Supplementary figures for

Isotopomer labeling and oxygen dependence of hybrid nitrous oxide production

Colette L. Kelly,[1,2*] Nicole M. Travis,[1] Pascale Anabelle Baya,[1] Claudia Frey,[3] Xin Sun,[4] Bess B. Ward,[5] and Karen L. Casciotti[1]

[1]Department of Earth System Science, Stanford University, Stanford, CA 94305, U.S.A.
[2]Department of Marine Chemistry and Geochemistry, Woods Hole Oceanographic Institution, Woods Hole, MA 02543, U.S.A.
[3]Department of Environmental Science, University of Basel, Basel, Switzerland
[4]Department of Global Ecology, Carnegie Institution for Science, Stanford, CA 94305, U.S.A.
[5]Department of Geosciences, Princeton University, Princeton, NJ 08544, U.S.A.

Correspondence to: Colette L. Kelly (email: colette.kelly@whoi.edu).

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Tables S1-S4 included separately as excel files in this .zip archive.

Supplemental Figures

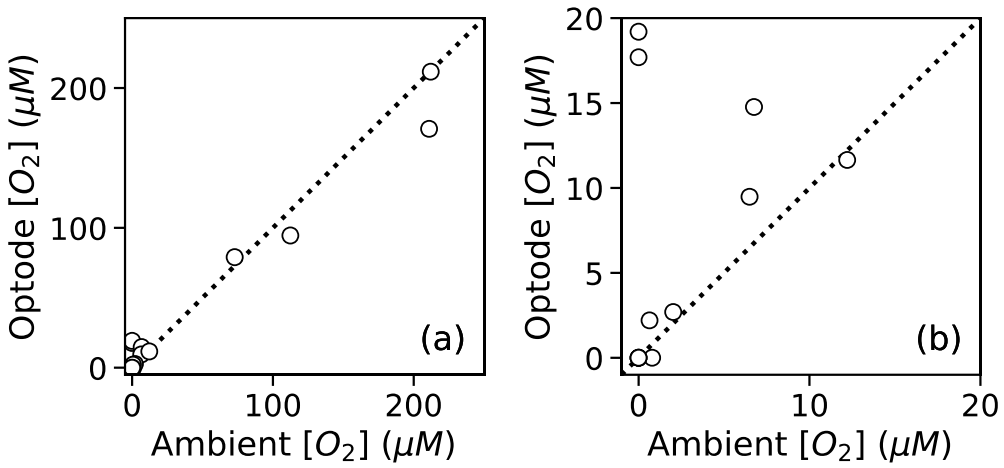

Figure S1. [O_2] measured by chemiluminescent optodes mounted inside sample bottles vs. ambient [O_2] measured by a Seabird sensor for the bottles from which samples were taken. Data (circles) are plotted along the full range of [O_2] (a) and zoomed in to 0-20 µM [O_2] (b). The dashed line in each plot is the 1:1 line. High values of optode [O_2] at 0 ambient [O_2] correspond to the two experiments at anoxic depths at station PS2 that were not purged before tracer addition.

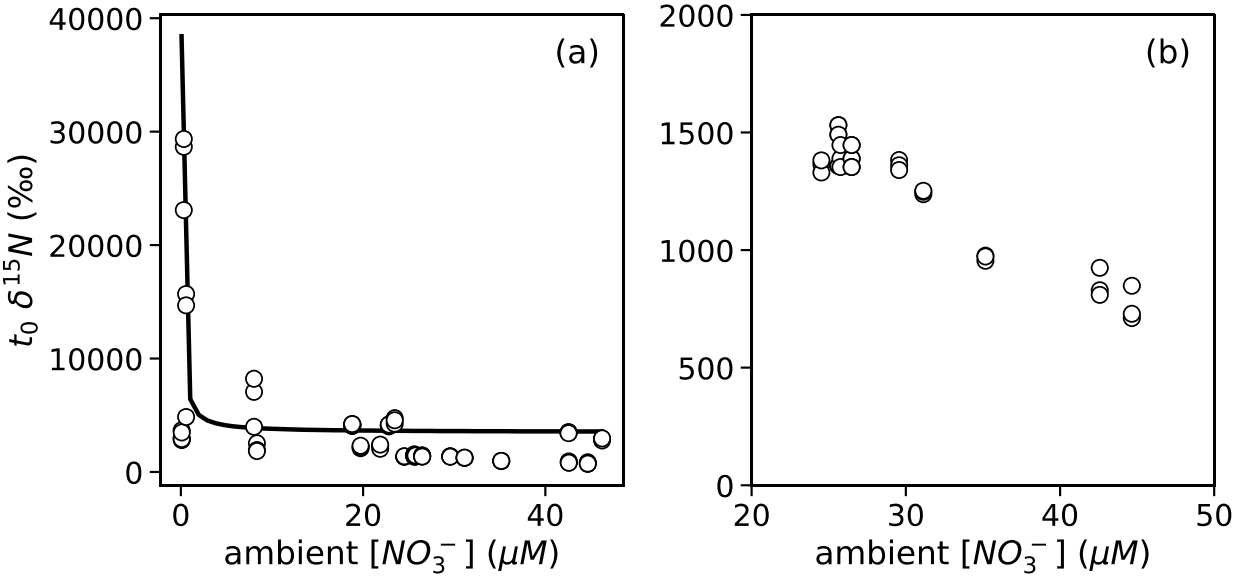

Figure S2. δ(^{15}N-NO_x^-) at t0 vs. ambient [NO_3^-] in ^{15}N-NO_2^- experiments across the full range of ambient [NO_3^-] (a) and from 20-50 µM [NO_3^-] (b).

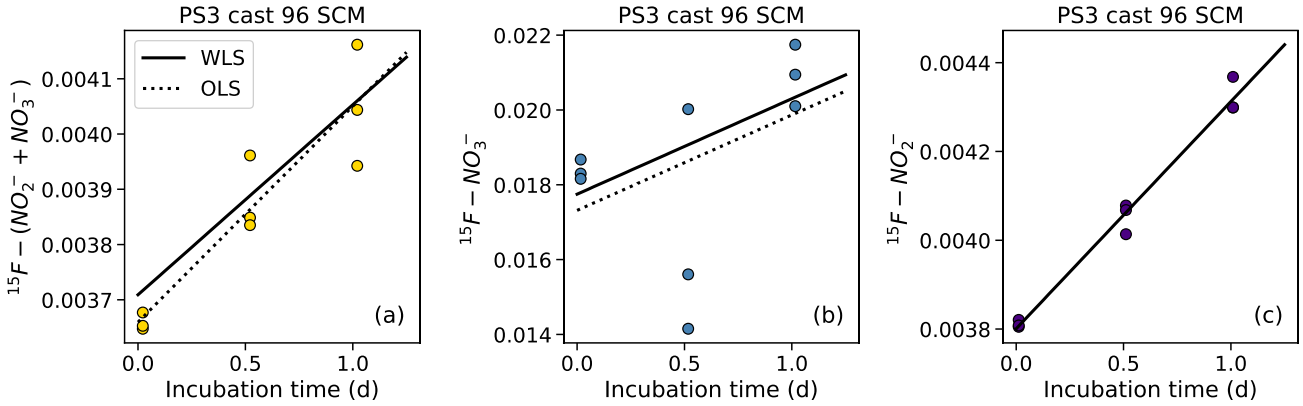

Figure S3. Example ^{15}N time course for the ^{15}N-NH_4^+ incubation experiment (a, yellow), ^{15}N-NO_2^- incubation experiment (b, blue), and ^{15}N-NO_3^- incubation experiment (c, indigo) in the secondary chlorophyll maximum at station PS3. Rates are calculated from the slope of the production atom fraction vs. incubation time. When production atom fraction was calculated with the denitrifier method (^{15}N-NH_4^+ and ^{15}N-NO_2^- experiments), slopes were calculated with a weighted least squares linear regression, weighting each point by its uncertainty. Weighted least squares (dashed line) and ordinary least squares (solid line) slopes are shown in each plot.

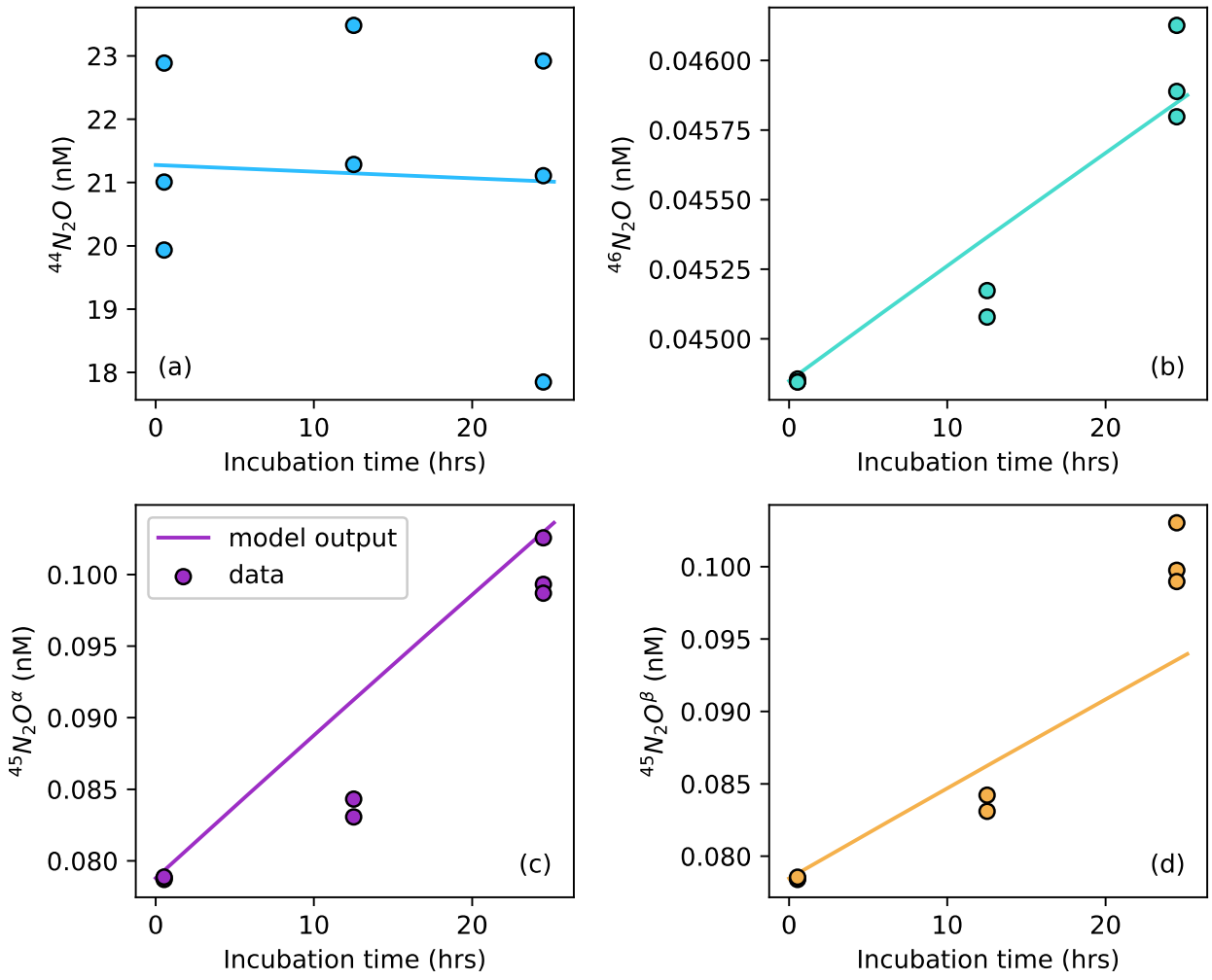

Figure S4. Example time-dependent numerical model fit through N_2O isotopocule data for the ^{15}N-NH_4^+ experiment in the secondary chlorophyll maximum at station PS3. Model output (solid lines) is optimized against the observed $^{44}N_2O$ (a), $^{46}N_2O$ (b), $^{45}N_2O^{\alpha}$ (c), and $^{45}N_2O^{\beta}$ (d) at each timepoint in each tracer experiment.

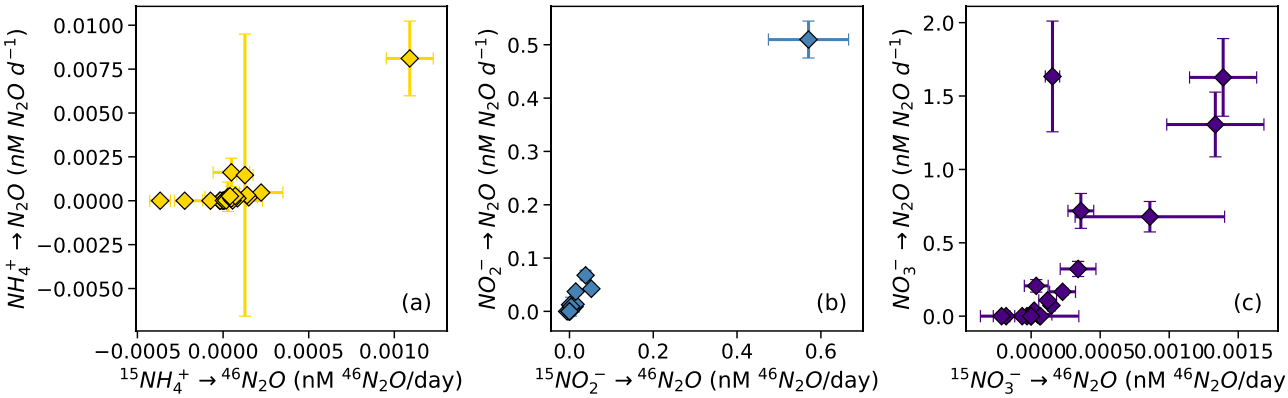

Figure S5. Modeled N_2O production processes are plotted against net $^{46}N_2O$ production to ground-truth the forward-running model. Total N_2O production from solely NH_4^+ is plotted against net $^{46}N_2O$ production from ^{15}N-NH_4^+ (a, yellow); modeled N_2O production from NO_2^- via denitrification is plotted against net $^{46}N_2O$ production from ^{15}N-NO_2^- (b, blue); and modeled N_2O production from NO_3^- via denitrification is plotted against net $^{46}N_2O$ production from ^{15}N-NO_3^- (c, indigo) at all stations and depths. The y-axes achieve greater rates than the x-axes because the y-axes represent the total N_2O production from a given process ($^{44}N_2O$+$^{45}N_2O$+$^{46}N_2O$), while the x-axes represent only the net production of $^{46}N_2O$ from a given ^{15}N-labeled substrate. Since the model cannot produce negative rates, negative net rates of net $^{46}N_2O$ production correspond to modeled N_2O production rates of zero.

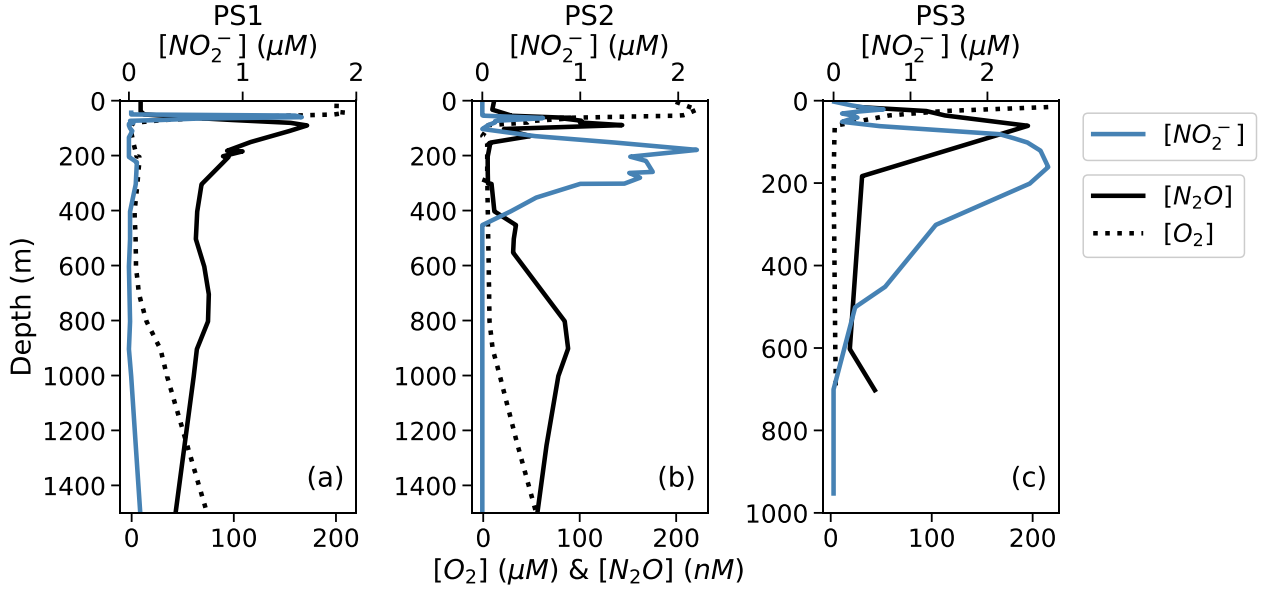

Figure S6. Dissolved [O_2] (dashed black lines), [N_2O] (solid black lines), and [NO_2^-] (solid blue lines) at station PS1 (a), PS2 (b), and PS3 (c). Data from Kelly et al., 2021.

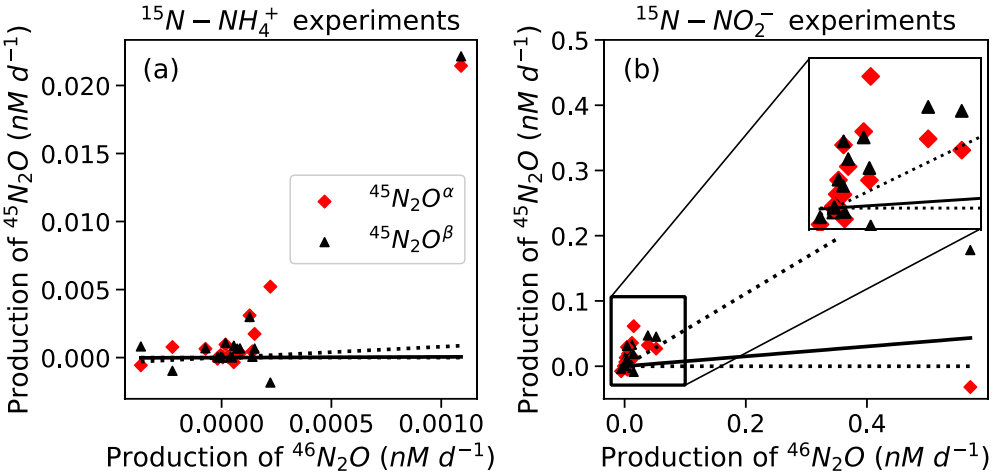

Figure S7. Net production of $^{45}N_2O^{\alpha}$ (red diamonds) and $^{45}N_2O^{\beta}$ (black triangles) vs. $^{46}N_2O$ from ^{15}N-NH_4^+ (a) and ^{15}N-NO_2^- (b). The insert in (b) shows a zoomed-in view of the data. The solid black lines indicate the expected production $^{45}N_2O^{\alpha}$ and $^{45}N_2O^{\beta}$ from a process drawing both N atoms in N_2O from the same substrate pool, based on the atom fraction of the labeled substrate (NH_4^+ or NO_2^-) and a binomial distribution of N_2O isotopocules. Dashed lines indicate the range of expected values, based on the range of atom fractions in each experiment. Production of $^{45}N_2O^{\alpha}$ and $^{45}N_2O^{\beta}$ above this expected production indicate the presence of a hybrid process.

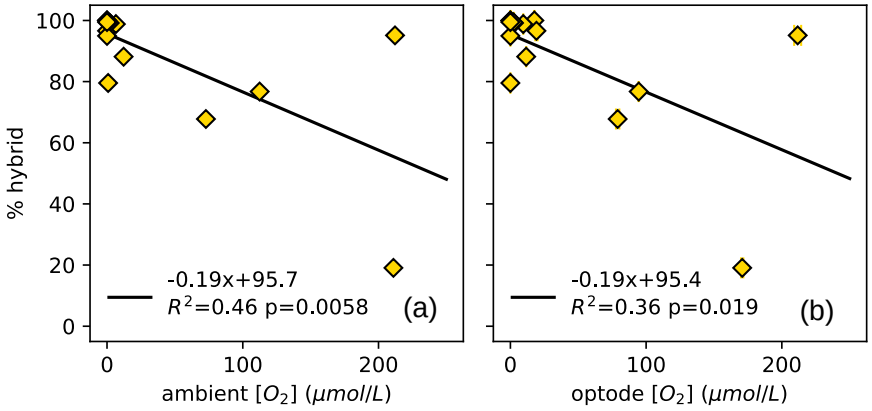

Figure S8. Hybrid production as a percentage of N_2O production from NH_4^+ along a range of ambient [O2] measured by a Seabird sensor for the Niskin bottles from which samples were taken (a) and [O_2] measured by chemiluminescent optodes mounted inside sample bottles (b). Slope, intercept, R^2, and p-values are displayed on each plot for the ordinary least squares regression through the data.

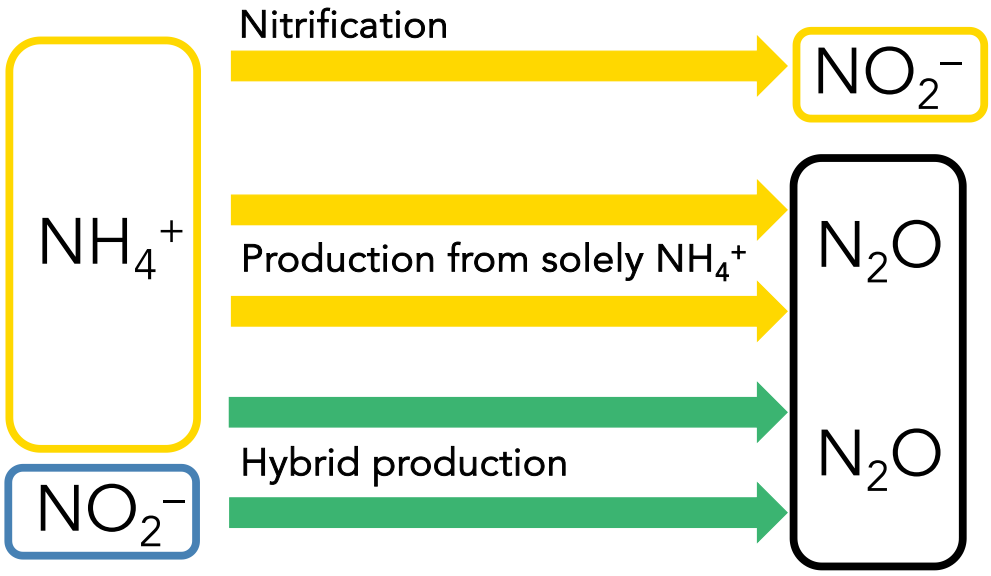

Figure S9. To ensure mass balance in terms of NH_4^+ consumption, N_2O yield (%) during production from NH_4^+ is calculated as {2*(N_2O production from NH_4^+, nM N_2O /day) /[2*(N_2O production from NH_4^+, nM N_2O /day) + hybrid N_2O production, nM N_2O /day + NH_3 oxidation, nM N/day]}*100. N_2O yield (%) during hybrid production is calculated as {hybrid N_2O production, nM N_2O /day /[2*(N_2O production from NH_4^+, nM N_2O /day) + hybrid N_2O production, nM N_2O /day + NH_3 oxidation, nM N/day]}*100.

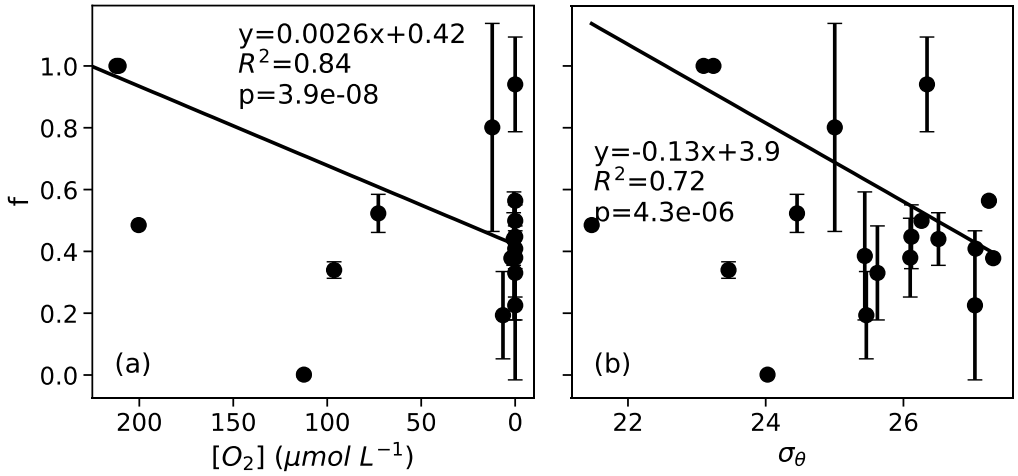

Figure S10. Weighted least squares regressions of *f* against ambient $[O_2]$ (a) and potential density σ_θ (b). Slope, intercept, R^2, and p-values are displayed on each plot for the weighted least squares regression through the data. The value of *f* indicates the proportion of each N atom in N_2O derived from NH_4^+ and NO_2^- during hybrid N_2O production; as approaches 1, more of N^α is derived from NO_2^-. Separation of $^{45}N_2O^\alpha$ and $^{45}N_2O^\beta$ production indicate values of *f* less than or greater than ½.