Ice Core and Aerosol Sample Preparation and Nitrate Isotopic Analysis by Electrospray-Orbitrap

Jack Saville & Julien Witwicky, Elsa Gautier, Nicolas Caillon, Joël Savarino

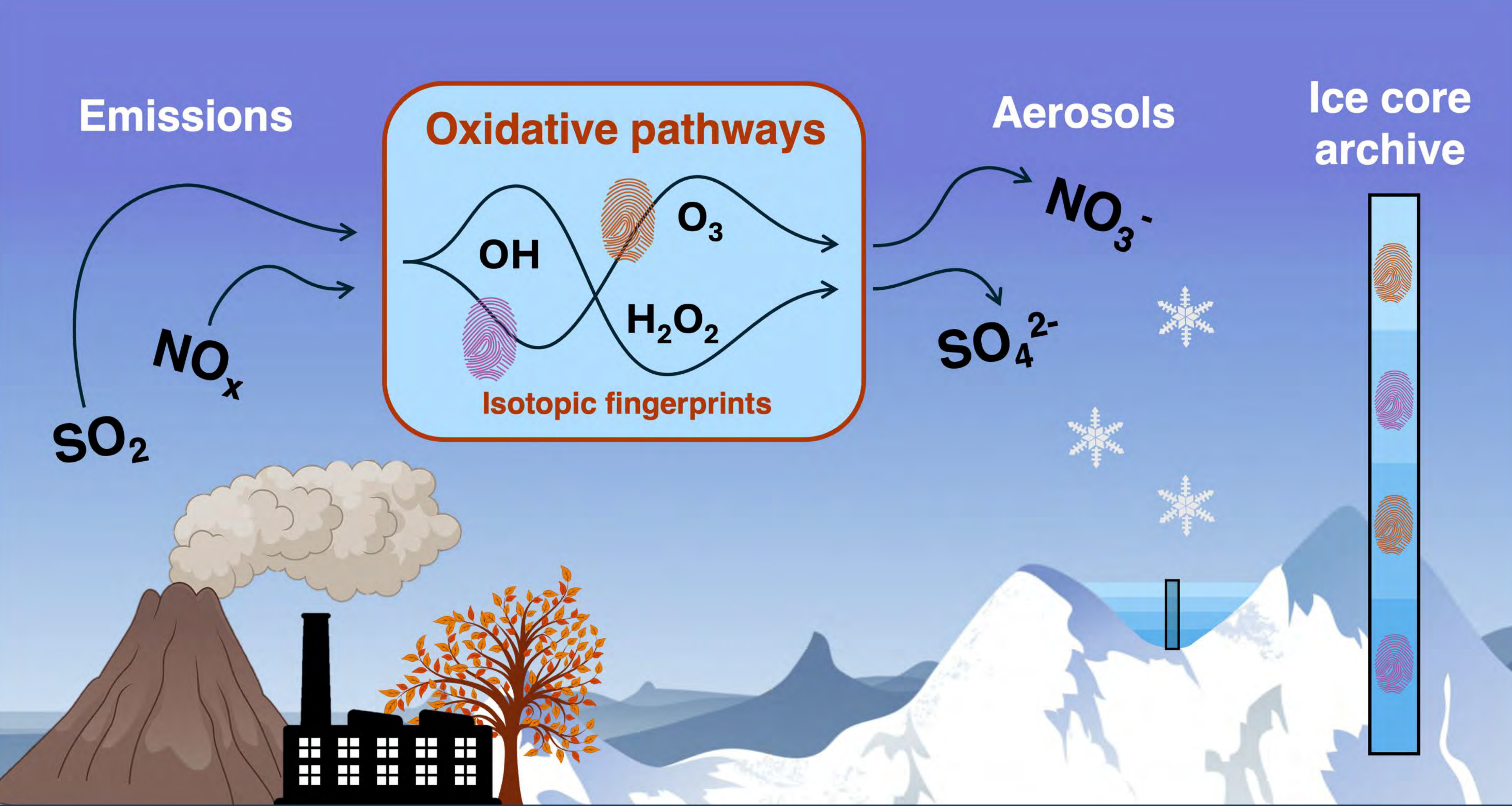
Motivation: Atmospheric Chemistry

pollutant	+	*oxidant*	→	*stable species*
CH_4	**+**	**oxidant**	→	**CO_2**
$C_xH_yO_z$ / SO_2 / NO_x	**+**	**oxidant**	→	**secondary aerosol**

Oxidants [OH], [O_3], [H_2O_2] & [NO_3] control:

- **pollutant** levels
- tropospheric **ozone** production
- **aerosol** and **cloud** formation
- **methane** lifetime

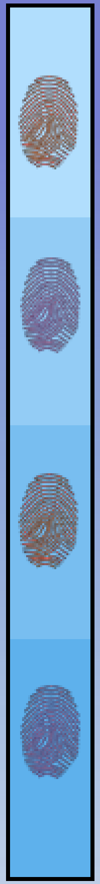
Air quality & Climate


Emissions
Oxidative pathways
OH
O_3
H_2O_2
Isotopic fingerprints
Aerosols
Ice core archive
NO_x
SO_2
NO_3^-
SO_4^{2-}

Traditional nitrate isotope method:
Denitrifier IRMS

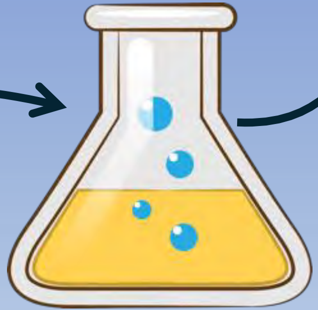
$$2NO_3^- \rightarrow N_2O \rightarrow N_2 + O_2$$

Ice core archive

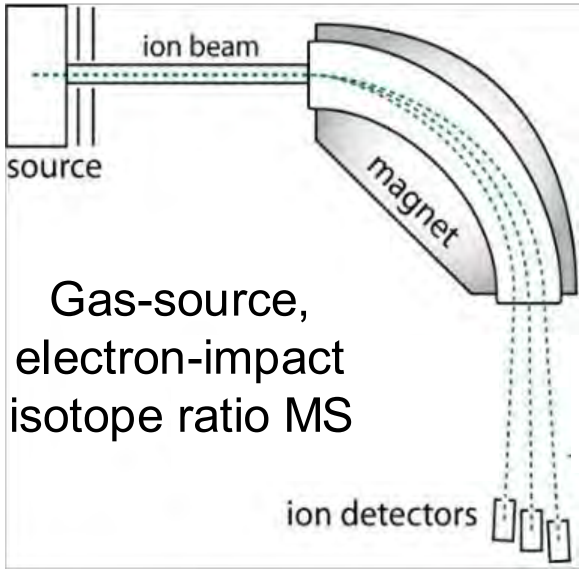


Ice core samples

Concentration
and/or purification



Chemical conversion of
oxyanions to simple gases



Gas-source,
electron-impact
isotope ratio MS

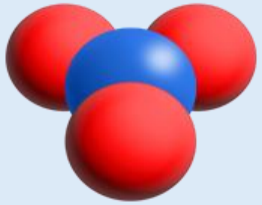
Traditional nitrate isotope method:
Denitrifier IRMS
$2NO_3^- \rightarrow N_2O \rightarrow N_2 + ½O_2$

<u>Limitations:</u>

1. **Sample size is large**. 100 nmol nitrate ~ 100g alpine ice, 1kg polar ice
2. **O atom recovery is non-quantitative** → accurate nitrate $\delta^{18}O$ requires careful calibration and reproducible bacteria performance, so can be imprecise
3. **Chemical bonds are broken** → intramolecular information is lost

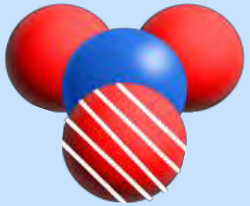
What are clumped isotopes?

c. 99%



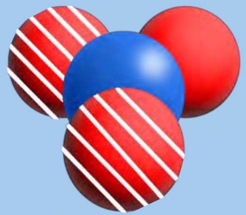
NO_3^-

c. 0.6%



$N^{18}OO_2^-$

c. 0.0024%



$N^{18}O_2O^-$

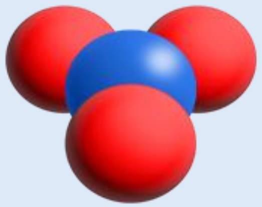
Are isotopes ***stochastically distributed*** between molecules?

$$R_{1818} = (R_{18})^2$$

Or do **heavy isotopes** prefer to ***clump*** together in the same molecule?

$$R_{1818} > (R_{18})^2$$

What are clumped isotopes?



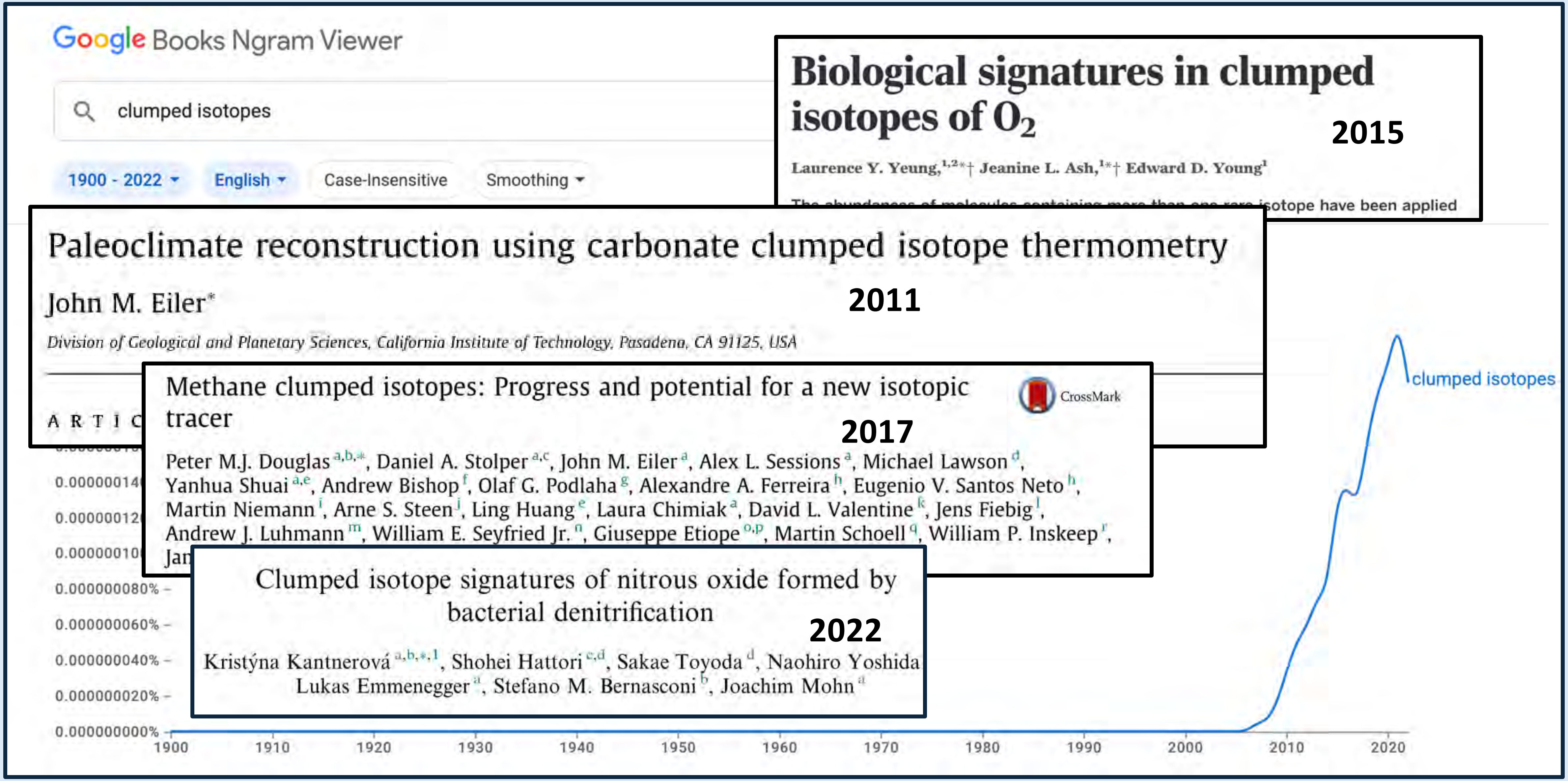
$$\Delta^{1518} = \frac{R_{1518}}{R_{15} * R_{18}} - 1$$

$$\Delta^{1818} = \frac{R_{1818}}{R_{18} * R_{18}} - 1$$

$$\Delta^{1718} = \frac{R_{1718}}{R_{17} * R_{18}} - 1$$

<u>Clumped isotopes signatures are:</u>

- imprinted during **molecule-forming reactions**
- dependent on reaction **energetics and kinetics**
- **insensitive to bulk isotopes**


Google Books Ngram Viewer
clumped isotopes
1900 - 2022
English
Case-Insensitive
Smoothing
Biological signatures in clumped isotopes of O2
2015
Laurence Y. Yeung, Jeanine L. Ash, Edward D. Young
Paleoclimate reconstruction using carbonate clumped isotope thermometry
John M. Eiler
2011
Division of Geological and Planetary Sciences, California Institute of Technology, Pasadena, CA 91125, USA
Methane clumped isotopes: Progress and potential for a new isotopic tracer
2017
Peter M.J. Douglas, Daniel A. Stolper, John M. Eiler, Alex L. Sessions, Michael Lawson, Yanhua Shuai, Andrew Bishop, Olaf G. Podlaha, Alexandre A. Ferreira, Eugenio V. Santos Neto, Martin Niemann, Arne S. Steen, Ling Huang, Laura Chimiak, David L. Valentine, Jens Fiebig, Andrew J. Luhmann, William E. Seyfried Jr., Giuseppe Etiope, Martin Schoell, William P. Inskeep,
Clumped isotope signatures of nitrous oxide formed by bacterial denitrification
2022
Kristýna Kantnerová, Shohei Hattori, Sakae Toyoda, Naohiro Yoshida, Lukas Emmenegger, Stefano M. Bernasconi, Joachim Mohn
clumped isotopes
0.000000080%
0.000000060%
0.000000040%
0.000000020%
0.000000000%
1900
1910
1920
1930
1940
1950
1960
1970
1980
1990
2000
2010
2020

Introducing Electrospray-Orbitrap

*A **high-sensitivity, liquid-source, soft-ionisation, high-resolution** mass spectrometer optimised for accurate-mass identification and quantification of biomolecules*

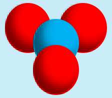
Thermo Scientific

Recently rebranded as an isotopologue-ratio MS

- **Sample demand 10x lower** than IRMS
- **No chemical conversions =** less chances for fractionation
- Intact molecules **= clumped isotopes** are accessible
- **Any soluble molecule** with m/z > 40 analysable

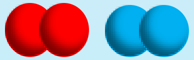
Electrospray-Orbitrap MS instrument

Denitrifier-IRMS

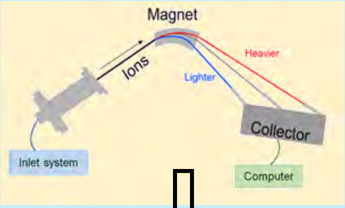
NO_3^-  100 nmol

Bacterial conversion

⇩

O_2  N_2

⇩

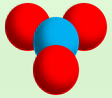


⇩

$\Delta^{17}O$ $\delta^{15}N$

$\delta^{18}O$

Electrospray-Orbitrap MS

NO_3^-  20 nmol

Intact nitrate ions

⇩

⇩

$\delta^{18}O$ $\delta^{15}N$ $\Delta^{17}O$

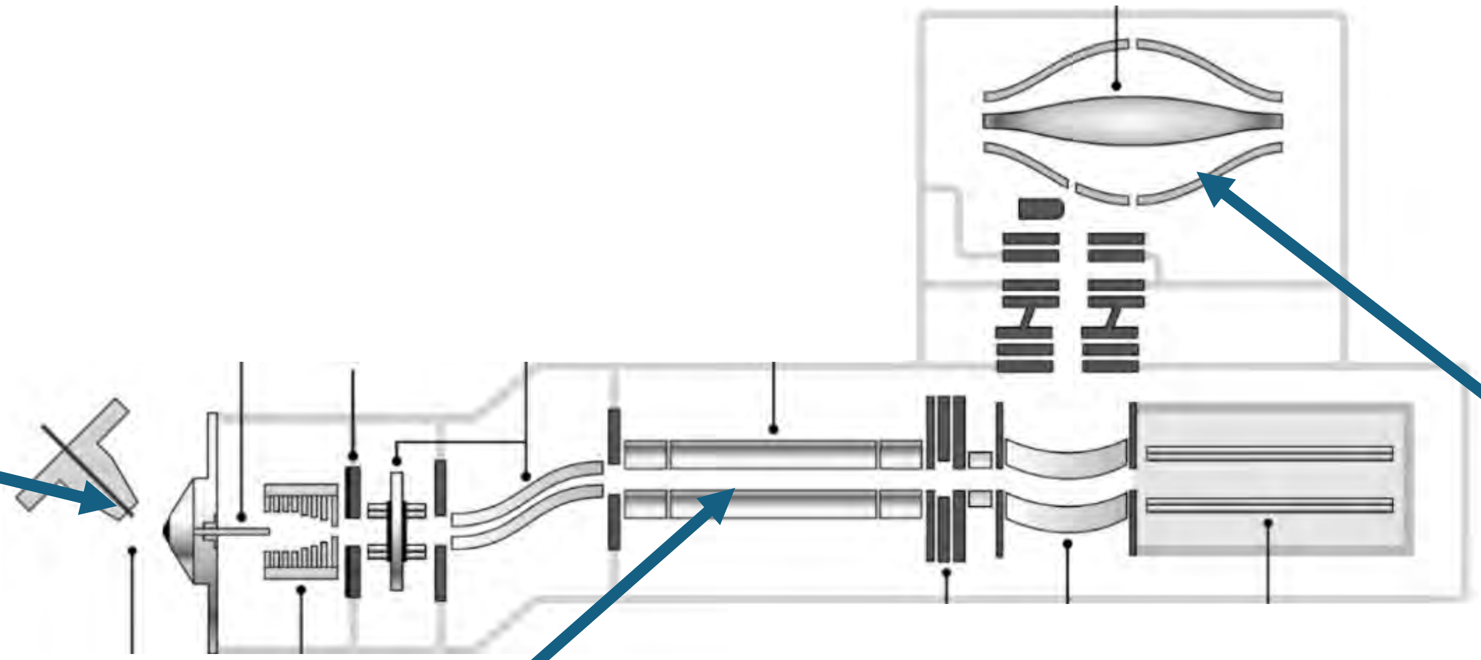
$\Delta\, ^{18}O^{18}O$ $\Delta\, ^{15}N^{18}O$

$\Delta\, ^{17}O^{18}O$

Electrospray-Orbitrap MS instrument

Pure **methanolic analyte solutions**
50 μM, 4 μL/min
stable delivery

Electrospray ionisation (ESI) produces gaseous molecular ions



Fourier-tranform of *image current* gives mass spectrum.
Mass resolution ~ measurement duration

Orbitrap analysis:
axial ion frequency
$\omega \propto \sqrt{m/z}$

Quadrupole filter isolates a **mass window** for analysis

Successive **fixed-size ion packages** are trapped for analysis.
Package size limited by space-charge effects

Orbitrap Exploris 240, Thermo Fisher; Kantnerovà, Kuhlbusch et al. Nature Protocols (2024)

Instrumental constraints

<u>ESI constraints:</u>

Matrix-sensitivity (salts and organics)

Requires **methanolic** samples

<u>Orbitrap constraints:</u>

Package size at least < 10^6 ions, or space-charge effects

Scan rate = 30 packages/sec (at 15k resolution)

→ c. **10^7 ions counted per second**

many minutes acquistion required for **1 ‰ precision** $\delta^{15}N, \delta^{18}O, \Delta^{17}O$,

→ LC-MS coupling difficult

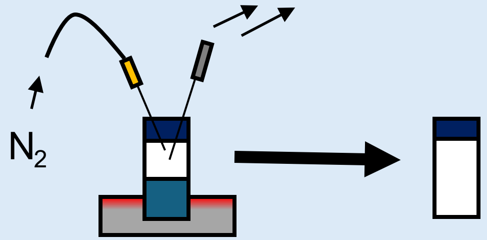
<u>Fun bonus constraint!</u>

Isotopologue ratio drift affects precision over **> 15 min** timescales

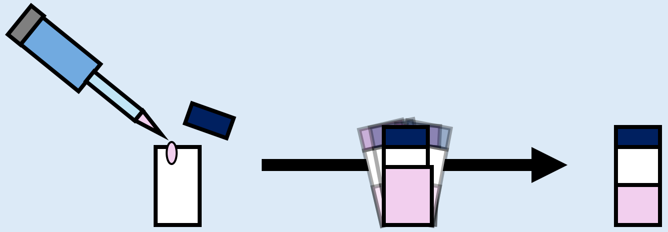
ESI-Orbitrap sample preparation

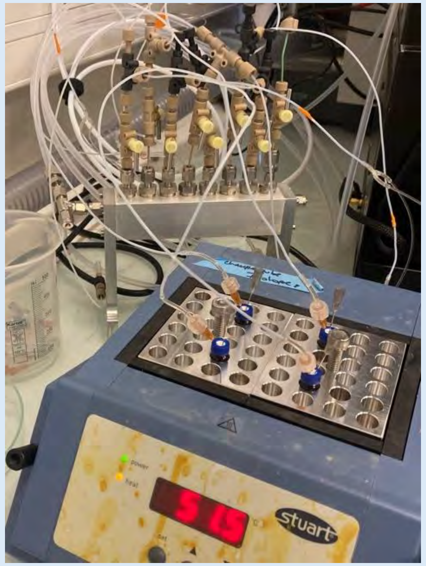
Separation, purification and concentration by IC

Precipitation by heating and N_2 headspace flushing



Redissolution in acidified methanol





- Aim for simple, consistent matrix
- Principle of identical treatment of samples and calibration standards

Ice core sample preparation

CFA and sampling
Melting, decontaminating, measuring and high resolution sampling

IC quantification
NO_3^- and SO_4^{2-}
other major ions

Recombine
the samples into bigger samples covering 6 or 12 years

Discard specific events

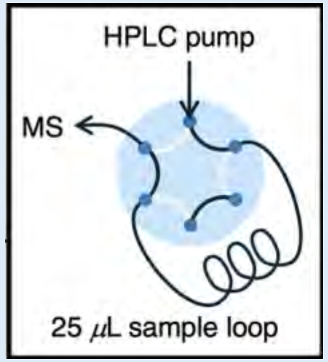
IC separation
into pure, concentrated NO_3^- and SO_4^{2-} samples

Change of matrix
Dry and redissolve the samples in methanol

Analysis by ESI-Orbitrap
NO_3^- or SO_3^-
isotopologues

PANDA
plateau analytique dédié aux archives glaciaires

Sample introduction



1. **Dual inlet**
   via twin syringes

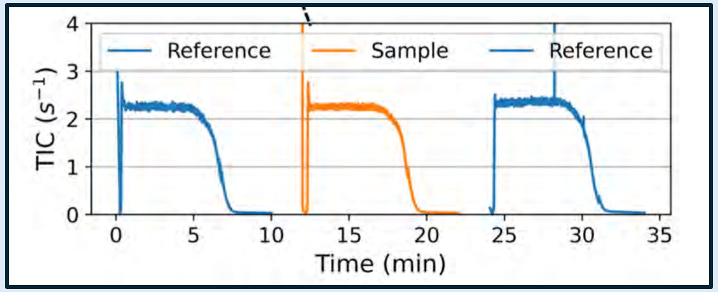
   + Alternating analysis of sample/ref
   + Requires no extra hardware
   + Simplest
   + Time-efficient = less drift = more precise

   – 2-point calibration impossible
   – Labour-intensive
   – No autonomy
   – 50 % of sample wasted
   – Low reproducibility
   – Blanks ~ syringe rinsing dedication

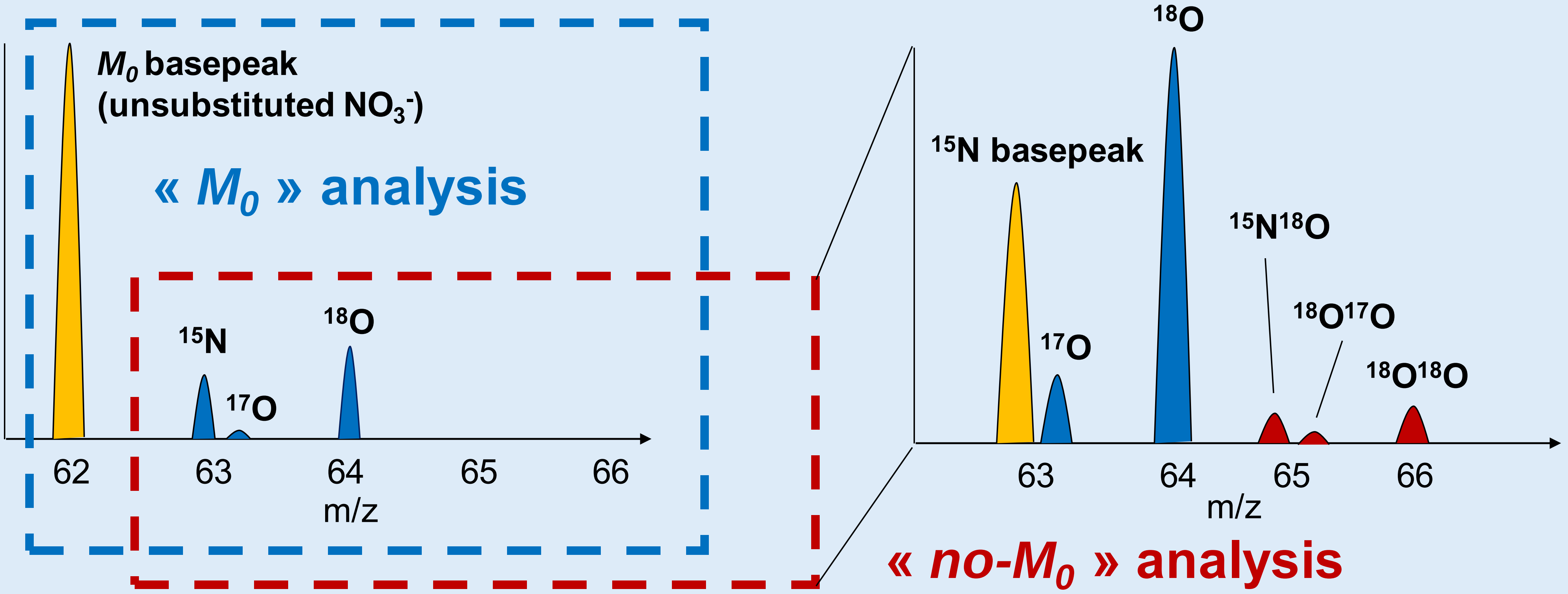
2. **In-flow injection**
   via HPLC autosampler

   + Long, autonomous sequences
   + Reproducible injections
   + Many standards and samples in one sequence
   + Controlled memory effect, low blanks
   + More sample-efficient

   – Time wasted rinsing sample loop
   – HPLC is over-qualified for the job



Isotopologue analysis



Removing the M_0 peak amplifies substituted isotopologue counting and allows quantification of doubly-substituted isotopologues

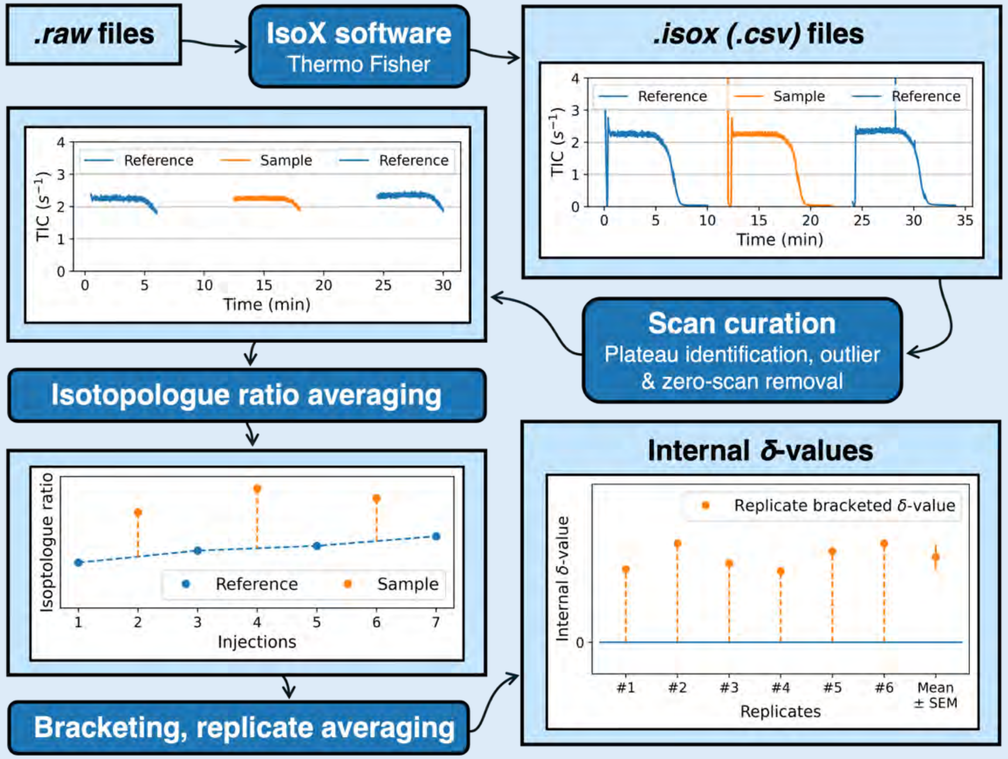
Data acquisition

Bracketed acquisitions

Repeated 6 times
in random order

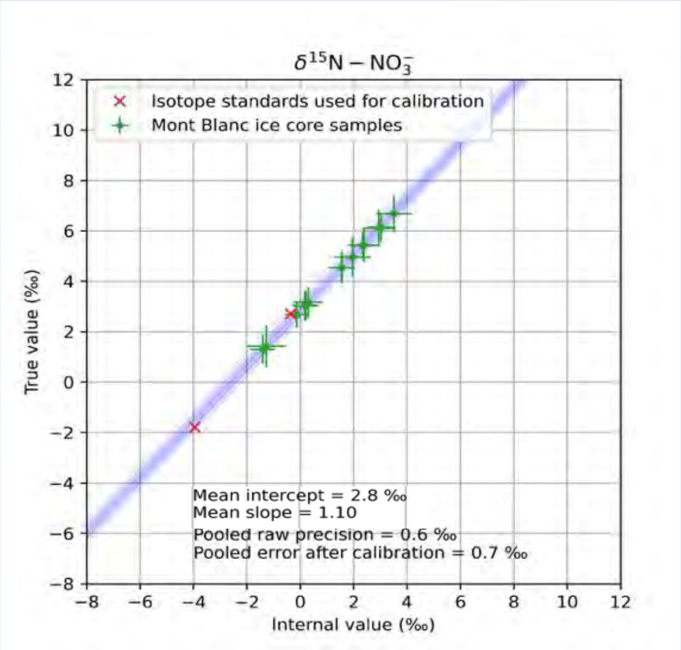
Internal standard	USGS35-ISD
Sample	CdM-28
Internal standard	USGS35-ISD
Sample	CdM-surf1
Internal standard	USGS35-ISD
Calibration standard	USGS32-2
Internal standard	USGS35-ISD
Sample	CdM-20
Internal standard	USGS35-ISD
Calibration standard	USGS35-1
Internal standard	USGS35-ISD
Calibration standard	USGS35-3
Internal standard	USGS35-ISD
Sample	CdM-3
Internal standard	USGS35-ISD
Calibration standard	USGS35-1
Internal standard	USGS35-ISD
Sample	CdM-15
Internal standard	USGS35-ISD

Raw data treatment



Isotopic calibration

Monte-Carlo linear regressions



Standard	$\delta^{15}N$ (‰)
USGS34	-1.8
USGS35	2.7
USGS32	180.0

Results

This is a preprint and has not been peer reviewed. Data may be preliminary.

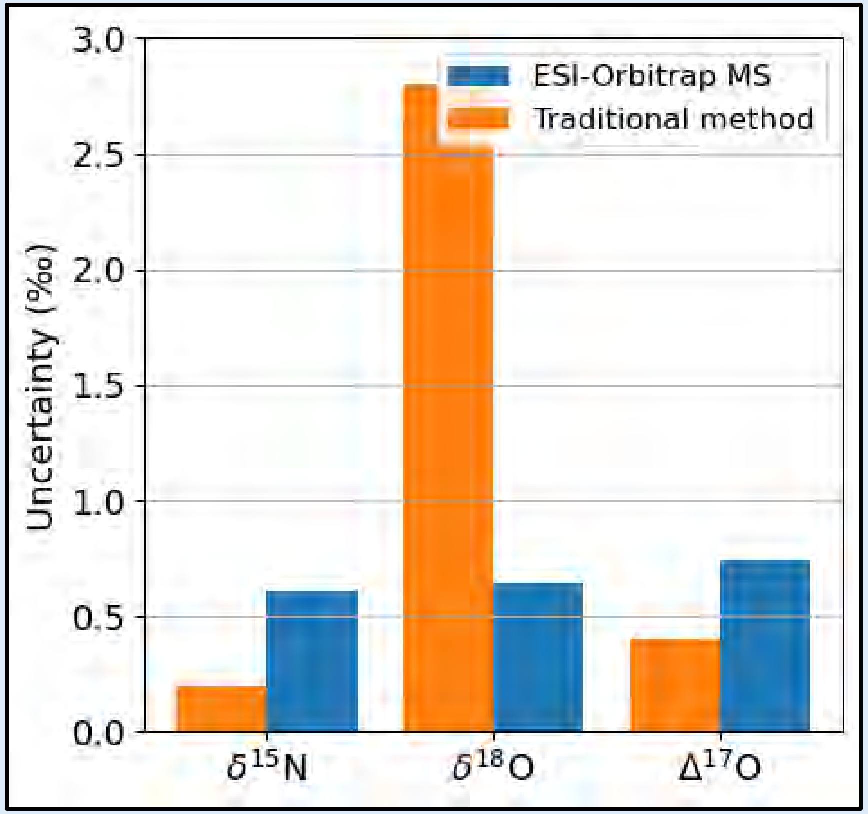
WORKING PAPER | 12 JANUARY 2026 | V1 LATEST VERSION

Oxyanion Isotopologue Analysis of Natural Samples by Electrospray-Orbitrap: Method, Validation and Clumped Isotopes

Authors: Mr. Jack Saville, Mr. Julien Witwicky, Dr. Elsa Gautier, Dr. Alexis Lamothe, Mr. Nils Johannes Kuhlbusch, Dr. Becky Alexander, Dr. Andrew Schauer, ... SHOW ALL ... , and Dr. Joël Savarino | Authors Info & Affiliations

https://doi.org/10.26434/chemrxiv-2026-80tdd

Results: nitrate precision

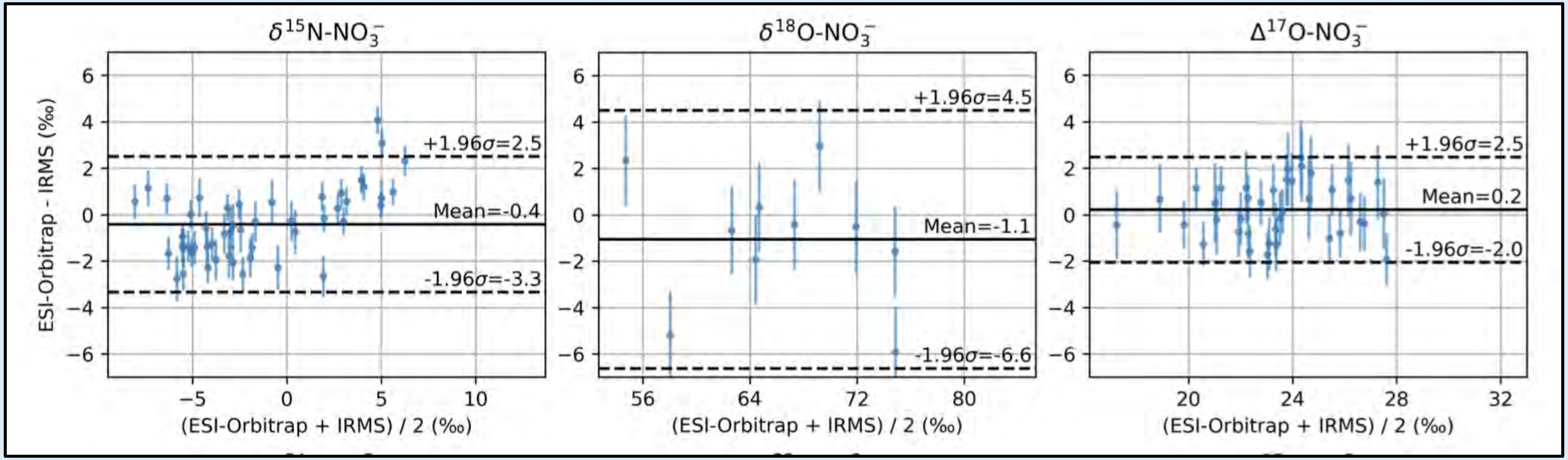


Uncertainty ^{2}
= standard error on mean raw value ^{2}
+ calibration uncertainty ^{2}

Now we perform even better than this:

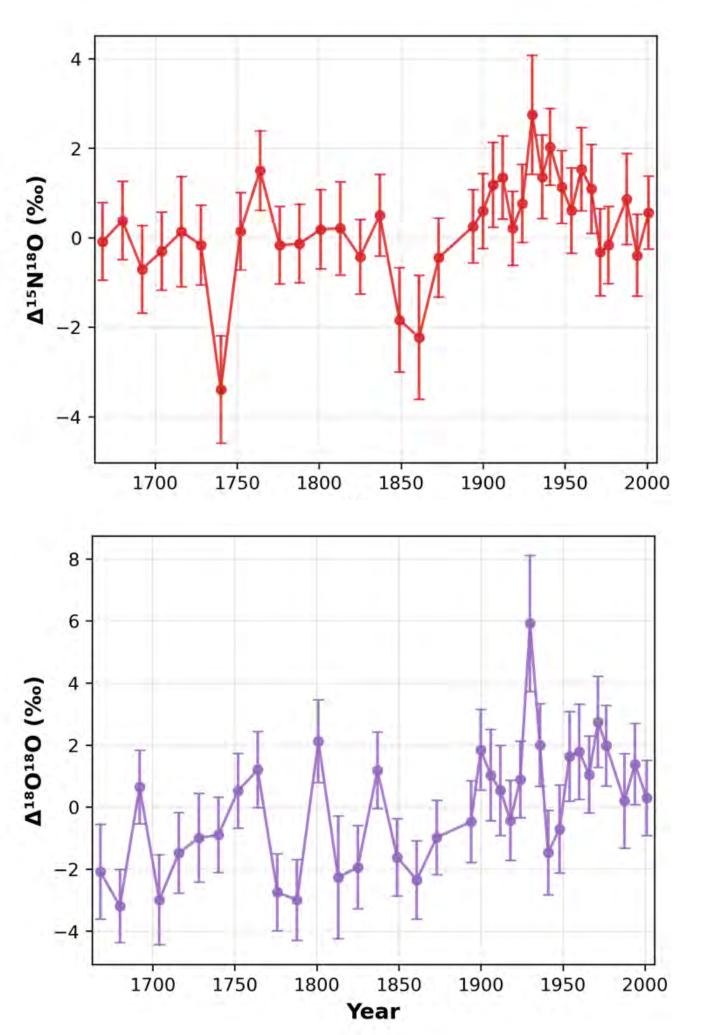
$\delta^{15}\mathrm{N} \rightarrow 0.3$ ‰
$\delta^{18}\mathrm{O} \rightarrow 0.5$ ‰
$\Delta^{17}\mathrm{O} \rightarrow 0.5$ ‰

Results: nitrate accuracy



	$\delta^{15}N$	$\delta^{18}O$	$\Delta^{17}O$
N ice samples	50	10	39
Mean difference | Orbitrap-IRMS | (‰)	1.1 ± 0.6	2.1±2.9	0.9±0.9
Mean bias (Orbitap-IRMS) (‰)	-0.4	-1.1	0.2

Results: nitrate clumped isotopes



Nitrate $\Delta^{15}N^{18}O$ and $\Delta^{18}O^{18}O$ are measured with **uncertainty 1 – 1.5 ‰** using 20 nmol samples from EGRIP ice core (Greenland)

Measurements are calibrated by assuming intramolecular stochasticity in existing isotope standards

Value are close to zero with variance roughly explainable by measurement uncertainty

→ **Ice core nitrate clumped isotope signals have probably < 1 ‰ amplitude**

Results

- Orbitrap nitrate **δ^{15}N** and **Δ^{17}O** agree with IRMS
- Orbitrap approaching IRMS **precision**
- **Sample size reduced** 100 nmol → 20 nmol
- Nitrate **clumped isotopes** are now measurable

Challenges

- Orbitrap – IRMS nitrate **δ^{18}O disagreement**
- **Matrix effects**: contamination control, coelutions
- Improve clumped isotope **precision**

Perpectives

- **Nanomole** nitrate samples = work in clean room
- Nitrate in **new matrices** (lakes, soils...) require new purification steps?
- **New analytes** (oxyanions, secondary organic aerosol, amino acids, lipids, metals…)
- **Position-specific** isotope analysis…

Other analytes

Stable Isotope Analysis of Intact Oxyanions Using Electrospray Quadrupole-Orbitrap Mass Spectrometry

Cajetan Neubauer,* Antoine Crémière, Xingchen T. Wang, Nivedita Thiagarajan, Alex L. Sessions, Jess F. Adkins, Nathan F. Dalleska, Alexandra V. Turchyn, Josephine A. Clegg, Annie Moradian, Michael J. Sweredoski, Spiros D. Garbis, and John M. Eiler

Oxygen Isotope Analyses of Phosphate and Organophosphorus Compounds by Electrospray Ionization Orbitrap Mass Spectrometry

Nora M. Bernet, Cleo Soldini, Timo M. O. Felder, Kristýna Lapčíková, Cajetan Neubauer, Wendy L. Queen, Ralf Kaegi, Federica Tamburini, and Thomas B. Hofstetter*

Simultaneous, High-Precision Measurements of δ^2H and δ^{13}C in Nanomole Quantities of Acetate Using Electrospray Ionization-Quadrupole-Orbitrap Mass Spectrometry

Elliott P. Mueller,* Alex L. Sessions, Peter E. Sauer, Gabriella M. Weiss, and John M. Eiler

Triple Oxygen Isotope Analysis of Methanesulfonate Using the SO_3^- Fragment in ESI-Orbitrap-MS

Yihang Hong, Longchen Zhu, Daniel R. Crocker, Tengyu Liu, Zhenfei Wang, Zhao Wei, Chen Yu, Yu Wei, Hao Yan, David T. Johnston, Issaku E. Kohl, Yongbo Peng, Andreas Hilkert, Cajetan Neubauer, and Shohei Hattori*

Metal Ion Isotope Ratio Using ESI-Orbitrap HRMS: Proof of Concept and Initial Performance Evaluation for Lead Isotopic Ratios

Gianluca Roncoroni, Davide Spanu, Gilberto Binda, and Damiano Monticelli*

Sub-10 Nanomole Perchlorate δ^{37}Cl, δ^{18}O, and Δ^{17}O Measurements by ESI-Orbitrap-MS

Longchen Zhu, Yihang Hong, Shohei Hattori, Zhenfei Wang, Zhao Wei, Yongbo Peng, Nils Johannes Kuhlbusch, and Huiming Bao*

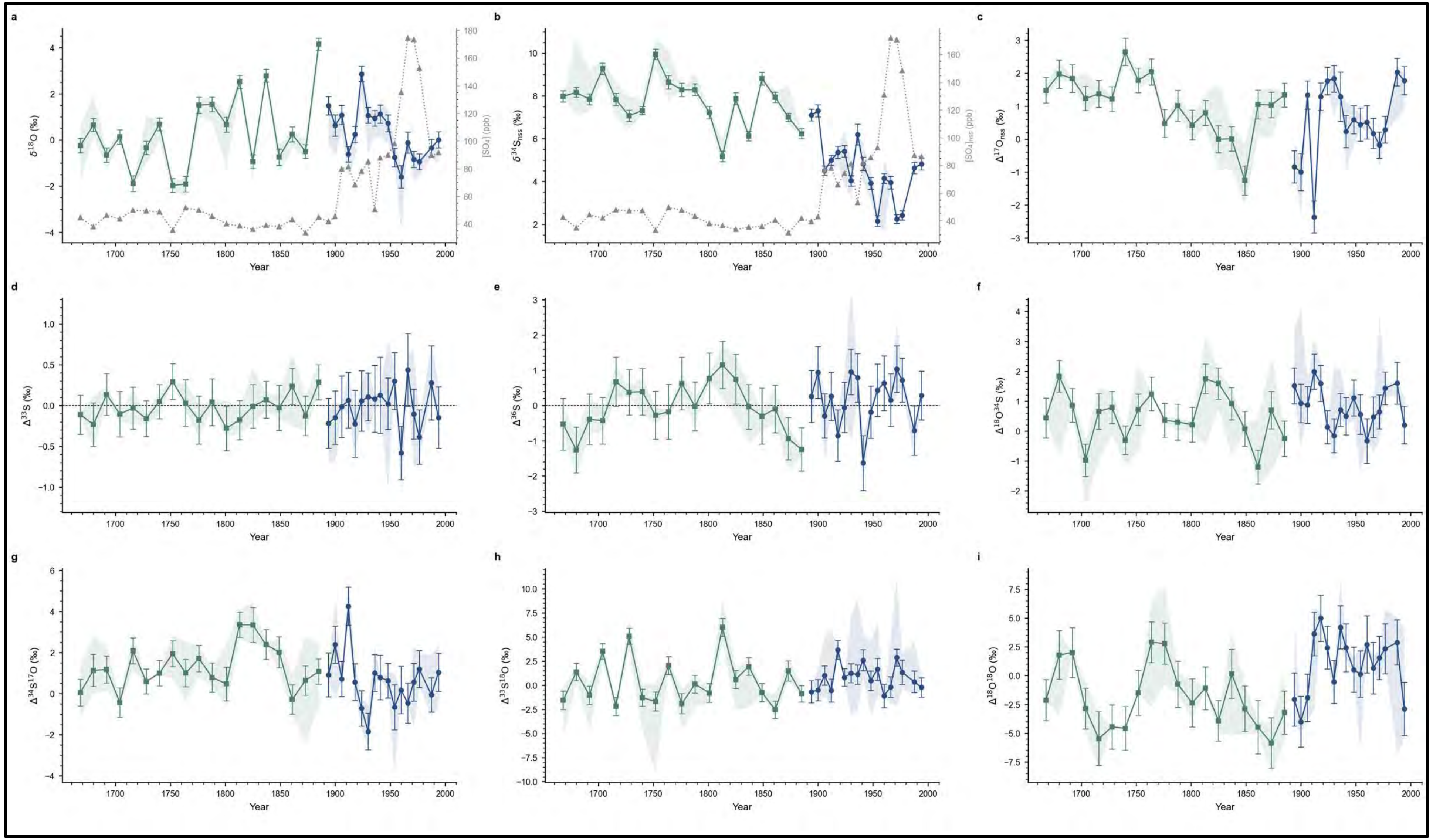
Advancing Stable Isotope Analysis with Orbitrap-MS for Fatty Acid Methyl Esters and Complex Lipid Matrices

Gabriel F. dos Santos,* Giovanni B. Bevilaqua, Alexis Gilbert, Hugo G. Machado, Maxime Julien, Gesiane S. Lima, Nerilson M. Lima, Júlio C. O. Ribeiro, Alexandre A. Ferreira, Ygor S. Rocha, and Boniek Gontijo*

Stable Carbon Isotope Analysis of PFOA and PFOS Using Orbitrap Mass Spectrometry

Hiroto Kawashima,* Tomoha Iezumi, Momoka Suto, and Sachi Taniyasu

Sulphate isotopologue analysis



Nitrate oxygen isotopes