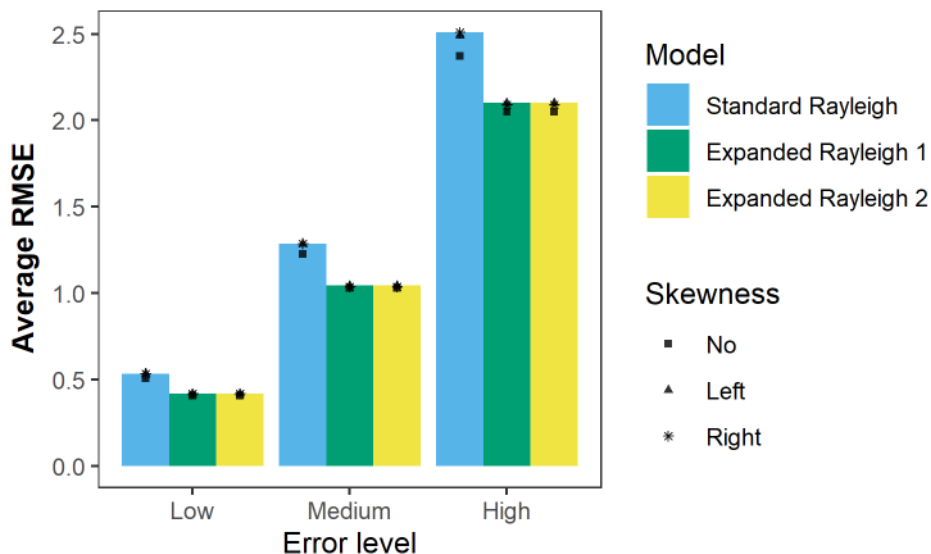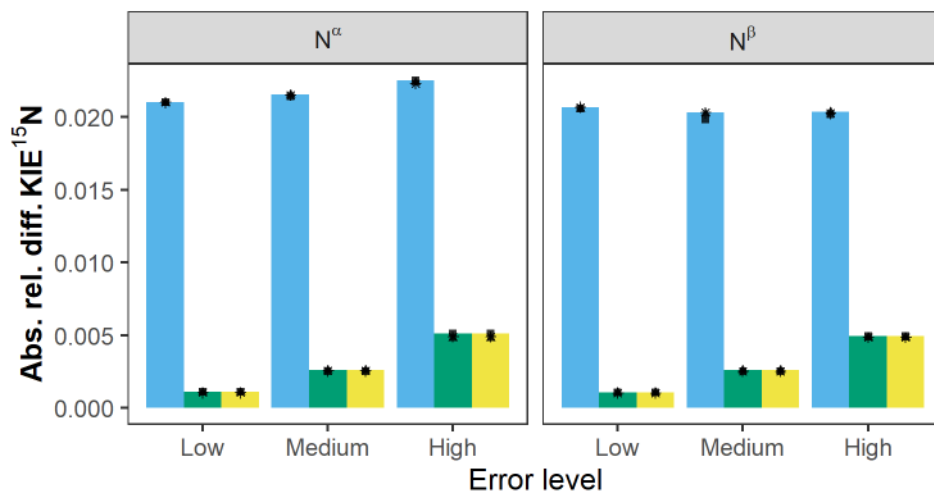
(b)

Figure S6. Dataset 3: Comparison of the accuracy and goodness of fit of the standard Rayleigh model and Expanded Rayleigh models 1 and 2.

A. Comparison of the accuracy of KIE $^{15}N^{\alpha}$ and KIE $^{15}N^{\beta}$ values. For each model, the absolute relative difference for KIE $^{15}N^{\alpha}$ and KIE $^{15}N^{\beta}$ values (average for 1000 simulated datasets derived from Dataset 3) are shown. Actual values for Dataset 3: $\delta^{15}N^{s0}$ = 0‰, $\varepsilon_{N\text{-bulk}}$ = -20‰, KIE $^{15}N^{\alpha}$ = 0.9810, and KIE $^{15}N^{\beta}$ = 1.0631. Absolute relative difference (Eq. (31)) is the absolute value of the difference between the estimated value and actual value divided by the actual value (|(estimate – actual)/actual|). B. Comparison of the average RMSE values for each set of 1000 simulated datasets derived from Dataset 3. Both the standard Rayleigh model and the Expanded Rayleigh model (1 and 2) use $\delta^{15}N^{bulk}$ as the

dependent variable, so average RMSE values can be compared directly. Lower RMSE values indicate a better goodness of fit. At each error level (low, medium, and high), the absolute relative difference value (A) or average RMSE (B) is depicted with a symbol that represents skewness type as shown in the legend. Note that in most cases these symbols overlap.
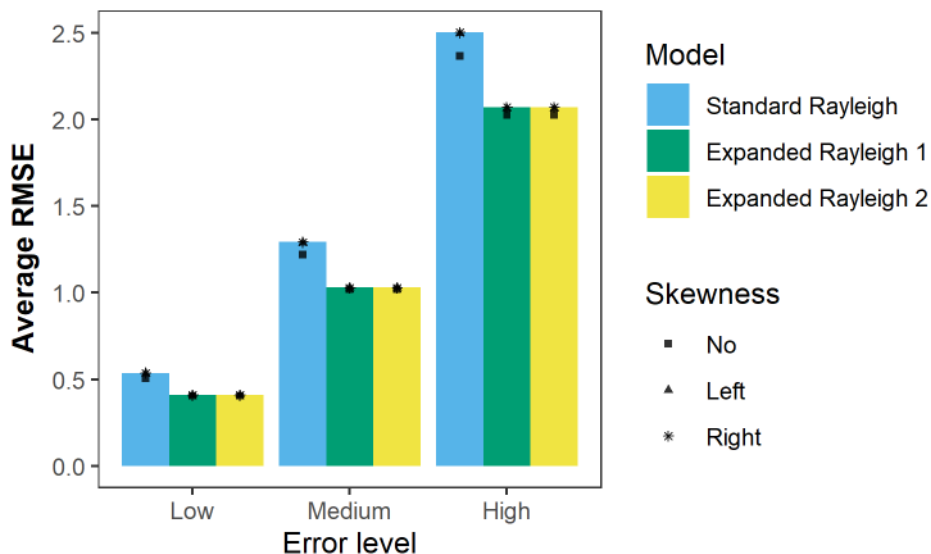
Figure S7. Dataset 4: Comparison of the accuracy and goodness of fit of the standard Rayleigh model and Expanded Rayleigh models 1 and 2.

A. Comparison of the accuracy of KIE $^{15}N^{\alpha}$ and KIE $^{15}N^{\beta}$ values. For each model, the absolute relative difference for KIE $^{15}N^{\alpha}$ and KIE $^{15}N^{\beta}$ values (average for 1000 simulated datasets derived from Dataset 4) are shown. Actual values for Dataset 4: $\delta^{15}N^{s0} = 0‰$, $\varepsilon_{\text{N-bulk}} = 20‰$, KIE $^{15}N^{\alpha} = 0.9706$, and KIE $^{15}N^{\beta} = 0.9903$. Absolute relative difference (Eq. (31)) is the absolute value of the difference between the estimated value and actual value divided by the actual value (|(estimate – actual)/actual|). B. Comparison of the average RMSE values for each set of 1000 simulated datasets derived from Dataset 4. Both the standard Rayleigh model and the Expanded Rayleigh model (1 and 2) use $\delta^{15}N^{\text{bulk}}$ as the

dependent variable, so average RMSE values can be compared directly. Lower RMSE values indicate a better goodness of fit. At each error level (low, medium, and high), the absolute relative difference value (A) or average RMSE (B) is depicted with a symbol that represents skewness type as shown in the legend. Note that in most cases these symbols overlap.

Figure S8. Dataset 5: Comparison of the accuracy and goodness of fit of the standard Rayleigh model and Expanded Rayleigh models 1 and 2.

A. Comparison of the accuracy of KIE $^{15}N^{\alpha}$ and KIE $^{15}N^{\beta}$ values. For each model, the absolute relative difference for KIE $^{15}N^{\alpha}$ and KIE $^{15}N^{\beta}$ values (average for 1000 simulated datasets derived from Dataset 5) are shown. Actual values for Dataset 5: $\delta^{15}N^{s0}$ = 0‰, $\varepsilon_{N\text{-bulk}}$ = -20‰, KIE $^{15}N^{\alpha}$ = 1.0417, and KIE $^{15}N^{\beta}$ = 1.0000. Absolute relative difference (Eq. (31)) is the absolute value of the difference between the estimated value and actual value divided by the actual value (|(estimate – actual)/actual|). B. Comparison of the average RMSE values for each set of 1000 simulated datasets derived from Dataset 5. Both the standard Rayleigh model and the Expanded Rayleigh model (1 and 2) use $\delta^{15}N^{bulk}$ as the

dependent variable, so average RMSE values can be compared directly. Lower RMSE values indicate a better goodness of fit. At each error level (low, medium, and high), the absolute relative difference value (A) or average RMSE (B) is depicted with a symbol that represents skewness type as shown in the legend. Note that in most cases these symbols overlap.

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