# 10th International Symposium on Isotopomers (ISI)

# 12th Isotopes Conference



## ISI+Isotopes

**Empa, May 29 – June 3, 2022**

**DÜBENDORF / ZÜRICH, SWITZERLAND**

## Organizing committee


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Empa, Switzerland

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**Béla Tuzson**
Empa, Switzerland

**Martin Elsner**
Technical University of Munich, Germany

**Ivan Prokhorov**
Empa, Switzerland


## Contact Empa


**Dr. Andrea Fischer**
Phone +41 58 765 46 59
andrea.fischer@empa.ch

**Dr. Joachim Mohn**
Phone +41 58 765 46 87
joachim.mohn@empa.ch



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## PROGRAM

## MONDAY, 30 MAY 2022

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|---|---|---|---|
| **8:00** | | **Registration & Coffee** | |
| **8:30** | | **Welcome** | |
| PROCESS TRACING IN ECOLOGY & PLANT SCIENCE<br>**Christiane Werner (University of Freiburg)**<br>**Marco Lehmann (WSL)** | **8:45** | **A. Kahmen – University of Basel – keynote speaker**<br>Using carbon and oxygen isotopes of herbarium specimen to infer long-term physiological responses of plants to global environmental change | |
| | **9:25** | **L. Wingate – INRAE – invited speaker**<br>Is a 'black box' approach sufficient to predict the exchange of $CO^{18}O$ and COS between soils and the atmosphere or do we need to dig deeper? | |
| | **9:45** | **Coffee Break** | |
| | **10:45** | **C.A. Stricker – US Geological Survey (online)**<br>Fat and fit: diet estimation, macronutrient assimilation, and nutritional implications for an iconic Arctic predator | |
| | **11:00** | **M. Julien – GFZ Potsdam**<br>Re-evaluation of the $^{13}C$ isotope fractionation associated with fatty acids biosynthesis by position-specific isotope analysis | |
| | **11:15** | **L. E. Daber – University of Freiburg**<br>Position-specific isotope labelling gives new insights into chiral monoterpene synthesis | |
| | **11:30** | **D. B. Nelson – University of Basel**<br>Historic European monthly precipitation isotope time series reconstructions using machine learning | |
| | **11:45** | **T. Röckmann – Utrecht University**<br>Exploring the potential of $\Delta^{17}O$ in $CO_2$ for determining mesophyll conductance | |
| **12:00** | | **Lunch** | |
| FOOD AUTHENTICITY, FORENSIC & BIOMEDICAL APPLICATIONS<br>Michèle Lees (Eurofins)<br>Illa Tea (University of Nantes) | **13:30** | **S. Kelly – IAEA – keynote speaker**<br>Improving accessibility to food authentication, using stable isotope analysis, in developing countries: The activities of the joint FAO/IAEA Centre's food safety and control laboratory | |
| | **14:10** | **M. Straub – University Hospital of Lausanne – invited speaker**<br>Distinct nitrogen isotopic compositions of healthy and cancerous tissue in mice brain and head & neck micro-biopsies | |
| | **14:30** | **M. Perini – Centro di Trasferimento Tecnologico**<br>Stable isotope ratio analysis to assess pharmaceuticals, cosmetics and dietary supplements authenticity | |
| | **14:45** | **H. Meijer – University of Groningen**<br>First Use of Triply Labelled Water analysis for energy expenditure measurements in mice | |
| **15:00** | | **Poster Session I (Coffee)** | |
| COMPUTATION OF ISOTOPE EFFECTS & ENZYME MECHANISMS<br>Agnieszka Dybala-Defratyka<br>(Lodz University of Technology) | **16:30** | **K. Świderek – Universitat Jaume I – keynote speaker**<br>Towards a new protocol for computer assisted biocatalysts design | Session on the occasion of the 70th birthday of Piotr Paneth |
| | **17:10** | **P. Paneth – Lodz University of Technology – invited speaker**<br>My 50 years with isotope effects | |
| | **17:30** | **V. Moliner – Universitat Jaume I**<br>Towards the design of an improved Retro-Aldolase based on QM/MM studies of the reaction catalyzed by different protein scaffolds | |
| | **17:45** | **I. H. Williams – University of Bath**<br>Computational simulation of kinetic isotope effects for enzymatic N-glycoside hydrolysis | |
| **18:00** | | **Poster Session II (Beverages)** | |

## PROGRAM

## TUESDAY, 31 MAY 2022

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|---|---|---|
| **8:15** | | **Welcome Coffee** |
| **ADVANCES IN ANALYTICAL INSTRUMENTATION AND METHODS**<br>Matthias Gehre (UFZ) and Béla Tuzson (Empa) | **8:45** | **A. Gilbert – Tokyo Institute of Technology – keynote speaker**<br>Isotopologues of organic molecules: method developments and applications |
| | **9:25** | **C. Neubauer – University of Colorado – invited speaker**<br>Discovering isotopic fingerprints anew on bioanalytical mass spectrometers |
| **9:45** | | **Coffee Break (Information on the Wednesday Afternoon excursion)** |
| | **10:45** | **C. Rennick – National Physical Laboratory (online)**<br>Calibration of Boreas: a new laser-based instrument for *in-situ* automated measurement of $\delta^{13}C$ and $\delta^{2}H$ in methane |
| | **11:00** | **T. Csernica – California Institute of Technology**<br>High-Dimensional Isotomics: Observation and Interpretation of Over 100 Isotopic Constraints on Methionine |
| | **11:15** | **R. G. H. Marks – University of Essen**<br>How to Couple LC-IRMS with HRMS – A Proof-of-Concept Study |
| | **11:30** | **S. Renou – University of Nantes**<br>Towards unbiased $^{13}C$ isotopic composition in PSIA |
| | **11:45** | **B. Tuzson – Empa**<br>Mid-infrared laser spectroscopy coupled to continuous sublimation extraction. A novel method for high-precision greenhouse gas measurements in ice cores |
| **12:00** | | **Lunch** |
| **CLUMPED ISOTOPES**<br>Stefano Bernasconi (ETH Zürich)<br>Ivan Prokhorov (Empa) | **13:30** | **J. Fiebig – Goethe University of Frankfurt – keynote speaker**<br>Benefits and perspectives of carbonate dual clumped isotope thermometry |
| | **14:10** | **M. Clog – University of Glasgow – invited speaker**<br>Robustness of clumped carbonate thermometry in carbonates from the Tara Deep, a large Irish orebody |
| | **14:30** | **M. Sivan – Utrecht University**<br>Characterization of microbial methane using clumped isotope measurements |
| | **14:45** | **J. Quade – University of Arizona**<br>Carbonate clumped isotope calibration from 6 to 1100°C using an isotope ratio laser spectrometer based on tunable infrared laser spectroscopy |
| **15:00** | | **Poster Session III (Coffee)** |
| **ORIGIN AND EVOLUTION OF THE EARTH (PLANETS) & LIFE**<br>Huiming Bao (Nanjing University) | **16:30** | **Y. Ueno – Tokyo Institute of Technology – keynote speaker**<br>Tracing oxygen in sulfate using $^{34}S$-$^{18}O$-clumping |
| | **17:10** | **J. Hemingway – ETH Zürich – invited speaker**<br>Interpreting triple-oxygen isotope compositions in the geologic sulfur cycle |
| | **17:30** | **I. Bobrovskiy – GFZ Potsdam**<br>Compound-specific isotope analysis on phylogenetically specific molecular fossils as a tool to deconvolve the stable carbon isotope record of the deep time |
| | **17:45** | **M. H. Thiemens – University of California San Diego**<br>Solar controls of radioactive sulfur isotopes |
| **18:00** | | **Poster Session IV (Beverages)** |

## PROGRAM

## WEDNESDAY, 1 JUNE 2022

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|---|---|---|
| **8:15** | | **Welcome Coffee** |
| **GLOBAL CHANGE, GREENHOUSE GASES & AEROSOLS** Thomas Röckmann (Utrecht University) and Sakae Toyoda (Tokyo Institute of Technology) | **8:45** | **S. Ono – MIT – keynote speaker**<br>A model for isotopologue signatures of microbial methane to improve source attributions |
| | **9:25** | **L. Yu – Tsingua University / Empa – invited speaker (online)**<br>Constraining global $N_2O$ budgets with decadal trends of multiple isotope signatures |
| | **9:45** | **A. Matson – Thünen Institute (announcement)**<br>Research Gate Discussion Group: Isotopic tools to study $N_2O$ in soil and aquatic systems |
| | **9:52** | **R. Hill-Pearce – National Physical Laboratory (online)**<br>Stable isotope reference materials for climate change monitoring |
| | **10:00** | **Coffee Break (Information on the Friday Mt. Rigi tour)** |
| | **11:00** | **P. M. Homyak – University of California Riverside**<br>Using isotopes to understand N-limitation in dry lands: Unexpected N loss pathways in systems with too little N |
| | **11:15** | **A. Hoheisel – University of Heidelberg**<br>Evaluation of six years of continuous $\delta^{13}CH_4$ measurements in Heidelberg, Germany |
| | **11:30** | **R. W. van Zwieten – Picarro, Inc. (sponsored)**<br>Committed to Science - Stable isotope analysis with CRDS – practical considerations and use cases |
| | **11:45** | **J. Kaiser – University of East Anglia**<br>Polyisotopic carbon dioxide ratios at the coastal Weybourne Atmospheric Observatory (Norfolk, UK) |
| | **12:00** | **S. L. Baartman – Utrecht University**<br>Isotopic measurements of carbonyl sulfide (COS): from biosphere to stratosphere |
| | **12:15** | **H. Bao – Nanjing University**<br>Atmospheric sulfate of prehuman time in inland northern China |
| **12:30** | | **Lunch** |
| **15:00** | | **Afternoon Excursion** |
| **18:00** | | **Conference Dinner – ETH Zürich** |

## PROGRAM

## THURSDAY, 2 JUNE 2022

**BIOGEOCHEMISTRY, ELEMENTAL CYCLES AND FATE OF CONTAMINANTS**
**Anat Bernstein (Ben-Gurion University of the Negev)**
**Moritz Lehmann (University of Basel)**

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|---|---|
| **8:30** | **Welcome Coffee** |
| **9:00** | **K. L. Casciotti – Stanford University – keynote speaker**<br>Tracing nitrous oxide biogeochemistry in marine oxygen deficient zones using isotopes and isotopomers |
| **9:40** | **C. L. Kelly – Stanford University**<br>Identifying a potentially variable site preference for hybrid nitrous oxide production via isotopomer labeling experiments |
| **9:55** | **Coffee Break** |
| **11:00** | **E. Harris – ETH Zürich**<br>Denitrifying pathways dominate nitrous oxide emissions from managed grassland during drought and rewetting |
| **11:15** | **B. Mayer – University of Calgary**<br>Isotopic tracing of sources and fate of nitrate, sulfate and methane in groundwater in Alberta (Canada) |
| **11:30** | **B. Wolf – Karlsruhe Institute of Technology**<br>Intramolecular $N_2O$ isotopic composition from grassland without preconcentration: interferences correction, nitrification inhibitors, freeze-thaw events and source process identification |
| **11:45** | **A. Danner & G. Rahe – Envicontrol (sponsored)**<br>Analysis of soil respiration with OA-ICOS technology |
| **12:00** | **Lunch** |
| **13:30** | **D. Hunkeler – University of Neuchatel – keynote speaker**<br>Does compound-specific isotope analysis contribute to a new conceptual understanding of the fate of contaminants in the environment? |
| **14:10** | **M. Wiggenhauser – ETH Zürich**<br>Fractionation of stable isotopes of metals and metalloids in plants - copper and cadmium as examples |
| **14:25** | **S.-L. Badea – ICSI**<br>Dehalogenation of α-hexachlorocyclohexane by iron sulfide nanoparticles: Study of reaction mechanism with stable carbon isotopes and pH variations |
| **14:40** | **P. R. Martin – University of Tübingen**<br>Manganese–driven oxidation of aminotris (methylene) phosphonate (ATMP) studied by carbon CSIA |
| **14:55** | **Coffee Break** |
| **15:55** | **S. G. Pati – University of Basel**<br>Oxygen kinetic isotope effects associated with reactions of singlet oxygen in aqueous solutions |
| **16:10** | **C. E. Bopp – EAWAG**<br>Tracing mechanistic adaptations of enzymatic oxygenations of aromatic contaminants using $^{13}C$ and $^{18}O$ kinetic isotope effects |
| **16:25** | **J. Hayles – NASA (online)**<br>Constraints on triple oxygen isotope kinetics |
| **16:40** | **M. Elsner – Technical University of Munich**<br>Isotope fractionation reveals limitations and microbial regulation of pollutant biodegradation at low concentrations |
| **16:55** | **Goodbye!**<br>**(to all not joining the Friday Mt. Rigi tour)** |

## POSTERS

## MONDAY, 15:00 – 16:30 & 18:00 – 19:00

### PROCESS TRACING IN ECOLOGY & PLANT SCIENCE (POSTER SESSION)

Christiane Werner (University of Freiburg) and Marco Lehmann (WSL)

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|---|---|
| **P1** | **C. Buchen-Tschiskale – Thünen Institute**<br>Using $N_2O$ isotopocule analysis and $^{15}N$ tracing approach to gain insights into $N_2O$ source processes in hydroponic tomato cultivation |
| **P2** | **R. Well – Thünen Institute**<br>Combining $^{15}N$ tracing and $^{15}N$ site preference of $N_2O$ to distinguish production by nitrification and fungal denitrification |
| **P3** | **F. Tamburini – ETH Zürich**<br>Oxygen isotopes in phosphate: defining potentials and limitations for environmental studies |
| **P4** | **R. A. Werner – ETH Zürich**<br>Intramolecular $^{13}C$ patterns of plant glucose convey environmental and metabolic information |
| **P5** | **F. Damak – Tokyo Institute of Technology**<br>Insights into nitrous oxide reduction by soybean inoculated with Bradyrhizobium from concentration and isotopocule analyses in a field |
| **P6** | **S. N. Ladd – University of Freiburg**<br>Leaf-level metabolic changes in daytime respiration and isoprenoid synthesis during drought determined by position-specific $^{13}C$-pyruvate labeling |
| **P7** | **C. Werner – University of Freiburg**<br>Whole ecosystem $^{13}CO_2$ and $^{2}H_2O$ Pulse-Labelling to investigate carbon allocation, $CO_2$ and VOC emissions and the role of deep water reserves during drought |
| **P8** | **J. Baan – University of Basel**<br>Comparing hydrogen isotope compositions of different lipid compounds across species to address possible origin of variation |

### FOOD AUTHENTICITY, FORENSIC & BIOMEDICAL APPLICATIONS (POSTER SESSION)

Michèle Lees (Eurofins) and Illa Tea (University of Nantes)

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|---|---|
| **P9** | **C. Citérin – Nantes Université**<br>Isotopic signature of $^{13}C$ and $^{15}N$ natural abundance in breast cancer patients |
| **P10** | **M. Couton – Nantes Université**<br>$^{15}N$-position-specific isotope analysis by isotope ratio mass spectrometry (PSIA-IRMS) |
| **P11** | **M. Perini – Centro di Transferimento Technologico**<br>Isotope ratio mass spectrometry to detect differences in four compartments of Simmental cows fed on C3 and C4 diets |
| **P11/2** | **P. Paneth – Lodz University of Technology**<br>The first oxygen stable isotopes assessment in 'in vivo' cancer tissues – a pilot study. |

### COMPUTATION OF ISOTOPE EFFECTS & ENZYME MECHANISMS (POSTER SESSION)

Agnieszka Dybala-Defratyka (Lodz University of Technology)

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|---|---|
| **P12** | **L. Chai – TU Munich**<br>Metabolic mechanism of sulfonamide cleavage: a combined computational and experimental study on sulfamethoxazole |
| **P13** | **A. Dybala-Defratyka – Lodz University of Technology**<br>Isotope effects on vaporization of organic compounds from aqueous solution – insight from experiment and computations |
| **P14** | **L. Pennacchio – University of Copenhagen**<br>First principles model of isotopic fractionation in formaldehyde photolysis: Wavelength and pressure dependence |

## POSTERS

## MONDAY, 15:00 – 16:30 & 18:00 – 19:00

### ADVANCES IN ANALYTICAL INSTRUMENTATION AND METHODS (POSTER SESSION)

Matthias Gehre (UFZ) and Béla Tuzson (Empa)

**P15** **F. Antritter – TU Munich**
Reducing the unwanted: selectivity of various solid phase extraction sorbents in relation to dissolved organic matter

**P16** **A. Canavan – TU Munich**
Position-specific isotope analysis using $^{13}C$-labels on sulfamethoxazole

**P17** **A. Tafa – TU Munich**
Suitability of passive sampling for compound-specific isotope analysis of micropollutants in aquatic environments

**P18** **R. Bakkour – TU Munich**
Universal vs. selective sorbents for targeted isotope analysis of aquatic contaminants

**P19** **C. Wabnitz – TU Munich**
Coupling a quartz crystal microbalance with liquid chromatography for online NOM monitoring

**P20** **S. Leitner – Institute of Soil Research**
A UAV-based sampling system to analyze greenhouse gases and volatile organic carbons encompassing compound specific stable isotope analysis

**P21** **E. P. Mueller– California Institute of Technology**
High-precision ESI-Orbitrap MS measurements of hydrogen isotope compositions from organic molecules

**P22** **A. Hilkert – Institute of Soil Research**
Comprehensive isotope ratio MS with electrospray-Orbitrap

**P23** **M. Öztopark – Royal Netherlands Institute for Sea Research**
Investigating the intramolecular isotopic structure of isoprenoids via ultra high resolution APCI - Orbitrap mass spectrometry

**P24** **G. S. Remaud – Nantes University**
Exploring the potential of $^{17}O$ NMR for intramolecular $^{17}O$ isotope profile: application to vanillin origin discrimination

**P25** **S. Renou – Nantes University**
How to determine the intramolecular $^{13}C$ composition on low amount of glucose using irm $^{13}C$-NMR

**P26** **R. P. J. Moonen – Utrecht University**
First results of $CO_2$ and $H_2O$ Isotope-Flux Measurements a semi-arid area with large scale irrigation

**P27** **G. A. Adnew – Utrecht University**
Temperature dependence of isotopic fractionation in the $CO_2$-$O_2$ isotope exchange reaction

**P28** **E. Safi – National Physical Laboratory**
Fractionation effects during methane separation from ambient air for high-precision optical analysis of $\delta^{13}C$ and $\delta^{2}H$

**P29** **A. Th. Aerts-Bijma – University of Gröningen**
Where do IRMS's go wrong? $\delta^{18}O$ SLAP determined at -56.3‰

**P30** **K. Huang – Empa**
A novel automated technique for simultaneous online analysis of $^{15}N$ in ammonium, nitrite, and nitrate

**P31** **K. Zeyer – Empa**
Real-time analysis of $\delta^{13}C$- and $\delta D$-$CH_4$ in ambient air with a QCL based absorption spectrometer: Method development

**P32** **M. Lehmann – WSL**
The hydrogen isotopic composition of plant carbohydrates – Advancement in methods and interpretation

**P33** **S. Hugger – University of Basel**
Method optimization for plant sugar purification and compound-specific hydrogen isotope analysis

**P33/2** **S. G. Pati – University of Basel**
δ-scale calibration for stable isotope analysis of $O_2$ by continuous flow IRMS from -10 to +95 ‰ with in-vitro photosynthesis experiments **P58**

## POSTERS

## TUESDAY, 15:00 – 16:30 & 18:00 – 19:00

### CLUMPED ISOTOPES (POSTER SESSION)

Stefano Bernasconi (ETH Zürich) and Ivan Prokhorov (Empa)

**P34** **A. Nataraj – Empa**
Quantum cascade laser absorption spectrometer with a low temperature multipass cell for precision clumped $^{12}C^{18}O_2$ and position specific isotope analysis

**P35** **I. Prokhorov – Empa**
Concordant optical clumped isotope thermometry of methane

**P36** **H. Eckhardt – University of Heidelberg**
Atmospheric $CO_2$ sources with specific $\Delta_{47}$ signals under mixing conditions

**P37** **N. Looser – ETH Zürich**
Clumped isotope reordering in belemnite and optical calcites: Towards material-specific reordering kinetics

**P38** **N. Zhang – Tokyo Institute of Technology**
Abiotic methane formation in nature: information from clumped isotope analysis of laboratory synthesized methane

### ORIGIN AND EVOLUTION OF THE EARTH (PLANETS) & LIFE (POSTER SESSION)

Huiming Bao (Nanjing University)

**P39** **L. Liu – Australian National University**
SHRIMP-SI quadruple sulfur isotopic compositions of two generations of pyrite in the 3.49 Ga dresser formation

### GLOBAL CHANGE, GREENHOUSE GASES & AEROSOLS (POSTER SESSION)

Thomas Röckmann (Utrecht University) and Sakae Toyoda (Tokyo Institute of Technology)

**P40** **H. A. Scheeren – University of Gröningen**
Measuring the stable isotopic composition of pure $CO_2$ samples on a dual-laser absorption spectrometer using a back-dilution method to obtain dry ambient conditions

**P41** **P. M. Steur – University of Gröningen**
A four-year record (2017-2021) of $\Delta^{17}O$ in atmospheric $CO_2$ from Lutjewad station (NL)

**P42** **M. Fatima – VTT**
Comparison of laser sources and driver electronics for optical isotope ratio spectroscopy

**P43** **S. Toyoda – Tokyo Institute of Technology**
Spacio-temporal distributions of atmospheric nitrous oxide and its isotopocules

**P44** **A. Matson – Thünen Institute**
Research Gate Discussion Group: Isotopic tools to study $N_2O$ in soil and aquatic systems

### BIOGEOCHEMISTRY, ELEMENTAL CYCLES AND FATE OF CONTAMINANTS (POSTER SESSION)

Anat Bernstein (Ben-Gurion University of the Negev) and Moritz Lehmann (University of Basel)

**P45** **K. Müller – TU Munich**
Applicability of a reverse stable isotope labeling approach to show biodegradation of microplastics on a single-cell level

**P46** **A. Matson – Thünen Institute**
Using depth profiles and natural abundance stable isotopes to determine $N_2O$ processes in agricultural soils

**P47** **K. Kourtaki – University of Tübingen**
Application of compound-specific carbon isotope analysis on aerobic biotransformation of glyphosate

**P48** **A. Röhnelt – University of Tübingen**
Heterogenous oxidation of aminopolyphosphonates and AMPA at manganese oxide surfaces studied by carbon LC-IRMS

**P49** **O. Boukaroum – Aix-Marseille University**
Significant $^{2}H$ and $^{13}C$ isotope fractionation during volatilisation and diffusion of hydrocarbons in soil

## POSTERS

## TUESDAY, 15:00 – 16:30 & 18:00 – 19:00

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|---|---|---|
| **P50** | **P. Höhener – Aix-Marseille University**<br>DECiSIvE - Tracking degradation of soil pollutants with multi-elemental compound-specific isotope analysis | |
| **P51** | **M. Alvarez-Salas – ETH Zürich**<br>Stable isotopes of oxygen: the key to understand the soil fate of fertilizer-derived phosphorus? | |
| **P52** | **E. Stoll – University of Innsbruck**<br>New insights into climate change-driven soil $N_2O$ production and emissions in managed montane grassland | |
| **P53** | **M. Vinyes-Nadal – University of Barcelona**<br>Assessing methoxychlor contamination and natural attenuation in a polluted aquifer using carbon compound specific isotope analyses | |
| **P54** | **D. Lewicka-Szczebak – University of Wrocław**<br>Combining isotope mixing and fractionation with a new modelling tool applying the Monte Carlo approach | |
| **P55** | **M. Bucha – University of Wrocław**<br>Tracing anaerobic decomposition of lactate, butyrate, propionate, and acetate by means of carbon isotopic analyses of products $CH_4$, $CO_2$, and DIC in the continuous-flow open systems | |
| **P56** | **P. M. Magyar – University of Basel**<br>Constraining interplay between kinetic and equilibrium isotope effects during anammox in a wastewater treatment system | |
| **P57** | **T. Einzmann – University of Basel**<br>Understanding biogeochemical controls on nitrous oxide production and consumption in Lake Lugano, Switzerland | |
| **P58** | **S. G. Pati – University of Basel**<br>δ-scale calibration for stable isotope analysis of $O_2$ by continuous flow IRMS from -10 to +95 ‰ with in-vitro photosynthesis experiments | **P33/2** |
| **P59** | **C. F. M. de Carvalho – University of Basel**<br>Oxygen isotope fractionation during enzymatic $O_2$ consumption reactions | |
| **P60** | **T. Kuder – University of Oklahoma**<br>Hydrogen isotope exchange between trichloroethene and water under mild environmental conditions – implications for the use of hydrogen CSIA in contaminated site assessment | |
| **P61** | **N. Gluschankoff – Stanford University**<br>Isotopic analysis of nitrous oxide during El Niño and La Niña in the Eastern tropical South Pacific | |
| **P62** | **J. Mohn – Empa**<br>Tracing $N_2O$ formation in full-scale wastewater treatment with natural abundance isotopes | |

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## Keynote: Using carbon and oxygen isotopes of herbarium specimen to infer long-term physiological responses of plants to global environmental change

*Ansgar Kahmen*, David Basler, Daniel Nelson, Jurriaan de Vos, Cristina Moreno Guiterrez*

Department of Environmental Sciences – Botany, University of Basel, Basel, Switzerland
*presenting author: ansgar.kahmen@unibas.ch

The isotope analysis of archived plant material offers the exciting opportunity to reconstruct the physiological responses of plants to environmental change. In this regard, the carbon isotope composition of plants is a good proxy of leaf-level intrinsic water use efficiency (which is the ratio of net photosynthesis over stomatal conductance), and the oxygen isotope composition can provide time-integrated information on leaf stomatal conductance. In combination, the stable isotope analysis of carbon and oxygen thus allows to explicitly determine integrated values for net photosynthesis and stomatal conductance.

In my presentation, I will show how carbon and oxygen isotope data of 3000 herbarium specimen, that have been collected across Switzerland over the past century, can be used to infer long-term physiological responses of plants to global environmental change for more than 70 different plant species that grew in a large range of different habitats. For our study we overcame the multi-signal problem of oxygen isotope signal by applying a multi-model approach allowing to disentangle environmental and biochemical from physiological effects on the oxygen isotope signals in plants. The analysis shows that plants from all taxa and irrespective of their native habitat improved their water use efficiency over the past century. However, the contribution of net photosynthesis or stomatal conductance to changes in intrinsic water use efficiency different dramatically among different plant functions groups (i.e. herbs, legumes, grasses and sedges). Our study demonstrates that the carbon and water relations of plants respond to long-term changes in the environment but that the physiological nature of these responses differ among plant functional groups.

## Invited: Is a 'black box' approach sufficient to predict the exchange of $CO^{18}O$ and COS between soils and the atmosphere or do we need to dig deeper?

*L. Wingate[1,*], S. Jones[1,2], A. Kaisermann[1], E. Van Schaik[3], S. Mondy[3], L. Meredith[4], S. Wohl[1], M. Lelievre[3], & J. Ogée[1]*

[1]INRAE, Villenave D'Ornon, France
[2]Max Planck Institute for Biogeochemistry, Jena, Germany
[3]INRAE, GENOSOL Platform, Dijon, France
[4]University of Arizona, Dept of Ecosystem Genomics, Arizona, USA
*presenting author: lisa.wingate@inrae.fr

Interest in the seasonal and spatial variability of atmospheric COS concentrations and the stable oxygen isotope composition of $CO_2$ ($\delta^{18}O$-$CO_2$) has intensified as their usefulness as independent tracers of biosphere productivity in the carbon (C) cycle has been demonstrated. The key link between the soil-air exchange of $CO^{18}O$ and COS is a family of enzymes called carbonic anhydrases (CA) that catalyse $CO_2$ hydration, but also the isotopic exchange between $CO_2$ and water and the hydrolysis of COS in plants and soil microbes. Recently laser spectrometry microcosm studies

simultaneously measuring the fluxes of $CO^{18}O$ and COS at the soil surface have demonstrated that the CA activity of soil communities differ considerably across soil types and are modified significantly by land use practices such as fertilisation with inorganic N. In this talk we will present our current understanding of how soil abiotic and biotic drivers regulate the CA activity of soil communities over the land surface and how shifts in community structure and CA enzyme classes affected by land use management maybe be important to take into account.

### Oral: Fat and fit: diet estimation, macronutrient assimilation, and nutritional implications for an iconic Arctic predator

*C.A. Stricker[1,*], K.D. Rode[2], C.T. Robbins[3], and B.D. Taras[4]*

[1]U.S. Geological Survey, Fort Collins Science Center, Fort Collins, Colorado, USA
[2]U.S. Geological Survey, Alaska Science Center, Anchorage, Alaska, USA
[3]Washington State University, Pullman, Washington, USA
[4]Alaska Department of Fish & Game, Fairbanks, Alaska, USA
*presenting author: cstricker@usgs.gov

Nutrition has clear implications on wildlife population demographics and fitness. The polar bear, a species of conservation concern, is a novel carnivore that selectively consumes the blubber of marine mammal prey. Sea ice provides critical habitat for polar bears and ice-associated prey, but a warming climate negatively impacts the extent of ice cover, threatening the health and functioning of this Arctic ecosystem. Contemporary diet estimates are therefore an important piece to understanding the ecology of this iconic predator while also providing context for the future where continued warming may restrict prey access. We approached this problem through ~10 years of systematic studies that relied on stable isotope tracers. Numerous captive feeding studies on ursids, including zoo polar bears were used to develop isotopic input functions needed for diet estimation. Multi-year capture efforts in the Chukchi and Beaufort Sea provided polar bear tissue samples. Dietary inference was focused on guard hair because the timing of growth integrates at-sea diet, a potential nutritional bottleneck as the sea ice minimum is approached annually. A large suite of prey tissue samples was assembled from field collections and subsistence harvests to produce a database of muscle and blubber isotopic compositions. These key pieces of information were used to implement a diet modeling framework that simultaneously accounted for protein and lipid macronutrient assimilation. The model was initially applied to the Chukchi Sea subpopulation to estimate lipid assimilation and diet composition over a 10-year period and subsequently applied to the Beaufort Sea subpopulation to contrast diet estimates across the range, while also exploring the role of nutrition on select population vital rates. Finally, the results from this work and other tangential studies allowed us to develop a better understanding of the health and evolutionary implications of protein overconsumption in ursids.

### Oral: Re-evaluation of the $^{13}C$ isotope fractionation associated with fatty acids biosynthesis by position-specific isotope analysis

*Maxime Julien[1,2*], Yu Zhao[3], Youping Zhou[3], Keita Yamada[4], Naohiro Yoshida[5], Gérald S. Remaud[6], Alexis Gilbert[2]*

[1]GFZ German Research Centre for Geosciences - Helmholtz Centre Potsdam, Potsdam, Germany.
[2]Department of Earth and Planetary Sciences, Tokyo Institute of Technology, Tokyo, Japan.
[3]Shaanxi University of Science and Technology, Xi'an, China.
[4]Department of Environmental Chemistry and Engineering, Tokyo Institute of Technology, Yokohama, Japan.
[5]Earth-Life Science Institute, Tokyo Institute of Technology, Tokyo, Japan.
[6]CEISAM Laboratory, University of Nantes, France.
*presenting author: mjulien@gfz-potsdam.de

Lipids can be preserved in sediments as different chemical species (free lipids, reduced to alkanes, macromolecular kerogens) and their carbon isotopic composition is thus of interest to trace present and past biogeochemical processes [1]. Long-chain fatty acids carry relatively depleted $\delta^{13}C$ signatures compared to other biomolecules and, from the limited analyses so far carried out, show an alternating pattern of relative enrichment/depletion in their $\delta^{13}C_i$ values along their carbon chain [2].

In the present study, $^{13}C$ position-specific isotope analysis (PSIA) was performed on fatty acids from vegetable oils using Nuclear Magnetic Resonance. Measured $^{13}C$ patterns are not in accordance with the conventional view of the relative $^{13}C$-depletion of acetogenic lipids and their alternation of $^{13}C$-enriched and $^{13}C$-depleted carbon positions. Whereas it is commonly accepted that the pyruvate dehydrogenase (PDH) is responsible for the $^{13}C$ distribution within fatty acids [3], data from the present work demonstrate that the conversion of acetyl-CoA to malonyl-CoA catalyzed by acetyl-CoA carboxylase (ACC) needs to be considered while explaining the measured non-stochastic $^{13}C$ pattern within fatty acids.

Combined with steady-state calculation, these data give a new description of metabolic steps responsible for typical $^{13}C$ intramolecular distribution patterns within acetogenic lipids.

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## Oral: Position-specific isotope labelling gives new insights into chiral monoterpene synthesis

*L. Erik Daber[1,*], Philipp Nolte[1], Jürgen Kreuzwieser[1] & Christiane Werner[1]*

[1]Chair of Ecosystem Physiology, Albert-Ludwigs-Universität Freiburg, Georges-Köhler-Allee 053/054, 79110 Freiburg, Germany
*presenting author: erik.daber@cep.uni-Freiburg.de

Plants produce and emit a plethora of biogenic volatile organic compounds (BVOCs) to communicate with their environment, e.g. to attract pollinators, repel herbivores or directly reduce effects of external stressors such as drought. Due to their high diversity and importance for plant defense, monoterpenes have been in focus of researchers for several decades. Even though general metabolic pathways for monoterpene synthesis are well-studied, there remain uncertainties regarding regulation of enantiomeric composition. Chemical properties and biosynthetic pathways of chiral monoterpenes, such as (+)/(-)-limonene can hereby greatly differ. We hypothesize that *de novo* synthesis of monoterpenes in *(i)* takes place in both cytosol and plastids; *(ii)* can be fueled by bidirectional carbon-exchange between cytosol and plastid and that *(iii)* enantiomeric composition of *de novo* monoterpene biosynthesis differs between cytosol and plastid. To get deeper insight into regulation of different chiral and non-chiral monoterpene synthesis from *de novo* or storage emissions and their biosynthetic pathways, we labelled branches of potted, two-year old *Picea abies* saplings with position-specific $^{13}$C-pyruvate ($^{13}$C2- and $^{13}$C1-labelled) or $^{13}CO_2$, respectively. Terpenoid emissions were measured with GC-MS-C-IRMS with a chiral separation column for identification, quantification of and $^{13}$C incorporation into chiral monoter-penes. We found distinct incorporation patterns for our three labelling approaches. Sabinene and (-)-α-pinene were labelled via all three treatments. $^{13}$C2-pyruvate label was found in both (+)/(-)-limonene, while $^{13}CO_2$-labelling via photosynthesis showed significant $^{13}$C-incorporation in all measured monoterpenes, with highest $\delta^{13}C$ values in (+)-limonene, (+)-α -pinene, myrcene and camphene. Our results indicate major differences in *de novo* synthesis of various monoterpenes depending on carbon source and raise questions about the role of refixation of $CO_2$ emitted during pyruvate decarboxylation, involved metabolic pathways and plastidial/cytosolic exchange of carbon precursors for monoterpene synthesis.

## Oral: Historic European monthly precipitation isotope time series reconstructions using machine learning

*D.B. Nelson[1,*], D. Basler[1] & A. Kahmen[1]*

[1]Department of Environmental Sciences – Botany, University of Basel, Basel, Switzerland
*presenting author: daniel.nelson@unibas.ch

Hydrogen and oxygen isotope values of precipitation are critically important quantities for applications in Earth, environmental, and biological sciences. However, direct measurements are not available at every location and time, and existing precipitation isotope models are often not sufficiently accurate for examining features such as long-term trends or interannual variability. This can limit applications that seek to use these values to identify the source history of water or to understand the hydrological or meteorological processes that determine these values. We developed a framework using gradient boosted regression tree-based machine learning, which we used to implement a procedure for calculating isotope time series at monthly resolution using available climate and location data at any coordinate location within the model spatial domain. Here we present two applications of our modelling framework, Piso.AI, one of which uses a diverse suite of climate and geographic data as predictor inputs to generate highly accurate monthly time series records beginning in 1950, and the second of which uses a restricted set of predictors to allow time series to be generated that begin in 1901 with slightly reduced accuracy compared to the 1950 model. Both products can be applied over most of Europe, and were trained on the historic archive of precipitation isotope data available from the Global Network of Isotopes in Precipitation. These model products facilitate simple, user-friendly predictions of precipitation isotope time series that can be generated on demand and are accurate enough to be used for exploration of interannual and long-term variability in both hydrogen and oxygen isotopic systems. These predictions provide important isotope input variables for ecological and hydrological applications, as well as powerful targets for paleoclimate proxy calibration, and they can serve as resources for probing historic patterns in the isotopic composition of precipitation with a high level of meteorological accuracy. Predictions from our modelling framework can be accessed at https://isotope.bot.unibas.ch/PisoAI/.

## Oral: Exploring the potential of $\Delta^{17}O$ in $CO_2$ for determining mesophyll conductance

*Getachew Agmuas Adnew[1], Thijs L. Pons[2], Gerbrand Koren[3], Wouter Peters[4,5], Thomas Röckmann[1,*]*

[1]Institute for Marine and Atmospheric research Utrecht (IMAU), Utrecht University, The Netherlands
[2]Institute of Environmental Biology, Utrecht University, The Netherlands
[3]Copernicus Institute of Sustainable Development, Utrecht, Utrecht University, the Netherlands
[4]Department of Meteorology and Air Quality, Wageningen University, The Netherlands
[5]Centre for Isotope Research, University of Groningen, The Netherlands
*presenting author: t.roeckmann@uu.nl

Mesophyll conductance to $CO_2$ from the intercellular airspace to the $CO_2$-$H_2O$ exchange site has been estimated using $\delta^{18}O$ measurements ($g_{m18}$). However, the $g_{m18}$ estimates are affected by the uncertainties in the $\delta^{18}O$ of leaf water where $CO_2$-$H_2O$ exchange takes place and the degree of isotopic equilibration between water and $CO_2$ in the leaf. Using $\Delta^{17}O$ ($\text{i.e.}\,\Delta^{17}O = \delta^{17}O - 0.528 \times \delta^{18}O$) for estimating $g_m$ ($g_{m\Delta17}$) can provide independent constraints on $g_m$ since the $\Delta^{17}O$ is less affected by fractionation processes during gas exchange. The $g_m$ calculations are applied to combined measurements of $\delta^{18}O$ and $\Delta^{17}O$, and gas exchange in two $C_3$ species, sunflower and ivy, and the $C_4$ species maize. The $g_{m18}$ and $g_{m\ \Delta17}$ estimates agree within the combined errors (p value is 0.876). Both approaches are associated with large errors when the isotopic composition in the intercellular air space becomes close to the one at the $CO_2$-$H_2O$ exchange site. Although values and changes of $\Delta^{17}O$ are small, it can be measured with much higher precision compared to $\delta^{18}O$. Measuring $g_{m\Delta17}$ has a few advantages compared to $g_{m18}$: i) it is

less sensitive to uncertainty in the isotopic composition of leaf water at the isotope exchange site and ii) the relative change in the $g_m$ due to an assumed error in the equilibration fraction $\theta_{eq}$ is lower for $g_{m\Delta17}$ compared to $g_{m18}$. Thus, using $\Delta^{17}O$ can complement and improve the $g_m$ estimates in settings where the $\delta^{18}O$ of leaf water varies strongly, affecting the $\delta^{18}O$ ($CO_2$) difference between the intercellular air space and the $CO_2$-$H_2O$ exchange site.

## POSTERS P1 – P8

### P1 Using $N_2O$ isotopocule analysis and $^{15}N$ tracing approach to gain insights into $N_2O$ source processes in hydroponic tomato cultivation

*C. Buchen-Tschiskale[1,*], R. Well[1], L. Odasso[2], D. Schwarz[2], S. Karlowsky[2]*

[1]Thuenen Institute of Climate-Smart Agriculture, Braunschweig, Germany
[2]Leibniz Institute of Vegetable and Ornamental Crops (IGZ), Großbeeren, Germany
*presenting author: caroline.buchen-tschiskale@thuenen.de

For greenhouse cultivation, knowledge about nitrous oxide ($N_2O$) production pathways and the contribution of $N_2O$ reduction is so far very limited. Although hydroponic cultivation in greenhouse systems has a high potential for $N_2O$ emissions due to the high application rates of nitrogen (N) fertilizers [3].

A promising tool to gain insights into $N_2O$ source processes is the combination of stable isotope analyses of emitted $N_2O$ ($\delta^{18}O_{N2O}$ and $\delta^{15}N^{SP}{}_{N2O}$) and data interpretation with the isotopocule mapping approach [4; 6; 2]. This approach was applied in combination with a $^{15}N$ tracing experiment for two different growth stages in a greenhouse hydroponic experiment with growing tomatoes (*Solanum lycopersicum*) on rock wool slabs.

Shortly before $^{15}N$ fertilizer application, natural abundance $N_2O$ gas samples were collected from static chamber measurements and analyzed by isotope ratio mass spectrometry (IRMS). $^{15}N$-enriched gas samples were collected 4h and 24h after $^{15}N$ fertilizer application ($^{15}NH_4{}^+NO_3{}^-$, $NH_4{}^+{}^{15}NO_3{}^-$) to the hydroponic system and also analyzed by IRMS. Calculations of the contributions of $N_2O$ originating from the labeled and non-labeled pools were based on the non-equilibrium distribution of $N_2O$ isotopocules, as described by Spott et al. [5] and Bergsma et al. [1].

Both approaches indicated a large contribution of denitrification and/or nitrifier-denitrification with high $N_2O$ reduction rates at both growth stages.

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### P2 Combining $^{15}N$ tracing and $^{15}N$ site preference of $N_2O$ to distinguish production by nitrification and fungal denitrification

*R. Well[1,*], L. Rohe[1] & J. Mohn[2]*

[1]Thünen Institute of Climate-Smart Agriculture, Braunschweig, Germany
[2]Empa, Laboratory for Air Pollution / Environmental Technology, Dübendorf, Switzerland
*presenting author: reinhard.well@thuenen.de

Isotopocule signatures of $N_2O$ such as $\delta^{18}O$, average $\delta^{15}N$ ($\delta^{15}N^{bulk}$) and $^{15}N$ site preference (SP = difference in $\delta^{15}N$ between the central and peripheral N positions of the asymmetric $N_2O$ molecule) can be used to constrain the atmospheric $N_2O$ budget and to characterize $N_2O$ turnover processes. However, due to overlap of endmember values of multiple source processes and to the impact of $N_2O$ reduction to $N_2$ on isotopocule values of residual $N_2O$, it is not possible to disentangle all of the processes (Yu et al., 2020). Especially the distinction of nitrification and fungal denitrification is not straightforward due to overlap in SP and in view of the large variability of $\delta^{18}O$ of $N_2O$ from bacterial denitrification (Rohe et al., 2017).

Theoretically, low level labelling with $^{15}N$ may allow both, a clear distinction of $NO_3^-$ and $NH_4^+$ derived fluxes by $^{15}N$ tracing, and the use of SP as additional constraint. This combination of $^{15}N$ tracer and site-specific isotopic information has the potential to complement and validate current natural abundance isotopocule mapping approaches including 3DI-model (Lewicka-Szczebak et al., 2020), but this has not yet been realized to our knowledge. As a first test of this approach, we conducted incubations of bacterial pure cultures applying $^{15}N$-labelled $NO_3^-$ (0.6 at% $^{15}N$), finding small deviations in SP (up to 5 ‰) from results of previous incubations with unlabelled N sources. To validate the analytics, we prepared $N_2O$ mixtures varying in $^{15}N$ enrichment up to 1 at% but with constant SP. Samples were/will be analysed by IRMS (Lewicka-Szczebak et al., 2017) and laser spectroscopy (Mohn et al., 2022). Finally, we conduct an incubation experiment

with soil amended with unlabelled $NH_4^+$ and $^{15}N$ labelled $NO_3^-$ (0.7 at% $^{15}N$) under dry and wet conditions to induce nitrification only or nitrification and denitrification, respectively.

If successful, low-level $^{15}N$ labelling will extend the "isotope–tool box" to distinguish fungal denitrification and nitrification and will be applied in our current project on fungal denitrification methods and dynamics (DFG project # 251282570, https://www.thuenen.de/en/ak/projects/control-of-fungal-denitrification-in-soils/?no_cache=1).

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## P3 Oxygen isotopes in phosphate: defining potentials and limitations for environmental studies

*F. Tamburini[1,*], C. Pistocchi[2], M. Siegenthaler[1, 3], C. von Sperber[4]*

[1]Group of Plant Nutrition, D-USYS, ETH Zurich, Zurich, Switzerland
[2]UMR Eco&Sols, INRAE, Montpellier, France
[3]Swiss Soil Monitoring Network NABO, Agroscope, Zurich, Switzerland
[4]Dept. of Geography, McGill UNiversity, Montréal, Quebec, Canada.
*presenting author: federica.tamburini@usys.ethz.ch

Phosphorus (P) is a limiting nutrient in many ecosystems around the world. Excess fertilization to overcome the limitation for crops can lead to the transfer of P from agricultural soils to aquatic ecosystems, resulting in eutrophication and toxic algal blooms. A great deal of research has been done in order to understand the cycling, the transfers and fluxes of P in the environment. Among the analytical tools used to deepen our understanding of the environmental P cycle, the ratio of oxygen isotopes in phosphate (reported as $\delta^{18}$O-$PO_4$ compared to VSMOW) has provided crucial new insights during the past two decades.

Under ambient conditions and in absence of biological activity the oxygen isotope composition of phosphate does not change. However, the activity of specific enzymes, i.e. phosphatases, promotes a phosphoryl transfer between phosphate and water. The associated oxygen exchange is subject to isotopic fractionations that have been determined for a range of phosphatases and substrates. The isotopic imprint caused by enzymatic reactions have been used by numerous studies studying P cycling, initially in aquatic ecosystems and then later in the soil-plant system. While there are undeniable spatial and temporal patterns in the $\delta^{18}$O-$PO_4$ in environmental systems (e.g. seasonal variation; decreasing $\delta^{18}$O-$PO_4$ values with soil depth; increasing $\delta^{18}$O-$PO_4$ values along riparian buffer strips), variations of $\delta^{18}$O-$PO_4$ values in different soil P pools and in controlled soil incubation experiments show that the current interpretation is far from conclusive. We have identified the following reasons for this persisting problem:

1: Disconnectedness of controlled laboratory conditions to complex environmental systems.

2: Crucial knowledge gaps of isotopic effects of metabolic pathways of microorganisms and plants.

3. Uncertainties associated with the determination of $\delta^{18}$O values of bioavailable soil water and intracellular metabolic water.

4: Imperfect identification and characterisation of relevant P pools in the environment.

5: Overlapping $\delta^{18}$O-$PO_4$ values of processes and endmembers.

Here we discuss these issues that obstruct the interpretation of $\delta^{18}$O-$PO_4$ data in environmental systems and we identify gaps in knowledge that should be addressed in future studies.

## P4 Intramolecular $^{13}$C patterns of plant glucose convey environmental and metabolic information

*T. Wieloch[1], R.A. Werner[2,*] & J. Schleucher[1]*

[1]Department of Medical Biochemistry and Biophysics, Umeå University, Umeå, Sweden
[2]Department of Environmental System Sciences, ETH Zürich, Zürich, Switzerland
*presenting author: roland.werner@usys.ethz.ch

The $^{13}C/^{12}C$ ratio of tree-ring cellulose is often used as proxy for past climate conditions. Whole cellulose is $^{13}C$ depleted with respect to air $CO_2$. This is commonly attributed to $^{13}C$ fractionation (denoted $^{13}\varepsilon$) associated with $CO_2$ assimilation (i.e., $CO_2$ diffusion vs. Rubisco carboxylation, Farquhar *et al.* model). Accordingly, $^{13}\varepsilon$ variability depends on stomatal conductance and the rate of $CO_2$ assimilation and their environmental influences (e.g., air vapor pressure deficit, VPD). Recently, Wieloch *et al.* [1] measured intramolecular $^{13}C/^{12}C$ ratios in glucose units of cellulose extracted from a tree-ring time series of *Pinus nigra*. They reported large positional deviations from the whole-molecule average of up to 10 mUr. Not all C atoms in glucose were sensitive to VPD. While C-1 to C-3 showed a negative correlation between VPD and $^{13}\varepsilon$ in line with the Farquhar *et al.* model, C-4 to C-6 were largely independent of VPD [1]. Since it can be expected that initially all C atoms were affected equally by $^{13}C$ fractionation of $CO_2$ assimilation, these observations point at $^{13}C$ fractionation in post-Rubisco metabolism. Removing the initial $^{13}C$ fractionation from C-4 requires an inverse $^{13}C$ fractionation of about 4 mUr [2]. An effect of this magnitude points to a reaction in central carbon metabolism. After assessing the potential of all reactions within

central metabolism to generate the C-4 effect, we propose glyceraldehyde 3-phosphate (GAP) in the cytosol of leaves as most plausible origin [2]. GAP is the substrate of two different glyceraldehyde-3-phosphate dehydrogenases (GAPDH): a reversible phosphorylating and a non-reversible non-phosphorylating GAPDH. We show that changes in carbon flux around these enzymes can introduce $^{13}C$ variability of substantial size. Our observations are consistent with a carbon flux pattern termed the cytosolic oxidation–reduction (COR) cycle [3]. We propose that this cycle constitutes a central hub in leaf energy metabolism and may help to maintain leaf-cytosolic redox balances under varying environmental conditions.

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### P5 Insights into nitrous oxide reduction by soybean inoculated with *Bradyrhizobium* from concentration and isotopocule analyses in a field

*F. Damak[1,*], S. Toyoda[1], M. Takeda[2], H. Akiyama[2], Y. Sasaki[2], K. Minamisawa[3]*

[1]School of Materials and Chemical Technology, Tokyo Institute of Technology, Yokohama, Japan
[2]Institute for Agro-Environmental Sciences, National Agriculture and Food Research Organization, Japan
[3]Graduate School of Life Sciences, Tohoku University, Japan
*presenting author: damak.f.aa@m.titech.ac.jp

Soybeans are leguminous plants that play an important role in nitrogen fixation and transformation. Large $N_2O$ emissions have been reported during the decomposition of the root nodules but the inoculation of *Bradyrhizobium sp.* with nosZ gene coding $N_2O$ reductase ($N_2OR$) has been reported to be effective in $N_2O$ emissions mitigation at field scale.

In this study, we report the $N_2O$ concentrations and isotopocule ratios ($\delta^{15}N$, $\delta^{18}O$ and SP= $\delta^{15}N^{\alpha}$ - $\delta^{15}N^{\beta}$ of $N^{\beta}N^{\alpha}O$) in plots of soybeans inoculated with indigineous *Bradyrhizobium sp.* isolated from Japanese agricultural soils. Treatments are (1) a mixture of 63 strains with nosZ+ stains (nosZ+), (2) a strain with higher $N_2OR$ activity (nosZ++) and soybeans without inoculation (Native). Gas samples were collected during four weeks of the decomposition phase. Furthermore, three gas sampling methods, namely soil probes made of silicon tube (Si) or stainless-steel tube (SUS) and the flux chamber (NC) are compared in order to determine the extent of $N_2O$ production and reduction with the best possible resolution.

The Si and SUS tube sampling methods resulted in similar concentrations and isotopomer ratios values, independently of the soybean inoculant and the date, and showed a siginificant correlation. However, the standard deviations pattern revealed that the Si-tube produces less variability between the replicate plots. This result could be attributed to the nature of the SUS-tube method being a single point sampler in time and space.

The $N_2O$ concentrations increased in the second week and were the highest in the Native plots, followed by nosZ++ and nosZ+ plots whichever the sampling method used, which shows that $N_2O$ reduction was more important in the latter plots.

Although the isotopomer ratios from the NC were significantly lower than those obtained by the Si method, the isotopomer ratios of the Native were always lower than those of nosZ+ and nosZ++ (except in the case of nosZ++ with Si) which confirms the concentrations data and is indicative of a higher progress of reduction of $N_2O$ in the nosZ+and nosZ++ compared with the Native.

### P6 Leaf-level metabolic changes in daytime respiration and isoprenoid synthesis during drought determined by position-specific $^{13}C$-pyruvate labeling

*S.N. Ladd[1,2,*], L.E. Daber[1], I. Bamberger[1,3], A. Kübert[1], J. Kreuzwieser[1], G. Purser[4,5], J. Ingrisch[1,6], J. Deleeuw[7], J. van Haren[7,8], L.K. Meredith[7,9] & C. Werner[1]*

[1]Ecosystem Physiology, University of Freiburg, Freiburg, Germany
[2]Environmental Sciences, University of Basel, Basel, Switzerland
[3]Atmospheric Chemistry, BayCEER, Bayreuth, Germany
[4]School of Chemistry, University of Edinburgh, Edinburgh, UK
[5]UK Centre for Ecology & Hydrology, Penicuik, UK
[6]Department of Ecology, University of Innsbruck, Innsbruck, Austria
[7]Biosphere 2, University of Arizona, Oracle, AZ, USA
[8]Honors College, University of Arizona, Tucson, AZ, USA
[9]School of Natural Resources and the Environment, University of Arizona, Tucson, AZ, USA
*presenting author: n.ladd@unibas.ch

Daytime respiration in leaves is an integral component of plants' metabolism, with $CO_2$ produced as a byproduct during the synthesis of many secondary metabolites, including volatile organic compounds (VOCs), such as isoprene and monoterpenes. It remains challenging to assess how the carbon fluxes associated with daytime respiration and VOC emission vary among mature trees, and how these fluxes are influenced by drought. To assess the metabolic processes responsible for daytime emissions of $CO_2$ and isoprenoid VOCs in mature individuals of four tropical species, and to determine if these carbon fluxes change with drought stress, we used position-specific $^{13}C$-pyruvate labeling to study leaf level $CO_2$ and VOC fluxes before and during drought in an enclosed rainforest at the Biosphere 2 research facility.

Although daytime respiration was always dominated by non-mitochondrial processes in all four species, the relative contribution of $CO_2$ produced from the tricarboxylic acid cycle during pre-drought conditions was highest in the legume tree *Clitoria fairchildiana*. The relative importance of the tricarboxylic acid cycle to daytime respiration increased for the other three species during the drought. For *C. fairchildiana*, our data also indicate that intermediates from the cytosolic mevalonic acid pathway are used to produce the monoterpene trans-β-ocimene, but not isoprene. This suggests that geranyl pyrophosphate is the more important intermediate for crosstalk between cytosolic and plastidial isoprenoid synthesis, not isopentenyl pyrophosphate. Crosstalk declined with drought, and although trans-β-ocimene emissions increased, they were primarily sourced from storage and not *de novo* synthesis. Overall, our

results demonstrate how position-specific $^{13}$C-pyruvate labeling can be an important tool to identify metabolic adjustments due to drought, and indicate how these adjustments affect daytime respiratory processes and isoprenoid synthesis.

## P7 Whole ecosystem $^{13}CO_2$ and $^{2}H_2O$ Pulse-Labelling to investigate carbon allocation, $CO_2$ and VOC emissions and the role of deep water reserves during drought

*Christiane Werner[1,*], Laura K. Meredith[2,3], S. Nemiah Ladd[1,4], B2WALD Team (author list of science paper)*

[1]Ecosystem Physiology, University of Freiburg, Germany.
[2]School of Natural Resources and the Environment, University of Arizona, USA
[3]Biosphere 2, University of Arizona, USA
[4]Department of Environmental Sciences, University of Basel, Switzerland, USA
*presenting author: c.werner@cep.uni-freiburg.de

Severe droughts are increasing worldwide, however, how physiological plant responses drive ecosystem water, carbon and biogenic volatile organic compound (VOC) fluxes during drought and recovery remains poorly understood. To disentangle complex ecosystem dynamics we imposed a 9.5-week drought on the Biosphere 2 (B2) tropical rainforest in the Water, Atmosphere, and Life Dynamics (B2WALD) experiment [1]. We continuously measured isofluxes of ecosystem exchange, soil and leaf $H_2O$, $CO_2$ and BVOCs, over five months. To trace changes in soil-plant-atmosphere interactions we labelled the ecosystem with a $^{13}CO_2$-pulse during pre-drought and drought. Subsequently, we introduced $^{2}$H-labelled deep-water label during severe drought, providing a unique opportunity to evaluate transit times and legacy effects during the recovery phase.

Ecosystem $^{13}CO_2$-pulse-labeling showed that drought enhanced the mean residence times of freshly assimilated carbon, indicating down-regulation of carbon cycling velocity and delayed transport form leaves to trunk and roots. Despite reduced ecosystem carbon uptake and total VOC emissions, plants continued to allocate a similar proportion of fresh carbon to de novo VOC synthesis, as incorporation of 13C into both isoprene and monoterpenes remained high. Maintaining carbon allocation into VOC synthesis demonstrates the fundamental role of these compounds in protecting plants from heat stress and photooxidative damage. VOC uptake increased immediately upon rain rewetting. Interestingly, all deep-rooted canopy trees taped into deep-water reserves, but spared deep water reserves until severe drought and exhibited long transit times until d2H-labelled water was transpired.

These data highlight the importance of quantifying drought impacts on forest functioning beyond the intensity of (meteorological) drought, but also taking dynamics response of hydraulic regulation of different vegetation compounds and soil microbial activity of the forest into account.

[1] Werner, C et al. (2021). Ecosystem fluxes during drought and recovery in an experimental forest. Science 374, 1514. DOI: 10.1126/science.abj6789

## P8 Comparing hydrogen isotope compositions of different lipid compounds across species to address possible origin of variation

*J. Baan[1,*], M. Holloway-Phillips[1], J. M. de Vos[1], D. B. Nelson[1], A. Kahmen[1]*

[1]University of Basel, Department of Environmental Sciences – Botany, Basel, Switzerland
*presenting author: jochem.baan@unibas.ch

Hydrogen stable isotope ($\delta^2$H) values of plant derived lipid compounds integrate environmental, as well as plant biochemical information. Large variation in lipid $\delta^2$H values among species, containing a phylogenetic signal, at a single geographic location suggests that species-specific plant biochemistry induces strong variation in lipid $\delta^2$H. The exact mechanism driving variation in lipid $\delta^2$H values is still poorly understood, but $\delta^2$H values of NADPH derived H has been suggested to be an important driver as it is a major contributor to H in lipid compounds. Various plant lipid compounds are synthesized through different biosynthetic pathways and may utilize similar or different H-sources depending on the cellular location of synthesis. We measured $\delta^2$H values of the acetogenic lipid compounds, *n*-alkanes and their chloroplast produced precursor molecules, palmitic acid, and the chloroplast produced isoprenoid lipid, phytol, across a large set of eudicot species grown in a botanical garden, and tested if phylogenetic relatedness structures variation in lipid $\delta^2$H values. Additionally, we compared $\delta^2$H values of these different lipid compounds to determine possible biochemical origins of variation in lipid $\delta^2$H values. We found that lipid $\delta^2$H values significantly evolved along the eudicot phylogeny, indicating that lipid $\delta^2$H values are likely driven by evolutionary relevant functional traits. Our results also show that species-specific variation in *n*-alkane $\delta^2$H values originates in the chloroplast. Lastly, species-specific $\delta^2$H values of different lipid compounds produced in the same cellular location did not strongly correlate, suggesting that processes within biosynthetic pathways, rather than $\delta^2$H values of H-sources, cause variation in lipid $\delta^2$H values across species.

## Keynote: Improving accessibility to food authentication, using stable isotope analysis, in developing countries: The activities of the joint FAO/IAEA Centre's food safety and control laboratory

*S. D. Kelly*, A. Abrahim, B. Maestroni, A. Mihailova, M. Islam, V. Golla*

Food Safety and Control Laboratory, Joint FAO/IAEA Centre of Nuclear Techniques in Food and Agriculture, Department of Nuclear Sciences and Applications, International Atomic Energy Agency, Vienna International Centre, Wagramer Strasse 5, PO Box 100, 1400 Vienna, Austria
*presenting author: s.kelly@iaea.org

Isotope ratio mass spectrometry has been used to detect economic fraud in food production since the early 1970s yet in many conferences dedicated to gas isotope mass spectrometry, presentations reporting the use of IRMS in food authentication have often been relegated to the 'miscellaneous sessions'. For the majority of users IRMS techniques are still the domain of geologists, ecologists and environmental scientists. However, the technique is gaining wider acceptance in food control laboratories, and is now very much 'front and centre' in terms of reliability and array of online techniques culminating in the use of IRMS analyses as 'another detection system for food labs'. Ultimately, IRMS can offer unequivocal evidence of food alteration such that it may be used as a practical everyday tool by control laboratories and enforcement agencies in the prosecution of dishonest traders. However, the fast evolving world of food fraud presents a unique set of challenges to developing countries due to the relatively high costs of state-of-the-art instrumentation and the lack of human capacity to implement analytical surveillance often imposed by markets such as the European Union. For developing countries to actively participate in food authenticity stable isotope testing of produce from their domestic markets, and for export, there is a need to develop appropriate, affordable, and rapid methods to screen foods for adulterants using accessible methods and instrumentation. Consequently, there is an increasing demand for strategic technical support that will enhance national food control systems and monitoring programs. Some examples will be given from the work of the FAO/IAEA Joint Centre of Nuclear Techniques in Food and Agriculture's Food Safety and Control Laboratory to target food authentication and origin as an important component of food quality and traceability. This presentation will also take a forward look at how stable isotope analysis might answer some of the more challenging questions around 'added-value claims' relating to food origin and food production methods, that are often important for the protection and promotion of foods produced in low and middle income countries.

## Invited: Distinct nitrogen isotopic compositions of healthy and cancerous tissue in mice brain and head&neck micro-biopsies

*M. Straub[1,*], D.M. Sigman[2], A. Auderset[3], J. Ollivier[4], B. Petit[4], B. Hinnenberg[3], F. Rubach[3], S. Oleynik[2], M.C. Vozenin[4], A. Martínez-García[3]*

[1]Institute of Radiation Physics, Lausanne University Hospital and University of Lausanne, Switzerland
[2]Department of Geosciences, Princeton University, Princeton, NJ, 08544, USA
[3]Max Planck Institute for Chemistry, 55128, Mainz, Germany
[4]Radiation Oncology Laboratory/DO/Radio-Oncology/CHUV, Lausanne University Hospital and University of Lausanne, 1011 Lausanne, Switzerland
*presenting author: marietta.straub@chuv.ch

Cancer cells have the ability to use $^{15}N$-depleted N by-products as ammonium ($NH_4$) as a source of N [1]. This recycling of a normally toxic waste product has been hypothesized to result in a measurably lower $^{15}N/^{14}N$ of tumors relative to the surrounding heathy tissue, in which low-$^{15}N$ metabolic N is not recycled but rather released to the extracellular environment [1-2]. In our study, measurements on micro-biopsies from mice brain and head&neck tumors, revealed a coherently lower $^{15}N/^{14}N$ ratio in tumor relative to neighboring healthy tissue from the same organ of individual mice [3]. Results are statistically significant for the cerebral tumor model, for the head&neck tumor model results are less conclusive. Cerebral tumors tissue were homogeneous and showed a high cancer cell density, as did the tumor bed and healthy tissue for healthy cell abundance. Contrary, in the head&neck model, the healthy tissue was partly infiltrated by tumor cells and vice versa. Therefore, discrimination by $^{15}N/^{14}N$ ratio was less conclusive due to intermingling of tissue types. Despite such challenges in tissue separation, our results confirm that cancerous and healthy tissue can be distinguished based on distinct isotopic signatures in $^{15}N/^{14}N$. The small samples required by our method greatly increase the potential of natural abundance nitrogen stable isotope variation for cancer diagnosis and cancer metabolism research where sampling size shall be kept as low as possible for patient well-being. Our study indicates that sampling technique, sampling size and appropriate tissue selection are crucial parameters to be able to determine differences in isotopic signatures on a cellular level. Different sampling strategies are currently being tested and ongoing work is elaborating possible applications of this technique in human tissue samples.

[1] Spinelli JB, Yoon H, Ringel AE, et al. (2017). Metabolic recycling of ammonia via glutamate dehydrogenase supports breast cancer biomass. Science 358(6365), 941–6. https://doi.org/10.1126/science.aam9305.
[2] Dang CV (2017). Feeding frenzy for cancer cells. Science 358(6365), 862–3. https://doi.org/10.1126/science.aaq1070.
[3] Straub M, Sigman DM, Auderset A, et al. (2021). Distinct nitrogen isotopic compositions of healthy and cancerous tissue in mice brain and head&neck micro-biopsies. BMC Cancer 21, 805. https://doi.org/10.1186/s12885-021-08489-x.

## Oral: Stable isotope ratio analysis to assess pharmaceuticals, cosmetics and dietary supplements authenticity

*M. Perini[1,*] & S. Pianezze[1]*

[1]Centro di Trasferimento Tecnologico, Fondazione Edmund Mach, San Michele all'Adige (TN), Italy
*presenting author: matteo.perini@fmach.it

The stable isotope ratio analysis of the mayor bio-elements (hydrogen, carbon, nitrogen, oxygen and sulphur) makes it possible to authenticate pharmaceuticals, cosmetics and dietary supplements. This technique, applied to bulk samples and/or to specific compounds, can be used to detect the origin of an ingredient (synthetic or natural), the substitution of one ingredient with another, as well as the geographical and/or botanical origin of the products. The $\delta^{13}C$ and $\delta^{2}H$ values of vanillin can determine whether this product is natural (deriving from the expensive CAM plant *Vanilla*), biotechnologically produced or synthetic [1]. Moreover, the $\delta^{13}C$ values of specific components of *Rosa damascene mill.*, one of the most expensive essential oils in the global market, can indicate the fraudulent addition of cheaper oil from C4 plants (e.g., *Cymbopogon martinii, palmarosa*) [2]. Finally, the $\delta^{13}C$ analysis is a suitable tool to discriminate between Monacolin K (contained in red yeast rice-based dietary supplements) and the marketed statin [3] and between natural L-theanine (extracted from *Camellia Sinensis*) and the biosynthetically produced one [4].

These examples show that the isotopic fingerprint represents an effective tool for the authenticity assessment of economically relevant pharmaceuticals, cosmetics and dietary supplements.

[1] M. Perini, S. Pianezze, L. Strojnik, F. Camin (2019). C and H stable isotope ratio analysis using solid-phase microextraction and gas chromatography-isotope ratio mass spectrometry for vanillin authentication. Journal of Chromatography A 1595, 168–173. DOI: 10.1016/j.chroma.2019.02.032.

[2] F. Pellati, G. Orlandini, K.A. van Leeuwen, G. Anesin, D. Bertelli, M. Paolini, S. Benvenuti, F. Camin (2013). Gas chromatography combined with mass spectrometry, flame ionization detection and elemental analyzer/isotope ratio mass spectrometry for characterizing and detecting the authenticity of commercial essential oils of Rosa damascena Mill. Rapid Communications in Mass Spectrometry 27(5), 591–602. DOI: 10.1002/rcm.6489.

[3] M. Perini, G. Carbone, F. Camin (2017). Stable isotope ratio analysis for authentication of red yeast rice. Talanta 174, 228–233. DOI: 10.1016/j.talanta.2017.05.057.

[4] M. Perini, S. Pianezze, L. Ziller, F. Camin (2021). Characterization of L-theanine in tea extracts and synthetic products using Stable Isotope Ratio Analysis. In Journal of Food and Drug Analysis 29(2), 312–319. DOI:10.38212/2224-6614.3349.

## Oral: First Use of Triply Labelled Water analysis for energy expenditure measurements in mice

*Xing Wang[1], Dehuang Kong[2], Gertjan van Dijk[2],*

*Harro A. J. Meijer*[*,1]*

[1]Centre for Isotope Research (CIO), Energy and Sustainability Research Institute Groningen, University of Groningen, Groningen, The Netherlands
[2]Groningen Institute for Evolutionary Life Sciences, Unit Behavioral Neuroscience, University of Groningen, Groningen, The Netherlands
*presenting author: h.a.j.meijer@rug.nl

The Doubly Labelled Water (DLW) method is widely used to determine energy expenditure of (free roaming) animals, and of humans. In this work, we demonstrate the addition of the third stable isotope, $^{17}O$, to turn it into Triply Labelled Water (TLW), exploiting the modern three isotopes measurement capabilities of optical spectrometry. We performed TLW measurements for the analysis of the $CO_2$ production ($r_{CO2}$) of mice on different diets. Triply highly enriched water (with abundances of 30%, 55% and 8% for $^{2}H$, $^{18}O$ and $^{17}O$, respectively) was injected into mice, and the isotope enrichments of the distilled blood samples taken after 2, 24 and 48 hours, respectively, were measured by an Off-Axis Integrated Cavity Output Spectroscopy instrument (LGR LWIA 912-0050). Typical $\delta$ -values for the 2-hours blood samples of the mice were 13000‰, 1800‰ and 1600‰ for $\delta^{2}H$, $\delta^{18}O$ and $\delta^{17}O$, respectively. Analysis of the measurements was done using a bespoke data analysis program (written in R), which includes a sophisticated memory correction algorithm [1]. For these enriched samples such an algorithm is indispensable. For calibration of the measurements, we extended the range of available DLW reference waters [2] by preparing $^{17}O$ enriched reference waters on a gravimetrical basis.
We found that the values of the $r_{CO2}$ (which are proportional to the energy expenditure), calculated based on $^{18}O$-$^{2}H$, and on $^{17}O$-$^{2}H$, agreed very well, increasing the reliability and redundancy of the measurements and lowering the uncertainty in the calculated $r_{CO2}$ to ±3%. However, like in a previous study using DLW [3], the TLW method overestimated the $r_{CO2}$ compared to the indirect calorimetry measurements that we also performed. Thanks to the addition of $^{17}O$, we could now unambiguously identify $^{2}H$ isotope effects as the culprit. We hypothesize an extra loss or exchange mechanism with a high fractionation for $^{2}H$ to explain this difference.

[1] S. Guidotti et al.(2013). Doubly Labelled Water analysis: Preparation, memory correction, calibration and quality assurance for delta H-2 and delta O-18 measurements over four orders of magnitudes. Rapid Communications in Mass Spectrometry 27, 1055–1066. https://doi.org/10.1002/rcm.6540.

[2] V. Faghihi et al. (2015). A new high-quality set of singly (2H) and doubly (2H and 18O) stable isotope labeled reference waters for biomedical and other isotope-labeled research. Rapid Communications in Mass Spectrometry 29, 311–321. https://doi.org/10.1002/rcm.7108.

[3] S. Guidotti, H.A.J. Meijer, G. van Dijk (2013). Validity of the doubly labeled water method for estimating CO2 production in mice under different nutritional conditions. American Journal of Physiology - Endocrinology and Metabolism 305, E317–E324. https://doi.org/10.1152/ajpendo.00192.2013.

## POSTERS P9 – P11

### P9 Isotopic signature of $^{13}C$ and $^{15}N$ natural abundance in breast cancer patients

*C. Citérin[1,*], S. Renou[1], R. Heng[2], A.M. Schiphorst[1], J. Dahlstrom[3], A.C. Blackburn[3], G. Tcherkez[2], I. Tea[1,2]*

[1]CEISAM UMR CNRS 6230, Nantes Université, Nantes, France
[2]Research School of Biology, Australian National University ANU, Canberra, Australia
[3]ANU College of Health and Medicine, Australian National University ANU, Canberra, Australia
*presenting author: maylis.couton@univ-nantes.fr

Breast cancer (BrCa) is the most prevalent cancer in women worldwide and is amongst the most important causes of mortality. There are important challenges: (i) finding new treatments, (ii) improving early diagnosis and cancer classification so as to anticipate treatment sub-type and life prognostic, and (iii) finding good markers to follow the response of cancer to treatment and anticipate cancer (here, the natural isotope abundance). There is currently a growing interest in the direct observation of metabolic fluxes (fluxomics), mostly using $^{13}C$-labelling. Carrying out $^{13}C$-labelling may be prohibited by clinical practice regulations and usually neglects differences in reaction rates between isotopic forms, because the isotopic signal used for diagnosing is far above small natural variations in $^{13}C$. One way to address these issues is to use stable isotope analyses at natural abundance, not requiring labelling. Stable isotopes reflect fluxes in metabolism and should provide a comprehensive and dynamic view of metabolism and help the definition of a relevant metabolic flux marker[1, 2, 3]. Our study design is based on a biomarker discovery process whereby we take advantage of 450 samples in total, which are provided by Australian Breast Cancer Tissue Bank. The samples are BrCa tissue, non-cancer tissue (paired) and serum from the same patients (3 samples/patient). Samples from 5 subtypes of BrCa which are: ductal carcinoma in situ (DCIS) and 4 subtypes of invasive BrCa (hormone receptor (HR)+HER2-, HR+HER2+, HR-HER2- and HR-HER2+) (n=30 per group, 5 groups = 150 patients, x 3 types of samples = 450 samples in total). We examined the bulk and compound specific isotopic differences between healthy and cancerous samples, and between different subtypes (e.g. receptor expression) by using both Elemental Analyser and Gas Chromatography coupled to an IRMS (EA-IRMS and GC-C-IRMS). We have found that the natural $^{13}C$ and $^{15}N$ abundance can discriminate normal and cancerous biopsies, the latter being significantly $^{13}C$-enriched and $^{15}N$-depleted. Different BrCa subtypes showed different isotopic fingerprints depending on hormone receptor expression. Using Compound Specific Isotopic Analysis, we have further demonstrated that the generation of $^{15}N$-depleted alanine, glycine, serine, aspartate and hydroxyproline are likely to be at the origin of the $^{15}N$ depletion in BrCa. These results will help to identify the key metabolic pathways involved in BrCa for the isotopic differentiation of BrCa subtypes and thus best-potential biomarkers in plasma samples.

[1] I. Tea *et al.*, (2016). $^{13}C$ and $^{15}N$ natural isotope abundance reflects breast cancer cell metabolism. Sci. Rep. 6, 34251. doi: 10.1038/srep34251.

[2] Tea, I.; Martineau, E.; Giraudeau, P.; Akoka, S.; Nion, S. International patent WO2012/123886

[3] I. Tea *et al.*, (2021). Stable Isotope Abundance and Fractionation in Human Diseases. A review. Metabolites 11(6), 370. doi: 10.3390/metabo11060370.

### P10 $^{15}N$-Position-Specific Isotope Analysis by Isotope Ratio Mass Spectrometry (PSIA-IRMS)

*M. Couton[1,*], M. Sanchez[1], V. Fargeas[1], I. Tea[1,2]*

[1]CEISAM UMR CNRS 6230, Nantes Université, Nantes, France
[2]Research School of Biology, Australian National University ANU, Canberra, Australia
*presenting author: maylis.couton@univ-nantes.fr

The development of new methods for early diagnosis, prognosis and therapeutic strategies relies heavily on biomarker discovery.

Using Compound Specific Isotope Analysis (CSIA), we have showed that the generation of $^{15}N$-depleted both arginine and urea in the Urea Cycle (UC) are likely to be at the origin of the $^{15}N$ depletion in breast cancer[1,2]. Although useful, the application of our isotopic technique to cancer diagnosis requires understanding of the metabolic origin of the $^{15}N$-depletion in UC substrates and intermediates. To reach this objective, we propose that measuring intramolecular isotopic compositions referred to as Position Specific Isotope Analysis (PSIA), of UC substrates and intermediates will provide a reliable descriptor of metabolic flux pattern and UC deregulation in cancer. That is, we suggest that the isotope composition of the four N atoms of arginine (Nα, Nδ 2Nε) differ and this can in turn be exploited for cancer diagnosis.

To do so, we have adapted an innovative approach using a new mass spectrometry (IRMS) techniques to access PSIA, which is under development. The PSIA setting includes fragmenting the molecule and separating the fragments before IRMS analyses. A number of approaches for arginine fragmentations are studied by using chemical, electrochemical and enzymatic reactions. The combined electrochemical and enzymatic approaches allow to measure $^{15}N$-PSIA of arginine.

Overall, these fragmentation methods will help to set up interfaces prior to IRMS (PSIA-IRMS on-line) in order to analyse biological samples in routine and understand the metabolic origin of the intramolecular isotope signature.

[1] Tea I., Martineau E., Antheaume I., Lalande J., Mauve C., Gilard F., Barillé-Nion S., Blackburn A.C., Tcherkez G., (2016). 13C and 15N natural isotope abundance reflects breast cancer cell metabolism. Sci. Rep. 6, 34251. doi: 10.1038/srep34251.

[2] Tea I., Martineau E., Giraudeau P., Akoka S., Nion S., International patent WO2012/123886

## P11 Isotope ratio mass spectrometry to detect differences in four compartments of Simmental cows fed on C3 and C4 diets

*S. Pianezze[1], M. Corazzin[2], L. Bontempo[3], A. Sepulcri[2], E. Saccà[2], M. Perini[1,*] & E. Piasentier[2]*

[1]Centro di Trasferimento Tecnologico, Fondazione Edmund Mach, San Michele all'Adige (TN), Italy
[2]Dipartimento di Scienze Agroalimentari, Ambientali e Animali, University of Udine, Udine (UD), Italy
[3]Centro Ricerca e Innovazione, Fondazione Edmund Mach, San Michele all'Adige (TN), Italy
*presenting author: matteo.perini@fmach.it

Fatty acids (FAs) can be found in adipose tissues and muscles of bovines. They may derive from the diet only, as for the essential FAs, and/or from *de novo* endogenous synthesis [1]. The FAs metabolic pathway starts in the rumen with the hydrolysation of dietary complex fats into long chain FAs [2]. Then, the FAs released during this process are converted into saturated ones through biohydrogenation [3]. The FAs reach the liver carried by the blood, whose flow, together with the FAs concentration, influences their supply to this organ [4]. The deposition of the FAs into the animal tissue represents the final step of their metabolism.

The aim of this study was to discriminate between two groups ($n_{TOT}$=13) of cows fed on different diets and to widen the knowledge about the FAs metabolic path in the bovine organism. The first group was fed on a C3 products-based diet ($\delta^{13}C_{C3\_BULK\_DIET}$ = *-32.55‰*) while the second one was fed on a C4 products-based one ($\delta^{13}C_{C4\_BULK\_DIET}$ = *-18.74‰*). Beside the diet, three compartments of the animals were considered: rumen, liver and meat. The fat extracted from the four matrices was both analysed through EA-IRMS, as a bulk sample, and through GC-C-IRMS, after a derivatization process which made it possible to measure the $\delta^{13}C$ of five FAs (C16:0, C18:0, C18:1n-9, C18:2n-6 and C18:3n-3). A good discrimination between C3 and C4 groups was achieved. Moreover, different trend of $\delta^{13}C$ passing from the diet to the loin were found as for C3 and C4 groups.

[1] C. Galli, P. Risé (2006). Origin of fatty acids in the body: endogenous synthesisversus dietary intakes. In European Journal of Lipid Science and Technology 108 (6), 521–525. DOI: 10.1002/ejlt.200600056.

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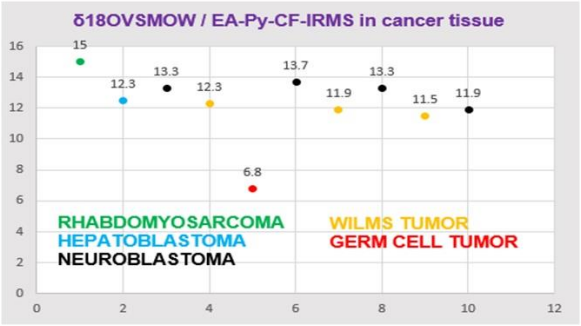
## P11/2 The first oxygen stable isotopes assessment in 'in vivo' cancer tissues – a pilot study.

*K. Taran[1], M. Cichon[1], K. Gasior[1], A. Hincz[1], K. Klajman[2], P. Paneth[2*]*

[1]Laboratory of Isotopic Fractionation in Pathological Processes, Department of Pathomorphology, Chair of Oncology, Medical University of Lodz, Poland
[2]Institute of Applied Radiation Chemistry, Lodz University of Technology, Lodz, Poland
*presenting author: piotr.paneth@p.lodz.pl

**Aim:** Assessment of oxygen stable isotope ratio for future cancer tissue studies with clinical impact.

**Introduction:** Oxygen plays a key role in tumor biology and hypoxia has been associated with angiogenesis and neovascularization in many cancers [1], however, there is no method to estimate or control this process in clinical practice. **Material and methods:** The spectrum of solid tumors of developmental age was evaluated. Oxygen isotopic composition was determined by the IRMS (Thermo Finnigan MAT 253) following pyrolysis at 1350°C and chromatographic separation (70°C) of $H_2$ and CO in a He gas stream. Measured values were calibrated to repeat analyses of gelatine (certified reference material provided by Elemental Microanalysis) and caffeine (measured and published by various research groups) standards and are reported vs. VSMOW on the VSMOW-SLAP scale. **Results:** The values of oxygen isotope ratio appeared different in Wilms tumors and neuroblastoma group (especially stroma poor), the highest value was identified in high-risk rhabdomyosarcoma and the lowest in germ cell tumor. The difference reaches as much as 8.2 ‰ as illustrated in the figure below:




**Conclusion:** Revealed differences between the examined cancers of different aggressiveness and prognosis indicate the need for the search for the potential clinical impact of oxygen stable isotope assessment.

[1] S. Soni, Y. Padwad. HIF-1 in cancer therapy: two decade long story of a transcription factor. Acta Oncol. (2017) 56, 503-515. DOI: 10.1080/0284186X.2017.1301680

## Keynote: Towards a new protocol for computer assisted biocatalysts design

*K. Świderek[1,*], A. Krzemińska[2], M. A. Galmés[1], I. Tuñón,[3] I. H. Williams,[4] J. Bertran,[5] V. Moliner[1]*

[1]Departament de Química Física i Analítica; Universitat Jaume I, 12071 Castellón, Spain
[2]Faculty of Chemistry, Lodz University of Technology, 90-924 Lodz, Poland
[3]Departament de Química Física, Universitat de València, 46100 Burjasot, Spain
[4]Department of Chemistry, University of Bath, Bath BA2 7AY, United Kingdom
[5]Departament de Química, Universitat Autònoma de Barcelona, 08193 Bellaterra, Spain
*presenting author: swiderek@uji.es

The origin of enzyme catalysis remains a question of debate despite much intense study. The biggest challenge is to define the common property responsible for speeding up chemical reactions in active sites of enzymes. In this presentation, we focus on three not related enzymatic models i.e protease of HIV-1 (PR),[1] glycine N-methyltransferase (GNMT)[2] and HG.3 and HG3.17, de novo design enzyme Kemp eliminase [3],[4] in order to investigate their common features crucial for catalysis.

Herein, we report the results of QM/MM theoretical studies for (a) peptide bond cleavage in a multi-step reaction catalyzed by HIV-1 PR, (b) the $S_N2$ methyl transfer reaction catalyzed by GNMT, and (c) kemp elimination catalyzed by two de novo enzymes, HG3.17 and HG.3. In general, our studies indicate the importance of the electrostatic properties of the protein in the active site. Thus, in the multi-step reaction catalyzed by HIV-1 PR, the electric field created by the protein in the active site of the enzyme emerges as being critical for the electronic reorganization required during the chemical process. Additionally, the decomposition of the electrostatic forces generated by the protein in the scissile peptide bond on the rate-limiting transition state would favor the peptide bond cleavage.

Finally, a rational QM/MM molecular dynamics strategy based on combining the best electrostatic properties of enzymes with activity in a common reaction is presented. The computational protocol has been applied to the re-design of the protein scaffold of an existing promiscuous esterase from *Bacillus subtilis* Bs2 to enhance its secondary amidase activity. After the alignment of Bs2 with a non-homologous amidase *Candida antarctica* lipase B (CALB) within rotation quaternions, a relevant spatial aspartate residue of the latter was transferred to the former to favor the electrostatics of transition state formation, where a clear separation of charges takes place. [5]

[1] A. Krzemińska, V. Moliner, K. Świderek J. (2016). Am. Chem. Soc. 138, 16283.

[2] K. Świderek, I. Tuñón, I. H. Williams, V. Moliner (2018). J. Am. Chem. Soc.140, 4327.

[3] K. Świderek, I. Tuñón, V. Moliner, J. Bertran (2017). Chem. Eur. J. 23, 7582.

[4] K. Świderek, I. Tuñón, V. Moliner, J. Bertran (2015). ACS Catalysis 5, 2587.

[5] M. À. Galmés, A. R. Nödling, K. Heb, L. Y. P. Luk, K. Świderek. V. Moliner (2022). Chem. Sci. just accepted, DOI: 10.1039/d2sc00778a.

## Invited: my 50 years with isotope effects

*P. Paneth[1,2,*]*

[1]Institute of Applied Radiation Chemistry, Faculty of Chemistry, Lodz University of Technology, Lodz, Poland
[2]International Center for Research on Innovative Biobased Materials (ICRI-BioM) – International Research Agenda, Lodz University of Technology, Lodz, Poland
*presenting author: piotr.paneth@p.lodz.pl

In the retrospective part of this presentation, I will discuss some aspects of measurements and calculations of isotope effects on enzyme-catalyzed reactions. These will include, among others, problems connected with the evaluation of the kinetic isotope effect when the rate of the spontaneous reaction and reversibility cannot be neglected [1], the dependence of the observed isotope effect on the concentration of the reagent that binds second [2], and mechanistic misinterpretations that may result from calculations carried out using low theory levels [3].

From among my recent involvements in isotope effects studies, I will discuss calculations of isotope effects on non-covalent interactions [4] and attempts to use isotopic fractionation of carcinomic cells for diagnostic and prognostic purposes [5].

[1] P. Paneth, M.H. O'Leary (1985). Carbon Isotope Effect on Dehydration of Bicarbonate Ion Catalyzed by Carbonic Anhydrase. Biochemistry 24, 5143-47.

[2] E. Gawlita, W.S. Caldwell, M.H. O'Leary, P. Paneth, V.E. Anderson (1995). Kinetic Isotope Effects on Substrate Association: The Reaction of PEP with Pyruvate Carboxylase and Pyruvate Kinase. Biochemistry 34, 2577-83.

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[4] M. Pokora, A. Paneth, P. Paneth (2022). Unprecedently large isotopic fractionation on nano-confinement of chloride anion. Sci. Rep. 12, 1768.

[5] A. Madej, E. Forma, M. Golberg, R. Kamiński, P. Paneth, J. Kobos, W. Różański, M. Lipiński. 13C Natural Isotope Abundance in Urothelium as a New Marker in the Follow-up of Patients with Bladder Cancer. submitted

## Oral: Towards the design of an improved Retro-Aldolase based on QM/MM studies of the reaction catalyzed by different protein scaffolds

*D. De Raffele, S. Martí & V. Moliner**

BioComp Laboratory, Institute of Advanced Materials (INAM), Universitat Jaume I, Castellón, Spain
*presenting author: moliner@uji.es

Designing an enzyme to catalyze a particular chemical reaction is a huge challenge. Different strategies adopted for this task have been developed in the last years but the achieved goals, and the real contribution of the computational studies, have

been argued. In this communication we present theoretical investigations of the multi-step Retro-Aldol reaction mechanism catalyzed by three different previously designed protein scaffolds; two de novo enzymes, RA95.5-8F and RA95.5-5, and one catalytic antibody, 33F12. Using different computational methods based on multiscale QM/MM potentials, the free energy landscape of the reactions were generated and key states along the reaction were identified and characterized.[1-3] Analysis of the results allow us to identify the roles played by the amino acids around the different reaction sites, their interaction with the species involved in the reaction, and their contributions in the stabilization of the transition states of the different chemical steps. Due to the multistep character of the reaction to be catalyzed, this is a challenging goal since the activation free energy of the rate-determining step of the reaction must be reduced but without harming the other chemical steps. We will show how our investigation has successfully led to the proposal of a new catalytic protein with a dramatically increased efficiency, by comparison with the most efficient aldolase.[4] This new strategy can significantly support and accelerate future experimental works on the design of new enzymes.

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[2] D. De Raffele, S. Martí and V. Moliner (2020). Understanding the Directed Evolution of De Novo Retro-Aldolases from QM/MM Studies. ACS Catal. 10, 7871–7883. DOI: 10.1021/acscatal.0c01165.

[3] D. De Raffele, S. Martí and V. Moliner (2021). A QM/MM study on the origin of Retro- Aldolase activity of a catalytic antibody. Chem.Commun. 57, 5306–5309. DOI: 10.1039/D1CC01081F.

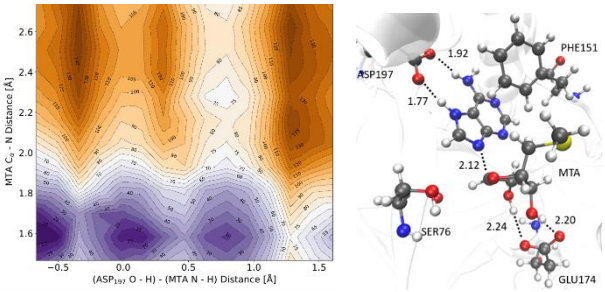
[4] D. De Raffele, S. Martí and V. Moliner, Computational (re)-Design of a Retro- Aldolases Based on the Comparison of Different Active de novo and Catalytic Antibodies. Manuscript in preparation

### Oral: Computational simulation of kinetic isotope effects for enzymatic *N*-glycoside hydrolysis

*J. H. Glancy*[1] & *I. H. Williams*[1,*]

[1]Department of Chemistry, University of Bath, Bath BA2 7AY, United Kingdom
*presenting author: i.h.williams@bath.ac.uk

Whilst use of kinetic isotope effects (KIEs) for transition-state analysis of mechanisms of glycoside hydrolysis is generally very successful, observed 2° α-D (or T) KIEs at the anomeric centre are often not reproduced by model QM calculations.[1] Previously we noted[2] that the reported lack of sensitivity[1] of these KIEs for methylthioadenosyl (MTA) hydrolysis to the dielectric environment arose from inappropriate use of spherical cavities in continuum solvation calculations. Now we present results of comparative QM/MM modelling of the MTA nucleosidases from *S. pneumoniae* and *E. coli* based upon 2D potential-of-mean-force surfaces computed using AM1/OPLS and M06-2X/6-31+G(d,p)-corrected methods applied to the complete proteins in explicit water. Multiple KIEs have been computed, as ensemble averages over many thermally accessible configurations, using QM/MM subset hessians. Alternative reactant states are considered, along with the role of leaving-group protonation, and mechanistic differences between the two variant enzymes will be discussed in terms of their transition-state structures and the influence of their protein environments.[3]




M06-2X:AM1/MM 2D PMF (left) and TS structure (right) for glycosidic cleavage by *S. pneumoniae* MTAN.

[1] Singh, V.; Schramm, V. L. (2007). Transition-State Analysis of *S. pneumoniae* 5′-Methylthioadenosine Nucleosidase. J. Am. Chem. Soc. 129, 2783-2795. DOI 10.1021/ja065082r.

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[3] Glancy, J. H. (2022). Kinetic Isotope Effects as Transition-State Probes for Glycosidic Reactions. Ph.D. thesis, University of Bath (*in preparation*).

## POSTERS P12 – P14

### P12 Metabolic Mechanism of Sulfonamide Cleavage: A Combined Computational and Experimental Study on Sulfamethoxazole

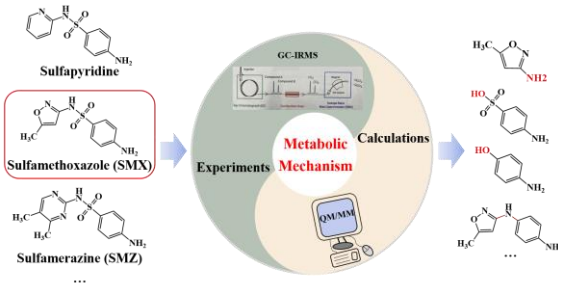
*Lihong Chai*[1,*], *Martin Elsner*[1] & *Etienne Derat*[2]

[1]Chair of Analytical Chemistry and Water Chemistry, Technical University of Munich, Munich 85748, Germany
[2]Institut Parisien de Chimie Moléculaire, Sorbonne Université, CNRS, 4 Place Jussieu, CC 229, 75005 Paris, France
*presenting author: Lihong.chai@tum.de

Antibiotics are widely used for human and veterinary medicine, especially in some developing countries where they are still used intensively. This, leads to a release of large amounts into the environment where they persist both in unchanged and in metabolized form as a result of uncomplete biotransformation. In recent years, antibiotics have attracted increased attention because of their potential to foster the development of antibiotic resistance[1]. Although many studies have been carried out in terms of biodegradation and metabolite detection, only limited information is available on their metabolic reaction mechanisms.

Sulfonamides is a particularly relevant class of antibiotics. To understand their enzymatic degradation, computational chemistry approaches based on quantum chemical calculations can characterize detailed potential energy surfaces (PESs) and electronic properties of reaction. Reaction paths involving putative short-lived intermediates and transition states can be predicted *in silico*, both to rationalize observed biotransformation pathways in the environment, as well as to guide directed evolution of even more proficient enzymes for contaminant degradation. In parallel, measurements of isotope can test for predicted transition states. The comparison of results from such gas chromatography-isotope ratio mass spectrometry

(GC-IRMS) analysis with predicted, kinetic isotope effects can therefore elucidate biochemical transformations.




Synergy between experiments and computations in elucidating metabolic mechanism of sulfonamides

On the basis of above, this study will focus on sulfamethoxazole (SMX) as a model substrate, to explore and optimize the metabolic mechanism of sulfonamide cleavage by combining calculations and experiments. We aim to use this mechanistic understanding to computationally focus directed evolution in an overarching pursuit to generate novel enzymes for curbing micropollutant contamination in drinking and waste water.

[1] Wang, J.; Wang, S., Microbial (2018). Degradation of Sulfamethoxazole in the Environment. Applied Microbiol Biotechnol 102 (8), 3573-3582. https://doi.org/10.1007/s00253-018-8845-4.

## P13 Isotope Effects on Vaporization of Organic Compounds from Aqueous Solution – Insight from Experiment and Computations

*A. Dybala-Defratyka[1,*], M. Rostkowski[1], A. Sowińska[1], L. Vasquez[1], M. Przydacz[1], H. Schürner[2] & M. Elsner[2]*

[1]Institute of Applied Radiation Chemistry, Faculty of Chemistry, Lodz University of Technology, Lodz, Poland
[2]Chair of Analytical Chemistry and Water Chemistry, Technical University of Munich, Munich, Germany
*presenting author: agnieszka.dybala-defratyka@p.lodz.pl

The predictability of water-air partitioning isotope effects from experiments and computations for benzene and trimethylamine (both H-bond acceptors) as well as chloroform (H-bond donor) has been explored. Isotope fractionation of different direction and magnitude was measured: $\varepsilon$ = -0.12‰ ± 0.07‰ (benzene), $\varepsilon_C$ = 0.49‰ ± 0.23‰ (triethylamine) and $\varepsilon_H$ = 1.79‰ ± 0.54‰ (chloroform) demonstrating that effects do not correlate with expected hydrogen bond functionalities. Computations revealed that the overall isotope effect arises from contributions of different nature and extent: weakening of intramolecular vibrations in the condensed phase plus additional vibrational modes from complexation with surrounding water molecules. Subtle changes in benzene contrast with stronger coupling between intra and intermolecular modes in the chloroform-water system and a very local vibrational response with few atoms involved in a specific mode of triethylamine. Careful evaluation of different computational approaches showed that the choice of the solvation model as well as the electronic structure method are important to mimic experimental results. Since the complexes of an organic compound and water molecules are governed by non-covalent interactions, interaction energy was computed for each system. Its decomposition revealed that each system was affected differently by electrostatics and dispersion where dispersion was dominating for benzene and electrostatics dominating for chloroform and triethylamine.[1]

[1] Rostkowski et al. (2021). Isotope Effects on Vaporization of Organic Compounds from Aqueous Solution – Insight from Experiment and Computations. J. Phys. Chem. B 125, 13868, DOI: 10.1021/acs.jpcb.1c05574.

## P14 First principles model of isotopic fractionation in formaldehyde photolysis: Wavelength and pressure dependence

*L. Pennacchio*, K. V. Mikkelsen & M. S. Johnson*

Department of Chemistry, University of Copenhagen, 2100 Copenhagen, Denmark
*presenting author: rkt505@alumni.ku.dk

Experimental studies show large isotope-dependent effects in the photolysis rates of formaldehyde isotopologues, that are both wavelength and pressure dependent. These effects are on the order of 10% for $^{13}C$ and $^{18}O$, and 40% for CHDO [1-4]. In this study we use computational chemistry to discover the origin of the fractionation. The method and mechanism are validated using available experimental studies and then used to make predictions about the isotope effects in additional systems including the amount of mass-independent fractionation. The following isotopologues of formaldehyde are investigated; HCHO, DCHO, DCDO, D$^{13}$CHO, H$^{13}$CHO, HCH$^{17}$O and HCH$^{18}$O. Rice–Ramsperger–Kassel–Marcus (RRKM) theory is used to calculate the rates for decomposition of the S0, S1 and T1 states with B3LYP, CAM-B3LYP, m06-2X and wB97XD and CCSD(T) or CASPT2 with the aug-cc-pVTZ basis set. Furthermore, the rates and likelihood of intersystem crossing are investigated for all isotopologues.

[1] K. L. Feilberg, B. D'Anna, M. S. Johnson, and C. J. Nielsen (2005). Relative Tropospheric Photolysis Rates of HCHO, H13CHO, HCH18O, and DCDO Measured at the European Photoreactor Facility. J. Phys. Chem. A 109, 8314-8319. https://doi.org/10.1021/jp0513723.

[2] K. L. Feilberg, M. S. Johnson, A. Bacak, T. Rockmann, and C. J. Nielsen (2007). Relative Tropospheric Photolysis Rates of HCHO and HCDO Measured at the European Photoreactor Facility. J. Phys. Chem. A 111, 9034-9046. https://doi.org/10.1021/jp070185x.

[3] A. Gratien, E. Nilsson, J. Doussin, M. S. Johnson, C. J. Nielsen, Y. Stenstrøm, and B. Picquet-Varrault (2007). J. Phys. Chem. A 111, 11506-11513. https://doi.org/10.1021/jp074288r.

## Keynote: Isotopologues of organic molecules: method developments and applications

*Alexis Gilbert**

Department of Earth and Planetary Sciences, Tokyo Institute of Technology
*presenting author: gilbert.a.aa@m.titech.ac.jp

Stable isotope analysis of light elements ($^{2}H$, $^{13}C$, $^{15}N$, $^{18}O$, $^{34}S$) provides detailed information regarding the origin and history of organic molecules [1]. The heavy/light ratios are traditionally measured on simple gases arising from conversion of the molecule to be analyzed: $H_2$ for $^{2}H$ analysis, $CO_2$ for $^{13}C$, $N_2$ for $^{15}N$, etc. While universally used, conventional methods result in a loss of information on the isotope distribution within molecules, thus omitting a large portion of available information. Isotope substitution within a molecule can occur at different positions or involve more than one heavy isotope and leads to a set of molecular species called isotopologues.

Recent years have seen the development of several methodologies for the precise measurement of isotopologues of organic molecules and have documented an ability to trace the origin of a given molecule in a way that is fundamentally different from traditional analyses [2,3]. Isotopologue analysis thus holds promise in several applications: food authentication, forensics, biogeochemistry, astrobiology, … The analysis of isotopologues is not trivial, however, and requires specific techniques involving either spectroscopy (Nuclear Magnetic Resonance spectroscopy; Tunable Infrared Laser Direct Absorption Spectroscopy), thermal/(bio)chemical degradation of the molecule or direct analysis using high resolution mass spectrometers [3].

This presentation will review the recent methodological developments for the analysis of doubly-substituted ('clumped') and position-specific isotopologues of organic molecules. Applications to natural sciences will also be introduced, with a particular focus on natural gas hydrocarbons (methane, ethane, propane). The latter have been the subject of a tremendous number of new method development enabling the precise measurement of clumped isotopes of methane ($^{13}CH_3D$, $CH_2D_2$), ethane ($^{13}CH_3$-$^{13}CH_3$) and position-specific isotope analysis of propane. Altogether, these methods have provided new insights into natural gas formation, including the temperature of formation [4], biological vs non-biological origin [5] and thermal vs microbial degradation [6].

[1] Hayes J. (2001). Fractionation of the Isotopes of Carbon and Hydrogen in Biosynthetic Processes. Reviews in Mineralogy and Geochemistry 43, 225

[2] Eiler J.M. (2013). The Isotopic Anatomies of Molecules and Minerals. Ann. Rev. Earth Planet. Sci. 41, 411

[3] Gilbert A. (2021). The Organic Isotopologue Frontier. Ann. Rev. Earth Planet. Sci. 49, 435.

[4] Stolper D. A. et al. (2014). Formation temperatures of thermogenic and biogenic methane. Science 344, 1500.

[5] Taguchi, K. et al. (2020). A fluorination method for measuring the $^{13}C$-$^{13}C$ isotopologue of $C_2$ molecules. Rapid Commun. Mass Spectrom. 34, 1.

[6] Gilbert, A. et al. (2019). Intramolecular isotopic evidence for bacterial oxidation of propane in subsurface natural gas reservoirs. Proc. Natl Acad. Sci. U. S. A. 116, 6653.

## Invited: Discovering Isotopic Fingerprints Anew on Bioanalytical Mass Spectrometers

*C. Neubauer** & *K. Kantnerová*

University of Colorado Boulder, USA
*presenting author: caj.neubauer@colorado.edu

It is becoming increasingly clear that mass spectrometers that are designed for use in life sciences can be fine-tuned for isotope analysis at the level of natural abundance variations. This talk provides an introduction to the technical possibilities of observing isotopic fingerprints from intact polar compounds by soft-ionization MS techniques. Research programs range from molecular surveys of complex mixtures to highly targeted analyses of purified polar solutes. These recent developments suggest that new routes are opening to precision measurements of position-specific and mass-independent isotope effects, the quantification of rare isotopic homologues, and gentle "soft labeling" strategies. We will examine the potential and tradeoffs of these emerging approaches, with the aim to outline their utility for basic research and practical applications.

## Oral: Calibration of Boreas: a new laser-based instrument for in-situ automated measurement of $\delta^{13}C$ and $\delta^{2}H$ in methane

*C. Rennick[1,*], E. Safi[1], A. Hillier, T. Arnold[1,2]*

[1]National Physical Laboratory, Teddington, Middlesex, TW11 0LW, UK
[2]School of GeoSciences, University of Edinburgh, Edinburgh, EH9 3FF, UK
*presenting author: chris.rennick@npl.co.uk

Methane ($CH_4$) has the second strongest anthropogenic contribution to radiative forcing, following carbon dioxide ($CO_2$). It is emitted by distinct sources such as agriculture, landfill, and fossil fuel use, and has a short atmospheric lifetime so is an attractive target for emission mitigation strategies. While the atmospheric amount fraction of methane is routinely measured by atmospheric monitoring stations for emissions estimates using regional-scale inversion modelling, it is not currently possible to disaggregate the relative proportion of each emission category. The different sources emit methane with distinct isotopic ratios, so continuous isotopic measurements will provide an important extra observable.

Optical instruments are capable of autonomous operation but are currently limited in the availability of reference materials traceable to international isotopic standards and the measurement precision. We will present our implementation of a calibration strategy based on synthetic gas reference materials prepared gravimetrically from a pure methane source with a single isotopic composition. These reference materials were produced under the EMPIR project "Stable isotope metrology to enable climate action and regulation". This approach is validated using different methane gas mixtures, and we quantify the contributions to the measurement uncertainty.

The laser spectrometer is coupled to Boreas, an automated preconcentrator sample preparation system that extracts methane from ambient air samples. Boreas increases the amount

fraction in the spectrometer, which improves the precision. The system can make hourly measurements of $\delta^{13}C(CH_4)$ and $\delta^{2}H(CH_4)$ from ambient air samples. The typical standard measurement uncertainty is 0.07 ‰ for $\delta^{13}C(CH_4)$ and 0.9 ‰ for $\delta^{2}H(CH_4)$, which is the lowest reported for a laser-based system.

Boreas has been deployed to a tall-tower atmospheric monitoring station, located south of London and we will present measurements that show the isotopic signature corresponding to pollution events from the region. Under the principle of identical treatment, the ambient air samples are calibrated against whole-air standards.

[1] C. Rennick, T. Arnold, E. Safi, A. Drinkwater, C. Dylag, E. Mussell-Webber, R. Hill-Pearce, D. R. Worton, F. Bausi, and D. Lowry. (2021). Boreas: A Sample Preparation-Coupled Laser Spectrometer System for Simultaneous High-Precision In Situ Analysis of $\delta^{13}C$ and $\delta^{2}H$ from Ambient Air Methane. Analytical Chemistry 93, 10141. https://doi.org/10.1021/acs.analchem.1c01103.

## Oral: High-Dimensional Isotomics: Observation and Interpretation of Over 100 Isotopic Constraints on Methionine

*T. Csernica[1,*], G. M. Weiss[2], A. L. Sessions[2], J. M. Eiler[2]*

[1]Division of Chemistry and Chemical Engineering, California Institute of Technology, Pasadena, CA, 91125,
[2]Division of Geological and Planetary Sciences, California Institute of Technology, Pasadena, CA, 91125
*Presenting author: tcsernic@caltech.edu

Orbitrap Mass Spectrometry is a developing tool for isotope ratio measurements applicable to a diverse range of compounds. For molecules with collisionally-induced fragmentation spectra, e.g. amino acids, Orbitrap methods can be especially valuable, as mass selection and fragmentation of different sets of isotopologues allows the observation of tens to hundreds of unique isotopologue ratios as constraints on isotopic composition. As many of these constraints lack an obvious relationship to the most commonly reported measures of isotopic composition, like molecular-average isotope ratios, they are often ignored. Here, we present a general framework for analyzing such measurements and apply it to observations of over 100 isotopic constraints on methionine. We mass select and fragment methionine isotopologues with different cardinal mass increases relative to the unsubstitued isotopologue; i.e., the 'M+1', 'M+2', 'M+3', & 'M+4' populations. We use our data both to fingerprint methionine via high-dimensional forensics and to reconstruct various singly- and multiply- (including triply-) substituted position-specific isotopic enrichments. Our methods are widely applicable to similar molecules.

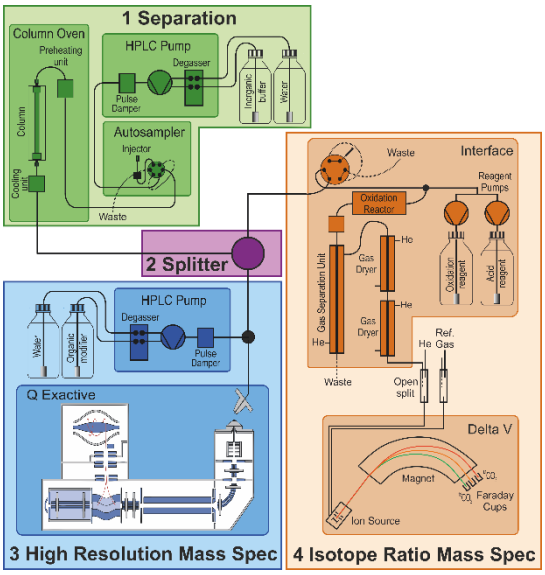
[1] T. Csernica and J. M. Eiler, (2022). High Dimensional Isotomics, Part 1: A Mathematical Framework for Isotomics. *In Review.*

[2] T. Csernica, G. M. Weiss, A. L. Sessions, and J. M. Eiler, (2022). High Dimensional Isotomics, Part 2: Observations of Over 100 Constraints on Methionine's Isotome. *In Preparation.*

## Oral: How to Couple LC-IRMS with HRMS – A Proof-of-Concept Study

*R.G.H. Marks[1,*], M.A. Jochmann[1], W. A. Brand[2], T.C. Schmidt[1,3]*

[1]Instrumental Analytical Chemistry, University of Duisburg-Essen, 45141 Essen, Germany
[2]Max Planck Institute for Biogeochemistry, 07745 Jena, Germany
[3]Centre for Water and Environmental Research (ZWU), University of Duisburg-Essen, 45141 Essen, Germany
*presenting author: robert.marks@uni-due.de




Compound-specific stable isotope analysis (CSIA) is a unique analytical technique for determining small variations in isotope ratios of light isotopes in analytes from complex mixtures. A problem of CSIA is that any structural information of the analytes is lost due to the process's inherent procedures. To obtain the isotopic composition of, e.g. carbon from organic compounds, all carbon in each analyte is quantitatively converted to carbon dioxide ($CO_2$). In the case of GC-IRMS, open split GC-IRMS-MS couplings have been described that allow additional acquisition of structural information of analytes and interferences. Structural analysis using LC-IRMS is more complicated and requires additional technical and instrumental efforts. In our study, LC was combined for the first time with simultaneous analysis by IRMS and high-resolution mass spectrometry (HRMS), enabling the direct identification of unknown or coeluting species. We thoroughly investigated and optimized the coupling and showed how technical problems, arising from instrumental conditions, can be overcome. To this end, it was successfully demonstrated that a consistent split ratio between IRMS and HRMS could be obtained using a variable post column flow splitter. This coupling provided reproducible results in terms of resulting peak areas, isotope values, and retention time differences for the two mass spectrometer systems. We demonstrated the applicability of the coupling, by addressing an important question regarding the purity of international isotope standards. In this context, we were able to confirm that the USGS41 reference material indeed contains substantial amounts of pyroglutamic acid as suggested previously in the literature. Moreover, the replacement material, USGS41a, still has significant amounts of pyroglutamic acid as an impurity, rendering some caution necessary when using this material for isotopic calibration.

[1] R.G.H Marks, M.A. Jochmann, W.A. Brand, T.C. Schmidt, (2022). How to Couple LC-IRMS with HRMS—A Proof-of-Concept Study. Analytical Chemistry, 94 (6), 2981-2987. https://doi.org/10.1021/acs.analchem.1c05226

## Oral: Towards unbiased $^{13}C$ isotopic composition in PSIA

*S. Renou[1,*], S. Akoka[1] & G. S. Remaud[1]*

[1]CEISAM, Nantes Université, Nantes, France
*presenting author: sophie.renou@univ-nantes.fr

$^{13}C$ PSIA (Position-Specific Isotope Analysis) gives an isotopic composition for each carbon position of a given molecule, allowing to obtain more information from the intramolecular isotopic distribution. Apart degradation (chemical or pyrolysis) PSIA is performed using analytical techniques which intrinsically distinguish between isotopomers/isotopologues concentration such as HRMS by Orbitrap or irm-$^{13}C$ NMR. MS-orbitrap offers a huge potential for PSIA -after calibration and using standards- but so far standards with known intramolecular compositions remain to be produced. NMR technique is a primary method meaning that the signal obtained can be described through a mathematical equation. Roughly the area's signal is directly proportional to the number of nuclei observed. However, in practice there is an instrumental response depending on the spectrometer (magnetic field, probe, hardware, etc.) and the molecule (as the spectral range to cover) [1], and that induces an error on the measured value of isotopic composition. This bias is not prejudicial to the use of NMR for isotopic measurements until precision is met and as long as the application requires relative comparison only as authentication purposes or isotope effect calculations. Correcting factors are therefore needed to harmonize the results obtained by NMR.

As a first objective the present work aims to give a protocol allowing the correction of any spectrometer with respect to a chosen one. The latest should have the configuration showing the less distortion sources [2]. The off-resonance phenomenon is one of these sources and it will be dependent on the spectral range of the molecule. A methodology has been established to give a fingerprint for each spectrometer and each configuration. Then, several molecules are used to consider different spectral width. A trend was obtained whatever the molecule allowing the determination of correcting factors.

It is expected that this first step could easily drive a study on the absolute $\delta$-scale allowing the calibration of standard with known $^{13}C$ intramolecular compositions. The last step could be achieved by using appropriate molecules for which PSIA can be performed by any methods. Alanine and vinyl acetate are suggested as putative molecules for playing that role.

[1] K. Bayle, *et al.* (2014). Conditions to obtain precise and true measurements of the intramolecular 13C distribution in organic molecules by isotopic 13C nuclear magnetic resonance spectrometry. Analytica Chimica Acta 846, 1-7. http://dx.doi.org/10.1016/j.aca.2014.07.018

[2] A. Chaintreau, *et al.* (2013). Site-specific 13C content by quantitative isotopic 13C Nuclear Magnetic Resonance spectrometry: A pilot inter-laboratory study. Analytica Chimica Acta 788, 108-113. http://dx.doi.org/10.1016/j.aca.2013.06.004

## Oral: Mid-infrared laser spectroscopy coupled to continuous sublimation extraction. A novel method for high-precision greenhouse gas measurements in ice cores

*B. Tuzson[1,*], B. Bereiter[1,2], A. Kupferschmid[3], P. Scheidegger[1,3], H. Looser[1], F. Krauss[2], D. Baggenstos[2], L. Mächler[2], J. Schmitt[2], L. Emmenegger[1], H. Fischer[2]*

[1]Laboratory for Air Pollution / Environmental Technology, Empa – Swiss Federal Laboratory for Materials Science and Technology, Dübendorf, Switzerland
[2]Climate and Environmental Physics, Physics Institute and Oeschger Center for Climate Research, University of Bern, Switzerland
[3]Transport at Nanoscale Interfaces, Empa – Swiss Federal Laboratory for Materials Science and Technology,
8600 Dübendorf, Switzerland
*presenting author: bela.tuzson@empa.ch

The European Partnership in Ice Core Sciences (EuroPICS) plans to drill an ice core extending over 1.5 million years (Myr), nearly doubling the time span of the existing greenhouse record. The oldest section from 1–1.5 Myr is expected to be close to the bedrock and, due to glacial flow, extremely thinned. Thus, a century-scale climate history is compressed into a few cm vertical extent of ice, containing about 1–2 ml STP of air.

Within the ERC Advanced Grant "deepSlice" we aim to unlock such atmospheric archives in extremely thinned ice by developing a coupled semi-continuous sublimation extraction / laser spectroscopy system. The custom-built high-precision laser spectrometer was specially designed to simultaneously measure $CO_2$, $CH_4$ and $N_2O$ concentrations as well as the $\delta^{13}C(CO_2)$ isotope ratios in such small air samples. The analytical approach is based on direct absorption technique using two quantum cascade lasers (QCLs) emitting at 4.34 and 7.87 µm, respectively. The main challenge of measuring small discrete sample volumes at the required high precision was addressed by a custom-developed multi-pass cell, optimized optical setup, and dedicated laser driver as well as data processing electronics [1].

In parallel, a near-IR laser assisted sublimation unit was developed that extracts 100 % of all gas species avoiding potential fractionation issues. With the target of reducing ice waste and increase sample throughput, the device uses a vertical sublimation of ice core sections with subsequent collection of the released air via cryo-trapping in dip tubes. Several challenges had to be addressed to optimize this approach to reach high reproducibility and repeatability between individual sublimation runs.

Laboratory assessments of the coupled system along the first results of ice core measurements will be presented.

[1] Bereiter, B., Tuzson, B., Scheidegger, P., Kupferschmid, A., Looser, H., Mächler, L., Baggenstos, D., Schmitt, J., Fischer, H., and Emmenegger, L. (2020). High-precision laser spectrometer for multiple greenhouse gas analysis in 1 mL air from ice core samples. Atmos. Meas. Tech., 13, 6391–6406. DOI 10.5194/amt-13-6391-2020.

## POSTERS P15 – P33

### P15 Reducing the Unwanted: Selectivity of Various Solid Phase Extraction Sorbents in Relation to Dissolved Organic Matter

*F. Antritter[1,*], A. Tholiotis[1], M. Elsner[1], R. Bakkour[1]*

[1]Chair of Analytical Chemistry and Water Chemistry, Technical University of Munich, Garching, Germany
*presenting author: felix.antritter@tum.de

Compound specific isotope analysis (CSIA) for aquatic pollutants is highly reliant on excellent separation of the pollutant from interferences. For carbon isotope analysis, these interreferences are mainly dissolved organic matter (DOM) that is co-extracted during sample preparation using solid-phase extraction (SPE) techniques. Being continuously developed for approximately 50 years, SPE nowadays provides a large variety of commercial sorbents. Nonetheless, they are often characterized for their performance in relation to capturing the target analyte but rarely for their co-extraction of unwanted interferences. Therefore, we investigate in this work several commercial sorbents and available protocols for their undesired co-extraction and elution of DOM during extraction of triazines in ground and surface waters.

Extraction parameters like sorbent type, pH and elution solvents have been investigated where the co-extracted DOM was determined using total organic carbon (TOC) analysis after evaporation of any organic solvent and reconstitution in water. Results for five selected commercial sorbents have shown extractions of DOM from highest to lowest for *Oasis HLB* 11.0 ± 0.5, *Sepra ZT* 8.2 ± 0.3, *Isolute ENV+* 4.3 ± 0.2 %, *LiChrolute EN* 3.9 ± 0.4 and *Bakerbond SDB* 3.6 ± 0.3. Furthermore, co-extraction of DOM decreased significantly as a function of increasing pH where full recoveries of triazines were still not compromised. These results show that an attentive selection of sorbent as well as the choice of the most suitable extraction parameters are crucial for reducing co-extraction of undesired DOM and thus enabling CSIA.

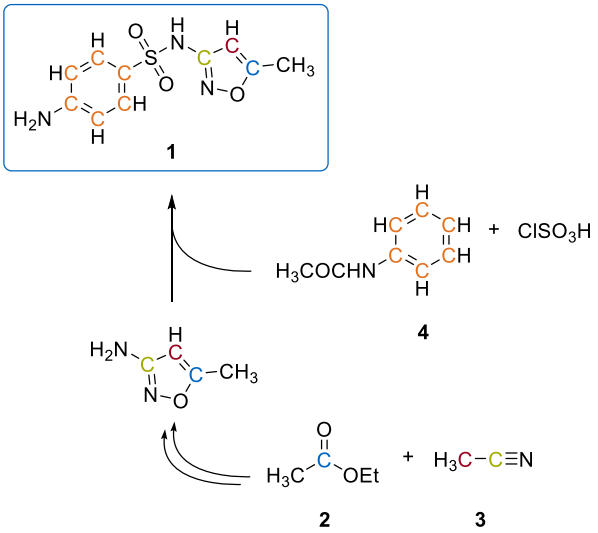
### P16 Position-Specific Isotope Analysis using $^{13}$C-Labels on Sulfamethoxazole

*A. Canavan[1,*], & M. Elsner[1]*

[1]Institute of Hydrochemistry and Chair of Analytical Chemistry and Water Chemistry, Technical University of Munich, Garching bei München, Germany
*presenting author: aoife.canavan@tum.de

The continuous introduction of organic micropollutants into the environment by livestock farming, agriculture, and wastewater is of great societal concern. Pollutants are often incompletely degraded, which can have detrimental effects on living organisms. Especially, the presence of antibiotics in the environment is thought to promote the spread of antimicrobial resistance.

Compound-specific isotope analysis (CSIA) is a powerful tool to characterize degradation pathways through occurring isotopic effects. CSIA offers the possibility of identifying putative degradation processes, such as biodegradation or photolysis even without knowing the exact pathways, and even when metabolites are not detected. Prior studies on biodegradation and photolysis of sulfamethoxazole, a common antibiotic in livestock farming, showed *normal* isotope fractionation.[1,2] However, it has not been possible to identify the changes in bonding contributing to these effects. Here, we present an approach to identify the specific bonds which contribute to the observed isotope fractionation during degradation by assigning stable isotope labels to different carbon positions within the analyte molecule. To this end, we are developing a total synthesis of sulfamethoxazole with $^{13}$C-labels at various positions. The total synthesis can be achieved by the formation of the isoxazole moiety with $^{13}$C-labeled versions of ethyl acetate (2) and acetonitrile (3), while the aromatic moiety can be synthesized




from commercially available $^{13}$C-labeled acetanilid (4) (Figure 1).

Figure 1: Synthesis scheme for potential position-specific $^{13}$C-labels (colored) sulfamethoxazole (1) using labeled ethyl acetate (2), acetonitrile (3), and acetanilid (4).

Degradation experiments with spikes of position-labeled sulfamethoxazole will subsequently inform about position-specific isotope effects. Interpretations of these effects for underlying mechanisms will foster a new level of understanding and put interpretations of stable isotope ratio analysis in degradation reactions of sulfamethoxazole on a firm basis.

[1] J. Birkigt et al. (2015). Carbon Stable Isotope Fractionation of Sulfamethoxazole during Biodegradation by *Microbacterium* sp. Strain BR1 and upon Direct Photolysis Environ. Sci. Technol. 49, 6029–6036. 10.1021/acs.est.5b00367.

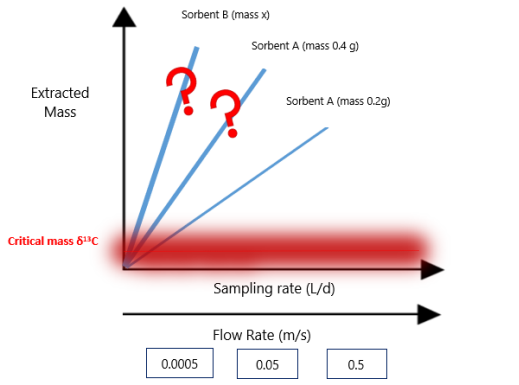
[2] S. Willach et al. (2018). Direct Photolysis of Sulfamethoxazole Using Various Irradiation Sources and Wavelength Ranges—Insights from Degradation Product Analysis and Compound-Specific Stable Isotope Analysis Environ. Sci. Technol. 52, 1225–1233. 10.1021/acs.est.7b04744.

## P17 Suitability of Passive Sampling for Compound-Specific Isotope Analysis of Micropollutants in Aquatic Environments

*Armela Tafa.[1,*], M. Elsner[1] & R. Bakkour[1]*

[1]Chair of Analytical Chemistry and Water Chemistry, Technical University of Munich, Garching, Germany
*presenting author: armela.tafa@tum.de

Compound-specific isotope analysis (CSIA) offers an important approach to track the biodegradation history of micropollutants, such as atrazine, in aquatic environments through measurement of significant changes in isotopic ratios (e.g. $^{13}C/^{12}C$ and $^{15}N/^{14}N$) inside the molecule. The low occurrence, however, of these micropollutants (i.e. ng/L range) combined with the limited sensitivity of gas chromatography hyphenated to isotope ratio mass spectrometers (GC-IRMS) render sampling of water a great challenge for CSIA. For example, active sampling of 60-210 L of water is necessary to provide the required amount of 1 nmol C and/or 3.5 nmol N to the GC-IRMS in order to accurately analyze $^{13}C/^{12}C$ and $^{15}N/^{14}N$, respectively, in atrazine at environmental concentration of 10 ng/L. In contrast, passive sampling offers a promising alternative approach to extract atrazine directly in the field alleviating thereby the tediousness of transporting and handling gigantic volumes of samples. This work intends, therefore, to explore possibilities and limitations of passive sampling for CSIA using a Polar Organic Chemical Integrative Sampler (POCIS) and atrazine as a model compound.




We reviewed available literature on POCIS for concentration measurements of atrazine and critically evaluate its feasibility for CSIA. To this end, we examine the dependence of extracted mass of atrazine on (i) sampling rates and water flow velocities, (ii) sorbent mass, (iii) sorbent types, and (iv) environmental occurrences of atrazine. The results show that the extracted mass of atrazine on a passive sampler does not correspond linearly as a function of the sorbent mass. Furthermore, the calculations highlight the importance of flow rates in the field and its impact on the accumulated mass of analyte on the sampler. Our study gives valuable hints for scaling up current procedures to suit isotope analysis and show the feasibility of combining passive sampling with CSIA for river water in contrast to significant limitations for groundwater regimes.

## P18 Universal vs. Selective Sorbents for Targeted Isotope Analysis of Aquatic Contaminants

*D. Glöckler[1], M. Harrir[2], P. Schmitt-Kopplin[2], M. Elsner[1], R. Bakkour[1,*]*

[1]Chair of Analytical & Water Chemistry, Technical University of Munich, Garching near Munich, Germany
[2]Research Unit Analytical BioGeoChemistry, Helmholtz Zentrum München, Neuherberg, Germany
*presenting author: rani.bakkour@tum.de

Isotope analysis of carbon in aquatic organic contaminants, such as pesticides and pharmaceuticals, may reveal invaluable information about their source and fate in the environment. Their typical low occurrences in the environment (i.e., ng/L range), however, require extensive enrichment from water to meet the requirements of gas chromatography hyphenated to isotope ratio mass spectrometers (GC-IRMS, i.e., mg/L range). To this end, non-volatile contaminants are transferred from a large volume of water to a solid sorbent phase, from which the contaminant can be recovered in a small volume of organic solvent for later isotope analysis on GC-IRMS. While a wide selection of commercial and/or tailor-made sorbents can be used for this purpose, it is a key for accurate isotope measurements to assess their selectivity not only toward the target contaminant but also toward the most abundant interfering constituent of the natural water, namely natural organic matter (NOM).

We assess in this work the accuracy of $\delta^{13}C$ analysis of selected pesticides after extraction by three commercially-available sorbents - Oasis HLB, LiChrolut EN, Supel-Select HLB - and compare it to selective tailor-made ones, namely crosslinked cyclodextrin polymers (CDPs). While the extraction procedure using CDPs maintained carbon isotopic integrity of the target contaminants, the reduced amount of co-enriched NOM led to significantly smaller matrix interferences during GC-IRMS analysis. In consequence, limits of accurate isotope analysis could be lowered by approx. half an order of magnitude for CDPs compared to the universally-used sorbent Oasis HLB. In addition, insights into molecular composition of extracted NOM by Fourier-transform ion cyclotron resonance mass spectrometry (FT-ICR MS) revealed evidence of a vast chemo-diversity of NOM extracted by the commercial sorbents. In contrast, the tailor-made sorbents showed polarity-driven selectivity which strongly discriminated against highly oxygenated as well as unsaturated, aromatic matrix compounds.

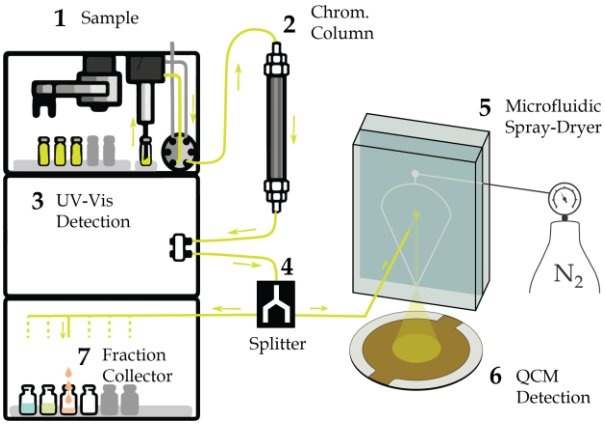
These results suggest that (i) commercially-available sorbents are not always the suitable choice for targeted isotope analysis of aquatic contaminants that occur in low concentrations, (ii) the presented selective materials may open applications that have hitherto not been possible, and (iii) molecular insights into the chemo-diversity of the extracted compounds may help design even more selective materials for targeted analytical approaches.

## P19 Coupling a Quartz Crystal Microbalance with Liquid Chromatography for Online NOM Monitoring

*C. Wabnitz[1,*], A. Canavan[1], W. Chen[1], Z. Toprakcioglu[2], M. Elsner[1], R. Bakkour[1]*

[1]Chair of Analytical Chemistry and Water Chemistry, Technical University of Munich, Garching, Germany
[2]Yusuf Hamied Department of Chemistry, University of Cambridge, Cambridge, Great Britain
*presenting author: christopher.wabnitz@tum.de

Accurate isotope measurements in compound specific isotope analysis (CSIA) for complex samples (e.g., environmental) often require extensive purification procedures to separate the target analyte (e.g., pesticide) from possible interfering matrices (e.g., natural organic matter, NOM). For an optimal and efficient purification, simultaneous online monitoring and quantification of both the pesticide and the NOM is highly valuable. While there are numerous methods for the online quantification of pesticides (e.g., UV/Vis or MS), online NOM monitoring poses a bigger challenge since (i) most used instruments can merely monitor certain fractions of NOM (e.g., chromophoric or ionizable), (ii) not compatible with organic solvents (e.g., TOC analyzer), or (iii) too expensive (e.g., FT-ICRMS).




In this work, we couple a quartz crystal microbalance (QCM) with an LC system through a custom-made microfluidic spray-dryer as a simple and efficient alternative for monitoring NOM amount during a chromatographic purification process. To this end, a flow splitter (**4** in Figure) was installed after the UV detection (**3**) and before a fraction collector (**7**). Using a split ratio of 200:1, the high flow (i.e., 99.5%) is collected (**7**) for later isotope analysis whereas the low flow (i.e., 0.5%) is directed to a microfluidic spray-dryer (**5**) centered above the QCM detector (**6**). Validated measurements of deposited masses on the QCM were accurate and demonstrated the ability of the built system to quantify NOM in environmental extracts using different solvents and chromatographic conditions. This system thus allows NOM online monitoring and opens new possibilities to optimize sample preparation procedures for CSIA.

## P20 A UAV-based sampling system to analyze greenhouse gases and volatile organic carbons encompassing compound specific stable isotope analysis

*S. Leitner[1,*], W. Feichtinger[2], S. Mayer[2], F. Mayer[2], D. Krompetz[3], R. Hood-Nowotny[1], A. Watzinger[1]*

[1]University of Natural Resources and Life Sciences, Vienna, Institute of Soil Research, Tulln, Austria
[2]Combinnotec GmbH, Alland, Austria
[3]M3 Consulting Group, LLC, DBA M3 Agriculture Technologies, Phoenix, Arizona, United States
*presenting author: simon.leitner@boku.ac.at

The study herein reports on the development of two sampling devices and the subsequent analytical setup for the sampling and analysis of atmospheric trace gases and their isotopic signature. Both samplers can be mounted to an unmanned aerial vehicle (UAV), the targeted compounds were the greenhouse gases (e.g. $CO_2$, $CH_4$, $N_2O$) and volatile organic compounds (VOCs, i.e. chlorinated ethenes), both were analyzed for their concentrations and stable isotope ratios (carbon, oxygen, hydrogen, nitrogen). In addition to the design of the measurement setups, compound calibration in the laboratory and the functionality test of the samplers, UAV-based sampling was tested in the field.

Atmospheric air was either sampled in glass vials for the analysis of greenhouse gases or flushed through sorbent tubes for the VOCs. The measurement setup for the sorbent tubes achieved analyte mass recovery rates of 63% - 100%, when prepared from gaseous or liquid calibration standards, and a precision of 0.1 to 0.9 ‰ for $\delta^{13}C$ in the concentration range of 0.35 – 4.45 nmol.

The precision of working gas standards achieved for $\delta^{13}C$ and $\delta^{18}O$ values of $CO_2$ was 0.2 ‰ and 0.3 ‰, and 2.4 ‰ for $\delta^{2}H$ of $CH_4$. The mid-term precision for $\delta^{13}C$ and $\delta^{15}N$ values of $CH_4$ and $N_2O$ working gas standards was 0.4 ‰ and 0.3 ‰, respectively. Injection quantities of working gas standards indicated a relative standard deviation of 1%, 5% and 5% for $CO_2$, $CH_4$ and $N_2O$, respectively. Measurements of manually taken atmospheric air samples demonstrated a standard deviation of 0.3 ‰ and 0.4 ‰ for the $\delta^{13}C$ and $\delta^{18}O$ values of $CO_2$, 0.5 ‰ for the $\delta^{13}C$ value of $CH_4$ and 0.3 ‰ for the $\delta^{15}N$ value of $N_2O$.

The precision of $CO_2$ measurements from samples taken at a field sampling campaign with the whole-air sampler mounted to an UAV was 0.4 – 5.6 mmol*mol$^{-1}$ and 0.03 – 0.21 ‰ ($\delta^{13}C$) while for $CH_4$ standard deviations were given at ±0.11 µmol*mol$^{-1}$ and ±0.4 ‰ ($\delta^{13}C$).

Overall results of conducted field sampling campaigns revealed the strengths of the presented sampling and measurement setup, which makes it a viable tool for monitoring atmospheric trace gas inventories and identifying emission sources.

## P21 High-precision ESI-Orbitrap MS measurements of hydrogen isotope compositions from organic molecules

*Elliott P. Mueller[1,*], Alex L. Sessions[1], Peter Sauer[2], Gabriella M. Weiss[1,3] & John M. Eiler[1]*

[1]Division of Geological and Planetary Sciences, California Insitute of Technology, Pasadena, CA 91125
[2]Department of Earth and Atmospheric Sciences, Indiana University, Bloomington, IN 47405
[3]Astrobiology Center of Isotopologue Research, Department of Geosciences, Pennsylvania State University, State College, PA 16802
*presenting author: emueller@caltech.edu

The diversity of molecular targets amenable to hydrogen stable isotope analyses has been limited by analytical challenges associated with gas-phase mass spectrometry (MS). Here, we demonstrate a novel technique for measuring $\delta^2$H values (vs. VSMOW) of organic molecules via electrospray ionization (ESI)-Orbitrap MS, using acetate as a model analyte. With only fifteen minutes of acquisition time and five nanomoles of acetate, we achieved precision and accuracy that match established isotope ratio MS techniques [1]. Moreover, this approach simultaneously captured the molecular average $\delta^{13}$C values (vs. VPDB) of acetate, enabling multidimensional isotopic analyses within a single acquisition.

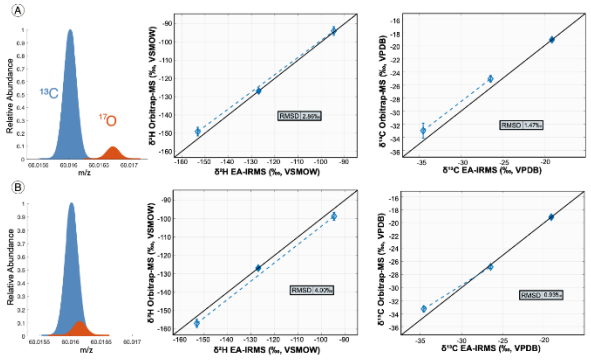



Figure 1: Accuracy experiments validating ESI-Orbitrap methods against EA-IRMS measurements.

Direct infusion ESI-Orbitrap measurements required off-line purification. In response, we developed a framework for preparative extraction of anions from complex matrices. This talk will discuss experiments that validate our preparative workflow, which will become increasingly important as Orbitrap techniques explore environmental samples. As an initial demonstration of our approach, we measured the acetate produced by bacterial fermentation and autotrophic acetogenesis, revealing a >200‰ difference in the fractionation between the metabolisms. These studies suggest that ESI-Orbitrap MS is poised to reveal novel microbial interactions in the environment and to diversify the molecular targets used in hydrogen isotope biogeochemistry.

[1] E.P. Mueller, A.L. Sessions, P.E. Sauer, G.M. Weiss & J.M. Eiler (2022). Simultaneous, High-Precision Measurements of δ2H and δ13C in Nanomole Quantities of Acetate Using Electrospray Ionization-Quadrupole-Orbitrap Mass Spectrometry. Anal. Chem. 94, 1092–1100. 10.1021/acs.analchem.1c04141

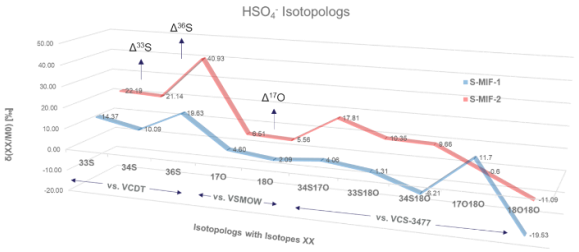
## P22 Comprehensive Isotope Ratio MS with Electrospray-Orbitrap

*Andreas Hilkert[1,*], Caj Neubauer[2] & Dieter Juchelka[3]*

[1]Thermo Fisher Scientific (Bremen) GmbH, Bremen, Germany
[2]INSTAAR, University of Colorado Boulder, Boulder, USA
[3]Thermo Fisher Scientific (Bremen) GmbH, Bremen, Germany
*presenting author: andreas.hilkert@thermofisher.com

A new, comprehensive approach to Isotope Ratio MS using an electrospray ionization (ESI) Orbitrap gives access to a wide range of isotopic information from wide variety of intact polar compounds in liquid samples. It delivers isotope ratios of singly substituted isotopologs, mass independent fractionation, clumped isotopes and, for organic molecules, position specific isotope analysis.

We have developed two sample introduction methods and automation, applying IRMS specific rules by using nitrate as a model compound. In total, 7 isotopologs of nitrate can be quantified simultaneously opening multiple pathways for calculating $\delta$ $^{15}$N, $\delta$ $^{18}$O, $\delta$ $^{17}$O and $\Delta$ $^{17}$O values with sub-‰ precision and accuracy [1]. It also opens a unique way to measure non-random isotopic distributions ("clumping") in oxyanions.




The general validity of this new development was proven by a full characterization of two recently published sulfates with mass independent sulfur isotope ratios, with in total 13 isotope ratios and triple isotope results.

We will present latest results and discoveries to describe the essential milestones of this new development.

[1] A.Hilkert, J.K. Böhlke, S.J. Mroczkowski, K.L. Fort, K. Aizikov, X.T. Wang, S.H. Kopf, C. Neubauer (2021). Exploring the Potential of Electrospray-Orbitrap for Stable Isotope Analysis Using Nitrate as a Model. Analytical Chemistry 93, 26, 9139–9148. DOI: 10.1021/acs.analchem.1c00944

## P23 Investigating the Intramolecular Isotopic Structure of Isoprenoids via Ultra High Resolution APCI - Orbitrap Mass Spectrometry

*M. Öztoprak[1,*], E. Hopmans[1], M. van der Meer[1], S. Schouten[1], L. Villanueva[1]*

[1]Royal Netherlands Institute for Sea Research, Texel, Netherlands
*presenting author: merve.oztoprak@nioz.nl

With >35.000 compounds, isoprenoids are the largest known group of biological products and can therefore be found ubiquitously in ancient sediments. One of the most prevalent sedimentary isoprenoids is phytane ($C_{20}$), which may be the

diagenetic product of either archaeol, a common archaeal core lipid with two phytanyl moieties, or the phytyl side chain of chlorophyll or bacteriochlorophyll. However, its sources are difficult to unambiguously establish[1]. The biosynthetic pathways producing isoprenoids are highly conserved in all Domains of Life. Bacteria almost exclusively synthesize isoprenoids through the methylerythritol phosphate (MEP) pathway, whereas Archaea use the mevalonate (MVA) pathway(s) and Eukarya utilize a modified MVA pathway, with photosynthetic eukaryotes harboring both pathways. These pathways not only determine the carbon isotope signature but also the intramolecular carbon isotopic composition of isoprenoids[2]. Characterization of these intramolecular isotopic signatures may thus reveals insight into sources of isoprenoid compounds found in modern, as well as ancient environments. Detection of intramolecular isotopic patterns of phytane and its precursors might therefore enable the assignment of sedimentary phytane to its biosynthetic sources. Recent advances in Fourier-transform mass spectrometers (FTMS) potentially allow for the first time an insight into the intramolecular isotopic structure of molecules with sufficient accuracy and precision for analysis of analytes at low abundance (~100 ng)[3].

Here, we establish a measurement method for the intramolecular isotopic analysis of phytol, a precursor of chlorophyll or bacteriochlorophyll derived sedimentary phytane, using a Q Exactive Orbitrap MS via atmospheric pressure chemical ionization (APCI). Stability and reproducibility was assessed with long duration infusion experiments and multiple replicate infusions. Intramolecular patterns of carbon isotopes were established by quantifying isotopolog ratios of molecular fragments of our phytol standard. Results of this study establish a novel framework for the establishing intramolecular isotope patterns of isoprenoids to reveal biosynthetic sources.

[1] Koopmans, M. P., Rijpstra, W. I. C., Klapwijk, M. M., de Leeuw, J. W., Lewan, M. D., & Damsté, J. S. S. (1999). A thermal and chemical degradation approach to decipher pristane and phytane precursors in sedimentary organic matter. Organic Geochemistry 30(9), 1089-1104. https://doi.org/10.1016/S0146-6380(99)00088-1

[2] Hayes, J. M. (2001). Fractionation of the isotopes of carbon and hydrogen in biosynthetic processes, pp. 225Ð277. Stable isotope geochemistry 43.

[3] Eiler, J., Cesar, J., Chimiak, L., Dallas, B., Grice, K., Griep-Raming, J., Juchelka, D., Kitchen, N., Lloyd, M., Makarov, A., Robins, R., Schwieters, J. (2017). Analysis of molecular isotopic structures at high precision and accuracy by Orbitrap mass spectrometry. International Journal of Mass Spectrometry 422, 126-142. https://doi.org/10.1016/j.ijms.2017.10.002

## P24 Exploring the potential of $^{17}O$ NMR for intramolecular $^{17}O$ isotope profile: application to vanillin origin discrimination

*E. Baguet[1], M. Grand[1], E. Martineau[1,2], G. S. Remaud[1,*]*

[1]CEISAM UMR 6230, Nantes Université, Nantes, France
[2]Capacités, Nantes, France
*presenting author: gerald.remaud@univ-nantes.fr

NMR offers the property to quantify, under specific conditions, heavy isotopomers of a given molecule for NMR active nuclei, i.e. $^{2}H$, $^{13}C$, $^{15}N$. Historically $^{2}H$-NMR, known as SNIF-NMR$^{TD}$, was first proposed, and then followed several tens of years after by $^{13}C$-NMR and very recently $^{15}N$-NMR [1] because of high precision requirement. Another isotope found in organic material, $^{17}O$ ($^{18}O$ being inactive), is difficult to study by NMR due to low abundance (0.038%) and low magnetogyric ratio (-3.63 $10^{-7}$ $rad.s^{-1}.T^{-1}$) leading to a very low receptivity as $6.5\times10^{-2}$ times lower than these of $^{13}C$.

It is therefore a deep challenge for establishing a $^{17}O$ intramolecular profile by NMR at natural abundance [2]. The present work aims to explore the capability to observe different position-specific $^{17}O$ contents in vanillin from several origins by NMR. For this purpose a new probe was built to increase the quality factor in the instrumental response. But it was installed on a common NMR spectrometer (500 MHz, $^{1}H$ frequency) with a 5 mm o.d. tube and 250 mg maximum of vanillin can be used, leading to sensitivity challenge.

Preliminary results will be shown on the potential discrimination of the origins of vanillin [3]: beans, synthetic, biotechnologic (ferrulic acid, eugenol, sugars, etc.) and compared with $^{18}O$ profiles [4]. The influence of the NMR parameters used as pulse sequence, signal/ratio, signal deconvolution, etc. is also discussed.

[1] S. Akoka, G.S. Remaud (2020). NMR-based isotopic and isotopomic analysis. Prog. Nucl. Magn. Reson. Spectrosc. 120-121, 1-24. https://doi.org/10.1016/j.pnmrs.2020.07.001.

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## P25 How to determine the intramolecular $^{13}C$ composition on low amount of glucose using irm $^{13}C$-NMR

*S. Renou[1,*], M. Grand[1], V. Daux[2], G. Tcherkez[3], S. Akoka[1], G. S Remaud[1]*

[1]CEISAM, Nantes Université, Nantes, France
[2]Laboratoire des Sciences du Climat et de l'Environnement, Université Paris-Saclay, Gif-sur-Yvette, France
[3]Research School of Biology, Australian National University, Canberra, Australia
*presenting author: sophie.renou@univ-nantes.fr

Quantitative NMR for isotopic measurements (irm-NMR) is a technique of choice to separate and quantify each isotopomer

(PSIA: Position Specific Isotope Analysis). Irm-$^{13}$C NMR was already used on glucose to study primary metabolism in plants [1]. However, the classical NMR approach, designed as 'Single pulse' sequence, requires a relatively high amount of sample or long experimental time making impossible many applications such as working with biological tissues or extracts.

To reduce the sample amount, a 2D NMR sequence has been developed for PSIA at low quantification limit [2]. This sequence was optimized on 10 mg of glucose with a standard deviation of precision less than 1‰ for each position. However, due to distortion associated to polarization transfer and spins manipulation during the NMR experiment, the obtained $^{13}$C isotopic compositions are expressed on an unusual δ-scale. A correction should be applied.

The present work aims to settle down a systematic methodology that allows expressing the results in the correct δ-scale. The bias is compensated with a correction factor obtained by analyzing a sample considerate as reference both by 'Single Pulse' and 2D sequences. Glucoses from different $CO_2$ assimilation metabolism of plants (namely $C_3$, $C_4$ and CAM) are analyzed with 2D sequence and the results are expressed on the usual δ-scale.

From these results it is conceivable to study now phenomenon where glucose is the isotopic molecular probe as in cellulose from tree rings to investigate climate change.

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## P26 First results of $CO_2$ and $H_2O$ Isotope-Flux Measurements a semi-arid area with large scale irrigation

*R.P.J. Moonen[1], G.A. Adnew[1], O.K. Hartogensis[2], J. Vilà-Guerau de Arellano[2], T. Röckmann[1]*

[1]Institute for Marine and Atmospheric Research, Utrecht University, The Netherlands
[2]Meteorology and Air Quality, Wageningen University, The Netherlands

Atmospheric $CO_2$ is the main inducer of climate change. Its budget is determined by the combination of all sources and sinks of $CO_2$. Importantly, the biosphere by far the largest source of atmospheric $CO_2$ while at the same time being the largest sink. Thus, to better constrain the budget of atmospheric $CO_2$, we need reliable data on the biosphere-atmosphere exchange. Currently, $CO_2$ exchange (flux) data is widely available but lacks separation into the counteracting sources and sinks as contemporary methods are unable to tell these apart. We aim to derive photosynthesis and respiration fluxes by exploiting differences within the isotopic composition of atmospheric $CO_2$ ($^{12}C^{16}O_2$, $^{13}C^{16}O_2$, $^{12}C^{16}O^{18}O$). As specific physical and chemical processes cause distinct isotopic fractionations, we can backtrack soil, plant, and atmospheric components of gross fluxes using local isotopic compositions. State of the art laser spectrometers let us derive isotopic compositions at temporal resolutions up to 10Hz, which allows for correlating isotope measurements to Scintillometer and Eddy Covariance fluxes. High-frequency $H_2O$ isotopologue measurements ($H_2{}^{16}O$, $DH^{16}O$, $H_2{}^{18}O$) are also used as fluxes of $CO_2$ and $H_2O$ are strongly linked and their isotopic fingerprints are interdependent. Together with local ecophysiology measurements giving us a ground truth, we get insight in the underlying processes at short temporal resolutions. In July 2021 we have been involved in the international LIAISE measurement campaign, aimed at better constraining changes in atmospheric circulation due to large scale irrigation. Here, we acquired insightful isotope-flux data and encountered several key issues which should be considered when performing such measurements (see Figure 1). In my presentation I hope to inform you on what these key issues are exactly, and I want to show you some unique first results from the data analysis.

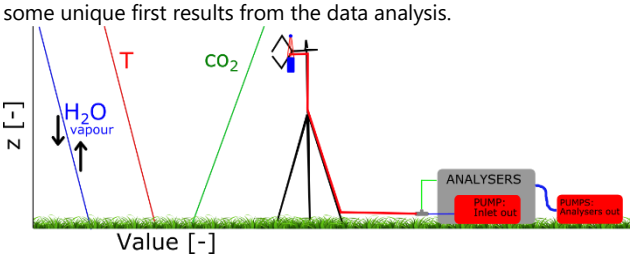


Figure 1: Visual description of isotope-flux measurements during the LIAISE field campaign (July 2021)

## P27 Temperature dependence of isotopic fractionation in the $CO_2$ - $O_2$ isotope exchange reaction

*Getachew Agmuas Adnew[1,*], Evelyn Workman[1], Christof Janssen[2], Thomas Röckmann[1]*

[1]Institute for Marine and Atmospheric research Utrecht (IMAU), Physics Department, Utrecht University, Princetonplein 5, Utrecht 3584CC, The Netherlands
[2]Laboratoire d'Etudes du Rayonnement et de la Matière en Astrophysique et Atmosphères (LERMA), Sorbonne Université, Observatoire de Paris, Université PSL, CNRS, 4 place Jussieu, 75005 Paris, France
*Presenting author: g.a.adnew@uu.nl

Oxygen isotope exchange between $O_2$ and $CO_2$ in the presence of heated platinum is an established technique for determining the $\Delta'^{17}O$ value of $CO_2$ [1,2,3]. However, there is not yet a consensus on the associated fractionation factors at the steady state. We determined experimentally the steady state $\alpha^{17}$ and $\alpha^{18}$ fractionation factors for Pt-catalyzed $CO_2$-$O_2$ oxygen isotope exchange at temperatures ranging from 500 to 1200 °C using $CO_2$ and $O_2$ differing in $\delta^{18}O$ value from -66 ‰ to +4 ‰. For comparison, theoretical $\alpha^{18}$ equilibrium exchange values by Richet et al. [4] have been updated using the direct sum method for $CO_2$ and the corresponding $\alpha^{17}$values are determined.

The experimentally determined steady state fractionation factors $\alpha^{17}$ and $\alpha^{18}$ are lower than those obtained updated from theoretical calculations. The offset is not due to scale incompatibilities between isotope measurements of $O_2$ and $CO_2$ nor to the neglect of non-Born-Oppenheimer effects in the calculations. The discrepancy between the theory and the

experimental $\alpha^{17}$and $\alpha^{18}$ may be due to thermal diffusion associated with the temperature gradient in the reactor. There is a crossover temperature at which enrichment in the minor isotopes switches from $CO_2$ to $O_2$. The direct sum evaluation yields $\theta$ value of ~ 0.54, i.e. higher than the canonical range maximum for mass-dependent fractionation process. This demonstrates the need to include anharmonic effects in the calculation and definition of mass-dependent fractionation processes for poly-atomic molecules.

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## P28 Fractionation effects during methane separation from ambient air for high-precision optical analysis of δ $^{13}$C and δ$^{2}$H

*E. Safi[1,*], C. Rennick[1] & T. Arnold[1,2]*

[1]National Physical Laboratory, Teddington, UK
[2]School of GeoSciences, University of Edinburgh, Edinburgh, UK
*presenting author: emmal.safi@npl.co.uk

It is recognised that reducing methane ($CH_4$) emissions is an important way to meet reduction targets such as those set out in the Paris Agreement and in achieving the aim of "net zero" carbon by the middle of the century. As such, individual countries and governments are increasing their efforts to reduce regional $CH_4$ emissions. To estimate $CH_4$ emissions from various sectors isotope ratio measurements are becoming a useful tool. In situ preconcentration instruments coupled to laser spectrometers are gaining attention as an option for making higher frequency precise isotope ratio measurements, however the volumes of air needed and the level of purity of the $CH_4$ extracted for such analyses are high.

We have developed Boreas, a field deployable preconcentrator coupled to a laser spectrometer capable of making these high precision, hourly measurements of regional scale $CH_4$ isotopologues in air. Boreas uses a HayeSep-D packed trap cooled cryogenically to separate $CH_4$ from ambient air.

We use this system to show how the separation process has potential to induce fractionation effects and we quantify these effects under various instrument operating conditions. We show how various combinations of trapping temperatures and flow rates influence fractionation and how a HayeSep-D based trapping system can be optimised to limit these effects.

## P29 Where do IRMS's go wrong? δ$^{18}$O SLAP determined at -56.3‰

*Anita Th. Aerts-Bijma[1*], Albert C. van Buuren[1], Dipayan Paul[1], Harro A.J. Meijer[1]*

[1]Centre for Isotope Research (CIO), Energy and Sustainability Research Institute Groningen, University of Groningen, Groningen, The Netherlands
*presenting author: a.t.aerts-bijma@rug.nl

The stable isotope scale of water has been successfully established and maintained by the two primary reference waters VSMOW and SLAP. In principle, only one reference material per isotope and per medium would be needed to define the isotopic scale, but two-point calibration leads to a dramatic improvement in inter-laboratory comparisons, due to various and variable scale contraction processes occurring in each measurement process.

For deuterium, it is possible to (re) produce the primary reference waters based on gravimetric mixtures of isotopically pure waters. In this way, the absolute deuterium abundances of VSMOW and SLAP has been precisely determined [1].

A similar experiment for oxygen is much harder, as pure $^{18}O$ and $^{16}O$ waters are not available. However, the 'absolute' determination of the $\delta^{18}O$ of SLAP versus VSMOW is feasible.

In this study, we quantify the difference in $\delta^{18}O$ between VSMOW and SLAP by gravimetrical mixing of a SLAP-like water with highly $^{18}O$ enriched water to mimic VSMOW and compared this with real VSMOW. The $^{18}O$ concentration of the highly enriched water, is precisely measured using an Extorr XT100 quadrupole mass spectrometer (Extorr Inc., USA). The isotope measurements were performed with an optical spectroscopy instrument (LGR LWIA 912-0050). The calculations of the isotopic abundances were done using a spreadsheet [2].

To our surprise this study resulted in a $\delta^{18}O_{SLAP}$ value of -56.33 ± 0.02 ‰ (average of 6 independent experiments). This is a much more negative value than the established value by consensus (-55.5‰) [3] and also more negative than we and some colleague laboratories measure nowadays, when taking scale contraction effects into account (around -55.8‰). Although this finding as such does not influence the use of the VSMOW-SLAP scale, it raises the intriguing question, what we actually measure with our instruments, and why even a well-corrected measurement can be so far off. It might have consequences for issues like the transfer of $\delta^{18}O$ from and to the VPDB scale, various fractionation factors, and the $\Delta^{17}O$ and other more sophisticated isotope measurements, such as clumped isotopes. The cause of this discrepancy needs to be found.

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## P30 A novel automated technique for simultaneous online analysis of $^{15}N$ in ammonium, nitrite, and nitrate

*Kun Huang[1,*], Wolfram Eschenbach[1,†], Jing Wei[1,‖], Damian Hausherr[2], Claudia Frey[3], André Kupferschmid[4], Jens Dyckmans[5], Adriano Joss[2], Moritz F. Lehmann[3], Joachim Mohn[1]*

[1]Empa, Laboratory for Air Pollution & Environmental Technology, 8600 Dübendorf, Switzerland
[2]Eawag, Process Engineering Department, 8600 Dübendorf, Switzerland
[3]Department of Environmental Science, University of Basel, 4056 Basel, Switzerland
[4]Empa, Transport at Nanoscale Interfaces, 8600 Dübendorf, Switzerland
[5]Centre for Stable Isotope Research and Analysis, University of Göttingen, 37077 Göttingen, Germany
*presenting author: kun.huang503@empa.ch
[†]Current affiliation: GeoInformationsDienst GmbH, Götzenbreite 10, 37124 Rosdorf Germany
[‖]Current affiliation: School of Atmospheric Sciences, Sun Yat-Sen University, Zhuhai 519082, China

$^{15}N$ tracer is widely applied to study biogeochemical nitrogen (N) transformation processes in various artificial and natural ecosystems, however, the analysis of $^{15}N$ fractions in inorganic N compounds, i.e. ammonium ($NH_4^+$), nitrite ($NO_2^-$), and nitrate ($NO_3^-$), is usually time and labor consuming. In this study, an automated sample preparation unit coupled to a membrane inlet quadrupole mass spectrometer was developed for the online, quasi-simultaneous analysis of $^{15}N$ fractions in $NH_4^+$, $NO_2^-$, and $NO_3^-$ in three separate reaction channels. Key to the method is the conversion of these aqueous N species to gaseous compounds, $N_2$ ($NH_4^+$) or NO ($NO_2^-$ and $NO_3^-$) by reaction with specific reagents (NaOBr, KI or $VCl_3$, respectively) at elevated temperature (75 °C).

The instrument is typically operated with a cycle time of 15 minutes, 5 minutes for each of the three reaction channels, to provide temporal trends in compound-specific N concentrations and $^{15}N$ fractions ($f_{15}$). Minimum N concentration and $^{15}N$ fraction ($f_{15}$) for an accurate (rel. error < 5%) and reproducible (rel. standard deviation (RSD) < 5%) determination of the $^{15}N$ fraction were 100 µmol/L and 1% for $NO_2^-$ and $NO_3^-$. $NH_4^+$ analysis is significantly affected by the high atmospheric $N_2$ background, and thus concentrations > 100 µmol/L (e.g., 200 µmol/L) and $f_{15}$ > 1% are required for accurate analysis. For the target application, i.e., tracing $NH_4^+$ cycling in wastewater processes, such sensitivity may still be sufficient. However, interference from organic nitrogen compounds (e.g., urea) is likely, and is the focus of ongoing investigations. For $NO_3^-$ analysis, the concentration and $f_{15}$ of $NO_2^-$ must be considered for corrections, since both N-species react with $VCl_3$ to form NO.

We will show results of a first application study, where our setup was applied in conjunction with a FTIR spectrometer to trace $N_2O$ formation pathways during partial $NH_4^+$ to $NO_2^-$ oxidation, as part of a two-stage anammox process in a lab-scale sequencing batch reactor with municipal waste water.

## P31 Real-time analysis of $\delta^{13}C$- and $\delta D$-$CH_4$ in ambient air with a QCL based absorption spectrometer: Method development

*K. Zeyer[*], I. Prokhorov, B. Tuzson, J. Mohn*

Laboratory for Air Pollution / Environmental Technology, Empa, Dübendorf, Switzerland
*presenting author: kerstin.zeyer@empa.ch

Methane ($CH_4$) is the second most important anthropogenically emitted greenhouse gas after carbon dioxide ($CO_2$). Over the last 150 years the $CH_4$ concentrations in the atmosphere has increased from around 772 ppb (parts-per-billion, nmole mole$^{-1}$) in pre- industrial times to 1906 ppb in 2021 (Ed Dlugokencky, NOAA/GML (gml.noaa.gov/ccgg/trends_ch4/)). High-precision analyses of the most abundant methane isotopoloques $^{13}CH_4$ and $^{12}CH_3D$ have been suggested as natural proxies to discriminate between different source categories.

For regionally focused studies, with large local fluxes, WMO/GAW suggests extended compatibility targets between laboratories of ±5 ppb, ±0.2 ‰, and ±5 ‰, for $CH_4$ mole fractions and isotope ratios, $\delta^{13}C$-$CH_4$, $\delta D$-$CH_4$, respectively [1]. This stringent compatibility levels can only be reached with an analytical technique demonstrating high precision and repeatability. Our previous research demonstrated that quantum cascade laser absorption spectroscopy (QCLAS) coupled to a trace gas extractor (TREX) can achieves these requirements [2, 3]. Only the $\delta^{13}C$-$CH_4$ data showed about 2 ‰ offset, which was attributed to enhanced $O_2$ levels after preconcentration.

In this work, we present an upgraded analytical system consisting of a new preconcentration device (TREX-III) equipped with cryo-focusing trap and a new dual-laser QCLAS (Aerodyne Research, Inc., USA). Within 1 h cycle TREX-III can process up to 18 liters (STP) of ambient air and quantitatively separate $CH_4$ from bulk air constituents ($N_2$, $O_2$, Ar) and trace gases ($CO_2$, $N_2O$). The QCLAS achieves precisions around 0.04 ‰ and 0.2 ‰ for $\delta^{13}C$- and $\delta D$-$CH_4$, respectively. Coupling to the TREX and laboratory validation experiments are ongoing. The instrument will be deployed for monitoring of $CH_4$ isotopes in the city of Zürich within the EURAMET funded metrology project (19ENV05 STELLAR).

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## P32 The hydrogen isotopic composition of plant carbohydrates – Advancement in methods and interpretation

*Marco M. Lehmann[1,*], Philipp Schuler[1], Marc-André Cormier[2], Shiva Ghiasi[3], Roland A Werner[4], Matthias Saurer[1], Guido Wiesenberg[5]*

[1]Forest Dynamics, Swiss Federal Institute for Forest, Snow and Landscape Research WSL, Birmensdorf, Switzerland
[2]Department of Earth Sciences, University of Oxford, UK
[3]Water Protection and Substance Flows, Agroscope, Zurich, Switzerland
[4]Institute of Agricultural Sciences, ETH Zurich, Zurich, Switzerland
[5]Department of Geography, University of Zurich, Zurich, Switzerland
*presenting author: marco.lehmann@wsl.ch

The hydrogen isotopic composition ($\delta^2H$) of plant organic matter is considered as a potential proxy for hydro-climatic conditions, plant physiology, and metabolic pathways. However, the information that is imprinted on $\delta^2H$ of photosynthetic assimilates (i.e., soluble mono- and disaccharides), carbon storage (i.e., starch) and structural plant compounds (i.e. cellulose) is barely constrained so far, mainly due to analytical issues. We recently developed a novel high-throughput method that allows accurate and precise analyses of $\delta^2H$ values in various plant carbohydrates [1]. The new method is based on an equilibration between water vapor of a known isotopic composition and hydroxyl groups of carbohydrate molecules at 130 °C in a metal chamber. The equilibrated samples are then measured with a TC/EA-IRMS system, which allows the estimation of the non-exchangeable carbon-bound hydrogen isotope ratio in plant carbohydrates that contains the actual meaningful information. First applications showed clear $\delta^2H$ differences among carbohydrates of plant types differing in their photosynthetic pathways (e.g., C3, C4, CAM) and carbohydrate family (e.g. soluble sugars, starch, and cellulose) within each plant type under controlled growth conditions. In addition, controlled tobacco experiments also showed large $\delta^2H$ variation in plant carbohydrates along an N nutrition gradient or with differences in genotype. We link this observed $\delta^2H$ differences to changes in metabolic processes, gas-exchange and growth. Given the recent methodological advancement and interpretation of hydrogen isotope patterns in plant carbohydrates, we predict a fast increase in $\delta^2H$ applications on various dateable geological and ecological archives such as tree-rings, lake-sediment plant macrofossils, or peatlands to investigate plant responses to climatic or non-climatic conditions or to use it as a proxy for changes in carbon allocation.

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## P33 Method optimization for plant sugar purification and compound-specific hydrogen isotope analysis

*Selina Hugger[1,*], Meisha Holloway-Phillips[1], Ansgar Kahmen[1,] Daniel B. Nelson[1]*

[1]Department of Environmental Sciences – Botany, University of Basel, Basel, Switzerland
*presenting author: se.hugger@unibas.ch

The hydrogen isotopic composition ($\delta^2H$) of plant organic material contains hydrological information about the environment and metabolic information about the plant. The metabolic component is of particular interest in the context of understanding how plant carbon metabolism responds to changing environmental conditions and in turn influences plant performance. Until recently, cellulose has been the only plant carbohydrate routinely measured, limiting the interpretation of cellulose $\delta^2H$ variation with respect to plant metabolism. Apart from that, $\delta^2H$ values of individual compounds like sugar molecules can be used to investigate specific metabolic pathways. However, measuring $\delta^2H$ values in plant sugars is complicated by the presence of hydroxyl hydrogen because this can exchange with hydrogen in surrounding liquid water or water vapor (e.g., during sample processing and analysis), obscuring the primary plant hydrological and metabolic information contained in the isotopic values of carbon-bound hydrogen. The contribution of exchangeable hydrogen can be accounted for using dual equilibration techniques, but in most cases these permit only analyses of bulk leaf extracted sugars. Thus, another difficulty lies in obtaining pure sugars from plants. An alternative to dual equilibration is therefore to derivatize the plant sugars prior to analysis with hydrogen of known isotopic composition to form new compounds that no longer contain exchangeable hydrogen, e.g., by acetylation. Acetylation makes sugar compounds amenable to gas chromatography, so this technique also allows for compound-specific analyses of multiple compounds in the same sample, such as different sugar types, while requiring low sample amounts.

Here, we present an update of our work on streamlining acetylation and purification of plant non-structural carbohydrates to permit analysis by gas chromatography-isotope ratio mass spectrometry (GC-IRMS). By acetylating whole extracts, we facilitate fast initial sample cleanup to partially purify sugars from the bulk extract. We then compare recovering the sugar acetates from the sample matrix by liquid-liquid separation with approaches using reverse phase solid phase extraction. The liquid-liquid separation uses water and organic solvents to separate sugar acetates from water soluble components of the sample matrix. In contrast, using solid phase extraction columns, the acetylating reagents as well as water soluble components of the sample matrix can be washed out, while the sugar acetates are retained on the column. The liquid-liquid separation is relatively labor-intensive, whereas the solid phase extraction is easy to learn, consumes less reagents, and permits a higher sample throughput. The procedures yield GC amenable sugar acetates that are soluble in acetone, allowing for sucrose octaacetate to be well resolved under normal GC measurement conditions.

## Keynote: Benefits and perspectives of carbonate dual clumped isotope thermometry

*J. Fiebig[1,*], A.J. Davies[1], M. Bernecker[1], M. Tagliavento[1], P. T. Staudigel[1], W. Guo[2]*

[1]Institute of Geosciences, Goethe University, Frankfurt, Germany
[2]Department of Geology and Geophysics, Woods Hole Oceanographic Institution, Woods Hole, USA
*presenting author: Jens.Fiebig@em.uni-frankfurt.de

The temperature dependence of the carbonate $\Delta_{47}$ clumped isotope thermometer depends on the internal fractionation of $^{13}C$ and $^{18}O$ isotopes amongst carbonate isotopologues. It addresses the excess of mass 47 isotopologues (mainly made up of $^{18}O$-$^{13}C$-$^{16}O$) in $CO_2$ evolved from acid digestion of carbonates relative to its stochastic abundance. It is superior to the heterogeneous oxygen isotope thermometer as it does not require knowledge of the oxygen isotope composition of the water from which the carbonate precipitated [1]. However, in addition to temperature, the $\Delta_{47}$ composition of carbonates can also be affected by kinetics, either occurring during original formation and/or during secondary alteration of the carbonate. If remaining unidentified, kinetics can compromise the accuracy of the $\Delta_{47}$ clumped isotope thermometer [2].

Recent advancements in gas source mass spectrometry enabled significant improvement in the external reproducibility of $\Delta_{47}$ measurements to less than 10 ppm. Moreover, precise measurements of $\Delta_{48}$ became possible, even though mass 48 isotopologues (mainly $^{18}O$-$^{12}C$-$^{18}O$) are one order of magnitude less abundant than those of mass 47 [3]. Dual clumped isotope thermometry, i.e. the simultaneous measurement of $\Delta_{48}$ alongside $\Delta_{47}$ in carbonate derived $CO_2$, allows us to identify the nature and extent of kinetics involved in carbonate formation [4, 5], just from isotopic analysis of the carbonate. Under certain circumstances, kinetic biases in $\Delta_{47}$ derived carbonate formation temperatures can be corrected, expanding our portfolio of natural carbonate archives for accurate paleotemperature reconstructions to include disequilibrium calcifiers [4, 5].

In the past three years, we have elaborated a routine protocol for processing and correcting mass spectrometric raw data [6], determined the temperature dependence of $\Delta_{47}$ and $\Delta_{48}$ of carbonate-derived $CO_2$ [6], and investigated the dual clumped isotope composition of brachiopods, mollusks, eggshells, belemnites, warm- and cold-water corals to test their suitability as temperature archives. We also heated biogenic aragonite in the absence of external fluids to study the response of the dual clumped composition to secondary alteration under low water-rock ratios. Based on these results the perspectives and limitations of dual clumped isotope thermometry will be discussed.

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## Invited: Robustness of clumped carbonate thermometry in carbonates from the Tara Deep, a large Irish orebody

*M. Clog[1,*], D. Drummond[1] & A. Boyce[1]*

[1]Scottish Universities Environmental Research Centre, University of Glasgow, Glasgow, United Kingdom
*presenting author: matthieu.clog@glasgow.ac.uk

Tara Deep (Ireland) is a recent discovery associated with the Navan Carboniferous carbonate-hosted base-metal orebody, which supports the largest underground Zn mine in Europe. Current understanding of this orebody is that it is the result of deep hydrothermal convection cells during an extension phase. Navan lacks workable fluid including, and so there is limited constrains on the temperatures of the circulating fluids which are key to understanding the processes which controlled metal transport and deposition of the base metal phases. Applying clumped isotopes on paragenetically associated calcite and dolomite phases thus offers a significant opportunity to develop the genetic model for this giant hydrothermal system, but to what extent do TΔ47 faithfully record the mineralization temperatures? The orebody formed ~345Ma ago but has been subsequently heated following the Zn-Pb mineralization to 120-150$^0$C for potentially millions of years. These temperatures may be high enough to cause re-distribution of isotopes in the lattice of carbonate minerals, at least for calcite.

Calcite samples have apparent temperatures ranging from 90 to 175$^0$C, with no grouping with sample petrology, which points to the role of both re-distribution of isotopes in the carbonate lattice and recrystallisation. Detailed petrography of textures support this with early diagenetic cements recording apparent temperatures of up to 150°C. The use of TΔ47 in calcites as a measure of the primary hydrothermal temperature during ore deposition is inhibited, as was anticipated from the burial history. Isotope redistribution has been achieved without inducing visible changes in crystal microstructure and trace element concentrations, metrics commonly used to gauge preservation quality. The temperatures recorded in our samples place a lower bound on the peak paleotemperatures at 175°C. Dolomites associated with the mineralization record lower apparent temperatures (82 to 146$^0$C), consistent at face value with previous studies showing a higher threshold for re-equilibration in dolomite. However, for dolomites as well, the dispersion of temperatures for similar facies points to the role of recrystallisation.

Apparent temperatures from carbonates at Tara Deep are not reflecting the initial deposition temperatures but a combination of solid-state reordering and recrystallisation that did not leave other readily identifiable markers. Final temperatures recorded can still give insights regarding heating and potentially cooling history, but the co-existence of both processes needs to be considered.

### Oral: Characterization of microbial methane using clumped isotope measurements

*Malavika Sivan[1,*], Anna J. Wallenius[2], Olga Zygadlowska[3], Thomas Röckmann[1], Carina van der Veen[1], Caroline P. Slomp[3], Mike S.M. Jetten[2], Maria Elena Popa[1]*

[1]Institute of Marine and Atmospheric Research, Utrecht University, Utrecht, Netherlands
[2]Department of Microbiology, Institute of Water and Wetland Research, Radboud University Nijmegen, Nijmegen, Netherlands
[3]Department of Earth Sciences, Faculty of Geosciences, Utrecht University, Utrecht, Netherlands
*presenting author: m.sivan@uu.nl

Atmospheric methane is the second most important greenhouse gas. Unfortunately, the concentration of methane in the atmosphere has been on a rise since the pre-industrial times. In order to mitigate methane emissions, it's crucial to understand all the sources and sinks of methane.

The measurement of bulk isotopic composition ($^{13}CH_4$ and $CDH_3$) is a widely used characterization technique. But due to the overlap of source signatures, it is often difficult to distinguish between all the different methane sources. With the advancement of high-resolution mass spectrometry, it is now possible to measure the clumped isotopologues of methane: $^{13}CDH_3$ and $CD_2H_2$. The clumping anomaly is temperature-dependent and can therefore be used as an additional information to constrain methane sources.

A significant fraction of biogenic methane emissions to the atmosphere is produced from the microorganisms in wetlands and other freshwater systems. However, there still exists a lack of experimental data to fully understand the different pathways of microbial methane production and consumption. To get more insights, we chose Lake Grevelingen in The Netherlands, one of the sites studied extensively for its methane production.

Methane in the water column and sediments from different depths of the lake were studied separately. For the analyses, we developed an efficient method to extract methane from large water samples. In addition to this, laboratory incubation experiments were also performed to study the effect on the isotopic composition of methane produced from the microbes when they are fed with different substrates. This is an ongoing study and we will present the first set of results and interpretation of the different controls on the isotopic signatures of methane.

### Oral: Carbonate clumped isotope calibration from 6 to 1100 °C using an isotope ratio laser spectrometer based on tunable infra-red laser spectroscopy

*Z. Wang[1], N. Yanay[1], D. Dettman[1,2], J. Quade[1,*], K. Huntington[3], A. Schauer[3], D. Nelson[4], J. McManus[4], K. Thirumalai[1], S. Sakai[5]*

[1]Department of Geosciences, University of Arizona, Tucson, Arizona, United States
[2]Estuary Research Center, Shimane University, Shimane, Japan
[3]Department of Earth and Space Sciences and IsoLab, University of Washington, Seattle, Washington, United States
[4]Aerodyne Research, Inc., Billerica, Massachusetts, United States
[5]Institute of Biogeochemistry, Japan Agency for Marine-Earth Science and Technology, Yokosuka, Kanagawa, Japan
*presenting author: quadej@arizona.edu

Clumped isotope abundance in carbonates is an important paleothermometer, but its application suffers from time-consuming measurement, large sample size, and common-mass (m/z) interferences using isotope ratio mass spectrometry (IRMS). We present our recent progress in developing a fully automated carbonate clumped isotope analyzer system based on tunable infrared laser differential absorption spectroscopy (TILDAS). This system follows widespread methods for carbonate-phosphoric acid reaction, purifies the $CO_2$ gas by cryogenically removing water vapor and non-condensable gases, and then directly measures the four $CO_2$ isotopologues involved in the clumped isotope calculation ($^{16}O^{12}C^{16}O$, $^{16}O^{13}C^{16}O$, $^{16}O^{12}C^{18}O$, and $^{16}O^{13}C^{18}O$). Our laser spectroscopic system achieves the same precision (0.01‰, 1 S.E.) as IRMS measurements, and surpasses typical IRMS systems in several key respects, such as rapid measurement (50 minutes per carbonate sample), small sample size (<20 µmol of $CO_2$, or <2 mg equivalent calcite), and requires no assumptions about $^{17}O$ abundance in the sample to correct for common-mass interference. An empirical $\Delta_{16O13C18O}$–temperature calibration, $\Delta_{638CDES} = 0.0409\pm0.0003 \times 10^6/T^2 + 0.1776\pm0.0031$ ($R^2$=0.997), which is based on 406 analyses of 51 synthetic carbonates equilibrated at 6 °C to 1100 °C, is consistent with results for natural carbonates and other published IRMS calibrations[1]. The clumped isotope values obtained with our system are indistinguishable from those produced by top-performing IRMS systems after replicating the recent InterCarb interlaboratory calibration effort[2]. Rapid and precise measurement by laser spectroscopy holds the potential to change the landscape for clumped isotope analysis.

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[2] S.M. Bernasconi, et al. (2021). InterCarb: A community effort to improve interlaboratory standardization of the carbonate clumped isotope thermometer using carbonate standards. Geochemistry, Geophysics, Geosystems 22, e2020GC009588. https://doi.org/10.1029/2020GC009588

## POSTERS P34 – P38

### P34 Quantum cascade laser absorption spectrometer with a low temperature multipass cell for precision clumped $^{12}C^{18}O_2$ and position specific isotope analysis

*A. Nataraj[1,*], M. Gianella[1], B. Tuzson[1], I. Prokhorov[1], J. Faist[2], L. Emmenegger[1]*

[1]Laboratory for Air Pollution / Environmental Technology, Empa, Dübendorf, Switzerland
[2]Institute for Quantum Electronics, ETH Zürich, Zürich, Switzerland
*presenting author: akshay.nataraj@empa.ch

High precision measurement of multiply substituted ("clumped") isotopologues of $CO_2$ and site-specific propane isotopomers is a topic of significant interest in the field of isotope geochemistry. The temperature-dependent behavior of $^{13}C$ and $^{18}O$ isotopes in gaseous carbon dioxide is widely used as a temperature proxy for paleoclimate reconstruction. Intramolecular isotope distributions can constrain source attribution, mechanisms of formation and destruction, and temperature histories of molecules. ($^{13}C/^{12}C$) fractionation between central "-$CH_2$-" (2-$^{13}C$) and terminal "-$^{13}CH_3$-" (1-$^{13}C$) positions of propane $C_3H_8$, a percent level component of natural gases, could potentially work as a geo-thermometer for natural gas systems. The established method to perform clumped isotope thermometry and position specific isotope analysis is Isotope Ratio Mass Spectrometry (IRMS) [1,2]. However, IRMS measurements, in particular for rare isotopologues, typically require several hours of analysis time and extensive sample preparation to separate isobaric interferences.

In this abstract, we present a quantum cascade laser-based absorption spectrometer (QCLAS) employing a low-volume segmented circular multipass cell (SC-MPC) [3]. The SC-MPC operated at cryogenic temperatures (153 K) to supress the hot band transitions of the principal isotopes, and low pressure (5 mbar) to work close to the Doppler limit, and avoid the overlap between neighbouring transitions. We optically measure the abundances of all three isotopologues involved in the reaction $^{12}C^{18}O_2 + {}^{12}C^{16}O_2 \leftrightarrow 2\cdot{}^{12}C^{16}O^{18}O$ simultaneously. We report a precision of 0.05 ‰ in the isotope ratios [$^{12}C^{18}O_2/^{12}C^{16}O_2$] and [$^{12}C^{16}O^{18}O/^{12}C^{16}O_2$] with 25 s integration time. In addition, we determine and resolve the tiny variation in the equilibrium constant, $K(T)$, of the above exchange reaction between room-temperature-equilibrated and 1273 K-equilibrated $CO_2$ [4]. We further demonstrate the capability of this instrument by acquiring the first high-resolution spectra of propane and its site-specific isotopomers. In mixtures containing $^{12}C$, 1-$^{13}C$ and 2-$^{13}C$ propane, we distinguish their contributions to the overall absorption spectrum. We demonstrate a precision of 0.03 ‰ and 0.06 ‰ for the isotopocule ratio (2-$^{13}C$)/$^{12}C$ & (1-$^{13}C$)/$^{12}C$, respectively. This versatile system can be extended to other chemical species where spectroscopic measurements are impacted by the hot-band transitions of abundant isotopologues e.g., methane and its deuterated isotopologues, $CH_3D$ and $CH_2D_2$, opening new perspectives in environmental sciences.

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### P35 Concordant optical clumped isotope thermometry of methane

*I. Prokhorov[1,*], B. Tuzson[1], N. Kueter[2], R. Rosskopf[2], L. Emmenegger[1], S. M. Bernasconi[2], J. Mohn[1]*

[1]Laboratory for Air Pollution / Environmental Technology, Empa, Dübendorf, Switzerland
[2]Geological Institute, ETH Zürich, Zürich, Switzerland
*presenting author: ivan.prokhorov@empa.ch

Methane clumped isotope thermometry deals with the relative abundance of doubly substituted isotopologues, whose formation probability in thermodynamic equilibrium increases as temperature decreases. However, kinetic effects occurring during the methane formation and transport, as well as the mixing of multiple gas sources, can drive the isotopologue signatures away from those expected in equilibrium. Hence, measurements of clumped isotopes might be misinterpreted in temperature reconstruction. Simultaneous measurements of $\Delta^{13}CH_3D$ and $\Delta^{12}CH_2D_2$, referred here as concordant clumped isotope thermometry, can help to avoid ambiguity in temperature interpretation by looking into departure from the equilibrium line in $\Delta^{13}CH_3D$ vs. $\Delta^{12}CH_2D_2$ space.

Currently, we are developing an analytical technique alternative to high-resolution isotope ratio mass spectrometry. Our all-optical approach is intrinsically free from isobaric interferences and allows simultaneous detection of all isotopologues involved in the isotope exchange reactions $^{12}CH_4 + {}^{13}CH_3D \leftrightarrow {}^{13}CH_4 + {}^{12}CH_3D$ and $^{12}CH_4 + {}^{12}CH_2D_2 \leftrightarrow 2\cdot{}^{12}CH_3D$. Compared to previously developed TILDAS analyzer [1], we achieve better selectivity for the low-abundant $^{12}CH_2D_2$ isotopologue by using an alternative spectral window and have an improved precision for $\Delta^{13}CH_3D$ analyzing all isotopologues within one laser scan, requiring smaller (1.4 ml STP) methane samples [2]. Within the framework of the SNSF funded project CLUMPME, the technique will be applied on $CH_4$ entrapped in rocks along a transect in the Alps, Apennines and Pyrenees to investigate the formation history of $CH_4$.

[1] Y. Gonzalez, *et al.* (2019) Precise Measurements of $^{12}CH_2D_2$ by Tunable Infrared Laser Direct Absorption Spectroscopy. Anal. Chem. 91, 23, 14967–14974, DOI:10.1021/acs.analchem.9b03412

[2] I. Prokhorov, J. Mohn (2022) CleanEx – A Versatile Automated Methane Preconcentration De-vice for High-Precision Analysis of $^{13}CH_4$, $^{12}CH_3D$, and $^{13}CH_3D$, *under revision*

## P36 Atmospheric $CO_2$ sources with specific $\Delta_{47}$ signals under mixing conditions

*H. Eckhardt*, M. Schmidt & V. Schmid*

Institute of Environmental Physics, Heidelberg University, Heidelberg, Germany
*presenting author: henrik.eckhardt@iup.uni-heidelberg.de

In many studies of the atmospheric carbon cycle and its exchange with other reservoirs, $CO_2$ mole fraction, $\delta^{13}C$ and $\delta^{18}O$ are widely used to partition between different processes contributing to sources or sinks. $\Delta_{47}$ is mainly used for applications as a paleothermometer, but was also proposed for atmospheric $CO_2$ studies[3,1]. The temperature dependency of $\Delta_{47}$ gives the possibility to differentiate between high temperature (e.g. by burning fossil fuels) and low temperature sources (e.g. soil respiration). In urban or local process studies, $\Delta_{47}$ could therefore support more common tracers such as $\delta^{13}C$ or $\delta^{18}O$. Combining multiple tracers provides the opportunity to better constrain and thus interpret results of this often underdetermined system. Here we present a theoretical study using synthetic data which examines the effect of mixing different source signals into an ambient air mass. Preliminary data will complement these studies. Furthermore, it is shown how to calculate $\Delta_{47}$ under two component-mixing conditions without producing nonlinearities, which have occurred in previous studies[3,1,2,4]. We will present examples where ignoring this effect may lead to wrong conclusions.

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## P37 Clumped isotope reordering in belemnite and optical calcites: Towards material-specific reordering kinetics

*N. Looser[1,*], P. Petschnig[2], J. D. Hemingway[1], A. Fernandez[2], L. Morales Grafulha[1], A. Perez-Huerta[3], M. Vickers[4], G. Price[5], M. W. Schmidt[1], S. M. Bernasconi[1]*

[1]Department of Earth Sciences, ETH Zurich, Zurich, Switzerland
[2]Andalusian Institute of Earth Sciences, CSIC-University of Granada, Granada, Spain
[3]Department of Geological Sciences, The University of Alabama, Tuscaloosa, USA
[4]Faculty of Science, Geology Section, University of Copenhagen, Copenhagen, Denmark
[5]School of Geography, Earth and Environmental Sciences, Plymouth University, Plymouth, UK
*presenting author: nathan.looser@erdw.ethz.ch

The application of carbonate clumped isotope ($\Delta_{47}$) thermometry in deep-time settings is often limited by the alteration of the original temperature signal by solid-state bond reordering. New modeling approaches to estimate the initial compositions of partially reordered calcites or maximal burial temperatures, however, open promising possibilities in temperature reconstructions. Such approaches strongly depend on laboratory-derived kinetic parameters of calcite materials. Calcites of different origin differ greatly in their microstructure, water content, and minor and trace elemental composition and it remains an open question whether they all share the same reordering kinetics. The rostra of belemnites, an extinct group of mollusks with a wide temporal and spatial occurrence in the Mesozoic, have been extensively used for deep-time paleoclimate reconstructions using oxygen isotope geochemistry. Belemnites are also important targets for clumped isotope-based temperature reconstructions, but in many cases, they have been found to have reordered $\Delta_{47}$ compositions. Here, we present new results from heating experiments on belemnite rostral calcite and optical calcite and provide belemnite-specific reordering kinetic parameters. We show that belemnite calcite reorders much faster and at lower temperatures than optical calcite and all other calcites reported in previous studies. We suggest that fast initial reordering results from oxygen isotope exchange of belemnite calcite with internal skeletal water and show that this process is completed within 2 minutes at experimental temperatures. Extrapolation to geological timescales using different reordering models shows that belemnite calcite reordering starts at lower burial temperatures than brachiopod, spar, and optical calcites. This susceptibility to solid-state reordering results in a measurable increase of the apparent $\Delta_{47}$ temperature even under moderate burial conditions (i.e., below 70 °C for millions to tens of millions of years). Similar to what is observed for cooling of carbonatites and marbles, the belemnite $\Delta_{47}$ during exhumation can re-equilibrate, resulting in a decrease of the apparent $\Delta_{47}$ temperature – which we term "retrograde reordering". This may overprint high $\Delta_{47}$ temperatures gained during preceding deep burial and result in underestimation of the amount of reordering a sample experienced during its geological history. Overall, our results argue for the use of material-specific kinetic parameters and urge caution when interpreting $\Delta_{47}$-derived temperatures from deep-time archives.

## P38 Abiotic methane formation in nature: information from clumped isotope analysis of laboratory synthesized methane

*N. Zhang[1,*], Y. Sekine[2], K. Yamada[3], A. Gilbert[1], M. Nakagawa[1], N. Yoshida[2,4]*

[1]Department of Earth and Planetary Sciences, School of Science, Tokyo Institute of Technology, Tokyo, Japan
[2]Earth-Life Science Institute, Tokyo Institute of Technology, Tokyo, Japan
[3]Department of Environmental Chemistry and Engineering, Tokyo Institute of Technology, Yokohama, Japan
[4]Terahertz Research Center, National Institute of Information and Communications Technology, Koganei, Japan
*presenting author: zhang.n.aa@m.titech.ac.jp

Natural abiotic methane has been widely observed in serpentinization systems such as hydrothermal vents, springs, seeps, and so on[1]. Understanding their formation mechanisms could be significative since it may play important role in supporting the origin and evolution of earliest life on Earth or elsewhere. Although numerous works including observations and laboratory synthesizes have been carried out during past two decades, our knowledge on pathways of its formation in nature reservoirs remains poor. Recent development of clumped isotope analytical approach, i.e. $\Delta^{13}CH_3D$ and $\Delta^{12}CH_2D_2$, provides new opportunity for us to answer this question.

We studied the clumped isotope signatures of abiotic methane synthesized via Fischer-Tropsch type (FTT) reactions ($CO/H_2$=1/4; Nickel; ~ 335 °C) using a 253 Ultra IRMS at Tokyo Tech[2]. Our results exhibit a dramatically disequilibrium pattern in $\Delta^{12}CH_2D_2$ (~-45‰) while the $\Delta^{13}CH_3D$ values present near-equilibrium values (+1.4‰) at the beginning of experiment. With reaction proceeding, $\Delta^{13}CH_3D$ decreased gradually and approached the lowest value (~-1.7‰) at 100% CO conversion rate; inconsistently, the trend of $\Delta^{12}CH_2D_2$ was not significant. After then, both clumped values increased to equilibrium with a $\Delta^{12}CH_2D_2$ vs. $\Delta^{13}CH_3D$ slope around a unit due to bond reordering catalyzed by Ni. We will discuss the potential mechanisms controlling bulk and clumped isotope patterns during laboratory synthesis of abiotic methane. In addition, by comparing with published clumped isotope values of natural 'abiotic' methane from various reservoirs, we will also discuss how could clumped isotope analytical technique be applied in understanding the origins of these natural samples.

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[2] N. Zhang et al. (2021). Doubly substituted isotopologues of methane hydrate ($^{13}CH_3D$ and $^{12}CH_2D_2$): Implications for methane clumped isotope effects, source apportionments and global hydrate reservoirs. Geochim. Cosmochim. Acta 315, 127-151. doi.org/10.1016/j.gca.2021.08.027.

## ORIGIN AND EVOLUTION OF THE EARTH (PLANETS)

### Keynote: Tracing oxygen in sulfate using $^{34}S$-$^{18}O$-clumping

*Y. Ueno[1,2,*], J. Surma[1,2,3], T. Katsuta[1], T. Ishimaru[1], M. Nakagawa[2], P.W. Crockford[4], H. Bao[5,6], N. Yoshida[2,7]*

[1]Department of Earth & Planetary Sciences, Tokyo Tech., Tokyo, Japan
[2]Earth-Life Science Institute (WPI-ELSI), Tokyo Tech., Tokyo, Japan
[3]Geoscience Center, Georg-August University, Göttingen, Germany
[4]Department of Marine Chemistry & Geochemistry, Woods Hole Oceanographic Institution, Woods Hole, USA
[5]International Center for Isotope Effects Research, Nanjing University, Nanjing 210023, China
[6]School of Earth Sciences and Engineering, Nanjing University, Nanjing 210023, China
[7]National Institute of Information and Communications Technology, Tokyo Japan
*presenting author: ueno.y.ac@m.titech.ac.jp

Sulfate oxygen ($\delta^{18}O$ and $\Delta^{17}O$) is a promising tracer for the redox evolution of the Earth, because atmospheric $O_2$ is incorporated into sulfate through the oxidative weathering of continental sulfides [1,2]. However, atmospheric isotopic signatures in sulfate can be erased via microbial sulfate reduction and re-oxidation processes [3]. Therefore, it is critical to evaluate the degree of preservation for the oxygen isotope signals in sulfate independently. We have developed a novel method based on the partial fluorination of sulfate into $SO_2F_2$ and subsequent analysis by means of high-mass-resolution gas source isotope ratio mass spectrometry of $SO_2F_2$. This approach allows us to simultaneously determine $\delta^{34}S$ and $\delta^{18}O$ values, in addition to measuring the $^{34}S$-$^{18}O$ clumping in sulfate ($\Delta^{34}S^{18}O$), which may provide new insights into Earth's sulfur cycle. Applications of this method to modern sulfate reveals that $\Delta^{34}S^{18}O$ value of modern seawater is homogeneous and is generally lower than river water sulfate, but is higher than those from volcanic lakes. The high $\Delta^{34}S^{18}O$ of river water sulfate has been reproduced experimentally by pyrite oxidation in the laboratory, even though the mechanisms require further understanding. On the other hand, microbial culturing experiments have demonstrated that high $\Delta^{34}S^{18}O$ signals can be erased via microbial sulfate reduction, which facilitates oxygen isotope exchange between sulfate and water. Therefore, measurement of $\Delta^{34}S^{18}O$ can be useful to constrain continental sulfate inputs into the ocean and subsequent microbial activity. Furthermore, we have measured Precambrian sulfates and found an interesting negative correlation between sulfate $\Delta^{34}S^{18}O$ and $\Delta^{17}O$, particularly in mid-Proterozoic samples. Analysis of $\Delta^{34}S^{18}O$ supports an atmospheric origin of the highly negative $\Delta^{17}O$ observed in mid-Proterozoic lacustrine sequences [1]. Our findings suggest that $\Delta^{34}S^{18}O$ analyses will likely provide a new insights into Earth's modern and ancient sulfur cycling and thereby the redox evolution of the Earth.

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### Invited: Interpreting triple-oxygen isotope compositions in the geologic sulfur cycle

*Jordon D. Hemingway[1,*], Anna Waldeck[2], Haley Olson[2], Kevin M. Sutherland[2], Weiqi Yao[2], David T. Johnston[2], Alexandra V. Turchyn[3], Edward T. Tipper[3], Mike Bickle[3], Aaron Bufe[4], Niels Hovius[4], Adina Paytan[5]*

[1]Geological Institute, ETH Zurich, Zurich, Switzerland
[2]Department of Earth and Planetary Sciences, Harvard University, Cambridge, USA
[3]Department of Earth Sciences, Cambridge University, Cambridge, UK
[4]GFZ German Research Center for Geosciences, Potsdam, Germany
[5]Department of Earth and Planetary Sciences, University of California at Santa Cruz, Santa Cruz, USA
*presenting author: jordon.hemingway@erdw.ethz.ch

Oxidation of the iron-sulfide mineral pyrite ($FeS_2$) during weathering forms sulfate ($SO_4^{2-}$), increases atmospheric carbon dioxide levels ($pCO_2$), and decreases oxygen levels ($pO_2$). This process represents a major control on Earth-surface redox state over geologic timescales. In addition to regulating atmospheric composition, the triple-oxygen isotope content ($\delta^{18}O$ and $\Delta'^{17}O$) of pyrite weathering-derived sulfate is thought to be a direct proxy for $pO_2/pCO_2$ due to the incorporation of anomalously $^{17}O$-depleted $O_2$ signals. Triple-oxygen isotope measurements of evaporites, barite, and carbonate-associated sulfate are thus becoming a common method to reconstruct $pO_2/pCO_2$ throughout Earth history. Despite this importance, the electrochemical mechanism of pyrite oxidation—including its impact on sulfate oxygen isotopes—remains largely unconstrained, hindering our ability to interpret the triple-oxygen isotope compositions of geologically preserved sulfate.

Here, I will discuss recent efforts to better constrain the mechanism and triple-oxygen isotope consequences of pyrite oxidation. Specifically, I will present: (i) sulfoxyanion isotope fractionation factor estimates derived using quantum chemical simulations, (ii) measurements from modern weathering environments draining pyrite-rich lithologies, and (iii) a new seawater sulfate $\Delta'^{17}O$ record for the Cenozoic and Cretaceous. Combined, these results imply a pyrite oxidation mechanism that incorporates an anomalously $^{17}O$-enriched signal—likely from reactive oxygen species such as hydrogen peroxide—but is overprinted by mass-dependent processes during fluvial transport and marine recycling. I will discuss how these results update our interpretation of geologic archives, focusing particularly on the (in)ability of sulfate oxygen isotopes to inform past atmospheric $pO_2$ and $pCO_2$ levels.

## Oral: Compound-specific isotope analysis on phylogenetically specific molecular fossils as a tool to deconvolve the stable carbon isotope record of the deep time

*I. Bobrovskiy[1,*], Yuta Isaji [2], Nanako O. Ogawa[2], Naohiko Ohkouchi[2], Jochen J. Brocks[3], Alex Sessions[4]*

[1]GFZ-Potsdam, Potsdam, Germany
[2]Biogeochemistry Research Center, Japan Agency for Marine-Earth Science and Technology, Yokosuka, Japan
[3]Research School of Earth Sciences, The Australian National University, Canberra, Australia
[4]Division of Geological and Planetary Sciences, California Institute of Technology, Pasadena, USA
*presenting author: ilya.bobrovskiy@gfz-potsdam.de

The stable carbon isotope record of carbonate minerals and organic matter of ancient rocks has long been used to obtain information about the carbon cycle in the oceans that existed hundreds of millions of years ago. The record documents large-scale carbon cycle perturbations as carbon isotope excursions – fluctuations from an expected standard value.

The largest of these excursions are found in Neoproterozoic deposits (1000 – 538 million years old). These excursions are extremely well studied as they may reflect global processes linked to the rise of complex life and the appearance and diversification of animals [1-4]. Yet, the origin of each Neoproterozoic isotope excursions remains unclear [5, 6]. The main reason for this is the large number of effects that influence the isotopic signals: carbonates might form not only in the water, but also within sediments during diagenesis, and the bulk organic matter may experience heterotrophic reworking or contain a large proportion of non-photosynthesizing organisms. Thus, both carbonates and organic matter may not reflect the isotopic composition of the water column and the atmosphere.

A potential approach to resolving the origin of Neoproterozoic carbon isotope fluctuations is to look at how they are reflected in the organic matter of various groups of primary producers. Compound-specific isotope analysis on molecular fossils, which are remains of biomolecules that are potentially stable in rocks for endless time, is an established tool for reconstructing ancient biogeochemical processes [7]. In addition to measuring $\delta^{13}C$ and $\delta^{15}N$ of individual porphyrins (geological remains of chlorophyll) [8, 9], we have expanded this approach to molecular fossils that are specific to certain groups of organisms, including steranes (remains of eukaryotic sterols), and hopanes (remains of bacterial hopanols). Even though steranes and hopanes in ancient rocks are only minor constituents of complex mixtures of molecules, a combination of preparatory HPLC steps allowed us to purify these compounds for further EA-IRMS measurements. Using one of the terminal Neoproterozoic excursions as an example, we show that such approach can pull apart not only the original and diagenetic isotopic signals, but also the composition of organisms that occupied different ecospaces within a marine paleobasin, allowing us to pinpoint the origin of the excursion that we studied.

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## Oral: Solar controls of radioactive sulfur isotopes

*M. Lin[1] & M.H. Thiemens[2*]*

[1]State Key Laboratory of Isotope Geochemistry, Guangzhou Institute of Geochemistry, Guangzhou, China
[2]Department of Chemistry and Biochemistry, University of California San Diego, La Jolla, United States
*presenting author: mthiemens@ucsd.edu

Radiosulfur ($^{35}S$; half-life: 87.4 days) is a cosmogenic isotope predominately produced in the stratosphere via the spallation of $^{40}Ar$ induced by high-energy cosmic rays. Recent combined measurements of all five sulfur isotopes ($\delta^{34}S$, $\Delta^{33}S$, $\Delta^{36}S$ and cosmogenic $^{35}S$) in modern aerosol samples provide new insights into the origin of sulfur mass-independent isotope fractionation [1]. Given the altitude-dependency of $^{35}S$ production and abundance, we preliminarily argued that the observed positive correlation between $^{35}S$ and $\Delta^{33}S$ indicated an altitude-dependent production of $\Delta^{33}S$ in the modern atmosphere, likely linked to stratospheric $SO_2$ photolysis [1]. We note that the production and abundance of $^{35}S$ at a global scale may also depend on other factors such as solar activity. Indeed, the reconstruction of past solar activity or high-energy events of our space environment is mainly based on long-lived cosmogenic radionuclides such as $^{14}C$ and $^{10}Be$. In this work, we compile a long-term dataset of atmosphere $^{35}S$ measurements made at La Jolla from 2009 to 2016 to test our hypothesis. We observe a clear inverse relationship between sunspot numbers and $^{35}S$

activities in Solar Cycle 24, which is consistent with a solar modulation effect in the atmospheric production of cosmogenic radionuclides. We also observe spikes of $^{35}S$ specific activities at the peak of 2015/2016 El Niño (the strongest and longest one in the Anthropocene), which may indicate significant changes in regional atmospheric circulation that controls $^{35}S$ budgets. Although a more quantitative conclusion must await for a more comprehensive dataset and more detailed time-series and climatology analysis, our findings highlight that solar and potentially climate controls must be considered in future $^{35}S$ studies. In addition, the reconstruction of past solar activity or high-energy events of our space environment using long-lived cosmogenic radionuclides is limited by our understanding of cosmogenic radionuclide production, transformation, and transport in the atmosphere. $^{35}S$ provides additional insights due to its ideal half-life, extensively studied atmospheric chemistry (gas and solid) and ubiquitous nature. Incorporating $^{35}S$ into a universal cosmogenic radionuclide model as an independent parameter facilitates better modeling of production and transport of other long-lived radionuclides with different atmospheric chemistries used for reconstructing past astronomical, geomagnetic and climatic events.

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## POSTER P39

### P39 SHRIMP-SI Quadruple Sulfur Isotopic Compositions of Two Generations of Pyrite in the 3.49 Ga Dresser Formation

*L. Liu[1,3,*], T.R. Ireland[1,2] & P. Holden[1]*

[1]Research School of Earth Sciences, The Australian National University, Canberra, Australia
[2]School of Earth and Environmental Sciences, The University of Queensland, Brisbane, Australia
[3]Department of Earth Sciences, University of Toronto, Toronto, Canada
*presenting author: emmaliu1016@gmail.com

The 3.49 Ga Dresser Fm. is well known for preserving evidence of Earth's earliest life. Microbial sulfate reduction (MSR) has been invoked based on bulk quadruple sulfur isotopic compositions of pyrite and barite (negative $\delta^{34}S$ and $\Delta^{33}S$)[1,2], while elemental sulfur disproportionation has been proposed in light of in-situ triple sulfur isotopic compositions of pyrite (negative $\delta^{34}S$ and positive $\Delta^{33}S$)[3]. Two recent SIMS studies have confirmed the $\Delta^{33}S$- and $\delta^{34}S$-positive and $\Delta^{33}S$- and $\delta^{34}S$-negative pyrite groups respectively[4,5]. However, how the two groups are linked has not been investigated. Based on the complex hydrothermal system forming the chert-barite unit of Dresser Fm., the pyrite are most likely multi-generation. Therefore, this study first established the pyrite generation combining NaOCl-etching and BSE imaging, and then measured the quadruple and triple sulfur isotopic compositions of pyrite grains and growth zones *in situ* in high resolution and precision using SHRIMP-SI. The core-rim internal textures and sulfur isotopic compositions indicate two generations of pyrite: G1 with positive $\Delta^{33}S$, G2 with negative $\Delta^{33}S$ and $\delta^{34}S$. G1 and barite plausibly represent the sequestered $\Delta^{33}S$-positive and $\Delta^{33}S$-negative photochemical products, respectively. G2 were formed via sulfide pathway, with $S^{2-}$ derived from sulfate reduction and magmatic $H_2S/S^{2-}/HS^-$. The $\delta^{34}S$-$\Delta^{33}S$-$\Delta^{36}S$ systematics suggests an abiologic origin for G1, and thermochemical sulfate reduction and possible (minor) MSR origin for G2.

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## Keynote: A model for isotopologue signatures of microbial methane to improve source attributions

*Shuhei Ono[1,*], Jeemin Rhim[2] & Ellen Lalk[1]*

[1]Department of Earth, Atmospheric, and Planetary Sciences, Massachusetts Institute of Technology, Cambridge, MA 02139
[2]Department of Earth Sciences, Dartmouth College, Hanover, NH 03755
*presenting author: sono@mit.edu

Methane is the second most significant long-lived greenhouse gas that contributes about a quarter of radiative forcing, but significant uncertainty exists in the atmospheric methane budget. Isotope ratios have been used to better constrain the methane budget, but studies reported conflicting results (e.g., Turner et al., 2017). A major limitation for the isotope budget is uncertainty in isotope signals of source methane.

This talk will discuss how precisely the source isotopologue signals ($^{13}CH_4$, $^{12}CH_3D$, $^{13}CH_3D$, and $^{12}CH_2D_2$) must be constrained to reduce the uncertainty in methane budgets. Then, we will present our recent efforts toward better parameterizing the source isotopologue signals by the model of microbial hydrogenotrophic methanogenesis (Ono et al., 2022). Based on thermodynamic and enzyme kinetic data, the model estimates the reversibility of 8 reactions from predicted *in vivo* concentrations of 17 metabolites and cofactors. The model explains the range of $^{13}C/^{12}C$ fractionation up to 80‰ between $CH_4$ and $CO_2$, increasing in magnitudes while decreasing in $H_2$ partial pressures. Relatively constant D/H fractionations of 300±40‰ between methane and water can be explained when methane is produced from three near-equilibrium H in methyl-coenzyme M with the addition of one kinetic D-depleted H during the last step of methanogenesis.

We apply our model to time-series measurements of methane isotopologue ratios from a local lake (Upper Mystic Lake in Arlington, MA) and a global dataset of $^{13}C/^{12}C$ and D/H ratios of methane from lakes and wetlands, towards improved parameterization of the methane isotopologue source signals.

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## Invited: Constraining global $N_2O$ budgets with decadal trends of multiple isotope signatures

*L. Yu[1,2*], E. Harris[3], S. Henne[1], J. Mohn[1]*

[1]Laboratory for Air Pollution & Environmental Technology, Empa, Swiss Federal Laboratories for Materials Science and Technology, Ueberlandstr. 129, CH-8600 Duebendorf, Switzerland.
[2]Institute of Environment and Ecology, Tsinghua Shenzhen International Graduate School, Tsinghua University, Shenzhen 518055, China
[3]Swiss Data Science Center, ETH Zürich, 8006 Zürich, Switzerland
*presenting author: longfei.yu@sz.tsinghua.edu.cn

Nitrous oxide ($N_2O$) is an important greenhouse gas and contributes to stratospheric ozone depletion. In recent decades, the mean $N_2O$ mixing ratio in the atmosphere has shown a steady growth by 1 ppb $a^{-1}$, which has raised worldwide concerns. Although agricultural $N_2O$ emission is believed to be the major contributor to atmospheric $N_2O$ growth, current understanding of global $N_2O$ sources is not enough to explain variabilities in long-term $N_2O$ trends and across individual monitoring sites.

Stable isotope signatures of $N_2O$ are effective in constraining atmospheric $N_2O$ sources. Recently, with a developed quantum cascade laser spectroscopy (QCLAS) system, we have achieved high-precision measurements of atmospheric $N_2O$ isotope signatures since 1980s from remote sites in both hemispheres (NH: East Greenland; SH: Cape Grim). Our results showed that the declines of $\delta^{15}N^{bulk}$ and $\delta^{18}O$ were associated with increase of $N_2O$ mixing ratio. $N_2O$ mixing ratios in the NH were on average higher by 1.5 ppb than in the SH while $\delta^{15}N^{bulk}$ was lower by 0.2‰, pointing to the stronger anthropogenic influences in the NH. The $\delta^{15}N^{sp}$ of $N_2O$ in the NH showed a significant decline along time, but such trend was insignificant for SH. We also looked into the seasonal patterns of isotope signatures based on a statistical approach, finding that $\delta^{15}N$ and $\delta^{15}N^{sp}$ varied accordingly with $N_2O$ mixing ratio in the SH, likely due to stratosphere-troposphere exchange. But in the NH, $\delta^{15}N^{sp}$ showed an opposite seasonal trend from $\delta^{15}N^{bulk}$. These findings suggest that anthropogenic sources may exert divergent impacts on $\delta^{15}N^{bulk}$ and $\delta^{15}N^{sp}$. Further, we used an atmosphere chemical transport model to quantify isotope signatures for the anthropogenic sources, indicating that both $\delta^{15}N^{bulk}$ and $\delta^{15}N^{sp}$ of anthropogenic sources may have decreased in the recent decades. Our work proves that multiple isotope signatures of $N_2O$ are highly valuable in constraining long-term $N_2O$ trends, although current estimate may still have limitations due to spatiotemporal coverage in the dataset.

## Announcement: Research Gate Discussion Group: Isotopic tools to study $N_2O$ in soil and aquatic systems

*A. Matson[1,*], E. Harris[2,*], Dominika Lewicka-Szczebak[3], Caroline Buchen-Tschiskale[1], Lena Rohe[1], Reinhard Well[1]*

[1]Climate-Smart Agriculture, Thünen Institute, Braunschweig, Germany
[2]Swiss Data Science Centre, ETH Zürich, Switzerland
[3]Laboratory of Isotope Geology and Geoecology, University of Wrocław, Poland
*Contact: amanda.matson@thuenen.de, eliza.harris@sdsc.ethz.ch

Isotopic methods using natural abundance or labelling techniques are indispensable to identify and quantify $N_2O$ production and consumption pathways. However, all methods are subject to limitations and possible biases, as well as underlying assumptions, which are not always fulfilled and are often difficult to verify. Moreover, stable isotope analysis of $N_2O$, $N_2$, and precursor compounds is challenging, particularly regarding calibration, instrumentation, and intercomparability between laboratories. Finally, methods that elucidate process dynamics by combining measurements from different compartments – such as soil air, soil solutes, and surface flux data – offer new possibilities, but limitations and assumptions are currently not well understood.

Despite these challenges, interest in the use of stable isotope methods to understand $N_2O$ production and consumption pathways is growing, and the number of publications per year is rising steadily. Communication channels for the discussion of methodologies – such as conferences, workshops, reviews and publications – are limited in access and continuity, and allow only for slow progress. This motivated us to start an open discussion group to enable rapid exchange on this topic.

We want to offer a forum to address four general areas:

- *Experimental Methods*: Discuss technical issues and present ideas for new methods
- *Analytical Methods:* Share data on performance and comparison of methods
- *Data analysis*: Discuss advantages, disadvantages, limitations, possible biases, and solutions relating to existing data analysis methods
- *Modelling*: Discuss modelling approaches to evaluate experimental data

We invite participation from interested researchers via: i) following the discussion group to stay up to date, ii) adding comments and ideas to ongoing discussions in the Project Log, and iii) contacting us with suggestions for new discussion topics.

We hope to see you soon!
https://www.researchgate.net/project/Discussion-on-isotopic-tools-to-study-soil-N2O

## Oral: Stable isotope reference materials for climate change monitoring

*R. Hill-Pearce[1,*], E. Mussell Webber[1], A. Hillier[1], C. Rennick[1], T. Arnold[1], D. Worton[1], P. Brewer[1], P. Steur[2], H. Meijer[2], F. Rolle [3], M. Sega [3], T. Tarhan [4], A. Boztepe[4], S. Persijn[5], H. Moossen[6], J. Mohn[7].*

[1]National Physical Laboratory (NPL), London, U.K.
[2]University of Groningen (RUG), Groningen, Netherlands
[3]Istituto Nazionale di Ricerca Metrologica (INRIM), Torino, Italy
[4]Scientific and Technological Research Council of Turkey (TUBITAK), Ankara, Turkey
[5]Dutch National Metrology Institute (VSL), Delft, Netherlands
[6]Max Planck Institute BGC stable isotope laboratory (BGC-IsoLab), JENA, Germany
[7]Swiss Federal Laboratories for Materials Science and Technology (EMPA), Zurich, Switzerland
*presenting author: ruth.pearce@npl.co.uk

To support governments verifying emissions and demonstrating national reduction targets, it is crucial to discriminate between the natural and various manmade sources of greenhouse gases. This requires accurate measurements of baseline amount fractions and contributions resulting from emission events. Separating man-made emissions from measured greenhouse gas amount fractions is challenging, requiring information on the isotopic composition.

Work [1] is presented towards the production of stable isotope reference materials of $CO_2$, $CH_4$ and $N_2O$, aiming at amount fraction and isotopic uncertanties meeting the world meteorological organization global atmosphere watch programme's compatibility goals. Reference materials of these most important greenhouse gases are prepared at a range of relevant amount fractions and isotope ratios for climate monitoring, with traceability to the SI, and existing scales including VPDB. The high-pressure gas reference materials are prepared at a number of national metrology institutes to ensure comparability and wide availability so as to fill the existing traceability gap for stable isotope reference materials. Particular importance is given to the matrix of the greenhouse gas reference materials so that it is tailored to the analytical technique used for monitoring. The trace amount fractions of the greenhouse gases are certified in the matrix and the effect of the trace gases on the uncertainty of the amount fraction and isotope ratio of the reference materials is calculated. The stability of the reference materials is also presented. Using the $CO_2$ reference materials developed, calibration techniques are demonstrated using two different spectroscopic techniques to certify the isotope ratio of an unknown gas sample with results compared to isotope ratio mass spectroscopy.

[1] The work presented forms part of the EMPIR project 19ENV05 STELLAR which has received funding from the EMPIR programme co-financed by the participating states and from the European Union's Horizon 2020 research and innovation programme.

## Oral: Using isotopes to understand N-limitation in dry lands: Unexpected N loss pathways in systems with too little N

*A. Peter M. Homyak[1,*]*

[1]Department of Environmental Sciences, University of California, Riverside
*presenting author: phomyak@ucr.edu

Moisture is a key factor governing soil nitrogen (N) biogeochemistry; it controls microbial activity and, therefore, the cycling of N. Ongoing climate change is altering precipitation regimes, increasing the frequency and intensity of droughts across regions with implications for ecosystem N retention. Disentangling how drought can alter the balance between ecosystem N retention and loss is challenging because both biotic and abiotic processes interact to control N availability and produce unexpected results.

Using drylands in southern California, I will show how drought affects soil N cycling given than: i) these drylands can experience >6 months without rain, facilitating drought studies; and 2) biotic–abiotic interactions can be interrogated in contrasting soils developing under the influence of plants (soils known as "islands of fertility") against soils developing in the bare interspaces between plants. Focusing on soil microscale processes, I'll show how N retention and loss trade off as dry conditions intensify using isotopes of both nitric oxide (NO) and nitrous oxide ($N_2O$) coupled with inhibition assays. In particular, I show how soil $N_2O$ emissions were undetectable from the interspaces between plants, but exceeded 1000 ng $N\text{-}N_2O$ $m^{-1}$ $s^{-1}$ in islands of fertility, rivaling emissions observed in global hotspots like tropical forests and temperate agroecosystems. Despite the hot and dry conditions, isotope tracers indicated $NO_3^-$ was reduced to $N_2O$ within 15 minutes of wetting dry desert soils and governed $N_2O$ emissions. In contrast to $NO_3^-$, $^{15}N\text{-}NH_4^+$ was not incorporated in $N_2O$, suggesting nitrifiers were not

producing $N_2O$ in these dry desert soils. Together with these findings, we found that $\delta^{15}N^{SP}$-$N_2O$ values averaged 22‰, suggesting chemodenitrification produced $N_2O$, but with some $\delta^{15}N^{SP}$-$N_2O$ values (~30-40 ‰) also suggesting fungal denitrification may be important. Overall, drought stress breaks down the transfer of metabolites between microorganisms, allowing "metabolic handoffs" to be lost from soils through processes operating outside of the control of biological N demand.

### Oral: Evaluation of six years of continuous $\delta^{13}CH_4$ measurements in Heidelberg, Germany

*A. Hoheisel[1,*] & M. Schmidt [1]*

[1]Institute of Environmental Physics, Heidelberg University, Heidelberg, Germany
*presenting author: antje.hoheisel@iup.uni-heidelberg.de

Recent instrumental developments in measurement techniques, such as cavity ring-down spectroscopy (CRDS), have made it possible to perform continuous in situ isotopic analyses of $\delta^{13}C$-$CH_4$ with high temporal resolution over several years.

At an urban station in Heidelberg, south-western Germany, the $CH_4$ mole fraction and its $^{13}C/^{12}C$ ratio in ambient air have been measured with a CRDS G2201-i analyser between 2014 and 2020.

These six-year atmospheric $\delta^{13}C$-$CH_4$ measurements are analysed for seasonal and long-term variations in regional and local $CH_4$ sources.

Therefore, different approaches based on the Keeling/Miller-Tans method were tested to determine the composition of $CH_4$ emissions in the catchment area of Heidelberg. The isotopic source signatures of methane vary between -77‰ and -30‰, with a mean value of (-52.5± 0.3) ‰. Within the last six years no significant trend and thus no significant change of the source composition in the catchment area of Heidelberg, has been detected. An annual cycle of the isotopic source mix is observed, with more depleted values (-56‰) in summer and more enriched values (-50‰) in winter, indicating a stronger biogenic $CH_4$ contribution in summer and stronger thermogenic (e.g. natural gas) emissions in winter. These isotopic source signature results, determined from atmospheric measurements, were then compared to regional emission inventories.

### Sponsored: Committed to Science Stable isotope analysis with CRDS – practical considerations and use cases

*R. W. van Zwieten[1,*], M. E. G. Hofmann[1]*

[1]Picarro Inc, Santa Clara, USA
*presenting author: rvzwieten@picarro.com

This presentation will discuss the analytical isotopic equipment options from Picarro and show in detail the latest improvements to the Picarro isotopic water system, enabling 2 additional measurements modes with unparalleled speed and precision.

In addition, we are going to discuss a selection of case studies for each of our main isotopic analyzers, highlighting the unique advantages of the CRDS platform and products.

Please find the improved technical specifications for the isotopic water system in the attached table.

L2130-*i* Technical Specifications

| L2130-*i* Liquid Specifications (with A0211 and A0325) | Specifications | Typical Performance* | |
|---|---|---|---|
| | | Standard mode | Express mode |
| Precision (1σ) | Guaranteed: $\delta^{18}O$ – 0.025‰ $\delta D$ – 0.1‰ | $\delta^{18}O$ – 0.010‰ $\delta D$ – 0.05‰ | $\delta^{18}O$ – 0.015‰ $\delta D$ – 0.05‰ |
| Zero Drift (24 hour) | Guaranteed: $\delta^{18}O$ – 0.2‰ $\delta D$ – 0.8‰ | $\delta^{18}O$ – 0.059‰ $\delta D$ – 0.30‰ | $\delta^{18}O$ – 0.100‰ $\delta D$ – 0.43‰ |
| Throughput (6 injections for each sample; for Express mode, 10 injections per sample) | 54 minutes per sample / 27 samples per day | 54 minutes per sample / 27 samples per day | 29 minutes per sample / 50 samples per day |
| Memory | Guaranteed: (after the 3rd injection) $\delta^{18}O$ – 99% $\delta D$ – 98% | (after the 3rd injection) $\delta^{18}O$ – 99% $\delta D$ – 98% | (after 15 min) $\delta^{18}O$ – 99% $\delta D$ – 98% |
| Total Dissolved Solids | <200 g/kg | N/A | N/A |

** Typical performance is defined as the median of testing results from a number of sequentially built L2130-i analyzers. Results available upon request.*

### Oral: Polyisotopic carbon dioxide ratios at the coastal Weybourne Atmospheric Observatory (Norfolk, UK)

*J. Kaiser[1,*], G. Forster[1], P. Pickers[1], A. Marca[1] A. Manning[1], L. Fleming[1]*

[1]Centre for Ocean and Atmospheric Sciences, School of Environmental Sciences, University of East Anglia, Norwich, United Kingdom
*presenting author: j.kaiser@uea.ac.uk

Polyisotopic carbon dioxide ($CO_2$) ratios are relatively new tools that can help improve our understanding of atmospheric greenhouse gas cycles. We define polyisotopic elements as elements with more than one minor isotope (e.g., $^{17}O$ and $^{18}O$ next to the most abundant $^{16}O$) and contrast them with polyisotopologues as compounds with two rare isotopes in the same molecule (e.g., $^{13}C^{18}O^{16}O$). Our recently funded research project POLYGRAM (POLYisotopologues of GReenhouse gases: Analysis and Modelling) will make targeted observations of both kinds of polyisotopic species across a small global sampling network, to quantify and understand their meridional and temporal variations, as well as characterise source fingerprints.

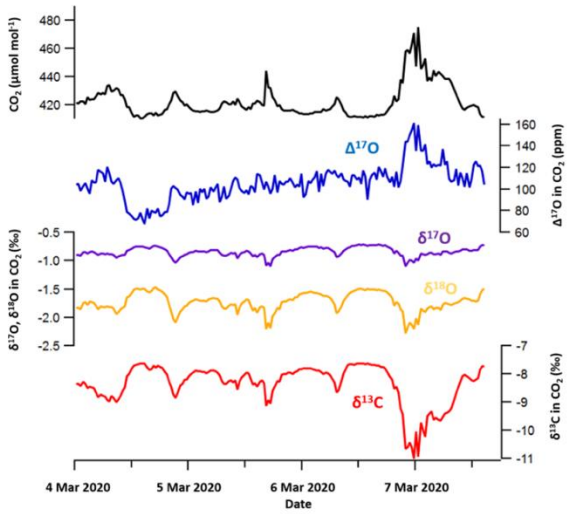


Figure: TILDAS $\delta^{13}C$, $\delta^{18}O$ and $\Delta^{17}O$ measurements (uncalibrated) show excellent precision to resolve fossil fuel and respiration signals in $CO_2$ fluxes.

In this presentation, we will focus on simultaneous $^{13}C/^{12}C$ and oxygen triple isotope ($^{16}O$, $^{17}O$, $^{18}O$) ratio measurements with an Aerodyne dual-laser tuneable infrared laser direct absorption

spectrometry (TILDAS) instrument at the University of East Anglia's Weybourne Atmospheric Observatory at the north Norfolk coast. These quasi-continuous *in situ* atmospheric measurements of the $\Delta^{17}O$ signature of $CO_2$, or "$^{17}O$ excess" will allow quantifying and assessing the terrestrial and oceanic processes driving $CO_2$ fluxes. Initial results gave a measurement precision of 4 ppm for $\Delta^{17}O$ aggregated over 28 minute-intervals (see figure below), in good agreement with the Allan deviation of 5 ppm for the same measurement interval.

### Oral: Isotopic measurements of carbonyl sulfide (COS): from biosphere to stratosphere

*S.L. Baartman[1,*], M.C. Krol[1,2], T. Röckmann[1], M.E. Popa[1], HEMERA 2021 team**, Plant chamber team****

[1]Insitute for Marine and Atmospheric Research Utrecht (IMAU), Utrecht University, Utrecht, the Netherlands
[2]Department of Meteorology and Air Quality, Wageningen University and Research Center, Wageningen, the Netherlands
*presenting author: s.l.baartman@uu.nl

Carbonyl sulfide (COS) is the most abundant sulfur-containing trace gas in the atmosphere, with an average mixing ratio of 500 parts per trillion (ppt). It has a relatively long lifetime of about 2 years, which permits it to travel into the stratosphere. There, it likely plays an important role in the formation of stratospheric sulfur aerosols (SSA), which have a cooling effect on the Earth's climate. Furthermore, during photosynthetic uptake by plants, COS follows the same diffusion pathway as $CO_2$, but is not emitted during respiration, and therefore it can be used to estimate gross primary production (GPP). However, significant uncertainties still exist in the sources, sinks and global cycling of COS, which need to be overcome. Isotopic measurements of COS could be a promising tool for constraining the COS budget, as well as for investigating its role in the formation of stratospheric sulfur aerosols.

At Utrecht University, within the framework of the COS-OCS project, we developed a measurement system using GC-IRMS that can measure $\delta^{33}S$ and $\delta^{34}S$ from $S^+$ fragment ions of COS from small air samples of 2 to 5 L. This system was recently expanded to also measure $\delta^{13}C$ using the $CO^+$ fragment ions of COS, which has never been measured before. Various samples have been measured using this method, ranging from a time-series of ambient air in Utrecht, traffic air samples collected in a highway tunnel, plant chamber experiments using C3 and C4 plants and samples collected in the stratosphere during the 2021 HEMERA campaign. Here we will present some of the (preliminary) measurement results, focusing on the plant chamber experiments and the stratospheric measurements.

** HEMERA 2021 Twin Gondola Team: A. Engel, H. Chen, S. M. A. C. van Heuven, T. Schuck, T. Wagenhäuser, A. Zanchetta, T. Keber, J. Laube, M. Ghysels-Dubois, N. Amarouche, K. Meixner, M. E. Popa

*** Plant chamber team: M. Wassenaar, L. M. J. Kooijmans, S. Driever, A. Cho, H. Chen, S. M. A. C. van Heuven, M. Krol, L. Mossink, M. E. Popa

### Oral: Atmospheric sulfate of prehuman time in inland northern China

*Yongbo Peng[1,2], Shohei Hattori[1,2], Pengfei Zuo[4], Haoran Ma[1,2], Huiming Bao[1,2*]*

[1]International Center for Isotope Effects Research, Nanjing University, Nanjing 210023, China
[2]School of Earth Sciences and Engineering, Nanjing University, Nanjing 210023, China
[3]School of Resources and Environment, Henan Polytechnic University, Jiaozuo 454000, China
*presenting author: bao@nju.edu.cn

Information on atmospheric sulfate in the prehuman time is essential to assessing uncertainties in global climate models. For much of the continental interiors, which are presently overwhelmed by anthropogenic atmospheric sulfate, the origin and nature of prehuman atmospheric sulfate remain pure speculation, which hampers our ability to quantify the multi-faceted impacts of anthropogenic perturbation on climate. Here we report the presence of prehuman atmospheric sulfate in weathered carbonate outcrops in arid to semi-arid regions today. The combination of sequential leaching and multiple isotope measurements allowed us to effectively distinguish sulfates of different origins being retained in carbonate outcrops on land. Our data from the interior of northern China show that one of the sulfates consistently has unusually positive $^{17}O$ anomaly ($\Delta^{17}O$ at ~+1.8‰) and characteristic $\delta^{34}S$ (~10‰) and $\delta^{18}O$ (4‰ to 8‰). We interpret this sulfate endmember to be an integrated atmospheric sulfate in much of the prehuman continental interiors of northern China, suggesting that sulfate aerosol chemistry over the continents in prehumen time resembled that over the oceans today, at least in northern China. The observation of higher $\Delta^{17}O$ and its interpretation are consistent with the result of a GEOS-Chem model implemented in $\Delta^{17}O$ calculation for year 1750 with negligible anthropogenic $SO_2$ emission. The same model also predicts spatial variations for $\Delta^{17}O$ of sulfate in prehuman time than today in many different regions, which can be tested by sampling carbonate outcrops worldwide. Our discovery of an unexplored archive on prehuman atmospheric sulfur chemistry will help constraining anthropogenic impacts and reducing uncertainties in global climate predictions.

## POSTERS P40 – P44

### P40 Measuring the stable isotopic composition of pure $CO_2$ samples on a dual-laser absorption spectrometer using a back-dilution method to obtain dry ambient conditions

*H. A. Scheeren[1,*], P. M. Steur[1], K. Chaniyara[1], H. A. J. Meijer[1], W. Peters[1, 2]*

[1]Center for Isotope Research (CIO), University of Groningen, Groningen, the Netherlands
[2]Meteorology and Air Quality Group, Wageningen University & Research Centre, Wageningen, the Netherlands
*presenting author: h.a.scheeren@rug.nl

We present a reliable and stable back-dilution method for pure $CO_2$ samples allowing for the analysis on laser absorption spectrometer systems for measurement of stable isotopes of

$CO_2$. We use a novel dual-laser infrared absorption spectrometer (Aerodyne Inc.) developed for the simultaneous measurement of $\delta^{13}C$, $\delta^{18}O$, and $\delta^{17}O$ of $CO_2$ in dry ambient air referred to as the SICAS (Stable Isotope of $CO_2$ Absorption Spectrometer) (Steur et al., 2021). The back-dilution method allows for scale intercomparisons between the SICAS and our Isotope Ratio Mass Spectrometry (IRMS) systems using pure $CO_2$ isotopic reference materials. In addition, it offers an alternative measurement method to IRMS for isotopic analysis of pure $CO_2$ samples extracted from ambient air.

In brief, the back-dilution method has the following steps: 1) The $CO_2$ sample from the storage container (e.g flame sealed tube) is released and let through a water trap (dry ice/ethanol) into a calibrated volume using $LN_2$; 2) The pressure reading in the calibrated volume at room temperature then determines how much dilutor gas (dry ambient air) will be required to make the final concentration in the flask of 400 ± 25 ppm; 3) The sample is transferred into a freeze finger using $LN_2$ attached to an evacuated 0.85 L sample flask; 4) Finally, the sample is recombined with the dilutor air that has been scrubbed of $CO_2$ (using Ascarite) and $H_2O$ traces (using Magnesium Perchlorate) at atmospheric pressure and left undisturbed for at least 12 hours to allow for an homogeneous mixture. As such, the system is able to measure amounts of $CO_2$ from 0.45 mg and up corresponding to >375 ppm of $CO_2$ in 0.85 L of ambient air. Figure 1 depicts an image of the back-dilution system.

The back-dilution method is currently applied to handle a large amount of flame sealed tubes containing $CO_2$ extracted from ambient air samples collected during an extensive sampling program called ASICA (Airborne Stable Isotopes of Carbon from the Amazon) that took place over the Brazilian Amazon from early 2016 until end of 2018. Here we present the set-up and test results of our back-dilution system using reference materials in comparison to our IRMS, as well as first measurement results of a number of ASICA samples on the SICAS.

Figure 1: Image of the back-dilution system at the CIO-laboratory.

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## P41 A four-year record (2017-2021) of $\Delta^{17}O$ in atmospheric $CO_2$ from Lutjewad station (NL)

*P. M. Steur[1,*], H. A. Scheeren[1], G. Koren[2], G. A. Adnew[3], W. Peters[1,4], H. A .J. Meijer[1]*

[1]Center for Isotope Research (CIO), University of Groningen, Groningen, the Netherlands
[2]Copernicus Institute of Sustainable Development, Utrecht University, Utrecht, the Netherlands
[3]Institute for Marine and Atmospheric research Utrecht, Utrecht University, Utrecht, the Netherlands
[4]Meteorology and Air Quality Group, Wageningen University & Research, Wageningen, the Netherlands
*presenting author: p.m.steur@rug.nl

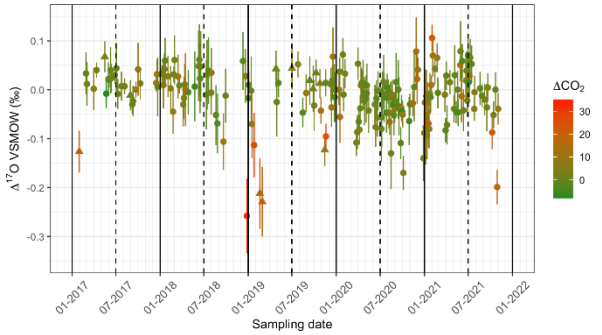


Figure: $\Delta^{17}O$ record from Lutjewad station. Colors indicate the deviation of $CO^{-}_2$ mole fractions from the background values.

We present a four-year (2017-2021) record of atmospheric $\Delta^{17}O$-$CO_2$ ($\Delta^{17}O = \ln(\delta^{17}O + 1) - \lambda * \ln(\delta^{18}O + 1)$) measurements from the Lutjewad atmospheric measurement station at the North coast of the Netherlands. Stable isotope measurements were conducted directly on dry air samples with a dual-laser absorption spectrometer, a novel technique for the simultaneous measurement of $\delta^{13}C$, $\delta^{18}O$ and $\delta^{17}O$ of $CO_2$. Samples were collected in flasks and measured at the University of Groningen with a mean final error of 0.03‰ for $\delta^{13}C$ and 0.04‰ for $\delta^{18}O$ and $\delta^{17}O$. From the measurements we deduced the $\Delta^{17}O$-$CO_2$ (mean final error of 0.04‰), a potential tracer for gross primary production. Elevated $\Delta^{17}O$ values originating from the stratosphere are eliminated when $CO_2$ equilibrates with water in plants (Hoag et al., 2005). We observe the expected declining values during the growing seasons in the years 2017, 2018 and 2020. A clear seasonal pattern is difficult to discern due to the complex signal of background air and local signals that are captured in the measurements. Peaks towards more negative values coincide with high $CO_2$ concentrations during winter months, indicating an $\Delta^{17}O$ signal dominated by the emissions from combustion processes. The record is compared with simulations from a 3-D global model describing the $\Delta^{17}O$ in atmospheric $CO_2$ (Koren et al., 2019).

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## P42 Comparison of laser sources and driver electronics for optical isotope ratio spectroscopy

*Mehr Fatima[1*], Thomas Hausmaninger[1], Ville Ulvila[1], Guillaume Genoud[1]*

[1]Optical Spectroscopy, National Metrology Institute VTT MIKES, Espoo, Finland.
*presenting author: mehr.fatima@vtt.fi

Carbon dioxide and methane emissions in the atmosphere have increased drastically overtime. It is crucial to monitor and reduce the greenhouse gases. This requires differentiation between anthropogenic and natural emission sources. The stable isotopes of carbon can be used for this since, different sources can have unique isotopic fingerprint in their emissions [1]. This calls out for increased detection sensitivity for carbon dioxide and methane isotopologues.

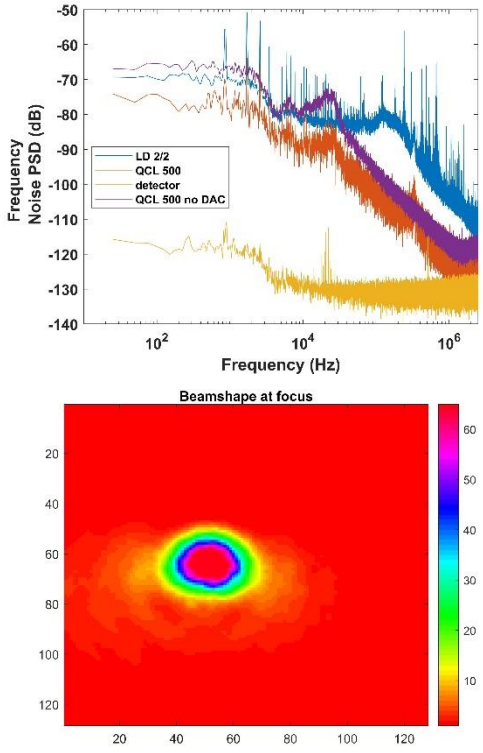


Fig. 1: The ICL beamshape at the focus is shown in the top figure. The figure on the bottom depicts the frequency noise for the different laser drivers and the detector noise.

Optical detection techniques have enabled low-cost and accurate measurements for isotope detection. In this work, we develop an optical isotope ratio spectrometer (OIRS) for carbon dioxide measurements. The main goal of the work is to identify and characterize the noise sources and increase the sensitivity of the system. OIRS-instruments are based on laser absorption spectroscopy (LAS) often in combination with multipass cells to enhance the signal strength. On short timescales the sensitivity of such systems is determined by the laser frequency and amplitude noise performance. However, on longer timescales the system performance is in general limited by background signals caused by optical interference between optical components of the system. In particular, multipass cells can give rise to significant background signals if the laser beam is not well collimated. Therefore, there are many tradeoffs between compactness of the optical setup, price and sensitivity. We have measured the noise performance of different commercially available lasers (QCL and ICL) with off-the shelf low-noise current drivers (see figure 1). Furthermore, we have compared different multipass cells, which include aerodyne cell, IRSweep cell etc. with simple collimation optics.

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## P43 Spacio-temporal distributions of atmospheric nitrous oxide and its isotopocules

*S. Toyoda[1,*], D. Goto[2], S. Morimoto[3], M. Sasakawa[4], T. Machida[4], Y. Tohjima[4], D. Worthy[5], & N. Yoshida[6,7]*

[1]School of Materials and Chemical Technology, Tokyo Institute of Technology, Yokohama, Japan
[2]Division of Advanced Research Promotion, National Institute of Polar Research, Tachikawa, Japan
[3]Center for Atmospheric and Oceanic Studies, Tohoku University, Sendai, Japan
[4]Earth System Division, National Institute for Environmental Studies, Tsukuba, Japan
[5]Climate Research Division, Environment and Climate Change Canada, Toronto, Canada
[6]Earth-Life Science Institute, Tokyo Institute of Technology, Tokyo, Japan
[7]Terahertz Research Center, National Institute of Information and Communications Technology, Koganei, Japan
*presenting author: toyoda.s.aa@m.titech.ac.jp

Nitrous oxide ($N_2O$) is one of the increasing greenhouse gases and is the most important stratospheric ozone-depleting gas emitted in the 21st century. Since secular trend of isotopocule ratios of atmospheric $N_2O$ can be used to deduce relative contribution from each source, several efforts have been made by measuring air trapped in the polar firn or analyzing air samples collected at monitoring stations. However, direct atmospheric measurements are reported for only a few stations in temperate region and Antarctica, and monitoring period is limited. Moreover, the north-to-south gradient of isotopocule ratios remains uncertain because published data by different laboratories cannot be compared directly due to calibration problems.

Here we present up to 23-year record of monthly or biweekly mixing ratio and isotopocule ratios of $N_2O$ obtained at three sites in the Northern Hemisphere: Hateruma, a southwestern island of Japan (24°N, 124°E) (since 1999), Novosibirsk in the western Siberia, Russia (55°N, 83°E) (since 2005), and Churchill, northern Canada (59°N, 94°W) (since 2011). We also analyzed surface air samples collected at Syowa station, Antarctica (69°S, 40°E) with 2–4 month interval in 1998–2020.

The bulk nitrogen isotope ratio ($\delta^{15}N^{bulk}$) is decreasing at similar rate (about $-0.04$‰ $yr^{-1}$) at the four sites while the $N_2O$ mixing

ratio are increasing (about 0.8 ppbv $yr^{-1}$). When compared at the same year, the value of $\delta^{15}N^{bulk}$ is about 0.2‰ higher in the southern hemisphere. The oxygen isotope ratio ($\delta^{18}O$) also shows decreasing trend with smaller rate at all the stations, but north-to-south gradient is not detectable with the precision of the analysis. The $^{15}N$-site preference in $N_2O$ (*SP*) does not show secular increasing nor decreasing trend, and north-to-south gradient is not detectable. Further analysis of isotopic signature of N/S sources with simple model approaches will be discussed.

### P44 Research Gate Discussion Group: Isotopic tools to study $N_2O$ in soil and aquatic systems

*A. Matson[1,*], E. Harris[2,*], Dominika Lewicka-Szczebak[3], Caroline Buchen-Tschiskale[1], Lena Rohe[1], Reinhard Well[1]*

[1]Climate-Smart Agriculture, Thünen Institute, Braunschweig, Germany
[2]Swiss Data Science Centre, ETH Zürich, Switzerland
[3]Laboratory of Isotope Geology and Geoecology, University of Wrocław, Poland
*Contact: amanda.matson@thuenen.de, eliza.harris@sdsc.ethz.ch

Isotopic methods using natural abundance or labelling techniques are indispensable to identify and quantify $N_2O$ production and consumption pathways. However, all methods are subject to limitations and possible biases, as well as underlying assumptions, which are not always fulfilled and are often difficult to verify. Moreover, stable isotope analysis of $N_2O$, $N_2$, and precursor compounds is challenging, particularly regarding calibration, instrumentation, and intercomparability between laboratories. Finally, methods that elucidate process dynamics by combining measurements from different compartments – such as soil air, soil solutes, and surface flux data – offer new possibilities, but limitations and assumptions are currently not well understood.

Despite these challenges, interest in the use of stable isotope methods to understand $N_2O$ production and consumption pathways is growing, and the number of publications per year is rising steadily. Communication channels for the discussion of methodologies – such as conferences, workshops, reviews and publications – are limited in access and continuity, and allow only for slow progress. This motivated us to start an open discussion group to enable rapid exchange on this topic.

We want to offer a forum to address four general areas:

- *Experimental Methods*: Discuss technical issues and present ideas for new methods
- *Analytical Methods:* Share data on performance and comparison of methods
- *Data analysis*: Discuss advantages, disadvantages, limitations, possible biases, and solutions relating to existing data analysis methods
- *Modelling*: Discuss modelling approaches to evaluate experimental data

We invite participation from interested researchers via: i) following the discussion group to stay up to date, ii) adding comments and ideas to ongoing discussions in the Project Log, and iii) contacting us with suggestions for new discussion topics.

We hope to see you soon!
https://www.researchgate.net/project/Discussion-on-isotopic-tools-to-study-soil-N2O

## Keynote: Tracing Nitrous Oxide Biogeochemistry in Marine Oxygen Deficient Zones using Isotopes and Isotopomers

*K. L. Casciotti[1,*], C. L. Kelly[1], N. Gluschankoff[1], and Patrick Monreal[2]*

[1]Department of Earth System Science, Stanford University, Stanford, CA, USA
[2]Earth Systems Program, Stanford University, Stanford, CA, USA
*presenting author: kcasciotti@stanford.edu

Nitrous oxide ($N_2O$) is a powerful greenhouse gas[1] and the dominant agent of stratospheric ozone destruction[2]. Eastern boundary upwelling areas are significant sources of $N_2O$ to the atmosphere, and thus $N_2O$ cycling there is particularly important to understand. Upwelling areas in the eastern tropical Pacific Ocean are underlain by significant expanses of $O_2$-depleted waters, known as marine oxygen deficient zones (ODZs). The dominant sources, and only sink, of $N_2O$ in both marine and terrestrial environments stem from microbial activities, and thus are sensitive to the presence of substrates that support or inhibit them, such as oxygen and organic carbon. Ammonia-oxidizing bacteria (AOB) and ammonia-oxidizing archaea (AOA), both linked to the nitrification process, are capable of producing $N_2O$[3,4], although recent experiments suggest that $N_2O$ production via the 'hybrid' mechanism associated with AOA is the dominant source of $N_2O$ production from ammonium ($NH_4^+$)[5,6,7]. Denitrification, the sequential reduction of nitrate ($NO_3^-$) through nitrite ($NO_2^-$) and $N_2O$ to dinitrogen gas ($N_2$) can represent either a net source or net sink of $N_2O$, depending on environmental conditions. Both $N_2O$ production processes can occur under low-oxygen conditions, leading to some of the highest marine accumulations of $N_2O$. Here we discuss the use of isotope-enabled models that synthesize isotopic and isotopomeric measurements of $N_2O$, $NO_3^-$, and $NO_2^-$ to characterize the biogeochemistry of $N_2O$ in and around ODZs in the Eastern Tropical North Pacific (ETNP)[8] and Eastern Tropical South Pacific (ETSP)[9] oceans. Although uncertainties still exist regarding the mechanisms and isotope? systematics of $N_2O$ production by both AOA and denitrifying bacteria, our isotopic data support the notion that processes linked to both nitrification and denitrification contribute to high $N_2O$ accumulations in the low-oxygen waters above oxygen deficient zones[8,9]. Within the oxygen-deficient zone itself, net consumption of $N_2O$ leads to local minima in $N_2O$ concentrations and maxima in $\delta^{15}N^{bulk}$-$N_2O$, $\delta^{15}N^{alpha}$, $\delta^{18}O$-$N_2O$, and site preference (SP = $\delta^{15}N^{alpha} - \delta^{15}N^{beta}$), while low $\delta^{15}N^{beta}$ values reflect concurrent $N_2O$ production[8,9]. Only by incorporating knowledge of $\delta^{15}N^{alpha}$ and $\delta^{15}N^{beta}$ separately, in addition to $\delta^{18}O$-$N_2O$ measurements and those of potential substrates ($NO_3^-$ and $NO_2^-$), can we separate the effects of $N_2O$ production and consumption in and around ODZs and better define the sources and controls of $N_2O$ cycling in these regions.

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## Keynote: Does compound-specific isotope analysis contribute to a new conceptual understanding of the fate of contaminants in the environment?

*D.Hunkeler[1,*]*

[1]Centre for Hydrogeology and Geothermics (CHYN), University of Neuchâtel, Neuchâtel, Switzerland
*presenting author: Daniel.Hunkeler@unine.ch

In recent years, the field of compound-specific isotope analysis (CSIA) applied to environmental contaminants has steadily expanded. CSIA has become feasible for additional elements such as chloride or bromide and multi-element isotope analysis has become increasingly common. Methods have been developed for a growing range of compounds and increasingly complex matrices. Method detection limits have been lowered expanding the range of application of CSIA to emerging and micropollutants. An increasingly complete understanding of

isotope fractionation associated with reactive and non-reactive processes has been achieved. The concept has been field tested in a broad range of environmental systems. A common motivation of the studies is it to track reactive processes that cannot be evaluated based on concentration data alone due to co-occurring non-reactive processes that alter concentrations as well. Thus, CSIA has the potential to reveal overlooked reactive processes and provide unique insight into the contaminant dynamics. The presentation aims at stimulating a discussion to what extent CSIA can and has indeed contributed to a new conceptual understanding of the fate of contaminants in the environment. In particular, contributions of CSIA to an improved understanding of the role of preferential flow and transport, rate-limiting mass transfer and geological/geochemical heterogeneity on the contaminant fate will be discussed.

### Oral: Identifying a potentially variable site preference for hybrid nitrous oxide production via isotopomer labeling experiments

*C.L. Kelly[1,*], N.M. Travis[1], P.A. Baya[1], C. Frey[2], X. Sun[3], B.B. Ward[4], K.L. Casciotti[1]*

[1]Department of Earth System Science, Stanford University, Stanford, U.S.A.
[2]Department of Environmental Science, University of Basel, Basel, Switzerland
[3]Department of Ecology & Evolutionary Biology, Yale University, New Haven, U.S.A.
[4]Department of Geosciences, Princeton University, Princeton, U.S.A.
*presenting author: clkelly@stanford.edu

Nitrous oxide ($N_2O$) is a potent greenhouse gas and ozone depletion agent, with a significant natural source from marine oxygen deficient zones (ODZs). Open questions remain, however, about the microbial processes responsible for this $N_2O$ production, especially hybrid $N_2O$ production when ammonia-oxidizing archaea (AOA) are present. Using $^{15}N$-labeled tracer incubations, we measured the rates of $N_2O$ production from ammonium ($NH_4^+$), nitrite ($NO_2^-$), and nitrate ($NO_3^-$) in the Eastern Tropical North Pacific ODZ, as well as the isotopic labeling of the central (alpha) and terminal (beta) N atoms of the $N_2O$ molecule. In general, we observed production of both doubly- and singly labeled $N_2O$ from each tracer, with the highest rates at the same depths as the near-surface $N_2O$ concentration maximum. Furthermore, at most stations and depths, the production of $^{45}N_2O$-alpha and $^{45}N_2O$-beta were statistically indistinguishable. Implementing these rates of labeled $N_2O$ production in a forward-running model, we found that $N_2O$ production from nitrate dominated at most stations and depths, with rates as high as 1.37±0.000349 nM-$N_2O$/day (uncertainty based on varying optimization initial guess for $N_2O$ production rate by up to 25%). Hybrid $N_2O$ production, in which one N atom each is derived from ammonium and nitrite, and which is posited to be a mechanism by which AOA produce $N_2O$, was modeled in two parts: one hybrid mechanism that always places the nitrogen derived from nitrite in the central, alpha position in the $N_2O$ molecule; and one mechanism that places nitrogen derived from nitrite and ammonium randomly in each N position in the $N_2O$ molecule. Hybrid $N_2O$ production was comprised mostly of this second mechanism, with rates as high as 0.136±0.000809 nM-$N_2O$/day that peaked in both the near-surface and deep $N_2O$ concentration maxima. Based on the random sorting of $^{15}N$ into the alpha and beta positions of the $N_2O$ molecule, as well as the 1:1 ratio in which $NH_4^+$ and $NO_2^-$ combine to form $N_2O$, we posit a two-step process for this hybrid mechanism, involving an initial bond-forming step that draws nitrogen atoms from each substrate to form a symmetric intermediate, and a second bond-breaking step that breaks the bonds in the symmetric intermediate interchangeably to form $N_2O$. From this posited process, we infer that hybrid $N_2O$ production by AOA may have a variable site preference that depends on the $^{15}N$ content of each substrate. This variable site preference may reconcile field experiments that identify a near-surface source with a site preference of 6-8‰, and culture experiments showing that AOA produce $N_2O$ with a site preference of ~30‰.

### Oral: Denitrifying pathways dominate nitrous oxide emissions from managed grassland during drought and rewetting

*E. Harris[1,2,*], E. Diaz-Pines[3], E. Stoll[1], M. Schloter[4,5], S. Schulz[4], C. Duffner[4,5], K. Li[6], K. Moore[6], J. Ingrisch[1], D. Reinthaler[1], S. Zechmeister-Boltenstern[3], S. Glatzel[8], N. Brüggemann[9], M. Bahn[1]*

[1]Functional Ecology Research Group, Department of Ecology, University of Innsbruck, Austria
[2]Swiss Data Science Centre, ETH Zürich, Switzerland
[3]Institute of Soil Research, University of Natural Resources and Life Sciences Vienna, Austria
[4]Research Unit Comparative Microbiome Analysis, Helmholtz Zentrum München, Germany
[5]Chair of Soil Science, Technical University of Munich, Germany
[6]Department of Materials, Photon Science Institute, The University of Manchester, UK
[8]Geoecology, Department of Geography and Regional Research, University of Vienna, Austria
[9]Forschungszentrum Jülich GmbH, Institute of Bio- and Geosciences, Agrosphere (IBG-3), Germany
*presenting author: eliza.harris@sdsc.ethz.ch

Nitrous oxide ($N_2O$) is a strong greenhouse gas released primarily from microbial nitrification (oxic) and denitrification (anoxic) in soils. The extent of these pathways – controlled by many factors, in particular soil moisture – is a key uncertainty in the N cycle. Future climate scenarios predict increased summer drought for European grasslands. The effects of such precipitation changes on $N_2O$ production pathways are unknown, complicating efforts to mitigate emissions. This study presents the first online isotopic measurements of $N_2O$ emitted from grassland soils subjected to drought and rewetting. Automated chambers were interfaced with laser spectrometers to monitor $N_2O$ fluxes and isotopic composition. The abundance of nitrifiers and denitrifiers, soil physicochemical properties, and NanoSIMS measurements of microscale soil N distribution were brought together with $N_2O$ isotope data to gain a detailed view of $N_2O$ production and consumption in drought-affected soils.

Unexpectedly, isotopic measurements showed that denitrifying pathways dominated $N_2O$ emissions under both drought and control conditions. Denitrification during drought was linked to a reversible, drought-induced enrichment in N-bearing organic matter on microaggregates, and suggested a strong role for the chemo-/co-denitrification pathways. Throughout rewetting, fluxes were highly variable and denitrification dominated emissions. Both total $N_2O$ flux and denitrification contribution

were significantly higher during rewetting than for control plots at the same soil moisture range. The observed feedbacks between precipitation changes and $N_2O$ emissions are sufficient to account for the accelerating atmospheric $N_2O$ growth rate observed globally over the past decade.

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### Oral: Isotopic tracing of sources and fate of nitrate, sulfate and methane in groundwater in Alberta (Canada)

*B. Mayer[1,*], P. Humez[1] & M. Nigthingale[1]*

[1]Applied Geochemistry group, Department of Geoscience, University of Calgary, Calgary, Alberta, Canada
*presenting author: bmayer@ucalgary.ca

Providing sufficient amounts of high-quality drinking water is of key importance for supporting an increasing population on planet Earth. Although in the province of Alberta in Canada approximately 80% of the 4.4 million inhabitants still obtain their drinking water from surface water sources, a steadily increasing portion of the population is becoming reliant on groundwater for its water needs. To support this development, we have investigated the quality and geochemical evolution of groundwater in Alberta throughout the last two decades. One of the key objectives was to determine the occurrence, the sources and the fate of nitrate, sulfate, and methane in groundwater in Alberta, using a combination of geochemical, isotopic and microbiological approaches.

Nitrate concentrations above the detection limit were only found in 20% of the groundwater samples. Only in 3 % of the samples did nitrate exceeded the maximum allowable concentration for drinking water of 10 mg/L $NO_3^-$-N [1]. The isotopic composition of nitrate revealed that high nitrate contents in groundwater are frequently the result of cattle manure applications. Denitrification was found to be the key nitrate removal process in aquifers in Alberta that often contain moderately to highly reducing groundwater. Sulfate is ubiquitous in Alberta groundwater with 16% of the samples exceeding the aesthetic objectives for drinking water of 500 mg/L [1]. The isotopic composition of sulfate revealed that elevated sulfate contents are predominantly caused by oxidation of sulfide minerals contained either in the glacial tills or in marine shales in various bedrock formations. Sulfate removal from groundwater can occur via bacterial sulfate reduction, establishing sufficiently reducing conditions in the aquifers to facilitate in-situ methanogenesis. Methane occurs in many aquifers in Alberta and isotopic analyses on methane revealed that this gas is predominantly of microbial origin. $\delta^{13}C$ values of methane and microbial data also revealed that methane oxidation is a frequently occurring process in groundwater in Alberta.

In summary, our study has revealed that the combination of geochemical, isotopic and microbiological approaches is a powerful tool to investigate the suitability of groundwater for drinking water purposes and its geochemical evolution on a regional scale.

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### Oral: Intramolecular $N_2O$ isotopic composition from grassland without preconcentration: interferences correction, nitrification inhibitors, freeze-thaw events and source process identification

*B. Wolf[1,*], L. Xia[1], Klaus Butterbach-Bahl[1], R. Kiese[1]*

[1]Karlsruhe Institute of Technology, Institute of Meteorology and Climate Research (IMK-IFU), Garmisch-Partenkirchen, Germany
*presenting author: benjamin.wolf@kit.edu

Within the linear N-N-O molecule, the rare stable isotopes $^{15}N$, $^{17}O$ and $^{18}O$ can substitute the abundant $^{14}N$ at central ($\alpha$) and/or terminal ($\beta$) positions and $^{16}O$. The intramolecular distribution of $^{15}N$ in $N_2O$ is referred to as site preference, SP, and defined as $\delta^{15}N^{\alpha} - \delta^{15}N^{\beta}$[1]. Especially SP was shown to provide information on $N_2O$ source processes [2–5].

The advent of laser spectrometers has spawned first datasets of $N_2O$ isotopic composition in daily resolution, but they have remained scarce. This is because, the precision of commercially available spectrometers did not allow direct determination of $N_2O$ isotopic composition without challenging cryogenic preconcentration of $N_2O$. The latest commercial spectrometers promised preconcentration free in-situ determination of $N_2O$ isotopic composition, but a recent instrument intercomparison showed that specific correction functions are necessary [6]. While the instruments were thoroughly characterized in the laboratory, instrument performance during long-term field deployment has remained unclear.

To support wide-spread acquisition of intramolecular $N_2O$ isotopic composition, we present results obtained during an eight months field deployment of a Picarro G5131i analyser. The analyser was coupled to a chamber system so that isotopic composition of $N_2O$ emitted from soil could be determined using the Keeling-plot approach.

Here we show that variability of trace gas concentration in chamber headspace can be corrected adequately and that isotopic composition of soil-emitted $N_2O$ can be determined based on the Keeling-plot approach for chamber headspace concentration increases of more than 150 ppb. Utilization of a nitrification inhibitor (NI) had no significant effect on SP and $N_2O$ isotopic composition. This suggests that there was no shift in predominant $N_2O$ production / consumption processes and that the contribution of nitrification to $N_2O$ emission was small. Compared to growing-season emissions, SP and $\delta^{18}O$-$N_2O$ during freeze-thaw cycles were distinctly different. SP for both ammonium sulfate (AS) fertilizer and AS+NI was ~0, indicating that $N_2O$ reduction to $N_2$ was negligible during freeze-thaw events.

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## Sponsored: Analysis of Soil Respiration with OA-ICOS technology

*Adrien Danner[1,*] & Geoffrey Rahe[1]*

[1]Envicontrol, Namur, Belgium
*presenting author: a.danner@envicontrol.com

ABB's patented Off-Axis Integrated Cavity Output Spectroscopy (OA-ICOS) technology, a cavity enhanced absorption technique, is a well-established analytical approach to a variety of samples from research areas. The measurement strategy is based on high-resolution laser absorption spectroscopy. A laser light is temporarily trapped inside a cavity.

Soil respiration refers to the $CO_2$ flux released from the soil surface to the atmosphere as a result of microbial and root respiration (heterotrophic and autotrophic). It is the second largest terrestrial carbon flux. Thanks to a careful choice of laser frequency, the OA-ICOS analyzers can provide several useful isotopic and concentration parameters for the analysis of soil respiration.

In this framework, stable $CO_2$ isotopes are extremely useful indicators to study biogeochemical processes and fluxes that occur at the soil-plant-atmosphere interface:

The $\delta^{13}C$ measurements can provide information on physiological history of soil carbon and help identify the sources of $CO_2$ soil emissions, be it photosynthesis, natural gas combustion, fossil fuels, landfills or other sources.

In parallel, oxygen isotopes can aid in inferring the interaction between $CO_2$ and soil waters as the $\delta^{18}O$ and $\delta^{17}O$ ratios of soil $CO_2$ are determined by its hydrological cycle.

Laser-based $CO_2$ carbon isotope analysis using instruments like ABB LGR–ICOS analyzers is a recent alternative approach that allows for real-time, *in situ* measurements of the concentration, $\delta^{13}C$, $\delta^{18}O$ and even $\delta^{17}O$ signature of $CO_2$. It is increasingly being used to monitor and track $CO_2$ emissions.

## Oral: Fractionation of stable isotopes of metals and metalloids in plants - copper and cadmium as examples

*Matthias Wiggenhauser[1,*], Rebekah E. T. Moore[2], Peng Wang[3], Gerd Patrick Bienert[4], Kristian Holst Laursen[5], Simon Blotevogel[6]*

[1]Group of Plant Nutrition, ETH Zurich, Zurich, Switzerland
[2]MAGIC Group, Imperial College London, London, UK
[3]College of Resources and Environmental Sciences, Nanjing Agricultural University, Nanjing, China
[4]Crop Physiology, Technical University of Munich, Freising, Germany
[5]Plant Nutrients and Food Quality Research Group, University of Copenhagen, Copenhagen, Denmark
[6]Laboratoire Matériaux et Durabilité des Constructions (LMDC), Université de Toulouse III, Toulouse, France
*presenting author: matthias.wiggenhauser@usys.ethz.ch

Non-traditional stable isotope ratios have been used to trace chemical and physical processes that control trace metal and metalloid uptake and translocation in plants. In this presentation the current knowledge of isotope fractionation of plant nutrients such as Zn, Fe, Cu, Ca, and Mg, beneficial elements such as Si and non-essential pollutants such as Cd and Tl is presented, based on a recent literature review.[1] The focus will be on the fractionation of the micronutrient Cu and pollutant Cd.

Copper isotope fractionation during root uptake in monocots and dicots plants differs. Monocots reduce Cu(II) to Cu(I) prior to root membrane transport which leads to a strong enrichment of light Cu isotopes in plants. In contrast, this fractionation is mostly absent in dicots which indicates that monocots may absorb Cu as Cu(II) in chelated form. In addition, Cu isotope fraction during root uptake varies with Cu supply. Therefore, Cu isotope fractionation could be used as a marker for different Cu uptake pathways under distinct environmental conditions. Cadmium is non-essential for plants and it readily enters the plant facilitated by membrane proteins that are thought to transport essential micronutrients such as Mn, Fe, and Zn. Typically, isotopically light Cd is preferentially retained in roots, while isotopically heavier Cd is translocated towards shoots. The retention of light Cd in roots is thought to be caused by membrane transport into the vacuole and chelation of Cd by thiol containing molecules such as phytochelatin. Compared to Zn, an element that is chemically similar to Cd, Cd is inversely fractionated within cereals. Hence, isotope fractionation of these two elements may be used to identify biochemical processes that separate Cd from essential micronutrients in plants. To further explore the scope of non-traditional stable isotope analysis in plants, isotope fractionation factors of individual processes such as membrane transport and complexation to organic ligands need to be investigated in more detail. Furthermore, research should shift towards hypothesis driven experiments e.g. by integrating contrasting nutrient supplies, using established model plants, genetic approaches, and by combining isotope analyses with complementary speciation techniques. Finally, concurrent use of traditional and non-traditional isotopes could further increase the potential of isotope process tracing in plants.

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## Oral: Dehalogenation of α-hexachlorocyclohexane by iron sulfide nanoparticles: Study of reaction mechanism with stable carbon isotopes and pH variations

*Silviu-Laurentiu Badea[1,*], Diana-Ionela Stegarus[1], Violeta-Carolina Niculescu[1], Stanica Enache[1], Amalia Soare[1], Roxana-Elena Ionete[1], Didier Gori[2], Patrick Höhener[2]*

[1]National Research and Development Institute for Cryogenic and Isotopic Technologies – ICSI Rm. Vâlcea, 4th Uzinei Street, 240050 Râmnicu Vâlcea, Romania
[2]Environmental Chemistry Laboratory (LCE), Aix-Marseille Université-CNRS UMR 7376, 3 place Victor Hugo - Case 29, 13331 Marseille Cedex 3, France
*presenting author: silviu.badea@icsi.ro

For many decades, remediation of chlorinated organics contamination was focused on their anaerobic and aerobic biodegradation. In this respect, the biodegradation of hexachlorocyclohexanes (HCHs) was proven in the last years to be accompanied by isotope fractionation of carbon ($^{13}C/^{12}C$). Nevertheless, with the successful application of permeable reactive barrier (PRB) technology in groundwater remediation this situation has changed. As a result, abiotic reductive dehalogenation by minerals [1] has attracted considerable attention within the research community but most of them on halogenated solvents and fuel additives and much less on chlorinated pesticides. In this study [2], we explored the carbon isotope fractionation of α-HCH during dechlorination by FeS nanoparticles at different pH values. The results of three different experiments showed that the apparent rate constants during dehalogenation of α-HCH by FeS increased with pH. Regardless of the pH used, the 1,2,4-trichlorobenzene (1,2,4-TCB), 1,2-dichlorobenzene (1,2-DCB), and benzene were the main degradation products of α-HCH. An enrichment factor ($\varepsilon_C$) of -4.7 ± 1.3 ‰ was calculated for α-HCH using Rayleigh model, which is equivalent to an apparent kinetic isotope effect ($AKIE_C$) value of 1.029 ± 0.008 for dehydrohalogenation, and of 1.014 ± 0.004 for dihaloelimination, respectively. The extent of isotope fractionation from this study suggests that abiotic isotope fractionation by FeS must be taken into account in anoxic sediments and aquifers contaminated with HCH isomers, when high concentrations of FeS are present in the above-mentioned anoxic environments.

**Funding.** This work was carried out with the support of Ministry of Research, Innovation and Digitization, project no. PN 19110302 "Research on the variation trends specific to stable isotopes in different tree species: deepening the fractionation mechanisms and the chemical processes interconnected on the soil-water-plant chain"

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## Oral: Manganese–driven oxidation of aminotris (methylene) phosphonate (ATMP) studied by carbon CSIA

*P. R. Martin[1,*], D. Buchner[1], M. A. Jochmann[2], M. Elsner[3], S. B. Haderlein[1]*

[1]Center for Applied Geoscience, University of Tübingen, Tübingen, Germany
[2]Instrumental Analytical Chemistry, University of Duisburg-Essen, Essen, Germany
[3]Chair of Analytical Chemistry and Water Chemistry, Technical University of Munich, Munich, Germany
*presenting author: philipp.martin@uni-tuebingen.de

Aminopolyphosphonates (APPs), such as aminotris(methylene) phosphonate (ATMP) are organophosphorus compounds of rising concern due to their increasing use in industrial and household applications and potential adverse effects on wastewater receiving surface waters. Homogeneous Mn(II)–catalyzed oxidation by dissolved oxygen and heterogeneous oxidation at manganese dioxide surfaces ($MnO_2$) were proposed as transformation pathways relevant for the fate of APPs in the environment.[1,2] However, a comprehensive assessment of the significance of these processes is hampered by lacking knowledge of the underlying transformation mechanism(s). To narrow down this research gap, we applied compound-specific carbon isotope analysis (carbon CSIA) in combination with speciation modeling and kinetic analysis to study manganese-driven oxidation of ATMP in laboratory batch experiments under varying conditions, such as pH or Mn-to-ATMP ratio.

Homogenous Mn(II)–catalyzed oxidation of ATMP was associated with a highly variable kinetic isotope fractionation (expressed as carbon isotope enrichment factors, $\varepsilon_C$) ranging from $\varepsilon_C \approx$ -1 ‰ to $\varepsilon_C \approx$ -10 ‰, which correlated – like the reaction rates – with the fraction of ATMP complexed with Mn(II). Based on these observations, a second – so far unknown – transformation pathway was proposed, namely oxidation of free ATMP by reactive Mn(III)ATMP–intermediates. Consequently, under environmentally relevant conditions ATMP might be (partially) trapped in Mn-complexes due to back-reduction of Mn(III)-intermediates by natural reductants, such as quinones.[3] Preliminary experiments on the oxidation of ATMP at $MnO_2$ in the presence of dissolved oxygen showed that the reaction is associated with a non-Rayleigh-type kinetic isotope fractionation. This behavior implies a shift in the underlying transformation mechanism, potentially from heterogeneous to homogeneous oxidation due to reductive dissolution of the mineral.

The presented work demonstrates the potential of carbon CSIA as a tool to study APP transformation and by this develop a more profound understanding of their environmental fate. Yet, to get further insights into manganese-driven oxidation of APPs, additional work on the kinetic isotope fractionation associated with the isolated heterogeneous oxidation is needed, such as experiments (i) in the absence of $O_2$ or (ii) with APPs resilient towards Mn(II)-catalyzed oxidation.

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## Oral: Oxygen kinetic isotope effects associated with reactions of singlet oxygen in aqueous solutions

*Sarah G. Pati[1,*], Martin Ley[1], Lara Brunner[1], Thomas B. Hofstetter[2,3]*

[1]Department of Environmental Sciences, University of Basel, Basel, Switzerland
[2]Eawag, Swiss Federal Institute of Aquatic Science and Technology, Dübendorf, Switzerland
[3]Institute of Biogeochemistry and Pollutant Dynamics (IBP), ETH Zürich, Zürich, Switzerland
*presenting author: sarah.pati@unibas.ch

Photochemical processes play an important role in the degradation of dissolved organic matter, the fate of organic contaminants, and the production of reactive oxygen species in aquatic environments. An oftentimes underappreciated fact is that photochemical processes can contribute substantially to the consumption of dissolved oxygen in the photic zone of oceans and lakes. If photochemical oxygen consumption leads to substantial isotope fractionation of dissolved oxygen, these processes need to be considered in models assessing gross primary production based on oxygen isotope signatures. There are several oxygen consuming photochemical reactions, but one of the most important ones is the formation of singlet oxygen by energy transfer from photochemically excited organic matter to ground state molecular oxygen. Upon formation, singlet oxygen can react with alkenes, sulfides, and phenols by cycloaddition, (hydro)peroxide addition, or electron transfer. In this study, we determined apparent kinetic isotope effects (AKIEs) for the reactions of four model compounds (furfuryl alcohol, histidine, methionine, tyrosine) with singlet oxygen in laboratory experiments. A visible light source and Rose Bengal, a well-studied photosensitizer, were used to generate singlet oxygen. Buffered, air-saturated solutions containing Rose Bengal and a model compound were exposed to light for different time periods in Exetainers without headspace. The decrease in oxygen concentrations was measured with optical oxygen sensors and changes in isotopic composition of dissolved oxygen were determined by GC/IRMS. We observed AKIEs between 1.000±0.001 and 1.030±0.001 without clear differences between the different model compounds. The magnitude of AKIEs correlated with the second-order reaction rate constant of the model compounds with singlet oxygen multiplied by the concentration of the model compounds. However, this correlation did not follow the expected commitment-to-catalysis model for a two-step reaction. The two KIE endmembers of 1.00 and 1.03 suggest that (i) the formation of singlet oxygen from ground state oxygen is not associated with any measurable isotope fractionation and (ii) the reaction of singlet oxygen with all tested model compounds occurs through a rate limiting single electron transfer step. Our results suggest that consumption of dissolved oxygen by singlet oxygen reactions can cause substantial and variable isotope fractionation in the photic zone of oceans and lakes under certain conditions.

## Oral: Tracing Mechanistic Adaptations of Enzymatic Oxygenations of Aromatic Contaminants using $^{13}C$ and $^{18}O$ Kinetic Isotope Effects

*C. E. Bopp[1,2,*], S. G. Pati[3], N. M. Bernet[1], H-P. E. Kohler[1], T. B. Hofstetter[1,2]*

[1]Department of Environmental Chemistry, Eawag, Swiss Federal Institute of Aquatic Science and Technology, Dübendorf, Switzerland
[2]Institute of Biogeochemistry and Pollutant Dynamics, ETH Zurich, Zürich, Switzerland
[3]Department of Environmental Science, University of Basel, Basel, Switzerland
*presenting author: charlotte.bopp@eawag.ch

Oxygenations of aromatic soil and water contaminants with molecular $O_2$ catalyzed by Rieske dioxygenases are frequent initial steps of biodegradation in natural and engineered environments. Many of these non-heme ferrous iron enzymes are known to be involved in contaminant metabolism. Yet the understanding of enzyme-substrate interactions that lead to successful biodegradation is still elusive. Here, we studied the mechanisms of $O_2$ activation and substrate hydroxylation of two wild-type nitrotoluene dioxygenases, as well as three mutant enzymes that have adapted to alternative substrates in laboratory evolution experiments to evaluate enzyme- and substrate-specific factors that determine the efficiency of oxygenated product formation. Experiments in enzyme assays with methyl-, fluoro-, chloro-, and hydroxy-substituted nitroaromatic substrates revealed that 20% to 100% of the enzyme's activity involves unproductive paths of $O_2$ activation with generation of reactive oxygen species. This so called $O_2$ uncoupling appears to be highly substrate-specific and could be a critical factor in the adaptation of mutant enzymes. The mechanistic events in the catalytic cycle of Rieske dioxygenases that cause $O_2$ uncoupling, however, are unclear.

To test enzyme mechanisms as the source of substrate- and enzyme-specific $O_2$ uncoupling, we determined $^{18}O$ and $^{13}C$ kinetic isotope effects (KIEs) for $O_2$ activation and nitroaromatic substrate hydroxylation, respectively. For all enzyme-substrate combinations tested, $^{18}O$ KIEs averaged around 1.016±0.002 suggesting a conserved generation of $Fe^{III}$-(hydro)peroxo species in the rate-determining step. This activated $O_2$ is either uncoupled as $H_2O_2$ or productively used for dioxygenation. Because $^{13}C$ KIEs of nitroaromatic substrate hydroxylation correlated with the extent of $O_2$ uncoupling in nitrobenzene dioxygenase (1.000-1.035) but not with 2-nitrotoluene dioxygenase (2NTDO, 1.000-1.011), we postulate that the two enzymes involve different Fe-oxygen species for the hydroxylation step. However, $^{13}C$ KIEs of the 2NTDO mutants remained between 1.000 and 1.010 excluding mechanistic differences as the source of substrate adaptation. Instead, distinct active site residues of the enzymes point to the substrate fit in the active site as an important factor determining $O_2$ uncoupling in non-heme iron dioxygenases.

## Oral: Constraints on triple oxygen isotope kinetics

*J. A. Hayles[1,*] & B. A. Killingsworth[2]*

[1]Jacobs-JETS, Astromaterials Research and Exploration Science, Johnson Space Center National Aeronautics and Space Administration, Houston, TX 77058, USA
[2]U.S. Geological Survey, Geology, Energy & Minerals Science Center, MS 954 National Center, 12201 Sunrise Valley Drive Reston, VA 20192, United States of America
*presenting author: justin.a.hayles@nasa.gov

The general assumption that systems are in equilibrium is convenient, but imperfect. Instead, the partial or full expression of kinetic reaction steps may need to be determined. Using an isotope fractionation Monte-Carlo model under the harmonic approximation (Figure 1 envelopes), we find the range of kinetic isotope effects (i.e., resulting from unidirectional chemical reactions) contains and exceeds the range of accessible equilibrium isotope effects. For the $^{16}O$-$^{17}O$-$^{18}O$ system, accessible kinetic isotope effects can yield $\Delta'^{17}O$ variations as large 0.8‰ from a single kinetic fractionation step. Reactions that yield the largest deviations from equilibrium are those with the largest molecular mass difference between decomposition fragments, typically the two products, such as a gas and solid generated from the thermal decomposition of an initial solid. For example, previously published experiments on the thermal decomposition of calcite ($CaCO_3$) and dehydroxylation of brucite ($Mg(OH)_2$) are matched by density functional theory models of each kinetic isotope effect, thus revealing their origin in mass-dependent reaction. Beyond oxygen, our approach applies to any system with more than two isotopes.

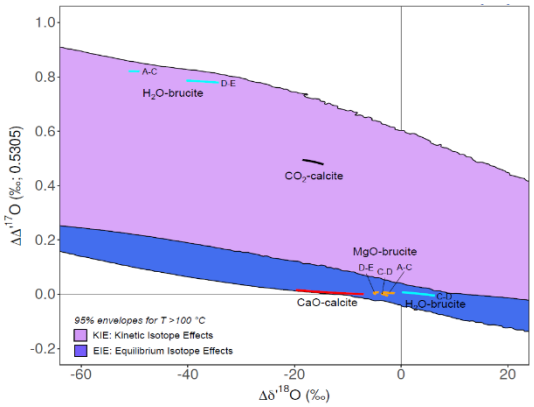


Figure 1: Density functional theory model results for kinetic Isotope effects for thermal decomposition of calcite and brucite. Model results are plotted with envelopes containing 95% highest model density fields of kinetic isotope effects and equilibrium isotope effects from Monte Carlo models for temperatures > 100°C. Kinetic and equilibrium isotope effect envelopes overlap. Calculated $CaCO_3$ thermal decomposition fractionations are plotted from 400-900°C and $Mg(OH)_2$ thermal decomposition fractionations A-C, C-D and D-E, corresponding to modelled transition states, are plotted from 150-450°C. **MgO: orange, $H_2O$: light blue, $CO_2$:black, CaO:red.**

## Oral: Isotope Fractionation Reveals Limitations and Microbial Regulation of Pollutant Biodegradation at Low Concentrations

*M. Elsner[1,2,*], F. Sun[1, 2], K. Kundu[2], B. Ehrl[2], M. Gharasoo[2], S. Marozava[2], A. Mellage[3], J. Merl-Pham[2], J. Peters[2], Z. Wang[2], R. Bakkour[1], A. Melsbach[1, 2], X. Cao[2], R. Zimmermann[2], C. Griebler[2], M. Thullner[4], O. Cirpka[3]*

[1]Chair of Analytical Chemistry and Water Chemistry, Technical University of Munich, Germany
[2]Helmholtz Zentrum München, Neuherberg, Germany
[3]Hydrogeology, University of Tübingen, Germany
[4]Helmholtz Center for Environmental Research UFZ, Leipzig, Germany
*presenting author: m.elsner@tum.de

Compound-specific isotope fractionation analysis (CSIA) of chemical trace contaminants ("micropollutants") could reveal bottlenecks of degradation at low, relevant (µg/L) concentrations. When enzyme-associated isotope effects were observable, this provided evidence that molecules diffused freely into and out of bacterial cells demonstrating that mass transfer was not limiting. In contrast, when isotope fractionation was pronounced at high concentrations, but isotope effects were masked at trace levels, this provided evidence that mass transfer into and out of the cell became limiting for biodegradation specifically at low concentrations [1]. An onset of masking was observed for atrazine when degraded by *Arthrobacter aurescens* TC1 at concentrations below 60 µg/L in chemostat [1,2] with complete rate control at 10 µg/L in retentostat [3]. Proteomics revealed that such mass transfer limitation served as trigger for physiological adaptation, where catabolic enzymes remained highly expressed, whereas other cellular functions were downregulated.

CSIA also demonstrated mass transfer limitations in a quasi-two dimensional sediment tank system mimicking realistic conditions of natural aquifers. High, unmasked isotope fractionation in the center of the plume indicated that 2,6-dichlorobenzamide degradation by *Aminobacter* sp. MSH1 was not limited by substrate availability, but rather by oxygen supply. In contrast masked isotope fractionation pinpointed rate-limiting mass transfer during cellular uptake towards the lower end of the concentration profile. [4] For bioremediation approaches of low-level concentrations, the direct observation of limitations offers an enabling tool to identify relevant bottlenecks and observe bacterial regulation / adaptation over time [4]. For Isotope Biogeochemistry these findings have, moreover, significant implications for the interpretation of isotope profiles at low concentrations: they imply that, based on isotopic evidence, turnover of substances at low concentrations may be widely underestimated.

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[4] Sun *et al.* (2021), Environ. Sci. Technol., DOI 10.1021/acs.est.0c08566

## POSTERS P45 – P62

### P45 Applicability of a Reverse Stable Isotope Labeling Approach to Show Biodegradation of Microplastics on a Single-Cell Level

*K. Müller*, M. Elsner & N. P. Ivleva*

Institute of Hydrochemistry, Chair of Analytical Chemistry and Water Chemistry, Technical University of Munich, Garching, Germany
*presenting author: kara.mueller@tum.de

Biodegradable polymers raise high expectations to curb plastic accumulation in the environment. However, biodegradation may depend on environmental conditions and must therefore be investigated in a microbiological context. Indirect methods (e.g. studies about polymer mass loss or changed polymer properties) typically fail to relate individual microorganisms to their role in biodegradation, although this link would be interesting to understand the effect of phenotypical heterogeneity, interactions within microbial communities and for environmental samples with non-culturable microbes.

Stable isotope probing (SIP) can be used to trace $^{13}C$-labeled carbon from polymers into the biodegradation products $CO_2$ and biomass [1]. So far only one study has been performed about microbial uptake into single cells, based on SIP-nanoscale secondary ion mass spectrometry (NanoSIMS) [1]. While highly sensitive and with high lateral resolution, NanoSIMS is a time- and cost-intensive technique. Here, we therefore pursue a combination of SIP with Raman microspectroscopy (RM) as a non-destructive, less expensive technique to demonstrate microbial degradation of microplastics (MP). RM allows to acquire vibrational fingerprint spectra of single cells *in situ*, in which the incorporation of $^{13}C$ can be identified by red-shifted Raman bands [2]. Due to the quick and simple sample preparation, the approach holds promise to provide a large amount of single-cell data as basis for a statistically reliable interpretation of microbial degradation potential and physiological heterogeneity.

As a cheap alternative to $^{13}C$-labelled polymers, we further explore the applicability of D-labelled polymers and a reverse labeling approach for screening bacteria for their biodegradation potential. In a first study, *Sphingomonas koreensis* cells, which were isolated from a suspension of polylactide (PLA) in an environmental sample, were initially labeled with $^{13}C$-glucose. After incubation in minimal medium without carbon source at 37 °C and 120 rpm, Raman spectra were obtained (20 s, 10 mW at the sample). Four characteristic Raman bands that were red-shifted by $^{13}C$-substitution (CH vibrations, amide I, phenylalanine, and cytosine / uracil) were fitted, and their peak positions were compared to $^{12}C$- and $^{13}C$-reference spectra for classification. No dilution of the $^{13}C$-label was observed for 3 months. In a next step we will incubate labeled bacteria with PLA-MP particles as sole carbon source and check whether the $^{13}C$-label is diluted by incorporation of carbon from unlabeled polymer.

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[2] Ivleva, N.P. et al. (2017). Exploring the Potential of Stable Isotope (Resonance) Raman microspectroscopy, surface-enhanced Raman scattering microspectroscopy, and stable-isotope Raman microspectroscopy for biofilm characterization. Anal Bioanal Chem 409, 4353-4375, DOI: 10.1007/s00216-017-0303-0.

### P46 Using depth profiles and natural abundance stable isotopes to determine $N_2O$ processes in agricultural soils

*A. Matson[1,*], R. Well[1], M. Maier[2], D. Lewicka-Szczebak[3]*

[1]Thünen Institute of Climate-Smart Agriculture, Braunschweig, Germany
[2]Department of Soil & Environment, Forest Research Institute Baden Württemberg, Freiburg, Germany
[3]Faculty of Earth Science and Environmental Management, University of Wroclaw, Wroclaw, Poland
*presenting author: amanda.matson@thuenen.de

Mitigating N-oxide emissions and optimizing N-use efficiency are important aspects of agricultural soil management. Studies that monitor net production of $N_2$ and $N_2O$, including the spatial/temporal heterogeneity of denitrification in soils, provide much-needed data to inform models that support management decisions. However, to more accurately model denitrification, we need to understand the pathways leading to N emissions.

Analysing isotopocules of $N_2O$ ($\delta$15N, $\delta$18O and 15N site preference) can be used to distinguish $N_2O$ production pathways and to quantify $N_2O$ reduction to $N_2$. However, the accuracy of this approach is limited during periods of low activity, and might be improved when combined with $N_2O$ isotopocules of soil air, provided that probes can avoid diffusive isotope effects during sampling. As part of the DFG-research unit "Denitrification in Agricultural Soils: Integrated Control and Modelling at Various Scales (DASIM)", we measured surface fluxes of $N_2O$ isotopocules using the closed chamber method and compared these with gas probe data. These were then assessed using a diffusion-reaction model.

Our results show the differences between isotopocule values of soil air and of produced $N_2O$, highlighting the need for data correction when using soil air values. We also show how well values can be corrected by modeling, and under which conditions soil air sampling might lead to better performance than closed chamber sampling.

### P47 Application of compound-specific carbon isotope analysis on aerobic biotransformation of glyphosate

*K. Kourtaki*, P. R. Martin, D. Buchner, S. B. Haderlein*

Center for Applied Geoscience, University of Tübingen, Tübingen, Germany
*presenting author: kleanthi.kourtaki@uni-tuebingen.de

Glyphosate (N-(phosphonomethyl)glycine) is a widely used herbicide commonly found in water and soil due to its excessive application in agriculture. Enzymatically catalyzed C-P or C-N bond cleavage is assumed to be the prevalent pathway leading to glyphosate transformation in the environment. Microorganisms capable of cleaving these bonds show high effectiveness giving biotransformation a great potential for glyphosate removal. Compound-specific carbon isotope analysis (carbon-CSIA) by liquid chromatography-isotope ratio mass

spectrometry (LC-IRMS) enables of proof glyphosate transformation even if its metabolites are not detected [1]. Even though numerous studies have successfully investigated the capability of different strains to scavenge C, N, or P from glyphosate, associated isotope effects have rarely been explored.

This work presents the first insights into carbon isotope fractionation occurring during the (aerobic) bacterial utilization of glyphosate as phosphorus source. The examined strains (genus Achromobacter) transform glyphosate to sarcosine as the primary metabolite under phosphorus limiting conditions. Our preliminary results indicate no significant shifts in the carbon isotope ratio ($^{13}C/^{12}C$) associated with the C-P bond cleavage. Since multiple enzymatic reactions can result in glyphosate metabolization, we aim to investigate potential changes in carbon isotope signatures associated with different biosynthesis pathways, i.e., by different bacterial strains. Hence, the observable isotope fractionation associated with aerobic biotransformation of glyphosate could provide information about the underlying transformation pathways that control the removal of glyphosate in the environment.

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## P48 Heterogenous oxidation of aminopolyphosphonates and AMPA at manganese oxide surfaces studied by carbon LC-IRMS

*A. Röhnelt[1,*], P. R. Martin[1], D. Buchner[1], M. A. Jochmann[2], S. B. Haderlein[1]*

[1]Center for Applied Geoscience, University of Tuebingen, Tuebingen, Germany
[2]Instrumental Analytical Chemistry, University of Duisburg-Essen, Essen, Germany
*presenting author: anna.roehnelt@uni-tuebingen.de

In recent years the use of aminopolyphosphonates (APPs) in household and industrial applications has increased – concomitant with their measured concentrations in wastewater systems. This has raised increasing interest in their environmental fate and potential transformation pathways [1]. One possible transformation pathway is the heterogeneous oxidation on manganese oxides, which are rated the strongest naturally occurring oxidants and show ubiquitous prevalence [2]. For the investigation of the transformation mechanism(s), compound-specific stable isotope analysis (CSIA) provides an ever more established tool [3].

In this study, we investigated the oxidation of iminodimethylenephosphonate (IDMP) and its transformation product (TP) aminomethylenphosphonate (AMPA) on amorphous manganese dioxide ($MnO_2$) by means of kinetic modelling in combination with carbon CSIA. IDMP served as a model compound, as it represents (i) the main TP of higher APPs, e. g., aminotrismethylenephosphonate (ATMP), and (ii) a potentially relevant source of AMPA in the environment [4]. The oxidation reaction comprises multiple steps, which include adsorption of a compound, electron transfer, bond cleavage and desorption. This is applicable for the parent compound as well as for TPs. We separately quantify the aqueous as well as the sorbed fractions of IDMP and the main TPs AMPA and *o*-phosphate, respectively. Preliminary results indicate that the electron transfer from IDMP to $Mn^{IV}$ (resp. $Mn^{III}$) is (partially) rate-limiting. The combination of kinetic data and carbon CSIA of aqueous and sorbed fractions of IDMP and AMPA will provide more information about the pre-bond cleavage (reversible) steps, competitive sorption and thereby more comprehensive insights into the simultaneous transformation of parent compound and TP.

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## P49 Significant $^{2}H$ and $^{13}C$ isotope fractionation during volatilisation and diffusion of hydrocarbons in soil

*O. Boukaroum[*], P. Höhener & D. Gori*

Laboratoire Chimie Environnement, Aix Marseille University, Marseille, France
*Presenting author: mohamed-ouassim.boukaroum@univ-amu.fr

In situ soil venting is a common remediation technique used to treat Volatile Organic Compounds (VOCs) contamination in the unsaturated zone. Before its application, it is essential to understand the volatilisation and the desorption mechanism to ensure effective implementation.

Compound Specific Isotope Analysis (CSIA) is nowadays a well-implemented technique to assess the progress of remediation after an oil spill. It assumes that non-degradative processes such as volatilisation, diffusion or sorption do not produce a significant isotope effect. However, studies in the unsaturated zone showed that some compounds can undergo a significant isotope fractionation after diffusion and volatilisation (Aelion et al., 2010).

Previous work has shown that at late stages of venting, desorption is controlled by intraparticle diffusion (Grathwohl and Reinhard, 1993). The latter can be modelled by postulating that diffusion occurs inside particles considered as homogeneous spheres and is delayed by equilibrium sorption within pores (Fig. 1). This model could explain the isotope effect observed in the

unsaturated zone. Nevertheless, CSIA has not yet been applied to study intraparticle diffusion.

The objective of this study is to get a better understanding of VOCs forced volatilisation and diffusion from unsaturated aquifer sediment. A stainless steel HPLC column was packed with VOC-contaminated sandy aquifer sediments. The later was connected through an autosampler to a CSIA system. Concentration and isotopes were monitored during the venting of the contaminated sediments.

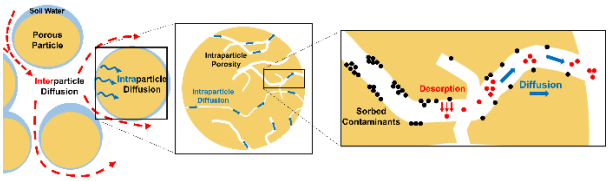


Figure: Intraparticular diffusion model

Early stage of volatilisation leads to a negative $\Delta\delta^{13}C$ shift down to -5‰ and $\Delta\delta^{2}H$ by more than -40‰ in the model compound toluene. Multistep partitioning has been identified to cause these shifts (Zamane et al., 2020). During later stages, enrichment up to 5‰ for $^{13}C$ and 50 ‰ for $^{2}H$ have been registered and associated to delayed intraparticular diffusion. Those isotope shifts are way larger than those usually reported in the literature and confirm the need to undertake further investigations on isotopic fractionation in the unsaturated zone.

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## P50 DECiSIvE - Tracking degradation of soil pollutants with multi-elemental compound-specific isotope analysis

*P. Höhener[1,*], O. Boukaroum[1], G. Imfeld[2], J. Masbou[2], S. Payraudeau[2], F. Martin-Laurent[3]*

[1]Aix Marseille University-CNRS, Laboratoire Chimie Environnement (UMR 7376), Marseille, France
[2]Strasbourg University, ENGEES, CNRS Institut Terre et Environnement de Strasbourg (ITES, UMR 7063), France
[3]INRAE, Institut Agro, University of Burgundy, (AGROECOLOGIE UMR 1347), Dijon, France
*Presenting author: patrick.hohener@univ-amu.fr

The project DECiSIvE is a collaboration of three laboratories funded by the French National research Agency ANR through grant ANR-18-CE04-0004-01 from 2019 to mid-2023. It aims at developing Multi-Elemental Compound-Specific Isotope Analysis (ME-CSIA) for an improved evaluation of the sources and transformation of synthetic pesticides in agricultural soils and their runoff. ME-CSIA is nowadays a routine tool for studies on industrial pollutants like chlorinated solvents and hydrocarbons in groundwater but is not yet widely used for the monitoring of pesticide residues in soils. This is because pesticide concentrations are generally low, and some soil constituents can pose difficulties for the chromatographic separation of pesticide residues.

To evaluate the initial stable isotope composition of pesticides, we collected more than 120 commercial formulations of pesticides currently used in France, containing approximately100 different active molecules. The commercial formulations were extracted, the extracts purified, and stable isotope analysis was made on the active molecules using GC-IRMS, mostly for $^{13}C$ and $^{15}N$. In parallel, isotopic compositions of pesticides were retrieved from the peer-reviewed literature. This still growing database contains until now around 550 isotopic compositions for 71 active ingredients issued from different manufacturers, mostly analytical standards, for the following isotopes: $^{13}C$ (61%), $^{15}N$ (14%), $^{37}Cl$ (14%), and $^{2}H$ (11%). These data reflect the overall isotopic variability and trackability of pesticide residues in the environment using this unique database.

To understand the environmental fate of pesticides which is governed by various transformation processes like bio- and photodegradation, or other abiotic degradations, isotope fractionation values were also retrieved from the peer-reviewed literature, resulting in a catalogue of approximately 260 fractionation values for 33 compounds. Most of them include legacy pesticides like *s*-triazines including atrazine, but also lindane, DDT, chlordecone, and some organophosphorus compounds. Based on these reference values, specific transformation processes may be identified in the environment, and hopefully quantified using the Rayleigh concept for isotope fractionation during reaction. To do this, protocols for extractions of pesticides from soil samples collected in fields regularly exposed to pesticides were elaborated in our project and their robustness is tested.

The main outcome of DECISIVE is the elaboration of a guideline for using ME-CSIA for the monitoring of pesticides in soils and soil runoff. This guideline will include the terminology and definitions of the isotope approaches, a collection of protocols for extraction of pesticides from soils or commercial formulations, an overview of analytical techniques for stable isotopes in pesticides, a detailed description of the Rayleigh concept. A guide for isotope data interpretation, and the above-mentioned databases as a tool for the data interpretation will be also included.

## P51 Stable isotopes of oxygen: the key to understand the soil fate of fertilizer-derived phosphorus?

*Mario Álvarez-Salas[1,*], Federica Tamburini[1], Jakob Magid[2], Astrid Oberson[1]*

[1]ETH Zürich, Institute of Agricultural Sciences, Group of Plant Nutrition, Eschikon 33, 8315 Lindau, ZH, Switzerland. * mario.alvarez@usys.ethz.ch
[2]University of Copenhagen (KU), Plant and Soil Science Section, Department of Agricultural Sciences, Faculty of Life Sciences, Thorvaldsensvej 40, DK-1871 Frederiksberg C, Copenhagen
*presenting author: mario.alvarez@usys.ethz.ch

Sourcing phosphorus (P) locally from wastes produced in urban or agricultural environments is important for achieving circular food systems. In the long-term field trial CRUCIAL (Magid et al., 2006) plots have been fertilized for over two decades with one of five bio-wastes (human urine, composted organic household waste, sewage sludge, cattle manure, cattle slurry, cattle deep litter) in doses that exceed crop P requirements (12-621 kg $P*ha^{-1}*a^{-1}$), and have positive P balances (Lemming et al. 2019). We have sampled soil plots one month before and one month after the yearly fertilization, as well as frozen samples of archived fertilizers applied in previous years. We determined total P concentration and P distribution in operationally defined fractions using an adapted sequential extraction protocol (Hedley & Stewart, 1982). We use a stable isotopic approach using oxygen isotopes in phosphate ($\delta^{18}$O-P) as indicators of the presence of P that has cycled through specific enzyme-driven soil microbial P turnover processes in the readily soluble P (anion exchange resin extractable P) and in the recalcitrant P fraction (1M HCl extractable P) of bio-wastes and bio-waste treated soils. We hypothesized that the fertilizer $\delta^{18}$O-P signal in the readily soluble P fraction of the soil will be overwritten by microbial processes but found in the recalcitrant P fraction as it contains P forms that are not easily accessible to microbial processes. However, preliminary results of the cattle-waste amended soils show that the fertilized soil $\delta^{18}$O-P in the readily soluble P ($\delta^{18}$O-P 16-19 ‰ VSMOW) resembles the fertilizer signal, and its clearly differentiated from the soil microbial P pool ($\delta^{18}$O-P 11-13 ‰ VSMOW) and recalcitrant P pool ($\delta^{18}$O-P 10-12 ‰ VSMOW). Our results indicate that microbial processes are not capable of overwriting the $\delta^{18}$O-P of the readily soluble P pool with the $\delta^{18}$O-P of the microbial P pool at the P fertilization level from the CRUCIAL field trial even one year after the last fertilization. This also suggests that this stable isotopic approach has a potential use as a tracer of fertilizer-P in soils.

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## P52 New insights into climate change-driven soil $N_2O$ production and emissions in managed montane grassland

*Elena Stoll[1,*], Eugenio Diaz-Pines[2], David Reinthaler[1], Jesse Radolinski[1], Michael Schloter[3,4], Stefanie Schulz[3], Clara Duffner[3,4], Stephan Glatzel[5], Julia Kurth[3], Ye Tian[6], Sophie Zechmeister-Boltenstern[2], Erich Pötsch[7], Wolfgang Wanek[6], Michael Bahn[1], Eliza Harris[1,8]*

[1]Functional Ecology, Department of Ecology, University of Innsbruck, Innsbruck, Austria
[2]Institute of Soil Research, University of Natural Resources and Life Sciences, Vienna, Austria
[3]Research Unit Comparative Microbiome Analysis, Helmholtz Zentrum München, Neuherberg, Germany
[4]Chair of Soil Science, Technical University of Munich, Freising, Germany
[5]Geoecology, Department of Geography and Regional Research, Faculty of Geosciences, Geography, and Astronomy, University of Vienna, Vienna, Austria
[6]Division of Terrestrial Ecosystem Research, Department of Microbiology and Ecosystem Science, Center of Microbiology and Environmental Systems Science, University of Vienna, Vienna, Austria
[7]Institute of Plant Production and Cultural Landscape, Agricultural Research and Education Centre, Raumberg-Gumpenstein, Austria
[8]Swiss Data Science Center, ETH Zürich, Zürich, Switzerland
*presenting author: elena.stoll@ubik.ac.at

Soils are dominant sources of atmospheric nitrous oxide ($N_2O$), a powerful greenhouse gas. $N_2O$ is produced by microbial N transformation processes, mediated mainly by nitrifiers under oxic conditions as well as denitrifiers, which catalyze the stepwise reduction of nitrate to $N_2$ under anoxic conditions. Thus, these processes depend heavily on soil conditions, including soil moisture, aeration, and substrate availability. Measurements of $N_2O$ isotopic composition can be used to distinguish between $N_2O$ emissions from denitrification, with low isotopic site preference, and nitrification, with high site preference.

Future climate projections in many regions, including the Alps, indicate warming-induced changes of the hydrological cycle, with impacts on soil moisture and increased occurrence of extreme summer droughts, which will most likely affect $N_2O$ emission rates. In addition, elevated carbon dioxide ($eCO_2$) and elevated air temperature will influence on N cycling and $N_2O$ emission rates, but it is unknown yet whether these effects are synergistic, antagonistic, or additive.

Here we tested how soil $N_2O$ fluxes, isotopic composition, and emission pathways in a managed montane grassland in Austria respond to environmental changes using a multifactorial global change experiment that combines warming, $eCO_2$, and summer drought. *In-situ* $N_2O$ flux measurements, online high time-resolution isotopic measurements using spectroscopy, and $N_2O$ isotope depth profiles were used to understand $N_2O$ emission pathways, supported by the molecular quantification of the nitrifiers and denitrifiers, soil and microclimate measurements.

We expect that in a future warmer climate under elevated $CO_2$, $N_2O$ emission rates will increase, due to increased denitrification rates and that the interactive effects are non-additive. The increased contribution of denitrification will be evident in low $N_2O$ site preference values. Furthermore, we hypothesize that drought has a greater effect than warming and elevated $CO_2$ on

$N_2O$ emissions. Our results provide unprecedented insights into the effects of global changes on soil N dynamics and $N_2O$ emission pathways in managed montane grasslands. The findings may help to improve modelling approaches to simulate soil N cycle dynamics at the atmosphere-biosphere interface, which could be used to further constrain terrestrial $N_2O$ production and consumption rates.

### P53 Assessing methoxychlor contamination and natural attenuation in a polluted aquifer using carbon compound specific isotope analyses

*M. Vinyes-Nadal[1,2,*], S. Gil-Villalba[1,2], A. Soler[1,2], N. Otero[1,2,3], C. Torrentó[1,2]*

[1]Grup MAiMA, Departament de Mineralogia, Petrologia i Geologia Aplicada, Facultat de Ciències de la Terra, Universitat de Barcelona, Barcelona, Spain
[2]Institut de Recerca de l'Aigua, Universitat de Barcelona, Barcelona, Spain
[3]Serra Húnter Fellowship, Generalitat de Catalunya Barcelona, Barcelona, Spain
*presenting author: martivinyesnadal@ub.edu

Methoxychlor is an organochlorine insecticide that has been widely used as an alternative for dichlorodiphenyltrichloroethane (DDT) since it has a lower toxicity and biodegradability[1]. Despite this, it was withdrawn from the European market in 2002 (91/414/EEC) due to its acute toxicity, bioaccumulation and endocrine disruption activity. Its high persistence and high tendency to adsorb in particles causes methoxychlor to be widespread and frequently detected in surface and groundwater. For these reasons, it is imperative to develop methods for monitoring methoxychlor and elucidating its degradation in the environment so water management and remediation actions can be improved.

The goals of this work are to detect the methoxychlor contamination hot spots and to find evidences of degradation in a polluted site located in Òdena, 50 km NW of Barcelona (NE Spain). To these ends, periodic concentration and carbon compound specific isotope analyses (CSIA) were conducted to relate temporal and spatial shifts in isotope ratios with degradation and to enable the track of those degradation processes[2].

The methods for extraction, preconcentration and analysis of methoxychlor in environmental water samples were set-up for concentration analysis and CSIA. A Solid Phase Extraction (SPE) method, adapted from EPA METHOD 525.33, was validated for 500 mL water samples, for concentration analysis, and upscaled and validated for 20 L water samples, for isotopic analyses. Hot-spots of methoxychlor contamination and different degradation products were detected in groundwater. Those degradation products may be the result of different degradation processes. The compound specific isotope ratios are currently being analyzed. The CSIA results may allow to confirm the degradation processes that are releasing those different metabolites and determine others. All this information will be useful in the future remediation decision making process.

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### P54 Combining isotope mixing and fractionation with a new modelling tool applying the Monte Carlo approach

*D. Lewicka-Szczebak[1,*], M. Lewicki[2] & G. Skrzypek[3]*

[1]Laboratory of Isotope Geology and Geoecology, Institute of Geological Sciences, University of Wrocław, Poland
[2]Institute of Nuclear Physics, Polish Academy of Science, Kraków, Poland
[3]West Australian Biogeochemistry Centre, The University of Western Australia, Perth, Australia
*presenting author: Dominika.lewicka-szczebak@uwr.edu.pl

The available stable isotope mixing models allow advanced quantification of mixing proportions but usually do not account for the stable isotope fractionation of the product. Here we present the newly developed model using Markov Chain Monte Carlo approach: isotope FRactionation And Mixing Evaluation (FRAME), with a user-friendly graphical interface that can simultaneously determine mixing proportions and progress of the fractionating process. The isotope fractionation may be defined by the user for specific requirements of the particular isotope system, e.g., open or closed system fractionation. The model can integrate up to three stable isotope signatures of each compound. This modelling approach has been designed and already validated for identifying $N_2O$ production pathways and quantifying $N_2O$ reduction progress in soil incubation experiments (Lewicka-Szczebak et al., 2020). However, it can be applied to other isotope systems where both isotope mixing and fractionation may coexist.

In this presentation, we show the model performance based on two different case studies: *(i)* the soil-derived $N_2O$ isotope studies and *(ii)* the river nitrate isotope budget. For *(i)* the model includes three isotope parameters ($\delta^{18}O$, $\delta^{15}N$, and *SP* – site preference – the difference in $\delta^{15}N$ value between central and peripheral position of the linear $N_2O$ molecule) to determine the contribution of four $N_2O$ production pathways and the progress of $N_2O$ reduction to $N_2$. For *(ii)* the model includes two isotope parameters ($\delta^{18}O$, $\delta^{15}N$) to determine fractions of three nitrate sources and the progress of nitrate fractionation associated with denitrification.

The model is potentially applicable across various study fields that employ isotope analysis. The open mathematical design allows for the implementation of additional processes that alternate the characteristics of the final mixture and can be applied to a broad range of mixing and fractionation models.

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## P55 Tracing anaerobic decomposition of lactate, butyrate, propionate, and acetate by means of carbon isotopic analyses of products $CH_4$, $CO_2$, and DIC in the continuous-flow open systems

*M.Bucha[1,*], Ł. Pleśniak[1], W. Drzewicki[1], M. Jakubiak[1], A. Trojanowska-Olichwer[1], A. Detman[2], A. Chojnacka, E. Łupikasza[4], A. Sikora[2]& M.O. Jędrysek[1]*

[1]Institute of Geological Sciences, University of Wroclaw, Wroclaw, Poland
[2]Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw, Poland
[3]Institute of Biology, Warsaw University of Life Sciences, Warsaw, Poland
[4]Institute of Earth Sciences, University of Silesia, Sosnowiec, Poland
*presenting author: michal.bucha@uwr.edu.pl

The common products of acidogenesis, as lactate, butyrate, propionate, and acetate, were decomposed in the Upflow-Anaerobic-Sludge-Blanket bioreactors working in the continuous-flow open systems. The observed $^{13}C/^{12}C$ ratios, reflected by $\delta^{13}C$ value, in substrates and the main products ($CH_4$, $CO_2$, and DIC) ranged within their natural abundances. Both, variations in isotopic ratios and concentrations of organic acids, in the effluents, were analyzed in order to better understand methanogenic processes.
Carbon isotope fractionation in the $CO_2$-$CH_4$ system reflected the $\alpha^{13}C_{CO2\text{-}CH4}$ factor, revealing a massive acetate decarboxylation. Moreover, the $CO_2$ reduction with the domination of butyrate and propionate was detected, although the acetic acid was present in the effluents from all the bioreactors. Butyric acid was the most resistant, and its decomposition resulted in $^{13}C$-enrichment of DIC. Lactic acid was the compound utilized almost completely. The above observations were also confirmed by means of the statistical analyses.
Comparative analysis of our $\delta^{13}C(CH_4)$ and $\delta^{13}C(CO_2)$ values with other based on natural substrates (detritic lignite, xylite, maize silage, cattle manure) showed that isotope fractionation differs significantly in closed system (potential thermodynamic processes). In the open systems, the isotope fractionation factor $\alpha^{13}C_{CO2\text{-}CH4}$ may be affected to apparent values mainly due to depletion or enrichment of the decomposed substance. Continuous acetate supply to bioreactors resulted in the formation of $CO_2$ enriched in light carbon isotopes and shifting the isotope fractionation factor $\alpha^{13}C_{CO2\text{-}CH4}$ to values typical for methane oxidation. Therefore, isotopic data gained even from constant temperature open systems must be very carefully considered due to dynamics of organic acids concentrations and their isotopic ratios. This conclusion is important for applications of stable isotope probing in the industry and biotechnology.

Funding: We acknowledge the support of The National Science Centre, Poland, through grant UMO-2015/17/B/NZ9/01718 and NAWA Project 3205/2003/21.

## P56 Constraining interplay between kinetic and equilibrium isotope effects during anammox in a wastewater treatment system

*P. M. Magyar[1,*], D. Hausherr[2], R. Niederdorfer[3], J. Mohn[4], H. Bürgmann[3], A. Joss[2] & M. F. Lehmann[3]*

[1]Department of Environmental Sciences, University of Basel, 4056 Basel, Switzerland
[2]Eawag, Swiss Federal Institute of Aquatic Science and Technology, Department of Process Engineering, 8600 Dübendorf, Switzerland
[3]Eawag, Swiss Federal Institute of Aquatic Science and Technology, Department of Surface Waters - Research and Management, 6047 Kastanienbaum, Switzerland
[4]Laboratory for Air Pollution/Environmental Technology, Empa, Swiss Federal Laboratories for Materials Science and Technology, 8600 Dübendorf, Switzerland
*presenting author: paul.magyar@unibas.ch

Anammox plays a pivotal role in both natural and engineered systems as a process that simultaneously converts fixed nitrogen to $N_2$ and regenerates $NO_3^-$. In aquatic and terrestrial ecosystems, isotopic measurements, especially of the $NO_3^-$ pool, provide an essential constraint on the processes that regulate the supply of fixed nitrogen, but the isotope effects of anammox remain poorly constrained. We present measurements of the $\delta^{15}N$ and $\delta^{18}O$ of $NO_3^-$, $NO_2^-$, and $NH_4^+$ as processed by anammox in a mixed microbial community enriched for N removal from wastewater. We find that oxygen isotope effects expressed in the $NO_2^-$ and $NO_3^-$ pools include substantial contributions from equilibration reactions with water superimposed on kinetic isotope effects. Equilibrium between water and $NO_2^-$ during processing by anammox is greatly accelerated over abiotic rates of exchange even when $NO_2^-$ is rapidly being consumed. The $\delta^{15}N$ values of $NO_3^-$ and $NO_2^-$ also show evidence of equilibration, raising the possibility that nitrite oxidation is partially reversible. Despite this complexity, $NO_2^-$ and $NO_3^-$ isotope measurements can be used to diagnose changes in the activity of anammox and related processes within the wastewater treatment system during a low-temperature perturbation, while the $\delta^{15}N$ of $NH_4^+$ appears to reflect physiological variation within the anammox cell.

## P57 Understanding biogeochemical controls on nitrous oxide production and consumption in Lake Lugano, Switzerland

*T. Einzmann[1,*], M. Lehmann[1], J. Zopfi[1], C. Frey[1]*

[1]Aquatic and Isotope Biogeochemistry, Department of Environmental Sciences, Basel, Switzerland
*presenting author: teresa.einzmann@unibas.ch

Nitrous oxide ($N_2O$) is a strong greenhouse gas and an ozone-destroying agent, the concentration of which in the atmosphere has been increasing significantly over the last few decades. The contribution of lacustrine systems to global $N_2O$ emissions is uncertain because of a lack of understanding of the environmental controls on the production and consumption processes involved in $N_2O$ cycling, and their spatial and temporal variability.
As part of my PhD project, seasonal $N_2O$ cycling in Lake Lugano, a eutrophic South alpine lake located at the Swiss-Italian border, will be investigated. Lake Lugano consists of two main basins with different mixing regimes. The North Basin is permanently

stratified with an anoxic and sulfidic deep hypolimnion, whereas the South Basin shows seasonal stratification and bottom-water anoxia during summer and complete mixing in winter. These contrasting mixing dynamics can potentially lead to different seasonal $N_2O$ emission patterns, which will be investigated in the current project. While there is significant interannual and seasonal variability in $N_2O$ concentrations particularly in the South Basin, the episodic occurrence of very high $N_2O$ concentrations (up to 900 nmol/l) in both basins have been observed, which begs a more detailed investigation of processes that produce or consume $N_2O$ in Lake Lugano. $N_2O$ can be formed as a byproduct during nitrification in the oxic environment, i.e., the stepwise oxidation of ammonium to nitrate. Another important process contributing to $N_2O$ production is nitrifier denitrification by ammonium-oxidizing bacteria at low oxygen levels, where nitrite is sequentially reduced to $N_2O$. Denitrification on the other hand is the main sink for $N_2O$ via the reduction of nitrate over nitrite over $N_2O$ to finally $N_2$ under anaerobic conditions. However, incomplete denitrification can result in the accumulation of $N_2O$ particularly at the suboxic-anoxic interface. Here, we study the isotopic composition of $N_2O$ throughout the water column over one year in order to identify reductive versus oxidative $N_2O$ production (the different $N_2O$ production pathways are known to produce distinct $N_2O$-isotopic/isotopomeric signatures). The occurrence of these processes in both basins of Lake Lugano will be further verified, and seasonal rate fluctuations will be quantified, via tracer incubation experiments using $^{15}N$ labeled nitrogen compounds. In addition, the microbial community composition in the lake and the enzymes catalyzing reactions involved in $N_2O$-transforming processes are studied to understand changes within the community structure with varying hydrographical conditions. This, in turn, will help to gain a better picture of $N_2O$ cycling in Lake Lugano and its role as a $N_2O$ source or sink to the atmosphere throughout the year, with general implications for lacustrine $N_2O$ emissions under different mixing regimes.

### P58 $\delta$-scale calibration for stable isotope analysis of $O_2$ by continuous flow IRMS from -10 to +95 ‰ with in-vitro photosynthesis experiments

*Sarah G. Pati[1,*], Carolina F. M. de Carvalho[1] &*

*Moritz F. Lehmann[1]*

[1]Department of Environmental Sciences, University of Basel, Basel, Switzerland
*presenting author: sarah.pati@unibas.ch

Molecular oxygen ($O_2$) is one of the most important electron acceptors for a large variety of biotic and abiotic processes in the environment, playing a vital role in ecosystem health and biogeochemical cycling of elements. Assessing sources and sinks of dissolved $O_2$ in aquatic environments is challenging, because multiple $O_2$ consuming and producing processes can occur simultaneously. Isotope analysis of $O_2$ has been shown to (i) help distinguish photosynthesis, respiration, and gas exchange in field studies and (ii) reveal reaction mechanisms of $O_2$ reduction in laboratory studies. For measurements of $\delta^{18}O$ values of $O_2$ by continuous flow isotope ratio mass spectrometry (CF-IRMS), however, one major challenge remains unsolved, and that is the lack of reference materials. For CF-IRMS measurements of $O_2$, only one reference standard, namely $O_2$ from ambient air ($\delta^{18}O_{VSMOW}$ = 23.88 ‰), is typically used to reference $\delta^{18}O$ values to the VSMOW scale. To investigate the potential bias introduced by this one-point calibration, we performed a series of in-vitro photosynthesis experiments in waters with $\delta^{18}O$ values from -10 to +95 ‰. We used thylakoids extracted from spinach leaves and an external, artificial electron acceptor to ensure that only water splitting occurs without biological $O_2$ consumption (photorespiration). In these experiments, $\delta^{18}O$ values of photosynthetically produced $O_2$ are expected to be identical to $\delta^{18}O$ values of the source water, because there is no isotope fractionation associated with photosynthesis. Therefore, deviations between $\delta^{18}O$ measurements of water by laser absorption spectroscopy with three reference standards and $\delta^{18}O$ measurements of $O_2$ by GC/IRMS with one reference standard will reveal $\delta$-scale compression or expansion of the latter. In our experiments, the linear regression between $\delta^{18}O$ values of the photosynthetically produced $O_2$ and $\delta^{18}O$ values of the source water produced a slope of 0.99±0.01 and an intercept of 1.4±0.4. The fact that the slope was unity within error indicates that there is no significant error introduced by the lack of a second reference standard for measurements of $\delta^{18}O$ in $O_2$ with our GC/IRMS system between -10 and +95 ‰. Preliminary results for $\delta^{17}O$ measurements of $O_2$ by GC/IRMS, on the other hand, indicate that there is a significant scale compression when using only $O_2$ from air as a reference standard. Together with oftentimes substantial argon interference, this scale compression further hinders accurate $\delta^{17}O$ measurements of $O_2$ by CF-IRMS.

### P59 Oxygen isotope fractionation during enzymatic $O_2$ consumption reactions

*Carolina F. M. de Carvalho[1,*], Moritz F. Lehmann[1] &*

*Sarah G. Pati[1]*

[1]Aquatic biogeochemistry, Department of Environmental Sciences, University of Basel, Basel, Switzerland
*presenting author: carolina.carvalho@unibas.ch

Molecular oxygen ($O_2$) is one of the most important electron acceptors for a variety of biotic and abiotic processes in the environment. Measuring changes in the stable isotopic composition of $O_2$ can be a valuable tool to help track and identify relevant $O_2$ consuming processes. To be able to track and identify an $O_2$ consuming process in the natural environment, it is necessary that the isotope enrichment factor (ε), for the given $O_2$ consuming process is known. A large range of oxygen isotope enrichment factors associated with biological respiration have been reported in field and laboratory studies (ε from -29 to -1 ‰). The observed variability in ε values has mainly been attributed to different types of organisms respiring. However, what ultimately modulates respiratory isotope fractionation of $O_2$ remains unclear. All biological $O_2$ consumption, including respiration, detoxification and biosynthesis, occur at the enzyme level. Only a few $^{18}O$ kinetic isotope effects ($^{18}O$-KIE) have been reported for isolated enzymatic $O_2$ reduction reactions. These laboratory-scale studies also display a relatively wide range of O isotope fractionation ($^{18}O$-KIE from 1.010 to 1.033), without clear correlation between $^{18}O$-KIE values and the type of enzyme, substrate, or $O_2$-reduction mechanism. Thus, the main drivers of the observed variation in the isotope fractionation of $O_2$ remain unclear, also at the enzyme level. In this study, we apply $O_2$ stable isotope analysis to a broader selection of $O_2$ consuming

enzymes, to improve our molecular understanding of the isotope fractionation of $O_2$ at the enzyme/enzyme-class level and as a basis to better understand and generalize oxygen isotope fractionation systematics at the organism level. We have conducted laboratory experiments with a series of commercially available oxidase enzymes with copper- and flavin-dependent active-site structures. Oxidases reduce $O_2$ to water (four-electron reduction), or to hydrogen peroxide (two-electron reduction), decoupled from substrate oxidation, and thus allow us to test the hypotheses that $O_2$ $^{18}$O-KIEs correlate: (i) with the number of electrons transferred to $O_2$ and/or (ii) with the type of active-site structure that is involved in the $O_2$ reduction. We will present preliminary results of experimentally determined $^{18}$O-KIEs for $O_2$ reduction by copper- and flavin- dependent oxidases that reduce $O_2$ to water or hydrogen peroxide. Our findings thus far suggest that the type of active site structure might not correlate with the $^{18}$O-KIE. However, this tentative suggestion is based on $^{18}$O-KIEs obtained solely for a few oxidases that reduce $O_2$ only to $H_2O_2$. More $^{18}$O-KIEs experiments are planned with more copper- and flavin- dependent oxidases, as well as other active site dependent oxidases, that reduce $O_2$ to $H_2O_2$ and to water.

### P60 Hydrogen isotope exchange between trichloroethene and water under mild environmental conditions – implications for the use of hydrogen CSIA in contaminated site assessment

*T. Kuder[1,*] & A. S. Ojeda[2]*

[1]Department of Geosciences, University of Oklahoma, Norman, USA
[2]Department of Geosciences, Auburn University, Auburn, USA
*presenting author: tkuder@ou.edu

Trichloroethylene (TCE) is a key legacy environmental contaminant and a major driver for contaminant remediation projects. Hydrogen compound-specific isotope analysis (CSIA) of halocarbons is a relatively recent technique, predominantly used as a line of evidence for apportionment of TCE sources. To date, no published data addressed the potential for hydrogen exchange between TCE and hydroxide in water solution under typical groundwater pH conditions. In this study, we determined the pH- and temperature-dependent rates of the exchange in controlled experiments, to constrain the scope of applications of hydrogen CSIA at field sites contaminated by TCE. Relatively high rates of the exchange were associated with above-neutral pH and slower, but not negligible, rates were associated with sub-neutral pH. Hydrogen exchange alters the hydrogen isotope composition of TCE in solution, masking initial sources signatures or degradation signatures over environmentally relevant time scales. In effect, the TCE-water exchange restricts the classical applications of hydrogen CSIA data to a subset of distinctly acidic environments. On the other hand, as the rates of the exchange can be predicted for specific combinations of pH and temperature, the process could be potentially utilized in age-dating of TCE contamination. Similarly to the effect on TCE, the hydrogen exchange process would also impact the utility of hydrogen CSIA in the assessment of other halogenated species acting as weak Brønsted acids, such as chloroform.

### P61 Isotopic Analysis of Nitrous Oxide During El Niño and La Niña in the Eastern Tropical South Pacific

*N. Gluschankoff[1,*], A. E. Santoro[2], C. Buchwald[3], K. L. Casciotti[1]*

[1]Department of Earth System Science, Stanford University, Stanford, USA
[2]Ecology, Evolution, and Marine Biology, University of California Santa Barbara, Santa Barbara, USA
[3]Department of Oceanography, Dalhousie University, Halifax, Canada
*presenting author: noahglu@stanford.edu

Nitrous oxide ($N_2O$) emissions from the Eastern Tropical South Pacific (ETSP) are highly sensitive to the interannual biogeochemical changes imparted by the warm and cold phases of the El Niño-Southern Oscillation (ENSO), known as El Niño and La Niña, respectively. When emitted to the atmosphere, $N_2O$ becomes a powerful greenhouse gas and the dominant degrader of stratospheric ozone. Thus, understanding what modulates its cycling underlying these emissions is imperative for constraining Earth's climate. Using isotopic measurements of $N_2O$, nitrate, and nitrite, we provide the first study directly investigating the alterations to the mechanisms and substrates mediating $N_2O$ cycling between both ENSO states. Results from our work corroborate previous findings that a larger accumulation of $N_2O$ in near-surface waters and greater atmospheric flux occur during La Niña compared to El Niño. The isotopomers and isotopocules of $N_2O$ provide nuanced insights into the "balancing act" of production and consumption in subsurface, low oxygen waters that lead to the observed differences between ENSO states. During La Niña, compared to El Niño, we observed a greater contribution of $N_2O$ formed by denitrification versus nitrification. Specifically, we attribute dentirification, with nitrite (as opposed to nitrate) as the starting substrate, to be a significant pathway of $N_2O$ formation in La Niña oxycline waters due to appreciably negative $\delta^{15}N$ values of the outer (beta) atom in $N_2O$ paired with low $\delta^{15}N^{bulk}$ values. Furthermore, a small, but significant fraction of $N_2O$ in oxycline waters must also be consumed in order to drive site preference $\delta^{15}N$ values to intermediate ranges. Due to ongoing ocean deoxygenation and projected increases in the frequency and intensity of ENSO events, we encourage further investigation into the mechanistic controls of $N_2O$ cycling in the tropical Pacific Ocean to better constrain future $N_2O$ cycling scenarios.

### P62 Tracing $N_2O$ formation in full-scale wastewater treatment with natural abundance isotopes

*J. Mohn[1,*], W. Gruber[2], P. Magyar[3], K. Zeyer[1], L. von Känel[4], E. Morgenroth[2], M. F. Lehmann[3], D. Braun[4], A. Joss[2]*

[1]Laboratory for Air Pollution / Environmental Technology, Empa, Dübendorf, Switzerland
[2]Department Process Engineering, Eawag, Dübendorf, Switzerland
[3]Aquatic and Isotope Biogeochemistry, University of Basel, Basel, Switzerland
[4]Department of Civil, Environmental and Geomatic Engineering, ETH, Zürich, Switzerland
*presenting author: joachim.mohn@empa.ch

Nitrous oxide ($N_2O$) dominates greenhouse gas emissions in wastewater treatment plants (WWTPs). Formation of $N_2O$ occurs during biological nitrogen removal, involves multiple microbial pathways, and is typically very dynamic. Consequently, $N_2O$ mitigation strategies require an improved understanding of

nitrogen transformation pathways and their modulating controls. Analyses of the nitrogen (N) and oxygen (O) isotopic composition of $N_2O$ and its substrates at natural abundance have been shown to provide valuable information on formation and reduction pathways in laboratory settings, but have never been applied to full-scale WWTPs.

Here we show that N-species isotope ratio measurements at natural abundance level, combined with long-term $N_2O$ monitoring, allow identification of the N2O production pathways in a full-scale plug-flow WWTP (Hofen, Switzerland). The proposed approach can also be applied to other activated sludge systems. Heterotrophic denitrification appears as the main $N_2O$ production pathway under all tested process conditions, while nitrifier denitrification was less important, and more variable. $N_2O$ production by hydroxylamine oxidation was not observed. Fractional $N_2O$ elimination by reduction to dinitrogen ($N_2$) during anoxic conditions was clearly indicated by a concomitant increase in SP, $\delta^{18}O(N_2O)$ and $\delta^{15}N(N_2O)$. The extent of $N_2O$ reduction correlated with the availability of dissolved inorganic N and organic substrates, which explains the link between diurnal $N_2O$ emission dynamics and organic substrate fluctuations. Consequently, dosing ammonium-rich reject water under low-organic-substrate conditions is unfavourable, as it is very likely to cause high net $N_2O$ emissions.

Our results demonstrate that monitoring of the $N_2O$ isotopic composition holds a high potential to disentangle $N_2O$ formation mechanisms in engineered systems, such as full-scale WWTP. Our study serves as a starting point for advanced campaigns in the future combining isotopic technologies in WWTP with complementary approaches, such as mathematical modelling of $N_2O$ formation or microbial assays to develop efficient $N_2O$ mitigation strategies.