

Title: Dataset associated with “Characterizing the importance of denitrification for N₂O production in soils using natural abundance and isotopic labelling techniques”

Abstract: Nitrous oxide (N₂O), a potent greenhouse gas that contributes significantly to climate change, is emitted mostly from soils by a suite of microbial metabolic pathways that are nontrivial to identify, and subsequently, to manage. Using either natural abundance or enriched stable isotope methods has aided in identifying microbial sources of N₂O, but each approach has limitations. Here, we conducted a novel pairing of natural abundance and enriched assays on two dissimilar soils, hypothesizing this pairing would better constrain microbial sources of N₂O. We incubated paired natural abundance and enriched soils from a corn agroecosystem and a subalpine forest in the laboratory at 10-95% soil saturation for 28 hr. The natural abundance method measured intramolecular site preference (SP) from emitted N₂O, whereas the enriched method measured emitted ¹⁵N₂O from soils amended with ¹⁵N-labelled substrate. The isotopic composition of emitted N₂O was measured using a laser-based N₂O isotopic analyzer, yielding two key findings. First, both methods revealed that denitrification was the primary source of N₂O in all soils: isotopic enrichment revealed clear NO₃⁻ reduction to N₂O, while SP indicated a likely combination of fungal and bacterial denitrification. Second, we quantified, to our knowledge for the first time, persistent (>55%) β-position-specific enrichment in N₂O emitted from ¹⁵NO₃⁻-amended soils. This counter-intuitive enrichment pattern could be indicative of co-denitrification, an understudied but potentially important contributor to N₂O emissions. Our work revealed the ubiquity of denitrification among the soils tested. Future pairings of natural abundance and enriched methods could better characterize diverse denitrification pathways.

Contact: Emily Stuchiner (emily.stuchiner@colostate.edu)

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<http://dx.doi.org/10.25675/10217/229664>

Associated publications:

Baron, J. S., H. M. Rueth, A. M. Wolfe, K. R. Nydick, E. J. Allstott, J. T. Minear, and B. Moraska (2000), Ecosystem responses to nitrogen deposition in the Colorado Front Range. *Ecosystems*, 3(4), 352–368, doi:10.1007/s100210000032.

Stuchiner, ER, Weller, ZD, von Fischer, JC. An approach for calibrating laser-based N₂O isotopic analyzers for soil biogeochemistry research. *Rapid Communications in Mass Spectrometry*, 2020;35(3): e8978. <https://doi.org/10.1002/rcm.8978>

Stuchiner, E. R., & von Fischer, J. C. (2022). Characterizing the importance of denitrification for N₂O production in soils using natural abundance and isotopic labeling

techniques. *Journal of Geophysical Research: Biogeosciences*, 127, e2021JG006555. <https://doi.org/10.1029/2021JG006555>

Stuchiner, E. R. (2021). Revealing the controls of microbial nitrous oxide (N₂O) production and consumption using stable isotope methods. Doctoral dissertation. Colorado State University.

Zhang, H., and K. Yemoto (2018), UAS-based remote sensing applications on the Northern Colorado Limited Irrigation Research Farm, *Int. J. Precis. Agric. Aviat.*, 1(1), 1–10, doi:10.33440/j.ijpaa.20190202.50.

Format of data files: Excel file (.xlsx), Comma delimited (.csv), .pdf

Location where data were collected: Loch Vale Watershed, Rocky Mountain National Park (specific details provided in Baron et al. 2000) & Limited Irrigation Research Farm, north of Greeley, Colorado (specific details provided in Zhang and Yemoto 2018) & Department of Biology, Colorado State University, Fort Collins, Colorado

Time period during which data were collected: 2018-06-12-2018-07-16

File Information: All the data presented in the study is included in the Excel file, “metadata_enrichment_study_data_repository_final.xlsx”. For preservation purposes, data tables have been converted to .csv files as well. All flux rates are processed data, wherein raw N₂O concentrations were analyzed to calculate flux rates. All delta values are processed from raw instrument output through a calibration algorithm (details provided in associated manuscript). Fractional abundance and atom % (AP) values are calculated from calibrated N₂O isotopomer concentration data (details provided in associated manuscript). Soil inorganic N and microbial biomass C and N are processed values wherein raw instrument output was taken and associated gravimetric soil moisture or other soil properties were used to calculate each value per gram of dry soil (details provided in associated manuscript). Soil fungi:bacteria ratios, pH, and soil organic C and N are all raw values.

There are 14 total files – the Excel file with all data, the corresponding .csv files, a .pdf file with a formatted data table, and this README file.

Natural abundance_SP – The intramolecular natural abundance site preference (SP) of the N₂O isotopomers $\delta^{15}\text{N}^{\alpha}$ - $\delta^{15}\text{N}^{\beta}$. All values are in ‰. These values were calculated by processing $^{15}\text{N}^{14}\text{N}^{16}\text{O}$ and $^{14}\text{N}^{15}\text{N}^{16}\text{O}$ isotopomer concentrations through the calibration algorithm (see associated manuscript), converting the calibrated concentrations into δ values, and then calculating SP by subtracting $\delta^{15}\text{N}^{\beta}$ from $\delta^{15}\text{N}^{\alpha}$.

Natural abundance_18Odata – The $\delta^{18}\text{O}$ values from the measured N₂O molecules. All values are in ‰. These values were calculated by processing $^{14}\text{N}^{14}\text{N}^{18}\text{O}$ isotopomer concentrations through the calibration algorithm (see associated manuscript) and then converting the calibrated concentrations into δ values.

Natural abundance_N2Ofluxrates – The N₂O flux rates were calculated by taking the instrument output N₂O concentrations, processing them through the calibration algorithm (see associated manuscript), and then converting the calibration N₂O concentrations into flux rates by accounting for the incubation period, the incubation vessel size, the amount of soil in the incubation vessel, the molar mass of N₂O-N, and then converting those values to a daily flux rate.

Natural abundance_logisticpreds – Predicted N₂O emission rates using logistic regression. (see Natural abundance_logisticpreds_chart for details)

Natural abundance_logisticpreds_chart – Each parameter in the chart corresponds to a component in the logistic regression equation that is used to predictively model N₂O emission rates for each soil type. The corresponding parameters differ by soil treatment because each soil has different N₂O production rates at each soil moisture, and these different values were used to parameterize the model for each soil treatment.

Enriched_N2Ofluxrates – Same as “Natural abundance_N2Ofluxrates” description, but this is with the isotopically enriched soils. Isotopic enrichment should not have impacted the N₂O flux rate.

Enriched_d15N and AP – This dataset includes the concentration of the α and β N₂O isotopomers, the fractional abundance (H/H+L) of each isotopomer, the α and β AP values, and the calculated δ values (as described in “Natural abundance_SP” and “Natural abundance_18O” above) for the isotopically enriched soils.

Soil properties – All soils were sampled for the below physical and chemical properties during the Summer of 2018.

- Inorganic nitrogen
- Microbial biomass C and N
- Fungi:bacteria ratios
- pH
- Organic C and N

Definitions of acronyms, site abbreviations, or other project-specific designations used in the data file names or documentation files:

Site abbreviations:

- LIRF – Limited Irrigation Research Farm
- LVWS – Loch Vale Watershed

Soil treatments:

- HNHW – High Nitrogen High Water
- HNLW – High Nitrogen Low Water

- LNHW – Low Nitrogen High Water
- LNLW – Low Nitrogen Low Water
- Subalpine – Subalpine forest in LVWS

Note: The numbers corresponding to each treatment for the LIRF soils are the plot replicates.

Enriched treatments:

- $^{15}\text{NH}_4$ – (Chemical formula $^{15}\text{NH}_4$) – Soils amended with 99 AP $^{15}\text{NH}_4$ aqueous solution
- $^{15}\text{NO}_3$ – (Chemical formula $^{15}\text{NO}_3$) – Soils amended with 99 AP $^{15}\text{NO}_3$ aqueous solution

Variable information: Measurement units are included in variable names. Please see the associated manuscript or email the dataset contact for clarification.

Uncertainty, precision, and accuracy of measurements: Each instrument used to measure the variables in this study have reasonably high analytical precision and we have taken care to perform sufficient technical replicates of each variable for our research questions.

Environmental or experimental conditions: NA – details about experimental setup included in associated manuscript.

Method(s): Included in associated manuscript.

Standards or calibrations that were used: See Stuchiner et al. (2020) –DOI: 10.1002/rcm.8978

Software: Microsoft Office Excel (version 16.46) and RStudio (version 4.0.2 (2020-06-22) -- "Taking Off Again" © 2020 The R Foundation for Statistical Computing

Date dataset was last modified: February 2021

Are there multiple versions of the dataset? yes/no