Conference Agenda

Session

1.1: Methodological Advances

Time: **Monday, 16/June/2025: 9:20am - 10:30am**
Session Chair: **Joachim Mohn**
Session Chair: **Heiko Moossen**

Location: **Ton Schoot Uiterkamp Zaal**

5159.0029 - Energy Academy Europe, Nijenborgh 6, 9747 AG Groningen

Presentations

9:20am - 9:50am

Keynote: A Beginner's Guide to Isotopocule Analysis Using Orbitrap IRMS

Kristyna Kantnerova[1], **Cajetan Neubauer**[2], **Sebastian Kopf**[2]

[1]University of Chemistry and Technology, Prague, Czech Republic; [2]University of Colorado Boulder, CO, United States of America; kristyna.kantnerova@vscht.cz

Isotopocule analysis is a transformative tool for environmental research, offering insights into isotopic distributions across many molecules, even as simple as oxyanions such as nitrate and sulfate. Traditional isotopic methods using isotope ratio mass spectrometry (IRMS) often rely on labor-intensive sample conversions, which can obscure site-specific isotopic information. This presentation introduces the application of electrospray ionization-Orbitrap IRMS, a robust technique for direct isotopocule analysis, circumventing the limitations of classical approaches.

The methodology employs low-flow solution inlets via dual syringe pumps or HPLC autosamplers, ensuring high sensitivity (down to 1 nmol nitrate) and tolerance to matrix interferences. The workflow includes advanced data processing with the open-source R package isoorbi, facilitating isotope ratio calculations, delta value determination, and isotopocule-specific visualizations. Demonstrated applications include precise nitrate isotope analysis from environmental samples, highlighting potential proxies for ecological and agricultural processes.

This talk will showcase method validation using reference materials, comparison with established IRMS protocols, and the potential for unattended long-term sample throughput. The integration of computational tools with Orbitrap technology underlines its promise for advancing isotopic studies in atmospheric, aquatic, and terrestrial systems, paving the way for novel insights into environmental dynamics.

9:50am - 10:10am

Electrospray-Orbitrap is a revolutionary tool for oxyanion clumped isotopologue analysis. How does it perform for natural samples?

Jack Saville[1], **Julien Witwicky**[1], **Elsa Gautier**[1], **Maria Page**[2], **Alexis Lamothe**[3], **Joël Savarino**[1]

[1]Institut des Géosciences de l'Environnement, UGA, CNRS, IRD, Grenoble, France; [2]Université Savoie Mont Blanc, INRAE, Bourget-du-Lac, France; [3]CEREGE, Université Aix Marseille, CNRS, IRD, INRAE, Aix-en-Provence, France; jack.saville@univ-grenoble-alpes.fr

For decades, isotope geochemists have quantified elemental isotope ratios using mass spectrometry. Traditional hard-ionisation, low mass resolution methods such as IRMS or ICP-MS break apart sample molecules for analysis, often destroying the sample's intramolecular isotopic detail.

In contrast, the recently repurposed, bioanalytical Electrospray-Orbitrap mass spectrometer employs soft ionisation and high-resolution mass analysis to directly measure isotopologue ratios on intact molecules. This innovation heralds a new era in stable isotope analysis, advancing beyond traditional elemental isotope ratios, to enable isotopologue-specific measurements, including isotope clumping quantifications.

We showcase the Electrospray-Orbitrap's unique capability to simultaneously quantify an extended range of singly- and doubly-substituted nitrate and sulphate isotopologue ratios, using nanomole amounts of ice core and aerosol sample. This breakthrough enables the first-ever clumped isotopologue analysis of natural oxyanion samples– offering crucial insights into Earth's past atmospheric oxidative capacity, as well as representing a potential new tool in the routine isotopic analysis of oxyanions.

Despite its potential, questions remain regarding the accuracy, precision and robustness of Electrospray-Orbitrap isotopic measurements compared to the established IRMS and ICP-MS methods. To validate its performance, we provide a comprehensive comparison of isotopic measurements obtained from the same alpine ice core and Antarctic aerosol samples using both Electrospray-Orbitrap and traditional techniques.

10:10am - 10:30am

ESI-Orbitrap-MS as a tool for isotopocule analysis on organic molecules

Nils Johannes Kuhlbusch[1,2], **Heiko Hayen**[1], **Andreas Hilkert**[2]

[1]University of Münster, Germany; [2]Thermo Fisher Scientific, Germany; nils.kuhlbusch@thermofisher.com

High precision natural abundance isotope ratio analysis of complex organic molecules has been an important tool in understanding environmental and planetary processes throughout the last decades. State of the art workflows often require the conversion of complex organic molecules to low molecular weight gases to perform a high precision isotope quantification using sector field MS. Recently, the high-resolution accurate-mass of Thermo Scientific^{TM} Orbitrap^{TM} MS has been shown to enable simultaneous, high precision analysis of intact isotopocules with isotopic substitutions from multiple elements, which bears new potential for gathering intramolecular isotopic information.

Here we highlight improvements in ESI-Orbitrap IRMS for the analysis of organic molecules using ^{13}C, ^{2}H, ^{18}O and ^{15}N substituted isotopocules of caffeine and vanillin as a model. Orbitrap results are compared and benchmarked against international reference materials and results obtained by sector field MS revealing accuracies and precisions down to <0.5 ‰ for carbon, 2 ‰ for oxygen, 10 ‰ for hydrogen and 1 ‰ for nitrogen isotopocule ratios. The presented data will show how challenges in Orbitrap-IRMS for complex organic molecules can be overcome by dedicated referencing and calibration procedures, highlighting the need for a wider availability of isotopically characterized reference materials. Performing efficient sample/standard comparison sets requirements on the sample introduction techniques. While many different techniques have been developed for reference introduction in the gas phase, liquid sample and reference introduction approaches still lack the efficiency and robustness of their gaseous counterparts. To this end, we have explored novel sample introduction techniques and online liquid chromatography for better automation and matrix tolerance specifically targeting high precision natural abundance isotopocule ratio quantification in complex organic mixtures. Among other application the developed methods aim to improve the analysis of isotope and isotopocule ratios of inorganic oxyanions and small organic molecules in water and soil samples.

Conference Agenda

Session

1.2: Methodological Advances

Time: **Monday, 16/June/2025: 11:00am - 12:40pm**
Session Chair: **Joachim Mohn**
Session Chair: **Heiko Moossen**

Location: **Ton Schoot Uiterkamp Zaal**

5159.0029 - Energy Academy Europe, Nijenborgh 6, 9747 AG Groningen

Presentations

11:00am - 11:20am

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

Nora M. Bernet[1,2], Federica Tamburini[3], Thomas B. Hofstetter[1,2]

[1]Department of Environmental Chemistry, Eawag, Dübendorf, Switzerland; [2]Institute of Biogeochemistry and Pollutant Dynamics, ETH Zürich, Zürich, Switzerland; [3]Institute of Agricultural Sciences, ETH Zürich, Lindau, Switzerland; nora.bernet@eawag.ch

Understanding the impact of human activities on the metabolic state of organisms from soil and aquatic environments is of paramount importance to implement measures for maintaining ecosystem services. Variations of natural abundance $^{18}O/^{16}O$ ratios in phosphate have been proposed as proxies for the assessment of variations in metabolic activity given the crucial importance of phosphoryl transfer reactions (i.e., the nucleophilic displacement of PO_3^{2-} groups between phosphate esters and water) in biological processes. However, limitations inherent to oxygen isotope analysis by isotope-ratio mass spectrometers have so far restricted a stable isotope-based evaluation of these metabolic processes.

To that end, we explore novel opportunities for oxygen isotope analysis of phosphate and organophosphorus compounds by electrospray ionization (ESI) Orbitrap mass spectrometry (MS) in three steps. First, we identified and optimised critical instrument parameters for accurate $^{18}O/^{16}O$ ratio analysis from the isotopologues of $H_2PO_4^-$. Second, we established the accuracy of $^{18}O/^{16}O$ ratio analysis after phosphate fragmentation to PO_3^- to enable oxygen isotope ratio measurements of organophosphorus compounds. Finally, we evaluate the selective phosphate extraction and analyte purification using Zr-based metal organic frameworks (MOFs).

Our results demonstrate that $^{18}O/^{16}O$ ratios of phosphate and organophosphorus compounds can be determined accurately from both $H_2PO_4^-$ and PO_3^- ions over the range of environmentally observed $^{18}O/^{16}O$ ratios. Measurements are reproducible over more than one year of repeated analyses on a standard ESI-Orbitrap-MS device at phosphate concentrations of 50 µM. Our data furthermore show that analyte extraction and sample purification protocols with MOF sorbents allow for phosphate enrichment from aqueous matrices for isotopic analyses. The procedures developed in our work provide promising avenues for stable isotope analysis of phosphate and organophosphorus compounds with potential applications in studies of microbial metabolisms and the assessment of organophosphorus pollutant sources and transformation.

11:20am - 11:40am

The fascinating world of hydrogen isotopes: innovative techniques illustrated with applications for geochemistry and archeology.

Francois Paul FOUREL[1], Christophe Lecuyer[2], Thibault Clauzel[3]

[1]CNRS UMR5023-LEHNA, France; [2]Laboratoire de Géologie de Lyon, UMR CNRS 5276, University of Lyon; [3]Centre de Recherche et de Restauration des Musées de France Département Recherche - Groupe Datation; francois.fourel@univ-lyon1.fr

Besides being considered as a promising source of energy for the future, the physical and chemical properties of hydrogen are often used to resolve complex scientific questions. In the old days, scientists used off-line vacuum lines, to generate hydrogen for dual-inlet-IRMS analyses. Waters were the first samples for 2H/1H determinations, then the evolution of techniques allowed applications to analyses of organic and inorganic matter. Various reduction techniques (U, Cr, V) were successfully developed. More recently, H2 was accessible to continuous flow techniques, especially using elemental analysers combined to EA-Py-IRMS techniques. Different types of reactors were developed to cover various types of hydrogen isotopic analyses (e.g. chromium reduction, glassy carbon pyrolysis). After a brief summary of technical evolutions, examples of applications for those techniques are presented. The first example is H analysis of refractory minerals where H is a trace element(1). Then a more recent illustration of the improvement for those techniques deals with analyses of δ^2H from bone collagen (δ^2H_{coll}), δ^2H of tooth enamel (δ^2H_{enamel}) and δ^2H of bulk bone material (δ^2H_{bone}) measured on specimens of humans, bovids, horses and a pig from the same archaeological site of Thézy-Glimont, France(2). Samples have previously been studied using other isotopic proxies: $\delta^{18}O$, $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$.We show that similar interpretations can be made from the hydrogen isotope analysis of mineral tooth enamel (δ^2H_{enamel}), which also provides information on children's dietary practices. These results open new prospects in a variety of research topics. Mineral body tissue δ^2H is now a useful proxy associated to the already-used panel of isotopic tools.

(1) Fourel et al. 2017 RCM, Volume31,Issue24,Pages2066-2072,https://doi.org/10.1002/rcm.7996.

(2) Clauzel et al. 2022 Journal of Archaeological Science 147:105676. DOI: 10.1016/j.jas.2022.105676

11:40am - 12:00pm

Development and Exploration of a ^{1}H NMR Spectroscopy Method for Position-Specific ^{15}N Isotope Analysis

Maylis COUTON[1], Valérie FARGEAS[1], Illa TEA[2]

[1]1CEISAM UMR CNRS 6230, Nantes Université, Nantes, France; [2]Universite Claude Bernard Lyon 1, CNRS, ISA, UMR 5280, 5 rue de la Doua, F-69100 Villeurbanne, France; illa.tea@univ-lyon1.fr

Quantitative nuclear magnetic resonance (NMR) is commonly used to determine isotope ratios within molecules (irm-NMR, isotope ratio measured by NMR). While irm-NMR is well-developed for **^{13}C** and **^{2}H**, and to a lesser extent for **^{15}N** in position-specific isotope analysis (PSIA), existing methods often require hundreds of milligrams of material and lengthy experimental times.

To address these limitations, we explored the potential of **^{1}H NMR** to determine the **^{15}N isotope ratio**, allowing position-specific nitrogen isotope analysis using only 5–30 mg of material. This new approach is the first demonstration of **^{1}H NMR** measuring position-specific **^{15}N isotope ratios** within molecules. However, the detection of nitrogen-bound ^{1}H peaks requires specialised sample preparation, particularly for amino acids.

The method involves acquiring **^{1}H NMR spectra** using a single-pulse sequence and quantifying the **^{1}H-^{14}N** and **^{1}H-^{15}N** peak areas by spectral deconvolution using an NMR fitting program. Initial tests on simple molecules, including ammonium chloride (NH_4Cl) and urea, established a calibration curve and demonstrated precision below 10‰ for both compounds. Trueness was achieved at <2‰ for NH_4Cl and <7‰ for urea, highlighting the potential of the method for more complex molecules such as amino acids. A poly-nitrogenous amino acid, tryptophan, was analyzed as a proof of concept.

In addition, we applied the method to a complex biological mixture—urine—to assess the **PSIA of ^{15}N** in a real-world matrix. These results further validate the robustness of this approach for isotope ratio measurements in diverse and challenging samples.

This innovative method paves the way for highly sensitive and position-specific nitrogen isotope analysis in small sample quantities, opening new perspectives for the study of complex nitrogen compounds.

12:00pm - 12:20pm

Recent Advances in Analytical Methods for Carbonate and Water Samples at McMaster University

Sang-Tae Kim

School of Earth, Environment & Society, McMaster University, Canada; sangtae@mcmaster.ca

The McMaster Research Group for Stable Isotopologues (MRSI) is a leading research group whose mission is to remain at the forefront of stable isotope science through innovative research and discovery. Its primary research areas include: (1) “Developing and Refining Geochemical Proxies”, which involves using laboratory-grown minerals to better understand past and current environmental changes, (2) “Reconstructing Local and Global Climate Histories”, where researchers analyze natural samples of various origins to provide essential information for understanding past and modern climate trends, and (3) “Innovating Analytical Techniques for the Stable Isotope Community”, which focuses on developing new and improved methods for stable isotope users.

MRSI’s state-of-the-art research facilities enable high-quality stable isotope analyses and support the group’s groundbreaking research. Over the years, MRSI has made significant contributions to analytical techniques, enhancing the accuracy, precision, and efficiency of stable isotope measurements.

At the upcoming meeting, two recent analytical advances from MRSI will be presented, highlighting its ongoing efforts in analytical geochemistry. These advancements have the potential to further enhance the performance of stable isotope measurements for carbonate and water samples - the most common types of samples used by stable isotope researchers - and help researchers gain deeper insights into Earth's complex environmental systems.

12:20pm - 12:40pm

Comparison of two approaches to quantify N2O reduction in wastewater treatment: N2/Ar analysis by QMS and N2O isotope analysis by OA-ICOS

Hannes Keck[1], Laurence Strubbe[2], Paul Magyar[1], Adriano Joss[2], Andreas Froemelt[2], Joachim Mohn[1]

[1]Empa, Laboratory for Air Pollution / Environmental Technology, Dübendorf, Switzerland; [2]Eawag, Department Process Engineering, Dübendorf, Switzerland; hannes.keck@empa.ch

Reducing nitrous oxide (N_2O) emissions of wastewater treatment is a pressing concern, as they dominate the sector's greenhouse gas footprint. N_2O is primarily produced during microbial denitrification and emitted to the atmosphere when N_2O reduction to atmospheric nitrogen (N_2) is limited. To reduce the climate impact of wastewater treatment the quantification of N_2O reduction, and related N_2 emissions, is essential. Here, we introduce a novel method to measure N_2 emissions from wastewater treatment based on the analysis of N_2/Ar ratios by quadrupole mass spectrometry (QMS) in a closed chamber setup. This novel approach is compared to on-line stable isotope analysis of the isotopic enrichment in residual N_2O emissions ($^{14}N^{15}N^{16}O$, $^{15}N^{14}N^{16}O$, and $^{14}N^{14}N^{18}O$) by off-axis integrated cavity output spectroscopy (OA-ICOS) to estimate N_2 formation. Both measurement techniques were implemented on-site during anoxic treatment of wastewater at the Eawag pilot wastewater treatment plant in Dübendorf, Switzerland. We compare N_2 emission estimates by QMS, OA-ICOS, and an aqueous N-balance and discuss their applicability for full-scale plants. On-line quantification of N_2O reduction and N_2 emissions has the potential to guide the development of process optimization strategies to reduce N_2O emissions and mitigate the climate impact of wastewater treatment, while meeting increasingly stringent N discharge criteria.

Conference Agenda

Session

1.3: Methodological Advances

Time: **Monday, 16/June/2025: 1:40pm - 2:20pm**
Session Chair: **Joachim Mohn**
Session Chair: **Heiko Moossen**

Location: **Ton Schoot Uiterkamp Zaal**

5159.0029 - Energy Academy Europe, Nijenborgh 6, 9747 AG Groningen

Presentations

1:40pm - 2:00pm

Method developments for the measurement of position-specific 13C isotope composition of amino acids

Alexis Gilbert[1,2], Keita Yamada[2], Maxime Julien[3], Mayuko Nakagawa[2]

[1]Utrecht University, The Netherlands; [2]Institute of Science Tokyo, Japan; [3]University of Nantes, France; a.r.gilbert@uu.nl

The stable isotope composition of amino acids records (bio)synthetic pathway and/or environmental conditions, and are thus of great importance in biogeochemistry [1]. While most isotope analysis are conducted at the molecular (or ‘bulk’) level, novel approaches such as position-specific isotope analysis (PSIA) could prove useful in deciphering different sources of amino acids.

Building up on the pioneering work of Abelson & Hoering (1961)[2], we used the ninhydrin reaction to form CO_2 and acetaldehyde (CH_3CHO) from alanine (CH_3-$CHNH_2$-COOH). The d^{13}C of the CO_2 evolved can be measured directly and that of each position in acetaldehyde can be determined through the on-line pyrolysis approach [3]. The method thus allows the determination of d^{13}C values of all positions in alanine.

The approach can be applied directly to a mixture of amino acids (typically a protein hydrolysate) where all amino acids are decarboxylated through the ninhydrin reaction, leaving CO_2 and aldehydes in the headspace of the vial. While the carboxyl position is averaged over all amino acids, the method allows PSIA of the remaining skeleton of the most volatile aldehydes, corresponding to non-polar amino acids: alanine, valine, leucine, isoleucine, phenylalanine, methionine.

We show that the difference between the methyl (CH_3) and the amine group ($CHNH_2$) in alanine from C_3, C_4 and CAM plants is correlated to that found between the two carbons of ethanol, as expected from their common precursor pyruvate. This high-throughput approach can be easily applied to recent sediments, cell cultures or hair, and shows great potential to refine our understanding of the sources of amino acids in natural samples.

[1] Hayes, “Fractionation of the Isotopes of Carbon and Hydrogen in Biosynthetic Processes,” 2001.

[2] Abelson and Hoering, *Proc. Natl. Acad. Sci.*, vol. 47, 1961

[3] Hattori et al., *J. Agric. Food Chem.*, vol. 59, 2011

2:00pm - 2:20pm

Into the ISOVERSE - open-source data tools for efficient, transparent, and reproducible processing of stable isotope data

Sebastian Kopf, Cajetan Neubauer

University of Colorado Boulder, Germany; sebastian.kopf@colorado.edu

Reproducible data processing is a key prerequisite for efficient data exchange, methodological progress, consistent training of new users, and productive discourse in stable isotope research. However, producing a faithful record of every step of the data reduction process from raw data to final results in a reproducible manner can currently be prohibitively time-consuming. All too often data processing that is both transparent and easy to communicate thus falls victim to the enormous effort required to design experiments well, run complex analytical procedures, and interpret the results in the proper geochemical/geologic/ecological context. While this is understandable, insufficiently documented data processing workflows create a high risk for errors to go undetected and become propagated. Additionally, they create barriers to sharing and discussing one’s approach to data reduction effectively. These drawbacks limit opportunities for exchange of ideas, methodological progress, and large-scale data repository efforts aimed at extracting maximum benefit from stable isotope data across the many disciplines that make us of it.

The goal of the ISOVERSE (www.isoverse.org) is to fill this gap by creating a comprehensive software ecosystem of free, open-source tools for efficient, transparent, and reproducible processing of stable isotope data from raw analytical measurements all the way to fully processed stable isotope data ready for publication and repository deposition. By building on best practices in modern data science and software engineering, the ISOVERSE seeks to empower scientists at all career stages and programming levels to share their work more easily and contribute fully reproducible data sets to data repository efforts. In this presentation, I will introduce some of the existing key capabilities of the ISOVERSE and discuss implementation plans for next stage of development over the coming 2+ (grant-funded) years with the goal of stimulating discussion and eliciting constructive feedback from the community of stable isotope experts at JESIUM.

Conference Agenda

Session

2.1: Progress in Reference Materials

Time: **Monday, 16/June/2025: 2:20pm - 3:10pm**
Session Chair: **Harro A.J. Meijer**
Session Chair: **Federica Camin**

Location: **Ton Schoot Uiterkamp Zaal**

5159.0029 - Energy Academy Europe, Nijenborgh 6, 9747 AG Groningen

Presentations

2:20pm - 2:50pm

Keynote: Recent developments concerning VPDB, SI-traceability and measurements of carbon isotope delta

Philip J H Dunn
National Measurement Laboratory, LGC Limited, Teddington, UK; philip.dunn@lgcgroup.com

Isotope delta (*d*) is a quantity that is not traceable to the SI because specific artefacts are used to define isotope delta scales. There are currently two carbon isotope delta scales that both have Vienna Peedee belemnite (VPDB) as the reference. The VPDB scale is defined by the exact carbon isotope delta value assigned to the NBS 19, while the VPDB-LSVEC scale is defined by both NBS 19 and LSVEC. This is not an ideal situation, but highlights the shortcomings of artefact-based measurement scales that evolve over time.

The carbon isotope delta scales could be made SI-traceable through determination “absolute” isotope ratio of VPDB. One might consider $R_{\mathrm{VPDB}}(^{13}\mathrm{C}/^{12}\mathrm{C})$ as the fundamental constant underpinning scales. To achieve this it is necessary to determine the SI-traceable isotope ratio and the VPDB-traceable carbon isotope delta of one or more specific materials.

The use of gravimetric mixtures of isotopes/isotopologues to correct for instrumental mass fractionation is a primary method for SI-traceable isotope ratios. It has been implemented several times using a variety of ^{12}C- and ^{13}C-enriched isotopologues with both IRMS and MC-ICP-MS instrumentation. SI-traceable carbon isotope ratios can also be determined by *ab initio* optical spectroscopy or using ^{13}C-satellites in ^{1}H-NMR.

The use of gravimetric mixtures has recently been improved through selection of ^{12}C- and ^{13}C-enriched glucoses as the starting compounds; careful planning of weighing steps; significant improvements in the determination of the presence of co-enrichment of oxygen and hydrogen; and external validation. This has afforded a reduction in uncertainty for $R_{\mathrm{VPDB}}(^{13}\mathrm{C}/^{12}\mathrm{C})$ by an order-of-magnitude.

This work has been recognized by the IUPAC CIAAW as the “best measurement” of the isotopic composition of carbon and prompted revision of their recommendation for the isotopic composition of VPDB for the first time since 1990 for carbon and 2010 for oxygen.

2:50pm - 3:10pm

Calcium carbonate and water pyrolysis measurements suggest minor adjustment to the VPDB and VSMOW-SLAP δ18O scale relation.

Anita Aerts-Bijma, Dipayan Paul, Albert van Buuren, Harro Meijer
University Groningen, Netherlands, The; a.t.aerts-bijma@rug.nl

Measurements of the isotopic oxygen composition of materials are widely used in many fields. These ^{18}O compositions are expressed using different scales. These scales are only linked indirectly, because water and calcium carbonate reference materials, used to establish these ^{18}O scales, have to be converted to CO_2 first, and the isotopic fractionation of these conversions has only been measured a few times in the past. The anchoring of the two ^{18}O scales is therefore currently sub-optimal.

Primary reference materials, both waters and calcium carbonates, were pyrolyzed within a single measurement sequence in a high-temperature elemental analyser–pyrolysis system connected to an isotope ratio mass spectrometer (IRMS) in continuous-flow mode. As calcium carbonate is difficult to pyrolyze completely, additives were added to reach a 100% yield. The d^{18}O of the calcium carbonates were normalised on the VSMOW-SLAP scale using VSMOW2 and in-house water references.

The average results of 6 international calcites references measured in five independent sequences of pyrolysis measurements showed a difference of 0.07 to 0.09‰ with the presently described relationship in literature between the d^{18}O VPDB and d^{18}O VSMOW-SLAP scale.

The outcome of the study made the direct comparison of the two different ^{18}O scales possible. Our results demonstrate a small discrepancy in the presently recommended relation between the two ^{18}O scales.

Conference Agenda

Session

2.2: Progress in Reference Materials

Time: **Monday, 16/June/2025: 3:40pm - 4:40pm**
Session Chair: **Harro A.J. Meijer**
Session Chair: **Federica Camin**

Location: **Ton Schoot Uiterkamp Zaal**

5159.0029 - Energy Academy Europe, Nijenborgh 6, 9747 AG Groningen

Presentations

3:40pm - 4:00pm

Evaluating the reliability of carbon isotope delta reference materials

Michelle Chartrand
Metrology Research Centre, National Research Council Canada, Canada; Michelle.Chartrand@nrc-cnrc.gc.ca

Isotope ratio analysis is used as a tool to distinguish between different sources of materials, and is applied in many scientific fields such as atmospheric sciences, food authenticity, and archeology. These small differences in isotope ratios require measurements of the highest precision. Unfortunately, several technical obstacles prevent us from reaching acceptable inter-laboratory measurement goals. One of the biggest impediments is the disagreement over the primary reference materials that set the measurement scales. Several recent studies, including a study performed at the NRC, have demonstrated that significantly different results for the same carbon dioxide sample can be obtained depending on which combination of calibration standards are used. Based on these studies, the scientific community has acknowledged that carbon isotope ratio measurements are indeed reported on two distinct measurement scales, depending on which calibration standards were used for the measurements.

Since the recommended discontinuation of the reference material LSVEC, reliable realization of the measurement scales has been questioned. The NRC undertook several measurement campaigns to provide additional data to address some of these questions, and to improve our knowledge about the isotopic composition of these widely used reference materials. First, the isotopic composition of both carbon and oxygen in the suite of IAEA reference materials IAEA-610-611-612 was measured to provide an independent isotope ratio data set for these materials. Second, we provide independent characterization of the NIST carbon dioxide reference gases SRMs 8562-8563-8564 to address the conflicting information about the traceability of the isotope delta values assigned to them. Finally, we will present preliminary results from a project aimed to evaluate the reliability of producing carbon dioxide reference gas from materials other than carbonates.

4:00pm - 4:20pm

New standards for isotope delta measurements of CO2 for atmospheric and biogeoscience applications

Joële Viallon, Edgar Flores, Tiphaine Choteau, Philippe Moussay, Robert Ian Wielgosz
Bureau International des Poids et Mesures (BIPM), Sèvres, France; jviallon@bipm.org

Progress in the development of pure CO_2 gas standards for $\delta^{13}C$, $\delta^{18}O$ and Δ47 measurements as well as CO_2 in air gas standards (with mole fractions in the range 350 to 800 μmol/mol) for $\delta^{13}C$, $\delta^{18}O$ measurements are described. Initial results indicate the potential to produce standards with internal consistencies at the 0.005 ‰ level for $\delta^{13}C$, and standard uncertainties of 0.015 ‰ in relation to the VPDB scale, with the magnitude of the latter principally limited by the homogeneity of primary carbonate reference materials.
Outputs of the project so far include:

- Establishment of a facility to produce stable pure CO_2 gas standards in 6L cylinders at 2 bar with $\delta^{13}C$ values from −1 ‰ to −45 ‰ vs VPDB, with internal consistency approaching the 0.005 ‰ level, and an effective calibration option for dual inlet IRMS systems as demonstrated in the international comparison CCQM−P204 completed in 2021;
- Studies of Δ47 values of mixtures of different pure CO_2 gas, and the reproducibility and stability of these and their potential to act as reference standards for clumped isotope ratio measurements with IRMS systems;
- The development and validation of a cryogenic Air Trapping system to extract CO_2 from air for determination of $\delta^{13}C$ and $\delta^{18}O$-CO_2 with IRMS, including a correction for the N_2O present in samples. The facility is currently being used for another international comparison (CCQM−P239) of 39 CO_2 in in air standards (gas cylinders) from 15 institutes containing CO_2 over the range of 380 $\mu mol\ mol^{-1}$ to 800 $\mu mol\ mol^{-1}$ and $\delta^{13}C$ and $\delta^{18}O$-CO_2 values from +1 ‰ to −43 ‰ and −1 ‰ to −45 ‰, respectively. The method demonstrates excellent reproducibility, with standard deviations of 0.005% and 0.05% for $\delta^{13}C$ and $\delta^{18}O$-CO_2, respectively, and will demonstrate the level of equivalence of new CO_2 in air isotope ratio standards currently being produced.

4:20pm - 4:40pm

First preparation of isotopic nitrous oxide in synthetic air reference materials for underpinning measurements of δ15N-N2O, δ15N-N2OSP and δ18O-N2O

Aimee Hillier[1], Freya Wilson[1], Ruth Hill-Pearce[1], Joachim Mohn[2], David Worton[1]
[1]NPL, United Kingdom; [2]Empa, Laboratory for Air Pollution/Environmental Technology, Dübendorf, Switzerland; aimee.hillier@npl.co.uk

The precise measurement of the nitrous oxide (N_2O) isotope ratio in atmospheric air is required to understand global emission trends. There are no existing internationally accepted reference materials for atmospheric amount fraction N_2O with characterised isotope ratio including uncertainties. There is an urgent need for the development of reference materials to fill this traceability gap and meet the requirements to underpin global measurements. Reference materials are required with traceability to existing international stable isotope ratio scales: AIR-N_2 and VSMOW (Vienna Standard Mean Ocean Water) for $\delta^{15}N$ and $\delta^{18}O$.

We will present progress towards the development of atmospheric amount fraction N_2O in synthetic air reference materials with characterised isotope ratios suitable for calibration of optical isotope ratio spectrometers (OIRS) operating in the field. The reference materials span a wide range of δ values. The pure N_2O used to prepare the N_2O in synthetic air reference materials was produced at Empa and has traceability to the primary AIR-N_2 and VSMOW scales. The N_2O in synthetic air reference materials were characterised for N_2O amount fraction and isotope ratio using OIRS. Nitrous oxide amount fractions in the range of 320-330 $nmol\ mol^{-1}$ were certified with combined (k=2) uncertainties of 0.5 %.

The reproducibility in the isotope ratio of dilutions of the pure N_2O reference materials to ambient amount fraction is shown to be less than 0.5 ‰ for $\delta^{15}N$, $\delta^{15}N^{\alpha}$, $\delta^{15}N^{\beta}$ and $\delta^{18}O$. A study on instrument calibration has demonstrated certification of reference materials with unknown isotope ratios with combined (k=2) uncertainties of 1.1 ‰ and repeatability within 0.3 ‰ for $\delta^{15}N$, $\delta^{15}N^{\alpha}$, $\delta^{15}N^{\beta}$ and $\delta^{18}O$. The sensitivities in the measured isotope ratios to commonly occurring synthetic air matrix impurities and to the N_2O amount fraction have been assessed to provide a full uncertainty budget for the N_2O in synthetic air reference materials.

Conference Agenda

Session

7: Geosciences and Hydrology

Time: **Tuesday, 17/June/2025: 9:00am - 10:30am**
Session Chair: **Jeroen Van der Lubbe**
Session Chair: **Paul Koeniger**

Location: **Ton Schoot Uiterkamp Zaal**

5159.0029 - Energy Academy Europe, Nijenborgh 6, 9747 AG Groningen

Presentations

9:00am - 9:30am

Keynote: Expanding the Hydrological Toolbox: Triple oxygen isotopes in precipitation and surface waters.

Stefan Terzer-Wassmuth[1], Leonard I. Wassenaar[1,2], Lucilena R. Monteiro[1,3], Astrid Harjung[1], Lucia Ortega[1], Yuliya Vystavna[1], Jodie A. Miller[1], Christine Stumpp[4]

[1]International Atomic Energy Agency, Department of Nuclear Sciences and Application, Division of Physical and Chemical Sciences, Isotope Hydrology Section, Vienna, Austria; [2]Andre E. Lalonde National Facility in Accelerator Mass Spectrometry, University of Ottawa, Canada; [3]IPEN - Instituto de Pesquisas Energéticas e Nucleares, São Paulo, Brazil; [4]BOKU University, Institute of Soil Physics and Rural Water Management, Vienna, Austria; s.terzer@iaea.org

For decades, stable isotope applications in hydrology have concentrated on ^{2}H, ^{18}O, and their first-order derivative, the deuterium excess. Only recently has the least-abundant oxygen isotope, ^{17}O, along with its relationship to ^{18}O — the so-called ^{17}O-excess (Δ'^{17}O) — started to attract interest in the hydrological sciences. Applications encompass surface water hydrology, lake evaporation, and any geochemical exchange process involving water O isotope signatures and other matrices such as carbonates or silicates. Nevertheless, its broader application is hindered by the “emerging tracer dilemma,” which includes analytical and metrological challenges, as well as the lack of a homogeneous precipitation baseline dataset to serve as an input function for the hydrological cycle.

Drawing on the reanalysis of over 4,000 samples from the GNIP archive, we present fresh insights into the spatial and seasonal distribution of precipitation Δ'^{17}O. This analysis incorporated Local Meteoric Water Lines from more than 80 locations worldwide, 22 of which are in Europe. The weighted regression slopes exhibited a broad range of distribution, although most were lower than that of the Global MWL. This establishes different starting metrics, such as those used in lake evaporation studies. Our research also included a six-year time series of daily sampled precipitation in Vienna, enabling us to correlate seasonal signatures of Δ'^{17}O and deuterium excess with specific source regions.

The analysis of Δ'^{17}O can provide an additional degree of freedom to hydrological studies, provided that the endmembers are sufficiently distinct relative to the analytical uncertainty. A systematic survey of surface waters, including lakes and wetlands, would enhance the understanding of ^{17}O/^{18}O patterns in evaporation-dominated settings. Strict quality control, such as the expansion of triple -isotope proficiency tests, will aid in benchmarking the precision and accuracy of the analytics, and assuring that data for hyrdological studies are fit for purpose.

9:30am - 9:50am

Clumped isotope thermometry and oxygen isotopes of soil water suggest soil grain size affects season bias of carbonate formation and water isotopes

Kathryn Elaine Snell[1], Rachel Elizabeth Havranek[2], Brett Davidheiser-Kroll[3], Sarah Brookins[1]

[1]University of Colorado Boulder; [2]University of Utah; [3]Thermo Fisher Scientific GmbH; kathryn.snell@colorado.edu

New paleoclimate proxies like clumped isotope thermometry have reinvigorated the investigation of modern soil carbonate; these studies are of use to the paleoclimate proxy community as well as those who study modern soils. To date, most clumped isotope thermometry studies (T(Δ_{47})) of modern soil carbonates have been on carbonates from coarse-grained soils, and show a warm season bias of carbonate formation. Most of these studies have also assumed that soil water d18O, which can be calculated by combining T(Δ_{47}) and pedogenic carbonate δ^{18}O, shares the same seasonal bias as carbonate formation, but few have independently measured soil water δ^{18}O with which to compare to these estimates. We applied clumped isotope thermometry to three fine-grained, clay-rich modern soils in Colorado and Nebraska, USA. At two sites, T(Δ_{47}) overlaps with fall soil temperatures and the timing of soil dry down, suggesting carbonate forms during the fall. At the third site, mean T(Δ_{47}) matches summer soil temperatures. Using a new soil water sampling device we built for this study, we independently measured soil water δ^{18}O, which matched well with the calculated soil water δ^{18}O for the two sites with cooler temperatures, while the calculated soil water δ^{18}O at the third site had a significantly higher isotope value than any observations of soil water δ^{18}O at that site. At all three sites, even in the fall season, measured soil water isotope values at carbonate bearing depths overlap with spring rainfall/snowmelt δ^{18}O, with little to no evaporative enrichment of δ^{2}H and δ^{18}O values. These results show that (1) grain size is an important control on the timing of carbonate formation and soil water dynamics, (2) that the seasonal bias of soil water δ^{18}O can differ from that of carbonate formation, and that (3) soil water δ^{18}O at carbonate-bearing depths is relatively unaffected by evaporative enrichment.

9:50am - 10:10am

Isotopic Disequilibrium in a Sub-Arctic Snowpack: Insights from Multi-Level Vapor Measurements

Shaakir Shabir Dar[1,3], Eric Klein[2], Pertti Ala-aho[1], Hannu Marttila[1], Jeffrey Welker[3]

[1]Water Energy and Environmental Engineering Unit, University of Oulu, Finland; [2]Department of Geological Sciences, University of Alaska Anchorage, United States of America; [3]Department of Biological Sciences, University of Alaska Anchorage, United States of America; shaakir.dar@oulu.fi

Water vapor transport plays a pivotal role in snowpack metamorphism. This study presents multi-level measurements of isotopic compositions (δ^{18}O, δ^{2}H, d-excess) of water vapor within the snowpack pore space and ambient air. By integrating these measurements with comprehensive meteorological data and periodic isotope profiles, we explore the complex dynamics of water vapor within the snowpack-atmosphere continuum. Our findings reveal a depth and time-dependent disequilibrium between the ice matrix and water vapor in the snowpack pore space. Notably, there is a significant isotopic disequilibrium at the base of the snowpack near the ground, contrasting with near isotopic equilibrium in the upper layer. A warming event triggers a widespread transition toward isotopic disequilibrium throughout the snowpack. We hypothesize that ground vapor, initially confined to lower layers in a stratified state—maintained by a snowpack temperature gradient and supported by stable, saturated atmospheric conditions—spreads throughout the snowpack under near-isothermal conditions. This redistribution is influenced by a mixture of factors including solar radiation, temperature, turbulence, and the saturation potential of the atmosphere, which, together with surface sublimation fluxes, drives the water vapor from the snowpack pore space into the atmosphere.

These observations underscore the necessity of incorporating non-equilibrium processes into isotope-enabled snowpack and climate models. This research provides essential insights into how dynamic environmental conditions, such as temperature fluctuations and phase transitions, rapidly influence isotopic variability in the snow-atmosphere continuum, offering significant implications for understanding and modelling water vapor transport in snowpacks.

10:10am - 10:30am

Isotope Studies in the Lusatian Lignite Mining District (Germany)

Maike Groeschke, Paul Koeniger, Anne Gädeke
Bundesanstalt für Geowissenschaften und Rohstoffe (BGR), Germany; maike.groeschke@bgr.de

In 2024, stable isotope samples (δ^2H, δ^{18}O) from ≈75 post-mining lakes, rivers, streams and ≈400 groundwater observations wells have been collected in the Lusatian lignite mining district, as an add-on to the long-term hydrological monitoring program of the mining administration company. The aim was to investigate, whether stable isotopes can be used as an additional tool to improve the understanding of groundwater- lake water interactions and to quantify evaporative loss from post-mining lakes. Samples from the post mining lakes were collected from different depths, and from different parts of the lakes, as well as in different seasons. Groundwater samples were collected from dump aquifers as well as from the surrounding natural aquifers. First results show that different parts of the Lusatian mining district can be discerned isotopically. While lake samples from the western (and northwestern part of the mining district show strongly negative deuterium excess (DE) values (up to -23), indicating higher evaporation loss, the DE values of groundwater samples of surrounding observation wells are consistently positive (with very few exceptions). In contrast, many groundwater samples from the southern part of the Lusatian mining district show comparably lower DE values, indicating lake water seepage into the groundwater. Further statistical analyses of the dataset will help to choose 3-4 representative lakes for in-depth studies, including a more thorough sampling program to study water balance and hydro-meteorological processes in more detail.

Conference Agenda

Session

6.1: Biogeochemistry: Carbon, Nitrogen, Sulfur and Other Cycles

Time: **Tuesday, 17/June/2025: 11:00am - 12:30pm**
Session Chair: **Pascal Boeckx**
Session Chair: **Lucia Fuchslueger**

Location: **Ton Schoot Uiterkamp Zaal**

5159.0029 - Energy Academy Europe, Nijenborgh 6, 9747 AG Groningen

Presentations

11:00am - 11:30am

Keynote: Revealing global patterns of gross N transformations – need for data harmonization

Tobias Rütting
University of Gothenburg, Sweden; tobias.rutting@gu.se

The availability of soil nutrients for plants is dominated by the internal recycling of the nutrient within the ecosystem via **mineralization**. Understanding and quantifying mineralization in soil is, hence, crucial for understanding ecosystem functioning and production. The actual dynamics of mineralization and other nutrient transformations is given by their **gross rates**, which cannot be measured directly but are quantified using an isotopic tracer in conjunction with kinetic modelling. This is one of the most powerful tools within biogeochemistry and has been applied for terrestrial nutrient cycling (mostly nitrogen) since the 1950's.

The use ^{15}N labelling techniques (pool dilution and tracing) to quantify gross nitrogen turnover in soils is widespread today. However, many researchers applying these techniques are seemingly not aware of critical assumptions and limitations of the ^{15}N labelling techniques, which unnecessarily compromises our understanding of ecosystem nitrogen dynamics. Also, differences in applied experimental protocols might hinder meaningful meta-studies on controlling factors for gross N transformations, if these methodological differences are not considered in interpretation.

In this presentation, I will give a historic review on the ^{15}N labelling methodologies for quantification of gross nutrient transformation rates and revisit the underlying assumptions. Based on examples and literature review we will discuss important knowledge gaps that future research should address. Moreover, we will discuss the need of **data harmonization** for meta-analyses and synthesis of gross nitrogen dynamics in soils. This will ultimately improve our understanding of ecosystem nutrient biogeochemistry and, hence, ecosystems functioning.

11:30am - 11:50am

Combining different methodological isotope approaches for estimating N2O processes and N2O reduction

Caroline Buchen-Tschiskale[1], Dominika Lewicka-Szczebak[2], Gianni Micucci[3], Jaqueline Stenfert Kroese[1], Thade Potthoff[4], Reinhard Well[1]

[1]Thünen Institute of Climate-Smart Agriculture, Braunschweig, Germany; [2]University of Wrocław, Institute of Geological Sciences, Wrocław, Poland; [3]McGill University, Department of Civiel Engineering and applied Mechanics, Montréal, Canada; [4]Julius Kühn Institute - Federal Research Centre for Cultivated Plants, Institute for Crop and Soil Science, Braunschweig, Germany; caroline.buchen-tschiskale@thuenen.de

Nitrous oxide (N_2O) emissions contribute significantly to greenhouse gas effect and are mainly produced through agricultural practices, particularly following the application of nitrogen-based fertilizers. The isotopic composition of N_2O can provide useful information for assessing N_2O sources. However, due to the co-occurrence of multiple N_2O microbial transformation pathways, it is challenging to use isotopic information to quantify the contribution of distinct processes. Nevertheless, identifying this is responsible for N_2O emissions is crucial to better understand the underlying mechanisms and develop targeted climate change mitigation strategies.

In recent decades, the analysis of abundance of the four most abundant isotopocules of N_2O ($^{14}N^{14}N^{16}O$, $^{14}N^{15}N^{16}O$, $^{15}N^{14}N^{16}O$, $^{14}N^{14}N^{18}O$) has been a promising tool to evaluate N_2O production pathways (heterotrophic bacterial denitrification, nitrifier-denitrification, fungal denitrification, nitrification) and N_2O reduction to N_2. To obtain a best estimate for N_2O reduction, this approach can be combined with the ^{15}N gas flux method with N_2-depleted atmosphere ($^{15}NGF^+$), which allows direct quantification of N_2 based on ^{15}N tracing. Nevertheless, the N_2O isotopocule approach cannot distinguish between heterotrophic bacterial denitrification and nitrifier denitrification, while the $^{15}NGF^+$ method cannot differentiate between nitrifier-denitrification and nitrification, but between fungal denitrification/heterotrophic bacterial denitrification and nitrifier denitrification/nitrification. Thus, the combination of both approaches provides values for heterotrophic bacterial denitrification and nitrifier-denitrification and thus improves our understanding of the N_2O processes.

We will present the calculation strategies and examples of combined datasets of N_2O isotopocules and $^{15}NGF^+$ from various laboratory and field studies.

11:50am - 12:10pm

Tracing the sources of nitrogen and phosphorous in alpine lakes

Maria Page[1], Ilann Bourgeois[1], Marine Souchier[1], Nicolas Caillon[2], Joel Savarino[2], Jean-Christophe Clément[1]

[1]Université Savoie Mont Blanc, INRAE, CARRTEL, Thonon-Les-Bains, France; [2]Univ. Grenoble Alpes, CNRS, IRD, INRAE, Grenoble INP, IGE, Grenoble, France; maria.page@univ-smb.fr

Alpine lakes are increasingly impacted by global environmental changes. Specifically, changing nutrient (nitrogen (N) and phosphorus (P)) inputs are of significant concern for the functioning of these ecosystems[1]. Long range transport from anthropogenic sources, such as agriculture and fossil fuel combustion, is an important nutrient source to alpine regions via atmospheric deposition[2]. However, local sources (e.g. glacial meltwaters, tourism) could increasingly subsidize nutrients to alpine lakes [3],[4]. This raises the question: which one of these changing sources predominantly drives nitrogen and phosphorus cycling in alpine lakes?

Eight lakes in the French Alps were selected along a gradient of anthropogenic and environmental pressures. Samples from the water column and sediment were taken over 3 seasons (winter, spring, summer) to trace the origin and fate of nutrients in the lakes. More specifically, stable isotope analysis is being conducted on nitrate ($\delta^{15}N$, $\delta^{18}O$ and $\Delta^{17}O$), ammonium ($\delta^{15}N$) and phosphate ($\delta^{18}O$). Preliminary results show that nitrate isotopic composition in water and sediment samples is similar regardless of the lake. This suggests that either all the lakes have a common driving source of nitrate, or that nitrate is biologically cycled in or before reaching the lakes. Combining these results with ongoing ammonium and phosphate isotopy analysis will give a novel multi-dimensional framework to interpret nutrient cycling in alpine lakes.

[1] Oleksy et al., (2021), Freshwater Science, DOI : 10.1086/713068.

[2] Galloway et al., (2003), *BioScience*, DOI : 10.1641/0006-3568.
[3] Saros et al., (2010), Environmental Science and Technology, DOI : 10.1020/es100147j.
[4] Baron et al., (2023), *Ecosphere*, DOI : 10.1002/ecs2.4504.

12:10pm - 12:30pm

Enhanced isotopic approach combined with microbiological analyses for more precise distinction of various N-transformation processes in contaminated aquifer – groundwater incubation study

Sushmita Deb[1], Dominika Lewicka Szczebak[1], Mikk Espenberg[2], Reinhard Well[3]

[1]University of Wrocław, Poland; [2]University of Tartu, Estonia; [3]Thünen-Institut für Agrarklimaschutz, Braunschweig, Germany; sushmita.deb@uwr.edu.pl

This study explores nitrogen transformations in groundwater from an agricultural area utilizing organic fertilizer (wastewaters from yeast production) integrating isotopic analysis, microbial gene abundance, and the FRAME (isotope FRactionation And Mixing Evaluation) model to trace and quantify nitrogen cycling pathways (1). Groundwater samples with elevated nitrate concentrations were subjected to controlled laboratory incubations with application of a novel low-level ^{15}N tracing strategy, to investigate microbial processes. Isotope analyses of nitrate, nitrite and N_2O, coupled with microbial gene quantification via qPCR (2), revealed a shift from archaeal-driven nitrification to bacterial denitrification post-incubation in suboxic conditions, stimulated by glucose addition. FRAME modeling further identified bacterial denitrification (bD) as the dominant pathway of N_2O production, which was supported by increased *nosZI, nirK and nirS* gene abundance and observed isotope effects.

Simultaneously to the intensive nitrate reduction, it was observed that the majority of nitrite is likely produced through nitrification processes linked to dissolved organic nitrogen (DON) oxidation. Nitrate reduction had minor contribution in the total nitrite pool. The results demonstrate the efficacy of integrating multi-compound isotope studies and microbial analyses to unravel nitrogen cycling mechanisms. This approach provides a robust framework for addressing nitrogen pollution in groundwater systems and improving water quality management strategies.

References:

1.Lewicki et.al.,. FRAME—Monte Carlo model for evaluation of the stable isotope mixing and fractionation. PLoS One. 2022;17(11):e0277204.

2.Deb et.al.,. Microbial nitrogen transformations tracked by natural abundance isotope studies and microbiological methods: A review. Science of the Total Environment. 2024:172073.

Conference Agenda

Session

6.2: Biogeochemistry: Carbon, Nitrogen, Sulfur and Other Cycles

Time: **Tuesday, 17/June/2025: 1:50pm - 2:30pm**
Session Chair: **Pascal Boeckx**
Session Chair: **Lucia Fuchslueger**

Location: **Ton Schoot Uiterkamp Zaal**

5159.0029 - Energy Academy Europe, Nijenborgh 6, 9747 AG Groningen

Presentations

1:50pm - 2:10pm

Soil texture matters: Deciphering the turnover of soil organic carbon and organic phosphorus in two C3/C4 field experiments

Layla M. San-Emetero, Marie Spohn

Department of Soil and Environment, Swedish University of Agricultural Sciences (SLU), Uppsala, Sweden; layla.marquez.san.emeterio@slu.se

Soil organic carbon (SOC) is crucial for soil fertility and climate regulation. Understanding its turnover helps predict responses to management. This study examines the turnover of organic carbon and phosphorus pools in soils with contrasting textures. We analyzed two agricultural experiments where vegetation shifted from C_3 to C_4. We hypothesize that the SOC pool will turn over more slowly in the clay-rich soil than in the clay-poor soil due to stronger sorption in the fine-textured soil.

We studied two replicated experiment sites in southeastern England; Rothamsted (Chromic Luvisol, 25% clay) and Woburn (Cambic Arenosol, 10–15% clay). The treatments included: (1) continuous barley (C_3 vegetation) (2) continuous maize (C_4 vegetation) and (3) continuous maize with additional incorporation of aboveground biomass (stubbles) after harvest. Soil samples were collected four times (1997–2015), and $\delta^{13}C$ was analyzed to calculate SOC turnover times.

We found that the SOC content was higher at Rothamsted (1.24 g kg^{-1}) than Woburn (0.79 g kg^{-1}), likely due to the silty clay loam texture. Treatments had no significant effect on the SOC content. Bulk $\delta^{13}C$ values were generally more enriched at Rothamsted ($\Delta^{13}C$ = 1.54‰) than Woburn ($\Delta^{13}C$ = 1.94‰). A significant difference between both C_4 treatments was observed at Woburn, where the $\delta^{13}C$ of the total soil organic carbon pool was up to 1.8 times more enriched with the stubble incorporation.

The soil at Rothamsted had a larger C stock (386 Mg·ha^{-1} vs 305 Mg·ha^{-1} in Woburn) and slower C turnover (mean turnover time = 192 years), likely due to the stabilization of organic matter on clay minerals. In contrast, the sandy soil texture at Woburn allowed for faster turnover and lower organic C retention (121 years). These differences highlight the influence of soil texture on carbon dynamics, with clay-rich soils promoting long-term carbon storage.

2:10pm - 2:30pm

KONATES: A Model Experiment on the Use of Contaminated Aquifers for Heat Management with ATES Plants - Microbiological and Isotopic Investigations

Jan Birkigt[1], Finn Höfgen[1], Raphael Hopp[1], Bruno Engelbrecht[2], Nina-Sophie Keller[1], Robert Köhler[1], Holger Weiß[2], Steffen Kümmel[1], Ivonne Nijenhuis[1], Carsten Vogt[1]

[1]Dept. Technical Biogeochemistry, Helmholtz Centre for Environmental Research - UFZ, Leipzig, Germany; [2]Dept. Environmental Informatics, Helmholtz Centre for Environmental Research - UFZ, Leipzig , Germany; ivonne.nijenhuis@ufz.de

ATES (Aquifer Thermal Energy Storage) is a CO_2-free technology for seasonal heating or cooling of buildings based on the storage and recovery of thermal energy in the aquifer. Since aquifers in urban areas are often contaminated with organic pollutants, the application of ATES raises the research question whether this type of heat management can accelerate natural attenuation processes. In the KONATES project, we are investigating how storage of hot water in an aquifer contaminated with trichloroethene (TCE) impacts the aquifer's microbiome structure and its potential for reductive dehalogenation of TCE. In laboratory experiments we could demonstrate that the native microbiome of the contaminated aquifer can reductively dehalogenate TCE within a temperature range typical for low-temperature ATES (12°C to 25°C). However, these processes are significantly inhibited or entirely absent at temperatures characteristic for intermediate- to high-temperature ATES (30°C to 70°C). In a pilot test, hot water (70°C) was injected into the TCE contaminated aquifer. The microbial community composition in the groundwater was investigated, with specific focus on thermophiles and organohalide respiring bacteria. To allow distinguishing the extent of biotransformation of TCE via reductive dehalogenation from e.g. dilution effects, dual-element ($\delta^{13}C/\delta^{37}Cl$) compound-specific stable isotope analysis was applied. However, as low concentrations of TCE at the pilot test site were observed, which were further decreased due to injection of hot water and mixing effects, a cryo-trap was integrated into the GC-analysis allowing a > six-fold increase in volume of injected gas samples, thus decreasing the limit of detection significantly.

Conference Agenda

Session

9.1: Ecology: Marine, Aquatic and Terrestrial

Time: **Tuesday, 17/June/2025: 2:30pm - 3:20pm**
Session Chair: **Loïc N Michel**
Session Chair: **Nemiah Ladd**

Location: **Ton Schoot Uiterkamp Zaal**

5159.0029 - Energy Academy Europe, Nijenborgh 6, 9747 AG Groningen

Presentations

2:30pm - 3:00pm

Keynote: Using stable isotopes as tools to solve the Rumsfeld matrix in ecology

Chris Harrod
University of Glasgow, United Kingdom; chris.harrod@glasgow.ac.uk

Although not the original thinker behind the philosophical approach, US hawk Donald Rumsfeld famously categorised information into "*known knowns, known unknowns, unknown knowns, and unknown unknowns*".

Although his comments were treated with disdain at the time, after 20 years I have come to grudgingly accept that this way of thinking parallels the experience of many environmental scientists, for example those working on problems of the function, evolution and conservation of the natural resources on which we rely on as humans. In this talk, I will introduce a series of field-based studies where stable isotopes have proven to be fundamental in helping us to identify and examine some *known knowns*, *known unknowns*, *unknown knowns*, and *unknown unknowns* in aquatic ecology.

3:00pm - 3:20pm

Using compound-specific stable isotope analysis to trace essential fatty acid bioconversion in invertebrates and fish

Matthias Pilecky[1,2], Sami Taipale[3], Patrick Fink[4], Travis Meador[5], Ursula Strandberg[6], Leonard Wassenaar[7], Martin Kainz[1,2]
[1]Wasser Cluster Lunz, Austria; [2]Universität for Continuing Education Krems, Research lab of Aquatic Ecosystem Research and -Health, Dr. Karl-Dorrek-Straße 30, 3500 Krems, Austria; [3]University of Jyväskylä, Department of Biological and Environmental Science, Survontie 9C, 40014, Jyväskylä, Finland; [4]University of Cologne, Institute for Zoology, Zülpicher Straße 47b, D-50674, Köln, Germany; [5]University of Southern Bohemia, Na Sádkách 7, 370 05 České Budějovice, Czech Republic; [6]University of Eastern Finland, Department of Environmental and Biological Sciences, Yliopistokatu 2, 80100 Joensuu, Finland; [7]University of Ottawa, National Facility in Accelerator Mass Spectrometry Laboratory, 25 rue Templeton Street (ARC411), Ottawa, ON, K1N6N5; matthias.pilecky@donau-uni.ac.at

Survival, growth, and reproduction of consumers depend on the acquisition of sufficient dietary energy and essential micronutrients. Organisms at the base of the aquatic food web synthesize essential polyunsaturated fatty acids (PUFA), which are transferred to consumers at higher trophic levels. Many consumers, requiring omega-3 long-chain (n-3 LC)-PUFA such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), have limited ability to biosynthesize them from the essential dietary precursor α-linolenic acid (ALA) and thus rely on dietary provision of LC-PUFA. If the dietary supply of such PUFA does not match the physiological needs, consumers may need to bioconvert dietary precursors into the required molecules. Such conversion may provide an ecological disadvantage over consumers that have direct access to required dietary compounds. We present a method, based on compound-specific stable hydrogen isotopes, to elucidate metabolic processing of fatty acids in both laboratory and field experiments. We show how compound-specific stable hydrogen isotopes of fatty acids can be used to study dietary limitations and bioconversion of n-3 PUFA in zooplankton and in fish. Furthermore, it can be used to discriminate between PUFA regulators and PUFA accumulators in ecosystems, whose functional diversity can have substantial impact on the availability of PUFA to higher trophic levels and thus influence ecosystem resilience.

Conference Agenda

Session

9.2: Ecology: Marine, Aquatic and Terrestrial

Time: **Tuesday, 17/June/2025: 3:50pm - 5:10pm**
Session Chair: **Loïc N Michel**
Session Chair: **Nemiah Ladd**

Location: **Ton Schoot Uiterkamp Zaal**

5159.0029 - Energy Academy Europe, Nijenborgh 6, 9747 AG Groningen

Presentations

3:50pm - 4:10pm

Effects of globally invasive fish on freshwater ponds food web structure and greenhouse gas emissions

Benjamin Lejeune[1,2], **Gilles Lepoint**[3], **Steven Bouillon**[4], **Alberto V. Borges**[2], **Mathieu Denoël**[1]

[1]Laboratory of Ecology and Conservation of Amphibians, Freshwater and OCeanic science Unit of reSearch (FOCUS), University of Liège, Liège, Belgium; [2]Chemical Oceanography Unit, Freshwater and OCeanic science Unit of reSearch (FOCUS), University of Liège, Liège, Belgium; [3]Laboratory of Trophic and Isotopes Ecology, Freshwater and OCeanic science Unit of reSearch (FOCUS), University of Liège, Liège, Belgium; [4]Department of Earth and Environmental Sciences, KU Leuven, Leuven, Belgium; Benjamin.lejeune@uliege.be

Ponds represent the most abundant freshwater lentic systems on Earth and provide essential ecosystem services including water supply, quality improvement, food provisioning or global biodiversity support. Despite their small size, they play a disproportionately important role in global carbon and nitrogen cycles, including fluxes of highly potent greenhouse gas (GHG) such as CO_2, CH_4 and N_2O. However, ponds suffer from a critical lack of consideration in management plans and legislations, leaving them especially vulnerable to anthropogenic disturbances such as alien species introduction. Alien fish introduction in naturally fishless ponds has been identified as a major issue for ponds biodiversity, but there is a lack of assessment of their consequences on food web dynamics and ecosystem functioning. By inducing different trophic cascades, alien fish have the potential to profoundly alter food webs in recipient ecosystems, with complex consequences on the services they deliver. In particular, there is a critical lack of data on the potential influence of biotic components such as predators on the dynamic of GHG fluxes in ecosystems. Here, we present findings from an ongoing project aiming to shed light on the impact of alien fish invasions on pond food webs and ecosystem functioning by relying on a wide array of techniques, including bulk $\delta^{13}C$ and $\delta^{15}N$ isotope analysis of biotic and abiotic ecosystem components, and field measurements of CO_2, CH_4 and N_2O fluxes. Data from 38 ponds in France, Slovenia and Croatia, collected in late Spring, reveal that alien fish collapse food web structures, shift primary producers equilibria toward phytoplankton domination, degrade water quality and significantly increase CO_2 partial pressure and CH_4 concentration in water relative to fishless ponds, but without apparent effect on N_2O fluxes. We discuss the implications of these results in a context of biological conservation and global changes.

4:10pm - 4:30pm

Nitrogen nutrition effects on δ13C of plant respired CO2 are mostly caused by concurrent changes in organic acid utilization and remobilization

Yang Xia[1,2], **Julie Lalande**[3], **Franz Badeck**[4], **Cyril Girardin**[5], **Camille Bathellier**[6], **Gerd Gleixner**[7], **Roland Werner**[8], **Guillaume Tcherkez**[3,9], **Jaleh Ghashghaie**[1]

[1]Université Paris-Saclay, CNRS, AgroParisTech, Ecologie Systématique et Evolution (ESE), Gif-sur-Yvette, France; [2]Shenzhen University, college of life science and oceanography, Shenzhen, China; [3]Institut de recherche en horticulture et semences, UMR 1345, Université d'Angers, SFR Quasav, Beaucouzé, France; [4]Research centre for Genomics & Bioinformatics (CREA- GB), Council for Agricultural Research and Economics, Fiorenzuola d'Arda, Italy; [5]Université Paris-Saclay, INRAE, UMR 1402 ECOSYS, Campus Agro Paris-Saclay, Palaiseau, France; [6]Centre d'affaires ATEAC, Elementar France, Lyon, France; [7]Max Planck Institute for Biogeochemistry, Jena, Germany; [8]Institute of Agricultural Sciences, ETH Zurich, Zurich, Switzerland; [9]Research school of biology, Australian National University, Canberra, Australian Capital Territory, Australia; catherinen_xia@hotmail.com

In the short- or mid-term, the variation of leaf-respired $\delta^{13}CO_2$ has important consequences for $\delta^{13}C$ of CO_2 in air in terrestrial ecosystems. Therefore, the isotope composition of plant respired CO_2 is of crucial importance for understanding plant and ecosystem carbon balance. It has previously been shown in tobacco (*Nicotiana tabacum*) that the balance between ammonium and nitrate has an influence on $\delta^{13}C$ of leaf-respired CO_2. However, uncertainty remains as to whether (i) the effect of N nutrition is observed in all species, (ii) N source also impacts on respired CO_2 in roots, and (iii), there is a relationship or equation predicting $\delta^{13}C$ of respired CO_2 that can be applied regardless of N conditions, species, and represents a hurdle in plant ^{13}C budget modelling.

Here, we carried out isotopic measurements of respired CO_2 and various metabolites using two species (spinach, French bean) grown under different NH_4^+:NO_3^- ratios. Both species showed a similar pattern, with a progressive ^{13}C-depletion in leaf respired CO_2 as the ammonium proportion increased, while $\delta^{13}C$ in root-respired CO_2 showed little change. Supervised multivariate analysis showed that $\delta^{13}C$ in respired CO_2 was mostly determined by organic acid (malate, citrate) metabolism, in both leaves and roots. We then took advantage of non-stationary, two-pool modelling that explained 73% of variance in $\delta^{13}C$ in respired CO_2. It demonstrates the critical role of the balance between the utilization of respiratory intermediates and the remobilization of stored organic acids, regardless of anaplerotic bicarbonate fixation by phospho*enol*pyruvate carboxylase and the organ considered. These findings underscore the role of nitrogen availability in shaping plant carbon metabolism and stable isotope signatures, with broader implications for modeling plant contributions to atmospheric CO_2.

4:30pm - 4:50pm

Experimental results on trophic discrimination factors for ectotherms: estimates and assumptions for the case of crocodiles

David X. Soto[1], **Frans G.T. Radloff**[2,5], **Alexander L. Bond**[3], **Keith A. Hobson**[4], **Alison J. Leslie**[5]

[1]Leibniz Institute for Zoo and Wildlife Research (IZW), Berlin, Germany; [2]Department of Conservation and Marine Sciences, Cape Peninsula University of Technology, Cape Town, South Africa; [3]Bird Group, Natural History Museum, Tring, Hertfordshire, United Kingdom; [4]Environment and Climate Change Canada, Saskatoon, Canada; [5]Dep. of Conservation Ecology and Entomology, Stellenbosch University, Stellenbosch, South Africa; soto@izw-berlin.de

Stable isotopes of carbon (δ^{13}C) and nitrogen (δ^{15}N) are progressively more employed to study the foraging ecology of ectotherms predators like crocodilians. However, accurate and precise estimations of trophic discrimination factors between diet and crocodile tissues (Δ^{13}C and Δ^{15}N, respectively) from captive experiments under controlled conditions are necessary to quantify the contribution of their preys in their feeding habits reliably. The issues of isotope equilibrium and isotopically constant diet are important factors that influence accurate estimations of diet-tissue discrimination factors. These assumptions influence the accuracy and reliability of ecological inferences based on stable isotopes. We raised Nile crocodiles (*Crocodylus niloticus*) fed with two isotopically distinct (but constant) diets and under controlled experimental conditions. They grew enough to precisely estimate the trend patterns that ultimately reach isotopic equilibrium. We sampled blood (plasma and red blood cells), scute keratin and collagen and nail tissues during the experiment and diet-tissue discrimination factors were estimated at isotope equilibrium with diet. Overall, our estimations of average diet-tissue discrimination factors (in ‰) were +0.1 for plasma, +0.1 for red blood cells (RBC), +0.3 for keratin, +1.9 for collagen, and +1.2 for nail tissue for δ^{13}C, and +0.2 for plasma, +1.9 for RBC, +1.6 for keratin, +2.3 for collagen, and +1.8 for nail tissue for δ^{15}N. We found no clear significant effect of crocodile size on these tissue estimates, but a slight influence for plasma Δ^{15}N. Understanding these differences in ectotherm isotope ecology is crucial for interpreting trophic relationships within food webs that include animals like reptiles.

[This abstract is dedicated to the memory of Prof. Keith A. Hobson, who united us in the quest of stable isotope ecology.]

4:50pm - 5:10pm

Mucopolysaccharides secreted by the sea slug Elysia crispata incorporate carbon from kleptoplast photosynthesis

Joana Filipa Barata[4], Diana Lopes[1], Paulo Cartaxana[2], Sónia S Ferreira[3], Maria Inês Silva[2], Margarida Nunes[2], Cláudia Nunes[5], Sónia Cruz[2]

[1]Laboratory for Innovation and Sustainability of Marine Biological Resources (ECOMARE), Centre for Environmental and Marine Studies (CESAM), Department of Chemistry, University of Aveiro, 3810-193 Aveiro, Portugal; [2]ECOMARE, CESAM, Department of Biology, University of Aveiro, 3810-193 Aveiro, Portugal; [3]Associated Laboratory for Green Chemistry of the Network of Chemistry and Technology (LAQV-REQUIMTE), Department of Chemistry, University of Aveiro, 3810-193 Aveiro, Portugal; [4]CESAM, University of Aveiro, 3810-193 Aveiro, Portugal; [5]Aveiro Institute of Materials (CICECO), Department of Materials and Ceramic Engineering, University of Aveiro, 3810-193 Aveiro, Portugal; jbarata@ua.pt

Some Sacoglossa sea slugs feed on macroalgae and sequester chloroplasts in the cells of their digestive diverticulum. In some species, mostly within the genus *Elysia*, the stolen chloroplasts – kleptoplasts – remain photosynthetically competent for weeks to months. These sea slugs, like other gastropods, produce a viscous secretion or mucus involved in protection, locomotion, and reproduction. In this study, we profiled the carbohydrate composition of the mucus of the kleptoplast-bearing sea slug *Elysia crispata* and tracked the incorporation of carbon and nitrogen via stolen chloroplasts. Mucopolysaccharides were mainly composed by uronic acids and galactose with 1→4 glycosidic linkages. Using inorganic ^{13}C-labelled sea water, incorporated carbon was found in the secreted mucus only in the presence of light, mainly in the form of 3-O-methylgalactose, galactose, and fucose. On the contrary, additional inorganic ^{15}N in the sea water could be traced in the mucus in both dark and light conditions. Results show that inorganic carbon was fixed through photosynthesis in the kleptoplasts, translocated to the animal tissue and incorporated in the secreted mucopolysaccharides. The present study pinpoints the biological relevance of photosynthesis to the metabolism of these remarkable animal-chloroplast symbiotic associations.

Conference Agenda

Session

3.1: Atmospheric Sciences: Greenhouse and Other Tracer Gases, Air Quality and Aerosols

Time: **Wednesday, 18/June/2025: 9:00am - 10:30am**
Session Chair: **Thomas Röckmann**
Session Chair: **Ulrike Dusek**

Location: **Ton Schoot Uiterkamp Zaal**

5159.0029 - Energy Academy Europe, Nijenborgh 6, 9747 AG Groningen

Presentations

9:00am - 9:30am

Keynote: Clumped isotopologues as tracers for atmospheric methane

Malavika Sivan, Maria Elena Popa, Thomas Röckmann
Institute for Marine and Atmospheric research, Utrecht University, the Netherlands; mals.sivan@gmail.com

The clumped isotopic composition ($\Delta^{13}CH_3D$ and $\Delta^{12}CH_2D_2$) of methane (CH_4) has recently been developed as additional tracers to constrain atmospheric CH_4 sources and sinks. Atmospheric CH_4 levels are controlled by a complex interplay of natural and anthropogenic sources, atmospheric OH levels, and other sink reactions. Understanding the contribution of each of these factors is crucial for a comprehensive understanding of the global CH_4 cycle.

Technical advancements in low-concentration sample extraction now facilitate direct measurements of the $\Delta^{13}CH_3D$ and $\Delta^{12}CH_2D_2$ of CH_4 from ambient air. The first such measurements reveal distinct signatures of approximately 1 ± 0.3 ‰ for $\Delta^{13}CH_3D$ and 44 ± 3 ‰ for $\Delta^{12}CH_2D_2$, with $\Delta^{12}CH_2D_2$ being higher than in all major source categories.

However, these measurements do not align with existing model predictions, highlighting significant knowledge gaps in the known clumped isotopic composition of CH_4 sources and the kinetic isotopic fractionation associated with sinks. Although the number of source-specific measurements remains limited, these discrepancies are unlikely to be attributed only to under-sampled sources. Instead, uncertainties in sink fractionation are more likely to explain the observed differences. However, precisely quantifying the fractionation effects in CH_4 sinks remains a challenge.

To further investigate this discrepancy, we reconstructed the first historical record of atmospheric $\Delta^{13}CH_3D$ and $\Delta^{12}CH_2D_2$ using firn air samples collected at East-GRIP. Our measurements show an unexpected increase of 10 ‰ in $\Delta^{12}CH_2D_2$ between 1993 and 2018. This dataset constrains a two-box atmospheric model of the five most abundant CH_4 isotopologues. Additionally, the optimized model assesses the influence of various sources and sinks on the clumped isotopic composition of CH_4 over time while also providing future projections.

Currently, we are working on expanding the database of the clumped isotopic composition of major CH_4 sources and measuring ambient air under different conditions to further constrain the clumped isotope budget.

9:30am - 9:50am

Triple oxygen isotope composition of stratospheric oxygen during the Last Glacial Maximum and implication to the global biosphere productivity

Lekshmi Mudra Bindhu[1], Ji-Woong Yang[1], Christof Janssen[2], Slimane Bekki[3]
[1]Laboratoire des sciences du climat et de l'Environnement, LSCE-IPSL (CNRS-CEA-UVSQ), Université Paris-Saclay, Gif-sur-Yvette, France; [2]de la Molécule aux Nano-Objets : Réactivité, Interactions et Spectroscopies, MONARIS-IPSL (CNRS-SU), Sorbonne Université, Paris, France; [3]LATMOS/IPSL, UVSQ Université Paris-Saclay, Sorbonne Université, CNRS, Guyancourt, France; lekshmi-mudra.bindhu@lsce.ipsl.fr

The triple isotope composition ($\Delta^{17}O$) of atmospheric oxygen (O_2) has been proposed as a tracer of the global (land + ocean) biosphere productivity. Yet, the $\Delta^{17}O$ of tropospheric O_2 is determined by both the productivity of the biosphere (i.e., photosynthesis) and stratospheric photochemistry (Luz et al., 1999). Previous studies reconstructed the global biosphere productivity from measurements of $\Delta^{17}O$ in O_2 from ice cores. They reported a reduced global biosphere productivity during Last Glacial Maximum (LGM) compared to the pre-industrial period (Blunier et al., 2002, 2012; Landais et al., 2007; Yang et al., 2022). However, our present understanding of isotope fractionation effects in the past stratosphere is limited. For example, climate-chemistry model experiments suggest that the LGM stratosphere was warmer, had reduced ozone levels, and experienced variable stratosphere-troposphere exchange (Wang et al., 2020; Fu et al., 2020). These changes could have altered oxygen isotope fractionation, which could, in turn, influence estimates of global productivity.

To address this issue, we conducted numerical experiments using a box model describing the $\Delta^{17}O$ budget of atmospheric O_2 and CO_2 under LGM boundary conditions. We explore how $\Delta^{17}O$ of O_2 is sensitive to factors such as stratospheric temperatures, concentrations of CO_2 and O_3, and the stratosphere-troposphere exchange flux. Preliminary results indicate that an LGM-like stratosphere reduces the magnitude of $\Delta^{17}O$ depletion in stratospheric O_2, which implies a global biosphere productivity lower than today. This illustrates that, if our current estimates of the past global biosphere productivity are to be refined, remaining uncertainties associated with past stratospheric photochemistry and isotope fractionation by biological/hydrological processes should be addressed and reduced.

Keywords: Triple isotope composition, biosphere productivity, Last Glacial Maximum, Stratosphere

9:50am - 10:10am

Inferring Urban CO_2 Sources Using Tall Tower $\delta^{13}C$ Measurements: Insights from the Vienna Urban Carbon Laboratory

Kathiravan Meeran[1], Bradley Matthews[2,3], Simon Leitner[1], Jia Chen[4], Andrea Watzinger[1]
[1]Institute of Soil Research, Department of Ecosystem Management, Climate and Biodiversity, BOKU University, Vienna, Austria; [2]Institute of Forest Ecology, Department of Ecosystem Management, Climate and Biodiversity, BOKU University, Vienna, Austria; [3]Environment Agency Austria, Vienna, Austria; [4]Technical University of Munich (TUM), Department of Electrical and Computer Engineering, Environmental Sensing and Modeling, Munich, Germany; kathiravan@boku.ac.at

Cities are major contributors to global anthropogenic carbon dioxide (CO_2) emissions. Accurately quantifying urban emissions at the local scale is therefore important for climate change mitigation. Traditional methods, such as emission inventories, often face significant uncertainties at the city-level, emphasizing the potential need for independent, observation-based approaches. Measurements of atmospheric CO_2 concentrations and the respective stable isotopic composition ($\delta^{13}C$) can characterize and quantify emissions from different sources. Within the *Vienna Urban Carbon Laboratory* project, we installed a cavity ring-down laser isotope spectrometer (Picarro G2131-*i*) on the Arsenal Radio Tower in Vienna. Since May 2022, this setup delivers continuous, high-resolution measurements of CO_2 mixing ratios and $\delta^{13}C$-CO_2 144 meters above ground level. Diel and seasonal variations in these parameters reveal distinct source contributions. In summer, increase in $\delta^{13}C$ coupled with daytime CO_2 reductions indicates strong photosynthetic activity. In autumn, elevated CO_2 levels and , decrease in $\delta^{13}C$ suggest increased contributions from ecosystem respiration. Bayesian inverse analyses of CO_2 sources shows a consistent contribution from gasoline combustion throughout the year. Emissions from natural gas combustion is particularly high during winter when winds originate from the east and southeast, which can be linked to heating systems in buildings and power plants. Our contribution will also present results from footprint analyses using the Stochastic Time-Inverted Lagrangian Transport (STILT) model, to infer the spatial distribution of emission sources.

10:10am - 10:30am

Unexpected variations in the clumped isotopic composition of O2 during the Holocene

Thomas Röckmann[1], **Amzad H. Laskar**[1,2], **Getachew Adnew**[1,3], **Sergey Gromov**[4], **Rahul Peethambaran**[1,5], **Benedikt Steil**[4], **Jos Lelieveld**[4], **Thomas Blunier**[3]

[1]Utrecht University, Netherlands, The; [2]Physical Research Laboratory, Ahmedabad, India; [3]University of Copenhagen, Denmark; [4]Max Planck Institute for Chemistry, Mainz, Germany; [5]National Institute of Water and Atmospheric Research, Wellington, New Zealand; t.rockmann@uu.nl

The recent geological epoch, the Holocene, is assumed to have been climatically stable, though this is challenged by discrepancies between climate proxies for temperature and model results. Furthermore, trends of the greenhouse gas methane are not understood. Atmospheric oxidants control the atmospheric methane abundance, but variations of oxidants in the past are not known since they are not preserved in paleo-climate archives. We present measurements of the clumped isotopes of atmospheric O_2 (abundance of $^{18}O^{18}O$ denoted by Δ_{36}) extracted from a Greenland ice core, covering the Holocene and the late glacial period, to provide new insights into past variations of temperature and oxidant levels. In the glacial period Δ_{36} was 0.07 ‰ higher than in the Late Holocene, attributed to low temperatures and a low tropospheric O_3 burden. Remarkably, Δ_{36} shows pronounced millennial-scale variations over the Holocene, with mid-Holocene Δ_{36} values being 0.06 ‰ lower than in the Late Holocene, and 0.03 ‰ below present-day conditions. Our analyses with an atmospheric chemistry-climate model and a box model suggest that the low Δ_{36} values in the mid-Holocene can be explained by a combination of high oxidant levels and high upper tropospheric temperatures, potentially augmented by changes in stratosphere-troposphere transport. The millennial scale variability of Δ_{36} matches the temporal evolution of CH_4, which suggests that the mid-Holocene minimum in CH_4 is largely driven by tropospheric oxidants. Our Δ_{36} data suggest that key atmospheric features, notably oxidant levels and temperature, have varied significantly during the Holocene.

Conference Agenda

Session

3.2: Atmospheric Sciences: Greenhouse and Other Tracer Gases, Air Quality and Aerosols

Time: **Wednesday, 18/June/2025: 11:00am - 11:40am**
Session Chair: **Thomas Röckmann**
Session Chair: **Ulrike Dusek**

Location: **Ton Schoot Uiterkamp Zaal**

5159.0029 - Energy Academy Europe, Nijenborgh 6, 9747 AG Groningen

Presentations

11:00am - 11:20am

First Coupled H_2-HD Inversion with a 3D Chemical Transport Model (TM5): Constraining the global hydrogen budget

Firmin Stroo[1], **Wouter Peters**[2], **Joram Hooghiem**[1,2], **Maarten Krol**[2,3], **Iris Westra**[1], **Harro Meijer**[1]

[1]Energy and Sustainability Research Institute Groningen (ESRIG), University of Groningen, Groningen, Netherlands; [2]Dept of Meteorology and Air Quality (MAQ), Wageningen University and Research, Wageningen, Netherlands; [3]Institute for Marine and Atmospheric research Utrecht (IMAU), Utrecht university, Utrecht, Netherlands; f.t.stroo@rug.nl

Hydrogen (H_2) is expected to become an increasingly important energy carrier during the energy transition. This will likely cause increased levels of atmospheric H_2, due to unavoidable losses during the production, transport, storage, and usage of hydrogen. Multiple studies have shown that through interaction with the hydroxyl radical, global tropospheric and stratospheric composition could be impacted, however a large uncertainty remains due to a lack of understanding of the global hydrogen budget. A key feature of the global hydrogen cycle is the strong kinetic isotope effects due to the large mass difference between H_2 and HD. The resulting large differences in $^2H/^1H$ isotope ratios help constrain constrain the magnitudes of sources and sinks in the global hydrogen budget.

For the first time, we present a comprehensive global hydrogen budget derived using a coupled H_2-HD inversion framework embedded within the three-dimensional chemical transport model TM5. This budget is obtained using a global set of 178,640 H_2 mole fraction measurements and 540 $\delta D(H_2)$ measurements, which are subsequently supplied to the CarbonTracker data assimilation system. Using its ensemble Kalman filter approach we estimate the magnitude and spatial distribution of monthly global hydrogen emissions, chemical production and losses for the period 2003–2023. To evaluate the robustness of our results, we compare optimized simulated hydrogen mole fractions with independent observational data from aircraft profiles collected during the IAGOS-CARIBIC, NOAA/ESRL, and ATom campaigns. We also compare optimized $^2H/^1H$ isotope ratios with available IAGOS-CARIBIC profiles but note that additional measurements would strengthen our evaluation.

11:20am - 11:40am

An isotope signature of photochemical aging of organic aerosol

Ulrike Dusek[1], **Haiyan Ni**[2], **Ellis de Wit**[1], **Stefan Grobbink**[1]

[1]Centre of Isotope Research (CIO), University of Groningen, The Netherlands; [2]School of Environmental and Municipal Engineering, Xi’an University of Architecture and Technology, Xi’an 710055, China; u.dusek@rug.nl

Organic aerosols (OA) account for a large fraction of particulate matter. Throughout their life-time the composition of OA is continually changed by chemical reactions in the atmosphere. Due to the complexity of the organic aerosol, there is no clear chemical tracer of these atmospheric transformations. In this work we investigate in a laboratory study, if stable carbon isotopic signatures can be used as a tracer of certain aging processes.

We produce organic aerosol by the combustion of biomass and coal and expose it to atmospheric aging in a Potential Aerosol Mass (PAM) Oxidation Flow Reactor. We collect organic aerosol on two filters, the primary filter collected directly after the combustion chamber and the second one after the aging reactor. We measure $d^{13}C$ in OA desorbed from filter samples at three different temperature steps, which correspond to different volatility fractions of the OA.

Aging in the PAM reactor strongly changes the $d^{13}C$ values of the organic aerosol. The aged filters have overall lower $d^{13}C$ values than the primary filters, which can be explained by the strong secondary organic aerosol (SOA) formation in the PAM reactor, with the reaction products (low $d^{13}C$) condensing on the primary aerosol. Moreover, we can see that characteristic changes in d13C at the different temperature steps, indicating that reaction products mainly accumulate at most volatile and least volatile OA fractions. The accumulation in the most volatile OA fraction can be explained by the fact that the condensing SOA is quite volatile. The accumulation of reaction products in the least volatile fraction can be explained by photolysis and oxidation reactions on the particles that produce less volatile organic compounds.

The experiments show the strong potential of carbon isotope analysis to unravel aging pathways in the ambient atmosphere.

Conference Agenda

Session

S1: Sponsor Presentations

Time: **Wednesday, 18/June/2025: 11:40am - 12:30pm**
Session Chair: **Anita Aerts-Bijma**
Session Chair: **Pharahilda Maria Steur**

Location: **Ton Schoot Uiterkamp Zaal**

5159.0029 - Energy Academy Europe, Nijenborgh 6, 9747 AG Groningen

Presentations

11:40am - 12:10pm

Showcasing the Elementar IRMS user community

Toby Boocock, **Mike Seed**
Elementar, United Kingdom; toby.boocock@elementar.com

Elementar, a global leader in Isotope Ratio Mass Spectrometry (IRMS) and complementary peripheral inlet systems, empowers scientists across diverse fields, including geochemistry, environmental science, food authenticity, and forensics. This presentation will showcase our dedication to advancing IRMS technology through recent innovations that enhance instrument performance and functionality. We will highlight advancements driven not only by our expert team, but also through close collaboration with our vibrant and dynamic user community. By fostering a collaborative environment, we integrate innovative user ideas, ensuring our instruments continually meet the evolving needs of scientific research.

12:10pm - 12:30pm

An online preparation system for carbonate analysis at elevated temperatures

Martin Moore
Nu Instruments, United Kingdom; martin.moore@ametek.com

Isotopic analysis of carbonates plays a pivotal role in various scientific fields, particularly in the study of paleoclimatology, geochemistry, and environmental science. Carbonates are valuable proxies in isotopic studies due to their widespread occurrence and their ability to record environmental conditions at the time of formation. The isotopic composition of carbon and oxygen in carbonates is utilized to infer past climatic conditions, diagenetic processes, and carbon cycling. Specifically, δ^13C values provide insights into carbon sources and sinks, while δ^18O values are indicators of paleotemperatures and palaeoceanographic changes. The incorporation of dual clumped isotope (Δ47 & Δ48) analysis has furthered our understanding of carbonate systems. Clumped isotope analysis involves measuring the abundance of ^13C-^18O bonds within CO_2 molecules released from carbonates, offering a temperature proxy independent of the water's isotopic composition. This method enhances the reconstruction of past temperatures and offers greater accuracy in paleoclimate studies. This presentation introduces a new preparation system developed by Nu Instruments to convert carbonate containing sample material to CO2 through acid digestion. The system has been developed to improve the digestion of impure carbonate materials such as dolomite, aragonites and magnesites. The preparation system enables routine analysis of these sample types within shortened time frames compared to conventional methods utilizing elevated temperatures and a stirring mechanism for each sample.

Conference Agenda

Session

5.1: Food Authenticity, Forensics, Isoscapes

Time: **Thursday, 19/June/2025: 9:00am - 10:30am**
Session Chair: **Eva de Rijke**
Session Chair: **Simon Douglas Kelly**

Location: **Ton Schoot Uiterkamp Zaal**

5159.0029 - Energy Academy Europe, Nijenborgh 6, 9747 AG Groningen

Presentations

9:00am - 9:30am

Keynote: From Food to Pharmaceuticals: Exploring Stable Isotope Ratios at the Food-Pharma Interface

Luana Bontempo, Alberto Roncone
Research and Innovation Centre, Fondazione Edmund Mach, San Michele all'Adige, Italy; luana.bontempo@fmach.it

Stable isotope ratio analysis has become a pivotal analytical technique, providing unique insights into the composition, origin, and processing of products across various disciplines. At the intersection of food and pharmaceuticals, its applications offer innovative solutions for authenticity verification, quality control, and process monitoring.

In the food industry, stable isotope analysis is indispensable for assessing product authenticity, detecting adulteration, and ensuring traceability. Similarly, these techniques are gaining traction in the pharmaceutical sector, particularly for verifying the origin and purity of bioactive compounds in supplements that blur the lines between food and medicine. By analyzing isotopic signatures of elements such as carbon, hydrogen, and oxygen, stable isotope ratio analysis can characterize raw materials, detect exogenous additives or active ingredients, and assess production process consistency.

This approach not only guarantees the integrity of products but also addresses growing concerns over consumer safety and regulatory compliance. Additionally, stable isotope data supports sustainability initiatives by tracing the geographic and biological origins of materials, ensuring ethical sourcing practices.

By bridging the realms of food science and pharmaceutical research, stable isotope ratio analysis provides a transformative tool at the food-pharma interface. It underpins innovation in product development, enhances consumer trust, and reinforces global efforts toward safer and more transparent supply chains. Stable isotope analysis contributes to the evolution of these interconnected fields, opening new possibilities for addressing challenges and ensuring product excellence.

9:30am - 9:50am

A Method to Determine the Carbon Isotope Ratios of Endogenous Steroids Found in Human Serum for Doping Control Purposes

Thomas Piper[1], Mario Thevis[1,2]
[1]German Sport University Cologne, Germany; [2]European Monitoring Center for Emerging Doping Agents (EuMoCEDA), Germany; t.piper@biochem.dshs-koeln.de

The determination of serum concentrations of testosterone (T) and 4-androstendione (A4) was implemented into the Steroidal Module of the Athlete Biological Passport in 2023 to complement current approaches relying solely on steroids excreted into urine. Monitoring T and A4 individually in a longitudinal manner enables to detect the misuse of low-dose T administrations especially in female athletes while urinary markers of the steroid profile may not be influenced significantly. In contrast to the urinary steroid profile, knowledge on confounding factors to serum concentrations of T and A4 is scarce. This may complicate the interpretation of measured values in the context of sports drug testing. Furthermore, no isotope ratio mass spectrometry-based confirmation method for serum steroids has been established so far which would enable to further investigate serum samples showing abnormal concentrations or concentration ratios in parallel to the approach already applied to urine samples for more than two decades.

In parallel to well established methods employed for urinary steroids, high performance liquid chromatography clean-up was tested and validated for serum steroids. Cholesterol and pregnenolone-sulfate were employed as endogenous reference compounds. As target analytes dehydroepiandrosterone-sulfate, 5-androstene-3β,17β-diol-sulfate, androsterone-sulfate, and epiandrosterone-sulfate were included into the test menu. The method was fully validated in line with current regulations issued by the World Anti-Doping Agency including linear mixing models, measurement uncertainty, and detection limits. Finally, a reference population encompassing 124 male and female athlete samples was investigated to enable the calculation of population-based thresholds as a basis to differentiate between endogenous and exogenous sources of serum derived steroids.

Keywords: endogenous steroids, doping controls, testosterone doping, GC/C/IRMS, reference population

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9:50am - 10:10am

Unlocking the full potential of natural vanillin through FT ICR MS-based analysis of carbon and oxygen isotopic ratios

José Ordaz-Ortiz[1], Leo Rocha-Burgos[1], Nayeli L. Romero-García[1], Jackie Mosely[2]
[1]Metabolomics and Mass Spectrometry Group, UGA-Langebio, CINVESTAV, Mexico; [2]Centre of Excellence in Mass Spectrometry, Chemistry Department, University of York, UK; jose.ordaz-ortiz@cinvestav.mx

Vanillin, the primary component of vanilla flavour, is predominantly artificially manufactured due to the high cost and time-intensive nature of cultivating natural vanilla pods. Isotope ratio mass spectrometry (IRMS) is the standard method, this approach has several limitations: it requires 1 g of sample, our research use Fourier Transform-Ion Cyclotron Resonance Mass Spectrometry (FT-ICR MS). This ultra-high mass resolution technique can achieve the precision of isotope ratio measurements needed to discern naturally occurring variability on intact molecular ions in complex natural mixtures. Our novel method for analyzing the $^{13}C/^{12}C$ and $^{18}O/^{16}O$ isotopic ratios of vanillin using FT-ICR-MS, focuses on a characteristic vanillin fragment that provides site-specific $^{18}O/^{16}O$ isotopic ratio data, we achieved superior clustering and discrimination of samples based on their botanical source and geographical origin. This innovative approach holds significant potential for vanillin authentication, requiring a mere 20 µg of pure vanillin and just 10 minutes of analysis time.

The isotopic ratio analyses carried out in the C_6H_4O vanillin fragment provided better clustering and discrimination of the samples based on botanical source (*Vanilla planifolia, Vanilla tahitensis*) and geographical location, including the separation between biosynthetic and synthetic samples compared to the vanillin-averaged isotopic ratio analyses. Thus far, our method can discriminate natural, authentic vanillin samples from their biosynthetic or synthetic counterparts, contrary to the analysis of $\delta^{13}C$ and $\delta^{2}H$ of vanillin methoxyl groups from vanilla pods, such as between vanillin derived from ex-eugenol or guaiacol and vanillin ex-glucose.

As proof of concept, our direct infusion FT-ICR-MS method demonstrates its ability to distinguish natural, authentic vanillin samples from biosynthetic or synthetic sources with superior separation. Ongoing work includes gathering samples from diverse geographical, botanical, and synthesis origins. Furthermore, the method's applicability in finished products such as ice cream and yoghurts is being explored, and we anticipate using fewer samples for analysis.

10:10am - 10:30am

Impact of ingredients and processing methods on the stable isotopic ratios (δ2H and δ18O) of wheat-derived noodles

Jingjie Yang[1,2], **Sara W. Eraumus**[2], **Boli Guo**[1], **Saskia M. van Ruth**[2,3]

[1]Institute of Food Science and Technology, Chinese Academy of Agriculture Sciences, Beijing, China; [2]Food Quality and Deign Group, Wageningen University & Research, Wageningen, The Netherlands; [3]School of Agriculture and Food Science, University College Dublin, Dublin, Ireland; janeyang95@126.com

Wheat is a globally important trade commodity, valued for its durability, longevity, and use in producing wheat-derived foods. The authentication of wheat and its processed products is critical to ensure quality and traceability. While stable isotope analysis (SIA) has been widely employed for authenticating wheat grains, the application of δ^2H and δ^{18}O isotopes to processed wheat-derived foods, such as noodles, presents additional challenges. This study investigates the influence of processing, specifically boiling and high-temperature heating (100°C, 180°C and 260°C), on the stable isotopic composition (δ^2H, δ^{18}O) of wheat-derived noodles produced with varying gluten-to-starch ratios. The results reveal that the gluten-to-starch ratio significantly impacts δ^2H and δ^{18}O values, with a more pronounced effect on δ^2H. Gluten exhibits greater temperature sensitivity, while starch demonstrates higher isotopic exchangeability. Heating induces temperature-dependent isotopic changes through organic substance denaturation, leading to notable δ^2H depletion. Boiling, in contrast, exerts a stronger influence on hydrogen isotope fractionation due to water uptake and exchange, with δ^2H values in noodles closely reflecting the isotopic composition of the cooking water. This study provides novel insights into the isotopic behaviour of food components in wheat-derived products and establishes a theoretical foundation for developing robust analytical methods in the future.

Conference Agenda

Session

5.2: Food Authenticity, Forensics, Isoscapes

Time: **Thursday, 19/June/2025: 11:00am - 11:40am**
Session Chair: **Eva de Rijke**
Session Chair: **Simon Douglas Kelly**

Location: **Ton Schoot Uiterkamp Zaal**

5159.0029 - Energy Academy Europe, Nijenborgh 6, 9747 AG Groningen

Presentations

11:00am - 11:20am

Tracking tomato processing with stable isotopes: a study on fractional composition and authenticity

Oana Romina Botoran[1], **Ana Maria Simionescu**[1,2], **Diana Costinel**[1], **Roxana Elena Ionete**[1]

[1]National Research and Development Institute for Cryogenic and Isotopic Technologies ICSI Rm. Valcea, Romania; [2]Doctoral School of Chemical Engineering and Biotechnologies, U.N.S.T. Politehnica of Bucharest; oana.dinca@icsi.ro

The isotopic fingerprint of food products serves as a crucial tool for assessing traceability and authenticity, offering insights into raw material origin and transformation processes. This study examines the stable isotope composition of carbon (^{13}C), nitrogen (^{15}N), oxygen (^{18}O), and hydrogen (^{2}H) in tomatoes and their derived fractions at different processing stages during their transformation into tomato passata (Brix ~36). The objective was to assess the persistence of isotopic signatures throughout processing and establish a direct isotopic link between raw material and final product. Six experimental trials were conducted using different tomato varieties, with isotopic ratios measured via Isotope Ratio Mass Spectrometry (IRMS). Despite significant water loss and concentration effects, isotopic values remained within a characteristic range, confirming the retention of the original raw material's isotopic fingerprint. The $\delta^{13}C$ values of bulk tomatoes ranged from -26.58‰ to -28.56‰, while $\delta^{15}N$ remained stable across processing stages. Expected variations in $\delta^{18}O$ and $\delta^{2}H$, due to evaporation, retained a discernible pattern linked to the initial material. A detailed isotopic analysis of tomato fractions, including pectin, pulp, sugar, and organic acids, further confirmed isotopic stability. $\delta^{13}C$ in pectins shifted slightly from -28.04‰ to -25.45‰ in juice and from -29.21‰ to -25.56‰ in passata, while $\delta^{15}N$ remained relatively constant. The pulp fraction exhibited minor isotopic shifts, with $\delta^{13}C$ ranging from -29.05‰ to -27.42‰ in juice and from -28.46‰ to -26.63‰ in passata. $\delta^{13}C$ in sugars and organic acids remained stable, demonstrating minimal alteration during processing. These findings highlight the robustness of stable isotope analysis as an effective tool for verifying the authenticity of processed tomato products. The observed isotopic stability across different tomato fractions supports the use of $\delta^{13}C$, $\delta^{15}N$, $\delta^{18}O$, and $\delta^{2}H$ as reliable markers for traceability, reinforcing their role in food authentication and quality control strategies.

11:20am - 11:40am

Latest applications of the LC-co-IRMS for food and dietary supplements authentication

Silvia Pianezze[1], **Luana Bontempo**[2], **Matteo Perini**[1]

[1]Technology Transfer Center, Fondazione Edmund Mach, Italy; [2]Research and Innovation Center, Fondazione Edmund Mach, Italy; silvia.pianezze@fmach.it

The LC-co-IRMS represents an innovative technique based on the oxidation in acid conditions of all the carbon-based compounds of a sample mixture, previously separated from each other through an appropriate analytical column. Since its introduction in the market in 2004, the LC-co-IRMS has been used to analyse various matrices [1]. Nevertheless, the potential of this techniques is still far from being fully exploited. In this work, we presented some of the latest LC-co-IRMS applications that our group developed for traceability purposes.

In a recent study, the LC-co-IRMS was applied to check for the fraudulent addition of exogenous sugars to Italian authentic wine must. A database of about 100 samples from 16 different Italian regions was considered to set reference values for the carbon isotopic ratio ($\delta^{13}C$) of glucose and fructose in this matrix [2].

Besides sugars, organic acids have also been considered. The addition of biosynthetic citric acid obtained though the fermentation of cheap starting materials like cane sugar by the fungus *Aspergillus Niger* was detected in matrices such as tomato sauce, lemon and orange juice.

Finally, dietary supplements and drugs have also been studied. Levodopa is an amino acid prescribed for Parkinson disease. Natural levodopa can be extracted from plants like the *Mucuna pruriens*, but cheaper analogues can be chemically synthesised or biochemically obtained from the fermentation of sugars by various fungi [3]. The LC-co-IRMS led to the characterisation of the different levodopa sources, pointing out the possibility to detect fraudulent additions of the biochemical active principle to products declared as natural.

[1] Perini, M.; Bontempo, L. *Trends in Analytical Chemistry* 2022, 147, 116515. doi.org/10.1016/j.trac.2021.116515

[2] Perini, M.; Pianezze, S.; Guardini, K.; Allari, L.; Larcher, R. *Molecules* 2023, 28, 1411. doi.org/10.3390/ molecules28031411

[3] Min, K.; Park, K.; Park, D.H.; Yoo, Y.J. *Appl Microbiol Biotechnol* 2015, 99, 575-584. doi: 10.1007/s00253-014-6215-4.

Conference Agenda

Session

8.1: Health, Nutrition, Medical Sciences

Time: **Thursday, 19/June/2025: 11:40am - 12:30pm**
Session Chair: **Dewi van Harskamp**

Location: **Ton Schoot Uiterkamp Zaal**

5159.0029 - Energy Academy Europe, Nijenborgh 6, 9747 AG Groningen

Presentations

11:40am - 12:10pm

Keynote: Post-prandial protein handling: You are what you just ate.

Lucas van Loon
Maastricht University, Netherlands, The; L.vanLoon@maastrichtuniversity.nl

Skeletal muscle protein is constantly being synthesized and broken down, with a turnover rate of about 1-2% per day. The rate of skeletal muscle protein synthesis is regulated by two main metabolic stimuli, food intake and physical activity. Food intake, or more specifically protein ingestion, directly elevates muscle protein synthesis rates. The dietary protein derived essential amino acids act as signaling molecules activating anabolic pathways and provide precursors for muscle protein synthesis. Ingestion of a meal-like amount of dietary protein elevates muscle protein synthesis rates for several hours, providing evidence that '*you are what you just ate*'. When food is ingested after a bout of physical activity the post-prandial muscle protein synthetic response is augmented, with higher muscle protein synthesis rates sustained over a more prolonged period of time. In other words, when you ingest protein following a bout of physical activity '*you become even more of what you just ate*'. In contrast, when protein is ingested following a period of inactivity the post-prandial muscle protein synthetic response is blunted, coined anabolic resistance. Therefore, disuse makes you '*become less of what you just ate*'. These concepts play a key role in the prevention and management of muscle loss in both health and disease.

12:10pm - 12:30pm

Development of a GC-C-IRMS Method for Quantitative and Isotopic Analysis of Fatty Acids in Breast Cancer Patient Samples

Louise Mangeon[1,2], Romain Le Balch[2], Olivier Mantha[3], Cyrille Guimaraes-carneiro[3], Michelle Pinault[3], Regis Hankard[3], Arnaud De Luca[3], Illa Tea[1]

[1]Université Claude Bernard Lyon 1, CNRS, ISA, UMR 5280, 5 rue de la Doua, F-69100 Villeurbanne, France; [2]CEISAM UMR CNRS 6230, Nantes Université, Nantes, France; [3]Nutrition, croissance et cancer UMR 1069, Inserm, Université de Tours, France; louise.mangeon@univ-nantes.fr

The discovery of biomarkers is essential for the development of novel methods for early diagnosis, prognosis, and therapeutic strategies. Building on our patented results, isotopomic analysis—a technique that determines high-throughput isotopic profiles or signatures from global, intra-, or inter-molecular measurements in natural or enriched abundance—shows great promise for identifying new biomarkers.

This study aims to develop a novel method for the quantitative and **^{13}C isotopic analysis of fatty acids** in biological samples from breast cancer patients, using gas chromatography coupled with isotope ratio mass spectrometry (GC-C-IRMS). Unlike conventional methods, the proposed approach eliminates the derivatization of fatty acids into methyl esters, reducing the consumption of polluting solvents and avoiding the introduction of exogenous carbon, which could alter **^{13}C values**. Lipids are extracted from biological samples, such as serum or adjacent/tumor tissues, followed by saponification to obtain free fatty acids, which are then analyzed by GC-C-IRMS.

This method simultaneously quantifies fatty acids in the biological matrix and determines their **^{13}C isotopic compositions ($\delta^{13}C$)**. Fatty acids, including myristic acid (C14:0), palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1), palmitoleic acid (C16:1), and linoleic acid (C18:2), were identified, quantified, and characterized isotopically across various biological matrices. Analytical validation parameters demonstrated the specificity, linearity, precision, accuracy, and robustness of the method in standard mixtures and biological samples.

The method was applied to adjacent, cancerous tissues and serum from breast cancer patients. Several breast cancer families were compared (HER2-, HER2+, triple positive, triple negative and in situ) and revealed differences in the concentration and isotopic composition of the analyzed fatty acids.

The GC-C-IRMS analytical method provides a reliable and efficient approach for identifying specific biomarkers, offering potential diagnostic, prognostic, and therapeutic applications in breast cancer management.

Conference Agenda

Session

8.2: Health, Nutrition, Medical Sciences

Time: **Thursday, 19/June/2025: 1:50pm - 2:30pm**
Session Chair: **Dewi van Harskamp**

Location: **Ton Schoot Uiterkamp Zaal**

5159.0029 - Energy Academy Europe, Nijenborgh 6, 9747 AG Groningen

Presentations

1:50pm - 2:10pm

A Novel GC-C-IRMS Method for Isotopic Profiling of Amino Acids in Biopsies

Fatmeh AL RAHAL[1], Anthony ANCHISI[1], Youssef Bakkour[2], Illa TEA[1]

[1]Universite Claude Bernard Lyon 1, CNRS, ISA, UMR 5280, 5 rue de la Doua, F-69100 Villeurbanne, France; [2]King Khalid University, Department of Radiological Sciences, Abha 61421, Saudi Arabia; fatmeh.al-rahal@isa-lyon.fr

In the context of advancing biomarker discovery tools in oncology, particularly for lung cancer, we propose to explore the potential of natural abundance stable isotopes of metabolites using gas chromatography-combustion-isotope ratio mass spectrometry (GC-C-IRMS). In this work, we investigated a derivatization method that enables GC-C-IRMS analysis, allowing the detection of methyl esters of pentafluoropropionic derivatives for a broad range of amino acids in lung cancer biopsies. This novel method provides highly sensitive measurements of $\delta^{13}C$ and $\delta^{15}N$, as well as amino acid concentrations, in lung cancer biopsies. We assessed the linearity, accuracy, precision, and detection limits of the method across several patient biopsies. The results demonstrated excellent analytical performance, confirming the method's applicability to lung cancer samples. This approach enhances the potential of isotopic profiling in clinical research, offering innovative pathways for non-invasive cancer detection and ongoing monitoring.

Conference Agenda

Session

S2: Sponsor Presentations

Time: **Thursday, 19/June/2025: 2:30pm - 3:20pm**
Session Chair: **Anita Aerts-Bijma**
Session Chair: **Pharahilda Maria Steur**

Location: **Ton Schoot Uiterkamp Zaal**

5159.0029 - Energy Academy Europe, Nijenborgh 6, 9747 AG Groningen

Presentations

2:30pm - 3:00pm

High accuracy and precision with Orbitrap-based Isotope Ratio MS

Andreas Hilkert[1], Nils Kuhlbusch[1,3], Issaku Kohl[2], Brett Davidheiser[1], Dieter Juchelka[1], Heiko Hayen[3]

[1]Thermo Fisher Scientific, Germany; [2]University of Utah, Department of Geology and Geophysics; [3]University of Muenster; andreas.hilkert@thermofisher.com

Orbitrap-based Isotope Ratio MS provides a unique and complimentary tool to classical Isotope Ratio MS techniques to measure the relative abundance of isotopologues. The soft Electrospray Ionization of the Thermo Scientific™ Orbitrap Exploris™ MS produces intact molecular ions that can optionally be fragmented by higher energy collisional dissociation. Combining these features with the high-resolution accurate mass of Orbitrap analyzers gives insights into compound specific as well as position specific isotope ratios.

Precision and accuracy of Orbitrap-based isotope ratio analysis can be significantly improved by utilizing sample-standard bracketing to correct for instrument drift and inaccuracies via isotope ratio calibration. This is currently achieved either by the dual syringe inlet approach, which uses a diverter valve switching between two syringes filled with a sample and a standard or using an HPLC autosampler for alternating injections of sample and standard solutions.

Here we present an optimized workflow to increase system productivity while improving isotope ratio standardization. This workflow utilizes two flows of liquid controlled by a switching valve with one flow from the HPLC autosampler for sample introduction and a second flow from a syringe for standard introduction. This fully automated setup allows the injection of a standard during the washout period of the HPLC autosampler loop, reducing idle time while improving the quality of the measurements. Precision and accuracy can now be verified more easily by drift correction and one- or two-point calibration.

Isotope ratio calibration using the novel strategy for sample and standard introduction will be demonstrated on latest applications for inorganic (e.g. sulfate) and organic (e.g. amino acids, vanillin) samples showcasing unique insights into the isotopic anatomy of molecules.

3:00pm - 3:20pm

The new Picarro Sage Gas Autosampler: Simple and efficient automation of discrete isotope and gas concentration measurements

Magdalena Hofmann[1], Jan Woźniak[1], Joyeeta Bhattacharya[2], Tina Hemenway[2], Keren Drori[2]

[1]Picarro B.V., Eindhoven, The Netherlands; [2]Picarro Inc., Santa Clara, United States of America; mhofmann@picarro.com

The greenhouse gas research community faces a growing demand for simple and efficient isotopic analysis of discrete gas samples (e.g., carbon isotopes of CO_2 and CH_4). Conventional techniques such as Isotope Ratio Mass Spectrometry (IRMS) often involve high initial and maintenance costs, complex deployment procedures, and limited fieldwork adaptability. In contrast, the new Sage Gas Autosampler from Picarro offers an efficient and reliable solution when paired with a Picarro isotopic carbon analyzer. This system features a 150-position vial rack designed for 12mL headspace vials, each pressurized with approximately 30mL of gas. The measurement of each vial takes about 5min. At atmospheric concentrations, the $\delta^{13}C$ precision for both CO_2 and CH_4 is approximately 0.3 ‰. Here, we detail the operational workflow of the Sage Gas Autosampler and present its performance data when integrated with the Picarro G2201-i carbon isotope analyzer. We will also discuss best practices for discrete isotope gas and concentration analysis when working with this analytical system.

Conference Agenda

Session

S3: Sponsor Presentations

Time: **Thursday, 19/June/2025: 3:50pm - 4:30pm**
Session Chair: **Anita Aerts-Bijma**
Session Chair: **Pharahilda Maria Steur**

Location: **Ton Schoot Uiterkamp Zaal**

5159.0029 - Energy Academy Europe, Nijenborgh 6, 9747 AG Groningen

Presentations

3:50pm - 4:10pm

Tracking Biogenic Carbon in Liquid Fuel Blends using Conventional Mass Spectrometry and Infrared Spectroscopy

Scott Herndon, David Nelson
Aerodyne Research, United States of America; herndon@aerodyne.com

This work demonstrates the analytical basis for an IR laser measurement system with the potential to perform routine quantification of biogenic carbon content in liquid fuel products at working refineries. The IR system results for $^{13}CO_2/^{12}CO_2$ compare favorably with gold-standard isotope ratio mass spectrometry (IRMS). The IR system employed a predilution stage that emploted transfer techniques to suppress fractionation.

We will present the performance potential for routine quantification for mixtures of C–3 and C–4 biogenic carbon sources mixed with fossil feedstock.

Finally, we will show how the IR based apparatus can directly quantify stable isotopologs that are isobaric in IRMS instruments.

Conference Agenda

Session

4.1: Paleoclimatology and Archaeology

Time: **Friday, 20/June/2025: 9:00am - 10:30am**
Session Chair: **Margot Kuitems**
Session Chair: **Marcel van der Meer**

Location: **Ton Schoot Uiterkamp Zaal**

5159.0029 - Energy Academy Europe, Nijenborgh 6, 9747 AG Groningen

Presentations

9:00am - 9:30am

Keynote: Wooden cultural heritage: a high-resolution archive for paleoclimate reconstruction and stable isotope tree-ring dating.

Kristof Haneca
Flanders Heritage Agency, Belgium; kristof.haneca@vlaanderen.be

Wood, the most abundant biological tissue in terrestrial ecosystems, has played a pivotal role throughout human history. Ancient timbers, shaped and crafted by our ancestors, are now part of our cultural heritage. The study of wooden cultural artifacts, including archaeological wooden remains and timber from historic buildings, allows us to document and answer questions about our cultural past, the organisation, and technological craftsmanship of (pre)historical societies and past forest management practices.

Dendrochronology, the science of analyzing tree-ring patterns, enables precise chronometric dating of wooden objects and constructions, offering a deeper understanding of historical contexts. When combined with archaeological and historical research, dendroarchaeology utilizes exact tree-ring dates of (pre-)historical wood to uncover details of past societies and their environments. But the same timbers also provide an archive of precisely dated wood tissues at an annual resolution, extending back millennia. The close relation between stable oxygen isotope ratios in tree-ring cellulose and hydroclimate, provides an invaluable tool for reconstructing past precipitation patterns.

Yet, this robust climate-signal in tree-ring cellulose has also opened new avenues for precise chronometric dating. Stable oxygen isotope dendrochronology now allows dating timbers that cannot be resolved through conventional ring-width dendrochronology. This advancement is particularly relevant in midlatitude regions with temperate climate, where moisture stress is typically low, what allows trees to grow fast, produce wide and sometimes invariant tree-ring series that challenge conventional dendrochronological approaches.

This keynote will explore how stable isotope dendrochronology contributes to the field of dendroarchaeology and cultural heritage studies, highlighting the contributions these fields make to one another.

9:30am - 9:50am

A hydroclimate reconstruction for medieval Flanders: insights from oxygen isotopes in oak timber tree rings.

Lise Meir[1,2], Kristof Haneca[3], Samuel Bodé[2], Pascal Boeckx[2], Jan Van den Bulcke[1]

[1]Laboratory of Wood Technology, Ghent University; [2]Isotope Bioscience Laboratory, Ghent University; [3]Flanders Heritage Agency, Brussels; Lise.Meir@UGent.be

Knowing past climatic patterns is essential for a better understanding of our history, and improves predictions of future conditions in a changing climate. Tree rings serve as natural archives of environmental conditions, providing annually resolved climate data. While tree-ring widths are widely used for climate reconstructions, they are less effective in temperate regions, where multiple factors influence growth. Instead, the stable oxygen isotopic composition in tree rings provides a more robust proxy, as it is strongly interlinked to a combination of temperature, precipitation and drought.

This study aims to establish a high-resolution hydroclimate reconstruction for Flanders (1100 to 1600 CE) using oxygen isotope ratios ($\delta^{18}O$) in oak tree rings from old timber. For calibration, increment cores are collected from living trees (*Quercus* sp.) along a transect stretching from the Belgian North Sea coast to southern Germany. The sampling locations represent diverse growing conditions, related to differences in soil type and elevation.

Up to 200-year-long time series of $\delta^{18}O$ in individual tree ring cellulose were processed. From these $\delta^{18}O$ time series, a composite chronology was developed. This chronology was then calibrated against instrumental climate data, linking it to key climatic variables such as temperature, precipitation and drought.

Preliminary results demonstrate strong inter- and intra-regional correlations between the $\delta^{18}O$ series. Initial calibrations reveal a significant positive correlation with temperature ($r = 0.52$) and a negative correlation with summer precipitation and the Standardized Precipitation Evapotranspiration Index (SPEI) ($r = -0.53$), highlighting the potential of this approach for reconstructing past climates.

Ultimately, this research aims to reconstruct summer climate conditions during the late Middle Ages (1100-1600), providing new insights into hydroclimate variability in Flanders. Historical oak samples will therefore be obtained from medieval timber constructions, which are abundant in Flanders' architectural heritage. However, the isotopic potential of these timbers for climate reconstructions remains unexplored.

9:50am - 10:10am

Assessing the influence of hydrology and ecology on stable carbon and nitrogen isotopes through time at three ombrotrophic raised bogs in Northern Ireland:

James Dill-Russell[1], Gill Plunkett[1], Graeme Swindles[1,2], Jo Smith[3]

[1]Queen's University Belfast, United Kingdom; [2]Ottawa-Carleton Geoscience Centre and Department of Earth Sciences, Carleton University, Ottawa, Ontario, Canada; [3]School of Biological Sciences, University of Aberdeen; jdillrussell01@qub.ac.uk

Hydrology plays a fundamental role in the development and function of ombrotrophic raised bogs, which rely exclusively on precipitation for water and nutrients. These peatland ecosystems are highly sensitive to climatic fluctuations, with changes in water table levels influencing the balance between carbon accumulation, and decomposition of organic matter. Due to a near constant rate of deposition peatlands are ideal archives of past environmental conditions, providing a record for understanding long-term hydrological and ecological responses to climate change. Stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotopes in peat deposits serve as valuable proxies for reconstructing past environmental conditions, offering insights into the balance between organic matter input and decomposition. Here we present isotopic records from three ombrotrophic raised bogs in Northern Ireland to evaluate how variations in hydrology and vegetation composition have influenced carbon and nitrogen dynamics over the period 6000-5000 BP. By integrating isotopic data with testate amoebae-based water table reconstructions and plant macrofossil analyses, we explore the interactions between shifts in bog hydrology and ecological responses with variations stable carbon and nitrogen isotopes. Our findings reveal variations in $\delta^{13}C$ and $\delta^{15}N$, corresponding to phases of decreasing bog surface wetness and vegetation shifts. This highlights how hydrological and ecological changes influence rates of carbon accumulation and decomposition providing insight into how future changes may alter carbon emissions from peatlands.

10:10am - 10:30am

Vegetation corrections facilitate precipitation reconstructions from Younger Dryas and Holocene plant wax δ2H records in central Switzerland

S. Nemiah Ladd[1], Ricardo N. Santos[1], Antonia Klatt[1], Daniel Nelson[1], Fatemeh Ajallooeian[1,2], Carsten J. Schubert[2,3], Nathalie Dubois[2,3], Cindy De Jonge[2]

[1]University of Basel, Switzerland; [2]ETH Zurich, Switzerland; [3]Eawag, Switzerland; n.ladd@unibas.ch

Hydroclimate reconstructions from periods of rapid change, like the Younger Dryas (12.9 – 11.7 ka), improve our dynamical understanding of how precipitation patterns vary with temperature. In central Europe, paleoclimate records from the Younger Dryas often display conflicting signals due to the region's complex alpine topography and variable moisture pathways, underscoring the need for high spatiotemporal resolution in climate reconstructions. Small lakes are widely distributed across the region and can provide continuous, high-resolution sedimentary archives. Within lake sediments, the hydrogen isotope ratios of plant waxes (δ^2H_{wax}) have been used as proxies of precipitation hydrogen isotope ratios (δ^2H_{precip}) and thus hydroclimate. However, δ^2H_{wax} values in small catchments are also influenced by vegetation changes, complicating their interpretation.

We deconvolved the influence of vegetation and δ^2H_{precip} values on sedimentary δ^2H_{wax} values in a sediment core spanning the past 13 kyr from Rotsee, a small lake in central Switzerland. We used molecular distributions and $\delta^{13}C_{wax}$ values to calculate the fraction of sedimentary plant waxes from woody plants and grasses over time. Using a Monte Carlo simulation, we then estimated past δ^2H_{precip} values, based on $^2H/^1H$ fractionation factors from each plant group.

We show that δ^2H_{precip} values decreased to -75 ‰ around Rotsee during the cold Younger Dryas. δ^2H_{precip} values rapidly increased to -55 ‰ in the early Holocene and were relatively constant around -60 ‰ throughout the Holocene. Importantly, our vegetation-corrected reconstructed δ^2H_{precip} values are stable through the widespread forest clearing caused by the ancient Romans ~2 ka, when the relative abundance of grasses increased. These results demonstrate how vegetation corrections may be required from small lake systems to reliably interpret δ^2H_{wax} values as hydroclimate proxies. With appropriate corrections, such records can be used to map past δ^2H_{precip} values across Europe through periods like the Younger Dryas, when both climate and vegetation changed dramatically.

Conference Agenda

Session

4.2: Paleoclimatology and Archaeology

Time: **Friday, 20/June/2025: 11:00am - 12:00pm**
Session Chair: **Margot Kuitems**
Session Chair: **Marcel van der Meer**

Location: **Ton Schoot Uiterkamp Zaal**

5159.0029 - Energy Academy Europe, Nijenborgh 6, 9747 AG Groningen

Presentations

11:00am - 11:20am

Feeding Through Time: δ15N Amino Acid Analysis Reveals Dietary Shifts in Ancient Green Turtles

Willemien de Kock[1], Marcel van der Meer[2], Canan Çakırlar[1], Philip Riekenberg[2,3]

[1]Groningen Institute of Archaeology, University of Groningen, Netherlands, The; [2]Department of Marine Microbiology & Biogeochemistry, NIOZ Royal Netherlands Institute for Sea Research, Den Hoorn, The Netherlands; [3]Center for Applied Isotope Studies, University of Georgia, United States of America; w.de.kock@rug.nl

Previously published bulk collagen $\delta^{15}N$ measurements of archaeological eastern Mediterranean sea turtles revealed a striking dietary contrast. Green turtles (Chelonia mydas) from the Iron Age site of Kinet Höyük (~2700 BP) exhibited isotopic values consistent with modern herbivorous counterparts, while Bronze Age green turtles from Tell Fadous (~4700 BP) showed signatures indicative of a higher trophic level. To investigate this discrepancy, we conducted $\delta^{15}N$ compound-specific isotope analysis (CSIA) on 12 archaeological specimens, comparing dietary patterns between sites and against the omnivorous loggerhead turtle (Caretta caretta). Trophic position calculations suggest that the Bronze Age green turtles fed at a higher trophic level, potentially incorporating more animal matter into their diet. To assess possible environmental drivers, we examined published Mediterranean sea surface temperature (SST) reconstructions, which indicate cooler conditions during the Early Bronze Age compared to the Iron Age. These lower SSTs may have necessitated a dietary shift toward increased animal protein consumption. Our findings suggest that Bronze Age green turtles displayed dietary flexibility, likely as an adaptive response to climate fluctuations.

11:20am - 11:40am

Reconstructing the paleoenvironment of the Gran Chaco by combining stable isotopes and zooarchaeology

María Macarena Zarza[1], Thomas Larsen[2], Guillermo Nicolás Lamenza[1], Luis Manuel del Papa[1], Jeroen van der Lubbe[3]

[1]National Scientific and Technical Research Council (CONICET), Argentine Republic; [2]Department of Archaeology, Max Planck Institute of Geoanthropology, Germany; [3]Earth Science Stable Isotope Laboratory, Vrije Universiteit Amsterdam (VU), Nederlands; macarena_zarza@hotmail.com

The South American Gran Chaco, a vast region spanning over one million km², currently lacks region-specific paleoclimatic models. To evaluate the validity of models extrapolated from neighboring regions, we conducted zooarchaeological studies on environmentally sensitive species. This work analyzes stable carbon (δ13C) and oxygen (δ18O) isotope variations in archaeological and modern shells of *Pomacea canaliculata*. Whole-shell analyses provide estimates of annual climatic conditions, while intra-shell analyses reveal seasonal variations, establishing the first isotopic baseline for this species in the region. These studies have refined our understanding of climatic periods and complemented morphogeometric analyses of *Lepidosiren paradoxa* and *Hoplosternum littorale*, offering an independent assessment of seasonality and environmental conditions at archaeological sites. The integration of isotopic and zooarchaeological data has enabled evaluation of the proposed model's reliability. Furthermore, isotopic analyses have revealed humidity and temperature fluctuations at various temporal scales, enhancing our understanding of resource seasonality stability and its relationship with long-term climate variability. This approach has also facilitated assessment of extraordinary events such as Mega ENSO on local ecology and human occupation patterns. Our study demonstrates the value of combining multiple lines of evidence in paleoclimatic and archaeological investigations, introducing new methodologies for reconstructing the Gran Chaco's climatic and environmental history.

11:40am - 12:00pm

Tracing the lives of Māori dogs by sampling kahukurī or dogskin cloaks in Aotearoa New Zealand

Priscilla Wehi

University of Otago, New Zealand; priscilla.wehi@otago.ac.nz

Domesticated species have travelled with humans throughout the world and are integrally woven into the development of culture. Māori dogs (kurī) arrived in Aotearoa New Zealand with the first Polynesian voyagers in around 1300AD, and their skins were used to create prestigious cloaks. The dogs were also highly valued as food, and pets. However, the dogs disappeared after European arrival in around 1800 AD. We provide a glimpse into kurī ecology, and its relationships with people, by sampling dog hair from cloaks made during the early stages of European settlement, that are now held in museum collections. We first constructed isotopic niches for kuri at a range of time-scales and locations, by sampling bones from kuri (and their likely prey) from 14th and 15th C South Island sites, and then from hair from dog-skin cloaks. We have used carbon and nitrogen stable isotopes to examine kuri diet changes that could be indicative of environmental change, and decoupled socioecological relationships. Isotopic results from South Island sites indicate a wide range of dietary items eaten by kurī, that are closely tied to the economy of these settlements and diet of their human owners. Doghair sampled from cloaks indicate these dogs also had a varied diet, and differ even within the same cloak, thus allowing us to estimate the number of dogs required to make a cloak. We compare our findings with historic accounts of kuri and their diet from early explorers, and discuss the results in relation to changing bio-economics and cultural worldviews. Finally, we also highlight some sampling challenges. In particular we highlight the importance of community involvement in these projects, and the need to return data to communities as part of our cultural responsibility, in ways that grow community connection to both science and the artefacts (taonga) themselves.