Fractionation of nitrogen and oxygen isotopic composition in N_2O produced by bacterial denitrification

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Introduction

- The isotopic composition of N_2O provides valuable information about the contributions from biological sources (e.g., denitrification) to N_2O accumulation in the atmosphere.
- We study $^{15}N/^{14}N$ and $^{18}O/^{16}O$ fractionation associated with N_2O production by pure cultures of different microbial and abiotic processes in real-time.
- First experiments are conducted with the denitrifier *Pseudomonas aureofaciens,* as it lacks N_2O reductase, which makes it possible to study N_2O production in isolation.

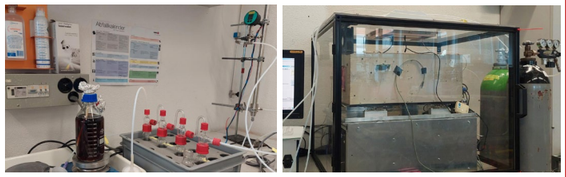

Fig. 1 – Pictures of the current setup.: bacteria culture with an alkaline CO_2 trap and a permeation dryer (left); QCLAS with mass flow controllers (MFC) (right).

Quantum cascade laser absorption spectroscopy (QCLAS)

- For on-line measurements of singly isotopically substituted species, we apply a spectrometer with a QC laser source emitting at 2203 cm^{-1}.
- For better temperature stability the spectrometer is enclosed in a Peltier cooled environment.
- In a second stage, doubly substituted isotopic species (Fig. 2) will be analyzed with a second spectrometer employing two laser sources (2142 and 2182 cm^{-1}).

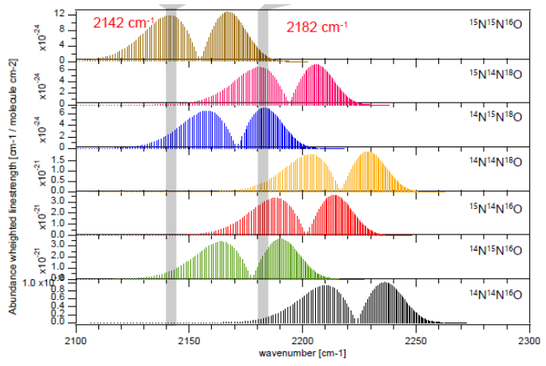

Fig. 2 – Absorption bands of eight most abundance isotopocules of N_2O; in this current setup, only the four most abundance isotopocules are analyzed

Experiments

- Pure culture stocks of *P. aureofaciens* are used to inoculate a tryptic soy broth medium with 10 mmol KNO_3, which is expected to yield 5 mmol of N_2O at 100% conversion of NO_3^- .
- N_2O production is quantified on-line by FT-IR spectroscopy.
- N_2O isotopic species ($^{14}N^{14}N^{16}O$, $^{14}N^{15}N^{16}O$, $^{15}N^{14}N^{16}O$, $^{14}N^{14}N^{18}O$) are analyzed with QCLAS.
- For optimal accuracy of isotopic analyses, we target the measurement of both sample and calibration gases as N_2O in N_2 with comparable compositions.
- Consequently, CO_2 and H_2O were removed with an alkaline trap solution / permeation drying.

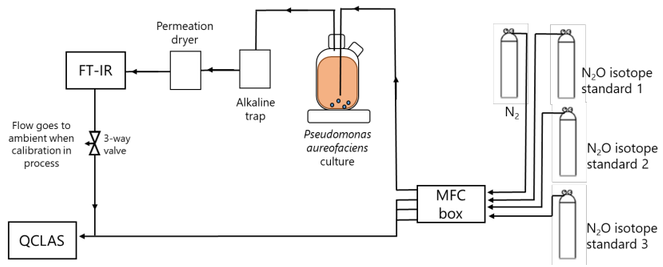

Fig. 3 – Current setup to analyze N_2O produced by *P. aureofaciens*

Results – evolution of N_2O

- First results indicate 3.5 mmol N_2O production by the bacterial culture within 11 days (29th March – 8th April) (Fig. 4).
- N_2O produced yields 70% of the supplied NO_3^-.
- N_2O isotopocules were measured for 5 days (3rd April – 7th April) (Fig. 4).

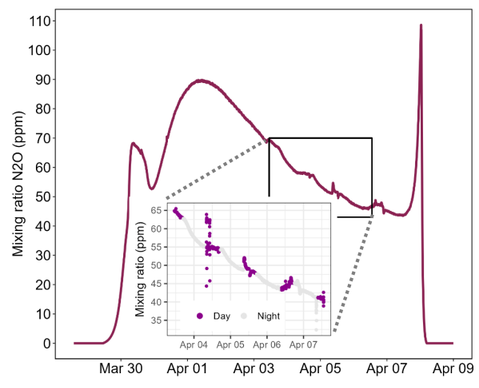

Fig. 4 – N_2O mixing ratio from *P. aureofaciens,* measured with the FT-IR. Zoom: timeframe, where QCLAS measurements were executed.

Results – isotopic measurements

- During daytime, isotopic measurements were referenced to calibration gases, while during nighttime no appropriate drift correction could be applied (Fig. 5).
- A general increase over time in $\delta^{15}N^{\alpha}$, $\delta^{15}N^{\beta}$ and $\delta^{15}N$ was observed.
- $\delta^{18}O$ and $\delta^{15}N^{SP}$ (= $\delta^{15}N^{\alpha}$ - $\delta^{15}N^{\beta}$) varied between 40 - 35 ‰ and 5 and -10 ‰, with no clear trends.
- Overall average of $\delta^{18}O$, $\delta^{15}N$ and $\delta^{15}N^{SP}$ are 37.4 ‰, -2.3 ‰ and -3.6 ‰.

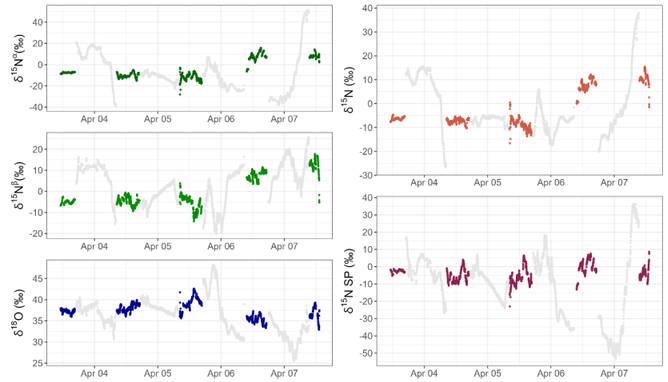

Fig. 5 – On the left $\delta^{15}N^{\alpha}$, $\delta^{15}N^{\beta}$, $\delta^{18}O$ (‰) vs. time; on the right $\delta^{15}N$ and $\delta^{15}N^{SP}$ (‰) vs. time. Colors distinguish night and day measurements (grey for nighttime, colored for daytime).

Discussion

- As correction factors change with time, the drift could not be corrected during the night, thus the bacterial signal during this period is unreliable.
- High quality daytime measurements of *P. aureofaciens* isotopic signatures fall into the expected ranges (Fig. 6), but remain quite variable within each day.

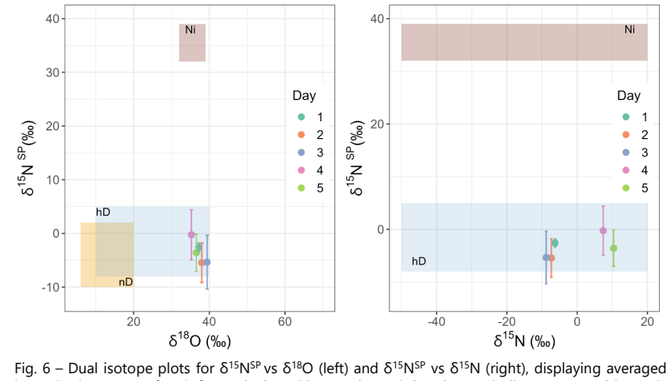

Fig. 6 – Dual isotope plots for $\delta^{15}N^{SP}$ vs $\delta^{18}O$ (left) and $\delta^{15}N^{SP}$ vs $\delta^{15}N$ (right), displaying averaged isotopic signatures of N_2O for each day with error bars. Colored areas indicate expected isotopic signatures for N_2O production pathways (Ni = hydroxylamine oxidation, nD = nitrifier denitrification, hD = heterotrophic denitrification) adapted from Yu et al. (2020).

Summary / Outlook

- N_2O isotopic species were continuously analyzed with QCLAS by culturing the N_2O-reductase-lacking denitrifier, *P. aureofaciens*.
- As measurements need to be referenced to calibration gases, only daytime measurements can be considered further.
- Isotopic signatures from *P. aureofaciens* fall into the expected ranges for heterotrophic denitrification (*i.e.*, $\delta^{15}N^{SP} \approx 5$ to -8 ‰).
- For further experiments, the measurements will be automated to have accurately standardized data day and night.
- We will explore other factors, *e.g.* growth rate, cell density, oxygen supply.

Next steps

- Compare bacteria that use similar Nor (i.e. NorB) enzymes and different Nir (i.e. NirS vs. NirK) enzymes (Fig. 7).
- This would be possible by comparing *Pseudomonas aureofaciens* with *Pseudomonas chlororaphis*: a similar SP would be expected vs. different $\delta^{15}N$ and $\delta^{18}O$ signatures and fractionation factors.

Fig. 7 – Denitrification pathway with the concerned enzymes

- Investigate other N_2O production pathways, including hydroxylamine oxidation reactions, ammonia oxidation by archaea and bacteria, fungal denitrification.
- Explore isotopologue-specific fractionation factors using biochemical kinetic modelling.
- Measure doubly-substituted isotopologues ($^{15}N^{15}N^{16}O$, $^{14}N^{15}N^{18}O$ and $^{15}N^{14}N^{18}O$) from all biogenic N_2O sources

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