JESIUM
2016

UNIVERSITEIT GENT

ISOFYS
Isotope Bioscience Laboratory

Benelux Association of
BASIS
Stable Isotope Scientists

Joint European Stable Isotope Users Group Meeting
JESIUM 2016 • 4–9 September 2016 • Gent • Belgium

Conference Programme
&
Book of Abstracts

Joint European Stable Isotope Users group Meeting

JESIUM 2016

4–9 September 2016
Ghent University
Gent, Belgium

Please fill in:

This JESIUM 2016 booklet belongs to

....

If found please return to conference desk and ask for chocolate.

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WLAN access

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Greetings of the organizers

JESIUM has been organized successfully in Vienna (2004), Presquile de Giens (2008) and Leipzig (2012). The objective of JESIUM is to serve as an excellent exchange forum for stable isotope users in Europe and worldwide. It is a pleasure for the Benelux Association of Stable Isotope Scientists (BASIS) and for us, the organizers in Gent to welcome you at JESIUM 2016. We'll bring together isotope scientists and companies with a broad range of experience and scientific interest, but with a shared passion for stable isotopes, providing the opportunity to discuss theoretical and methodological approaches in application of stable isotopes, exchanging ideas for further projects encouraging communication and collaborations across disciplines and country boundaries.

The scientific program, based on your engagement to present new ideas, first data and methodological advances, cover a broad range of application of stable isotopes. We were very happy to see how the topics we had proposed for the program resulted in more than 220 contributions. We are convinced that all of us will make JESIUM 2016 a successful meeting with a lot of interactions. Gent is a dazzling city with historical places, restaurants and bars, and an authentic heart (it ranked third on the National Geographic list of the most authentic cities worldwide).

We wish you a joyful time here in Gent and an inspiring JESIUM 2016.

On behalf of the organizing committee and BASIS,

Pascal Boeckx, BASIS chairman, Head of the Isotope Bioscience Laboratory at Ghent University, Belgium

JESIUM 2016 Committees

Scientific Committee

- Pascal Boeckx
- Eric Boschker
- Tom N. P. Bosma
- Federica Camin
- Isla Castañeda
- Jasper M. de Goeij
- Pedro Hervé
- Eric Kerstel
- Dwight Matthews
- Jeffrey McDonnel
- Harro A. J. Meijer
- Ivonne Nijenhuis
- Lars O. Hedin
- Henk Schierbeek
- Frans Stellaard
- Marcel van der Meer
- Gerard van der Peijl

Organizing Committee

- Pascal Boeckx
- Samuel Bodé
- Tom N. P. Bosma
- Pierre Cartigny
- Paul Dennis
- Nada Horvatincic
- Henk Schierbeek
- Florian Stange
- Frans Stellaard
- Katja Van Nieuland

Keynote speakers

Session: Advances in instrumentation and analytics –
Erik Kerstel

Session: Ecology –
Jasper M. de Goeij

Session: Environmental pollution –
Ivonne Nijenhuis

Session: Forensics –
Federica Camin

Session: Paleoclimatology & archeology –
Isla S. Castañeda

Session: Geosciences & hydrology –
Jeffrey McDonnell

Session: Nutrition, biochemistry & medicine –
Dwight E. Matthews

Session: Biogeochemistry –
Lars O. Hedin

Conference Programme

JESIUM 2016 Programme at a glance

	MON	TUE	WED	THU	FRI
08:00	Registration	Registration			
08:15					
08:30				Registration	Registration
08:45			Registration		
09:00	Opening	Environmental pollution		Nutrition, biochemistry & medicine	Biogeochemistry
09:15	Advances in instrumentation and analytics				
09:30			Geosciences & hydrology		
09:45					
10:00					
10:15					
10:30	Coffee	Coffee	Coffee		Coffee
10:45				Coffee	
11:00		Forensics			
11:15				Sponsor talks	
11:30					
11:45					Closing & awards
12:00					
12:15			Sponsor talk Elementar		
12:30	Lunch time	Lunch time		Lunch time	
12:45					
13:00					
13:15					
13:30					
14:00	Ecology	Paleo-climatology & archeology		Biogeochemistry	
14:15					
14:30					
14:45					
15:00			15:00–17:00 **City Walk** starts at Aula, ends at Town hall		
15:15					
15:30	Coffee	Coffee		Coffee	
15:45					
16:00					
16:15					
16:30					
16:45		Sponsor talk thermo			
17:00		Switch poster sessions	17:00–18:00 **Reception Town Hall**		
17:15	**Poster social I** *Advances in instrumentation and analytics, Ecology, Environmental pollution, Paleoclimatology & archeology, Forensics*			**Poster social II** *Geosciences & hydrology, Biogeochemistry, Nutrition, biochemistry & medicine*	
17:30					
17:45					
18:00			18:00–22:00 **Thermo event**		
18:15					
18:30					
18:45					
19:00		Conference dinner at Parnassus			
20:00					
21:00					
22:00					

Sunday, September 4

16:00 ***Registration***

17:30 ***Icebreaker reception at the conference venue Aula*** 17:30 – 19:00 h

Monday, September 5

08:00 ***Registration***

09:00 ***Opening***

Advances in instrumentation and analytics |

Convener: H. A. J. Meyer, co-convener and keynote: E. Kerstel

09:15 KEYNOTE: Water isotope ratio measurements using cavity enhanced absorption spectroscopy in field applications
Eric Kerstel

09:45 Correcting laser-based water stable isotope readings biased by carrier gas changes
Benjamin Gralher

10:00 Recent developments of position-specific isotope analysis for determining ^{13}C intramolecular composition
Gerald Remaud

10:15 A novel high-temperature combustion interface for compoundspecific stable isotope analysis via high-performance liquid chromatography/isotope ratio mass spectrometry
Eugen Federherr

10:30 ***Coffee break***

11:00 Extending the boundaries of isotope ratio MS – Latest technological improvements
Andreas Hilkert

11:15 Isotope Analysis of Sulfur, Bromine and Chlorine in Individual Anionic Species by Ion Chromatography/Multicollector-ICPMS
Faina Gelman

11:30 "Purge and trap" technology: new approach to continuous flow IRMS
François Fourel

11:45 A comparison of methods for triple isotopocule N_2 analysis
Jens Dyckmans

12:00 Challenges in measuring $\delta^{15}N$ in inorganic nitrogen forms: results from a ring test comparing three measurement approaches show large variation
Christina Biasi

12:15 Present status and future developments of the stable isotope reference materials at the IAEA
Sergey Assonov

12:30 ***Lunch break*** *(see page 20)*

Monday, September 5

Ecology

Convener: E. Boschker, co-convener and keynote: J. M. de Goeij

14:00 KEYNOTE: How fast and efficient sponge engines drive and modulate the food web of shallow and deep reef ecosystems
Jasper M. de Goeij

14:30 Hydrogen isotopes as an aquatic provenance tracer
David Soto

14:45 Mechanisms mediating the effect of organic matter quality on the role of aquatic fungi and bacteria in carbon turnover
Jenny Fabian

15:00 Facultative paedomorphosis in newts: A heterochrony promoting trophic niche differentiation at the intraspecific level
Benjamin Lejeune

15:15 Trophic position estimates from amino acid nitrogen isotopic analyses: the implications of intra-individual variability
Tamsin O'Connell

15:30 ***Coffee break***

16:00 Invesigating N_2O emissions from plants by using high resolution measurements of N_2O mixing ratio and isotope signatures (^{15}N, ^{18}O)
Katharina Lenhart

16:15 Evaluation of the importance of water isotope variations in precipitation versus plant source water for the interpretation of O and H isotopes in plant tissues
Rolf Siegwolf

16:30 A stable isotopic view on lianas' and trees' below ground competition for water
Hannes De Deurwaerder

16:45 Hydrogen isotopes in plant organic compounds indicate strong shifts in the carbohydrate metabolism of plants over the past century
Ansgar Kahmen

17:00 Mechanisms of seedling facilitation through mycorrhizal links with adult plants – carbon costs and nutritional benefits in common mycelial networks
Stephan Unger

17:15 ***Poster social*** *(see page 22)****:***

Advances in instrumentation and analytics
Ecology
Environmental pollution
Paleoclimatology & archeology
Forensics

Environmental pollution

Convener: T. N. P. Bosma, co-convener and keynote: I. Nijenhuis

09:00 **KEYNOTE: Characterisation of microbial reductive dehalogenation of halogenated organic contaminants using compound-specific stable isotope analysis**
Ivonne Nijenhuis

09:30 **Carbon and chlorine isotope fractionation during anaerobic biodegradation of chlorinated ethanes by Dehalogenimonas**
Mònica Rosell

09:45 **Lichen nitrogen and carbon isotopic composition as indication for atmospheric nitrogen pollution – a synopsis**
Stefanie Boltersdorf

10:00 **Model-based integration of enantiomer and stable isotope fractionation for chiral pesticides degradation**
Biao Jin

10:15 **Soil and water extraction methods for Compound Specific Isotope Analysis (CSIA) of aromatic anilide pesticides and application to agricultural soils**
Charline Wiegert

10:30 ***Coffee break***

Forensics

Convener: G. van der Peijl, co-convener and keynote: F. Camin

11:00 **KEYNOTE: Stable isotope ratios for food authentication and traceability**
Federica Camin

11:30 **The potential of IRMS and GC-IRMS analysis of TATP in forensic explosives investigations**
Karlijn Bezemer

11:45 **Global spatial distribution of human carbon isotope ratios**
Frank Huelsemann

12:00 **Compound-specific stable isotope analysis of gamma-hydroxybutyrate (GHB) in urine: a powerful tool to prove drug facilitated assults**
Balázs Horváth

12:15 **Identifying different origin of tropical timber from West- and Central African countries**
Micha Horacek

12:30 ***Lunch break*** *(see page 20)*

Tuesday, September 6

Paleoclimatology & archeology

Convener: M. van der Meer, co-convener and keynote: I. S. Castañeda

14:00 **KEYNOTE: Hydroclimate variability in central North Africa during the Plio-Pleistocene**
Isla S. Castañeda

14:30 **From Prehistory to History: change and continuation in the dietary habits from the inhabitants of the Balearic Islands based on ^{14}C and stable isotope research (δ ^{13}C and δ ^{15}N)**
Mathieu Boudin

14:45 **Reconstruction of sea surface salinity changes of the Sea of Okhotsk during the past 1.5 Ma using δD of long-chain alkenones**
Julie Lattaud

15:00 **Assessment of ns-LA-MC-ICP-MS for Pb and Sr isotopic analysis of archaeological glass – mass bias correction strategies and Corning glass standards**
Alicia Van Ham-Meert

15:15 **Relative humidity changes during the Younger Dryas in the Western Eifel region based on a biomarker-isotope paleohygrometer**
Johannes Hepp

15:30 ***Coffee break***

16:00 **The stable hydrogen isotopic composition of sedimentary plant waxes as quantitative proxy for rainfall in the West African Sahel**
Eva Niedermeyer

16:15 **Preliminary investigation of seasonal patterns recorded in the oxygen isotpe compositions of theropod dinosaur tooth enamel**
Jean Goedert

16:30 **Modelling oxygen isotope ratios in tree-rings: From process-based studies to large spatial and temporal scales**
Matthias Saurer

16:45–17:00 Improvements in EA – IRMS for low µg to mg sample amounts
Chris Brodie, Thermo Fisher Scientific | at conference venue Aula

17:00 **Switch of poster sessions:** Session I authors, please remove your posters, Session II authors, please mount your posters!

19:00 ***Conference dinner at Parnassus*** *(see page 22)*

Geosciences & hydrology

Convener: P. Fernandez-Hervé, co-convener and keynote: J. McDonnel

09:30 **KEYNOTE: Isotope tracers in catchment hydrology: Opportunities and challenges**
Jeffrey J. McDonnell

10:00 **Sub-catchment size as a limiting factor for nitrogen transformation recognition in a meso-scale river basin**
Christin Müller

10:15 **Isotope memory effects between soil water pools influence the isotopic composition of available plant water**
Sarah Newberry

10:30 ***Coffee break***

11:00 **Resolving eco-hydrological dynamics of soil–plant–atmosphere interactions**
Maren Dubbert

11:15 **Long-term isotope series in large river basins indicating groundwater contribution and climate variability, examples from the Weser River in Germany**
Paul Koeniger

11:30 **Compound specific stable isotope for sediment fingerprinting in Chitlang catchment (Nepal): From biotracer isotopic proportions to sources soil contribution**
Hari Ram Upadhayay

11:45 **Worldwide interlaboratory comparison of cryogenic water extraction systems for stable water isotope analysis**
Natalie Orlowski

12:00 **Determining the origin of raining air masses in Iran and the Middle East Countries using isotopic composition (^{18}O and ^{2}H) of Precipitations**
Hossein Mohammadzadeh

12:15–12:45 IonOS: A new synthesis of IRMS software
Elementar Analysensysteme | at conference venue Aula

15:00 ***Free guided city tour*** 15:00 – 17:00 h (starts at Aula, ends at Town Hall, *page 23*)

17:00 ***Reception at Gent Town Hall*** 17:00 – 18:00 h *(see page 23)*

18:00–22:00 IRMS Users Appreciation Event
Thermo Fisher Scientific

Thursday, September 8

Nutrition, biochemistry & medicine

Convener: H. Schierbeek, co-convener and keynote: D.E. Matthews

09:00 **KEYNOTE: Measuring protein synthesis and breakdown rates using stable isotopes and mass spectrometry**
Dwight E. Matthews

09:30 **Following the impact from an overfeeding on Human metabolism using stable isotope tracers**
Valérie Sauvinet

09:45 **Metabolic fluxes, compartments and carbon-isotope fractionation in androgen metabolism**
Ulrich Flenker

10:00 **Primary Hyperoxaluria: unraveling the clinical pathophysiology by means of stable isotopes**
Sander Garrelfs

10:15 **Carbon isotope values of non-essential amino acids in mice reflect macromolecular compostion of diets**
Thomas Larsen

10:30 **Fructose or sucrose co-ingestion increases exogenous carbohydrate oxidation during exercise in trained cyclists**
Jorn Trommelen

10:45 ***Coffee break***

11:15 ***Sponsor Talks***

Agilent Technologies (10 min)
Extend your metabolomics insight with VistaFlux Stable Isotope tracing software
Martin Haex, Product Specialist Mass Spectrometry

Elementar Analysensysteme GmbH (10 min)
Precision and Vision: The new generation of stable isotope analysers from Isoprime

Nu Instruments Ltd (10 min)
Helen Atkinson

BUCHEM: Film (7 min)

Picarro (10 min)

Sercon (10 min)

12:30 ***Lunch break*** *(see page 20)*

Biogeochemistry

Convener: P. Boeckx, co-convener and keynote: L. O. Hedin

14:00 **KEYNOTE: Grand challenges in understanding global land biogeochemical cycles: How can isotopes help?**
Lars O. Hedin

14:30 **Nitrogen transformation and retention in temperate forest edges**
Elyn Remy

14:45 **Hydrogen dynamics in soil organic matter as determined by ^{13}C and ^{2}H labeling experiments**
Alexia Paul

15:00 **Simulating the isotopic composition of terrestrial N cycle components with the stable isotope model of N-cycle evolution (SIMONE)**
Tobias Denk

15:15 **Response of subalpine vegetation to N deposition and land-use changes from a new isotopic perspective**
Ilann Bourgeois

15:30 ***Coffee break***

16:00 **Quantification of enzyme-mediated hydrolysis by means of the oxygen isotope ratio of phosphate in grassland and forest soils**
Yvonne Oelmann

16:15 **Continental-scale spatiotemporal patterns of plant water isotope values as a tool for improved understanding of hydroclimate proxies from plants**
Daniel Nelson

16:30 **Nitrogen cycle inferred from N and O isotopes in a changing Arctic Ocean**
François Fripiat

16:45 **Interacting effects of climate variation and site conditions on tree growth and physiology of beech and spruce across Central Europe**
Rosemarie Weigt

17:00 **Do cable bacteria enhance dissimilatory nitrate reduction to ammonium in coastal sediments?**
Perran Cook

17:15 ***Poster social*** *(see page 22)****:***

Geosciences & hydrology
Biogeochemistry
Nutrition, biochemistry & medicine

Friday, September 9

09:00 The influence of atmospheric water vapour on the oxygen isotope ratio of organic molecules in plants
Marco M. Lehmann

09:15 Plant-plant interactions and environmental factors explain high small scale variation in foliar $\delta^{13}C$ and $\delta^{15}N$: insights from a combined isoscape – remote sensing approach
Christine Hellmann

09:30 Can the two-pool model explain oxygen isotope variations in needles and tree rings of drought-stressed Scots pine trees?
Galina Timofeeva

09:45 The emission of CO_2 from a forest floor on natural soil temperature gradient in a volcanic area in southwest Iceland and the contribution of geothermal CO_2 measured by $\delta^{12}C/\delta^{13}C$ fractionation
Marja Maljanen

10:00 Isotopic characterization of N_2O emissions and N_2O soil concentrations from hotspots in sub-Arctic permafrost peatlands
Jenie Gil

10:15 Stable carbon isotopes indicate high importance of vascular plants for methane emissions from subarctic wetlands in Northeast European Russia
Maija Marushchak

10:30 ***Coffee break***

11:00 Quantification of organic and inorganic nitrogen dynamics in soil using the ^{15}N tracing model Ntrace
Tobias Rütting

11:15 C:N:P:K stoichiometric constraints on litter and soil organic matter decomposition
Jennifer Soong

11:30 Bidirectional fluxes in microbial metabolism: The next step towards quantitative soil fluxomics
Michaela Dippold

11:45 ***Closing & awards***

Events
General Information

UPLOAD OF SLIDES FOR ORAL PRESENTATION

- Come to the meeting room latest in the break before your session starts, or, if your session is in the morning, latest in the last break of the day before to upload you contribution. An assistant will be present to help you to save your file to the presentation notebook, etc.
- Bring your file on a USB stick, please DO NOT bring your own notebook.

TECHNICAL INFORMATION FOR MOUNTING A POSTER

Posters will be presented in two sessions:

POSTER SESSION I:

Monday–Tuesday evening (Poster Social: Monday evening)

Topics:
- *Advances in instrumentation and analytics*
- *Ecology*
- *Environmental pollution*
- *Paleoclimatology & archeology*
- *Forensics*

If your poster belongs to Poster Session I, your poster number starts with I-...

POSTER SESSION II:

Wednesday–Friday (Poster Social: Thursday evening)

Topics:
- *Geosciences & hydrology*
- *Biogeochemistry*
- *Nutrition, biochemistry & medicine*

If your poster belongs to Poster Session II, your poster number starts with II-...

Please note: The switch of poster sessions is scheduled on Tuesday after the end of the lectures. Poster session I authors, please remove your posters asap to allow the Poster session II authors to mount their posters.
There are poster lists on display for quick reference where to find your poster number.

AWARDS

The best student talk and poster will be awarded during the closing session on Friday.

Lunch

Lunch will not be served at the Aula and it is not included in the conference fee. However, hence the Aula is right in the middle of the city centre, you will find plenty of opportunities to spend a nice lunchtime not far from the conference venue.

In the following, please find 15 restaurants that are within a 10 min walk (or less) from the conference venue Aula serving lunch for less than € 15.

Thanks to Emer. Prof. Oswald Van Cleemput for sharing this list to the JESIUM 2016 participants.

No	Restaurant	Address	Walk | min
1	New Maximilian	Mageleinstraat 50	2
2	De Foyer	St-Baafsplein 17	3
3	Lepelblad	Onderbergen 40	3
4	Belfort restaurant	E Braunplein 40	3
5	Kapittelhuis	Korte Kruisstraat 4	3
6	Du Progrès	Korenmarkt 10	4
7	Het Pand	Onderbergen 1	4
8	The Celtic Towers Irish Pub	St-Michielshelling 5/6	5
9	Café Theatre	Schouwburgstraat 5-7	5
10	De Graslei	Graslei	5
11	Faim de toi	Belfortstraat 8	6
12	Korenlei Twee	Koornlei 2	7
13	Godot	Hooiaard 8	7
14	Thalia	St-Pietersnieuwstraat 18	8
15	Kaai 14	Kuiperskaai 14	9


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Aula
Tourist Information Centre
Design museum Gent
Galerie Pont & Plas
Graslei
Korenmarkt Gent
Groentenmarkt
Arteveldehogeschool campus Hoogpoort
Graffiti Street
Gent Watertoerist
Sint-Jacobskerk
Sint-Niklaaskerk
City Pavilion
Emile Braunplein
Het Belfort van Gent
Sint-Baafsplein
MIRY Concertzaal
Sint-Baafskathedraal
Sint-Michielskerk
Gent Korenmarkt Perron 1
Gent Korenmarkt Perron 3
Gent Korenmarkt Perron 5
Balls & Glory
De Kastart
Galerie Jan Dhaese
Complex Voldersstraat
Gent Duivelsteen
Gent Zonnestraat
Gent Korte Meer
Gent Oude Houtlei
Handelsbeurs Concertzaal
Center Office
Poppentheater Pedrolino VZW
Gent Savaanstraat
Sint-Barbaracollege
Pain Perdu
Minard
REWIND
Vooruit
Pipoos
Wasbar
Leie
Hoogpoort
Zwartezustersstraat
Hoornstraat
Voldersstraat
Onderbergen
Veldstraat
Korte Meer
Mageleinstraat
Universiteitstraat
Kouterdreef
Vogelmarkt
Koestraat
Schouwburgstraat
Kuipgat
Ketelvest
Savaanstraat
Jeruzalemstraat
Bagattenstraat
Lammerstraat
Brabantdam
Nederkouter
Coupure Rechts
Google

Events

Icebreaker

Sunday, 4 September 2016 • 17:30 | at the conference venue Aula

Poster Socials I & II

- at the conference venue Aula

Please note: The switch of posters will be Tuesday (September 6) evening after the sessions.

Poster Social I: Monday, 5 September 2016 • 17:15

Poster Social II: Thursday, 8 September 2016 • 17:15

Don't miss these poster socials ! For the posters, sure, though also for the social part which will be enlighten with the most exclusive Trappist beer you can get; it was nominated as the best beer in the world in 2002 and 2007, by Michael Jackson (no not the popstar, *http://beerhunter.com/*) and is only available by personal appointment with the monks in the very remoted abbey (*http://sintsixtus.be/*), it's probably the one chance in your live to taste it!

Conference Dinner at Parnassus

Wednesday, 7 September 2016 • 19:00–22:00

- at PARNASSUS (address: Oude Houtlei 122, *http://www.parnassus.be*, € 60.00 | limited places!

Have a seated dinner with music at a stunning place – a fantastic old church that serves as restaurant and concert hall today.

Guided City Walk

Thursday, 8 September 2016 • 15:00–17:00

- starts at the conference venue Aula (Voldersstraat 9, 9000 Gent) and ends at the Town Hall for the reception (Botermarkt 1); subscription mandatory

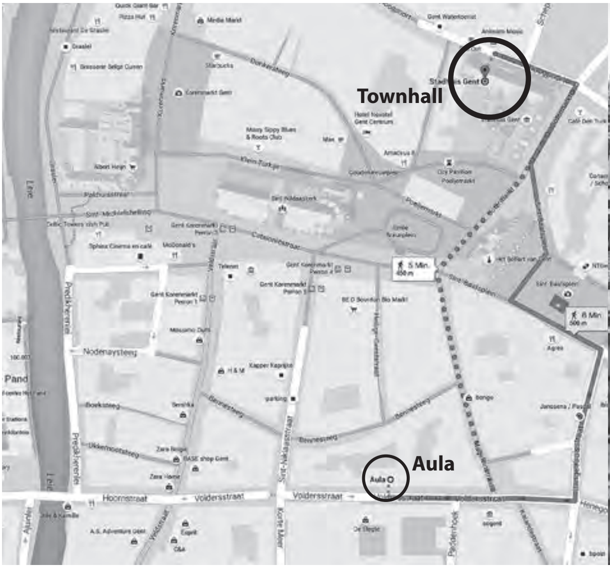
Discover the history and the beauty of Gent. You'll be mesmerized by its quays, its buildings and squares. Every house has its own story, every facade is hiding some legends behind.

Reception at the Townhall (Stadhuis Gent)

Thursday, 8 September 2016 • 17:00–18:00

- Town hall (Botermarkt 1), reserved for participants of the guided city walk

Enjoy a warm welcome by the Gent representatives and a reception in this beautiful historical building.



Editors of **Isotopes in Environmental and Health Studies** are interested in organizing a Special Issue JESIUM 2016 as we did in JESIUM's history.

Isotopes in Environmental and Health Studies (IEHS) provides a unique platform for stable isotope studies in geological and life sciences, with emphasis on ecology.

Manuscripts from all topics of JESIUM 2016 are welcome. All manuscript submissions are subject to initial appraisal by the Editor, and, if found suitable for further consideration, to peer review by independent, anonymous expert referees. All peer review is single blind.

SUBMISSION

Submitting deadline is **December 01, 2016.**

Submission is online via ScholarOne Manuscripts linked on the journal's web site, and further "Submit".

(*http://www.tandfonline.com/toc/gieh20/current*). Assign the submission to "Special Issue JESIUM2016".

GUIDELINES

The manuscripts should be in English (UK), meet the style of IEHS (see for instructions & submission on the journal's web site) and are limited on 10 manuscript pages (please see instructions journal's web site).

Please note the IEHS copyright terms and conditions.

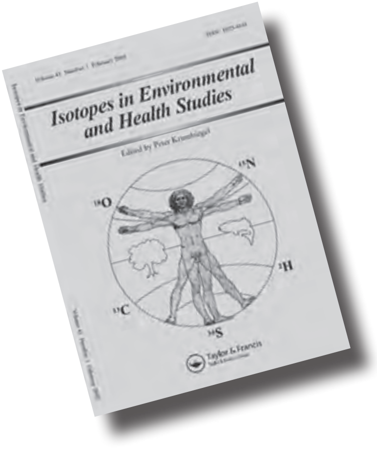
Submission is free except of figures in colour in the print version.

CONTACT

For any contact, please use our e-mail address: **editoriehs@ufz.de**

Cordially,

Gerhard Strauch, Editor-in-Chief, and Peter Krumbiegel, Co-Editor



Abstracts of Talks

Water isotope ratio measurements using cavity enhanced absorption spectroscopy in field applications

Erik Kerstel[1], Daniele Romanini[1], Samir Kassi[1], Mathieu Casado[2], & Amaelle Landais[2], Roberto Grilli[3] & Jerome Chappellaz[3]

[1] Laboratory of Interdisciplinary Physics (LIPhy), CNRS/University of Grenoble Alps, France
[2] Laboratory of Environmental and Climate Sciences (LSCE), CNRS, Gif-sur-Yvette, France
[3] Laboratory of Glaciology and Environmental Geophisics (LGGE), CNRS, Grenoble, France

In this presentation we will briefly review laser based isotope ratio measurements and identify the major issues when attempting to make reliable measurements at low concentration in extreme environments. In particular, we will detail our efforts to develop a precise and sensitive water vapor isotope spectrometer based on the principle of Optical Feedback Cavity Enhanced Absorption Spectroscopy and its accompanying calibration device. Furthermore, a similar spectrometer was miniaturized in order to fit into a 10 cm diameter ice-coring probe [1]. In this way, the probe will be able to perform an in-situ analysis of a fraction of the melted ice for the deuterium isotope ratio, and measure the methane concentration of air inclusions, during its descent through the ice-pack. Recent and future field applications in Antarctica of these two instruments will be discussed.

Reference

[1] http://www.iceandlasers.org, last accessed on May 26, 2016.

Correcting laser-based water stable isotope readings biased by carrier gas changes

Benjamin Gralher[1], Barbara Herbstritt[2], Markus Weiler[2], Leonard I. Wassenaar[3] & Christine Stumpp[1]

[1] Institute of Groundwater Ecology, Helmholtz Zentrum München, Neuherberg, Germany
[2] Hydrology, University of Freiburg, Freiburg, Germany
[3] Isotope Hydrology Section, International Atomic Energy Agency, Vienna, Austria

Recently, laser-based water stable isotope spectrometers have become popular as they enable previously impossible approaches of environmental observations. Consequently, they have been subjected to increasingly heterogeneous atmospheric conditions. However, the impact of non-standardized gas matrices on analyzer performances has not yet been rigorously tested. Against this background, we investigated the influence of changing proportions of N_2, O_2, and CO_2 in the carrier gas on the isotope readings of a typical laser-based water stable isotope analyzer (Picarro L2120-*i*). We combined environmentally relevant mixtures of N_2, O_2, and CO_2 with referenced, flash-evaporated water and found that isotope readings of the same water were altered by up to +14.57 ‰ for $\delta^{18}O$ and -35.9 ‰ for $\delta^{2}H$. All tested relationships between carrier gas changes and respective isotope readings were strongly linear correlated ($R^2>0.99$). Furthermore, an analyzer-measured variable allowed for reliable post-correction of the biased isotope readings, which we additionally tested on field data. Our findings affect the trustworthiness of all environmental data obtained by analyzers based on the same technology. They are crucial for assays where inconsistent gas matrices or a mismatch in this regard between unknown and reference analyses cannot be excluded, which is in particular common when investigating the soil-vegetation-atmosphere continuum.

Recent developments of position-specific isotope analysis for determining ^{13}C intramolecular composition

Gérald S. Remaud, Kevin Bayle, Didier G. Diomande, Maxime Julien, Noelle Merchak, Richard J. Robins & Serge Akoka

EBSI team, CEISAM UMR CNRS 6230, Unversity of Nantes, France

The development of isotopic NMR spectrometry at natural abundance enables the quantification each isotopomer constituting a given molecule for a given element.

The possibility of measuring position-specific ^{13}C/^{12}C ratios directly by using ^{13}C NMR has been established and tested over the last few years. The main difficulty in obtaining the intramolecular ^{13}C composition by ^{13}C NMR is meeting the requirement for a high level of precision: better than 1‰! Advanced protocols have been developed that overcome several obstacles and enable its successful application in a number of fields (Bayle *et al.* 2014). Recent technological developments have made further improvements, specifically by the exploitation of polarization transfer techniques, in which the abundance of the ^{1}H atom is exploited to enhance sensitivity (Tibaudeau *et al.* 2010). Thus, ^{13}C isotopic profiles of targeted molecules were obtained with the required precision and within a reasonable measurement time. The prospect of carying out ^{13}C isotope analysis by 2D NMR has also been demonstrated with an ^{1}H-^{13}C HSQC experiment (Martineau *et al.* 2013).

A serie of examples are presented in order to delineate the step-wise experimental scheme required for measuring intramolecular ^{13}C distributions. In the Food Industry, theobromine is a good target for traceability of chocolate but, due to its poor solubility, theobromine (from cocoa) cannot be studied directly by NMR. However, its ^{13}C profile can be obtained *via* caffeine after a suitable conversion. Thus, caffeine is a single molecular probe which can be used to assess the geographical origin of Coffee break, tea and cocoa (Diomande *et al.* 2015). In the Pharmaceutical Industry, ^{13}C isotopic profiles of ibuprofen and naproxen were obtained, again as a clue for traceability: different manufacturing processes and precursors should each lead to a unique isotopic constitution (Remaud *et al.* 2013). Isotope profiling can be considered as an 'isotopomic' study (Merchak *et al.* 2015). This is a promising generic approach complementary to metabolomics.

Pollutants can be naturally eliminated via different ways such as bioremediation, oxidation or volatilization. Position-specific ^{13}C analysis provides greater detail of isotope fractionation during such processes. As an example, position-specific ^{13}C analysis has clarified the isotope fractionation during evaporation, i.e. liquid to vapour tranformation (Julien *et al.* 2015).

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A novel high-temperature combustion interface for compoundspecific stable isotope analysis via high-performance liquid chromatography/isotope ratio mass spectrometry

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In aqueous samples compound-specific stable isotope analysis (CSIA) plays an important role. No direct method (without sample preparation) for stable nitrogen isotope analysis (δ^{15}N SIA) of non-volatile compounds is known yet. The development of a novel HPLC/IRMS interface based on high-temperature combustion (HTC) for both δ^{13}C and δ^{15}N CSIA and its proof of principle are described in this study.

To hyphenate high-performance liquid chromatography (HPLC) with isotope ratio mass spectrometry (IRMS) a modified high-temperature combustion total organic carbon analyzer (HTC TOC) was used. A system to handle a continuously large amount of water (three-step drying system), favorable carrier and reaction gas mix and flow, an efficient high-temperature-based oxidation and subsequent reduction system and a collimated beam transfer system were the main requirements to achieve the necessary performance.

The proof of principle with caffeine solutions of the system succeeded. In this initial testing, both δ^{13}C and δ^{15}N values of tested compounds were determined with precision and trueness of ≤0.5‰. Further tests resulted in lower working limit values of 3.5 µgC for δ^{13}C SIA and 20 µgN for δ^{15}N SIA, considering an accuracy of ±0.5‰ as acceptable.

The development of a novel HPLC/IRMS interface resulted in the first system reported to be suitable for both δ^{13}C and δ^{15}N direct CSIA of non-volatile compounds. This highly efficient system will probably open up new possibilities in SIA-based research fields.

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Extending the boundaries of isotope ratio MS – Latest technological improvements

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Isotope ratio mass spectrometry has a long history, which started with the analysis of the isotopes of CO_2. Over several decades a broad range of IRMS techniques has been derived like multi-collector high resolution ICP-MS, TIMS, noble gas static MS and gas IRMS. These different flavors of IRMS are now building a technology tool box, which allows to derive new applications build on new capabilities by combination of specific features of these sister technologies.

In the 90's inductive coupled plasma ionization was added for the high precision analysis of rare elements. In 2000 extended multicollection opened the way into clumped isotopes. In 2008 the concept of a high resolution gas source IRMS was layed out to revolutionize stable gas IRMS recently followed by the combination of this static multicollection mode with fast mass scans of the single collector double focusing high resolution GCMS. Recently new technologies were created, like the mid infrared analyzers (IRIS) based on difference frequency generation lasers, the combination of a collision cell with HR MC ICPMS as well as the use of a high resolution electrostatic ion trap for extended stable isotope analysis on individual compounds.

All these building blocks for IRMS address selected requirements of sample preparation, sample introduction, referencing, ionization, mass separation, ion detection or signal amplification. Along these lines new technological improvements and applications will be shown and discussed.

Isotope Analysis of Sulfur, Bromine and Chlorine in Individual Anionic Species by Ion Chromatography/Multicollector-ICPMS

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The isotope composition of sulfur, chlorine, and bromine in organic and inorganic species can provide unique data for studying natural biogeochemical processes, water contamination, and natural attenuation mechanisms. During the last decades, isotope analysis of carbon, hydrogen, and nitrogen inorganic compounds by gas chromatography (GC) coupled to an isotope ratio mass spectrometer (IRMS) has become a strong analytical tool in environmental, forensic, biochemical, and geochemical sciences. In recent years, compound-specific isotope analysis of Hg, S, Br, and Cl by GC-MC-ICPMS has been established. However, isotope analysis of inorganic S, Br and Cl still remains a complex task requiring a tedious off-line sample preparation.

We developed an analytical method for precise and accurate analysis of $\delta^{34}S$, $\delta^{81}Br$, and $\delta^{37}Cl$ in individual anionic species by coupled ion chromatography (IC) to MC-ICPMS. The method is based on the on-line separation and purifications of ions by IC prior to their isotope analysis by MC-ICPMS. The developed technique significantly simplifies $\delta^{34}S$, $\delta^{81}Br$, and $\delta^{37}Cl$ analysis in environmental samples. In cases when several anionic species of the same element are present in the samples, they might be analyzed in a single analytical run. Major isobaric interferences for the analyzed elements were reduced by using "dry" plasma conditions and applying sufficient mass resolution power. The sample-standard bracketing technique was used for instrumental drift correction. In the case of $\delta^{34}S$ analysis, precisions up to 0.15‰ (1sd) have been achieved for analytes containing down to 5 nmol of S; for $\delta^{81}Br$, the attained precision was 0.1‰ (1sd) for analytes containing down to 0.6 nmol of Br. Precisions of 0.2‰ have been obtained for $\delta^{37}Cl$ with analytes containing 0.7 µmol of Cl. Robustness of the developed analytical method, as well as high precisions and accuracies, has been demonstrated for the laboratory standard solutions and for environmental samples.

"Purge and trap" technology: new approach to continuous flow IRMS

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Since the early days of continuous flow IRMS analyses by Preston and Owens (1985) analyte gas separations have been mostly done through conventional gas chromatography, first with packed columns then using capillary columns and this for various types of automated preparation systems. Since the end of the 2000's another type of gas separation system in continuous flow mode has been made available to IRMS users first for EA-IRMS applications.

In this presentation we describe the technological differences between conventional GC separation and purge and trap separation systems for EA-IRMS techniques both in combustion mode and pyrolysis mode (Fourel et al 2014[a,b]).

As far as pyrolysis mode is concerned we emphasise the significant differences observed using purge and trap technology for $^{18}O/^{16}O$ analyses of various types of samples but mainly focusing on silver phosphates, generally considered as diffcult samples to pyrolyse. We discuss the important N_2-CO separation issue and the solutions offered by purge-and-trap technology. We also demonstrate how reliable those measurements can be even when going down in sample sizes down to 50 micrograms of Ag_3PO_4. We illustrate the new possibilities this technique might offer in terms of selecting adequate Reference Calibrated Materials.

Dealing with combustion mode we emphasize the interest of using purge-and-trap technology for simultaneous determinations of N, C and S isotopic signatures as well as concentrations. We insist in particular on the high quality of $^{34}S/^{32}S$ determinations for samples with low S content as well as high C/S ratios.

We discuss the new requirements generated by this technique for peak integrations and blank subtraction procedures.

Finally, based on the results obtained using purge-and-trep technology for EA-IRMS techniques we discuss the potential use of this separation method for other types of preparation systems.

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A comparison of methods for triple isotopocule N_2 analysis

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For the determination of N_2 isotopic composition, usually only masses m/z 28 and 29 are used, because m/z 30 determination is impeded by NO, which is formed in the mass spectrometer source during the measurements. The calculation of $^{15}N/^{14}N$ isotope ratios from these data is possible as long as the measured N_2 is in isotopic equilibrium (i.e. the isotopes are distributed statistically among all molecule masses m/z 28, 29 and 30).

When measuring a mix of N_2 from two different sources with different isotopic abundances, this simple approach is no longer possible because the isotopes are not distributed statistically among the molecules in the mixture.

Two different approaches have been used to determine the triple isotopocule distribution of N_2:

1) Measuring all three masses m/z 28, 29 and 30 while taking great care to reduce contribution of NO formation on m/z 30 (Lewicka-Szczebak et al. 2013)

2) Measuring the masses m/z 28 and 29 before and after establishing statistical distribution of the isotopes among all molecules (Well et al. 1998). The statistical distribution is accomplished by disintegrating N_2 molecules and subsequent instantaneous reformation of N_2. Isotopes then are statistically distributed among the molecules. A set-up was developed, where measurements with and without equilibration are performed from the same sample in one run.

Here we present a comparison of the two methods with regard to handling and accuracy of results.

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Challenges in measuring δ¹⁵N in inorganic nitrogen forms: results from a ring test comparing three measurement approaches show large variation

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The negative environmental effects of excess nitrogen (N) on soil, water and air quality, and in consequence on human health, have intensified studies on the role and fate of inorganic N forms in ecosystems in recent years. To assess the sources and sinks of bioavailable nitrogen forms, modern stable isotope tools are increasingly applied and many exciting new discoveries were made in that area over the last decades. All these studies rely heavily on precise measurements of the isotopic signals (δ^{15}N or at%^{15}N) of NO_3^- and NH_4^+.

Here, we conducted a ring-test involving 10 European laboratories to compare different methods commonly used to measure δ^{15}N in inorganic nitrogen forms (NO_3^- and NH_4^+). The approaches tested were 1) the microdiffusion method (MD), 2) chemical conversion methods (CM) and 3) the biological denitrifier method (DN). Standards (natural ^{15}N abundance, ^{15}N labelled) and environmental samples were centrally prepared (in Vienna) and sent out to the different laboratories. We also included each one standard (mixed standard) with the target compound at natural ^{15}N abundance and other N forms being ^{15}N labelled to assess possible cross-contamination.

The ring test showed that standards were reasonably well replicatedand analysed by the different methods, though variability increased with ^{15}N enrichment. The analysis of the mixed standards revealed that MD is largely hampered by cross-contamination (by up to 60%), only some DN and CM methods showing no cross-contamination issues. The results of the environmental samples were generally all highly variable with differences in reported isotope values up to 50‰ between the labs. The variability was larger when the concentration of the target compound was low and the concentration of the additional N sources (e. g. organic N) was high, but this was not always the case. We identified incomplete recovery and associated fractionation, issues with background corrections and isotope calibrations as the prime sources of error and will discuss this in the presentation. Obviously, more studies are needed to optimize the methods in terms of selectivity and precision in order to reliably analyse the isotopic signal of inorganic N sources. This is crucial for interpreting analytical data on the isotopic composition of NO_3^- and NH_4^+.

Present status and future developments of the stable isotope reference materials at the IAEA

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The presentation will give the overview of the stable isotope reference materials (SI-RMs) under distribution by the IAEA, its laboratory and capacities related to material testing & production as well as future plans, with the main focus on delta-^{13}C materials. The future plans include several directions as follows:

- Maintaining the scale-defining SI-RMs at the highest level and introducing adequate replacements when needed;
- Monitoring existing SI-RMs for potential alteration(s) of isotopic values assigned;
- dentifying and then addressing the needs for new SI-RMs, with the priority to address the most critical applications (environmental and climate related applications, human health, food safety studies) and newly emerging analytical isotope techniques;
- Performing all measurements aimed for characterisation of new SI-RMs and the corresponding uncertainty evaluation in accordance to the modern metrological concepts;
- Promoting metrological approaches on traceability and uncertainty evaluation in every day practice of stable isotope measurements;
- Expanding the IAEA capacities for SI-RMs by (i) planning a renewed laboratory at IAEA; (ii) enlarging collaboration with expert laboratories aimed to help IAEA in production and characterisation of new SI-RMs.

These major directions will help to address the increasing demand for Stable Isotope Reference Materials.

How fast and efficient sponge engines drive and modulate the food web of shallow and deep reef ecosystems

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Ever since Darwin's early descriptions of reefs, it has been a mystery how one of earth's most productive and diverse ecosystems thrives in oligotrophic seas, as an oasis in a marine desert. We recently discovered the so-called sponge loop pathway using (^{13}C- and ^{15}N-) stable isotope tracers in *ex situ* mesocosm and *in situ* reef experiments (De Goeij et al. 2013). This pathway revealed that, within the food web of coral reefs, sponges efficiently retain and transfer energy and nutrients on reefs, recognizing their (so far neglected) role as key ecosystem drivers. Evidence now accumulates on sponge loops in other ecosystems, such as deep-sea coral reefs (Rix et al. 2016). As a result, current reef food web models lacking sponge-driven resource cycling are incomplete and need to be redeveloped. However, mechanisms that determine the capacity of sponge 'engines', how they are fuelled, and drive communities are unknown.

I will discuss the experimental tracer-isotope approach leading to the discovery of the sponge loop and subsequently the establishment of a novel reef food web framework, integrating sponges as key ecosystem drivers. To this end, existing critical knowledge gaps at both organismal and community scale will be identified. Sponges will be evaluated on functional traits (morphology, associated microbes, pumping capacity) in the processing of dissolved food, the main fuel of the sponge engine. To what extent these different functional traits are a driving force in structuring reef ecosystems, from fuel input (primary producers), to engine output (driving and modulating the consumer food web) will be assessed at the community level. The resulting framework will be implemented in a sponge-driven food web model, a much-needed foundation to test and predict future scenarios of changes in shallow tropical and temperate as well as cold-water deep-sea reef communities.

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Hydrogen isotopes as an aquatic provenance tracer

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Hydrogen isotope (δ^2H) measurements of consumer tissues in food webs are useful tracers of diet and provenance, and may be combined with δ^{13}C and δ^{15}N analyses to evaluate trophic relationships in complex aquatic systems. These isotopes (δ^2H) are widely adopted in terrestrial ecology for animal migration and provenance studies. However, they do behave differently in aquatic experimental settings (Soto et al. 2013a) and the use of H isotopes in aquatic ecology has been limited due to an inconsistency among published analytical methods and the lack of well-defined isotope models.

Measurements of δ^2H of organic tissues are complicated by analytical issues (e.g. H exchangeability, lack of matrix-equivalent calibration standards, lipid effects) and physiological mechanisms, such as H isotopic exchange with ambient water during protein synthesis, and the influence of metabolic water. We obtained δ^2H (and δ^{15}N) values from fish muscle samples from Lake Winnipeg, Canada, 2007-2010, and we assessed the effects of species, feeding habits, and ambient water δ^2H values. After lipid removal, we used comparative equilibration to calibrate muscle δ^2H values to non-exchangeable δ^2H equivalents, and controlled for H isotopic exchange between sample and laboratory ambient water vapour. We then examined the data for evidence of trophic ^{2}H enrichment by comparing δ^2H values to δ^{15}N values. Ultimately, we evaluated the importance of water δ^2H in controlling δ^2H values of fish tissues and, consequently, the potential of H isotopes as a tracer of provenance by taking account of confounding variables such as body size and trophic effects.

Our results showed a significant correlation between fork length and δ^2H values, and no strong relationships between δ^{15}N and δ^2H were found. This suggests the so-called apparent trophic compounding effect (Solomon et al. 2009; Soto et al. 2013a) and the influence of metabolic water into tissue H (Soto et al. 2013b) were the potential mechanisms for δ^2H enrichment. The δ^2H values of fish appear to be a good tracer for tracking provenance, when confounding effects – known previously through experimental settings - were considered. We here present some recommendations in the application of these isotopes in fish provenance studies and these should be also applicable to other aquatic species. Specifically, we recommend assessing size effects or working with fish of relatively similar mass when inferring fish movements by using δ^2H measurements.

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Mechanisms mediating the effect of organic matter quality on the role of aquatic fungi and bacteria in carbon turnover

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Aquatic microbes play a fundamental role in terrestrial-aquatic carbon (C) cycling through the recycling of terrestrial organic matter (OM) to CO_2. Hence, evaluating mechanisms that drive microbial C turnover of terrigenous substrates in aquatic ecosystems is fundamental for understanding C cycling across ecosystem boundaries.

In nature, microbial communities face OM pools as mixtures of substrates with distinct origin and quality towards microbial demands. As such, the substrate composition of OM pools is considered to effect the contribution of fungi and bacteria to OM degradation differently. Using a ^{13}C stable-isotope approach, we aimed to elucidate mechanisms underlying the effect of substrate quality on microbial carbon turnover. Surface sediments from a stream were incubated with OM pools of particulate substrates with distinct quality: terrestrial derived, leaf litter of complex molecular structure and low nutrient content and two aquatic algae mixtures of distinct nutrient stoichiometry and less complex molecular structure. We combined stable-isotope analysis of phospholipid fatty acids (^{13}C-PLFA) with analytical chemistry (^{13}C-DIC, DOC) to determine the associations between C turnover of microbial groups and OM quality. OM quality affected microbial community composition and C-turnover differently, while C availability was primarily controlled by the consumers' cellular nutrient demand and capacity to breakdown C bonds. However, the results from our study indicate that additional mechanisms such as N-mining and ecological stoichiometry play a role in the modification of microbial substrate availability, and that these processes are largely controlled by the substrate composition of OM pools.

Our results shed light on the complex mechanisms linking OM quality to microbial C cycling and improve our understanding of the role of microorganisms in global carbon cycling. Further, the combinations of microbial and biogeochemical analyses with stable isotopes enable a more detailed view of the underlying processes of microbial carbon cycling, hence should be applied for further research.

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Facultative paedomorphosis in newts: A heterochrony promoting trophic niche differentiation at the intraspecific level

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Facultative paedomorphosis is a heterochrony allowing some individuals in a population to retain larval traits at the adult stage. It is considered as an important evolutionary process as it can generate new phenotypes without the need for extensive genetic modification. In newts, which have a complex life-cycle involving an aquatic and a terrestrial life-stage, facultative paedomorphosis lead to radical differences in the morphology of adults. In such populations, metamorphic adults undergo complete metamorphosis, adapted for a terrestrial life-stage, while paedomorphs retain external gills at the adult stage and are therefore strictly aquatic, but both phenotypes cohabitate in the aquatic environment during the reproductive period. This process has been classically viewed as an adaptation to environmental features with paedomorphs thriving in favourable aquatic environments and metamorphs in unfavourable aquatic conditions. However, because facultative paedomorphosis affects trophic structures and feeding mechanism of the newts, one hypothesis is that it may be maintained in some populations as a trophic polymorphism, with the advantage to lessen intraspecific competition. We explored this hypothesis in facultatively paedomorphic populations of two species occurring in contrasting environments: *Ichthyosaura alpestris*, inhabiting deep alpine lakes and *Lissotriton helveticus*, inhabiting small permanent ponds. We determined the trophic niche and regime of both phenotypes using stomach contents, SIBER niche modelling and SIAR mixing models on carbon and nitrogen stable isotopes. Our results show that paedomorphs and metamorphs differed in their trophic niches in both species and environments during the aquatic life-stage. Interestingly, we observed different patterns of niche differentiation between morphs and sexes that could be linked to differences in food resources and microhabitat use. Our results support the hypothesis that facultative paedomorphosis promotes trophic niche differentiation during the aquatic life-stage of newts.

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Trophic position estimates from amino acid nitrogen isotopic analyses: the implications of intra-individual variability

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Isotopic values of body proteins have been used to investigate individuals' dietary intake across a range of fields, particularly archaeology and (palaeo)ecology. Analyses of bulk tissues have proved informative, yet compound-specific work at the amino acid level potentially offers even greater precision, particularly using nitrogen isotopic values as a trophic level marker. The application of this technique has increased very rapidly in popularity in ecological foodweb studies over the last five years.

Work has shown that various factors influence the isotopic values of each amino acid, and the degree to which their isotopic values change with increasing tropic level (the trophic discrimination factor or TDF), particularly at the inter-species level. So far, less attention has been paid to intra-individual factors, although intra-individual variability has long been observed in different bulk tissue and amino acid isotopic values (e.g. Gaebler et al. 1966).

Here we present bulk and compound-specific nitrogen isotopic data of multiple tissues from four model species raised long-term on isotopically controlled diets. We show that there are significant non-random intra-individual nitrogen isotopic differences between collagens extracted from different tissues (skin, heart, muscle, bone). The isotopic ranges observed for each species and tissue are variable, yet there was some consistency of patterning. Similarly, we show that there is considerable variability in isotopic values of different amino acids across different tissues within an individual, for all four species.

We explore the potential implications of these observations for isotopic trophic biomarker estimates in ecology and archaeology, as well as for insights into the underlying mechanisms by which such isotopic variation occurs.

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Invesigating N_2O emissions from plants by using high resolution measurements of N_2O mixing ratio and isotope signatures (^{15}N, ^{18}O)

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Plant-derived nitrous oxide (N_2O) emissions have been identified from cryptogamic covers (Lenhart et al., 2015) and higher plants (Hakata et al., 2003; Bruhn et al., 2014).

As shown for lichens and mosses, N_2O emissions were related to respiration rates over a broad range of environmental conditions (Lenhart et al., 2015). A robust coupeling of N_2O emission rates to respiration allows the global estimation of plant-derived N_2O emissions based on respiration data.

Therefore, we determined N_2O and CO_2 emission rates from several plant species and sterile tobaco plants (*N. tabacum*). We tested the effects of temperature, N availability, light, and injury on N_2O emission rates. Mixing ratios of CO_2 and N_2O were measured in a closed chamber system via GC analysis and an Off-axis ICOS laser-based gas analyzer for N_2O mixing ratio, site-specific $\delta^{15}N$ and ^{18}O.

In real time resolution we observed large changes in the isotopic signatures which might be indicative for the processes involved in plant derived N_2O formation.

Our measurements showed – analogous to the findings for cryptogamic species- a robust relationship between N_2O emissions and respiration. Based on this ratio, we estimated the contribution of plant-derived N_2O emissions to the N_2O emissions of a grassland ecosystem and to the global N_2O budget.

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Can the two-pool model explain oxygen isotope variations in needles and tree rings of drought-stressed Scots pine trees?

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Ecologically and commercially, Scots pine (*Pinus sylvestris* L.) is a key species. Recent increases in the frequency, duration and intensity of drought may have contributed to Scots pine decline and mortality in some regions, including dry inner-Alpine valleys, but the detailed mechanisms triggering decline still remain unclear (Rigling et al. 2013). Because of their relationship to ecohydrological processes, $\delta^{18}O$ ratios in tree-rings are potentially very useful for understanding past tree-physiological responses and the adaption of Scots pine to increasing drought. However, there are still large gaps in the mechanistic understanding of $\delta^{18}O$ fractionations in plants that currently limit our ability to use $\delta^{18}O$ in tree-rings as a proxy for past environmental changes. Several models to estimate needle water enrichment were developed, but critically discussed such as the Craig-Gordon model for its overestimation (Barbour et al. 2007) and the widely used the Péclet correction for its unrealistic assumptions (Roden et al. 2015).

The focus of our study was to improve our understanding of environmental effects on $\delta^{18}O$ variations in tree rings of stressed *vs*. healthy (irrigated) trees, including a group of trees that recently died. We investigated isotope variations in soil water, needles and tree rings in a 10-yrs irrigation experiment in a mature Scots pine forest in Pfynwald (Valais, Switzerland), one of the driest parts of the Alps. We applied a recently proposed two-pool approach (Song et al. 2015), which considers the fraction of unenriched (xylem) water in the needles and the total needle water (xylem + cell water). This fraction is higher in drought-stressed needles due to shrinkage of the mesophyll tissue, reflected also in smaller needles, resulting in lower overall enrichment compared to the needles from irrigated trees.

Our findings suggest that the two-pool approach is able to precisely predict the bulk needle water enrichment in needles of stressed and healthy Scots pine trees. These results are consistent with observed lower $\delta^{18}O$ in tree-rings from trees that recently died compared to surviving trees over the past few decades and are among the first results showing evidence that needle anatomical changes resulting from drought stress are clearly reflected in $\delta^{18}O$ ratios in tree-rings.

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A stable isotopic view on lianas' and trees' below ground competition for water

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Recent studies highlight distinct liana abundance and biomass increase in the neotropics in the last decades. Despite several explanatory hypothesis have been proposed, the dominant underlying mechanism of this liana proliferation is still unknown. One hypothesis ascribes lianas, in comparison to trees, being able to adapt better to increased drought conditions resulting from climate change. Moreover, lianas are indicated of having a deeper root system compared to tropical trees, providing access to deeper soil layers less susceptible for dehydration during drought events, increasing the lianas belowground competitiveness for water.

In order to test this hypothesis, water stable isotopes (δ^2H and $\delta^{18}O$) were measured in precipitation, bulk soil (at different depths), stream, and xylem water from lianas and trees. This was done in two catchments with different soil texture (sand and clay) in the close vicinity of the Guyana flux tower at Paracou (French Guyana) during October 2015. Our results show that lianas and trees in the white sand catchment use different sources of water, with lianas tapping water with a heavier weighted isotope signature, from an water unknown source. However, in the oxisol (clay) catchments, lianas and trees compete for the same water sources.

Recent studies using water stable isotopes (δ^2H and $\delta^{18}O$)have described an ecohydrological separation of water between streams and the LMWL (i.e. soil mobile water) and plants (i.e. soil static water) indicated as the "two water world hypothesis", suggesting that vegetation is using water that is not contributing to stream water. Based on this concept, we further characterized all isotopic data by estimating the precipitation offset (Pp-offset) which represents the distance between the local meteoric water line (LMWL) and xylem δ^2H and $\delta^{18}O$ signature. Our results support this two-water-world hypothesis

In conclusion, our study shows that xylem water from lianas has a heavier weighted isotopic signature than those observed in trees xylem water. Our results thus indicate that belowground competition for water between lianas and trees might be less strong than previously expected and seems soil texture dependent.

Hydrogen isototes in plant organic compounds indicate strong metabolic shifts in pants over the past century

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In contrast to the stable C, N and O isotope composition of plant materials, the hydrogen isotope composition of plant organic compounds has not yet been established as a tool in ecological or biogeochemical research. With the development of new analytical instruments that allow the hydrogen isotope analysis of selected plant compounds there is, however, growing interest to explore the power of hydrogen isotopes as tools for ecological and biogeochemical research. In my talk I will summarize recent work from my lab where we show that the hydrogen isotope composition of lipids and carbohydrates reflect the carbon and energy metabolism in plants: Our experiments revealed that that autotrophic and carbon-autonomous plant tissue is ^{2}H depleted while heterotrophic or non-carbon-autonomous tissue is ^{2}H enriched. We were able to identify various biochemical processes during the biosynthesis of plant tissue that contribute to these patterns and can show that these patterns apply for various levels of organization: across plant species (e.g. heterotrophic parasitic plants and their autotrophic hosts), across different organs within an individual plant (e.g. autotrophic leaves vs. heterotrophic roots), and even within a plant organ (e.g. following the transition of a leaf from a carbohydrate sink to a carbohydrate source during ontogeny). As such, the hydrogen isotope composition of plant tissue seems to be a robust proxy for the carbohydrate metabolism in plants and could serve thus as an important new tool in plant ecology, plant breeding, biogeochemistry, and paleo applications. In fact, we investigated a historic collection of grass samples from the famous 150 year old Park Grass Experiment in Rothamsted and were able to show, that the hydrogen isotope composition of grasses has become ^{2}H depleted by about 40 per mil over the past decades. This suggests that global environmental change leads to significant and previously unresolved shifts in the carbohydrate dynamics of plants and that the hydrogen isotope composition of carbohydrates and lipids is a fascinating new tool to identify these changes.

Mechanisms of seedling facilitation through mycorrhizal links with adult plants – carbon costs and nutritional benefits in common mycelial networks

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Common mycelial networks (CMNs) are known to affect the outcome of plant-plant interactions through a variety of mechanisms. Particularly in the seedling stage, plants may be facilitated through neighbouring host plants in a CMN. These facilitative CMN-effects may be attributed to accelerated mycorrhizal colonization and the resulting nutritional benefits ('Inoculation effect'), as well as fungal carbon drain alleviation through shared costs for CMN-maintenance ('Maintenance effect'). However, these effects may be overlaid by competition for nutrients between the CMN-partners ('Competition effect'). The interplay between positive and negative CMN-effects results in a variety of observed growth responses of mycorrhizal seedlings in the presence of adult plants with host plant traits such as functional group, mycotrophy, productivity, and others, potentially affecting the outcome of the interaction. However, neither the importance of host plant C-supply to a CMN for seedling facilitation nor the question how certain host plant traits affect these parameters, have yet been given much scientific attention. In consequence, disentangling the extents of positive and negative CMN-effects driving net facilitation of seedlings has not been achieved to date.

In the present study we used a novel compartmented pot approach with selective elimination of host plant CMN-connections in combination with pulse chase labelling and nutritional analyses. We tried to disentangle, if certain 'key species' maintain a CMN by disproportionately high C supply, thereby reducing seedling CMN-costs.

Although net seedling facilitation through the CMN was observed neutral in the present study, we showed that CMN-growth and maintenance can be promoted by productive, highly mycotrophic plants, thereby accelerating inoculation of seedlings. Thus, we found evidence for the hypothesized existence of host key species for CMN maintenance, with these species ideally being both, highly mycotrophic and productive. However, surprisingly carbon costs to the seedling for CMN-maintenance were not reduced, but increased by maintaining CMN-connections of adult hosts, opposing the hypothesis of a positive maintenance effect. This increase was independent of species identity and mycotrophic degree, with the driving factor for seedling CMM C-drain being CMN-size. However, our results also suggest that compensation of the positive 'inoculation effect' was rather due to negative effects by CMN-mediated competition, mainly for nitrogen, than by the increased seedling C-costs to the CMN, and that presence of a CMN may cause a shift from P- to N-limitation in seedlings, explaining neutral CMN-effects. We conclude that CMN-maintenance effects are probably much less important than the provision of inoculum and the mediation of competition for the outcome of net facilitation of seedlings by CMN-hosts.

Characterisation of microbial reductive dehalogenation of halogenated organic contaminants using compound-specific stable isotope analysis

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Over the last decades, concepts involving compound-specific stable isotope analysis (CSIA) have been developed allowing the qualification and quantification of in situ (bio)transformation of common groundwater contaminants such as the chlorinated ethenes. The advance in methods now enables, besides carbon, also the analysis of chlorine, bromine and hydrogen isotopes of halogenated organic substances, allowing detailed insights into biotransformation reactions. The observed variability in carbon stable isotope fractionation during microbial reductive dechlorination of chlorinated ethenes was attributed to rate-limitating steps, such as membrane transport and uptake of the substrate, prior to bond cleavage (Nijenhuis *et al.*, 2005, Cichocka *et al.*, 2008). The dual-element isotope analysis of chlorinated and brominated ethenes and ethanes, however, revealed also a suprising variability in the correlated carbon-halogen fractionation (Renpenning *et al.*, 2014). In this presentation, results from microbial reductive dechlorination of chlorinated ethenes and 1,2-dichloroethane will be compared and contrasted to their brominated analogues. Underlying factors potentially influencing the observed isotope fractionation, such as substrate hydrophobicity, intrinsic reaction rates and microbial cell structure, will be addressed (Renpenning *et al.*, 2015). Finally, implications for the application of CSIA to quantify *in situ* biotransformation will be discussed.

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Carbon and chlorine isotope fractionation during anaerobic biodegradation of chlorinated ethanes by *Dehalogenimonas*

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Chlorinated ethanes like 1,2-dichloroethane (1,2-DCA) and 1,1,2-trichloroethane (1,1,2-TCA) are common groundwater pollutants found at many contaminated sites around the world. Anaerobic enrichment cultures containing *Dehalogenimonas spp.* derived from 1,2-dichloropropane-to-propene cultures established with sediments from the Besòs River Estuary, Spain (Martín-González et al., 2015) showed capability for reductive dechlorination of these two chlorinated ethanes. The dihaloelimination of 1,2-DCA by this organohalide-respiring bacterium resulted in stoichoimetric accumulation of ethene without the detection of undesireable toxic vinyl chloride (VC) which was often observed in *Dehalococcoides*-cultures, whereas VC was the main degradation product during 1,1,2-TCA transformation.

Foccusing on 1,2-DCA dihaloelimination, previous carbon isotope fractionations (ε_C) showed a wide variation (from -7.3 to -32‰, Fletcher et al., 2011), which was not conclusive to ensure different reaction mechanisms (e.g. stepwise versus concerted). In the present study, chlorine together with carbon isotope analyses were performed in laboratory batch experiments in order to get new insights into the reaction mechanism used by *Dehalogenimonas spp.* during 1,2-DCA dechlorination.

The obtained ε_C (-23 ± 2)‰ was significantly lower than the ones assignated to *Dehalococcoides* (around -30‰, Schmidt et al., 2014), but still within the above mentioned range. However, the quite high ε_{Cl} = (-12 ± 1)‰ observed in our *Dehalogenimonas*-culture might point to a simultaneous cleavage of both C-Cl bonds via a concerted mechanism in the first reaction step, differently to the stepwise previously suggested (Schmidt et al., 2014). Moreover, the obtained dual C-Cl isotope slope (Λ = 1.9±0.02) was clearly different from previous C-H bond oxidation (0.78±0.03) and S_N2 (7.6±0.2) reaction mechanisms observed for different aerobic degrading strains (Palau et al., 2014). So, this study highlights the chances of dual isotope approach to distinguish aerobic from anaerobic chlorinated ethanes biodegradation pathways at contaminated sites.

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Lichen nitrogen and carbon isotopic composition as indication for atmospheric nitrogen pollution – a synopsis

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Mankind has dramatically influenced the N fluxes between soil, vegetation, water and atmosphere – the global N cycle. Many problems are caused by the human-accelerated environmental change. Long-term ecological monitoring programs are fundamental to evidence-based environmental decision-making. Biomonitoring is essential, particularly where less deposition measurement sites exist. In Germany still key societal threats of excess reactive nitrogen (N_r) are on hand. One tool that allows quantification of the exposure and the effects of atmospheric N impacts on an ecosystem is the use of bioindicators. Due to their specific physiology and ecology, especially lichens are suitable to reflect the atmospheric N input at ecosystem level.

The studies focused on verifying if it is possible to characterize and map the N impacts by analysing the N content of lichens on a regional scale (western part of Germany). Furthermore, we investigated whether $\delta^{15}N$ ratios of lichens can be used to differentiate the main N sources and types of deposition along an altitudinal gradient (from lowland to submountainous areas) and through different land use affected areas. Besides, we specified that lichens, under currently increased N deposition rates, react species-specific with different stable N and C isotopic compositions in their tissues, stimulated by environmental N stress (nationwide survey of Germany).

The N content of epiphytic lichens reflects species-specific, agriculture-related circumstances of N deposition at various sites in Germany. Simultaneously, $\delta^{15}N$ values of the different investigated epiphytic lichen species are highly depleted in ^{15}N under high ammonium deposition. The verification of highly negative $\delta^{15}N$ values at sites with local and regional emitters shows that source attribution is possible by comparing different $\delta^{15}N$ values in areas with a wide range of different N deposition types and the corresponding differences in tissue N contents among various source N pools (Boltersdorf et al. 2014). Especially nitrophytic lichens can support the on-site instrumentation measuring N deposition by qualification and quantification the N deposition. By grid-based mapping of tissue N content and $\delta^{15}N$ values with geostatistics we verified this (Boltersdorf & Werner 2014). Besides, isotope specifity of lichen species was investigated relating to $\delta^{15}N$ and $\delta^{13}C$ values. Lichens react with specific physiological responses to N impact which point to N deposition as a stress factor.

In conclusion, bioindication with lichens is an alternative to physico-chemical measurements to comprehensively assess different prevailing N doses and sources of N pools on a regional scale.

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Model-based integration of enantiomer and stable isotope fractionation for chiral pesticides degradation

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Understanding the environmental distribution and the degradation processes of chiral pesticides is essential for risk assessment and for evaluating the hazardous effects of these organic compounds in both wastewater treatment systems and natural aquatic environments (Wong, 2006). The combination of stable isotope and enantiomer analysis techniques has been recently proposed to better characterize the fate of chiral pesticides. Different reaction pathways are characterized and effectively visualized by plotting enantiomer ratios together with stable isotope ratios.

First-principle based numerical modeling is an important tool to describe the evolution of concentration and isotope data during contaminant degradation (Aeppli et al., 2009), as well as to provide a quantitative description of different reaction pathways (Jin & Rolle, 2014). In this study, we introduce a modeling approach with the aim of unifying and integrating the interpretation of isotopic and enantiomeric fractionation. The model is based on the definition of enantiomer-specific isotopologues and jointly predicts the evolution of concentration, enantiomer fractionation, as well as changes in stable isotope ratios of different elements. The method allows evaluating different transformation pathways and was applied to investigate enzymatic degradation of dichlorprop (DCPP), enzymatic degradation of mecoprop methyl ester (MCPPM), and microbial degradation of α-hexachlorocyclohexane (α-HCH) by different bacterial strains and under different redox conditions. The proposed modeling approach was tested and validated with isotopic and enantiomeric data observed in previous experimental studies (Bashir et al., 2013; Jammer et al., 2014; Qiu et al., 2014). The model results reproduce the observed isotope and enantiomer ratios and precisely capture the dual-dimensional trends characterizing different reaction pathways. Furthermore, the model allows testing possible combinations of enantiomer analysis (EA), compound specific isotope analysis (CSIA), and enantiomer specific isotope analysis (ESIA) to identify and assess isotope and enantiomer selective reaction mechanisms.

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Soil and water extraction methods for Compound Specific Isotope Analysis (CSIA) of aromatic anilide pesticides and application to agricultural soils

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The global use of pesticides makes them widespread contaminants in all environmental compartment, including soils, where they can accumulate and persist over decades (Fenner et al., 2013). There is therefore a need to understand their degradation behavior, the only process allowing their complete removal. Compound-specific isotope analysis (CSIA) has proven efficient in assessing sources and transformation pathways of pesticides at the sub-µg scale and over space and time (Elsner and Imfeld, 2016). Yet, CSIA studies on the fate of pesticides in complex environmental matrices are scarce, mostly due to the lack of validated extraction methods (Ivdra et al., 2014).

Water and soil extraction methods of acetanilide herbicides, e.g. acetochlor, alachlor, butachlor and S-metolachlor and the acylanilide fungicide metalaxyl were developed. Extraction from synthetic and field water was performed by solid phase extraction (SPE), which allows pre-concentration and purification of water samples in e.g. runoff at low ng·L^{-1} concentrations. Up to 100% recovery was achieved for acetochlor, alachlor and S-metolachlor without significant carbon isotope ($\delta^{13}C$) fractionation, regardless of the initial pesticide concentration (0.2 to 15 mg·L^{-1}) or volume extracted. Soil was extracted three consecutive times with ethyl-acetate, followed by a cleanup with primary-secondary amine (PSA) sorbent. Metalaxyl showed the highest extraction yields (up to 100%) while extraction of butachlor was below 15%, in three different soils. The highest yields were observed for the highest water content. No significant $\delta^{13}C$ shift was observed during the procedure for initial concentrations ranging from 10 to 50 µg·g^{-1}, whereas butachlor showed 1 to 4‰ shift compared to the standard's signature.

The method was applied to investigate the degradation of butachlor and S-metolachlor, spiked at two concentrations in a paddy soil from Guilan Province (Iran) and a crop soil from Alsace (France) and incubated for 85 days. Butachlor and S-metolachlor showed 90% degradation after 30 and 60 incubation days, respectively. The $\delta^{13}C$ signatures fit the Rayleigh model yielding isotope enrichment factor (ε) ranging from -0.7 to -2.5 and -1.0 to -3.1 ‰, for the low and high concentrations, respectively. This demonstrates in situ pesticides degradation in soils. Further, DNA extractions will allow the identification of the microbial communities associated with the degradation of these compounds.

These results confirm the potential of CSIA to evaluate pesticides degradation and persistance in soils. Current analytical developments include nitrogen isotope analysis ($\delta^{15}N$) to decipher degradation pathways, and enantiospecific isotope analysis (ESIA) to differenciate degradation among chiral isomers in agricultural soil samples, which open doors to field applications.

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Stable isotope ratios for food authentication and traceability

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Determining the authenticity of foods means uncovering misdescription of foods not meeting the requirements for adopting a particular name, substitution with cheaper but similar ingredients, undeclared processes and/or extension of food using adulterants, incorrect origin (e.g. geographical), species or production method (e.g. organic vs conventional). Nowadays, the objective assessment of food authenticity is of paramount importance as consumers come into daily contact with a wide variety of foods. Indeed, globalization means that more and more foods are traded around the world. Traceability has thus become a cornerstone of the EU's food safety policy. This need led the European Union to institute a Traceability Regulation (178/2002/EC), which came into force in January 2005, and defines 'food and feed traceability'.

Methods for testing authenticity and providing analytical data on traceability require robust analytical techniques that can be utilised by the various regulatory authorities. Of the many techniques available, one of the most widely-used is isotope ratio mass spectrometry (IRMS), applied since around 1975 to detect adulteration of products like wine, honey, fruit juice, maple syrup, vinegar with cheaper extenders, such as sugar, or simply water. Those "traditional" applications of stable isotopes in food control rely on the analysis of the isotopic ratios of only one or two elements ($^{13}C/^{12}C$ and/or $^{18}O/^{16}O$, $^{13}C/^{12}C$ and $^{2}H/^{1}H$) and several of these methods have been officially validated and acknowledged as AOAC, CEN, EU or OIV methods. More recently multi-isotope ratio analysis ($^{13}C/^{12}C$, $^{18}O/^{16}O$, $^{2}H/^{1}H$, $^{15}N/^{14}N$, $^{34}S/^{32}S$, sometimes combined with $^{87}Sr/^{86}Sr$ and elemental profiling) and GC- or HPLC- IRMS, have been successfully applied for verifying geographical origin and organic production of food (e.g. cereal, tomato, meat, cheese) and for identifying the natural origin of flavours (e.g. vanillin) and bioactive molecules (e.g. Monacolin K).

The potential of IRMS and GC-IRMS analysis of TATP in forensic explosives investigations

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Triacetone triperoxide (TATP) is an organic peroxide classified as a primary high explosive. It is relatively easy to synthesize and its precursors are widely available and inexpensive. Therefore, TATP is of interest as home-made explosive and popular among terrorists. The explosive TATP is frequently used in terrorist attacks all over the world, e.g. the Israeli embassy bombing in London (1994), the attempted "shoe-bombing" on Flight 63 of American Airlines (2001), the suicide bombings in Casablanca (2003), the London subway attacks (2005), the attempted bombing on Flight 253 of Northwest Airlines from Amsterdam (2009), the Paris attacks (2015) and the Brussels bombings (2016).

Beyond detection and identification of the explosive TATP, another important goal of forensic analysis is chemical profiling, i.e. investigate possible links between samples from a crime-scene and samples related to a specific person or location (e.g. bomb maker or manufacturing place). Most analytical techniques used for identification usually cannot distinguish between different sources of the same substance. Stable isotope analysis has the potential to differentiate between chemically identical substances based on differences in their isotopic compositions.

In this profiling study, isotope ratio mass spectrometry (IRMS) has been used to determine the isotopic compositions of TATP samples and its precursors. A set of the TATP precursors, acetone and hydrogen peroxide, has been analyzed to determine their carbon, hydrogen and oxygen isotopic compositions. The sample set mostly consisted of NFI related casework samples collected in The Netherlands. Precursors with distinct compositions have been selected for the synthesis of TATP and small amounts of the explosive were prepared in two sample sets. The first sample set used standard synthesis conditions in combination with different precursors to investigate possible linkage. The second sample set used a fixed set of precursors in combination with varying synthesis conditions to investigate the effects on isotopic fractionation. In addition to bulk-IRMS, compound-specific carbon and hydrogen isotope measurements were performed using gas chromatography isotope ratio mass spectrometry (GC-IRMS) to examine the differences in isotopic compositions when possible by-products and impurities are either included or excluded.

The results of the study show that it is possible to discriminate between TATP samples when different precursors and synthesis conditions are used. The variation in isotope values mostly depends on the isotopic range of the precursor acetone. During the synthesis of TATP significant isotopic fractionation occurs. A robust linear relationship was found for the carbon isotope values of the acetone-TATP combinations and the reaction yield. This shows that carbon IRMS has practical use in forensic TATP investigations to investigate possible linkage between TATP samples and their acetone precursors. GC-IRMS measurements were very comparable to the bulk IRMS data, due to the high purity of the in-lab produced TATP-samples. However, in real cases where additional substances are likely to be encountered the use of GC-IRMS might be beneficial.

Global spatial distribution of human carbon isotope ratios

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Natural stable carbon isotope ratios of humans are related to individual dietary habits and environmental and physiological factors. In nutritional research, archaeology, forensic science as well as in doping analysis the stable isotope ratios of human tissues or metabolites are used for dietary analysis, geographical and source allocation or information about the metabolism of an individual. For substantial interpretation of analytical results the knowledge of the global spatial distribution of individual isotope ratios is an essential component.

For more than a decade so-called *isoscapes* (*iso*tope land*scapes*), which are derived from models based on elemental fractionation processes and distributions, have been used to answer scientific and forensic questions regarding sources, partitioning or provenance of materials and organisms.

The amount of data on modern human's carbon isotope ratios (mainly hair, nail, and urinary steroids) has increased significantly within recent years. In this study literature and experimental data on human global carbon isotope ratios were summarized in order to achieve detailed information on their global spatial distribution.

The current available dataset of human stable isotope ratios is biased towards Europe and North America with only limited data for countries in Africa, Central and South America and Southeast Asia. The global spatial distribution of carbon isotopes is related to latitude and supports the fact that human carbon isotope ratios are dominated by the amount of C4 plants in the diet, either due to direct ingestion as plant food, or by its use as animal feed. However, additional factors like the geographical position and altitude as well as climatic influences have to be take into consideration if modern human carbon isotope ratios are to be interpreted. By use of substantial global data bases it is possible to simulate the global spatial distribution of carbon isotope ratios for modern human urinary steroids as well as for other matrices like hair or nail. Lowest values were found for Northern Europe inhabitants in contrast to the most enriched values for individuals from Southern Africa or Central America.

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Compound-specific stable isotope analyis of gamma-hydroxybutyrate (GHB) in urine: a powerful tool to prove drug facilitated assults

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GHB is known for euphoric and sedative properties and its potential for amnesia in higher concentrations. It became known as "date rape drug", used in crimes of drug facilitated sexual assault. (LeBeau et al, 2000). Peroral administrated GHB metabolizes extremely fast in the human organism: the terminal half-life in blood is around 40–60 min. Thus, 6 hours after administration usually there is no elevated GHB concentration in blood (Haller et al, 2006). This makes it very difficult to prove drug misuse in crime victims by standard gas-chromatographic methods. Therefore, we follow the novel approach of analysing stable isotopes of GHB in the urineof victims, presented by Saudan et al. (2007).

Saudan et al (2007) analyzed carbon isotope composition of GHB in urine after GHB administration. They found a significant difference in $\delta^{13}C$ signatures between *in-vivo* and administered GHB. They could prove administration of the drug – even under the cut off concentration of 10 µg mL^{-1}. These results can substantially extend the detection window for GHB misuse. However, there are two major challenges concerning this method. (1) An extremely elaborate cleaning process prior to the GC-C-IRMS measurement. (2) Marclay et al. (2010) investigated the $\delta^{13}C$ signature of GBL (precursor for GHB) which shows a major overlap with the *in vivo* $\delta^{13}C$ signature of GHB. This limits the traceability of administration of this incapacitating agent.

We addressed the first problem with two approaches. On the one hand, headspace GC-IRMS analysis provides the lowest background and allows the direct analysis of GHB (converted to GBL) from urine without any purification steps. In this case the GC-IRMS system must be optimized for maximum sensitivity, to achieve measurable signals. The other approach is cold injection of the purified (SPE) and extracted (DCM) GBL converted GHB. PTV large volume injection GC-IRMS method can provide larger signal intensities even at low concentrations with an acceptable background.

To address the second problem, we tested – beside $\delta^{13}C$ – the $\delta^{2}H$ signature of GHB in urine and in different external sources. This approach could also facilitate tracing GHB misuse in crimes.

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Identifying different origin of tropical timber from West- and Central African countries

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Controlling the declared provenance of timber is an important topic in view of illegal logging. Globally 20-40% of logging is estimated to be illegal. A large international project regarding the investigation of origin of tropical timber from Western and Central Africa has just been completed. The aim of this project was to establish DNA- and isotope methods to differentiate between the origins and to feed the produced data into a database to serve as a base for the identification of true origin. This shall serve as a reference for questions of provenance control in the future and combat illegal logging of tropical timber. Here we report the investigations of stable isotope patterns of Ayous (Triplochiton scleroxylon). Ayous is one of the major timber logged in this region and intense harvesting resulted in over-exploitation. The measured samples came from five countries in Western and Central Africa: Ivory Coast, Ghana, Cameroon, Congo and Democratic Republic of Congo (DRC). The samples were measured for their isotopic ratio of the elements hydrogen, oxygen, carbon, nitrogen and sulphur by IRMS (Isotope Ratio Mass Spectrometry). Sr isotopes were measured by TIMS (Thermal Ionisation Mass Spectrometry).

The results show a strong influence of geology and climate on the isotope signatures. Samples from DRC can be identified due to the climatic situation of the Congo Basin and the western African countries possess a pattern dominantly influenced by the Precambrian bedrock.

The results furthermore document a significant influence of the type of investigated sample material (drill core taken with a drill, wood chips, tree bark, the latter two types harvested with a bush knife).

The reliability of the produced reference data was controlled by a blind-test, giving satisfying results for the isotope data.

Hydroclimate variability in central North Africa during the Plio-Pleistocene

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The North African continent underwent major hydroclimate fluctuations in the past, most recently during the early Holocene when increased precipitation allowed vegetation, lakes and human populations to occupy a "green Sahara", a region that is desert today (Kuper and Kröpelin, 2006). The Plio-Pleistocene climate of the African continent is of much interest because hominin speciation, extinction and migration events have been linked to changes in global or regional climate (e.g. Maslin et al., 2014). To date, much of our knowledge of Plio-Pleistocene African hydroclimate comes from marine dust flux records, which suggest a progressive aridification of the North African continent occurring since 3 Ma (deMenocal, 1995; deMenocal, 2004). However, a more recent study that re-examines the same dust flux records using different statistical techniques questions this interpretation (Trauth et al., 2009). Here, we investigate the Plio-Pleistocene hydroclimate of central North Africa by examining the hydrogen isotope composition of plant leaf waxes (δD_{wax}) at ODP site 660 (10°N). This site is located offshore Guinea in the tropical Eastern Atlantic and is situated underneath the Saharan dust plume, which recieves material sourced from central and northwest Africa. We find that leaf wax δD values at ODP 660 fluctuate by over ca. 70‰, attesting to dramatic fluctuations between wet and arid conditions in central North Africa from 4.0 and 0.5 Ma, the interval examined in this study. Interestingly, the most depleted values of the entire record occur at ca. 3.3-3.2 Ma, coinciding with Marine Isotope Stage M2 (~ 3.3 Ma), suggesting wet conditions at this time. The M2 event is recognized globally as a cooling event, which interrupted an otherwise relatively warm Pliocene. Prior to 2.5 Ma, our δD_{wax} records reveal several major fluctuations between wet and arid conditions occurring within the Pliocene. The Pleistocene portion of the record is also characterized by variable hydroclimate conditions with a progressive enrichment in δD_{wax} noted from ca. 2.5-1.7 Ma, reflecting an overall trend to increasing aridity. Subsequently, a gradual depletion in δD_{wax} occurs until 0.4 Ma, suggesting a return to wetter conditions. Our new leaf wax records reveal that dynamic hydroclimate shifts in central North Africa, which are believed to have influenced hominin and faunal migration patterns, characterize the past 4.0 Ma, with especially variable conditions noted during the Pliocene. Instead of progressive aridification since 3 Ma, our δD_{wax} record instead suggests that a shift to wetter conditions has occurred since ca. 1.7 Ma.

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From Prehistory to History: change and continuation in the dietary habits from the inhabitants of the Balearic Islands based on ^{14}C and stable isotope research ($\delta\ ^{13}C$ and $\delta\ ^{15}N$)

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In a previous study the dietary habits from the inhabitants of the Balearic Islands (Mallorca & Menorca) were studied (Van Strydonck et al. 2005). The results showed that these island dwellers hardly ate any marine food and that there was no real trade in dairy products between the settlements. In this study the prehistoric data set is compared with data from Roman and Medieval sites (Christian as well as Muslim sites) and a sub recent cemetery. Data for more than 10 sites are compared. Some sites have only a few data points while others have more than 15.

There are major differences between the examined sites. The data from some smaller Islamic sites don't overlap. This is a strong indication for a lack of food trade between the sites. The Christian sites have, on the average, a higher $\delta\ ^{13}C$ and a higher $\delta\ ^{15}N$ value. Probably because of a somewhat higher consumption of marine products. The data of the Christian sites overlap each other, an indication that food is much more distributed than in the Islamic or prehistoric sites. Also the dispersion of the data is much more outspoken. Even sites with a very narrow range in isotope data show an important number of outliers, this is probably due to immigrants from other parts of the islands or from elsewhere. Differences in dietary habits based on social status can be ruled out in some cases, but not in all. In the Islamic sites we see statistically no difference between the data from female and male skeletons. The same is true for the Christian sites, but this conclusion can be invalid due to the lack of sufficient data. At two sites a possible immigrant, probably from North Africa could be detected because of the high $\delta\ ^{13}C$ value (C4 plant). The monks from the small island of Cabrera were immigrants as well and this is reflected in a large dispersion between the data points.

The city of Pollentia, the largest city in Roman times, has the largest dispersion of data points. On the average the $\delta\ ^{15}N$ values are the highest of all investigated sites. The dataset holds also some individuals with a very high $\delta\ ^{13}C$ value (C4 plants) and others with a very high $\delta\ ^{15}N$ value (marine fish consumption). These data are the reflection of a rather cosmopolitan city. It has also be proven that during the Muslim rule of the island Christians continued to have their own burial rites, proof of an open and tolerant society (Cau Ontiveros et al. 2016).

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Reconstruction of sea surface salinity changes of the Sea of Okhotsk during the past 1.5 Ma using δD of long-chain alkenones

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The Sea of Okhotsk is a semi-closed marginal sea located in the northwestern part of the Pacific, and is the sea at the lowest latitude covered with seasonal sea ice in the Northern Hemisphere. In this area, salinity, along with temperature, determines the density of seawater and thereby controls the intensity of downwelling. Almost no salinity record exist from this area, and none covers the Holocene and the mid to late Pleistocene.

Here we use the hydrogen isotope composition of the $C_{37:2}$ and $C_{37:3}$ alkenones ($\delta D_{alkenones}$ measured on the two alkenones) as a proxy for relative sea surface salinity changes during the past 1.5 Ma in the Sea of Okhotsk. Alkenones are produced by certain Haptophyte algae, *i.e. Emiliana huxleyi* in the Sea of Okhotsk, that thrive in the upper part of the water column. Culture studies have shown that in *E. huxleyi,* and other Haptophytes, the $\delta D_{alkenones}$ is related with salinity (Schouten et al., 2006; M'Boule et al., 2014). Several environmental studies have demonstrated the application of this proxy in sedimentary settings (e.g. Kasper et al., 2015).

The $\delta D_{alkenones}$ paleo-record from the Sea of Okhotsk shows a substantial change towards more D-depleted values during interglacials (*e.g.* the Holocene, MIS 5e, MIS 11), with the most negative value of -215 ‰ for MIS 5e. These decreases from glacial to interglacial are not due to changes in temperature because of the combination of the two alkenones (Schouten et al., 2006; van der Meer et al., 2013) but can be explained by factors such as changes in global ice volume and in salinity, and potentially species composition and growth rate. The two last possibilities can, most likely, not explain the substantial differences observed (10 to 30‰) between glacial and interglacial stages. These shifts are larger than the expected changes in δD_{water} due to the global ice volume change (5 to 8‰). Therefore, it seems likely that salinity was responsible for the remaining change of 5 to 15‰. This suggests that the Sea of Okhotsk was more saline during glacial stages than interglacial, likely due to the local formation of sea ice. The positive correlation between the BIT index (a measure for soil derived organic matter) and $\delta D_{alkenones}$ indicates a link between formation of sea ice and influx of terrestrial matter brought in by ice rafted debris during glacial stages.

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Assessment of ns-LA-MC-ICP-MS for Pb and Sr isotopic analysis of archaeological glass – mass bias correction strategies and Corning glass standards

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Isotopic analysis of lead (Pb) and strontium (Sr) is of particular importance to archaeometry (Stos-Gale,1992; Aggarwal et al. 2008). Lead isotopic signatures of metallic objects are commonly used for provenancing purposes, i.e. to determine the geologic origin of the Pb-bearing ore and possibly the associated manufacturer workshop locations. Lead was also used as a flux in lead-glasses and glazes, or as an opacifying agent in glass. Low levels of Pb in glass can originate from the heavy mineral fraction of sand, the source material of the network former. The study of Pb isotope ratios in glass has proven useful in either determining the sand source, or the source of the Pb added to the glass (Degryse, 2013).

The ^{87}Sr/^{86}Sr isotope ratio is measured to determine the source of lime in glass, though the Sr might also come from some minerals in the silica source. Quantification of Sr can help discriminating between those two cases (Degryse, 2013).

When analysing archaeological objects, (quasi-) non-destructive techniques are preferable. The damage inflicted on the sample surface by laser ablation sampling is substantially smaller in scale than that caused by micro-coring followed by digestion, hence the former is preferred for the present study. Furthermore, sampling by laser ablation allows spatially resolved isotopic data to be obtained for samples with multiple glass types. Finally, it improves throughput by circumventing time-consuming digestion procedures.

Two main challenges need to be addressed in this approach: (i) instrumental- and laser-induced mass bias, which needs to be adequately corrected for, and (ii) the necessary availablity of matrix-matched standards of glass with accurately and precisely known isotopic compositions. Matrix matching is especially important when sampling relies on lasers with nanosecond pulse duration, as they produce more melting and larger condensed particles (than lasers with femtosecond pulse duration), inducing isotopic fractionation.

This paper will compare different mass bias correction strategies:

a. internal correction using either the response for a pair of isotopes of the target element or for a pair of isotopes of a nebulized internal standard, whilst relying on the exponential or power law correction methods

b. external correction using a sample standard bracketing approach

c. combination of both internal and external correction

High-precision Pb and Sr isotope ratio measurements of Corning glasses A, B, C, and D (Corning Museum of Glass, United States, New York) were also performed. These glasses, which are widely used in EPMA or XRF analysis, were specifically synthesized to mimick the matrix of ancient glasses and, hence, are appropriate candidates to serve as matrix-matched reference materials in an archaeological context.

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Relative humidity changes during the Younger Dryas in the Western Eifel region based on a biomarker-isotope paleohygrometer

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Leaf wax-derived biomarkers, e.g. long chain *n*-alkanes and fatty acids, and their $^2H/^1H$isotopic composition are proved to be of a value in paleoclimatology/-hydrology research. However, the alteration of the isotopic signal as a result of the often unknown amount of leaf water enrichment challenges a direct reconstruction of the isotopic composition of paleoprecipitation. The coupling of $^2H/^1H$ results of leaf wax-derived biomarkers with $^{18}O/^{16}O$ results of hemicellulose-derived sugars has the potential to overcome this limitation and additionally allows reconstructing relative air humidity (Zech et al., 2013; Tuthorn et al., 2015).

We investigated lacustrine sediment samples from Lake Gemündener Maar, Germany, located in the Western Volcanic Eifel region. High resolution samples from the late Glacial, Younger Dryas and the early Holocene are analysed for their $^2H/^1H$ (*n*-alkanes) and $^{18}O/^{16}O$ (sugars) isotopic composition.

The established chronostratigraphy is based on (i) a ^{14}C dated piece of charcoal at 7.27 m depth, (ii) the Laacher See Tephra at 6.80 m depth and (iii) the wiggle-match between the distinctive TOC shift at 6.41 m depth with the onset of the Holocene in the Greenland icecore $\delta^{18}O$ curve. The sugar biomarker pattern indicates a relatively high terrestrial constribution of leaf biomass to the sedimentary organic matter in the lake, which is in agreement with the catchment area being small, steep and densely vegetated with broad-leaved trees.

The reconstructed relative air humidity, based on the coupled biomarker-isotope approach, indicates a two-phase development of the Younger Dryas in Western Europe. The expected clearly drier conditions in the Younger Dryas compared to the Allerød are not confirmed. However, the early Holcene seems to be markedly drier and is characterized by large and rapid relative air humidity changes. Additionaly, the reconstructed isotopic composition of precipitation allows the direct comparison of a Western Europe and Greenland precipitation curve.

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The stable hydrogen isotopic composition of sedimentary plant waxes as quantitative proxy for rainfall in the West African Sahel

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Various studies have demonstrated that the stable hydrogen isotopic composition (δD) of terrestrial leaf waxes tracks that of precipitation (δD_{precip}) both spatially across climate gradients and over a range of different timescales. Yet, reconstructed estimates of δD_{precip} and corresponding rainfall typically remain largely qualitative, due mainly to uncertainties in plant ecosystem net fractionation, relative humidity, and the stability of the amount effect through time. Here we present δD values of the C^{31} n-alkane (δD_{wax}) from a marine sediment core offshore the Northwest (NW) African Sahel covering the past 100 years and overlapping with the instrumental record of rainfall. We use this record to investigate whether accurate, quantitative estimates of past rainfall can be derived from our δD_{wax} time series. We infer the composition of vegetation (C^3/C^4) within the continental catchment area by analysis of the stable carbon isotopic composition of the same compounds ($\delta^{13}C_{wax}$), calculated a net ecosystem fractionation factor, and corrected the δD_{wax} time series accordingly to derive δD_{precip}. Using the present-day relationship between δD_{precip} and the amount of precipitation in the tropics, we derive quantitative estimates of past precipitation amounts. Our data show that a) vegetation composition can be inferred from $\delta^{13}C_{wax}$, b) the calculated net ecosystem fractionation represents a reasonable estimate, and c) estimated total amounts of rainfall based on δD_{wax} correspond to instrumental records of rainfall. Our study has important implications for future studies aiming to reconstruct rainfall based on δD_{wax}; the combined data presented here demonstrate that it is feasible to infer absolute rainfall amounts from sedimentary δD_{wax} in tandem with $\delta^{13}C_{wax}$ in specific depositional settings.

Preliminary investigation of seasonal patterns recorded in the oxygen isotpe compositions of theropod dinosaur tooth enamel

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Oxygen isotope compositions of tooth enamel increments in theropod dinosaurs are investigated as potential proxies of climate seasonality. Six teeth of large carnivorous theropods collected from four Cretaceous formations deposited under contrasted climates have been sampled. These teeth have been analyzed for the oxygen isotope compositions of their apatite phosphate ($\delta^{18}O_p$) through incremental sampling performed along the major growth axis. Significant fluctuations in oxygen isotope compositions along the growth axis of each tooth are observed and interpreted as reflecting seasonality in ingested local surface water $\delta^{18}O_w$ values. Fluctuations in $\delta^{18}O_p$ values of theropod teeth from the Aptian of Thailand and Cenomanian of Morocco vary similarly to meteoric water $\delta^{18}O_{mw}$ values occurring today in sub-tropical regions subjected to large seasonal amounts of precipitations. A dinosaur tooth recovered from the more inland and mid-latitude Nemegt Formation of Mongolia shows a seasonal pattern similar to present-day cold temperate and continental climate. Finally, the high latitude and coastal Kakanaut Formation (Russia) experienced strongly dampened seasonal variations, most likely due to the influence of warm Pacific oceanic currents. Such conditions occur today in high latitude regions submitted to marine influence. These results further highlight the potential of using the oxygen isotope compositions of large theropod teeth to reconstruct past seasonal variations of terrestrial climates. Increased knowledge of past seasonality may help to better understand the complex interactions between climate and the dynamics of land biodiversity in terms of ecological adaptations, biogeography and the evolutionary history of organisms.

Modelling oxygen isotope ratios in tree-rings: From process-based studies to large spatial and temporal scales

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Oxygen isotope analysis of organic matter is a widely applied method to infer physiological and environmental information from plants, which can be readily extended to the past by the use of tree-rings. For interpreting the isotope signal reliably, however, a detailed understanding and modelling of the isotope ratio is necessary. The isotope ratio in tree-rings is influenced on the one hand by source water isotope variations and on the other hand by fractionations within the plant, mainly through leaf water enrichment and biochemical fractionations in the leaf and stem (Saurer et al. 2012). Given a detailed analysis of various ecosystem compartments at high temporal resolution, process-based models can quite well explain the isotope variations in the tree-rings. However, one particular difficulty in modelling $\delta^{18}O$ of tree-rings is the source water isotope ratio, which is often not known, but may vary through time. We discuss different approaches for modelling $\delta^{18}O$ in tree-rings, starting from simple Craig-Gordon-based approaches to a more complex integration in the framework of a global dynamic vegetation model (Keel et al. 2015). Finally, the climatic signal of a well replicated 1200-yr long oxygen isotope chronology from the Alps is discussed, which is based on wood from living larch trees in combination with wood from old buildings.

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Improvements in EA – IRMS for low µg to mg sample amounts

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Bulk stable isotope analysis (BSIA) is the most widely used continuous flow technique for organic and inorganic sample matrices. By coupling an elemental analyser to an isotope ratio mass spectrometer, it is possible to analyze NCS and OH isotopes in single or multi-element mode on sample amounts in the µg to mg range. The success of the EA Isotope Ratio Mass Spectrometry (EA – IRMS) systems during the past 30 years has been based on meeting application challenges in routine and research environments concerning sample size, data quality and reproducibility, system automation, sample throughput, and cost of ownership.

This presentation will demonstrate highly improved performance and reliability of the EA – IRMS for low µg to mg analyte concentrations. Here, we highlight the strength and importance of continuous flow gas chromatography for data reproducibility, and chromatographic peak shape quality and baseline separation. In particular, the system performance will be shown for multi-element isotope analysis on sample types with challenging analyte concentration, for example C/S ratio of >7000:1 alongside very large and very small sample sizes in single and multi-element analysis mode. The application of the SmartEA^{TM} on a large dynamic range of sample sizes, is shown. We present data from single (N, C, S, O and H) and multi-element (NC, NCS and OH) isotopic analysis on sample matrices from different application fields, such as geosciences, ecology, biology, food and forensics.

Isotope tracers in catchment hydrology: Opportunities and challenges

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Headwater catchments are the building blocks of larger watersheds. While headwater catchments have been gauged and studied for more than a century, only in recent years has it been recognized that the celerity of hydraulic potentials (that control the storm hydrograph response) can exceed by orders of magnitude the velocity of the water itself (that controls the mean transit time of water in the stream). However, the mechanisms controlling these responses remain poorly understood. Here I review some of the recent work that uses isotope tracers for the study of flowpaths, flow sources and transit times. I explore both stream water and plant transpiration as water balance outflows and how stable isotope tracers are opening up new possibilities for insights into catchment exit time distributions. I also discuss some the current methodological limitations and opportunities for new research on scaling relations and their controls and the overall storage and release of water at the headwater catchment scale.

Sub-catchment size as a limiting factor for nitrogen transformation recognition in a mesoscale river basin

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Nitrate in surface and groundwater has increased in the last decades due to landuse change, the application of different fertilizer for agricultural landuse and industrial dust in the atmospheric deposition. Increasing nitrate concentrations have a major impact on eutrophication, especially for coastal ecosystems. Therefore it is important to quantify potential nitrate sources and determine nitrate process dynamics with its drivers. The Bode River catchment (total size of 3200 m^2) in the Harz Mountains in Germany was intensively investigated by a monitoring approach with 133 sampling points representing the same number of sub-catchments for a period of two years. The area is characterized by a strong anthropogenic gradient, with forest conservation areas in the mountain region, grassland, and intensively mixed farming in the lowlands (Mueller at al. 2015). Consecutive discharge simulations by a mesoscale hydrological model (mhM) allow a quantitative analysis of nitrate fluxes for all observed tributaries. The investigation of nitrate isotopic signatures for characteristic landscape types allows the delineation of dominant NO_3^- sources: nitrified soil nitrogen, fertilizer, and manure. Besides source delineation, the relationship between runoff and nitrate dynamics was analyzed for the entire Bode river catchment and, more detailed, for one major tributary with minor artificial reservoirs (Selke River). Thereby, it becomes apparent that nitrate isotopic variations increase with decreasing discharge. This effect might be due to a local, more intense impact of bacterial denitrification under low discharge conditions (higher residence time) in the anoxic soil zone, in the groundwater that discharges into the river and in the hyporheic zone. Generally, $\delta^{15}N_{NO3}$ and $\delta^{18}O_{NO3}$ decrease with increasing runoff at each sampling point, which can be caused by a preferential wash-out of more easily mobilizable, isotopically lighter fractions of the soil nitrate pool.

For streams with runoff larger than 5 m^3s^{-1} nitrogen isotopic signatures converge to a stationary value and cannot be assigned to particular nitrogen turnover processes and sources. Thus, clear conclusions on the origin of nitrate and the overall impact of microbial processes are possible up to a certain watershed size only. This information is relevant for understanding the process dynamics and has to be considered for future monitoring and modeling approaches.

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Isotope memory effects between soil water pools influence the isotopic composition of available plant water

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Stable oxygen and hydrogen isotopes have become an established tool in plant ecohydrology. Cryogenic vacuum extraction is typically the method of choice for the extraction of soil water for isotope analysis of waters moving through the soil-plant-atmosphere continuum. Increasing research has suggested there could be critical issues with this method that require urgent investigation (Orlowski, Breuer et al. 2015).

We tested under controlled conditions if tightly bound water in soils can alter the isotopic composition of water added to them. For that, oven dried soils with differing physicochemical properties were re-wetted with water of known isotopic composition and subsequently extracted by cryogenic water distillation. Relative to the initial re-wetting water, extractable soil water was significantly depleted in $\delta^{18}O$ (up to 1 ‰). Further experiments revealed that not all soil water is removed during cryogenic vacuum extraction and this remaining water imprints an isotopic memory effect on any water added. These findings suggest two water pools exist in soils: tightly bound pore water and loosely bound mobile water. Extractions only remove the loosely bound mobile water, and only oven drying soils removes the tightly bound water. Kinetic isotopic fractionation is likely responsible for the ^{18}O-depletion of water extracted from water added during laboratory re-wetting experiments. Additional re-wetting events suggest tightly bound and loosely bound soil waters are in dynamic isotopic equilibrium.

In a further tests we assessed if the isotopic composition of water cryogenically extracted from a variety of different soil types, i.e. the mobile soil water, is representative of the isotopic composition of the plant available water pool we conducted a greenhouse growth experiment. We grew 100 *Salix viminalis* cuttings, irrigated with water of a constant isotopic composition, grown in 5 different soil types, under two different watering regimes (arid vs. wet), and restricted soil water evaporational isotopic enrichment. Results suggest cryogenically extractable soil water represents biologically available plant water for $\delta^{2}H$ under both "arid" and "wet" conditions, while the situation is more complex for $\delta^{18}O$.

Collectively these findings provide a mechanistic understanding of the processes that lead to the isotopic depletion of cryogenically extracted soil water from water added during laboratory testing of cryogenic vacuum distillation systems. We show that two water pools exist within soils, that these pools are not isotopically separate, and that equilibrium isotopic fractionation can influence the isotopic composition of these pools, and therefore plant available water. These findings have significant implications for ecohydrological and paleoenvironmental studies.

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Resolving eco-hydrological dynamics of soil–plant–atmosphere interactions

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Water is the main driver of ecosystem productivity in most terrestrial ecosystems worldwide. The predicted increase in rainfall variability and extreme climatic events under future climate conditions is therefore anticipated to strongly affect plant and ecosystem functioning. Given that extreme events such as floods and droughts are predicted to increase in frequency in many regions, dynamic responses in soil-vegetation-atmosphere feedbacks play a privotal role in understanding the ecosystem water balance and functioning. In this regard, more interdisciplinary approaches, bridging hydrology, ecophysiology and atmospheric sciences are needed. Water stable isotopes are a powerful tracer of water transfer in soils and at the soil–plant interface (Werner and Dubbert 2016). Differences in stable hydrogen and oxygen isotope compositions (δ^2H and $\delta^{18}O$) in different source water pools (i.e. rain, groundwater), within the soil matrix, and subsequently in different plant species provide vital insights into the terrestrial water cycle.

Here, we present observations from a Portuguese cork-oak woodland. It is a bi-layered system of widely spaced cork-oak trees and an herbaceous layer dominated by native annual forbs and grasses. Ecosystem water fluxes, atmospheric concentrations and their isotopic compositions were measured using laser spectroscopy. Soil moisture and its isotopic composition in several depths as well as further water sources in the ecosystem (precipitation, groundwater, lake water) were monitored throughout the year. Using these isotopic approaches we disentangled soil-plant-atmosphere feedback processes controlling the ecosystem water cycle including vegetation effects on soil water infiltration and distribution, event water use of vegetation and soil fluxes, vegetational soil water uptake depths plasticity and partitioning of ecosystem water fluxes. In this regard, we review current strategies of *ET* partitioning. We highlight pitfalls in the presented strategies and show uncertainty analyses for the different approaches. We show that the isotopic composition of evaporation is very sensitive to sampling strategy (Dubbert *et al.* 2013). The isotopic composition of transpiration, on the other hand, is not in steady-state, most of the time under natural conditions (Dubbert *et al.* 2014a). Moreover, both vegetation layers strongly influenced water cycling in the ecosystem, altering infiltration and distribution of precipitation and in the long run ground-water recharge potential.

In conclusion, application of stable water isotope tracers delivers a process based understanding of interactions between soil, understorey and trees governing ecosystem water cycling necessary for prediction of climate change impact on ecosystem productivity and vulnerability.

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Long-term isotope series in large river basins indicating groundwater contribution and climate variability, examples from the Weser River in Germany

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Water isotopes (deuterium, oxygen-18) in river water primarily reflect the input concentrations from precipitation, melt water from snow and glaciers and time delayed contributions of runoff components (direct flow, subsurface flow). Information on recharge areas (altitude), time of recharge (season) as well as residence times and influencing processes (e.g., evaporation and mixing) of such systems can be derived if corresponding input signals are known (Wyhlidal et al., 2014). During base flow conditions river water samples carry mainly groundwater isotope signals, which is the most valuable water resource nowadays. Whereas in Germany continuous series of isotope composition in precipitation exists for more than 20 stations (Stumpp et al., 2014), isotope series from large river basins are rather rare.

We evaluate and compare stable isotope and tritium series from seven sub-basins of the Weser basin (46,240 km^2) available from monthly discharge-weighed river water samples over 13 years (2003–2015) and 35 years (1980–2015) respectively. Long-term isotope data from precipitation (GNIP stations Wasserkuppe, Bad-Salzuflen, Braunschweig, and Cuxhaven) are used to construct input series to better understand runoff behavior and residence time contributions of the system. Black box modelling approaches (FLOW-PC) were used to estimate residence times and relative amounts of direct flow, young and old groundwater. Long-term isotope patterns are used for a comparison and validation of earlier results (Koeniger et al., 2010).

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Compound specific stable isotope for sediment fingerprinting in Chitlang catchment (Nepal): From biotracer isotopic proportion to sources soil contribution

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Catchment managers require specific information on sediment sources to mitigate the multi-faceted effects of soil erosion. In particular, what are the major sources of eroded soils and how persistent are these sources over time? A new forensic stable isotope technique, using the compound specific stable isotope (CSSI) signatures of plant derived fatty acids (FAs) biotracers, can discriminate and apportion the source soil contribution in the sediment from different land uses. However, challenges exist in conversion of biotracer isotopic proportions obtained from Bayesian isotopic mixing model (IMM) to soil proportions. This research focuses on the critical evaluation of three scenarios of soil proportions calculation i.e. concentration based IMM and biotracer isotopic proportions upscaled using organic carbon content and fatty acids concentration.

Erodible top soil from different land uses and deposited stream sediments in a year 2013 from Chitlang catchment Nepal, were analysed for $\delta^{13}C$ of FAs using gas chromatography-combustion-isotope ratio mass spectrometry (GC-c-IRMS). The contribution of each sediment source to stream sediments was calculated using both with and without concentration based Bayesian IMM (MixSIAR). Concentration based IMM showed the majority of sediments was attributed to broad leaf forest (45-55 %) followed by low land and upland with similar contribution (8-18 %). This proportion of contribution slightly changed while upscaling biotracer isotopic proportions to soil proportions using FAs concentrations. In contrast, the pattern and proportions completely changed while upscaling using organic carbon content of the sources. The sediment was dominated all year round by dirt road (33-43 %) followed by lowland (15-25 %) and upland (10-20 %). Biotracer isotopic proportions correction approaches introduced additional uncertainty and increased credible intervals. The results indicated that the connectivity between land uses and drainage networks is important to consider while proper interpreting the soil source contributions. By linking $\delta^{13}C$ of FFAs fingerprints of land use to the sediment in deposition zones, CSSI approach has been shown to be a useful technique for determining the source of eroded soil and thereby identifying areas prone to soil degradation at catchment scale.

Worldwide interlaboratory comparison of cryogenic water extraction systems for stable water isotope analysis

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For more than two decades, research groups worldwide performed cryogenic water extractions for the analysis of ^{2}H and ^{18}O isotopes of soils and plants. However, recent studies have shown that cryogenic extraction conditions (extraction time, temperature, vacuum threshold) along with physicochemical soil properties can affect extracted soil water isotope results. Here we explore the development of a community standard operating procedure (defined temperature, extraction duration, vacuum) in order to obtain comparable results from different cryogenic extraction lines and to overcome potential isotope effects caused by the extraction method itself. We present results from a worldwide round robin test. We shipped 16 laboratories two different standard soils (silty sand and clayey loam) along with reference water. Participants oven-dried and rewetted the soils to two different gravimetric water contents (8 and 20%) with the reference water of known isotopic composition. One batch of soil samples was extracted via predefined extraction conditions which were common to all laboratories (silty sand: 45 min; clayey loam: 240 min, temperature: 100°C, vacuum: 0.3 Pa for both soils, respectively) and the second batch via extraction conditions considered routine in the respective laboratory for the specific soil type and water content. The obtained extracted water was isotopically analyzed using both a laser spectroscope and a mass spectrometer for cross-checking isotope results and ruling out potential analytical differences. Our results indicate large differences between participating laboratories and between applied extraction approaches, soil types, and water contents. This would suggest that defining any sort of standard extraction procedure might be challenging. Our results question the usefulness of this method as a standard for water extraction since results are difficult to compare across labs.

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Determining the origin of raining air masses in Iran and the Middle East Countries using isotopic composition (^{18}O and ^{2}H) of Precipitations

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The isotopic composition of precipitation is essential to determine the origin of raining air masses as well as to study the surface water and groundwater resources. In this paper, the GNIP (global network of isotopes in precipitation) data obtained from 58 stations belonging to the International Atomic Energy Agency (IAEA, 2015) as well as from conducted studies in different spots of Iran (Mohammadzadeh & Ebrahipour, 2012; Shamsi & Kazemi 2014; Najjar Saligheh 2014) were used to generate the Middle East meteoric water line (MEMWL - $\delta^{2}H = 6.86\ \delta^{18}O + 9.4$) and Iran meteoric water line (IMWL - $\delta^{2}H = 6.56\ \delta^{18}O + 4.25$), and to recognize the origin of raining air masses in Iran and the Middle East (ME) Countries. The wide range of isotopic compositions observed in Iran (-8.3 to -0.4‰ and -53.6 to 8.5‰ for $\delta^{18}O$ and $\delta^{2}H$, respectively) and in the ME Countries (-6.8 to 0.7‰ and -42.6 to 16.1‰ for $\delta^{18}O$ and $\delta^{2}H$, respectively) indicates the existence of different origin of raining air masses for the precipitations in these areas. Due to similarity between d-excess of the ME precipitations and East of Mediterranean Sea (MS), the major origin of ME precipitations is Mediterranean dry rain-producing air masses (Lambs & Moussa 2013). Discrepancy in d–excess, slope and intercept of LMWLs in different parts of Iran, represents the climate diversity and difference of precipitations origins. The evaporation rate of Iran's rainfall due to less slope of IMWL is more than that of the global and the ME. The map of oxygen-18 distribution confirms the entrance of Sudan warm air masses from the south, Siberia cold mass from the north and Mediterranean dry mass from the west and northwest of Iran.

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Measuring protein synthesis and breakdown rates using stable isotopes and mass spectrometry

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Scientists have been developing methods to measure in vivo synthesis and breakdown rates of protein since the 1930's. These early methods used isotope ratio mass spectrometry (IRMS) and stable isotopes, but these methods were very difficult and tedious to apply and were surplanted with the introduction of radioisotopes in the 1940's. It was not until development of new IRMS instruments in the 1960's that there was a resurgence in the use of stable isotope tracers. Although IRMS has extreme sensitivity for measuring isotope abundances, it requires purification of the analyte species to be measured. The development of gas chromotography (GC) combustion (C) IRMS dramatically changed how IRMS was used by providing GC separation of analytes before meaurement of ^{13}C as CO_2 or ^{15}N as N_2 by IRMS. Pyrolysis (P) was later added to provide measurement of ^{2}H as H_2, ^{13}C and ^{18}O as CO by a modified IRMS. The inherent problem for both GC-C-IRMS and GC-P-IRMS is that all atoms in the whole analyte molecule are measured. Unless the whole molecule is labeled with ^{13}C or ^{2}H, the GC-C-IRMS and GC-P-IRMS sensitivity are reduced for ^{13}C and ^{2}H due to dilution of unlabeled C or H, abundant in most analytes, that is also measured. For these reasons, GCMS techniques, developed in the 1970-80's, became prominent for isotope abundance measurements. The key advantages of GCMS was much better sensitivity, reuquring less sample and the ability to measure multiple isotopes and isotopomers in the same analyte molecule. The introduction of electrospray ionization (ESI) revolutionized mass spectrometry providing liquid chromatography (LC) MS and the introduction of both high resolution MS and tandem ESI-LCMS/MS techniques for extreme senstivity of multiple analyte measurement in complex matrices. Simultaneously GCMS/MS with high resolution MS also becamse commercially available, giving us now multiple methods to measure isotope and isotopomer abundances.

The classic problem for use and measurement of stable isotope abundances is assessment of rates of protein synthesis and breakdown of individual proteins in tissues using stable isotopic labeling. Although these measurements have been made by conventional IRMS as early as the 1930's, it would not be until the 21st century that modern bottom-up proteomic ESI-LCMS/MS methods would be applied for isotope abundance measurements to determine rates of synthesis and breakdown of individual proteins. The ESI-LCMS/MS methods are not only specific for individual proteins, but also can determine the turnover rate of specific protein posttranslational states. The latter is particularly important to understand, but measurements of individual protein posttranslational states have required specialized protein fractionation methods in conjunction with ESI-LCMS/MS. The inherent advantage of proteomic methods remains its limited ability to measure low enrichment levels of stable isotopes, which is typically found in slower turnover proteins that will incorporate limited enrichment of stable isotope tracers during the time course of the experiment. Thus, we are faced with improving our ESI-LCMS/MS methodology with a specific focus on determining small enrichment increases of stable isotopes in proteins. These methods move away from global measurements of proteins and protein turnover and focus on directed highly specific measurements of selected proteins. The current state-of-the-art of determining stable isotope enrichments in peptides from proteins by ESI-LCMS/MS will be discussed.

Following the impact from an overfeeding on Human metabolism using stable isotope tracers

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Obesity is a major public health problem. Deciphering the early processes occurring during weight gain is a major issue for understanding the development of obesity. Experimental overfeeding in humans is a unique situation to tackle these events.

Our aim was to study changes in metabolic fluxes during the initial phase of weight gain during an overfeeding experiment performed in Humans.

Twenty healthy men (18-55y; 23<BMI<27 kg.m^{-2}) were submitted to a high fat high sucrose diet for 31 days. The high fat high sucrose diet corresponded to a daily caloric excess of +50% from energy needs. The subjects added crisps, chocolate breads, Mars and Coca-Cola, to their usual diet in the aim to simulate "junk food" snacking.

Before and at the end of the nutrition intervention, subjects underwent clinical explorations:

- to evaluate the evolution of the insulin sensitivity before and after the overfeeding period (respectively D0 and D31), using the gold-standard method of the hyperinsulinemic-euglycemic- clamp. The use of [6,6-$^{2}H_2$]-glucose during the clamp allowed to measure the rate of glucose appearance during the basal condition and during the steady state of insulin infusion period.
- to follow the metabolic fate of ingested lipids using [1,1,1-$^{13}C_3$]-tripalmitin and [1,1,1-$^{13}C_3$]-triolein integrated in a test-meal respectively at D-7 and D28.

Plasma [6,6-$^{2}H_2$]-glucose analyses were performed using GC-MS. [1,1,1-$^{13}C_3$]-tripalmitin and [1,1,1-$^{13}C_3$]-triolein were followed in plasma lipids, non esterified fatty acids and triglycerides using GC-C-IRMS. Oxidation of labelled triglycerides was measured with $^{13}CO_2$ breath test coupled to indirect calorimetry.

In this context, the analytical aspect of the hyperinsulinemic-euglycemic-clamp with [6,6-$^{2}H_2$]-glucose and of the test-meal with [1,1,1-$^{13}C_3$]-tripalmitin and [1,1,1-$^{13}C_3$]-triolein will be presented from the sample collection. The overfeeding protocol led to an average weight gain of 2.7±0.3 kg. Measurements of [6,6-$^{2}H_2$]-glucose associated to the other parameters of the hyperinsulinemic-euglycemic-clamp do not show any alteration of insulin sensitivity after 31 days of overfeeding. Concerning the fatty acid kinetics following the ingestion of the test-meal containing labelled triglycerides, no difference in the apparition of the labelling in the non esterified fatty acid fraction before and at the end of the overfeeding was observed, ruling out an increase in the spill over phenomenon. On the contrary, an increased of the labelled fatty acid concentration was observed between D-7 and D28 in the triglyceride fraction. This suggests that more dietary lipids were incorporated in the triglycerides after overfeeding. The observed changes were similar for the saturated [1-^{13}C]-palmitate or the mono-insaturated [1-^{13}C]-oleate. This study suggests an increase in the hepatic synthesis of triglycerides coming from the diet.

If information provided by stable isotopically labelled tracers is not an end in itself, this study demonstrates the real interest of their use in human clinical studies for the understanding of mechanisms occurring during weight gain.

Metabolic fluxes, compartments and carbon isotope fractionation in androgen metabolism

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Androsterone (AND) and etiochlonaolone (ETIO) by far represent the most abundant androgen metabolites in the human. AND is a so-called 5α steroid while ETIO is its 5β isomer. In the liver, AND and ETIO are formed by different enzymes from identical substrates in a branched pathway. Both reactions feature irreversible reduction of the Δ^4-double bond in the A-ring of the steroid backbone. This is the rate-limiting and initial step of steroid metabolism. A single steroid 5β reductase (AKR1D1) has been described yet. By contrast, three isoforms of steroid 5α reductase (SRD5A1 to 3) are known.

ETIO is ^{13}C-depleted as compared to AND, but to varying degrees (Flenker et al., 2008). This suggests presence of an isotope effect during 5β reduction. Expectedly the $^{13}C/^{12}C$ ratio of ETIO linearly increases with its fractional yield. However, the effect is not fully consistent with theory: Intercept and slope are different and isotopic fractionation is predicted to disappear at yields less than one. Moreover, the $^{13}C/^{12}C$ ratio of AND is independent from fractional yield.

Traditionally, the bigger part of steroid metabolism has been assumed to occur in the liver. However, there is substantial evidence, that considerable amounts of 5α steroids are formed by so-called "intracrine" processes in peripheral tissues (Labrie et al., 2005). Peripheral steroid reduction is unbranched because AKR1D1 is absent. Consequently, no isotope fractionation may occur.

Therefore there are two sources of AND which differ in isotopic composition: Non-fractionated AND from the periphery and ^{13}C enriched AND from the liver. In turn, the combination of $^{13}C/^{12}C$ ratios and fractional yields allows to model the proportions of hepatic and peripheral androgen metabolism. We calculated average hepatic AND contributions of merely 25 % in a population of more than 100 subjects.

Longitudinal data from young women suggest hepatic AND contributions of ca. 15 %. The proportion is even significantly lowered in women using birth control pills. This can well be explained by synthetic estrogens and progestins present in these preparations. Both are known to inhibit steroid 5α reductases. Oral administration inevitably results in large hepatic concentrations of the pharmaceutical.

Our model facilitates interpretation of steroid $^{13}C/^{12}C$ data in sports drug testing. The results strongly support the view of predominant 5α steroid production by intracrine processes in the periphery. Hepatic steroid 5α reduction is probably limited. We suggest that natural abundance $^{13}C/^{12}C$ ratios and relative excretion rates of urinary steroids can be employed to infer peripheral and hepatic androgen fluxes. This is of utmost interest for the investigation of many hormone related diseases. The data can be obtained from a single GC-IRMS analysis.

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Primary Hyperoxaluria: unraveling the clinical pathophysiology by means of stable isotopes

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The primary hyperoxalurias (PH) are rare inborn errors of glyoxylate metabolism characterized by an increased endogenous oxalate production, which leads to the development of urolithiasis, nephrocalcinosis and ultimately renal failure. Once PH patients develop renal failure systemic deposition of oxalate accelerates, resulting in oxalosis, a devastating and life threatening condition affecting multiple organs most notably the skeleton, heart, veins, eyes and skin.

To date, three types have been defined on a molecular basis (PH1 – 3). Despite many decades of research into the metabolic pathways leading to oxalate synthesis in normal and PH individuals, uncertainties still remain. With the current promising research into new therapeutic modalities for PH1, the understanding of the molecular pathophysiology of all subtypes (PH1-3) could revitalize the research in this field and provide optimal targets for therapy and hence, optimal treatment for all PH patients. Recently, a new drug based on RNA interference has been developed and the first-in-man study is awaiting, which stresses the necessity for an accurate marker to evaluate therapeutic efficacy.

In the Academic Medical Center (AMC) in Amsterdam we have developed a stable isotope dilution protocol, which could not only be applied to evaluate therapeutic efficacy of new (drug) therapies but is also the ideal tool to explore the glyoxylate metabolism. Since the AMC is specialized in both the treatment of Primary Hyperoxaluria (PH) and the application of stable isotope techniques, we are able to be the first to unravel the complex glyoxylate metabolism and consequently enable the development of new treatments that were previously considered impossible.

Primary objective: Unravel the mechanisms underlying the increased endogenous oxalate production in both PH1, PH2 and PH3 patients.
Secondary objective: Quantification of oxalate, glycolate and glycine production in both normal healthy volunteers and PH patients in order to obtain reference values for the upcoming clinical trials to evaluate therapeutic efficacy.

Tertiary objective: To obtain insight into the contribution of different pre-cursors (e.g. glycolate, glycine and hydroxyproline) to endogenous oxalate production.

Study population: PH1 patients (n = 4) and normal healthy volunteers (n = 4)

Measurements: Both [^{13}C] stable isotope dilution as incorporation techniques will be used in order to quantify oxalate and glyoxylate kinetics. Subjects will be admitted [AMC] for two days in a row (duration of visit 10 and 6 hours respectively). On each visit, a baseline sample will be collected to determine the background enrichment of the [^{13}C] stable isotope after which the primed continuous infusion will be started. Subjects will be required to follow a diet low in oxalate for 3 days and start fasting 12 hours prior to the measurements.

Most important parameters:

- Ra and endogenous production of Oxalate, glycolate and hydroxyproline.
- [U-^{13}C]/ [1-^{13}C]oxalate and glycolate enrichment.

These parameters will allow us to perform further kinetic analysis, most importantly, the calculation of the fractional synthesis rate (FSR) and consequently the incorporation of tracer in oxalate.

Work in progress...

Carbon isotope values of non-essential amino acids in mice reflect macromolecular compostion of diets

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Analysis of stable carbon isotopes (^{13}C) has long been an indispensable tool in both ecology and archaeology for tracing dietary sources to consumers. In addition of being a source tracer, ^{13}C has the potential of informing about macromolecular composition of diets because ^{13}C fractionation during trophic transfer reflects the relationship between molecules derived directly from the diet and those synthesised de novo by the consumer or its symbionts. The building blocks for de novo synthesized molecules are 'scrambled' from dietary carbohydrates, fat, and proteins. To test how those macronutrients would affect ^{13}C values of non-essential amino acids, we analysed 10 groups of mice fed varying proportions of carbohydrates (20 – 75%), fat (20 – 75%), and proteins (5 – 60%). We analysed proteins extracted from bone collagen because we were interested in tissues that preserve well. Our results show that ^{13}C values of glutamic acid were particular informative of the relative proportion of carbohydrates. In terms of dietary protein, several non-essential amino acids became more ^{13}C enriched when the diets were protein limited. ^{13}C values of total collagen protein were less informative of macromolecular composition compared to ^{13}C values of individual amino acids. Our findings show that ^{13}C values of non-essential amino acids are promising for reconstructing the macromolecular composition of diets *in situ*, and for characterizing metabolic processes underlying variable bulk ^{13}C fractionation during trophic transfer.

Fructose or sucrose co-ingestion increases exogenous carbohydrate oxidation during exercise in trained cyclists

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Peak exogenous carbohydrate oxidation rates typically reach ~1 g/min during exercise when ample glucose or glucose polymers are ingested. Fructose co-ingestion has been shown to further increase exogenous carbohydrate oxidation rates. The purpose of this study was to assess the impact of fructose co-ingestion provided either as monosaccharide or as part of the disaccharide sucrose on exogenous carbohydrate oxidation rates during prolonged exercise in trained cyclists.

Ten trained male cyclists (VO_2peak: 65±2 mL·kg BM min^{-1} cycled on 4 different occasions for 180 min at 50% Wmax and consumed a carbohydrate solution providing 1.8 g/min of glucose (GLU), 1.2 g/min glucose + 0.6 g/kg fructose (GLU+FRU), 0.6 g/min glucose + 1.2 g/min sucrose (GLU+SUC), or water (WAT). Breath samples were collected to determine the $^{13}C/^{12}C$ ratio in expired air and combined with indirect calorimetry (VO_2 and VCO_2) to calculate oxidation rates of total fat, total carbohydrate, and exogenous carbohydrates. Repeated measures ANOVA with treatment as within-subjects factor was used to determine differences in exogenous carbohydrate rates between treatments.

In the GLU, GLU+FRU, AND GLU+SUC trials, the $^{13}CO2$ enrichments of expired breath increased from -26.9±0.5, -26.7±0.4 and -26.8±0.6% versus PDB at rest to –22.4±1.1, -20.7±0.6, and -21.0±1.1% versus PDB by the end of the 180-min exercise, respectively. Peak exogenous carbohydrate oxidation rates did not differ between GLU+FRU and GLU+SUC (1.40±0.06 vs 1.29±0.07 g/min, respectively, $P=1.000$), but were approximately 40 % higher when GLU+FRU or GLU+SUC were compared to GLU (0.96±0.06 g/min, $P<0.05$). In line, exogenous carbohydrate CHO oxidation rates during the last 120 min of exercise were approximately 40 % higher in GLU+FRU or GLU+SUC compared with GLU (1.19±0.12, 1.13±0.21, and 0.82±0.16, respectively, $P<0.05$).

Fructose co-ingestion (0.6 g/min) provided either as monosaccharide or as sucrose results in approximately 40 % higher exogenous carbohydrate oxidation rates during prolonged exercise in trained cyclists.

Grand challenges in understanding global land biogeochemical cycles: How can isotopes help?

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Stable isotopes offer a remarkable tool for understanding biogeochemical cycles. At the global scale, they have helped us constrain and understand cycles of carbon and major nutrients/elements, and to infer the mechanisms that govern the dynamical behavior of complex ecosystems.

Despite this progress, there are classes of processes and dynamics for which isotope approaches thus far have provided only limited resolution and insight. By focusing on land ecosystems, I will highlight a set of "grand challenge" questions, for which isotope approaches hold exceptional promise to offer new insights. I will also address questions, conditions, and timescales for which isotopic approaches appear subject to fundamental limitations, or for which innovative new isotopic tools are needed.

Central to my argument is the urgent need to understand the coupling of carbon, nitrogen and phosphorus across land ecosystems worldwide, and the challenge of resolving fundamental mechanisms across highly complex and heterogeneous biogeochemical systems. I will compare these challenges to progress in ocean biogeochemical systems, in which isotope systems are subject to different constraints and opportunities. It is my hope that my presentation will generate a broader discussion about needs, opportunities, and limitations in the pursuit of broader theories about land biogeochemical systems.

Nitrogen transformation and retention in temperate forest edges

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Forest edges are increasingly important landscape features worldwide, but they have largely been ignored in assessments of forest ecosystem functioning. Compared to interior forest zones, edges suffer increased nitrogen (N) input through atmospheric deposition but lose less N with percolating soil water, which challenges the current N-saturation paradigm. The overall aim of this study is to scrutinize the effect of edge proximity on N cycling and N sequestration in temperate forest ecosystems and therefore it is important to know the factors that determine N transformation and retention (Staelens et al., 2012).

Enriched ^{15}N solutions, containing $^{15}NH_4Cl$ and $Na^{15}NO_3$ were sprayed on the forest floor and movement of ^{15}N was followed in time through the different soil layers (litter, fragmented and humified litter and mineral soil) along edge-to-interior transects in an oak, a pine (Belgium) and a spruce forest (Denmark). Moreover, gross N transformation rates were quantified by the ^{15}N pool dilution technique. The principle is based on the dilution of the product pool that has been labelled with ^{15}N. Here, we used the *in situ* ^{15}N soil-labelling method, developed by Rütting et al. (2011), called the 'virtual soil core' injection, which studies undisturbed soils with live roots and their associated microbial communities. In this study, we investigated the fate of ^{15}N in throughfall and used analytical equations from Griffin (2007), adapted from Kirkham and Bartholomew (1954) to quantify gross mineralization and nitrification rates of the labelled N pools,. Our results indicate an edge effect on mineralization and a tree species effect on nitrification. Mineralization rates are higher in the forest edge than in the forest interior, while nitrification rates do not differ between forest edge and interior, but are higher in the oak forest than in the pine and spruce forest. Furthermore, ^{15}N enrichment and N retention differ between forest edge and interior in the three examined forests, irrespective of the treatment ($^{15}NH_4$ or $^{15}NO_3$).

This research indicates the importance of incorporating forest edges in programs monitoring N assessment on a landscape scale, as forest edges significantly influence N cycling.

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Hydrogen dynamics in soil organic matter as determined by ^{13}C and ^{2}H labelling experiment

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Soil organic carbon and water dynamics are of major importance in the prediction of ^{14}C and ^{3}H behaviour in terrestrial environments, at local or global scales. Whereas soil organic carbon cycle has been extensively studied, organic hydrogen in soil and its recycling in the environment have been rarely studied due to the high mobility of the element. Bound to N, O and S, hydrogen is usually exchangeable with the ambient water and water vapour whereas hydrogen bound to carbon is considered to be stable, non-exchangeable, due to the strong covalent bonds. Since closely linked to carbon, we hypothesized that the acquisition of the non-exchangeable hydrogen isotope signature is determined by the dynamics of carbon in soils and is thus impacted by two main processes: the partial preservation of the isotopic composition of the organic material decomposed in the soil, and the microbial biosynthesis incorporating water-derived hydrogen.

To decipher and quantify both processes, we designed incubation experiments with labelled compounds, with three scenarios: 1- $^{13}C^{2}H$-double labelled molecules (glucose, phenylalanine, palmitic acid and isoleucine) in presence of $^{1}H_2O$, 2- ^{13}C-labelled molecules in presence of $^{2}H_2O$, 3- no molecule addition but presence of $^{2}H_2O$. Substrates were introduced in three topsoil samples chosen for their various pedologic properties: one podzol, one luvisol and one cambisol. From 0 to one year, ^{13}C content of bulk samples were analysed by Elemental Analyser-Isotope Ratio Mass Spectrometry and the simultaneous ^{13}C and ^{2}H content of bulk soil, and lipid fraction of some samples, with a Combustion Module-Cavity Ring-Down Spectroscopy Isotope Analyser.

Our results highlight that the mineralisation of hydrogen and carbon of the substrate was similar whatever the added molecule. The preservation of added substrate derived hydrogen after one year of incubation (ca. 5% in most cases) is lower than the preservation of its carbon counterpart (30% in average). The isotopic composition of the non-exchangeable hydrogen appears to be mainly driven by the incorporation of water hydrogen during microbial biosynthesis. Indeed, the results of incubation show that the incorporation of water hydrogen is linearly correlated with the amount of carbon that is degraded in the soil.

Soil properties play a significant role in the mineralisation of organic hydrogen and in the incorporation of water hydrogen. The isotopic results of the lipid fractions showed that the "biodegradation-driven" water hydrogen incorporation into the non-exchangeable pool is not only organically bound. The increase in incorporation of water hydrogen, with soil clay content, suggests that part of the hydrogen is bound, in non-exchangeable position, to clay or organo-mineral complexes.

From these experiments, the fate of hydrogen at ecosystem scale will be predicted by modelling and extrapolated to the fate of tritium in organic matter.

Simulating the isotopic composition of terrestrial N cycle components with the stable isotope model of N-cycle evolution (SIMONE)

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The excessive use of Nitrogen (N) fertilizers on the one hand has boosted global population growth, but on the other hand had detrimental effects on the environment. For instance, the surplus of reactive N, i.e. inorganic and organic N forms, in terrestrial ecosystems has led to increased soil emissions of nitrous oxide (N_2O), which depletes stratospheric ozone and increases radiative forcing. Process-based biogeochemical models are increasingly used to assess the fate of N compounds in the environment, to track soil N_2O emission sources and to develop targeted strategies to mitigate soil N_2O emissions as well as nitrate leaching from agricultural systems. Although such models consider all relevant N cycling processes, they are usually calibrated and validated with individual, measurable quantities at ecosystem scale such as exchange rates of N compounds or N in harvested plant products. However, the improvement and validation of biogeochemical models requires information on processes underlying soil N_2O emissions. The isotopic composition of large N pools, e.g. the soil N pool, reflect the dominant N cycle processes in a specific environment and, thus, may be used as additional integrative validation quantity.

With SIMONE we present a module that allows tracking changes in the isotopic composition of the various N compounds due to physico-chemical and biological N cycling in ecosystems. This isotope model builds upon the biogeochemical model LandscapeDNDC and uses isotopic fractionation factors for isotope calculations. Here we present a review of available data on N fractionation and assess the sensitivity of the simulated isotopic composition of different N compounds in view of the uncertainty of fractionation factors for different processes. The performance of SIMONE will be discussed based on a comparison of simulated and measured $\delta^{15}N$ in soil emitted N_2O using a dataset obtained during a field campaign conducted at the intensively managed grassland site Chamau, central Switzerland. During this campaign, also the isotopic quantity “site preference” was determined, i.e. the difference of $\delta^{15}N$ of N_2O substituted with ^{15}N at the central and terminal position, respectively, which is considered a powerful tool for partitioning soil N_2O emissions to the contributions of nitrification and denitrification. The dynamics of measured and simulated site preference will be discussed in view of management action and meteorological conditions.

Response of subalpine vegetation to N deposition and land-use changes from a new isotopic perspective

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Atmospheric nitrogen (N) deposition has been affecting remote environments for decades, thus changing N availability and consequently the critical ecosystems services provided (Aber et al, 1998, Baron et al, 2000, Voss et al 2006). Changes in land management have also been shown to alter N biogeochemical cycle as well as composition and functioning of upland vegetation (Robson et al 2010, McGovern et al 2013, Gill et al 2014) but the potential synergetic effect between both phenomena has yet to be understood.

Here we studied the response of endemic vegetation to both N-deposition and land-use changes at the Lautaret Pass in the French Alps. We focused on two plants, *Dactylis glomerata* and *Festuca paniculata*, which constitute most of the green biomass of respectively "mown+fertilized" and "unmown" grasslands on terraces, and "grazed" unterraced grasslands. $\Delta^{17}O$, $\delta^{15}N$, $\delta^{18}N$ of NO_3^- as well as $[NO_3^-]$, $[NH_4^+]$ and TDN were measured in plants and soils extracts collected from early summer 2015 throughout the end of autumn 2016 (ongoing work) in above listed subalpine meadows, coupled to aerosols and streams samples. We particularly aimed to trace the changes in the fate of atmospheric NO_3^- that deposited based on its $\Delta^{17}O$ value, as this specific isotopic signature is a conservative tracer.

Preliminary results highlight different responses to N deposition and land management and point out the usefulness of the $\Delta^{17}O$ tracer in an ecological study as a unique tool to further investigate transformations affecting remote ecosystems in conjunction with human activities.

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Quantification of enzyme-mediated hydrolysis by means of the oxygen isotope ratio of phosphate in grassland and forest soils

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Organic P (Po) contributes 30-50 % to total P concentrations in soil. Plants and microorganisms developed strategies to contribute to the transformation of Po to inorganic P (Pi) by synthesizing enzymes capable of hydrolyzing Po. Commonly, assays with substrate additions are used to characterize potential enzyme activity which differs from actual enzyme activity. We present an approach based on $\delta^{18}O$ values of resin-extractable Pi ($\delta^{18}O_P$) in soil. This approach relies on the incorporation of O atoms of ambient water into phosphate that results from the hydrolysis of Po by enzymatic activity. We added waters differing in the O isotope composition to soil prior to laboratory (grassland soils) or field (forest soils) incubation. The slope of the linear regression of $\delta^{18}O_P$ values on $\delta^{18}O$ values in soil water was used to quantify the incorporation factor (a) of water-O into Pi in soil and to identify pathways based on apparent fractionation factors.

In the laboratory incubation of grassland soils, the actual incorporation of water-O into Pi was small (a = 5.7±2.1%) calling for cautious interpretations of potential enzyme activities. Apparent fractionation factors associated with extra- and intra-cellular enzymatic reactions suggest that between 0.6% and 2.3% of Pi in soil originated from extra-cellular Po mineralization. Between 3.4% and 5.1% of Pi in soil was released by living cells.

For the in-situ incubation of forest soils, the incorporation factor of water-O into resin-extractable Pi was 0.25 (± standard error 0.05) indicating that, averaged across all resin-extractable phosphate molecules, one out of four O atoms originated from enzymatic incorporation of water-O atoms. Potential monoesterase activity and the incorporation factor a were significantly related ($p = 0.03$) with 84% of the variation in a remaining unexplained. This might be caused by the contribution of phosphate molecules without any water-O incorporation and of enzymatic reactions of other Po compounds (pyrophosphatase, diester etc.). Labile and moderately labile Pi fractions ($NaHCO_3$-extractable + NaOH-extractable) explained nearly half of the variation in the incorporation factor a ($p = 0.001$). In general, we found no significant effects of tree species or land use intensity on potential or actual enzyme activities in the forests under study ($0.05 < p < 0.83$). In conclusion, our approach offers unique insights into actual enzymatic processes involved in Pi release in soil.

Continental-scale spatiotemporal patterns of plant water isotope values as a tool for improved understanding of hydroclimate proxies from plants

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The hydrogen and oxygen isotopic composition of water available to plant biosynthetic processes plays an important role in shaping the isotopic composition of organic compounds that these organisms produce, including leaf waxes, leaf-, and tree ring-cellulose. Characterizing changes in large scale spatial patterns of precipitation, soil water, stem water, and leaf water isotope values over time is therefore useful for evaluating the extent to which plants reflect changes in the isotopic composition of these source waters in different environments, which can in turn provide improved calibration targets for understanding the environmental signals that plants preserve. The pathway of water through this continuum is associated with several isotopic fractionations, but the extent to which the isotopic composition of each of these water pools varies under normal field conditions and over space and time has not been systematically evaluated at large spatial scales.

Two season-long sampling campaigns were conducted at twenty sites throughout Europe over the 2014 and 2015 growing seasons to track changes in the isotopic composition of plant-relevant waters, and to test the capacity to provide quantitative estimates of these values from climate observations. Samples of precipitation, soil water, stem water, and leaf water were collected over more than 200 field days and include more than 500 samples from each water pool. Measurements were used to validate continent-wide leaf water isotope values predicted from a combined mechanistic and statistical modeling approach run with gridded temperature, precipitation, and relative humidity data. Data-model comparison shows good agreement for summer leaf waters, and substantiates the use of such models to evaluate how plants respond to hydroclimate changes. This information can then be used to assess the controls on the isotopic composition of organic compounds that are produced by plants, and that can be preserved in the environment over geologic timescales. This approach allows for the diversity of water sources on plant compounds to be tested at higher temporal and spatial resolution, and over longer durations than is possible even with dense field sampling networks.

Nitrogen cycle inferred from N and O isotopes in a changing Arctic Ocean

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The rapid decline of sea ice extent in the Arctic Ocean will likely impact the overall Arctic ecosystem and associated biogeochemical cycles. As a first indicator, Arctic primary production has significantly increased during the last decades. All evidences highlights the necessity to better constrain the modern, yet overlooked, Arctic nitrogen (N) cycle to make future primary production projections more reliable. We analysed nitrate $\delta^{15}N$ and $\delta^{18}O$ and water $\delta^{18}O$ on full water column profiles during two summer cruises (July-August 2014) in the Arctic Ocean: SWERUS (R. V. Oden) consisting of across-slope sections over the East-Siberian and Laptev shelves; and ARK-XXVII (R. V. Polarstern) in the vicinity of Fram Strait. The coupling of the N and O isotopes is especially useful to constrain nitrification, the microbially-mediated oxidation of ammonium into nitrate. Preliminary interpretations indicate a strong decoupling between nitrate $\delta^{15}N$ and $\delta^{18}O$ in the upper ocean, increasing and decreasing upward, respectively. This feature is unique in comparison to the other oceanic basins, and indicates that most of the nitrate in the upper ocean has been produced by nitrification within the Arctic Ocean, either over the shelves or in the deep basins.

Interacting effects of climate variation and site conditions on tree growth and physiology of beech and spruce across Central Europe

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Trees' responses and adaptation to climate change is more clearly detectable at the edge of their ecological distribution than within their growing optima. Yet, at temperate sites, long-term shifts and abrupt responses of the physiological performance due to changing environmental conditions can be retrieved from stable C and O isotopes of tree rings and – in combination with growth data – provide hints for future optimum ranges and limits for growth.

In this large-scale study comprising 10 sites across climate gradients (temperature, precipitation) throughout Central Europe, we investigated tree-ring widths and stable isotope ratios (C and O) of European beech (*Fagus sylvatica*) and Norway spruce (*Picea abies*). The sampling design included larger and smaller trees. The short-term, i.e. year-to-year, variability of the isotope time series over 100 yrs was analyzed in relation to tree-ring growth and climate variation. Variability in the isotopic ratios consistently responded to summer climate, particularly vapor pressure deficit, but was weakly correlated to annual stem growth. In contrast, climate sensitivity of radial growth of both species was rather site-dependent, and was strongest at the driest (in terms of soil water capacity) site.

The generally strong positive correlation between the year-to-year differences in $\delta^{13}C$ (corrected for the atmospheric shift due to ^{13}C-depleted CO_2 from fossil combustion) and $\delta^{18}O$ across most sites emphasized the role of stomatal conductance in controlling leaf gas exchange. However, the correlation between both isotopes decreased during some periods, at several sites particularly pronounced during recent decades. This suggests a decoupling of stomatal and photosynthetic responses from environmental conditions. Depending on the site, we found both positive and negative relationships between $\delta^{13}C$-derived intrinsic water use efficiency (iWUE) and tree ring width. This further indicates the site-specific potentials of either growth benefit from increasing C-assimilation or growth limitations in response to water shortage.

Overall, our preliminary results revealed that under mesic or moist site conditions, tree growth seemed to benefit from increases in photosynthesis as shown by increased ring width despite enhanced iWUE in recent decades. At rather dry sites, reduced growth was likely due to limited C assimilation and allocation to stem growth, unless the trees' adaptation capacity to dry conditions allowed for more sustainable growth as indicated from the warmest site. Understanding the underlying physiological mechanisms controlling the short-term variation in tree-ring records helps constrain the possible range of growth performance of these ecologically and economically important tree species under future climate conditions.

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Do cable bacteria enhance dissimilatory nitrate reduction to ammonium in coastal sediments?

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The recent discovery of cable bacteria has challenged the way biogeochemical processes are conceptually understood in sediments (Pfeffer et al. 2012). Recent studies have shown that these organisms can have a marked effect on the cycling of iron, phosphorus, sulfur, oxygen and carbonate in marine sediments (Risgaard-Petersen et al. 2012, Seitaj et al. 2015, Sulu-Gambari et al. 2016). How these organisms affect the nitrogen cycle in marine sediments remains unknown. It has been shown that cable bacteria can directly use nitrate as an electron acceptor, however the final product (N_2 or NH_4^+) remains to be determined. It has recently been hypothesised that cable bacteria stimulate dissimilatory nitrate reduction to ammonium rather than denitrification (Marzocchi et al. 2014). The postulated mechanism is that cable bacteria enhance Fe^{2+} liberation within the sediment through acidification. The liberated Fe^{2+} is then oxidised by NO_3^- which is reduced to NH_4^+ in preference to N_2 (Roberts et al. 2014, Robertson et al. 2016). To date the direct involvement of cable bacteria in this process remains circumstantial. To directly test the role of cable bacteria in this process we set up a series of repacked cores using sediment from the Yarra River, Australia, which has been shown to harbour *in situ* metabolic activity of cable bacteria. Half of the cores had filters inserted into them to prevent the growth of cable bacteria, which was verified with pH profiles. Isotope tracer experiments using $^{15}NO_3^-$ were subsequently undertaken to measure rates of denitrification and dissimilatory nitrate reduction to ammonium. This presentation will outline the results of this study and the implications for nitrogen cycling in periodically hypoxic sediments colonised by cable bacteria.

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The influence of atmospheric water vapour on the oxygen isotope ratio of organic molecules in plants

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Oxygen stable isotopes of organic molecules in plants hold potential information on environmental and climatic conditions, physiological processes, and biochemical reactions. While the influence of source water, precipitation, and processes that cause ^{18}O-enrichment in leaf water are well understood, we still lack knowledge about the influence of atmospheric water vapour on the oxygen isotope ratio (δ^{18}O) of organic molecules.

Here we labelled young Oak trees (*Quercus robur*) under wet and dry soil moisture conditions with ^{18}O-depleted water vapour in high relative humidity conditions (~90%) for 5 hours, simulating atmospheric conditions prevalent during rain or fog. To follow the label from the atmosphere into leaves and to their ultimate incorporation in plant biomass, we traced the oxygen isotope ratio of water and organics from the leaves to the stem during a 24 h period.

Our results clearly demonstrate that the ^{18}O-label from the water vapour caused a strong depletion in leaf water within the first hour. After 5 h, the label was observed in leaf and twig phloem sugars and subsequently in the stem phloem after 20 h. Compound-specific isotope analysis of the leaf sugars revealed that fructose, glucose, and sucrose were strongly labelled, but not the sugar alcohol quercitol. Nevertheless, leaf sugars did not show high temporal variations or any treatment differences after labelling, while δ^{18}O of twig phloem sugars progressively declined. The latter effect was stronger for wet plants than dry plants, which may indicate the retardation of phloem loading in leaves from trees experiencing drought.

In summary, we demonstrate that ^{18}O-labelling via water vapour is a useful tool to investigate O allocation in plants. We provide clear evidence that high humidity events are incorporated into the oxygen isotope ratio of organic molecules, demonstrating that this parameter deserves more attention in future studies.

Plant-plant interactions and environmental factors explain high small scale variation in foliar δ^{13}C and δ^{15}N: insights from a combined isoscape – remote sensing approach

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The composition of foliar C and N isotopes may vary considerably at small spatial scales, potentially reflecting the spatial range of functional processes and interactions within plant communities. Environmental factors such as micro-topography or vegetation cover may cause additional small scale spatial variation. Yet, spatially explicit stable isotope studies with high resolution in the range of meters are rare and the degree of variation has seldom been evaluated.

In a Portuguese dune ecosystem invaded by an N_2-fixing invasive tree, *Acacia longifolia*, we tested whether δ^{13}C and δ^{15}N isoscapes of native species' foliage with high spatial resolution could be used to detect the spatial range and extent of changes in C and N cycling within the native community. Isoscapes revealed strong spatial patterns with pronounced species-specific differences. A coniferous tree (*Pinus pinaster*) and an ericacean dwarf shrub (*Corema album*) showed significant enrichment in δ^{15}N within a range of 5-8 m surrounding the canopy of *A. longifolia*, indicating input of N originating from symbiotic N_2-fixation by the invader. In the highly susceptible dwarf shrub, enrichment in δ^{13}C additionally suggested spatially explicit changes to water use efficiency, while a native N_2-fixer (*Stauracanthus spectabilis*) was unresponsive to the presence of the invader.

To test in how far variation due to further environmental factors influences small scale spatial variability and thus, the ability to interpret isoscapes as tracers of plant-plant-interactions, we used the distance to *A. longifolia* and airborne LiDAR (laser scanning) derived vegetation cover, elevation and topographic indices to model foliar δ^{15}N of the highly responsive shrub *C. album* in 35 transects of 20 m length across 5 different sites along the coast of South-West Portugal. The model explained 58% of the variation in δ^{15}N, which showed a large total range of 14.3‰. Proximity to *A. longifolia* was consistently associated with enriched δ^{15}N values of *C. album*, confirming its applicability as a spatial tracer for invader influence. Furthermore, vegetation cover was identified as an important predictor of δ^{15}N.

We conclude that isoscapes are highly suitable to analyze spatial processes within plant communities. At the same time, pronounced small scale spatial variability demands caution when attempting to use foliar isotopic data as community-wide tracers. Values can vary by several per mill at a scale of meters due to biotic interactions and abiotic factors even when using standardized sampling designs within individual species. Generally, linking remotely sensed topographic and vegetation cover data with spatial information significantly increased the predictability of foliar δ^{15}N.

Evaluation of the importance of water isotope variations in precipitation versus plant source water for the interpretation of O and H isotopes in plant tissues

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The isotopic ratio of precipitation is frequently used as a proxy for plant source water in place of either the isotopic ratio of soil water or even xylem water for the prediction of O and H isotope ratios in plant organic matter.

In general, the variation in precipitation is likely to greatly exceed that of soil water, which in turn exceeds that of xylem tissue. Additional variation in water isotopes is introduced through transpiration, which results in the evaporative enrichment of leaf water used for metabolic processes. Thus, using precipitation isotopes as a direct measure of the water used by plants for metabolic processes such as photosynthetic carbon assimilation neglects intermediate steps and may bias estimates of water isotopes in assimilates.

We carried out a spatially explicit sampling of water isotopes in precipitation, throughfall, soil water, xylem tissue and leaves in order to constrain the extent of variation in a 1 ha plot in central Switzerland, as well as a second sampling of soil water and xylem tissue at 190 sites across Switzerland. A significant decrease in isotopic variation in soil and xylem water was observed at the local, as well as at the regional scale, although to a lesser extent but with similar variation between soil and xylem water. Consequently, relying solely on precipitation would lead to the overestimation of the isotopic variability of xylem water in most cases and thus bias estimates of the $\delta^{18}O$ and δD in plant organic matter. We discuss the consequences for the interpretation of the $^{18}O/^{16}O$ and D/H isotope ratios in plant organic matter and particularly in tree rings.

The emission of CO_2 from a forest floor on natural soil temperature gradient in a volcanic area in southwest Iceland and the contribution of geothermal CO_2 measured by $\delta^{12}C/\delta^{13}C$ fractionation

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We studied carbon dioxide (CO_2) emissions along a natural geothermal soil temperature (T_s) gradient in a volcanic area in southwest Iceland. The gradient was recently formed after a major earthquake in May 2008 in the middle of a planted Sitka spruce forest. The variation in soil temperature along the gradient ranged from 8.4 to 61.2 °C at depth of 10 cm. Carbon dioxide emissions (ecosystem respiration) from the forest floor was measured along the temperature gradient using the static chamber method. CO_2 concentration in the gas samples were analyzed with a gas chromatograph (GC). In addition to CO_2 concentration analysis also the isotope ratio ($\delta^{13}C$) was measured from the CO_2 samples with GC-IRMS (isotope-ratio mass spectrometry).

With a moderate soil temperature increase (up to +5 °C) there were no change in CO_2 emission rates but the temperature raise from 20 to 45 °C induced a significant increase in CO_2 emissions. The measured CO_2 emissions (up to 2500 mg CO_2 m^{-2} h^{-1}) from the warmest plots were ten times higher compared with those from the coolest plots (250 mg CO_2 m^{-2} h^{-1}). Similarly as with the CO_2 emissions the changes in the isotope ratio of CO_2 ($\delta^{13}C$ %o of soil CO_2 efflux) was not significant below 5°C warming, but increased significantly and linearly from ca. -26%o to -5 % above that threshold point up to the highest temperature sampling point. This strongly suggests an alternative carbon source contribute to the CO_2 emissions, namely abiotic CO_2 which is enriched in ^{13}C such as CO_2 from geothermal sources (Klusman et al. 2000). Our isotope source partitioning approach (simple 2 pool mixing model) revealed that up to 90% of CO_2 originates from geothermal sources at higher temperatures. When accounting for that additional C source and calculating biotic (vs. abiotic) CO_2 emissions on an aerial basis, we found that ecosystem respiration shows an optimum in the mid temperature range (T_s increase about 10°C) rather than a linear increase with increasing temperature. This study demonstrates that isotope approaches are needed to disentangle sources of respiration if we want to understand underlying mechanisms and temperature responses on such sites. This can substantially impact on the results in volcanic areas where natural temperature gradients are increasingly explored, but also e.g. in soils rich in carbonates. We conclude that if temperature gradients on volcanic soils are used for studying temperature responses in the ecosystem (O'Gorman et al. 2014) also the effects of geothermal CO_2 should be taken into account.

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Isotopic characterization of N_2O emissions and N_2O soil air concentrations from hotspots in sub-Arctic permafrost peatlands

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Nitrous oxide (N_2O) is a potent greenhouse gas contributing to global warming. Soils make up for about 70% to the global annual emissions of N_2O, with most of the N_2O emissions originating from tropical and agricultural soils. Typically N_2O emissions from remote terrestrial ecosystem on Arctic and sub-Arctic soils have been neglected due to the nitrogen-limited nature of these biomes. Nevertheless, recent findings have shown that there are habitats in the sub-Arctic region emitting N_2O at high rate [Repo *et al.,* 2009] and that permafrost soils have the potential for the N_2O emissions after thawing [Elberling *et al.*, 2010]. All these latest results suggest that nitrogen has to be considered together with carbon when estimating climate feedbacks of Arctic ecosystems.

In order to improve our understanding of the microbial production and consumption processes active in these N_2O hotspots and their controlling factors we measured *in situ* N_2O emissions and stable isotope composition from bare peat surfaces in sub-Arctic permafrost peatlands and N_2O stored in the peat profile (Seida, NW-Russia; 62°57'E, 67°03'N). Soil N_2O bulk isotopic composition ($\delta^{15}N^{bulk}$ and $\delta^{18}O$) and the "site preference" (SP) [which is the difference between intramolecular ^{15}N isotopic composition, i.e., the $^{15}N/^{14}N$ ratio at the central (α) or terminal (β) nitrogen position] have been used as tool to identify the microbial pathways of N_2O production and consumption

The $\delta^{15}N^{bulk}$ and $\delta^{18}O$ of the N_2O emitted by the bare peat soil range was -17‰ to -3‰ and -6‰ to 30‰, respectively, with an emission-weighted average value for $\delta^{15}N^{bulk}$ of -13‰ ± 2 ‰ and for $\delta^{18}O$ of 17 ± 3‰ (mean ± s.e; n=8). Emission-weighted $\delta^{15}N^{bulk}$ value of N_2O from the bare peat is within the range of the emission-weighted average values from other natural ecosystems. The enrichment factors of $\delta^{15}N^{bulk}$ of the N_2O relative to $\delta^{15}N^{bulk}$ values of NO_3^- in soil extracts for our data (-10 to -27‰) falls within the range of values indicative of N_2O production from denitrification (-13 to -28‰; Perez *et al.,* 2011). However, there are several short-comings in this partitioning approach based on $\delta^{15}N^{bulk}$.

The individual SP values of the N_2O emitted presented a large range over the season (from -36‰ to 52‰) and were in the range of SP values reported from soil N_2O fluxes and N_2O concentrations from *in situ* measurements (chamber and profile) and laboratory incubations [Toyoda *et al.,* 2015] (SP=-40‰ to 90‰). The emission-weighted average SP value for N_2O of -10 ± 6‰ (mean ± s.d; n=8) is the lowest one reported so far from *in situ* studies and is overlapping with SP values for nitrification/nitrifier denitrification (−17‰ ± 8‰) obtained by Perez *et al.* [2006] in soil incubation experiments, suggesting that the larger N_2O emission in sub-Arctic tundra are more likely to be produced by nitrifier denitrification in the relatively drier study year. However, due to variable published SP values for N_2O production processes in soils this interpretation has to be taken with caution. On the other hand. The SP values from bare peat soil profile (-0.7‰ to 23.0‰) suggested that in deeper soil layers denitrification-related mechanisms were the main N_2O production pathway and up to 70 % of the produced N_2O was reduced to N_2 within the soil. This study revealed important mechanisms behind the factors controlling N_2O release from the Arctic, but also highlights the complexity in interpreting ^{15}N results in N_2O studies.

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Stable carbon isotopes indicate high importance of vascular plants for methane emissions from subarctic wetlands in Northeast European Russia

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Wetlands represent hot-spots of methane (CH_4) emissions in subarctic tundra landscapes. The anticipated warming at northern latitudes together with associated permafrost thaw may increase the proportional area of these wet ecosystem types, which would be seen as increased CH_4 release (Johansson et al. 2006). Therefore, there is a need for more accurate estimations of the total CH_4 release from tundra as well as for better understanding of the mechanisms behind the CH_4 emissions. Stable isotope composition of emitted and porewater CH_4 has potential for both: constraining the global CH_4 budget based on isotopic signatures of CH_4 from different sources, and assessing the importance of various production, consumption and transport mechanisms for the net CH_4 release.

Here we report the emissions and pore water profiles of CH_4 together with stable isotopic composition of C-CH_4 in subarctic wetlands in the Northeast European Russia. The research was conducted during two growing seasons on three wetland types: willow stands, *Carex* fen and *Eriophorum* fen. These ecosystem types cover ~15% of the studied landscape, but are responsible for 98% of the regional mean (± SD) CH_4 release of 6.7 (± 1.8) g CH_4 m^{-2} y^{-1} (Marushchak et al. 2016). The annual CH_4 release from these wetlands ranged from 34 to 53 g CH_4 m^{-2} yr^{-1}. The ground water level stayed permanently at high level due to floating wetland surface and the seasonal variability of the emissions was strongly driven by temperatures, whereas vascular leaf area index explained as much as 88% of the spatial variability. Stable isotope analysis gave further evidence on the important role of vascular plant cover for CH_4 emissions: The ^{13}C in the CH_4 emission correlated negatively with the vascular plant cover (LAI) and CH_4 released to the atmosphere was lighter than that in the surface porewater. This implies that the dominant CH_4 release pathway from wetlands to the atmosphere was plant mediated diffusion through aerenchyma, a process that discriminates against ^{13}C-CH_4. The ^{13}C of CH_4 flux did not show much variability among the wetland types, years, or sampling dates. The bulk mean $\delta^{13}C$ value (± SD) for the emitted CH_4 was -68.2 (± 2.0) ‰.

Our data suggests that future higher temperatures together with possible increase in vascular biomasses would increase the CH_4 emissions from the studied subarctic wetlands on unit area basis. Together with expansion in their surface area at the expense of permanently frozen peatland, this would mean higher CH_4 emissions in regional scale.

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Quantification of organic and inorganic nitrogen dynamics in soil using the ^{15}N tracing model *Ntrace*

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Depolymerization of soil organic nitrogen (SON) into monomers such as amino acids is thought to be the rate limiting step for the terrestrial nitrogen (N) cycle. The production of free amino acids (FAA) is followed by FAA mineralization to ammonium (NH_4^+), which is an important fraction of the total N mineralization. Accurate assessment of depolymerization and FAA mineralization rate is important for a better understanding of the rate limiting steps.

Recent developments in the ^{15}N pool dilution techniques, based on ^{15}N labelling of FAA's, allow the quantification of gross rates of SON depolymerization and FAA mineralization (Wanek et al., 2010; Andersen et al., 2015), in addition to quantifiction of gross N transformation rates of inorganic N species. However, it is well known that the ^{15}N pool dilution approach has limitations; in particular that gross rates of consumption processes (e.g. FAA mineralization) are overestimated due to substrate addition. This has consequences for evaluating the rate limiting step of the N cycle, as well as for estimating the nitrogen use efficiency (NUE).

Here we present a novel ^{15}N tracing approach, which combines ^{15}N labelling of FAAs with an advanced version of the ^{15}N tracing model *Ntrace* (Müller et al., 2007; Andresen et al., 2016) explicitly accounting for FAA turnover in soil. This approach (1) provides a robust quantification of gross depolymerization, FAA mineralization and total N mineralization, which are simultanesouly quantified, and (2) suggests a realistic estimate for the microbial NUE of amino acids. Advantages of the new ^{15}N tracing approach will be discussed and further improvements will be identified.

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C:N:P:K stoichiometric constraints on litter and soil organic matter decomposition

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A long history of agronomic research has revealed the importance of Nitrogen, Phosphorus and Potassium to plant productivity, litter mass loss and overall soil health. However, we know much less about the relative constraints of these nutrients on the fate of decomposing litter and soil organic matter during decomposition. Deposition of mineral nitrogen from anthropogenic activities, as well as through the acceleration of soil organic matter decomposition due to global warming, is leading to an enrichment of nitrogen availability in many ecosystems. Meanwhile, soil erosion is depleting phosphorus resources critical to agricultural production and P-limited ecosystems such as the humid tropics. Thus, the stoichiometric balance of ecosystems is being altered with potential implications on ecosystem functioning such as decomposition and soil carbon sequestration. The physiological stoichiometric demands of microbes affect their carbon use efficiency as well as the potential soil organic matter priming effects of litter decomposition.

Here we will present results from a laboratory incubation where we incubated nutrient poor soil from the tropics with additions of +N, +NK, +K, +PK, +NPK or no addition along with ^{13}C and ^{15}N enriched leaf litter. We measured ^{12}C- and ^{13}C-CO_2 fluxes throughout the incubation to partitioned CO_2 fluxes from litter or soil organic matter decomposition. At the end of the incubation we measured soil extractable C, N and P as well as microbial biomass C, N, and P. We quantified litter and soil contributions to microbial biomass C and different microbial groups using phospholipid fatty acid stable isotope probing.

We found nutrient effects on the magnitude of soil organic matter priming due to litter, and slight effects on overall C lost as CO_2 from litter decomposition. Microbial biomass stoichiometry and the implications for litter mass loss partitioning into CO_2 fluxes vs. microbial biomass and dissolved organic matter will also be discussed. Our studies provides a mechanistic understanding of the direct effects of multiple nutrients and stoichiometric balances on microbial functioning and C fluxes due to litter and soil organic matter decomposition.

Bidirectional fluxes in microbial metabolism: The next step towards quantitative soil fluxomics

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Biogeochemists' view on the C transformation of microorganisms in soil has rarely exceed a strongly simplified concept assuming that C gets either oxidized to CO_2 via the microbial catabolism or incorporated into biomass via the microbial anabolism. Biochemists, however, thoroughly identified in the past decades the individual reactions of glycolysis, pentose-phosphate pathway and citric acid cycle underlying the microbial catabolism. At various points within that metabolic network the anabolic fluxes feeding biomass formation branch off. Recent studies on metabolic flux tracing by position-specific labeling allowed tracing these C transformations in soils in situ (Dijkstra et al., 2011a; Dippold and Kuzyakov, 2013). Metabolic flux modeling complements this approach by quantifying the underlying fluxes (Dijkstra et al., 2011b). However, up to now these approaches are based on fluxes from more reduced C sources and assume a succesive oxidation of these compounds during their metabolization. Although pathways transfering C from the oxidative citric acid cycle back into the basic metabolic network, even up to the pool of hexoses are fully described in biochemistry, such pathways, mainly the gluconeogenesis, did not enter soil biogeochemical considerations, yet.

Combining position-specific labeling with compound-specific isotope analysis revealed that such energy intensive pathways, reducing C for anabolic purposes, exist in soil in parallel to the oxidative catabolism. Position-specific labeling of precursors and quantification of isotopic enrichment in the product (i.e. glucose and glucoseamine) revealed that 55% of the incorporated glucose C was not used intactely but first entered via glycolysis the oxidative pathways and was then allocated back via gluconeogenesis to the glucose pool. Furthermore, recovery of ^{13}C from glutamate in fatty acids demonstrated a similar upcycling of C from the citric acid cycle to the fatty acid anabolism. Position-specific labeling revealed that this glutamate C is metabolized via an anaplerotic pathway. This demonstrates that such upcycling pathways are switched on in those organisms suffering from C starvation, which are forced to refill their anabolism from unfavorable substrates (Apostel et al., 2013). Such bidirectional fluxes are unlikely to occur in pure cultures, where constant growth conditions allow a straight unidirectional regulation of C metabolism. However, spatial heterogenity on smallest scales, unstable environmental conditions and interactions between a still unknown diversity of microorganisms in the soil are likely to induce the observed pattern of bidirectional C fluxes through the microbial metabolism in soil.

This fundamentally revised view on soil microbial C metabolism requires adaptation of the existing quantitative flux models. Based on incorporation of single C positions from metablic tracers into CO_2, such models are currently able to trace the whole microbial communities' C transformation. The ratio of anabolic to catabolic fluxes provides the carbon use efficiency (CUE), which represents a parameter for energy and C efficiency of the investigated microbial community. Including bidirectional fluxes will allow for calculating furthermore an upcycling factor, which will serve as an indicator for microbial starvation, representing the necessity to use unfavorable oxidized C sources to maintain C supply for biomass production. Consequently, these results allow for adaptation of existing approaches of microbial fluxomics towards consideration of the heterogenous environmental conditions soil microbial communities are exposed to. Novel flux-based parameters derived from that approach will allow for the evaluation of C supply of microbial communities in soils in situ.

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Abstracts of Posters

The separation characteristics study for intermediate components

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Using a quasi-idea cascade as a model, the separation characteristics for intermediate component in multi-component isotope in single step separating is analyzed by solving system of equations in this paper. According to the analysis, the abundance of the target component has an extremum in single step separating process for separation of multi-component isotope. When the cascade is enough long, the abundance of the target component is related to the feed abundance and is independent on other parameters.

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Optimization of the chromium-based high-temperature conversion (Cr/HTC) technique for on-line hydrogen isotope analysis in halogen- and sulfur-bearing organics

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The stable isotope analysis of hydrogen in halogen- and sulfur-bearing organics using high-temperature conversion (HTC) has often produced erroneous results in the past. The formation of reaction products other than molecular hydrogen (H_2) has been shown as a cause of incomplete H_2 yield and hydrogen isotopic fractionation [1, 2]. In our previous description of the chromium-based Elemental Analyzer – Isotope Ratio Mass Spectrometer (EA-Cr/HTC-IRMS) technique the primary focus was on nitrogen-bearing compounds [1, 3]. Chromium-based approach was extended to a compound-specific application using Gas-Chromatography – Isotope Ratio Mass Spectrometry (GC-Cr/HTC-IRMS) [2]. Several issues with halogen-bearing samples, however, remained unresolved and required a more detailed evaluation and improvement of the reactor system.

The chromium reactor was significantly modified and optimized for the conversion of halogen- and sulfur-bearing samples. The conversion performance of the revised setup was evaluated using a dual-detection system of simultaneously operating IRMS and MS. Quantitative conversion and the absence of unfavorable by-products could be demonstrated for F-, Cl-, Br-, I- and S-bearing compounds. The accuracy of the hydrogen isotope analysis was validated in different laboratories using the online EA-Cr/HTC-IRMS, GC-Cr/HTC-IRMS and offline dual-inlet IRMS. Our results suggest good precision and inter-laboratory reproducibility of hydrogen isotope analysis for selected organics. The optimized Cr/HTC reactor setup for EA and GC interfaces can be implemented in existing analytical equipment using commercially available reactor materials. The sensitivity and simplicity of the chromium-based HTC approach provides a promising routine tool for hydrogen isotope analysis for a wide range of hetero-element bearing compounds.

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A comparison of methods for triple isotopocule N_2 analysis

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For the determination of N_2 isotopic composition, usually only masses m/z 28 and 29 are used, because m/z 30 determination is impeded by NO, which is formed in the mass spectrometer source during the measurements. The calculation of $^{15}N/^{14}N$ isotope ratios from these data is possible as long as the measured N_2 is in isotopic equilibrium (i.e. the isotopes are distributed statistically among all molecule masses m/z 28, 29 and 30).

When measuring a mix of N_2 from two different sources with different isotopic abundances, this simple approach is no longer possible because the isotopes are not distributed statistically among the molecules in the mixture.

Two different approaches have been used to determine the triple isotopocule distribution of N_2:

1) Measuring all three masses m/z 28, 29 and 30 while taking great care to reduce contribution of NO formation on m/z 30 (Lewicka-Szczebak et al. 2013)
2) Measuring the masses m/z 28 and 29 before and after establishing statistical distribution of the isotopes among all molecules (Well et al. 1998). The statistical distribution is accomplished by disintegrating N_2 molecules and subsequent instantaneous reformation of N_2. Isotopes then are statistically distributed among the molecules. A set-up was developed, where measurements with and without equilibration are performed from the same sample in one run.

Here we present a comparison of the two methods with regard to handling and accuracy of results.

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High resolution and precision sulfur isotope ($\delta^{34}S$, $\delta^{33}S$ and $\Delta^{33}S$) analysis by UV-femtosecond laser ablation with isotope-ratio-mass spectrometry

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The spatial resolution of sulfur isotope ratios in sulhides provides a new knowledge into geological processes that cannot be reached using traditional bulk analytical techniques (Ono et al., 2006) High precision $\delta^{34}S$ and $\delta^{33}S$ data are needed in to constrain of atmospheric processes, to studies genesis of ore deposits and early life (Thiemens, 2006). We present a new fs-laser technique that accepted advantage of continuous He flow gas chromatography – isotope-ratio-mass spectrometry and to achieve precise analysis of stable sulfur isotopes (^{32}S, ^{33}S, and ^{34}S) at $n \times 10^{-9}$ mol level of SF_6. Our system consisted of a commercially available laser ablation system (NWR Femtosecond UC) and includes ablation chamber, reactor, cryogenic trap, cryofocusing loop, gas chromatograph, modified open split and mass spectrometer MAT-253. We used a 50 – 60 µm laser spot diameter with varied number of pulses delivered at 100Hz for sample ablation. With ablation, removal of material from the sample surface, forming clusters of nanometer-sized sprayed into helium. From the chamber nanoparticles are injected into the reactor in helium flow. SF_6 is produced inside nickel tube reactor at a temperature 350 ^{0}C with vapor of BrF_5 in He flow. This system allows you to simultaneously place in a chamber collection of samples and standards. The proposed system advantageously differs from the previously used laser systems. Exit of reactor is connected to cryogenic traps for SF_6 treatment. Final purification of the sulfur hexafluoride is carried out on chromatographic capillary column (Q-PLOT OD 0.32, 25 m long) at 60 ^{0}C and flow of helium-3 cm^3/min. Pure SF_6 are condensed in the loop at temperature of liquid nitrogen. After condensation of SF_6 on the loop, the open split is closed and the helium is pumped out to high vacuum. Loop are heated, and the released sulphur hexafluoride are injected into the ion source of the mass spectrometer MAT-253. Mass spectrometer is equipped with a multicollector system for simultaneous measurement of the ion beams at masses 127 ($^{32}SF_5^+$) 128 ($^{33}SF_5^+$) и 129 ($^{34}SF_5^+$). The results obtained for reference materials Ag_2S (IAEA-S1, S2, S3), elemental sulfur(IAEA-S4), sphalerite (NBS-123), and natural sulfides (pyrite, chalcopyrite, sphalerite and galena) show excellent agreement with the accepted values. The isotope composition results for the standards and natural sulfides demonstrate the accuracy and reproducibility of our method $\delta^{34}S \pm 0.15‰$ and $\Delta^{33}S \pm 0.05‰$ (2σ).

One of the most important applications of our technique will be to analyse sulfides hosted in Archean rocks that can provide information relevant to the genesis ore deposits. Our method may be useful of local analysing of sulfur isotope composition in many geological and cosmochemical materials.

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An analytical system for oxygen isotope analysis of H_2O_2 using continuous-flow isotope-ratio mass spectrometry

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An analytical system has been developed to determine $\delta^{17}O$ and $\delta^{18}O$ values in hydrogen peroxide (H_2O_2) dissolved in water at various concentrations. This system can be used for measurement of $\delta^{17}O$ and $\delta^{18}O$ of H_2O_2 in natural water, e.g. atmospheric hydrogen peroxide dissolved in rainwater. Atmospheric hydrogen peroxide is a major atmospheric oxidant which is mas-independent fractionated (Savarino & Thiemens 1999). Concentration of hydrogen peroxide in the atmosphere is very small that is a consequence of H_2O_2 high reactivity. Hydrogen peroxide efficiently oxidizes a number of trace gases in cloud droplets including oxidation of sulfur dioxide to sulfuric acid, and it is also involved in Fenton and photolysis processes. A key role of oxidants in tropospheric chemistry is understood as a regulator of atmospheric composition. In order to develop a better understanding of peroxide chemical transformations in the atmosphere, the oxygen isotopic composition $\delta^{17}O$ and $\delta^{18}O$ of H_2O_2 in rain water was perfomed by Savarino & Thiemens (1999). These authors have reported an experimental technique to determine both $\delta^{17}O$ and $\delta^{18}O$ of H_2O_2 in natural water. Oxygen isotope analysis was carried out with offline extraction system, which required large sample sizes of the order of 4 L, and the extraction and purification procedures were time-consuming. We reported here an analytical system which used continuous-flow techniques to measure $\delta^{17}O$ and $\delta^{18}O$ values that requires a smaller sample volume. A single measurement can be performed in less than 15 min. The design of our developed extraction and purification system was optimized to operate with samples of H_2O_2 solutions (typically sample size of 0.3 mL) which contain the H_2O_2 in the amount of at least 0.5 µmol, approximately. An extraction and purification procedure which was used in our system, followed the same principal steps as that described by Savarino and Thiemens (1999) to obtain O_2 from H_2O_2. A sample of H_2O_2 water solution was acidified with sulfuric acid at pH~1 and was degassed by three cycles of freezing-pumping. Potassium permanganate was added to this aqueous solution to oxidize H_2O_2 to O_2. The extracted O_2 was passed through the cold trap (-196°C) and then completely collected in the 13X molecular sieve trap at liquid nitrogen temperature. For isotope measurement, only 1/20 part of the total volume of extracted oxygen was transported to a mass spectrometer. The O_2 to be analysed was filled into a calibrated loop (100µL) on a 6-port /3-channel Valco valve from where the O_2 was transported by He carrier gas into a gas chromatography column (silica capillary column, molecular sieve 5A, length of 30 m, i.d. of 0.32mm, temperature of 40°C) and then to the mass spectrometer MAT 253. The typical reproducibility of the system was obtained to be 0.16‰ and 0.24‰ for $\delta^{17}O$ and $\delta^{18}O$ measurements.

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SPIN-MIMS simplifying the SPIN-MAS instrumentation for online measurement of ^{15}N-abundances of ammonium, nitrite and nitrate in aqueous solutions

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Common methods for measuring selectively the ^{15}N abundances in individual N-species such as NH_4^+, NO_2^- and NO_3^- in samples with multiple N-species are laborious and time consuming.

The SPIN-MAS technique (Stange et al. 2007) offers an automated, rapid and selective determination of ^{15}N abundances in NH_4^+, NO_2^- and NO_3^- in aqueous samples. During a **SPIN-MAS** measurement one of three different reaction solutions is mixed with the aqueous sample in a **S**ample **P**reparation unit for **I**norganic **N**-species (**SPIN**). The reaction solution is chosen in dependence on the N-species of interest. The gaseous reaction products (N_2 or NO) are then conducted to a quadrupole mass spectrometer (**MAS**) in a helium stream. This measurement technique is not commonly used due to its complex instrumentation.

The instrumentation can be significantly simplified by the use of a membrane inlet mass spectrometer (**MIMS**). The presented SPIN-MIMS approach relies on the use of a reaction capillary in which the sample containing the N-species of interest is mixed with the corresponding reaction solution. The mixture of reaction solution and sample is pumped from the reaction capillary directly to the membrane inlet of the mass spectrometer. The reaction products (N_2 or NO) formed during the reaction of NH_4^+, NO_2^- and NO_3^- with the reaction solutions are passed through the gas-permeable membrane of the inlet directly into the ion source of the mass spectrometer.

^{15}N standards with different at% ^{15}N (NH_4^+, NO_2^- and NO_3^- respectively in dist. Water) were used to assess the performance of the system. Overall, SPIN-MIMS measurements showed a good agreement between measured and expected ^{15}N abundances (range 0.36 – 10 at% ^{15}N deviations: <0.5 at% ^{15}N for NH_4^+-, <0.23 for NO_2^-- and <0.15 at% ^{15}N for NO_3^-- standards).

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The precisION. IRMS as a versatile foundation for novel isotopic analysis

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Isoprime now introduce the precisION stable isotope ratio mass spectrometer with the highly versatile IONOS software. This innovative combination delivers useful instrument flexibility to pursue isotope science across the broadest spectrum of research. The ability to acquire up to 10 ion beams simultaneously at a resolution of 110 with large dynamic range 100V amplifiers permits samples to be analysed at the highest level of performance. The instrument and software presents a straightforward user interface whilst offering complete control of every hardware parameter. Automation of tasks, flexibility with interfacing and ease of analytical data output are enabled with the inclusion of the centrION interface and IONOS software.

Direct connection with the fully integrated centrION continuous flow interface maximizes the advantages of the precisION and handles the full range of peripheral inlet systems allowing the user to simply transfer from one analysis type to another seamlessly. The centrION permits up to six monitoring gases and five inlets to be connected to an individual precisION system.

IONOS brings intelligent automation to the stable isotope laboratory with a powerful and flexible platform for control of the entire analytical process. From initial manual or automated system performance monitoring through to data acquisition, IONOS provides a completely new, yet simple experience to the user. With automatic multipoint, multi-isotope calibration stable isotope data processing of complex data sets is simple and robust within a secure auditable environment.

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Interfacing with the full range of peripherals for IRMS, Isoflow, Liquiface, GC5, and the full range of cube elemental analysers and TOC cube the precisION is enabling isotopic research in any direction.

iso FLOW: A cryogen-free inlet for the isotopic analysis of headspace gases from trace to pure concentrations

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Stable isotope applications that call for preconcentration (i.e. greenhouse gas measurements, small carbonate samples, etc.) universally call for cryogenic fluids such as liquid nitrogen, dry ice slurries, or expensive external recirculation chillers. This adds significant complexity, first and foremost in the requirements to store and handle such dangerous materials. A second layer of complexity is the instrument itself – with mechanisms to physically move either coolant around the trap, or move a trap in or out of the coolant. Not to mention design requirements for hardware that can safely isolate the fluid from other sensitive areas. In an effort to simplify the isotopic analysis of gases requiring preconcentration, we have developed a new separation technology, UltiTrap^{TM} (patent pending), which leverages the proprietary Advanced Purge & Trap (APT) Technology employed in elemental analysers from Elementar Analysensysteme GmbH products. UltiTrap^{TM} has been specially developed as a micro volume, dynamically heated GC separation column. The introduction of solid-state cooling technology enables sub-zero temperatures without cryogenics or refrigerants, eliminates all moving parts, and increases analytical longevity due to no boiling losses of coolant . This new technology makes it possible for the system to be deployed as both a focussing device and as a gas separation device. Initial data on synthetic gas mixtures (CO_2/CH_4/N_2O in air), and real-world applications including long-term room air, solid carbonates and a comparison between carbonated waters of different origins show excellent agreement with previous technologies.

Determination of $\delta^{33}S$ and $\delta^{34}S$ on SO_2 gas using negative ion mass spectrometry (N-IRMS)

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We report on the development of a new type IRMS for simultaneous determination of $\delta^{33}S$ and $\delta^{34}S$. The mass spectrometer is a 60° sector analyzer with dual inlet and triple collector in which the Nier-type ion source is replaced by a high efficiency negative ion source (Hałas et al. 2015). The negative ion species produced from SO_2 gas are: SO^-, S^- and O^-, whereas the most intense ion species produced from SF_6 is SF_5^- (i.e. likewise in the positive mass-spectrum of SF_6). We have also tested H_2S gas, but we noted a strong production of HS^- ion species which excludes possibility of ^{33}S analysis.

The high efficiency negative ion source will be described in detail elsewhere (Pieńkos and Hałas, in preparation). Briefly, it can be made from the Nier type ion source by the emplacement of the electrons emitting filament to the position of electron beam in the ionization chamber. By adjustment strength of the external magnetic field and appropriate polarization of the filament and the ion repeller with respect to the ionization chamber, it is possible to obtain a strong negative ion beam the intensity of which is comparable to that obtained by common IRMS operated on positive ions.

The novelty of our analytical method is the use of SO_2 gas for simultaneous $\delta^{33}S$ and $\delta^{34}S$ determination in natural samples what is possible directly on the ion beams $^{32}S^-$, $^{33}S^-$ and $^{34}S^-$ without any corrections for interfering ions. So far such analysis was only possible with the use of sulfur hexafluoride on positive mass spectrum of: $F^{32}S_5^+$, $F^{33}S_5^+$ and $F^{34}S_5^+$ at masses 127, 128 and 129, respectively (fluorine has only stable isotope ^{19}F). However the use of SF_6 gas requires a fluorination line for its preparation and a higher resolution of mass spectrometer. In contrast to SF_6, the SO_2 gas can be easily prepared from both sulfides (Robinson and Kusakabe 1975) and sulfates (Halas and Szaran 1999).

Initial tests of delta measurements indicate a high possible precision, better than ±0.1‰. This may lead to the detection of minor anomalies in sulfur isotope composition ($\Delta^{33}S = \delta^{33}S - 0.52\delta^{34}S$) which may exist in geologically young minerals, particularly those formed during big $\delta^{34}S$ sulfate excursions of ocean in the Phanerozoic (Claypool et al. 1980). Studies of such samples are in progress.

This study is supported by National Science Center (Polish) according to the decision no. DEC-2013/11/B/ST10/00250. We are grateful to Dr. Keith Hackley from Illinois State Geological Survey for his kind donation and assistance in the shipment of the old Nuclide mass spectrometer, from which we use the electromagnet with its excellent movement assembly.

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The high accuracy of stable isotope analysis of chlorine

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The most common method used in chlorine isotope ratio analysis employs the conversion of a chlorine sample to chloromethane (CH_3Cl) prior to the mass spectrometric analysis.
For CH_3Cl preparation the exchange reaction between the iodomethane (CH_3I) and silver chloride (AgCl) is applied (Eggenkamp 2004). This reaction requires at least 10-fold molar excess of CH_3I to AgCl, which makes difficult purification procedure of CH_3Cl from CH_3I due to their similarity in physical properties. So far two purification methods of chloromethane are used: (i) gas chromatography and (ii) cryogenic separation. In both methods CH_3I has been admitted to the preparation line by the injection through a septum and by this operation also some impurities (air, water vapour) are contributed to the sample. Moreover in above mentioned methods sealing and cracking operations of the tubes with sample are needed.

Therefore to eliminate these drawbacks the new triple-stage cryogenic system with two different cryogenic substances was constructed, similar to that described in ref. (Wu & Satake 2006). The most important novelty in our preparation line is a new and simple pipette system, comprising of two glass containers separated by two stopcocks with Teflon plug, for CH_3I introduction to the cryogenic separation line. Additionally we considerably simplified separation procedure of both chloro- and iodomethanes by replacement break-seal tubes by ampoules having stopcocks with Teflon plug. Such modification of the chloromethane purification method allowed shortening of the time needed for completion of the CH_3Cl separation.

The described improvements of the chloromethane preparation together with the applying of the recently developed method of chlorine isotope ratio analysis based on the negative ion mass spectrometry [Pelc & Hałas 2008, in preparation; Hałas & Pelc 2009) leads to enhanced reproducibility of the measurements. The attained uncertainty of single $\delta^{37}Cl$ determination was reduced to ±0.05‰, which is crucial in most of chlorine isotope studies. Examples of such accurate results will be presented.

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Storage in polyethylen bottles and the multi isotope composition of water: The impact of time and temperature

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Storage of natural water samples in polyethylen vials for later hydrogeochemical analysis is a common practice in laboratories world-wide. It is, however, known for a long time to geochemists that aqueous solution loose water as a function of time due to the diffusion of water molecules (similar to CO_2 or H_2S) through the polymer membrane, a process that is suspected to increase with rising temperature. First observations on the impact of storage on $\delta^{18}O$ and δ^2H data were reported by Spangenberg & Vennemann (2008) and Spangenberg (2012), but no study considered systematically the effect of temperature, sofar.

In the present study we carried out long-term experiments and stored deonized water for up to 18 months at 4 different temperatures (4, 10, 23 and 60°C). The loss of water was followed gravimetrically, and the stable isotope composition of the water sample was analyzed with a Picarro CRDS 2140-i system.

Whereas, at low temperatures of 4 and 10°C, no loss of water was observed during a storage time in LDPE up to 1.5 years at room temperature and 60°C a substantial loss of water was found. This was associated with, for instance at 60°C: an increase in $\delta^{18}O$ (up to 10 ‰), δ^2H (up to 28 ‰), $\delta^{17}O$ (up to 5 ‰). The deuterium excess was shifted to lower values by up to 55 ‰). The magnitude of the isotope effects mostly depend on the extend of evaporation from the bottles, allowing for an estimate of the fractionation factor by considering a closed Rayleigh-type system.

A comparison of traditional EA-IRMS and nano-EA-IRMS – a case study

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Combining an elemental analyzer (EA) with an isotope ratio mass spectrometer (IRMS) has been common practice for many decades. Few results have been reported using a cryotrap to reduce the sample size required in an EA-IRMS system by increasing the amount of sample transferred to the ion source of the IRMS (Fry et al, 1996, Polissar et al, 2009). We present a case study performed using a modified Nano-EA from NU-Instruments (cryotrap setup) coupled to an EA-IRMS used to evaluate nano- and micro- molar quantities of particulate organic matter (POM) and zooplankton (*Limacina*) collected from field sites and algae (*Spirulina/Fucus Vesiculosus)* purchased as a composition standard and compare the results to traditional EA-IRMS analysis.

Sample analysis was performed using a Nu Instruments Nano cryotrap connected to a EuroVector EA3028 elemental analyzer and a Nu Horizon isotope ratio mass spectrometer. Samples were dried overnight in an 80°C oven prior to being packaged in acetone-washed tin capsules. Samples were combusted at 1030°C in the presence of chromium (III) oxide and silvered cobalt (II, III) oxide prior to reduction at 650°C by reduced copper wires. The resulting CO_2 and N_2 gasses are either trapped by a cryogenic trap at high flow and released from the trap at low flow (low split) before being admitted to the IRMS for determination, or sent directly to the IRMS at high flow (high split). The resulting data is subjected to a scale correction as well as a blank correction.

Both POM trapped on a glass fibre filter and juvenile zooplankton proved difficult to study on the EA-IRMS system due to difficulty collecting sufficient quantities, so data is limited for some samples. There were no such limitations for *Spirulina* and *Fucus Vesiculosus*, so a complete data set is presented for those. A comparison of isotopic and composition values is presented, along with a discussion on homogeneity and differences in accuracy and precision between EA-IRMS and Nano-EA-IRMS. In general, NANO-EA-IRMS and EA-IRMS values are comparable when amounts analyzed are in the linear response range of the IRMS, with N response being more sensitive to sample mass than C and evidencing a smaller linear response range.

We conclude that it is possible to use a Nano-EA-IRMS system with minimal modifications to an EA-IRMS system to lower the sample size necessary and that the results are comparable to EA-IRMS results. The system we present is shown to be suitable for routine analyses of samples with in excess of 1 µg C and N content.

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On-line separation of strontium or magnesium from matrix and determination of $^{87}Sr/^{86}Sr$ or $^{26}Mg/^{24}Mg$ ratio by Ion Chromatograph/Multicollector-ICPMS

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In this work a high throughput, robust and sensitive method for the precise isotopic analysis of $^{87}Sr/^{86}Sr$ or $^{26}Mg/^{24}Mg$ by coupling Ion Chromatography (IC) and Multicollector Inductively Coupled Plasma Mass Spectrometry (MC-ICPMS) is presented. The effective separation of Sr or Mg from matrix by IC enables *on-line* isotopic analysis of $^{87}Sr/^{86}Sr$ or $^{26}Mg/^{24}Mg$ in transient signal by MC-ICPMS, without the need in a laborious *off-line*, time consuming sample preparation and clean room facilities. Mass discrimination and instrument drift were corrected for Sr by the use of the natural constant $^{86}Sr/^{88}Sr$ ratio as an internal standard and for Mg using "bracketing technique". The precision (2 σ) of the $^{87}Sr/^{86}Sr$ ratio of 0.003% was achieved in natural fresh water samples and water with the heavy matrix, e.g. Dead Sea sample, as well as carbonates and silicates rocks and for $^{26}Mg/^{24}Mg$ precision.

Robustness, relatively high precision and accuracy, as well as minimum possibility of sample contamination of the developed analytical method have been demonstrated in complex natural samples of water and rocks as well. This method for the first time reports a possibility of isotopic analysis of cations by *on-line* IC separation with precision close to this obtained by *off-line* technique.

Analysis of sugar, amino acid, and caffeine ^{13}C by using liquid chromatography/isotope ratio mass spectrometry

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In 2004, liquid chromatography coupled with isotope ratio mass spectrometry (LC/IRMS) has been developed1, which allows the measurement of stable carbon isotope ratio ($\delta^{13}C$) composition of highly polar and non-volatile analyses without the need for derivatization. The interface is based on a wet oxidation of organic compounds in aqueous solution to produce CO_2 gas, and separate CO_2 from the liquid phase. Today, LC/IRMS system has been used for various compounds e.g., sugar, amino acid, alcohol and pesticide. Stable isotope analysis has proved to be a powerful tool for source apportionment of various compounds. However, the research of precision and accuracy for LC/IRMS were very limited until now. In addition, the effect of including nitrogen has not been investigated.

We analyzed $\delta^{13}C$ of sugars, amino acid and caffeine samples using direct injection mode and online LC/IRMS mode. Firstly, accuracy and precision in this method were investigated in the concentration range of L-alanine and L-histidine from 200 to 6,500ngC and 110 to 7,000ngC, respectively. Secondly, we measured sugar, amino acid and caffeine samples using direct injection mode. We compared direct injection mode with EA/IRMS. Finally, we measured amino acid and caffeine using online LC/IRMS mode. This study presents a new application using common anion exchange column to the determination of $\delta^{13}C$ of amino acid and caffeine samples.

For direct injection, the determination coefficient was 1.00 (sugar and amino acid). In addition, the standard deviation of all samples were <0.26‰. The difference between EA/IRMS and direct injection mode was lighter, 0.41‰ (sugar), 2.01‰ (amino acid), 5.09‰ (caffeine, Wako Pure Chemical Industries), and 7.41‰ (caffeine, IAEA). The reason of the difference seemed to be incomplete of nitrogen. The amino acid and caffeine samples have nitrogen from 11.0 to 29.0 % of total molar weight.

When 1% phosphoric acid was not added, the differences between the EA/IRMS value and online LC/IRMS were from 2.54‰ to 9.93‰. In the case of online LC/IRMS mode, the eluent for LC was used 5mM Sodium tetraborate decahydrate (pH=9.51). The 1% phosphoric acid was not added, the oxidation potential of sodium peroxodisulfate was decreasing. In contrast, 1% phosphoric acid was added, the differences between the EA/IRMS value and online LC/IRMS value was from 0.09‰ to 2.09‰. Thus, when the eluent of alkalinity eluent was used, phosphoric acid should keep acidic liquid. However, the difference of caffeine between EA/IRMS and online LC/IRMS were approximately 8‰ regardless with or without phosphoric acid. Thus, the reason of caffeine seemed to be rate of oxidation reaction rather than with or without phosphoric acid.

Online Carbon Isotope Dilution-Isotope Ratio Mass Spectrometry for Absolute Quantifiacation of Fatty Acid Methyl Esters in biodiesel

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Online combustion reactions used in compound-specific Isotope Ratio Mass Spectrometry (IRMS) are capable of transforming all organic compounds eluted from Gas Chromatography (GC) system into carbon dioxide. Recently, this capability of GC-IRMS instruments has been used for compound-independent calibration of C-containing compounds using online Carbon Isotope Dilution. Firstly introduced in 2009, this new concept allows the generic quantification of any organic compound present in a sample without the need of individual standards, obtaining results that are directly traceable to the International System of Units (SI).

In a first step, in order to apply the above mentioned procedure, some simple instrumental modifications of the system were done. Compounds eluted from the gas chromatographic column were online mixed with the continuously added isotopically enriched $^{13}CO_2$. This addition was carried out through the splitter holder located inside the chromatographic oven of the instrument. Online combustion reaction ensured quantitative conversion of every organic compound into carbon dioxide reaching the required isotopic equilibrium between the organic compounds eluting the column and the $^{13}CO_2$ used as tracer before the ionization step. The online measurement of the $^{12}CO_2/^{13}CO_2$ in the mass spectrometer led to quantitative data without resorting to compound-specific standards. In this case, the same signal amplification was used in both Faraday cups at m/z 44 and 45 in contrast to the conventional mode of operation of GC-IRMS instruments. Interestingly, the complete removal of the 13C-enriched tracer required from the instrument after the isotope dilution experiments could be easily achieved. In fact, the transition of the instrument from the online isotope dilution mode to the standard IRMS mode can be achieved overnight. In a second step,, the analytical development was focused on one important limitation to this online carbon IDMS procedure: any loss of substance occurring before isotopic equilibration is not corrected for. So, online carbon isotope dilution applications have been developed when a direct analysis of the sample can be implemented or suitable recovery correction procedures can be employed. Therefore, a careful control of all possible sources of isotopic fractionation, especially the injection step, is essential.

Biodiesel is a new clean-burning alternative fuel defined as mono-alkyl esters of long chain fatty acids derived from vegetable oils, animal fats or microbial oils (algae, bacteria and fungi). The determination of FAMEs (Fatty Acids Methyl Esters) profile content is a key parameter for the characterization of biodiesel. In this work we present the results obtained for both quantification of FAMEs in pure biodiesel samples by online Carbon Isotope Dilution, and carbon delta determination using the same GC-IRMS instrument. Both parameters are crucial and complementary, not only for the evaluation of physical-chemical properties of biodiesel samples but also for origin determination and fraud detection. As biodiesel is a simple matrix, only sample dilution was required before its injection into the system.

Furthermore, the proposed quantification methodology was also evaluated in comparison with EN 14103 procedure based on GC-FID analysis, and the use of alternative internal standards was also studied.

Optimization of stable isotope analysis of dissolved inorganic carbon by continuous flow isotope ratio mass spectrometry

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The isotopic composition of dissolved inorganic carbon (DIC) in natural waters is of particular interest in geological, biological, and environmental studies. Today, the analysis of the stable carbon isotope ratio ($\delta^{13}C$) by continuous flow isotope ratio mass spectrometry (CF-IRMS) is a routine analytical technique. However, a recent inter-laboratory comparison showed that variations of analytical results were larger than expected for a routine technique (van Geldern et al., 2013). To date, many aspects with respect to DIC samples have not been thoroughly evaluated and still lack a standard operational procedure. This starts with field sampling protocols, the selection of suitable sample vials, and analytical issues for very low DIC concentrations.

Here we present a study that explores many of the aspects that eventually influence the final analytical results. Starting with field sampling procedures, we evaluated the effect of filtration and the suitability of various vial-septa combinations. For the latter, the influence of storage time on the $\delta^{13}C$ value was investigated. Subsequently, laboratory analytical issues such as the preparation of DIC-free water for the preparation of in-house reference materials and reaction times were systematically evaluated. Low DIC concentrations of <50 µmol L^{-1} are especially challenging for IRMS analysis. Such low concentrations require large sample volumes to attain the minimum required signal height. From theoretical considerations large sample volumes should also cause an isotope partitioning effect that influences the $\delta^{13}C$-DIC value. We present and evaluate different approaches to extend the limit of stable isotope analyses for samples with ultra-low DIC concentrations.

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IsoMemo.com – a database of isotopic data for archaeology, ecology and environmental science

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Isotopic tracers are used across a variety of research fields to provide a rich diversity of scientific information, but lack of centralized storage limits efficient use os isotopic data. To overcome this limitation, we are currently developing the isotope database IsoMemo.com in collaboration with a number of leading isotope and radiocarbon laboratories.

The two main goals of IsoMemo are i) to archive isotopic data from past and modern contexts, and ii) to provide a user-friendly graphical interface allowing for the simple and fast access to required data. This database is intended as a collaborative platform for researchers to contribute and extract data for advancing archaeological, ecological and environmental studies.

IsoMemo will serve isotope data producers and users from multiple research disciplines with vastly different isotope tracers, research questions, measurement techniques, sample types, environmental settings, time period of interest, and availability of complementary sources of data.

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A novel tool for natural abundance stable nitrogen analysis in in aqueous samples

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The bulk stable isotope analysis (BSIA) of dissolved matter (e.g. dissolved organic carbon, total nitrogen bound (TN_b), etc.) is of particular importance since this pool is a prime conduit in the cycling of N and C. Studying the two elemental pools is of importance, as the transformation and transport processes of N and C are inextricably linked in all biologically mediated systems. No system able to analyze natural abundance stable carbon and nitrogen isotope composition of dissolved nitrogen directly (without offline sample preparation) and simultaneously has been reported so far. Extension of the high temperature combustion (HTC) total organic carbon (TOC) analyzer, to the ability to measure TN_b stable nitrogen isotope composition is described in this study.

To extent the TOC analyzer to the ability to measure TN_b, modifications from the HTC high performance liquid chromatography/isotope ratio mass spectrometry (HPLC/IRMS) interface were implemented and expanded. Reduction reactor for conversion of NO_x to N_2 was implemented into the new developed system. Extension addresses mainly the development of the focusing unit for nitrogen and a degassing device for online separation of TNb from in the sample solved N_2 prior to injection.

The proof of principle of the system with different compound solutions succeeded. In this initial testing, $\delta^{15}N_{AIR-N2}$ values of tested compounds were determined with precision and trueness of typically ≤0.5‰. Further tests aimed at the working range investigation. Good results (U ≤ 0.5‰) could be achieved down to a TN_b concentration of 40 mgN/L and sufficient results (U ≤ 1.0‰) down to 5 mgN/L. Additionally the development resulted in the first system reported to be suitable for simultaneous $\delta^{13}C$ and $\delta^{15}N$ direct BSIA of aqueous samples.

The expansion of a TOC analyzer, specially designed for coupling with isotope ratio mass spectrometry (IRMS) to the ability to measure TN_b resulted in the first system reported to be suitable for both $\delta^{13}C$ and $\delta^{15}N$ direct BSIA in aqueous samples. This system could open up new possibilities in SIA based research fields.

Century long assessment of herbaceous plants' physiological responses to climate change in Switzerland

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The isotopic analysis of archived plant material offers the exceptional opportunity to reconstruct the physiological activity of plants over long time periods and thus, to assess plant responses to environmental changes during the last centuries. In addition, the stable isotope analysis of herbarium samples offers the opportunity to reconstruct the physiological processes of a large range of different plant species and from different environments. Interestingly, only few studies have to date assessed these archives.

We will present a novel analysis of leaf nitrogen and carbon isotope ratios of more than a thousand herbarium specimen from the unique herbaria hold at the University of Basel. The objective of our study was to assess century-long physiological responses of herbaceous plant species and to test if these responses differ in different plant functional groups and in plants that live in different habitats across Switzerland. The goal of our study was to determine with our investigations the long-term responses of plants to climate change. Such investigations are important as they allow to assess long-term processes of acclimation and adaptation in plants to global environmental change.

In our study we found that the N isotopic composition of sedges and grasses showed contrasting trends during the last 150 years. Sedges (*Carex* species) showed a decreasing N isotopic composition with time, in contrast to grasses that did not show any change with time in their N isotopic composition. We interpret the N isotopic composition of plants as indications of the available nitrogen source for the plant. This is in particular with respect to N deposition that exceeds critical loads (10–20 kg N/ha/year) in 90% of the Swiss forest area, including the Swiss Plateau. N deposition can have an effect on plant communities' composition as it can promote growth of certain species, but also cause nutrient imbalances (shortage of P) in other species. Our N isotope data suggest that sedges appeared to be more effective than grasses in their ability to use this new anthropogenic source of N that is being deposited. Grasses were not able to get advantage of this new source of N to such an extent as sedges, probably due to other nutrient imbalances. In addition, plants from contrasting altitudes showed contrasting responses as well: sedges from alpine environments, where N deposition is below the critical threshold, did not show any trend in their N isotopic composition. In the long term, the growth and physiological performance of sedges may be favored by N deposition and their dominance could increase at expenses of grasses and forbs in grassland communities. Our findings have implications for understanding plant physiological responses to N deposition in various habitats and thus to asses the long term consequences of environmental change on ecosystem biogeochemistry.

Use of isotopic discrimination ($\delta^{13}C$ and $\delta^{15}N$) for screening drought tolerance of grassland species

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As a consequence of Global Changes, perennial pastures have to face more and more extreme climatic conditions such as longer drought periods which impact forage production and, as a consequence, reduce the sustainability of livestock systems.

In that context it is important to evaluate the capacity of grassland species to tolerate water shortage. This point is of major interest for *Fabaceae*, the most frequent family after *Poaceae* in grassland ecosystems, since they allow application of N fertilizer to be reduced while maintaining acceptable production levels.

In that purpose, we set 2 experiments which aimed at screening the drought tolerance of grassland species (i) in 2012 we grew, on soil columns, 14 *Fabaceae* and 6 grasses under controlled environment in a greenhouse under four growth conditions, by crossing two levels of both Phosphorus and water availability; (ii) in 2013, 24 *Fabaceae* and 4 *Poaceae* were seeded in a common garden on 2 m^2 plots and grown under full water availability; in both experiments, species were randomly distributed in 4 repetitions blocks.

Stable isotopes as $\delta^{13}C$ values are good indicators for drought tolerance which could bring information to select plant species in function of their water use efficiency, productivity and photosynthetic yield. As we screen *Fabaceae* species, the $\delta^{15}N$ values have been used to monitor their productivity and nutrient origin.

The first results in the green-house on the forage legumes, shows a significant impact of the growth conditions on the N% and $\delta^{15}N$ (mean values from -1.15 to -0.36 ‰), but no on the grass specie (mean value range from $\delta^{15}N$ = +3.12 to +3.62 ‰). Water was a higher limitation factor compared to Phosphorus. This data will be discussed with available functional traits (as SRL, SLA, TMS) measured during the experiment and the calculated %Ndfa (N_2-fixation).

On the field side, the surprise was that same Mediterranean species, like *Anthyllis montana, Astragalus monspessulanus* and *Coronilla minina,* present highly positive $\delta^{15}N$ values (from +5.54 to +9.24 ‰). This result was find for both 2014 and 2015. These plants also displayed the higher C/N values (>15).

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Determining the geographical origin of Asian longhorn beetle (*Anoplophora glabripennis*) specimens

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An outbreak of EU quarantine listed pest *Anoplophora glabripennis* (Asian Longhorn Beetle = ALB) in Kent (UK) resulted in environmentally and financially costly eradication action being taken. Monitoring at the outbreak site continues but this pest is also, on occasion, entering the UK with imported materials. In this study the potential of using multi-element stable isotope or trace element (TE) analyses to determine the geographical origin of individual specimens has been investigated.

The isotope ratios of ALBs for hydrogen varied within and across 5 locations. For carbon, the isotope ratios varied between -20.72 and -25.78 ‰, which fell within the expected values for C3 plants (trees using the photosynthetic pathway common for moderate climates). For nitrogen, isotope ratios indicated separation of European from American beetles; whilst sulfur isotope ratios distinguished ALBs from New York against the other 4 locations. Three - manganese, arsenic and strontium - out of 19 selected TEs separated UK laboratory-reared beetles from American beetles (Ohio and New York) with ~ 68% confidence.

Stable isotope and trace element analyses show potential to differentiate between newly arrived ALB individuals and those from previously undetected in-country populations, which would be of immediate practical benefit in making appropriate strategic decisions on surveillance and eradication. Analyses of additional samples (i) from the same populations, (ii) different locations and (iii) variety of host trees will enhance the overall picture.

Further experiments, e.g. effect of solvent storage on specimens and monitoring isotopic signature from tree into larvae stages, will also be presented.

Trophic Ecology of Howler Monkeys – Insights from Stable Isotope Analysis

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The progressive loss of habitat of howler monkeys (*Alouatta spp.*), mainly due to anthropogenic activity, has led to a reduction of their original populations of up to 90%. Habitat disturbance changes the quality of resources according to the size of the forest fragments, so that survival depends on the level of dietary flexibility (Arroyo-Rodríguez & Dias, 2010). It has been observed that these primates are able to modify their trophic niche, thus persisting in small forest fragments (Bicca-Marques 2003). Under this scenario, the study of howler monkeys' feeding habits and use of resources has received special attention from the primatological community, in order to explain the wide distribution of this gender through environments with different levels of perturbation. Until now, most of the current knowledge about their trophic ecology is based on direct observations. However, the effective trophic niche can be quite different from that suggested by direct observations, owing to the inherent limitations of this method, and those of the observer abilities. Nevertheless, in the last decades the description of the isotopic niche measured by stable isotope analysis has been used to explore changes in the trophic niche breadth of wild arboreal primate populations in response to the heterogeneity of the environment. The aim of this study is to explore the trophic variability within and between wild populations of howler monkeys in response to different levels of environmental disturbance where their populations still persist, in order to understand how habitat fragmentation affects the trophic niche breadth of these primates. Analysis of stable isotopes of C and N was performed on hair samples from 204 individuals captured in different rainforests of Mexico in 2008, 2010 and 2012. Isotopic niches of each population are described through Standard Ellipse Areas. No significant differences were found between sexes. However, differences between juveniles and adults were found. Also, there are differences in the isotopic niche at species level; this is especially interesting in sympatric sites. Similarly, seasonal differences (rainy vs. dry) were detected. Overall, it was observed a negative relationship between the values of C and N along populations. The application of stable isotopes is a novel complementary analytical tool to observational studies made so far on the ecology of these primates. Undoubtedly, acquiring more knowledge about the trophic ecology of these monkeys at different scales, both spatial and temporal, will allow a better understanding concerning the impact of human activities on their populations and to identify key points for their conservation.

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Trophic niches within a black coral meadow (*Hexacorallia: Antipatharia*) near the Great Reef of Toliara, Madagascar

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Black corals are deep-sea organisms characterized by a long lifespan, a very slow growth rate and late maturity. Generally, they are difficult to study as more than 75% of species are found below 100 meters. They have very diverse morphologies, from a single stem to a bushy tree shape. These morphological differences have an impact on the polyp morphology, which is linked to the general shape and size of the colony. Mouth and tentacle sizes can be very different between species, and these features can have an effect on the capacity to capture food, either particulate organic matter or large zooplanktonic prey.

Studies about stable isotope ratios of black corals are few and mainly based on deep sea species. Here, for the first time, we study the isotopic composition of carbon, nitrogen and sulphur of a multispecific black coral meadow located in the shallow waters of the northern limit of the Great Reef of Toliara, Madagascar. The objective of this study was to compare diet and isotopic niche of different black coral species in relation to colony morphology and polyp size. Twelve species of black corals were sampled by removing tissues from the skeleton underwater to avoid killing entire colonies. Potential food sources were also collected with different trapping techniques during the day and night to determine their diet. Morphological data about the colony and the polype size were also recorded.

Using statistical comparisons, as well as SIBER and SIAR models, we found that these corals have a certain trophic diversity and gather in 3 different groups, showing a division in the assimilation of their food. Their diet is mainly based on microplankton, mesoplankton and particulate organic matter and not on zoobenthos. As hypothesized, the general morphology of the colony influences food assimilated, as branching corals with very small polyps tend to have a significantly lower value of $\delta^{15}N$. Moreover, these species showed different feeding strategies regarding to a diurnal or nocturnal feeding activity, as values of $\delta^{13}C$ were significantly different for mesoplankton according to the time of their capture. These results are consistent with observations made during dives, as some species were observed with expanded tentacles during nighttime.

Seasonal physiological interpretation of intra-ring δ¹³C variation in temperate species: experimental and modelling approach

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Ring $\delta^{13}C$ is a physiological and climatic archive that offers resolution at the annual and seasonal time scales. In this contribution, we synthesize our results relative to the functional understanding of the intra-ring $\delta^{13}C$ variations. Our experimental approach consists in studying processes that could influence the seasonal $\delta^{13}C$ variations. We focus on two deciduous European species: *Quercus petraea* and *Fagus sylvatica*.

One main result in oak is the identification of a recurrent pattern of the seasonal variations of the $\delta^{13}C$. These variations were mainly related to the mobilization of carbon reserves at the beginning of growth (high $\delta^{13}C$ values) and to the influence of environmental conditions on the photosynthetic discrimination during the rest of the growing season. Latewood $\delta^{13}C$ was correlated with climatic conditions (especially vapor pressure deficit, soil water content). For *Fagus*, there was no apparent reserve effect at least in healthy trees. However, in declining beech trees, $\delta^{13}C$ values tended to be lower (Granda et al. in prep) and an intra-ring $\delta^{13}C$ decrease reveals a difference in starch storage dynamics compared to healthy trees (Michelot et al. in prep).

The $\delta^{13}C$ intra-annual variations of the ring bulk organic matter were little influenced by variations in biochemical composition (in particular, lignin and holocellulose contents, Eglin et al. 2009). Comparisons between water use efficiency (WUE, the ratio of carbon assimilation and stomatal conductance to water) calculated from a leaf carbon isotope discrimination model applied to $\delta^{13}C$ ring data and simulated or measured WUE at the tree or ecosystem levels suggest that latewood $\delta^{13}C$ is a seasonal recorder of WUE with a time lag corresponding to the mixing time of sugars in the phloem (Michelot et al. 2011). Recent results at the inter-annual scale over 8 years indicate that the maximal correlation between WUE deduced from $\delta^{13}C$ and that obtained from C and H_2O flux is obtained with a time lag of 1-20 days. Moreover, intra annual $\delta^{13}C$ patterns exhibited important inter-annual and inter-individual variability which were mainly attributed to inter-annual climate variations and competitive status of the trees respectively.

These experimental data have been used to elaborate a mechanistic model simulating the carbon isotopic balance at tree-scale (Eglin et al. 2010). This model has been coupled with CASTANEA, a model simulating the carbon, water and energy balances at stand-scale, then calibrated and validated on measurements of intra-annual variations of $\delta^{13}C$ within oak rings, sampled in the Fontainebleau-Barbeau (FR-Fon) forest (ICOS network, http://www.barbeau.u-psud.fr/). Results from ISOCASTANEA showed a strong attenuation of the carbon isotopic signal from the leaf to the ring. Key parameters influencing the simulated whole-tree-ring $\delta^{13}C$ were tested through a sensitivity analysis: the budburst date, growth rate and discrimination during respiration are particularly important. From a physiological point of view, the use of inter-annual intra-ring δ^{13}C associated with other variables like anatomy, microdensity could particularly relevant to examine the importance of the functional life history of the trees to explain their vulnerability to climate change.

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Mass-independent oxygen isotope effect as an indicator of anthropogenic and natural sources of hydrogen peroxide

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One of the most important factors affecting the environmental conditions of natural aquatic ecosystems, is the redox potential which characterizes the content of oxidizing agents such as hydrogen peroxide (H_2O_2) in natural water. H_2O_2 is involved in the biogeochemical cycle, in which the processes of natural formation and consumption of H_2O_2 are balanced and control the H_2O_2 content in aqueous system at stationary stage – of the order of 10^{-7} mol/L – physiological optimum for aquatic habitat. Disbalance of redox processes leads to negative environmental impacts. The problem of changing in the oxidation state of the aquatic environment has not been studied completely. On the one hand, increasing the concentration of H_2O_2 in natural waters may occur due to rainwater H_2O_2, a major atmospheric oxidant which is formed in photochemical atmospheric processes. On the other hand, the increase of H_2O_2 can be attributed to anthropogenic impacts due to wastewater containing H_2O_2, in particular waste water of the textile, chemical, medical and other industrial sectors. Both H_2O_2 sources of natural and anthropogenic genesis are factors of the impact on the oxidative balance of the aquatic environment. Identifying of the H_2O_2 origin is not an easy task. The solution to this problem is possible by using a stable isotope method.

In this study we measured the triple isotopic composition of oxygen ($\delta^{17}O$ and $\delta^{18}O$) in H_2O_2 to examine a difference in the triple isotopic signature of oxygen for H_2O_2 originated from different sources. We used H_2O_2 which was formed in our laboratory experiments by gas-phase radical chemical reactions (an analogue of the natural source of atmospheric H_2O_2), and commercially available H_2O_2 which was produced by ordinary chemical reactions (the anthropogenic source of H_2O_2). Data obtained here showed that both H_2O_2 originating from different sources are mass-independent fractionated. We found that $\delta^{17}O$ of H_2O_2, which was formed by gas-phase radical chemical reactions, lies above a mass-dependent fractionation line on the three-isotope plot by ~1.4 to 2.5‰. The obtained $\Delta^{17}O$ value falls within the range of $\Delta^{17}O$ variations that was identified by Savarino & Thiemens (1999) for H_2O_2 in rainwater. The ^{17}Oexcess can be interpreted as a consequence of non-mass-dependent fractionation during the synthesis of H_2O_2. The $\delta^{17}O$ of commercial hydrogen peroxide, however, lies below the mass-dependent fractionation line by ~ -0.4‰.The ^{17}O deficit was expected due to the fact that atmospheric oxygen, which is used in the H_2O_2 manufacture, is anomalously low in $^{17}O/^{16}O$ ratio. This ^{17}O deficit in atmospheric O_2 would be transferred to H_2O_2 during the peroxide formation. As a result, H_2O_2 has unique the triple isotopic signature of oxygen that can be indicative of their source or the processes that formed them. We assume that this unique isotopic signature would be a useful indicator for distinguishing among natural and anthropogenic H_2O_2 that are chemically identical but originated from different sources.

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Dark respiratory carbon isotope fractionation in maize (C_4) plants during early ontogeny: comparison with C_3 plant species

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Carbon isotope discrimination during respiration has received increasing interest in plant ecophysiology during the past decade mainly because of its relevance for ecosystem carbon balance studies. A particularly relevant aspect is the difference between autotrophic (leaves) and heterotrophic (roots) organs, with leaves being in general ^{13}C-depleted compared to all other organs. Despite huge variability in isotopic signature of both leaf- and root-respired CO_2 (depending on species, environmental conditions as well as plant developmental stages), it is now well established that respiratory fractionation operates in opposite direction in leaves *versus* roots in C_3 herbs; leaf-respired CO_2 is ^{13}C-enriched while root-respired CO_2 is ^{13}C-depleted compared with the respective organic matter as well as putative respiratory substrates (*i.e.* carbohydrates), but not in C_3 woody species for which a ^{13}C-enrichment is also reported for root-respired CO_2. The opposite respiratory fractionation between leaves and roots could partly explain the observed isotopic difference in bulk organic matter (OM) between these organs in herbaceous species.

We have already shown that the opposite respiratory fractionation between leaves and roots starts only upon leaf autotrophy onset: leaf-respired CO_2 becomes highly ^{13}C-enriched (i.e. respiratory fractionation becomes negative), while root-respired CO_2 becomes progressively ^{13}C-depleted (i.e. respiratory fractionation become slightly positive) during early ontogeny in bean and peanut plants. However, the literature data on the respiratory fractionation in C_4 plants are scarce showing only a small fractionation with either ^{13}C-depletion or ^{13}C-enrichment in respired CO_2 compared to plant OM with no clear difference between organs (see our recent review Ghashghaie & Badeck, 2014).

The main objectives of the present work were to examine if (i) the opposite respiratory fractionation between leaves and roots as well as (ii) the isotopic pattern during early ontogeny already observed in two C_3 species (bean and peanut) can also be observed in a C_4 plant. The experiments were done on maize plants (different leaf ranks, stem, and roots) during ontogeny until the leaf of rank 5 was fully developed.

The results clearly show that the maize roots behave similarly to those of C_3 plants with respired CO_2 becoming progressively ^{13}C-depleted during ontogeny (i.e. respiratory fractionation becomes positive). Surprisingly, the maize leaves (whatever the leaf rank) show opposite time trajectory compared to C_3 leaves, with leaf-respired CO_2 becoming progressively ^{13}C-depleted too during ontogeny (i.e. respiratory fractionation in maize leaves changes in parallel to that of maize roots). Interestingly, the leaf-respired CO_2 in maize leaves shows a clear and rapid isotopic change after darkening (from -4 to -16 per mil after 10 to 80 min in the dark) suggesting, similarly to C_3 leaves, a transitorily increased organic acid (malate) decarboxylation.

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Unusually high sea ice cover influences resource use by benthic invertebrates in coastal Antarctica

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Antarctica currently undergoes strong and contrasted impacts linked with climate change. While the West Antarctic Peninsula is one of the most rapidly warming regions in the world, resulting in sea ice cover decrease, the sea ice cover of East Antarctica unexpectedly tends to increase, possibly in relation with changes in atmospheric circulation. Changes in sea ice cover are likely to influence benthic food web structure through modifications of benthic-pelagic coupling, disruption of benthic production and/or modifications of benthic community structure (i.e. resource availability for benthic consumers).

Here, we studied shallow (0–20 m) benthic food web structure on the coasts of Petrels Island (Adélie Land, East Antarctica) during an event of unusually high spatial and temporal (two successive austral summers without seasonal break-up) sea ice cover. Using stable isotope ratios of C and N and the SIAR mixing model, we examined importance of 4 organic matter sources (benthic macroalgae, benthic biofilm, sympagic algae, suspended particulate organic matter) for nutrition of dominant primary consumers and omnivores. 14 invertebrate taxa including sessile and mobile polychaetes, gastropods, bivalves, sea stars, sea urchins and sea cucumbers were studied.

Our results indicate that most benthic invertebrates predominantly relied on sympagic algae. Despite its very high abundance, trophic role of benthic biofilm seemed limited. However, interpretation of data was complicated by the peculiar ecophysiological features of Antarctic invertebrates, whose very low metabolic rates could be associated to low isotopic turnover and long time to reach isotopic equilibrium with their food items. Resource use by consumers from Adélie Land markedly differed from literature data about invertebrate diet in coastal Antarctica, suggesting 1) important influence of increased sea ice cover on benthic food web structure and 2) high spatial and/or temporal variation in the feeding habits of studied organisms, likely linked with a high degree of trophic plasticity. Our results provide insights about how Antarctic benthic consumers, which have evolved in an extremely stable environment, might adapt their feeding habits in response to sudden man-driven changes in environmental conditions and trophic resource availability.

Valorisation of agriculture for the water supply in context with climate change (ClimAgro project proceedings)

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Almost 90% of agricultural land in South Tyrol is composed of extensively and intensively used meadows und pastures. Land-use, such as managed grasslands, in mountain environments has strong influence on water supply in the lower regions, soil stability and hydro power (Körner et al. 1989). With the alpine region not only being influenced by large-scale changes in land-use during the last decades, but also being subject to climate change more than the global mean (Beniston 2006), it is essential to further understand the hydrological mechanisms of alpine grassland ecosystems.
This abstract reports the current proceedings of the "ClimAgro" project, a research cooperation of the European Academy Bozen-Bolzano (EURAC), the University of Innsbruck and the Free University of Bolzano sponsored by the the Province of South Tyrol (Autonome Provinz Bozen – Südtirol, Abteilung Bildungsförderung, Universität und Forschung). The project aims at analysing the impact of different agricultural management types of grasslands (intensification and extensification) and changing climate (higher mean temperatures, lower precipitation) on water use efficiency (WUE) of grasslands in alpine environments.

The WUE is evaluated by performing a common garden experiment, established in 2014. The field plot is located in Stubai Valley in Tyrol, Austria, where local soil monoliths together with others from Matscher Valley in South Tyrol, Italy, are transplanted into smart-field lysimeters on the valley floor. These monoliths are sheltered to allow the control of environmental parameters such as precipitation and temperature. To quantify WUE in this experiment we use stable isotope analysis of Carbon ($^{13}C/^{12}C$) and Oxygen ($^{18}O/^{16}O$).

Concurrently we investigate the sources of water for grasslands in relation to location, altitude and vegetation type. We analyse the Hydrogen ($^{2}H/^{1}H$) and Oxygen isotopic signature of water to trace the supply of water from the different possible sources (ground water, rain, snow and glacier water) into plants. In our presentation we will present new isotope data of seasons 2015/16 on WUE (Farquhar et al. 1982+1989), photosynthetic activity and stomatal conductance (Scheidegger et al. 2000).

With our approach of combining proven ecological techniques such as species transplantation experiments and state-of-the-art technology like smart-field lysimeters and stable isotope analysis, we will contribute to a better understanding of the future development of agricultural ecosystems in mountain environments in a changing world.

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How hungry are carnivorous plants? Investigating dependence on heterotrophic nutrition amongst carnivorous flora of Western Australia, using stable isotope techniques

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True carnivorous plants are able to attract, trap, and absorb nutrients from prey. Although numerous studies describe the biology and ecology of many carnivorous species, few *in situ* studies quantify the degree to which these plants are *reliant* upon heterotrophic nutrition (i.e. carnivory). To address this knowledge gap we apply modern isotopic methodology to some of the pioneering ecological concepts developed by Charles Darwin over a century ago.

The nutrition of nearly all carnivorous genera will be assessed through international collaborations with numerous research institutions, with samples from genera including *Drosera*, *Utricularia*, *Byblis*, *Cephalotus*, *Aldrovanda, Drosophyllum*, and *Nepenthes* collected from natural populations. This presentation will focus particularly on the comparison between carnivorous plant species (*Drosera, Utricularia, Byblis*) occurring in two distinct and highly biodiverse regions of Western Australia: the Southwest (Mediterranean climate) and the Kimberley (monsoonal climate).

To measure the relative importance of active (the capture and digestion of animal prey) and passive (root uptake) nutrition in these carnivorous genera, an end-member mixing model will be used to compare their $^{15}N/^{14}N$ isotopic ratios with those of co-occurring non-carnivorous plants and prey insects from their natural habitats. Comparisons will be made between species of the same genera (e.g. various growth forms of *Drosera*) and between different genera (e.g. *Byblis* vs. *Drosera*), across a variety of habitats within the Southwest and Kimberley regions.

This study will explore the efficiency of prey capture for several carnivorous genera, providing the first empirical evidence of the reliance upon carnivory *in situ* for nearly all *Byblis spp* and several *Utricularia spp*, as well as additional evidence for the great diversity of *Drosera spp* occuring in Western Australia, expanding upon the work of Schulze *et al* (1991). The role of carnivory as a convergent ecological driver for sticky-leaved carnivorous plants (i.e. *Byblis* and *Drosera*) and the influence of habitat features (climate/latitude, soil nutrition, surrounding vegetation structure) on the plant's dependence upon carnivory will also be explored.

Empirically determining reliance on carnivory is important from an ecological perspective, and in developing an understanding of the biotic relationships that these highly specialised (and often sensitive) species have with other organisms. This holistic understanding of the role of carnivorous plants in fragile ecosystems will allow for improved management and conservation of these unique plants, not only in the globally/nationally significant regions of Western Australia but throughout the world.

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Does soil microbial biodiversity affect soil C stabilisation? Molecular and isotopic approaches

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Diversity at the macroscale is recognised as important for ecosystem function. In grasslands, it has been demonstrated that increased plant diversity increases biomass production (Tilman et al. 1996). In soils, microbial diversity and health are often used interchangeably and seen as positives for many soil functions. However, given the huge complexity of soils, the true importance of soil microbial diversity on functional competence is difficult to probe. A small number of studies have manipulated microbial diversity directly in soil and found variable impacts on both the direction and scale of ecosystem function. Unfortunately, many of these studies were performed before the advent of modern sequencing tools, and therefore likely grossly underestimated the microbial diversity in their treatments. A more recent study (Philippot et al. 2013) found strong negative effects of reduced soil microbial diversity on nitrogen (N) cycling functions. Here, our aim was to probe the more complex processes surrounding stabilisation of organic matter in soils, and to identify whether microbial diversity was important for this function.

We took advantage of management-induced differences in soil microbial biodiversity between a remnant forest and an adjacent perennial pasture system on the same soil type on the Fleurieu Peninsula, South Australia. Here, we varied the diversity in soil microcosms by sterilization and re-inoculation with native microbial communities with varying levels of species richness through a dilution series. Litter was labelled with ^{13}C, and this was added to the soils at a rate of 100 mg litter C / g soil C. We tracked the fate of the ^{13}C from the litter during decomposition into $^{13}CO_2$, ^{13}C dissolved organic carbon, and stable soil fractions using IRMS techniques. We used ^{13}C CP/MAS NMR spectroscopy to quantify changes in organic matter chemistry, and we quantified soil bacterial and fungal community diversity using Ion Torrent next-generation sequencing of the ^{16}S and ITS genes (Whiteley et al. 2012) at specific time intervals.

Though the experiment is still ongoing, our preliminary results indicate that after three months of incubation, pasture soil C loss (measured as respired CO_2) increased in the decreased biodiversity treatments ($P<0.001$), while no effect was observed in the forest soils. NMR analysis revealed little change in the organic C chemistry of the soils under different diversity treatments. Data are currently being collected to investigate partitioning between soil- and litter-derived C in soil C fractions, CO_2 released, and extractable C. These will be coupled with microbial community structure analysis at each time point to understand how these changes in litter and SOM processing relate to microbial community composition.

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Use of stable isotopes to characterize symbiotic associations

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Organisms belonging to different species which are closely and durably associated together form a symbiotic association that involves a host and one or several symbionts. Depending on the relative duration of the interaction and the impact on host fitness, three categories of symbiotic association can be distinguished: parasitism, commensalism and mutualism (Parmentier and Michel, 2013). It is difficult to categorize a symbiotic association because the estimation of the costs and benefits for each organism depends on many different factors. One of the most important concerns the potential trophic relationship existing between both symbiotic partners. Indeed, a symbiont that eats its host in a large proportion will be qualified of parasite while a symbiont that consume its host predators usually defines a mutualistic symbiosis.

This study describes how we compared the carbon and nitrogen stable isotopes of the food contained in the digestive system of different symbionts to the isotopic signatures of their hosts and different organisms that can be found in their ecosystem. Particularly, we focus on multimodal analyses allowing the characterization of the diet of crustaceans which are obligatory symbionts of echinoderms (Caulier et al, 2014). The integration of morphologic, genetic and isotopic data provide a large overview that helps us to better understand symbiotic associations.

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Origin of woodcocks hunted in Austria

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The woodcock (Scolopax rusticola) in the EU is a protected species. Still, in several countries, among them Austria, its hunt is allowed. EU regulations, however, request these countries to protect their native woodcock population and to prove that the woodcock hunt is sustainable and does not reduce the local woodcock population. As the woodcock is a migratory animal, the woodcocks hunted in Austria might belong to different origin with respect to their breeding areas.

For this purpose primary feathers of hunted woodcocks in Lower Austria have been collected and measured for their stable isotope signature. From the condition of the feathers juvenile and adult individuals are distinguished.

Expected are differences in isotope pattern between juvenile and adult woodcocks, as adult woodcocks have their moult in winter and thus report patterns representing the conditions in the wintering areas. Juvenile woodcocks report the conditions of the breeding sites in summer.

The results show the expected differences between juvenile and adult individuals. Five different origins of the juvenile individuals are identified and three different ones for the adult ones. Furthermore there is evidence that woodcocks might not have a very high site-fidelity.

Food web baseline in a subtropical tidal flat, Atlantic south-western: spatio-temporal variations in the d^{13}C and d^{15}N isotope values

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To understand on the main primary carbon sources for consumers of a food web, stable isotopic values of the baseline are required. Based on this knowledge, this work was developed with the objectives of identifying the main baseline components, and analysing the variability in time and space of d^{13}C and d^{13}N in the food web of Araçá Bay (23°49'S; 45°24'W). This relatively small intertidal flat (750 m wide and 750 m long) is located on the continental side of a channel adjacent to the São Sebastião Island; it is also adjacent to the port and the oil terminal of the city of São Sebastião, and of the mouth of the submarine sewage outfall. The physical properties of water present a sharp seasonal change in the temperature and salinity. The present work is part of a multidisciplinary and interdisciplinary project with the objective of carrying out an integrated investigation to understand the functioning of a coastal system, such as trophic interactions and flux of matter and energy, among other subjects. Sampling was conducted between January (austral summer) and July (austral winter) 2013, in the continental (Cont), intertidal (Intert) - mangrove, soft and rocky bottoms, and sublittoral (Subl) regions. Samples (n=193) of sixteen primary sources were collected for analysis of d^{13}C and d^{15}N. They were collected and treated according to the usually used protocol for isotope analysis. The first results for suspended organic matter (SPM), surface sediment organic matter (SSOM), and microphytobenthos are presented. For SPM, samples of water were collected in the continental region (stream), in the intertidal region (upper and intermediate zones), and along the 2-4, 8-10, and 20-24 m-deep isobaths of the sublittoral region. SSOM and Microphytobenthos samples were collected in the upper and intermediate zones of the intertidal region, and along the 8-10 and 20-24 m-deep isobaths of the sublittoral region. A Minitab®17.0 routine was applied to perform parametric and non-parametric hypothesis tests (*p-value*<0.05), in order to verify time (summer and winter) and space effects (Cont, Intert, and Subl) on the mean d^{13}C and d^{15}N. The first results of the covariance analysis (GLM-ANCOVA), and grouping information using Tukey's method are presented. The values range and the mean values of d^{13}C and d^{15}N of these producers are in the range of the reported data in literature. For SPM, sampled in the three regions, d^{13}C was higher in summer than in winter, but there was no difference among regions; on the contrary, two groups of regions were formed (Subl>Cont=Intert) for d^{15}N, which showed no time difference. Microphytobenthos and SSOM, collected in the intertidal and sublittoral regions, showed similar results, i.e., effect only of the region on d^{13}C (Intert>Subl). The time effect on the SPM d^{13}C (>summer) is, most likely, related to biophysical processes associated to the seasonal dynamics. The region effect on the SPM d15N (Subl>Inter=Cont) could be related to the continental nitrogen load. And the region effect on the d^{13}C SSOM and microphytobenthos (>Intert) is, probably, due to the higher values of the intertidal microphytobenthos.

A contribution to the trophic levels and trophic diversity of fungi genus *Hygrocybe* (wax caps)

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Most of the fungi species from the genus *Hygrocybe* grow on unfertile meadows like lean oat grassland, red fescue prairie and Nardus grassland (Ruthsatz & Boertmann 2011). The wax cap species were investigated under the aspect of biodiversity in Western Germany, Rhineland-Palatinate, in the region of Trier (Mosel, Eifel, Hunsrück). The nomenclature of the fungi species follows Boertmann (2010). In this investigation, also the total $\delta^{15}N$- and $\delta^{13}C$-ratios as well as nitrogen and total carbon concentrations were measured. Fruiting bodies of the fungi were taken as samples. 31 different species were found on 110 different extensively managed hay meadows with variable wax cap species composition. The samples were collected in different years (2012-2015) with repeated sampling in preferably every year in autumn, if they showed fruiting bodies. So the variance source of the measured values could be examined as species-specific, site-specific and vegetation period specific.

The measured values of $\delta^{15}N$-ratios ranged from ca. +2 to +18‰, those of the $\delta^{13}C$-ratios from ca. -27 to -31‰. The range in $\delta^{15}N$ is 3.5 times higher than the variation in $\delta^{13}C$-ratios. The values of the different species show a high overlapping in $\delta^{15}N$- and $\delta^{13}C$-ratios, but there is at the same time a clear differentiation of species groups for $\delta^{15}N$. That indicates that the nitrogen source of the species within the genus differs highly in cause of different trophic level. In literature, it is pointed out, that the span of trophic levels of wax caps probably reaches from parasitic over symbiotic mycorrhiza to saprophagous, coprophagous, necrophagous, carnivorous especially nematophagous diet or trophic levels, so that they are called biotrophic (Gebauer & Tayler 1999, Halbwachs et al. 2013a, 2013b, Tello et al. 2013). This may cause the surprising wide span of $\delta^{15}N$-ratios in comparison to a smaller span of $\delta^{13}C$.

In the presented poster some hypothesises to trophic levels of the genus would be discussed, how to find out, if the span of $\delta^{15}N$ base on different substrates like phytomas, phyto- or animal-necromas, animal excretion, animal biomass or humus of different decomposition stage. Moreover the span of enriched ^{15}N in some Hygrocybe species may base on selected and specialized utilization of nitrogen containing substances of different species only (alimentation only from fungiphagous Collembola or Nematoda or Humus material what is highly decomposited and often metabolised).

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Carbon isotope discrimination as a surrogate of grain yield in drought stressed triticale

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Water availability in semi-arid regions is increasingly becoming threatened by frequent droughts due to climate change and variability. Crop adaptation to climate change particularly to drought has become a priority in Africa. Water use efficiency (WUE) is one important trait in adapting crops to water limited environments but its direct measurement remains a challenge. Hence, there is need for surrogate traits.

A two-year study was carried out with four moisture levels, ranging from well-watered to severe stress (SS), combined with four commercial triticale genotypes. The study evaluated the use of carbon isotope discrimination ($\Delta^{13}C$) as a proxy of WUE in triticale. The study revealed strong influence of soil moisture on $\Delta^{13}C$, intrinsic WUE and grain yield of triticale. Higher soil moisture levels significantly increased $\Delta^{13}C$ while lower values of $\Delta^{13}C$ were observed under water stressed conditions. The relationship between $\Delta^{13}C$ and grain yield was positive ($P<0.01$), but only significant under water stressed conditions, indicating dependence of the relationship on moisture level. Intrinsic WUE and $\Delta^{13}C$ showed a negative relationship for combined data of all moisture levels.

Overall, the study showed that $\Delta^{13}C$ could be useful as a triticale grain yield predictor under drought conditions. In addition, $\Delta^{13}C$ also offered potential as a proxy of intrinsic WUE and could be used for breeding towards improved drought tolerance in triticale.

Uniformly ^{13}C-Labelled Biomass Tracers: Advances in studying changes in soil microbial processes and communities

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Stable isotopes like ^{13}C and ^{15}N are used in ecology for tracing microbial activities in organic environments in both natural and agricultural soils. They are applied for quantifying C & N fluxes and transformations in complex ecosystems where decomposition processes of soil organic matter (SOM) are driven by soil microbial populations.

Since 2005, IsoLife produces a large range of ^{13}C and/or 15N labelled plant materials – from 1.2% up to 98 atom %. These have opened up new ways in unravelling key processes and functional relationships and in studying decomposition of plant residues and formation of soil organic matter. Their application also improved the sensitivity of analytical techniques such as Stable Isotope Probing (SIP).

Recent advances are:

i) SIP enables detection of functional, and often uncultivable, organisms in complex ecosystems at species level using U-^{13}C substrates and density gradient centrifugation to separate ^{12}C- from ^{13}C-DNA or -RNA. Subsequent sequencing of the ^{13}C-bands reveals the functional species active in decomposition of polymers like chitin, cellulose, lignin, etc. This technique alos works in complex soil ecosystems with high background ^{12}C. Trophic interactions in food webs, biodiversity issues, carbon fluxes in symbiotic associations, and formation of SOM can be studied with a renewed focus (Rime et al. 2016).
ii) Methods for studying in situ ecological relations enable tracing C and nutrient transfer between hosts and symbiotic organisms or the utilisation of biomass-^{13}C by single cells in soil. These can be measured and visualised microscopically by high resolution imaging mass spectrometry (NanoSIMS) using ^{13}C-labelling at high enrichment levels (>97 atom %; Eichhorst et al. 2015).
iii) Decomposition studies have been carried out with partially or uniformly ^{13}C-labelled plant parts (whole leaves or roots; intact or ground) or extracted plant materials like (hemi)cellulose, yielding detailed information about fractions respired, organic matter transformations, and distribution among or turnover of different SOM fractions (German & Allison 2015; Wild et al. 2016).
iv) Atmospheric labelling in m^3 volumes with $^{15}N_2$ to demonstrate the presence of N_2 fixation.

IsoLife's poster gives an overview of the latest achievements of using in situ uniform ^{13}C-labelling and uniformly ^{13}C-labelled substrates in soil ecology.

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Isotopic turnover and trophic fractionation in two species of European freshwater fishes: an experimental approach

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To improve the understanding of trophic relationships in aquatic ecosystems, scientists aim to identify the link between preys and consumers by quantifying diet. However, many complications could make harder this investigation as consumer diets vary over time with life stage[1] or individual length. Each species is able to ingest a large variety of preys[2–4] and spatiotemporal factors influence physiological and/or behavioural responses of species[5–7]. Stable isotope ratios of carbon ($^{13}C/^{12}C$) and nitrogen ($^{15}N/^{14}N$) are commonly used to analyse animal diets and trace pathways among consumers. However, dietary analyses require knowledge of fractionation factor and turnover rate of the study elements. These variables could fluctuate according to the environment, the stage or the diet of the consumer. Although it is theoretically possible to calculate isotope fractionations of many elementary chemical reactions, the biological reality is much more complex and requires an experimental approach. To decrease the variability, it is advised to determine the fractionation factor corresponding to the study species in laboratory with a diet shift experiment[8,9]. Controlled feeding experiment using 2 kinds of preys can also be used to determine the metabolic turnover rate, the time required for the body tissues to reflect the isotopic composition of the new diet[10].

Food acquisition and assimilation occupy an important part for growth and survival of fish, particularly during early stages when individuals are highly vulnerable to competition, food availability, perturbations[11,12]. Furthermore, feeding ecology of young-of-the-year from resorbed yolk sac larvae to juveniles are poorly understood especially for freshwater teleost fish[12]. In this paper, we have selected two cyprinid taxa, chub (*Squalius cephalus*) and roach (*Rutilus rutilus*), which are common freshwater species widely distributed in Western Europe[13,14]. During their larval stage chub and roach mainly feed on zooplankton but their diet shifts at the juvenile 0^+ stage, with chub starting to feed on invertebrates and roach becoming omnivorous[15]. The aim of this paper was to examine the turnover rate and the fractionation factor of nitrogen and carbon stable isotopes of two freshwater fish larvae following a dietary shift under laboratory conditions. Roach and European chub gametes were collected from adults sampled in the Meuse River at the fish pass during the spawning period. Eggs were fertilized and incubated at 12°C under static conditions. After yolk sac resorption, 6000 larvae of each species were distributed into 6 tanks at constant temperature. The initial $\delta^{13}C$ and $\delta^{15}N$ of larvae before initial feeding was determined by sampling 3 larvae in each tank (t0). During 5 weeks (t0 to t5), larvae were daily fed in excess 5 times a day with freshly hatched *Artemia* nauplii. Dietary shift begun from t5 to t10 (5 weeks), larvae were daily fed in slight excess with frozen larvae of chironomidae sp. coarsely chopped. During the dietary shift experiments, 3 larvae per tank were randomly selected each week for isotopic analysis after 24h of food privation for gut clearance and one sample of prey sources was daily collected. Stable isotope ratios measurements ($\delta 13C$ and $\delta 15N$) were performed via continuous flow isotope ratio mass spectrometry, using a Vario Micro Cube elemental analyser coupled to an Isoprime 100 mass spectrometer. The change in isotopic composition strongly followed predictions of the exponential model of Hobson & Clark ($R^2>0.88$). Half-life of nitrogen and carbon was between 10.6-11.3 and 8.2-12.6 days respectively mainly due to production of new tissue.

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Hidden robbery: A new approach (δ^2H) discloses organic nutrient-gain effectiveness of C_3-hemiparasites from C_3-host plants

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Parasitic plants are intensively studied as they have large economic (Westwood *et al*. 2010) and ecological impacts (Press & Phoenix 2005). For very few hemiparasites following C_3-photosynthesis and utilizing C_4- or CAM-plants as hosts carbon stable isotope analyses have shown a profit from two different carbon sources: autotrophic gain (photosynthesis) and heterotrophic gain. Heterotrophic carbon gain among hemiparasites is caused by an increased water potential gradient through higher transpiration. Nutrient flow (minerals, water, xylem mobile organic carbon) is induced by a physical and physiological bridge (haustorium).

Unfortunately, hemiparasites and the majority of their host plants follow C_3 photosynthesis. For all C_3-C_3 host-parasite interactions carbon stable isotope abundance alone is not suited to estimate the origin from either of the two potential carbon sources. However, a recent approach with mycoheterotrophic orchids has demonstrated the suitability of hydrogen stable isotope natural abundance as a substitute for carbon isotope abundance to distinguish carbon gain from two different sources (Gebauer *et al.* 2016).

The aim of this study is to establish this new approach for efficiency estimates in nutrient gain of C_3-hemiparasites (Orobanchaceae, Santalaceae) from fully autotrophic potential C_3-host plants. Thereby simultaneous analyses of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotopes (EA-IRMS) as well as hydrogen (δ^2H) and oxygen ($\delta^{18}O$) isotopes (TC-IRMS) will be realized. Efficiency is calculated with an application of the two-source linear mixing model. Autotrophic reference plants are setting the lower endpoint (0% heterotrophy) and holoparasitic plants (*Lathraea squamaria*, *Orobanche*) are setting the upper endpoint (100% heterotrophy) of the model. The approaches of the intermediate C_3-hemiparasite signatures (δ^2H) to one or the other site represent the parasitic effectiveness.

Due to higher transpiration and lower water-use efficiency in general a depletion in δ^2H, $\delta^{13}C$ and $\delta^{18}O$ should be expected for tissues of hemiparasites. With this expectation in mind the following hypotheses will be tested:

(i) The transpiration-driven 2H-depletion in hemiparasites is counterbalanced by a 2H-enriched gain of organic matter from the host (Yakir 1992).
(ii) The difference in tissue $\delta^{18}O$ values of hemiparasites and their hosts is exclusively driven by the higher parasite transpiration and thus, can be used to correct δ^2H values for the transpiration effect.
(iii) Hemiparasites within the Orobanchaceae differ whether they are generalists (high host plant range, unspecialized haustoria) or specialists (low host plant range, specialized haustoria) and therefore different efficiencies are assumed.

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Feeding with fondness? Obtaining a higher precision in nitrogen gain estimates among carnivorous Pinguicula species by considering the trophic level of their prey

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Carnivorous plants evolved a special habit to obtain essential nutrients that allows them to most often inhabit moist and nutrient-limited habitats: the trapping of animals (Givnish 2015). While many tropical carnivorous plants of different genera have been studied, there is a significant knowledge gap about the success of alpine *Pinguicula* species (butterworts) to gain nutrients from prey.

Pinguicula vulgaris and *P. alpina* were sampled in the Austrian Alps to calculate nutrient gain effectiveness according to the two-source linear mixing model. Fully autotrophic reference plants and potential prey animals (Insecta, Arachnida) were collected nearby the carnivorous plants. Prey animals were graded corresponding to their trophic level. Stable isotopes ($\delta^{13}C$, $\delta^{15}N$) of plants and animals were analysed with EA-IRMS.

Nutrient gain effectivity was estimated for *P. vulgaris* (*24% / 54%) and *P. alpina* (*3% / 31%) by comparing their $\delta^{15}N$-values with autotrophic plants and potential prey animals. Stepwise enrichment per trophic level of about 3‰ in $\delta^{15}N$ as expected was confirmed. Trophic levels of photo-autotrophic and carnivorous plants as well as herbivorous and zoophagous prey were successfully estimated ($r^2 = 0.72$) and different efficiencies in nutrient supply were calculated ($p < 0.01$). Using this approach a clearer resolution of the prey derived nutrient gain of a carnivorous plant was achieved. Costs of carnivorous structures could be detected by higher transpiration ($\delta^{13}C$).

Interestingly, the reference plant *Tofieldia calyculata* revealed a higher enrichment in $\delta^{15}N$ compared to the other reference plants ($W = 50$, $p = 0.003$) resulting in consequences for the efficiency calculation (*). *Tofieldia calyculata*, a member of one typical plant association of temperate *Pinguicula* species (Oberdorfer *et al.* 2001), should be analysed for the reasons of the unexpected enrichment in $\delta^{15}N$. Divergent isotopic values should be considered when using *Tofieldia* as reference plant.

Carnivorous plants play an important role in many biotic interactions and ecosystem functioning. The calculation of nutrient gain efficiencies and influences of different trophic levels of temperate montane species aims to complement knowledge about nutrient flows in carnivorous species and their importance for our ecological understanding. The study showed moderate nutrient gain efficiencies of *Pinguicula vulgaris* and *P. alpina* which are similar to further sticky-leaves-rosette plants (e.g. certain species of sundews: *Drosera*). The trophic level reveals a distinct impact on the isotopic values, so that the procedure of earlier studies to use a pool of prey animals to calculate the efficiency of a carnivorous plant can be improved by considering the trophic level of the respective prey animals.

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Which fungi taste good to orchids? Diversity of carbon and nitrogen gaining strategies in mycoheterotrophic orchids of Southeast Asia

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Because of lacking endosperm, all of the > 22000 orchid species start their lives as mycoheterotrophs fully relying on carbon and nutrient supply from their mycorrhizal fungi. Later, the majority of orchids develops green leaves and becomes photosynthetic, yet remaining mycorrhizal. There are, however, > 200 orchid species from at least 43 genera that remain achlorophyllous and fully mycoheterotrophic (Merckx et al. 2013). Because they use a ^{13}C and ^{15}N enriched fungal source for nutrition, mycoheterotrophic orchids are enriched in ^{13}C and ^{15}N, when compared with autotrophic plants from the same microhabitats. Based on their isotopic positioning between autotrophic non-orchids and achlorophyllous orchids, during the last decade several orchids among the chlorophyllous orchids have been found, that acquire carbon from both autotrophic photosynthates and associated fungi. Thus, there is a wide diversity of carbon and nitrogen gaining strategies within orchids: initial mycoheterotrophy, full mycoheterotrophy, partial mycoheterotrophy and putatively autotrophy. The type of mycorrhiza also varies: Orchids are not only mycorrhizal with representatives of the ubiquitary and saprotrophic polyphyletic *Rhizoctonia* group, but also with fungi that simultaneously form ectomycorrhizas with forest trees or with fungi that have long been considered as exclusively saprotrophic and non-mycorrhizal wood- or litter-decaying.

A center of biodiversity of mycoheterotrophic orchids is found in Southeast Asia, where they grow on dark floors of broadleaf or coniferous forests or in bamboo forests (Merckx et al. 2013; Lee et al. 2015). In Taiwan, more than 50 fully mycoheterotrophic orchids can be found within over 300 native orchid species (Su, 2000). However, little is known about their mycorrhizal fungi. First investigations among fully mycoheterotrophic orchids indicate association with saprotrophic non-*Rhizoctonia* fungi, that decay either wood or litter, or less often with ectomycorrhizal fungi (Lee et al. 2015). Despite the large number of fully mycoheterotrophic orchids in Taiwan, so far no reports about partially mycoheterotrophic orchids exist. Here we analyze the carbon and nitrogen stable isotope abundances of both green and fully mycoheterotrophic orchids from Taiwan, to identify potential partially mycoheterotrophic orchids in Taiwan and to reveal the orchids' fungal partners. Five green mycoheterotrophic orchid species suspected to be partially mycoheterotrophic from the genera Liparis, Malaxis, Nervilia and Neottia and eight aclorophyllous, fully mycoheterophic species from the genera Didymoplexis, Lecanorchis, Gastrodia, Stereosandra and Yoania from different forest sites in Taiwan are currently analyzed.

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Drought and defoliation impacts on nitrogen dynamics in branches of 10 year old beech trees investigated through several short ^{15}N urea pulses

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Nitrogen (N) is a major element in metabolism and growth of trees. To our knowledge, its specific role in forest dieback and the loss of resilience of a tree under harsh environments, has been poorly explored. Nowadays, the scientific debate about tree death is to understand if a tree becomes unhealthy and dies due to a lack of carbohydrates or due to a lack of water. In addition to these two hypotheses we propose that a strong disturbance in a tree of its internal nitrogen cycle could participate to the cascade of physiological events leading to the the tree death in response to a stress.

To feed our hypothesis and to progress in the understanding of N pathways responses of trees to stress, we designed an original experiment with more than 1000 10-years-old beech (*Fagus Sylvatica*) grown under a rain exclusion system. Three treatments were installed during two years: i) a drastic drought treatment, ii) an extreme defoliation (75%) carried out each year in june on irrigated trees and iii) a control treatment with irrigated trees. A N shortage was expected to occur in the drought treatment due to limiting water and mineral absorption by roots and in the defoliated treatment because in the case of beech, 30 % of nitrogen is located in leaves in June. To follow the impacts of the artificial N loss on **N internal cycle of trees during the vegetation season** and in particular on N dynamics inside branches of beech trees, several short ^{15}N-urea pulses have been applied on tree foliage at three times during the second vegetative season of constraints: in June, at the end of the main shoot growth period (before the second defoliation), in July (one month after the second defoliation) and in September. Then ^{15}N dynamics were followed in leaves and branch compartments of control, water-stressed and defoliated trees by collecting leaves 24 h, 48 h, 4 days, 7 days after labelling and leaves and branches 14 days after labelling. We showed that ^{15}N dynamics in beech shoots were markedly altered in response to constraints compared to control trees indicating that modifications in the internal N cycle of beech trees occur under harsh environments. The potential role of this alteration of N functions in tree death will be discussed.

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Feeding ecology of Southern Ocean sea stars inferred from stable isotopes ratios

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The Southern Ocean is currently subjected to strong and contrasted impacts of climate change. The Western Antarctic Peninsula is one of the most rapidly warming regions of the world, resulting in sea ice cover decreases. Increasing seawater temperature and sea ice cover reduction in Western Antarctic Peninsula and associated regions will likely impact food web functioning through temperature-related changes in consumer physiology, modifications of benthic community structure (e.g. expansion of exogenous species such as predatory crabs), modifications of benthic-pelagic coupling intensity or disruption of benthic production. Asteroids (Echinoderms) are an important group of southern benthos. This group also has a great trophic variability and is potentially more resistant than other organisms to temperature changes (Peck et al. 2008). Consequently, they will be likely impacted by modifications in food webs functioning rather by direct warming and investigating their trophic ecology is necessary to infer how climate change will impact them.

In this context, the aim of this study is to use stable isotopes ratios of C, N and S to infer sea stars trophic ecology. 16 species of sea stars spanning 10 different families sampled in multiple and contrasted habitats across Subantarctic (South Georgia, South Sandwich Islands, Falkland Islands) and Antarctic (South Shetland Islands, South Orkney Islands, Western Antarctic Peninsula) locations. In total, tegument samples from 213 specimens was analysed.

Diversity and plasticity of asteroid diet along Southern Ocean coasts were explored through isotopic niche parametrisation (e.g. niche width and overlap between species and/or populations; Jackson et al. 2011). The data will also be used in a larger scale research project on the trophic ecology of Antarctic sea stars. This project will notably compare trophic resources supporting asteroid communities in Western Antarctic Peninsula, where sea ice cover is decreasing, and in Terre Adélie, where sea ice cover is increasing (Parkinson & Cavalieri 2012). Ultimately, this project will help understanding which ecological processes determine how an animal group copes with environmental modifications linked to climate change.

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Using Natural Air to Increase the Accuracy of Environmental Measurements

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Human activity is increasing the concentrations of green house gases (GHG) in the atmosphere which has resulted in substantial temperature increases. Many countries have entered into agreements to limit and / or decrease GHG emissions. This requires precise measurements by region to clearly evaluate GHG emissions, sinks and evolution as well as mitigation strategies. High precision measurements are a key requirement to study and evaluate the global carbon cycle and its effect on climate change.

Calibrating the analytical instruments used to make atmospheric measurements are often done using standards prepared in synthetic air. There are significant differences between synthetic air and natural air which introduce bias into some measurement; therefore natural air is preferred. This presentation will examine the natural air and isotopic mixture preparation process and the role of precisely characterized materials. Emphasis will focus on adjustment of isotope ratios to more closely bracket sample types without the reliance on combusting naturally occurring materials, thereby improving analytical accuracy.

Tracing sources of nutrient (NO_3) loading in the Lake Winnipeg watershed

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Anthropogenic nitrate inputs into the global nitrogen cycle have increased considerably and are reducing water quality. Lake Winnipeg is the tenth largest freshwater lake in the world, and the nutrient inputs have increased considerably in the lake and its watershed during last decades resulting in eutropication. These inputs include loadings from agriculture (inorganic and organic fertilizer and animal waste) and urban sources (sewage, wastewater discharge). The main nutrient inputs into the Lake Winnipeg are the Red and Winnipeg Rivers in the South basin, and the Saskatchewan River on the west side of the North basin – although the major source of elevated nutrient loadings comes from the Red River. The aim of this study was to use stable isotope methods to evaluate sources and seasonal patterns of nitrates into the South Basin.

Sources of dissolved nitrate can be determined using stable nitrogen ($\delta^{15}N$) and oxygen ($\delta^{18}O$) isotopic compositions of nitrate. Nitrate isotopes in water from the Assiniboine and Red River were analysed using a method based on the isotopic analysis of N_2O produced from the conversion of NO_3- by cultured denitrifying bacteria that lack N_2O-reductase activity. We conducted the measurements of $\delta^{15}N$ and $\delta^{18}O$ from the resultant N_2O in an isotopic N_2O laser analyzer (Los Gatos Research, Inc.) that employs cavity enhanced laser-absorption spectroscopy (Soto et al. 2015). Values of $\delta^{15}N$ and $\delta^{18}O$ were used to estimate the relative contribution of potential nitrate sources (fertilizer, nitrification and manure/septic waste) using Bayesian isotope mixing models.

Overall, $\delta^{15}N$ values ranged from −2 ‰ to +20 ‰, and $\delta^{18}O$ values ranged from −20 ‰ to +20 ‰. These values indicated a variable contribution of nitrate sources depending on the river reach and period of sampling. Nitrates in water samples collected in 2015 from the Assiniboine River, which connects with the Red River at the City of Winnipeg, appear to come from animal waste sources (i.e. manure and sewage) predominantly, but those from the Red River seem to originate largely from fertilizers. The relative contributions of nutrient sources in the watershed changed over time, with reduction of agricultural inputs from fertilizers following spring melt. This study promises to help managers to improve water quality and to assess nutrient changes in the Lake Winnipeg system.

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Characterization of 1,2-dichloroethane dehalogenation in *Dehalococcoides mccartyi* using multi-element compound-specific stable isotope analysis

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Chlorinated ethenes and ethanes belong to the most common ground water and soil contaminants as they were widely applied in agriculture, e.g. as pesticides, or produced industrial and even naturally. While certain compounds, as e.g. *cis*-dichloroethene (cDCE), accumulate in the environment as a result of (incomplete) reductive dehalogenation of higher chlorinated ethenes under anoxic conditions, 1,2-dichloroethane (1,2-DCA) is an exclusively man-made contaminant released due to improper waste treatment at versatile production sites. Nevertheless, both are persistent, toxic or carcinogenic and influence the ground water and soil quality. In the last years, however, several *Dehalococcoides mccartyi* strains were described, known to be capable of complete dehalogenation of these compounds to non-toxic ethene, under strictly anoxic conditions.[1]

Compound-specific stable isotope analysis (CSIA) can be applied to gain knowledge about microbial transformation reactions, as the technique is based on measurements of changes in isotopic ratios of selected substrates and products, which occur during reactions. The rate of the bond-cleavage is thereby affected by the nature of the isotope – heavy or light. Therefore, with the state-of-the-art technique, carbon, hydrogen, and chlorine isotope ratios can be used to investigate transformation mechanisms on a multi-element basis.

In this study we aimed to investigate the anaerobic transformation of 1,2-DCA by *Dehalococcoides mccartyi* strain 195, isolated from an anoxic digester sludge, and BTF08, which was enriched from a contaminated groundwater in Bitterfeld (Germany). Dihaloelimination of 1,2-DCA to ethene was observed with relatively higher conversion rates for strain 195 compared to strain BTF08, similar to previous observations. Furthermore, the compound-specific carbon, chlorine and hydrogen stable isotope composition was analyzed for both the substrate, 1,2-DCA, and ethene as product to obtain information on the reaction. The experiments showed unexpected similarities of this anaerobic reaction to aerobic nucleophilic substitution reactions but significant differences with monooxygenase reactions involving a primary hydrogen abstraction [2]. Therefore it could be concluded, that the carbon-chlorine bond cleavage is the rate determining step on the way from 1,2-DCA to ethene in both investigated *Dehalococcoides mccartyi* spp. The investigation of hydrogen isotope effects should provide further insights into the new mechanism.

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New insights about the fate of atrazine in the environment by compound specific isotope analyses – A field study from karst aquifers of the Franconian Alb, Germany

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The herbicide atrazine, and its metabolite desethylatrazine (DEA) rank among the most frequently detected contaminants in German groundwaters, even though their use has been forbidden for more than 20 years. Karst aquifers are particular vulnerable to contaminations due to their characteristic flow paths consisting of fractures and a conduit network, with the latter one representing the fast water movement in kast systems. Detecting atrazine degradation over such long time spans in karst systems is a challenging task to characterize their self-purification potential. Not all metabolites are typically detected in karst systems which are characterized by a high mixing and dilution potential, and mass balances are difficult to close.

Here, compound specific isotope analysis (CSIA) can provide more conclusive evidence since it relies changes in naturally occurring isotope ratios (e.g., $^{13}C/^{12}C$, $^{15}N/^{14}N$) within the parent and the daughter compound during transformation reactions. This study aims to give new insight into transport- and degradation processes of atrazine and DEA at two different karst springs- the Mühlbach spring (mean residence time of ca. 80-100 a) and the Kinding spring (mean residence time of less than 10 a) – which are located in the Franconian Alb, Germany, by the use of CSIA. Identification of DEA indicated that degradation of atrazine has taken place at some time at the field-sites. At the Kinding spring concentrations of atrazine and DEA did not change over the observation period (from spring to winter 2015) of a few months (0,08 ± 0,01 µg/L, 0,09 ± 0,02 µg/L, respectively). However, carbon isotope ratios of atrazine varied at the Kinding spring. The $\delta^{13}C$ values of atrazine were heavier (-22,1 ± 1 ‰) during winter months than during summer and autumn (-24.8 to -24.1 ± 1 ‰). This may indicate that during groundwater recharge conditions atrazine with a different carbon isotope signature was mobilized from a more immobile part of the karst system shifting $\delta^{13}C$ to less negative values. However, at the Mühlbach spring concentrations of atrazine and DEA (0.21 ± 0.02 µg/L and 0.24 ± 0.02 µg/L) and stable carbon isotope values ($\delta^{13}C_{atrazine}$ = -23,5 ± 1 ‰ and $\delta^{13}C_{DEA}$ = -19,5 ± 1,5 ‰) did not change significantly over the observation period, indicating that further degradation does not occur in the karst groundwater with a calculated mean residence time of about 100 years. The stable isotope values of this aquifer may also lead to the conclusion that ongoing degradation in the karst aquifer is likely to be inhibited by limiting conditions (e.g. scarcity of nutrients, bioavailibilty). Similiar, at the Kinding spring, $\delta^{13}C$ values of DEA (-19.1 ± 0.8 ‰) did not differ significantly. This may give evidence that DEA is not further degraded in the karst system.

Exploring the bottlenecks of micropollutant degradation at low concentrations by isotope fractionation

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Micropollutants such as pharmaceuticals, solvents, and pesticides are frequently discharged into the environment. These compounds are degraded at high concentrations (mg/l), but the degradation often stops at ng/L to µg/L concentrations even though competent bacterial degraders are present. This raises the question what bottlenecks prevent micropollutant degradation at low concentrations and whether they can be overcome by adequate bioremediation strategies. At micropollutant threshold concentrations the bottleneck can potentially be caused by two limiting factors: (i) mass transfer imitations (bioavailability, cell uptake) or (ii) physiological limitations (enzyme down-regulation). Current observations based on micropollutant concentration measurements, however, can only distinguish between "degradation" or "no degradation" scenarios, but are not suited to pinpoint underlying bottlenecks. Compound-specific isotope analysis (CSIA) holds promises as an alternative approach. Since CSIA measures changes in naturally occurring stable isotope ratios (e.g., $^{13}C/^{12}C$, $^{15}N/^{14}N$) within a parent compound during degradation, the method can provide information that is complementary to concentration measurements. Molecules with light isotopes are typically slightly preferred during degradation and therefore, an enrichment of heavy isotopes in the parent compound can be observed. However, this strong isotope fractionation can only be observed when the enzyme reaction is rate limiting. In this case a fast exchange of micropollutant molecules occurs between cell and the surrounding solution corresponding to a situation of full bioavalatility. If bioavailability becomes limiting, however, this scenario must change: diffusion of micropollutant molecules into the cell becomes rate-limiting (mass transfer limitation) so that every molecule which enteres the cell is quickly degraded and the changes in isotope ratios (which still occur next to the enzyme) can no longer be observed outside the cell in solution (where samples are taken). Results from previous experiments suggest that mass transfer is not limiting at high concentrations (mg/L) (Rennicke et al. 2012). However, they have not addressed the question "At what concentration does mass transfer become rate-limiting step for MPs degradation?" Answering this question is challenging due to difficulty of compound-specific isotope analysis at µg/L concentration: the requirement of sufficiently large sample volumes, the cultivation of bacterial degraders on recalcitrant MPs in a suitable engineered systems, etc. To overcome these challenges, we chose to investigate micropollutant degradation for the first time in chemostats as experimental model system. In chemostats, micropollutants can be continuously added and removed at equal flow rates. This allows collecting large volumes of samples from the outflow for isotope analysis and at the same time offers the flexibility of studying the degradation at different concentrations of micropollutants by varying the inflow rate. Our results bring forward i) the first chemostat cultivation of *Arthrobacter aurescens* TC1 on atrazine as highly recalcitrant pesticide, (ii) the first isotope fractionation study of organic micropollutants in a chemostat setup .

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Comparison of Trichloroethene and *cis*-1,2-Dichloroethene Reductive Dechlorination with Different Bacterial Cultures Using Dual Element Isotope Analysis ($\delta^{13}C/\delta^{37}Cl$)

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Chlorinated ethenes are among the most common detected contaminants in groundwater systems throughout the US and Europe. Trichloroethene (TCE) in particular can be observed in very high concentrations in groundwater because of its widespread use in industry. In the environment TCE can be degraded under anoxic conditions via reductive dechlorination which in some cases results in the accumulation of more toxic *cis*-1,2-dichloroethene (*cis*-DCE) (Bradley 2000). Having a detailed knowledge about the fate of these contaminants and understanding their microbial degradation pathways and the underlying transformation mechanisms in groundwater and the subsurface is crucial for contaminated site remediation and water management.

Conventional protocols often fail to detect degradation. To study the degradation mechanisms of these contaminants a very promising alternative is compound-specific isotope analysis. The principle behind it is that molecules with exclusively lighter isotopes typically react faster than molecules with heavier isotopes. In case of degradation, the substrate thus becomes gradually preferentially enriched with molecules containing heavier isotopes. Additionally, analyzing different elements of bonds involved in the transformation mechanism results in dual isotope plots, which show different slopes for different reaction mechanisms. Consequently, multi-element isotope analysis of the parent compound can pinpoint different degradation pathways. Recently, this approach was advanced for chlorine isotope analysis. Now it is possible to produce dual (C, Cl) isotope plots for the contaminants stated above, which should allow deeper insights into their reaction mechanisms (Elsner & Hofstetter 2011, Renpenning et al. 2014).

The aim of this project was to investigate the variability of carbon vs. chlorine isotope fractionation during anaerobic biodegradation of TCE and *cis*-DCE. For *cis*-DCE degradation *Dehalococcoides mccartyi* strain 195 and strain BTF08 were used. For TCE degradation the mixed cultures KB-1 RF (subculture of dehalogenating consortium KB-1) and Donna II were used. Both were routinely maintained on TCE and both contain strains of the genus *Dehalococcoides*. All cultures are able to degrade TCE/*cis*-DCE via reductive dechlorination (Taş et al. 2010). Additionally, a TCE degradation study was conducted with KB-1 subcultures previously maintained on *cis*-DCE, *trans*-DCE, vinyl chloride or 1,2-dichloroethane prior to TCE exposure. The results indicate significant differences regarding TCE isotope fractionation.

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Aerosol source (biomass, traffic and coal emission) apportionment in Vilnius using stable carbon and radiocarbon analysis

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We present carbonaceous aerosol source apportionment results in Eastern European city Vilnius (capital of Lithuania) using stable carbon isotope ratio (^{13}C) and radiocarbon (^{14}C) methods. The aerosol sampling campaigns were performed in 2014-2016 winter and spring seasons in Vilnius. PM1 particles were collected on quartz fiber filters using high volume sampler, while PM10 and size segregated aerosol particles were collected using low volume and MOUDI 128 cascade impactor respectively. ^{13}C values were measured with EA-IRMS system while radiocarbon analysis was performed using Single Stage Accelerator Mass Spectrometer (SSAMS). For the AMS analysis, filters (or aluminium foils from cascade impactor) were graphitized using Automated Graphitization Equipment. It was estimated that dominant carbonaceous aerosol source in Vilnius was of biogenic/biomass origin (60-90 %). Fossil fuel sources accounted for up to 23 % of total carbon fraction. Combining stable carbon and radiocarbon isotope analysis we were able to quantify the amount of coal derived aerosol particles. The contribution of coal burning emissions were up to 14 %. We will present the applicability of dual carbon (^{13}C and ^{14}C) isotope ratio method for the aerosol source apportionment in different regions of Europe, also the perspectives of using MOUDI cascade impactors to make source apportionment in size segregated aerosol particles.

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Using compound-specific isotope analysis to evaluate the effect of temperature and soil water content on pesticides degradation in agricultural soils

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Synthetic pesticides and their degradation products can persist in soils following pesticide application. They can be mobilised after rainfall events and reach aquatic systems. In these environmental matrices, pesticides and degradation products can become toxic to microorganisms, therefore hampering further degradation. Hence, assessment of pesticide degradation and controlling factors are necessary. Compound specific isotope analysis (CSIA) enable to assess pesticides degradation over long time scales without analyses of degradation products (Elsner and Imfeld, 2016). However reference experiments involving stable isotope analysis to assess key-factors controlling pesticides' fate in soils are missing.

Using CSIA we assessed the effect of 20 and 40% water contents and 20 and 30 °C incubation temperatures on pesticides degradation extent. We used Calcareous soils from crop and vineyard catchments. The crop soil (pH 7.2) is composed of 29.5 % clay, 64.2 % silt, 6.4 % sand and 1.1 % organic matter while the vineyard soil (pH 8.1) contains 68.5 % of clay, 23.1 % of silt, 8.4 % of sand and 3.9 % of organic matter (Duplay et al., 2014). These soils were spiked at environmental concentrations with a mix of 9 fungicides (anilinopyrimidine, benzimidazole, cyanoacetamide oxide, morpholine, phenylamide and strobilurin ones), 10 herbicides (benzamide, chloroacetamide, dinitroaniline, phenylpyrazole, phenylurea, triazine and urea ones) and 1 benzoylurea insecticide typically used by farmers in the studied catchments. These soils were incubated for 200 days. We developed a soil extraction method including three consecutive times with ethyl-acetate, followed by a cleanup with primary-secondary amine (PSA) sorbent. Larger degradation extent of pesticides could be observed in incubations at the highest temperature and water content, as shown by the results for S-metolachlor (chloroacetamide herbicide), emphazing different extent of reaction. Enrichment factors (ε), for soils at 20 % water content and incubated for 100 days (about 90% degradation), raised from -1.6 ‰ to -3.6 ‰ for temperature of 20 °C and 30 °C, respectively. Calculated ε was -2 ‰ for the soils at 40 % water content incubated for 100 days at 20 °C. After 10 incubation days, S-metolachlor showed less than 1 ‰ difference in carbon isotopic signatures ($\Delta\delta^{13}C$) between the vineyard and the crop soils incubated at 20 °C for both investigated water contents. In contrast, up to 2 ‰ shift was observed between the two soils when incubated at 30 °C.

Our results demonstrate the potential of stable isotope analysis to assess degradation of pesticide mixtures in soils according to the ambiant conditions, in order to understand the corresponding reaction mechanisms and quantify the degradation extent. Further, the evolution of microbial communities and of pesticides partitioning among soil fractions will be investigated to assess temporal pesticides mobility in soils.

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Stable isotope fractionation of nitrate and sulfate indicates 'recycling' of sufate during petroleum hydrocarbon degradation

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The assessment of petroleum hydrocarbon (TPH) degradation using compound specific isotope analysis is impeded by their chain length. Alternatively, in-situ biodegradation of petroleum hydrocarbons (TPH) can be investigated using isotops shifts in terminal electron acceptors (TEA) nitrate and sulfate involved in anaerobic TPH oxidation. Biodegradation of a paraffin-rich crude oil was studied in anaerobic aquifer microcosms with nitrate (NIT), sulfate (SUL), nitrate plus sulfate (MIX) and nitrate under sulfate reduction suppression by molybdate (MOL) as TEA. After 8 months, TPH biodegradation was not different (around 33%) in experiments receiving only nitrate (NIT, MOL) versus under mixed TEA-conditions (MIX). Molybdate addition effected higher nitrate depletion, possibly by increasing the production of nitrate reductase. Additional sulfate depletion under mixed conditions suggested bioconversion of polar intermediates. Microcosms only receiving sulfate (SUL) showed no significant TEA and TPH decrease. A Rayleigh kinetic isotope enrichment model for ^{15}N nitrate and ^{34}S sulfate gave apparent enrichment factors $\varepsilon_{N\text{-}NIT}$ and $\varepsilon_{N\text{-}MOL}$ values of -16.7 to -18.0 ‰ for nitrate as sole TEA and $\varepsilon_{N\text{-}MIX}$ of-6.0‰ and $\varepsilon_{S\text{-}MIX}$ of -4.1‰ under mixed electron accepting conditions. ^{18}O measurements of nitrate and sulfate are currently conducted to complement existing data. The stable isotope shifts correlated well ($R^2 > 0.83$) with TPH degradation, allowing the translation of ^{15}N nitrate and ^{34}S sulfate measurements into TPH biodegradation. The presence of two enrichment factors for nitrate, depending on the presence of a competing TEA-process indicates the occurrence of lithotrophic or organotrophic denitrification. The smaller ^{15}N fractionation in the presence of sulfate suggests sulfide-dependent denitrification, leading to sulfate recycling. This theory was supported by TEA concentration measurements and the presence of key microbial players. This is, to our knowledge, the first characterization of sulfur and nitrogen isotope shifts associated to concurrent organotrophic and lithotrophic denitrification in a hydrocarbon-contaminated environment, and offers the prospect of improved understanding of biogeochemical cycles including in situ hydrocarbon biotransformation.

Application of compound specific and enantiomer specific stable isotope analysis for tracing the fate of HCHs at a landscape scale

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In recent years, compound-specific isotope analysis (CSIA) has become a routine approach for monitoring and evaluation of the in situ transformation of contaminants at polluted sites. Hexachlorocyclohexane (HCH) isomers and enantiomers are pollutants of particular concern because of their widespread distribution, toxicity, and persistence in the environment. Since only γ-HCH (Lindane) has pesticide activity, the purification of Lindane resulted in the production of other waste isomers which were mostly dumped near the production site. These waste HCH isomers with nearly no degradation were defined as muck. Thus, the muck samples can represent the original mixture of waste material from HCHs production. One of the HCH isomers is α-HCH possessing two enantiomers. Enantiomer selectivity can be used to distinguish biotic and abiotic degradation. Therefore, enantiomer specific analysis and CSIA can be combined as enantiomer specific isotope analysis (ESIA) for evaluating the transformation of α-HCH. In this study, we selected Bitterfeld, Germany, as the model field site for investigation. Technical HCH mixtures and Lindane had been produced in Bitterfeld for 30 years until 1988. In the vicinity of the former dump sites and the production areas, large plumes of HCHs persist in two aquifer systems. The soil and wetland in the north part of the production and dump area were also contaminated by the HCH containing materials due to periodic flooding. We aimed to evaluate the degradation of HCHs in soil, sediment, groundwater, and also the transfer of HCHs from soil to plants using CSIA and ESIA.

In this study, muck samples from dump site were analyzed to get the isotope signature and enantiomer ratio from the original source. Samples of groundwater, soil and sediments were analyzed for investigating the reactive transport processes in a catchment area using the muck as a reference. Plants and the corresponding soil were used to investigate uptake process of HCHs from soil to plants.

Compared to the muck samples, isotope fractionation and enantiomeric fractionation were observed in groundwater, soil, sediments and plant samples. Especially in groundwater, carbon isotope enrichment up to 8.6‰ and enantiomeric fractionation of α-HCH up to 0.35 suggested the transformation of HCHs. Based on isotope enrichments in the groundwater, HCHs degradation was estimated from 15% to over 90% by using the Raleigh equation approach. Compared to the muck, in the soil and sediment samples, the enrichment in carbon isotope composition is up to 5.8‰ which indicate transformation in the field site. Only α-HCH and β-HCH were detected in plant samples and the isotope composition were all shifted compared to the corresponding soil. This suggested degradation of HCHs during uptake process.

This study shows that CSIA and ESIA can be applied to investigate *in situ* (bio)degradation as well as to evaluate uptake into plants which is an entrance point for the investigation of the food chain. The isotope study provides information about the status of biodegradation on a catchment level and might be used for managing contaminated catchment areas.

Application of compound-specific stable isotope analysis to characterize photolysis of tributyl phosphate (TBP)

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In recent years, compound-specific isotope analysis (CSIA) has become a routine method to characterize the degradation mechanisms of organic compounds. Tributyl phosphate (TBP) is the most common organic phosphate esters (OPEs), which were detected in the surface water, due to their widely usages as flame retardants, plasticizers, and extraction solvents. TBP is expected to undergo very slow hydrolysis in the environment due to estimated hydrolysis half-lives of 9.9 to 11.5 years at pH 5 to 9. This means hydrolysis of TBP will be too slow under typically neutral or slightly acidic environmental conditions to reduce TBP in a reasonable time frame. However, photolysis may be fast enough to be an important degradation pathway in the aquatic environment. In this study, we aim to identify the pathways of indirect photolysis (UV/H_2O_2) and direct photolysis of TBP using CSIA. We used 280 nm long pass filter during all experiments in order to simulate the sunlight wavelength at earth surface. Within 5.4 h, more than 94 % degradation was observed during inoxidation reaction with OH radicals generated by UV irradiation with H_2O_2. The direct phototolysis is slower as 93 % of TBP was photolyzed after 144 h as well. Our preliminary results showed that the degradation of TBP conformed to the first-order kinetics and also accompanied with a slight isotope fractionation in both indirect and direct photolysis. The carbon isotope enrichment factors (ε_C) determined for direct photolysis and oxidation by OH radicals were $-0.5 \pm 0.1‰$, $-0.7 \pm 0.2‰$ respectively, which indicated the potential to identify specific reaction in the environment. To have a better understanding on the photolysis pathways of TBP, hydrogen isotope fractionation will be investigated in next step.

Iron solubility and isotopic fractionation in cloud-processing of industrial aerosols

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Iron (Fe) is an essential micronutrients in surface ocean waters. Fe in atmospheric dust deposition could represent the main source of bioavailable Fe in HNLC (High Nutrient Low Chlorophyll) zones (e.g. the Southern Ocean). If natural emissions (soil dust) are the most important sources of atmospheric Fe, the aqueous solubility of natural Fe-bearing particles is very limited. Conversely, anthropogenic Fe-rich particles, as fly-ashes (combustion aerosols), display an higher solubility (Sholkovitz et al., 2012). Then, how human activities impact the transport of bioavailable iron to the oceans remains a fundamental question addressed in atmospheric and oceanographic studies.

With the recent advances in mass spectrometry, it is now possible to measure precisely small variations in Fe isotopic composition, as they are controled by many physical, geochemical and biological processes. If the isotopic signature of radiogenic isotopes, such as lead, has been widely used to assess the contribution of pollution aerosols for transport and deposition fluxes in the low troposphere, the non-traditional heavy stable isotopes, such as Fe, are still understudied for atmospheric processes. Now, if reported values for Fe isotopic fractionations in natural aerosols (soil dust: δ^{56}Fe ≈ +0.08‰ / IRMM-014) are close to those of the average signature of igneous rocks (Beard et al., 2003), a previous work (Flament et al., 2008) showed δ^{56}Fe reaching +0.14‰ in urban aerosols influenced by industrial emissions. In the present study we tentatively assess the isotopic fingerprint of atmospheric transport on anthropogenic Fe, by simulating cloud processes affecting industrial Fe-bearing particles.

The studied sample was collected on the industrial filter located in the chimney stack of a metallurgy plant (cooling area of the sintering unit). This sample was fully characterized by X-Ray diffraction, laser diffraction particle sizing and scanning electron microscopy (SEM-EDX). Weathering processes were simulated using 300-400 µg of industrial particles into 10 mL of a leaching solution (13µM oxalic acid solution, pH≈4.5) mimicking cloud droplets. This represents 15-20 µg of total Fe. Each leaching experiment was performed for 60 minutes under simulated insolation The Fe concentrations in leachates were measured by Quadripole Inductively Coupled Plasma Mass Spectrometry (ICP-MS) at the ULCO/LPCA laboratory and Fe isotopic compositions by Multiple Collection-ICP-MS (Nu Plasma II) at the ULB/G-Time laboratory.

The results of Fe fractional solubilities, under simulated solar irradiation, range from 2.3 to 7.8 %, which is clearly higher than solubilities reported for natural dust (0.5-2.0%, Sholkovitz et al., 2012). Variations in Fe isotopic composition of processed particles are compared to the measured fractional solubilities and Fe isotopic data reported in the literature for natural aerosols.

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Compound-specific Isotope Analysis (CSIA) of pesticides on the catchment scale – *S*-metolachlor pulses in agricultural runoff

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The recurrent detection of pesticides in groundwater underscores the difficulties in extrapolating laboratory tests to environmental conditions. Current approaches are often limited in their ability to evaluate the prevailing zones and periods (“hot/cold-spots and moments”) of pesticide dissipation at the catchment scale. Compound-specific isotope analysis (CSIA) may provide a complementary line of evidence to tackle pesticide degradation in agricultural systems at different temporal and spatial scales (Elsner and Imfeld).

The purpose of this study was to use CSIA of pesticides in agricultural catchments to delineate the dynamics of “hot spots” and “hot moments” of degradation and transport after pesticide use and during rainfall-runoff events. We evaluated the concentrations and carbon isotope ratios ($\delta^{13}C$) of the widely used pre-emergence and chiral herbicides *S*-metolachlor in soils and runoff of a 47-ha agricultural catchment (Alsace, France) during the corn growing season (March-June 2012). Concentrations of *S*-metolachlor in runoff remained above 10 $\mu g.L^{-1}$ throughout the study period, which underscored its persistence in the soil. *S*-metolachlor concentrations at the catchment outlet were highest (62 μg L^{-1}) following an intense rainfall-runoff event a few days after its application in the fields. The degradates MESA and MOXA accounted for a mass equivalent load of 7.3% of the total exported mass of *S*-metolachlor. The enantiomeric excess values were generally similar to those in the commercial products (0.72 to 0.74), suggesting unsignificant enantioselective degradation. We developped water and soil extraction methods for CSIA of acetanilides herbicides using solid phase extraction (SPE) to pre-concentrate large water volumes. No significant $\delta^{13}C$ shift was observed during the water or soil extraction procedure.

During the first runoff events that followed application in May, $\delta^{13}C$ of *S*-metolachlor at the catchment outlet (-32.4 to -31.6‰) were in the same range as the $\delta^{13}C$ of the commercial product (-31.9±0.31‰). In contrast, *S*-metolachlor became isotopically enriched during the growing season (June and July), which indicated degradation between the source and the catchment outlet. The $\delta^{13}C$ of *S*-metolachlor gradually increased by 2.5‰ ($\Delta\delta^{13}C$) at the outlet. Following *S*-metolachlor application, the bulk of the applied herbicide might have sorbed and only become rapidely mobilized during the first intense rainfall event of May 21, thereby limiting the reaction time for degradation. $\delta^{13}C$ values suggest that degradation mainly occurred from June on, which may reflect reduced microbial activity and biodegradation rates when temperatures were lower in April and May.

To assist the interpretation of discharge and herbicide concentrations, a reactive model for pesticides sorption, mobility and transport based on the distributed hydrological model BEACH (Sheikh et al., 2009), is currently developed, which incorporates the information from CSIA and enantiospecific stable isotope analysis (ESIA).

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Assessment of Degradation Potential of Atrazine in Deep Aquifers by Isotope Analysis

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Atrazine (ATZ) is still detectable in the environment even though it is prohibited in the EU since 2004. Its degradation takes mostly place within the topsoil, where the microbial activity is highest. However, since the transit time of pesticides in aquifers can be orders of magnitude higher than in soil, the question arises whether on-going attenuation of ATZ is significant, and can be still observed in groundwater.

Conventional assessments rely on parent compound/metabolite ratios. Such ratios, however, can be distorted if target analytes are subject to sorption. Further, such assessments are not possible if the transformation product of the pesticide is not known. Finally, they give wrong results if the transformation product gets further degraded resulting in a biased ratio. To circumvent this problem, compound-specific stable isotope analysis (CSIA) is an alternative analytical method to identify degradation processes. Analysis of natural isotope abundances and isotopic shifts within an analyte can help to determine the extent of pesticide degradation (Meyer and Elsner, 2013). These shifts are caused by kinetic isotope effects when reactive bonds containing the heavy isotope (e.g., ^{13}C) react more slowly than bonds containing its lighter counterpart (e.g, ^{12}C). However, CSIA by GC-IRMS is challenged by concentrations of ATZ and its metabolite desethylatrazine (DEA) that are within the lower sub µg/L range. Extraction from large amounts of water is necessary, and interfering matrix effects require the development of non-isotope discriminating enrichment and clean-up step of the samples for robust CSIA by GC-IRMS (Schreglmann K. et al., 2013). Hence, the aim of this study is to validate sample enrichment of large volumes of water (up to 100L) by solid phase extraction and consecutive clean-up by HPLC (fraction collection) to exclude method-induced isotope fractionation. With these results we characterize for the first time the extent of ATZ and DEA degradation derived from observed carbon isotope shifts of groundwater samples taken from a spring of a deep water sandstone aquifer. With a groundwater mean residence time of 10 to 20 years and a detectable ATZ concentration, the Luxembourg Sandstone formation is used as a prime example for this case study. Compared to commercially available atrazine standards, which usually have a $\delta^{13}C$ value of -24‰ to -33‰, the isotopic signature of ATZ determined in this study shows a significant enrichment of the heavier isotope ($\delta^{13}C$ values up to -16‰), indicating the degradation of ATZ within this aquifer.

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Microcosm studies indicate masking of stable isotopic effects during reductive dechlorination of chlorinated ethenes by sorption processes

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Chlorinated ethenes like Perchloroethene (PCE) or cis-1,2-Dichloroethene (cis-DCE) are xenobiotic volatile organic compounds (VOC) representing a serous group of groundwater and soil contaminants (toxic and carcinogenic). These compounds can be naturally degraded by dehalorespiration of soil- and groundwater bacteria. Degradation capability of dehalorespiring bacteria at a contaminated land has to be quantified in order to prospect natural attenuation. Biotic degradation processes can be quantified via stable isotope fractionation of the contaminant, parameterised by the isotope enrichment factor (ε). The methodology to determine ε is mainly performed by microcosm studies in batch scale observing the changes in concentration and isotope ratio of a compound (e.g. PCE, cis-DCE) in time. The presented project was performed in order to enhance the general understanding of isotopic effects during the degradation of chlorinated ethenes by endemic groundwater bacteria in sediment-water microcosms.

The aim of this project was to evaluate the influence of sorption processes, time scale, bacteria species and degradation rates on the magnitude of ε for PCE and cis-DCE. In addition, an algorythm to calculate ε of intermediate degradation compounds was tested. Data arised from 180 anaerobic microcosms monitored over two years as well as sorption experiments for PCE. Data acquisition was mainly done by Purge & Trap GC-MS and GC-C-IRMS for the microcosm studies and GC-ECD for the sorption experiments. Sediment and groundwater samples were taken from a geologically homogeneously contaminated land in Upper Austria showig reductive dechlorination of PCE (source contaminant) and its metabolite. The microcosms were inoculated with PCE or cis-DCE in pure phase after sediment samples were mixed with anoxic media generally comprising of sampled uncontaminated groundwater, sodium sulphide as reducing agent and sodium acetate as carbon source. The sorption experiments contained dried soil samples mixed with sampled groundwater amended with sodium azide to inhibit bacterial activity.

Results of the microcosm study showed a heterogeneous distribution of degradation end products and ε magnitudes of PCE within the spacial origin of the sediment samples. These findings could be linked to different potential dehalorespiring bacteria (measured via 16S-rDNA analysis) within the sediment samples. Furthermore, these bacteria showed difference in the magnitude of isotope enrichment factors. Sorption experiments showed low fraction of organic carbon (0-4%), where only 4 of 23 sampling spots exceeded the 1% level, resulting in low sorption coefficients. Evaluating time constant mass balances for isotope ratios and concentrations of all chlorinated ethenes within microbially active microcosm showed that increasing bacterial multiplication could be responsible for a decreasing ratio between actually measured and target concentration of PCE in the liquid phase. Explanations refer to the non-polarity of PCE promoting sorption to lipophillic layers, like cell membranes. These mass balance effects could not be observed for cis-DCE, as water solubility is higher and sorption capacity is lower. In addition, degradation rates and ε of PCE showed an inverse correlation suggesting that transport processes over bacteria membranes are not a limiting process increasing isotopic effects. The verification of the algorithm to calculate ε of the inoculum and its intermediates is being finalized. Summarised, isotopic effects appear to depend on characteristics of dehalorespiring bacteria.

The interface between the satuarted and unsaturated zone represents biodegradation hot-spots of BTEX

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Dissimilatory sulphate reduction represents an important process in the anaerobic degradation of organic contaminants in groundwater ecosystems. We therefore studied the sulfur cycle in a BTEX (*i.e.* benzene, toluene, ethyl-benzene, and xylenes) plume which interacts with the groundwater table. We found that the transition zone between the saturated and unsaturated zone represents a zone of variable redox conditions, in which redox cycling processes complicate the interpretation of concentration measurements and the microbial community structure to the point that reactive transport models have previously failed to reproduce observed trends. This geochemical complexity is studied through a suite of observations, including the use of sulfur intermediates such as S^0, SO_3^{2-}, and $S_2O_3^{2-}$, and microbial community analysis in conjunction with high-resolution sampling of the porous aquifer. In addition stable isotopes measurements of dissolved sulphate, hydrogen sulphide, and elemental sulphur were performed to shed light on sulphur cycling processes at this interface. Our results suggest that the biochemical gradient zone within the saturated-unsaturated interface may represent a hot-spot of aerobic and anaerobic BTEX degradation.

Induced biodenitrification by a dairy industry by-product as an electron donor source: Flow-through experiment

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Nitrate (NO_3^-), one of the most common groundwater pollutants, can cause health problems in humans and contributes to the eutrophication of surface water bodies. NO_3^- pollution sources are linked to extensive application of synthetic and organic fertilizers, inappropriate placement of animal waste and spills from septic system effluents. Denitrification is an effective process by which NO_3^- is reduced to nitrogen gas (N_2) under anaerobic conditions. However, in natural systems, denitrification is mainly limited by the availability of electron donors (Knowles, 1982). To overcome this natural restriction, biostimulation of heterotrophic denitrifying bacteria by means of adding an external organic electron donor has been commonly used. Frequently tested electron donors include pure compounds such as alcohols (ethanol, methanol) or sugars (glucose, sucrose) among others (Akunna et al., 1993, Fernandez-Nava et al., 2010). During last years, there has been an increasing interest in alternative sources of organic carbon such as compost or sawdust (Trois et al., 2010). Hence, the use of industry by-products as electron donors could provide important economic and environmental benefits compared to pure compounds. In this context, this work aims to evaluate the viability of whey, a dairy industry by-product, as an organic C source to promote denitrification and to prevent the generation of adverse compounds such as nitrite (NO_2^-). The secondary goal is to determine the isotopic fractionation (ε) of N and O of dissolved NO_3^- and to study the C isotopic evolution of the organic carbon source during the induced denitrification reaction. The isotopic characterization will be applied in future field-scale studies to quantify the efficiency of the in situ bioremediation treatment, as in Vidal-Gavilan et al. (2013).

This study is performed at laboratory scale by means of a flow-through experiment. Whey is injected periodically at the bottom of the column which operates with a constant NO_3^- input. During the experiment, different C/N ratios are tested. Preliminary results suggest that whey can act as organic carbon source to promote NO_3^- attenuation. NO_3^- concentration reduces after the first injection while NO_2^- significantly accumulates. However, NO_2^- accumulation decreases with time and complete NO_3^- depletion without NO_2^- occurrence is reached at day 16th. At day 24, whey injection is stopped to evaluate the role of biomass growth into the system as an alternative organic carbon source to promote denitrification.

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Isotopic signature of 4-nitrotoluene oxidation

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Remediation of the environment from nitroaromatic pollutants remains a constant challenge. Chemical and enzymatic oxidation can be used to deal with this problem. In substituted toluenes two competing reactions can occur; oxidation of the aromatic ring and oxidation of the methyl group. We have addressed the problem of this competition with the aid of two-dimensional compound specific isotopic analysis (CSIA) using isotopes of hydrogen and carbon. Simultaneously, the processes were modeled theoretically using standard DFT calculations for chemical oxidation with permanganate anion and enzymatic oxidation by nitrotoluene dioxygenase. We obtained good agreement for a number of mono- and disubstituted nitrotoluenes except 4-nitrotoluene. In order to find out the source of this inconsistency we have carried out extensive search for the appropriate functional suitable to describe the process of 4-nitrotoluene oxidation. Furthermore, we have started measurements on the chemical reaction using positions specific isotopic analysis. The results of these studies will be presented.

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Hydrogen isotopic fractionation of benzo(a)pyrene exposed to climate change conditions in a Mediterranean soil

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Benzo(*a*)pyrene (BaP) is a polycyclic aromatic hydrocarbon (PAH), semi-volatile compound, composed by five benzene rings. These compounds are produced due to anthropogenic pyrolytic processes such as combustion of fossil fuels and wood, industrial processes and traffic and also due to natural events such as volcanic eruptions and bush fires (Sander et al., 2013). In the environment, PAHs are transported over long distances in air before they settle on soil surfaces where they are exposed to solar radiation, and therefore, they can experience photodegradation (Van Jaarsveld et al., 1997).

Since the Intergovernmental Panel on Climate Change has predicted an increase in the global temperature of approximately 4°C by 2100 (IPCC, 2013), the aim of this study was to investigate the effect of this change on the photodegradation of BaP in a Mediterranean soil by exposing a Calcaric Regosol soil to a light with solar radiation spectrum (including UV-B) and at a temperature of 24°C (average Mediterranean area temperature of 20 °C, plus the 4 °C predicted) by using a climatic chamber. In order to investigate not only the climate effect, but also the potential photocatalysing effect of the soil (due to metal oxides or humic substances), this study investigated four different conditions –"soil + light", "soil + darkness", "no soil + light", "no soil + darkness"–. Samples were exposed to these conditions during different periods of time in order to observe the changes in concentration and hydrogen isotope composition (δ^2H) of BaP along 28 days.

Results showed a decrease in BaP concentration under all tested conditions, however it was faster, achieving complete removal, under "no soil + light", showing a potential major protective effect of soil matrix against light. Certain shifts in δ^2H (SD:±20‰) but without an obvious trend under "no soil + darkness" conditions could be attributed to potential experimental volatile losses, while under "soil + darkness" and "no soil + light" conditions, the decrease in BaP concentration was associated to a certain normal hydrogen isotope fractionation. However, the poor lineal correlation obtained might be linked to the coexistence of different non-predominant processes under these conditions. On the contrary, "soil + light" samples showed a clearer inverse hydrogen isotope fractionation allowing to calculate an epsilon (ε_H) of +137‰ ($R^2 = 0.78$). Inverse hydrogen isotope fractionation has been also recently associated to naphthalene oxidation by aerobic strains (ε_H up to +71‰) (Kümmel et al., 2016). As biodegradation was in principle excluded from our experiments by UV-C sterilization and dryness of the soil, this fractionation might be related to an oxidation of BaP by heterogeneous photocatalysis due to metal oxides present in the tested soil under light radiation (including UV-B).

Most of all, large inverse ε_H obtained would be useful to discriminate different BaP degradation processes in environmental samples in the future and proved the potential of the compound specific isotope analysis (CSIA) technique also to quantify and monitor the degradation of larger PAHs.

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Revealing the catastrophic events of AD 536 and AD 541 in Siberian tree-rings using a multi-parameter approach

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Stratospheric sulfate aerosols originating from large explosive volcanic eruptions may have far-reaching impacts on the radiation budget, atmospheric and surface temperatures, and regional weather patterns, and can thereby result in global climatic changes (Robock 2000).

Here we examine the response of Siberian larch trees to climatic and environmental changes associated with the volcanic events in AD 536 and AD 541 using a multi-parameter approach. The larches analyzed are growing at two high-latitude sites in northeastern Yakutia (69°N, 148°E, YAK) and eastern Taimyr (70°N, 103°E, TAY) as well as at a high-altitude site in the Russian Altai (50°N, 89°E, ALT). We analyzed $\delta^{13}C$ and $\delta^{18}O$ values from tree-ring cellulose, cell wall thickness of annual tree rings (CWT), latewood density (MXD) and tree ring widths (TRW) to better understand growth responses of trees to these catastrophic events. We hypothesize that major volcanic eruptions during the period of AD 520–560 have led to depressions of tree growth and that these depressions were due to a combination of reduced incoming solar radiation, temperature decrease and decreased vapor pressure for both the high- latitude and -altitude sites. We reveal that the years AD 536 and AD 541 are indeed associated with different, but marked changes in tree-ring parameters. Sharp declines observed in TRW, MXD, and CWT in AD 536, which are accompanied by simultaneous drops in $\delta^{13}C$ of cellulose for YAK and TAY. A significant decrease in $\delta^{13}C$ also indicates cold conditions with low vapor pressure deficits during the growing season, which is strongly enhanced by light reduction due to the presence of dust veils after volcanic eruption, which in turn led to a reduction in photosynthesis. A more gradual decline of in $\delta^{13}C$ is visible in the ALT series and goes along with decreasing TRW, caused by the same delayed effectiveness of light reduction. Very low $\delta^{18}O$ values are measured at ALT in AD 536, and reduced CWT and MXD values are seen in AD 536 and AD 537, in addition to frost-ring formation between AD 536 and 538. The significant and rapid decrease in $\delta^{18}O$ values (-4.8 σ relative to the mean) are a result of low condensation temperatures of atmospheric water vapor to precipitation, together with a low vapor pressure deficits due to low temperatures, which in fact controls needle water fractionation, thereby leading to a diminished needle $H_2{}^{18}O$ enrichment. The discrepancy in $\delta^{18}O$ between high-latitude and high-altitude sites most likely has its cause in a different hydraulic regime. While trees in ALT react predominantly to precipitation water throughout the growing season, the northern trees from YAK and TAY utilize to a large part a mix of precipitation water, and water originating from thawed permafrost. Therefore, the variability in $\delta^{18}O$ values is somewhat dampened in TAY and YAK. The recently published, improved chronologies for volcanic sulfate deposition in Greenland and Antarctic ice cores (Plummer et al. 2013; Sigl et al. 2013) point to a possible volcanic explanation for the catastrophic events recorded in Siberian trees.

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From Prehistory to History: change and continuation in the dietary habits from the inhabitants of the Balearic Islands based on ^{14}C and stable isotope research ($\delta\ ^{13}C$ and $\delta\ ^{15}N$)

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In a previous study the dietary habits from the inhabitants of the Balearic Islands (Mallorca & Menorca) were studied (Van Strydonck et al. 2005). The results showed that these island dwellers hardly ate any marine food and that there was no real trade in dairy products between the settlements. In this study the prehistoric data set is compared with data from Roman and Medieval sites (Christian as well as Muslim sites) and a sub recent cemetery. Data for more than 10 sites are compared. Some sites have only a few data points while others have more than 15.

There are major differences between the examined sites. The data from some smaller Islamic sites don't overlap. This is a strong indication for a lack of food trade between the sites. The Christian sites have, on the average, a higher $\delta\ ^{13}C$ and a higher $\delta\ ^{15}N$ value. Probably because of a somewhat higher consumption of marine products. The data of the Christian sites overlap each other, an indication that food is much more distributed than in the Islamic or prehistoric sites. Also the dispersion of the data is much more outspoken. Even sites with a very narrow range in isotope data show an important number of outliers, this is probably due to immigrants from other parts of the islands or from elsewhere. Differences in dietary habits based on social status can be ruled out in some cases, but not in all. In the Islamic sites we see statistically no difference between the data from female and male skeletons. The same is true for the Christian sites, but this conclusion can be invalid due to the lack of sufficient data. At two sites a possible immigrant, probably from North Africa could be detected because of the high $\delta\ ^{13}C$ value (C4 plant). The monks from the small island of Cabrera were immigrants as well and this is reflected in a large dispersion between the data points.

The city of Pollentia, the largest city in Roman times, has the largest dispersion of data points. On the average the $\delta\ ^{15}N$ values are the highest of all investigated sites. The dataset holds also some individuals with a very high $\delta\ ^{13}C$ value (C4 plants) and others with a very high $\delta\ ^{15}N$ value (marine fish consumption). These data are the reflection of a rather cosmopolitan city. It has also be proven that during the Muslim rule of the island Christians continued to have their own burial rites, proof of an open and tolerant society (Cau Ontiveros et al. 2016).

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Exploring social variability by stable isotope (C, N) ratios within the monastic and lay population buried at the post-medieval Carmelite friary of Aalst (Flanders, Belgium)

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The Carmelite friary (16th–18th c. AD) in Aalst, Belgium, was excavated in two phases, in 2004/5 and 2011, revealing about 240 human burials. In 2014, a pilot study was published using stable isotope ratios ($d^{13}C$ and $d^{15}N$) to explore the dietary patterns of 39 adult individuals excavated during the 2004/5 excavations (Quintelier et al. 2014). Samples derived from a mixed monastic and lay population buried in three different locations, i.e. the church, the cloister garth, and the northern and western cloister alley, reflecting groups with differing social status based on historic records and osteological data. The data of this pilot study showed significant differences in diet between females and males, as well as dietary differences between individuals buried at the different locations within the friary, likely reflecting social stratification. In 2011, the eastern and the southern part of the cloister alley and a larger part of the cloister garth were excavated, revealing another 160 human burials. This created the opportunity to enlarge the existing dataset by sampling and analysing individuals from all burial locations within the cloister walls for stable isotope ratios, including adult as well as non-adult individuals, in order to further explore social variability in diet within the post-medieval population buried with the Carmelite friars. This paper presents the results of the 62 new samples and provides an overview and interpretation of the combined analyses (n=101).

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$\delta^{18}O$ composition of archaeological collagen: promise and problems

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Ecological and forensic work on hair and feather keratin regularly includes determination of protein $\delta^{18}O$ composition, which is linked to diet/drinking water composition, and therefore to location of origin. Only a few groups have studied $\delta^{18}O$ in the principal protein of archaeological interest, bone collagen, which could potentially yield similar information, though over a different timeframe in relation to the life of the organism.

Preparatory laboratory workup for collagen is however very different from workup for keratin, as it involves demineralisation (and typically gelatinisation) in addition to defatting. The potential effects of demineralisation reagents, most commonly aqueous solutions of HCl or EDTA, must therefore be characterised, especially given that proteins are known to exchange carboxylic O at low pH. It is therefore possible that laboratory workup for collagen may introduce exogenous O into the collagen molecule.

This presentation presents the results of an experiment on four bone collagen materials with a range of $\delta^{18}O$ values to examine the effects of (1) the workup methodology (EDTA/HCl) and (2) the isotopic composition of workup solutions ($\delta^{18}O$ range -21 to +10‰) on resultant collagen $\delta^{18}O$ values. The data demonstrate that O exchange does occur during collagen workup, and that this effect is greater with HCl methodologies. The presentation will discuss the magnitude of these effects relative to endogenous $\delta^{18}O$ variability, and how O exchange may be affected by taphonomic degradation of collagen molecules.

Stable hydrogen isotope signatures of lignin methoxyl groups of four tree species across Germany

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The δ^2H signature of the lignin methoxyl groups (δ^2H_{LM} values) of tree wood have been shown to reflect δ^2H signature of precipitation (δ^2H_{precip} values) modulated by a large uniform apparent fractionation (ε_{app}) (Keppler et al., 2007). Thus, the δ^2H_{LM} values were suggested as a potential paleotemperature proxy since δ^2H_{precip} values are dominantly controlled by air temperature in the mid-latitudes. A recent tree ring study that investigated the δ^2H_{LM} values of various tree species across Europe strengthened this suggestion: a strong linear relationship between δ^2H_{LM} values and mean annual temperatures was found (Greule et al., *in revision*). However, using δ^2H_{LM} as a paleotemperature proxy requires further understanding of, for instance, noise emerging from site and species-specific influences in order to improve tree sampling strategies. This may lead to an increased temperature significance of the δ^2H_{LM} proxy which is particularly required to resolve smaller temperature changes of lower than 2 °C.

Here, we collected tree ring sections of four different species (Norway spruce, European beech, English oak, Scots pine) at 15 sampling sites across Germany with MAT differences of Δ 5 °C. At every sampling site ≥ 3 trees of the four 4 aforementioned species were used to retrieve 3 core samples. In total this led to 660 tree ring cores. Beside the spatial temperature variations every sample site experienced also a small temperature increase from 1981-1990 to 1991-2011 (~ Δ 0.7° C). Each core was dissected for both time periods and subsequently homogenized prior to δ^2H_{LM} measurements. Hence, the sampling design allowed a detailed assessment of four tree species for the (i) 'within tree' and 'within site' variability of the δ^2H_{LM} values (ii) species-specific differences in ε_{app} and (iii) the relationship between δ^2H_{LM} values and both spatial and temporal temperature variations.

For the δ^2H_{LM} values we found a mean 'within tree' variability of <6‰ and is considered negligible and was also evident for the 'within site' variability for some locations. Nonetheless, most sampling sites showed an elevated 'within site' variability of >20‰, regardless of tree species, that is suggested to reflect hydrologically heterogeneous micro-environments. Among the four investigated tree species differences have been noted in ε_{app} particularly for Norway spruce which suggests that single tree species should be preferred in future studies. The general pattern of the spatial δ^2H_{LM}-MAT relationship as found for the European data set (Greule et al., *in revision*) has been reproduced for the German data set but lacks a significant correlation. We explain this by a poor spatial δ^2H_{precip}-MAT correlation ($R^2 = 0.2$) found for Germany. The temperature difference between 1981-1990 and 1991-2011 was much lower (~ Δ 0.7° C) when compared to the range of MAT for the sampling sites (Δ 5 °C). However, δ^2H_{LM} values of European beech trees indicated temperature changes correctly within ± 2 °C. Thus, the δ^2H_{LM} values are considered to possess the highest potential of reconstructing temperature changes when applied on tree ring chronologies (single site and single species) and, thus, may particularly be interesting for Late Holocene climate studies.

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253 Plus – New 10 kV Isotope Ratio Mass Spectrometer: Robust Precision and Accuracy for Low Abundance Isotope Research

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The clumped isotope application requires precise and accurate measurements of rare isotopologues on small sample sizes (Eiler et al. 2003). The 253 Plus is a further development of the successful MAT253 10 kV mass spectrometer, which has been the instrument of choice since clumped isotope science developed and low abundance continuous flow applications.

Careful ion optical design investigations led to reshaping of the magnet which significantly improved peak shapes. Building on the experience of the MAT253 the surfaces of the gas inlet system and the ion source are machined to minimize scrambling and isotope exchange effects. All along the ion optical path special care has been taken to minimize any scattering, which could disturb the analytical baseline and possibly affect Δ47-CO_2 linearity effect. A Faraday cup between mass positions in the collector array is introduced to monitor the baseline during analysis. A new software workflow, LIDI (Long Integration Dual Inlet), is integrated into ISODAT to support higher productivity, reduced sample consumption, improved sample utilization and hence increased sensitivity. The LIDI workflow allows analysis of smaller samples to enable high resolution studies. The benefits of advanced signal integration with all new features of the 253 Plus will be presented.

High-gain 10^{13} ohm amplifier technology is integrated into the 253 Plus, to further reduce the amplifier noise by a factor of 3. These instrumental improvements will push the boundaries for clumped isotope applications as well as for GC-IRMS where sensitivity, precision, stability and low noise are key features for high precision measurements.

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The integration of n-alkanes δ²H values into sedimentary archives at the landscape level scale

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The compound-specific δ^2H values of terrestrial plant derived leaf wax *n*-alkanes have emerged as potentially powerful paleoclimate proxy. These long-chained hydrocarbons (typically nC_{27}, nC_{29}, and nC_{31}) persist in the environment over geologic time due to the bonding structure between the hydrogen and carbon atoms, carrying with them a δ^2H value that can be traced back to the hydrological cycle, and therefore the climate during synthesis. Large areas of the biotic and abiotic processes that drive *n*-alkane δ^2H values at the scale of the individual leaf have already been described. One critical area that has received little attention is how the processes that operate at the individual leaf level become integrated at landscape level scales to produce the sedimentary *n*-alkane δ^2H value chronologies we make our paleoclimate reconstructions from.

Significant variability can often be observed in the δ^2H values for homologous alkane chains between different leaves of the same individual, between different individuals of the same species, and in particular between different species subject to the same environmental conditions. We selected 6 self-contained alpine lake catchments in the Alps of Ticino, Switzerland, on the basis of variability in altitude, vegetation coverage, species composition, size and, topography. Our goal was to gain a quantitative insight into how precipitation δ^2H values and leaf water evaporative enrichment shape *n*-alkanes δ^2H values in the extant vegetation and how these in turn relate to δ^2H values preserved in the surface soils, and then to δ^2H values preserved in the lacustrine surface sediments. By separating each lake catchment into sub-sites on the basis of topographical features and different vegetation communities. We mapped the 5 dominant plant species and their % coverage, and sampled them for leaf water δ^2H, xylem water δ^2H, *n*-alkane δ^2H and *n*-alkane concentration. With this information we build *n*-alkane concentration- plant species biomass weighted- integrated spatial analyses of *n*-alkane δ^2H values from the existing vegetation and compare these to the *n*-alkane ^{2}H values recovered from soils and sediments.

Preliminary results suggest the δ^2H values of extent vegetation are highly variable with 120 ‰δ^2H variability observed for nC_{29} from a single site, collected at the same time. However, it is possible to disentangle this variability and when we step back to the landscape scale distinct patterns emerge. For example, grass species average -220 ‰δ^2H for nC_{29} while woody plant species average -150 ‰δ^2H, therefore separating the vegetation by broad plant functional groups already explains large portions of the variability observed. In my presentation, I will assess (i) if the lacustrine surface sediment *n*-alkane ^{2}H values integrate an entire catchment signal or simply the riparian vegetation signal, (ii) if *n*-alkane δ^2H values of some plant species were preferentially incorporated into the sediment record over others and (iii) how precipitation and leaf water evaporative enrichment shape these *n*-alkane δ^2H values.

Cave bat guano $\delta^{13}C$ and $\delta^{15}N$ values as proxy for climate and anthropogenic activity

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Understanding the complex drivers behind Earth's past climate changes may help predict the likelihood, extent, and patterns of future changes. Cave deposits are of special interest because caves provide somewhat isolated environments that are physically and chemically more stable on short time scales, allowing for a better preservation of deposits that archive environmental conditions (speleothems, guano, sediments, etc.) (Bradley, 2015). Geochemical studies of cave guano using stable isotopes may provide valuable paleoecological and paleoclimate (precipitation) records (e.g., Wurster et al., 2007; Onac et al., 2014).

Cave guano $\delta^{13}C$ values reflect the dietary preferences of bats that are controlled by local vegetation dynamics, which in turn depend on local climatic conditions.

The aim of the present study is to demonstrate the usefulness of stable carbon and nitrogen isotopes in bat guano as proxies for paleoclimate changes and anthropogenic activity. We show a 2500-year record of environmental change in Romania using $\delta^{13}C$, $\delta^{15}N$, and (C:N) derived from precisely ^{14}C-dated bat guano cores recovered from Gaura cu Muscă (GM), Zidită (ZC), and Măgurici (MC) caves (Onac et al., 2015; Forray et al., 2015).

$\delta^{13}C$ values in guano at the Medieval Warm Period (MWP) - Little Ice Age (LIA) transition changed markedly only in the GM record. In the other two caves the carbon isotopic values indicate a steady onset of LIA at around AD 1200 but without significant climatic changes before ~ AD 1450 and rapidly ending between ca. AD1870 and 1900. The beginning of the 20th century is characterized by an average $\delta^{13}C$ value of –25.2‰ up to ca. AD 1965 (which indicate a moderate change toward warmer period). More rapid changes in $\delta^{13}C$ values are recorded after AD 1970. These rapid changes by 0.5 to 1.5‰ were recorded within a few years indicates swings between several cold and warm events.

$\delta^{15}N$ values of guano can be utilized as an integrator of past states of the local nitrogen cycle. A $\delta^{15}N$ record from a 1.5-m core of bat guano deposit from Zidită Cave (Romania) provides a record of climatic and anthropogenic influence on the regional nitrogen pool. The $\delta^{15}N$ values of guano has decreased from 12.5‰ to 7‰ since AD 1950, suggesting the N-cycle has been trending towards a more conservative state in response to lower water availability.

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Development and validation of a PALEOHYGROMETER based on the coupling of $\delta^2H_{n\text{-alkane}}$ and $\delta^{18}O_{sugar}$ results

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The hydrogen isotopic composition (δ^2H) of leaf waxes, especially of *n*-alkanes ($\delta^2H_{n\text{-alkanes}}$), is increasingly used for paleohydrological and paleoclimate reconstructions. However, it is challenging to disentangle past changes in the isotopic composition of precipitation and changes in evapotranspirative enrichment of leaf water, which are both recorded in leaf wax δ^2H values (Zech et al., 2015).

In order to overcome this limitation, Zech et al. (2013) proposed a coupled $\delta^2H_{n\text{-alkanes}}$–$\delta^{218}O_{sugar}$ biomarker approach. This coupled approach allows for calculating (i) biomarker-based "reconstructed" $\delta^2H/\delta^{18}O$ values of leaf water ($\delta^2H/\delta^{18}O_{leaf\ water}$), (ii) biomarker-based reconstructed deuterium excess (d-excess) of leaf water, which mainly reflects evapotranspirative enrichment and which can be used to reconstruct relative air humidity (RH) and (iii) biomarker-based reconstructed $\delta^2H/\delta^{18}O_{precipitation}$ values. This approach was validated by Tuthorn et al. (2015) by applying it to topsoils along a climate transect in Argentina. Accordingly, both the reconstructed RH and $\delta^2H/\delta^{18}O_{precipitation}$ values correlate highly significantly with actual RH and $\delta^2H/\delta^{18}O_{precipitation}$ values.

Here we present for the first time also coupled $\delta^2H_{n\text{-alkane}}$-$\delta^{18}O_{sugar}$ results obtained for leaf material from a climate chamber experiment, in which *Eucalyptus, Vicia* and *Brassica* were grown under controlled conditions (Zech et al. 2014). The biomarker-based reconstructed RH values correlate well with the actual RH values, validating the proposed "paleohygrometer". Moreover, the proposed coupled $\delta^2H_{n\text{-alkanes}}$–$\delta^{18}O_{sugar}$ biomarker approach will allow more robust $\delta^2H/\delta^{18}O_{precipitation}$ reconstructions in future paleoclimate research.

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Environmental records in river carbonates: reliable proxies or erratic data? (example from Krka, Slovenia)

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River carbonates (tufas) as chemical precipitates have been extensively studied as palaeoenvironmental and palaeoclimate indicators, however, the interpretation of elemental and isotopic records is often biased because of the complex and dynamic nature of river systems, in particular those in humid temperate karstic regions. To understand the geochemical and stable isotopic relationships between the tufa precipitate and parent water is of crucial importance for the interpretation of environmental conditions revealed from (fossil) tufa deposits. The focus of the study was to investigate the relationship between the temperature, precipitation rate and the content of detrital material in tufa on one hand, and its isotopic composition and elemental ratios as the most common palaeoclimate proxies on the other.

Tufa and river water in a groundwater-fed karstic river (Krka, Slovenia) were studied during a 3 year period at 16 consecutive barrages along a 13 km river section. Physicochemical and isotopic parameters in water were monitored seasonally, while tufas were analysed for their petrographical, mineralogical, elemental and stable carbon (δ^{13}C) and oxygen (δ^{18}O) isotope composition. The attemps to measure the net precipitation rate failed because we were not able to quantify the accumulation and erosion with reasonable certainty, therefore the carbonate precipitation rate was calculated using the diffusion-boundary layer (DBP) model (Liu & Dreybrodt 1997). It was found that carbon and oxygen isotope exchange between tufa and river water are controlled by disequilibrium (kinetic) isotope fractionation. Biological activity and water temperature had a minor influence on the carbon isotope fractionation, while rapid CO_2 degassing and consequent tufa precipitation control the isotopic enrichment of the remaining dissolved inorganic carbon (DIC) reservoir, with progressive tufa precipitation downstream. Similarly, the high precipitation rate of tufa and high pH control the oxygen isotope fractionation, being ~2 ‰ lower than the assumed equilibrium. Nevertheless, the δ^{18}O proxy showed good agreement between measured and calculated water temperatures for the spring–summer period using the commonly used palaeotemperature equations (e.g. that of Anderson & Arthur (1983) or Hays & Grossman (1991), showing the importance of the fractionation factor choice. On the contrary, the elemental proxies performed rather poorly. It seems the effect of the precipitation rate on δ^{18}O values was much stronger than that of temperature, which is in line with experimental results of Dietzel et al. (2009).

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Multi element (HCNS) stable isotope analyses along hair strands

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Continuously growing hair can be used as an informational source to recognize changes of living circumstances over time. In general, stable isotope data of the bio elements (H, C, N, O, S) reflect geographical information due to regional nutrition habits and climate conditions in the living area of an individual. Isotope signals of local foods and drinks are incorporated into hair keratin almost immediately after dietary intake (Lehn et al. 2015).

Human scalp hair was used to receive geographical information over the past months or years of an individual's life. Hair strands with a length of 54 cm or 15 cm were obtained from two women of German and Japan respectively who stayed abroad in different countries for some weeks or months. Hair segments of 0.5 cm, which span a growth period of about two weeks, were cut along the strands and analysed for δ^{2}H, δ^{13}C, δ^{15}N and δ^{34}S.

The results of the isotope analyses along the hair strands stated that the most conclusive parameters related to the changes of whereabouts were δ^{13}C and δ^{34}S, their values indicated a high-resolution power connected to the stays abroad. But clear isotope signals were only visible for the most recent part of the hair strand, particularly over the proximal hair length of about 10 cm. With increasing hair length, isotope signals became blurred, probably caused by the presence of different growth phases or growth velocities of single hairs. A growth rate variation in individual hairs of 30% can be reasonably concluded. The fastest growing hair mark the date of change of e.g. geographical whereabouts or living circumstances. To specify the precise date by isotope analyses along a strand, the mean hair growth rate plus 30% should be taken into account.

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Determining the geographical origin of chicken eggs in the United Kingdom

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The UK Food labelling regulations (1996), ultimately amended by Regulation (EU) 1169/2011, mandate the country of origin labelling requirements for specific commodities including eggs. To enforce labelling claims, analytical methods are required that are able to confirm country of origin claims. Intensive farming practises, applied in e.g. poultry industry, might require non-local feed. Studies examining the relationship between isotopic signature of an animal and that of the food and water source (Rhodes, C. N. et al.(2010), Ben-David, M. et al. (2012)) have been performed for selected isotopes.

The initial multi-stable isotopes approach – measuring HCNS of freeze-dried egg white and feed – and the latest approach of comparing oxygen isotopes of drinking water with liquid egg white, in order to detect possible produce extension in UK eggs, will be presented.

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Improved Discrimination of Duct Tapes using Carbon and Hydrogen Isotope Ratio Variations in Duct Tape Polyester Fibers

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At the Netherlands Forensic Institute (NFI) isotope ratio mass spectrometry (IRMS) is used in combination with (LA-)ICPMS to investigate potential relations between different materials in forensic casework investigations (various tape types, adhesives, paper, safe wall filling materials, jeans, motor oils, polyester trousers, polypropylene rope, poly methyl methacrylate objects, candles, polyethylene jerry cans,…).

A prominent example material is (grey/silver) duct tape as it is commonly used in Dutch households and thus often found at crime scenes if tape is encountered. If a visually similar tape is retrieved during *e.g.* a search at a suspect's house, a request is almost always made to compare the tape materials. If tapes are not discriminated using visual comparison (color, thickness, width) and FT-IR, other techniques such as LA-ICPMS and IRMS are used to investigate chemical and isotope composition characteristics.

Duct tape mainly consists of three different layers: 1) a backing film, 2) a network of cotton or polyester fibers (web) and 3) an adhesive layer (glue). These tapes can be used for several years with intermediate storage of the tape roll. Previous NFI R&D investigations showed that the separation of the adhesive layer from the backing film increases the value of forensic evidence and evaluated the background variation of carbon and hydrogen isotope ratios from the backing from 44 duct tape rolls randomly purchased in the Netherlands.

With this subsequent study $\delta^{13}C$ and $\delta^{2}H$ data are presented from the backing of 120 duct tape rolls from various duct tape products as sold in the Netherlands in order to evaluate the discrimination power of this approach. Secondly, the added forensic value of carbon and hydrogen isotope ratios from the cotton/polyester layer in duct tapes will be demonstrated.

Since the isotope discrimination between different batches produced during a short production period was weak using the backing material only, an analytical procedure was developed and validated to determine $\delta^{13}C$ and $\delta^{2}H$ from the isolated web material. The added level of discrimination provided by polyester fiber isotope ratios ($\delta^{2}H$ and $\delta^{13}C$) was evaluated using the pairwise comparison of averaged isotope ratios. Within the study population of duct tapes polyethylene fibers, the values vary substantially (ranges of 38.8 ‰ and 3.6 ‰, respectively) within brands and product lines, but being homogeneous within tape rolls. The $\delta^{2}H$ and $\delta^{13}C$ isotopic ratios differ between lengthwise and crosswise polyethylene threads.

The comparison showed that 21 out of 23 tapes can be discriminated allowing for within and between brands and batches differentiation of the duct tapes. The remaining non discriminated pair of grey duct tapes were both rolls of a single Pattex tape product as acquired on the same day in two different shops from a single chain of stores. It can therefore not be excluded that these two rolls originate from a single production batch.

The method developed in this study is now applied in forensic duct tape casework applications. A special application is the use of duct tape in some arson investigations where Molotov cocktails are used as an incendiary device and duct tape is used for the device. From the remnants of a Molotov cocktail burnt duct tape materials were retrieved (backing layer and adhesive had mostly disappeared but polyester web material was still present and even partly not melted. These duct tape remnants provided enough characteristics for a comparison with a duct tape roll as found with a suspect and resulting in a strong link between the Molotov cocktail and the suspect.

EU funded project 'SPICE Profiling' (2015-2017) – Isotopic profiling of new psychoactive substances (NPS): A case study on MDMB-CHMICA

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Since 2008, the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) observed a dramatic increase in new psychoactive substances (NPS) on the European drug market. These substances are mainly represented by synthetic cannabinoids; they are typically added to herbal blends and bath salts. With each consecutive year rising numbers of NPS are reported by forensic and clinical institutes around the world (14 in 2005 to 81 in 2013 (EMCDDA, 2014)]). Many of these substances are highly potent and produce unpredictable effects on consumers. Therefore, the NPS phenomenon is one of the most urgent drug-related problems in the European Union.

The project SPICE-profiling is funded within the EU's ISEC 2013 programme (JUST/2013/ISEC/DRUGS/AG/ISEC/4000006421). The knowledge about the production and the supply chain of NPS is very limited and is assumed to be quite different from classic synthetic drugs (amphetamine-type-stimulants). One goal of the project is to obtain information about the manufacturing procedures, the required key chemicals, the origin of adulterants and plant matrices and the chemical relations between different products. Therefore, test purchases of NPS samples in internet shops, authentic samples from the chemical manufacturers (typically from China) and NPS samples from seizures by custom and police will be collected and subsequently analyzed by different analytical methods: chemical impurity profiling by gas chromatography-mass spectrometry and isotopic profiling procedures by isotope ratio mass spectrometry (IRMS). For reference purpose, controlled syntheses of selected NPS with different batches of precursor chemicals will be conducted.

In December 2014, the custom of Luxembourg made a large seizure of 40 kg (40 bags à 1 kg) pure MDMB-CHMICA (Methyl (S)-2-(1-(cyclohexylmethyl)-1H-indole-3-carboxamido)-3,3-dimethylbutanoate). This NPS is mainly added to herbal blends and it is highly potent. Because the amount of MDMB-CHMICA was so high in many Spice products, it was responsible for several fatal casualties. Since November 2015, it is banned in Germany.

In the case study, the 40 pure MDMB-CHMICA samples from Luxembourg and over 100 CHMICA samples extracted from Spice products were/will be analyzed using IRMS. The isotope ratios of $\delta^{13}C$, $\delta^{15}N$ and $\delta^{2}H$ will be determined using elemental analyzer (FlashEA), high temperature conversion elemental analyzer (TC/EA) and gas chromatography (GC) coupled to an isotope ratio mass spectrometer. Differences and similarities of the isotope ratios will be discussed. Prospectively, the IRMS techniques will help to link different seizures and link seizures to clandestine producers.

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Isotope profiling in food and alcoholic beverages authentication

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Product Authentication Laboratory, a new laboratory located in Lodz, is mostly involved in the food control by employing isotope profiling and NMR profiling of organic compounds. We are working on the implementation of isotopic methods enabling quality control and detection of frauds in alcoholic beverages, honey and juice. The Quality Management System according to the ISO/IEC 17025:2005 norm for some methods will be introduced soon.

In the case of isotope profiling there are two methods of the isotope ratio measurement: isotope ratio mass spectrometry (IRMS) and nuclear magnetic resonance spectroscopy (NMR). Our laboratory is equipped with isotope ratio mass spectrometer (MAT 253) with elemental analyser (Flash 2000 HT) and nuclear magnetic resonance spectrometer (Bruker 500 MHz) for liquid analysis (one probe with ^{19}F lock channel is dedicated to the deuteron measurement).

Currently we are working on the implementation of isotopic methods enabling quality control and detection of frauds in alcoholic beverages. We introduce an innovative control method of rye spirits in order to ensure the quality of Polish vodka. The approach was holistic by using advanced technology such as SNIF-NMR, IRMS, ICP-MS.

Our research interests are also in the application of isotope analysis for authentication of wine. We employ SNIF-NMR combined with metabolomics (^{1}H and ^{13}C NMR). We develop also pyrolysis technique for the direct determination of oxygen isotope ratios ($\delta^{18}O$) of wine water and ethanol.

Traceability of Mozzarella di Bufala Campana by means of multi-element stable isotopes analysis

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Nowdays, the identification of the geographical origin of food products results very important for both producers and consumers and it is closely related to the quality of foodstuffs. In this context, stable isotopes ratio analysis is confirmed as a valued technique to discriminate the production area of foods, of particular relevance in the case of PDO (Protected Denomination of Origin) products.

In this study, we applied this methodology to characterize the Mozzarella di Bufala Campana, a typical dairy product of South Italy, made with buffaloes milk, produced exclusively in the areas designated in the production disciplinary of the Ministero delle Politiche Agricole e Forestali.

The aims of the project are: i) to characterize the whole farm making process of different agricultural company, starting from the carbon, nitrogen and oxygen isotopic signal of buffalos fodder, through the milk one and finally that of the mozzarella produced; ii) to compare the stable isotopes ratios of Mozzarella produced in the different provinces approved by the disciplinary.

Preliminary results showed a discrimination of samples by carbon and oxygen isotopes, while not from nitrogen signals. In addiction, the $\delta^{13}C$ of Mozzarella resulted directly related to the carbon isotopes signal of fodder.

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Food forensics – Multi-elemental and stable isotope analysis for authenticity testing of high-value plant products

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Adulteration of foods and beverages is an increasing global problem and new fraud cases are often reported. Several plant products are at risk of adulteration as they are sold at premium prices due to quality claims regarding unique geographical origins or specific methods of cultivation, processing or production. Consequently, analytical methods that can verify the authenticity of plant products are increasingly requested.

Some of the most promising methods for authenticity testing of plant products are based on Inductively Coupled Plasma – Mass Spectrometry (ICP-MS) or Isotope Ratio – Mass Spectrometry (IR-MS). In combination, these analytical techniques enable analysis of numerous elements of the periodic table as well as stable isotope ratios of selected elements at the natural abundance level. Traditionally, multi-elemental analysis of plant tissue has focused on the essential plant nutrients (H, B, C, N, O, Mg, P, S, Cl, K, Ca, Mn, Fe, Ni, Cu, Zn, Mo) and selected non-essential heavy metals such as Cd and Pb using complementary analytical techniques. However, it has recently been shown that rather unexplored analytical methods such as semi-quantitative ICP-MS and compound-specific isotope ratio analysis (CSIA) can generate hitherto unseen elemental coverage and superior classification power when evaluating the authenticity of plant based food products [1-2]. When combined with chemometrics, these analytical advances constitute promising tools for determining where and how plants have been grown. At the conference, an overview of climatic, edaphic and biological factors controlling elemental and isotopic plant fingerprints will be given. Emerging analytical approaches such as compound-specific stable isotope ratio analysis of plant-derived sulphate and nitrate will be presented, and its suitability for authenticity testing of high value plant products will be demonstrated and discussed.

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Two-dimensional nitrogen isotope analysis of ammonium nitrate

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Stable isotope analysis is a suitable method for tracking and discriminating sources of explosives and related substances. Compared to other explosive nitro-organic compounds, the natural abundance range of ^{15}N in ammonium nitrate (AN) is rather narrow (Benson *et al.* 2009). For this reason, the ability to discriminate among AN samples is highly reliant upon accurate measurements and/or multi-element isotope analysis. Previous studies suggest that separate analysis of component ions would provide additional discriminatory power (Howa *et al.* 2014; Aranda *et al.* 2011). This study aimed to validate a recommended method for separate AN component ion analysis by Howa *et al.* Therefore, various nitrate and ammonium salts, extracts from fertilizers and evidence objects were examined.

Isolation of ammonium ions in dissolved AN was achieved by precipitation using sodium tetraphenylborate solution. For the analysis of fertilizers, AN was extracted prior to the precipitation. Both AN and ammonium tetraphenylborate were tested for their $\delta^{15}N$ values using an elemental analyzer (EA) coupled to an isotope ratio mass spectrometer (IRMS). The $\delta^{15}N$ values of nitrate ions were calculated using mass balance.

In this study the precipitation method sufficiently separated the AN component ions without influencing the nitrogen isotope ratios of the ammonium ions. There was no correlation between $\delta^{15}N$ values of ammonium and nitrate within the 21 fertilizer AN samples. In difference to bulk AN analysis, separate analysis of AN component ions revealed potential isotope ratio variation in both ions. For all samples, $\delta^{15}N$ values of ammonium and nitrate differed from the theoretical atmospheric source value of 0‰.

In conclusion, isolation of component ions using sodium tetraphenylborate proves a valid technique for stable isotope analysis of AN containing substances. It is simple to perform and does not require specialized equipment. Furthermore the procedure adds forensic value, as the bulk nitrogen isotope analysis might obscure potential isotope ratio variation.

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Analysis of stable isotope ratios ($\delta^{13}C$ and $\delta^{15}N$)in fat tissue and organs as an authentication tool to determine the feeding regime in Iberian pigs

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Iberian pork products have earned a reputation for having exceptional organoleptic characteristics. This differential quality is based on the feeding regime (acorns) followed by the animals at the end of its fattening period and it is related to the dehesa ecosystem(Rodríguez-Estévez et al. 2009). The legislation defines the quality categories of Iberian pigs as *bellota*, *cebo* and *cebo de campo*, where animals are fed acorns, concentrates or a combination of both, respectively.

Nowadays, the only control method is the supervision of the farms and fields by authorized controlling enterprises. Therefore, it is necessary to assure the traceability of these products to protect consumers against fraud.

The feeding strategy may affect the adipose tissue growth, reducing body adiposity or intramuscular fat or modifying the proportion of unsaturated fatty acids (Skiba 2010, Wiecek et al. 2011). Moreover, it not only affects the amount, distribution and fat composition but may also condition proteolytic activity (Čandek-Potokar & Škrlep 2012). This fact should be evaluated in the protein fraction of the organs with higher turnover rates like the liver or kidney.

The current problem is the difficulty to establish a unique analytical method to differentiate among animals fed under different regimes. The application of stable isotope ratio mass spectrometry (IRMS) has become a promising technique to study the impact of animal feeding (Moreno-Rojas et al. 2008). In this sense, this research shows the isotopic ratios of stable isotopes of carbon and nitrogen from different pig tissues, such as muscle, liver, kidney (fat extracted and protein residue), and hypodermis fat layers to evaluate the impact of the feeding regime on their isotopic values.

Our results showed differences among the isotopic signatures of organs and fats, revealing a clear impact of the feeding regime. These preliminary data demonstrated the potential of this technique for achieving good classification parameters for the models built (i.e. 100% and 94% of correct classification for *bellota* and *cebo* classes respectively). The importance of $\delta^{15}N$ values of the protein fraction showed the impact of the proteolytic-nitrogen isotope signature on the correct classification of feeding regimes in Iberian pigs.

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Nitrogen isotopic signature of organic and conventional fertilizers used in intensive agricultural systems

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In the last decade, the demand for organic products has increased constantly (Proporsal Regulation EC-2014). These products tend to retail at a higher price than conventionally grown ones due to certification costs and yield gap. In this sense, the premium price and the increasing demand make organic food susceptible to fraud.

Spain is the most important organic producer in the European Union (EU) in terms on cultivated area (1.61 million hectares of a total of 11.5) (Willer and Schaack 2015). Intensive organic farming has been extended in the Mediterranean basin due to market demand. The transformation of greenhouses from intensive conventional agriculture to organic has become an important issue for certifiers and farmers.

The isotopic signature $\delta^{15}N$ (‰) of synthetic fertilizers differs markedly from that of organic ones (Bateman et al. 2007). At present, there is not a robust technique implemented to differentiate between organic and conventional plant products. It is important to note that there is not enough information about isotopic fractionation during crop development, and especially, fruit ripening fractionation steps. Moreover, it is necessary to obtain a local perspective of the different agrosystems (pedoclimatic characteristics), inputs (fertilizers and plant protection products) and cultural practices. Therefore, studies are required into the stable nitrogen isotope signature from fertilizers in order to obtain a better background of inputs used in organic farming. The objective of this paper was to provide an updated database containing the different commercial fertilizers used in intensive organic agriculture in the Mediterranean basin. The results obtained in this database represent the first step to characterize the main factor of nitrogen fingerprinting from a local perspective.

This database will make it possible to show the organic control in the most important organic productive area in the world. Moreover, this database highlights the importance of organic plant fertilizers based on leguminous plants.

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A new tool to human provenancing? Neodymium in a forensic and archaeological context

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The addition of another isotope system to the human provenance repertoire can potentially provide more precise information on the region of origin of a person, as well as address some of the limitations that are related to currently used isotopic techniques: oxygen (O), hydrogen (H), strontium (Sr) and lead (Pb). This study reports on the viability of using neodymium isotopes ($^{143}Nd/^{144}Nd$) as a possible tool to track the origins of humans. Nd is a light rare earth element (REE), which is, in contrast to what the name suggests, widely distributed in the Earth's crust at parts per million levels. Due to the isotopic variations in the crust Nd isotopes have previously been successfully applied to identify the production centres of glass archaeological artefacts (Ganio et al. 2013). The tendency of the human body to filter out non-essential elements means that REE concentrations are very low in human teeth (Nd 10-x 100 ppb), making previous Nd isotope analysis of human tissue impossible. Due to recent developments in mass spectrometry, the analysis of Nd has become achievable on small sample sizes (> 50 pg) and results can be used to complement information derived from other isotope systems. We report the optimisation of chromatographic methods to separate Nd from bio-apatite. Sub-nanogram amounts of Nd were analysed using a latest generation thermal ionization mass spectrometer (TRITON-Plus) equipped with 10^{13} Ω resistors at the Vrije Universiteit Amsterdam (Koornneef et al. 2014; 2015). This study presents the neodymium results of third molars from modern Dutch residents. These results will then be compared to O, Sr and Pb data, demonstrating the possibilities for Nd as a new forensic and an archaeological human provenancing tool.

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Study of the Milo river basin (French Guinean) from isotopes and dendrochronology measurements

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The Milo river is a tributary of the Niger river, and over the last decades undergoes discharge change due to the fluctuation of the West-African monsoon. Moreover, anthropogenic activity as bank management and water pumping for crops, increasing population with little waste water treatment and mining activity affect the water availability and water quality.

The river Milo has been study over a two years period in the area of Kankan and 80 km upstream, in view to understand the seasonal pattern and the effect of the monsoon. For this purpose, river water and ground water were sampled to determined their $d^{18}O$ and $d^{2}H$ values. The calculation of the d-excess, enable to determine the evaporation process. The first results show a strong influence of the monsoon in this savannah landscape (mean annual rainfall 1480 +/- 240 mm) and limited groundwater reservoir.

Riparian trees species have been determined and core to determine their age, growth rate and also to follow the Milo river discharge over time. The oldest trees enable us to go back 50 years back, and a second sampling this summer will try to get older trees more away of the river. The main limitation of this study is that very few trees under the tropic make distinctive annual rings. Precise anatomic determinations are needed and results are check against teak wood.

The land-use change act as a general factor of hydrological evolution of soils and basins. It is time to follow local hydrodynamic and collect precise climate data in view to understand the local water cycle and to preserve the water quality.

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Ground water isotopic specificity at the tree limits in Northern Mali, West Africa

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The Sahel has undergone drastic climatic change since the seventies characterized by a long rainfall deficit, with records of extreme droughts in 1973 and 1984, which lead to an important bush and trees mortality. From the 90's, amount of rainfall has increased but does not reach values observed before the drought period.

The present study aims to investigate the response and adaptation of trees to such a climatic variability. We report here our first results of the ground water isotopic survey since mid-2014 within the AMMA-CATCH Observatory near the village of Hombori, North-East of Mali. This program collects meteorological data and soil humidity in this area since the sixties. From 2013, dendrochronology and dendro-isotopic studies also look on the water uptake by the trees and their water regulations.

The first results of the groundwater survey on a dozens of wells, show that the mean values from April 2014 to January 2016, remain close to $\delta^{18}O$=-4.55 +/- 0.34 ‰. This is in good accordance with the weighted mean rainfall values for the surrounding GNIP stations, as Bamako with $\delta^{18}O$=-4.46 +/- 1,44 ‰. The calculated d-excess values present more variation, with some values above 15.

The depth of the ground water range from 2 to 49 m and the seasonal variation can be high. There can be also a high variation of the $\delta^{18}O$ values, and some wells located mainly East dry quite rapidly after the monsoon and display evaporated values. On the contrary, a few wells localised more closed to the Hombori peaks (1155 m asl) on the west part, present more depleted value, with $\delta^{18}O$ from -6.9 to -6.2 ‰. These more negative values present also high d-excess values and could show localised amount effect or older groundwater reservoir.

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A transect across the Western Ghats to understand the dual monsoon system in South India

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The Western Ghats range exerts a complex and important 'gatekeeper' effect on the climate mosaic of South India. The coastal areas in the west of the Western Ghats (Arabian Sea side) experience abundant unimodal southwest monsoon whereas the eastern part is influenced by the drier multimodal northeast monsoon. The annual rainfall can drop from 6000 to 600 mm in ca. 80 km across the Western Ghats. Major rivers in South India originates at the high altitudes of the Western Ghats and flows further into west and east coasts respectively. This makes the Western Ghats as a water tower of South India, and in turn supports the rich biodiversity forming a green backbone to Southwest India.

Earlier studies on groundwater, river and rainfall in this region has raised crucial points in the functioning of the hydrological cycle in the west coast of India[1,2]. In this study, a west to east transect was established with reference wells from the Arabian Sea coast line to the inland, at the level of Kozhikode and across the Wayanad plateau, Kerala. Rain gauges were also installed for recording the water amount and seasonality. A fog collector was installed to identify and quantify the local moisture contribution. In addition, teak cores were sampled along this transect to study the monsoon change over time. This study refines our understanding about the water and moisture cycling in the region with a focus on the present microclimate to the past climate in South India.

The initial results show that the narrow coast line and the small hilly areas in the west coast display a mean $\delta^{18}O$ value around -1.6 ± 0.5 ‰ and a d-excess close to the GMWL line. A rapid shift in the $\delta^{18}O$ value to -3.0 ± 0.4 ‰ with higher d-excess value is observed at the foothills of the Western Ghats representing high water vapor recycling. Depending on the valley location, there is a strong variability of rainfall (2500 to 4500 mm), and a possible contribution of winter monsoon up to 20%. There is no amount or altitude effect on the isotopic ratio up to 2000 m asl, certainly due to the high air moisture and nebulosity throughout the year. On the foothills of higher peaks, the ^{18}O exhibits relatively higher depletion between -4.0 and -5.0 ‰.

When approaching the Deccan plateau, the rainfall amount drops rapidly to 1200-1600 mm, or even lower, and the proportion of the northeast monsoon enhances. The mean $\delta^{18}O$ value is around -4.0 ± 0.5 ‰ with d-excess value greater than that of the GMWL. This reflects higher contribution from the northeast winter monsoon.

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In situ sulfur isotope analysis of pyrites from Archean metasedimentary rocks Sharyzhalgai complex southwest of the Siberian craton

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The thermodynamic and kinetic fractionation processes of sulfur isotopes ^{32}S, ^{33}S, ^{34}S and ^{36}S is subject to mass-dependent isotope fractionation (MDF), and is described by the simple equation $\delta^{33}S = 0.515 \times \delta^{34}S$ (Hulston & Thode, 1965). In the study of the isotopic composition of minerals and rocks of the Earth believed that strictly observed the mass-dependent fractionation of sulfur isotopes. The first violation of the law of mass-dependent fractionation of sulfur isotopes was found in the Archean rocks (Farquhar et al., 2000), which was associated with mass-independent fractionation of sulfur isotopes in photochemical reactions with SO_2 in an oxygen-free atmosphere of the Archean (Pavlov & Kasting, 2002). The discovery of anomalous contents of ^{33}S isotope in the Archean rocks and no anomalies in rocks younger than 2.4 billion years (Farquhar et al., 2000) has stimulated a series of new MIF sulfur isotope research in the Archean rocks on different continents. Given that $\Delta^{33}S$ anomalies have only recently been discovered in a number of regions of the world, we can assume that in Russia may be present rocks that also have mass-independent fractionation of sulfur isotopes. Here we present evidence that the Archean and Paleoproterozoic rocks of the Siberian craton contains $\Delta^{33}S$ anomalies.

We carried out research sulphide samples Sharyzhalgay granulite complex, which is one of the main structural elements of margin salient of the Siberian craton. We have studied more than 20 samples of rocks his major complexes (Cheremshanskaya suite, Kitoi granulite complex and Onot greenstone belt) and was discovered mass independent fractionation of sulfur isotopes with $\Delta^{33}S$ ($\Delta^{33}S=\delta^{33}S-0.515\times\delta^{34}S$) values ranged from -1.17‰ to +2.75‰. In the samples we studied the isotopic composition of sulfur $\delta^{34}S$ changes in a relatively narrow range of values from -2.55‰ to 5.58‰ and $\delta^{34}S$ grouped around zero, to a first approximation corresponds to the mantle source of sulfur. At the same time reliably established in a number of sample values $\Delta^{33}S$ ‰ deviates from zero and excludes mantle source of sulfur. Deviations from zero in the Archean rocks are derived from mass-independent isotope fractionation of sulfur SO_2 in atmospheric photochemical reactions in oxygen-free atmosphere of the Archean. When photochemical dissociation of SO_2 formed S_8 aerosols with positive values $\Delta^{33}S$ in elemental sulfur and SO_3^- with negative values. During sedimentation negative $\Delta^{33}S$ sulfides formed in the reduction of seawater sulfate, and positive values to form sulfides with the participation S_8 aerosol. Consequently, $\Delta^{33}S$ sign values can be used as a specific source of sulfur label sedimentary rocks in Archean. We observed anomalous values ^{33}S isotope can be highly informative tool for further research, and undoubtedly will be useful in solving the fundamental problems of evolution and genesis of ore deposits.

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Isotopic composition of daily precipitation at high-mountain site Kasprowy Wierch, southern Poland: Impact of moisture source regions and rainout history of moist air masses

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Thorough understanding of factors controlling isotopic composition of precipitation on various temporal and spatial scales under present-day climate is a necessary step towards proper interpretation of water isotope records preserved in diverse climatic archives (ice cores, lake sediments, speleothems, etc.). The IAEA/WMO Global Network of Isotopes in Precipitation (GNIP) played a central role in characterizing spatial and temporal variability of δ^2H and δ^{18}O of precipitation on the global scale (Dansgaard, 1964; Rozanski et al., 1993). As samples analyzed in the framework of GNIP represent cumulative monthly precipitation, there is a need to better understand the link between the origin and rainout history of moist air masses and the isotopic signatures of precipitation recorded on the continents.

A 5-year record of deuterium and oxygen-18 isotope composition of daily precipitation collected at high-altitude mountain station Kasprowy Wierch (49°14'N, 19°59'E, 1989 m a.s.l.) located in north-western High Tatra mountain ridge, southern Poland, is presented and discussed. In total 660 precipitation samples have been collected and analysed. Weighted monthly mean ^{2}H and δ^{18}O values of daily precipitation collected at Kasprowy Wierch were compared with analogous data available for the GNIP station Krakow-Balice (50°04'N, 19°55'E, 220 m a.s.l.) located approximately 100 km north of the Tatra Mountains.

Stable isotope composition of the analysed precipitation events varied in a wide range, from -2.9 to -26.6‰ for δ^{18}O and from -7 to -195 ‰ for δ^2H. The local meteoric water line (LMWL) defined by daily precipitation data ($\delta^2H=(7.86\pm0.05)\delta^{18}O+(12.9\pm0.6)$ deviate significantly from the analogous line defined by monthly composite precipitation data available for IAEA GNIP station Krakow-Balice ($\delta^2H=(7.82\pm0.11)\delta^{18}O+(6.9\pm1.1)$. While slopes of those LMWLs are statistically indistinguishable, the intercept of Kasprowy Wierch line is almost two times higher than that characterizing Krakow monthly precipitation. This is well-documented effect associated with much higher elevation of Kasprowy Wierch sampling site. Single event isotope data for Kasprowy Wierch precipitation correlate significantly with air temperature, with the slope of the regression line being equal 0.35±0.02 ‰/°C for δ^{18}O, and (2.90 ± 0.16‰/°C for δ^2H, whereas no significant correlation with precipitation amount could be established.

The impact of moisture source regions and rainout history of the moist air masses on the isotopic composition of daily precipitation collected at Kasprowy Wierch site was analyzed using HYSPLITE back trajectory model (Draxler and Rolph, 2011). Five-days back trajectories were calculated for all analyzed precipitation events and seasonal maps of trajectory distribution were produced. Five-days back trajectories were calculated for all analyzed precipitation events and seasonal maps of trajectory distribution were produced. They illustrate the changes in the prevailing moisture transport patterns to the sampling site. In addition, the events yielding extreme isotopic composition of precipitation collected at Kasprowy Wierch (see above) were analyzed in detail.

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Validation of a simple and rapid method for $\delta^{15}N$ determination of ammonium for natural water samples using sodium tetraphenylborate

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We tested the applicability of the tetraphenylborate method to determine the $\delta^{15}N$ signature of dissolved ammonium in natural water samples. Existing methods used up to now are both work and time-consuming because they are based on the separation of ammonium through the gas phase as ammonia. The investigated, rapid and simple method uses the insolubility of ammonium tetraphenylborate in water, which is already known for the qualitative and quantitative detection of ammonium. In Howa, Lott, & Ehleringer (2014) the salt was tested for its suitability in the isotope analysis. The obtained results showed that the method is in principle suitable for isotopic analysis, but only a very specific scenario (very low volumes of distilled water) was investigated.

The ammonium-containing (about 50-60 µmol NH_4-N) water sample is concentrated by freeze-drying or completely dried – suspended matter containing samples have to be filtered before freeze-drying – and then treated with a sodium tetraphenylborate solution. After half an hour the precipitate can be isolated by filtration from the liquid phase. The filter residue is subsequently oven-dried (60 °C) and then carefully removed from the filter. The dry ammonium tetraphenylborate can be stored in closeable glass vials for an extended period. For the isotope analysis the ammonium tetraphenylborate is weighed directly into tin capsules and then combusted in an elemental analyzer (EA) coupled to an IRMS system for analysis. The residue of a filter allows thereby at least one measurement in triplicate.

The method was tested on three different ammonium inhouse-standards with different signatures: ammonium sulfate ($(NH_4)_2SO_4$) = +0.2 ±0.2‰, n=8), ammonium chloride (NH_4Cl = -0.71 ±0.2‰, n=8) and ammonium bromide (NH_4Br = + 12.25 ±0.2‰, n=8). The influence of nitrate and potassium was investigated and the reliability of the method in a highly mineralized matrix. The concentration of nitrate and potassium was increased up to the ratio of 4:1 to ammonium in each case (this means NO_3:NH_4 and K:NH_4 was ≤ 4:1) and there was found no significant effect. Since potassium just like ammonium precipitates as a white salt upon contact with tetraphenylborate the dosage of Natriumtetraphenyborats needs to be adjusted and increased. Accordingly, the test sample for the EA-IRMS measurement must be increased so there are still about 0.1 mg of nitrogen per capsule. The measurements showed good accordance with the expected values. In total there were 82 samples analyzed by the tetraphenylborate method from which 94% had a deviation from the expected value of less than 0.4‰. However, it was found a consistent ^{15}N enrichment of +0.6‰, which must be corrected accordingly.

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Using Isotope Ratio Infrared Spectrometer to determine carbon ($\delta^{13}C$) isotopic composition from $CaCO_3$ carbonate and DIC samples

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Precise and accurate determination of isotopic composition of carbon (^{13}C) and oxygen (^{18}O) from carbonate or DIC sample with proper referencing and data evaluation algorithm presents a challenge for scientists. Mass spectrometry was the only widely used technique for this kind of analysis, but recent advances make laser based isotope ratio infrared spectroscopy (IRIS) a viable alternative. It is now possible to perform this analysis reliably with IRIS with sample amounts as low as 200 µg. We present measurements of three international reference materials, and one of them treated as an unknown. Five samples each of approximately 1mg each were acidified using a few droplets of 43% H_3PO_4 and left for equilibration overnight at 25°C. The standard deviation was less than 0.15‰ $\delta^{13}C$.

To achieve these kind of results, the Universal Reference Interface (URI) Connect was developed. CO_2 free synthetic air is used to flush out the contents of a sample container into a variable volume. If necessary, the sample is further diluted before entering the analysis chamber. Reference gas measurements are automatically performed at the same concentration as sample measurements to compensate for instrument drifts and non linearity. In a test experiment with 1% CO_2 in 12 ml vials, we achieved an internal precision of better than 0.07‰ and 0.1‰ for $\delta^{13}C$ and $\delta^{18}O$, respectively.

The URI Connect can handle about 100 samples per day from an autosampler, or samples can be injected one at a time through a septum on the front of the instrument. Gas samples collected in flasks, bags, syringes, or vials can be analyzed. The system only needs the equivalent of about 80µg - or 40µL – of pure CO_2 gas to complete an analysis. Due to it's small weight and robustness, sample analysis can be performed in the field, e.g. aboard a research vessel.

Intercomparison of lab-based soil water extraction methods for stable water isotope analyses in the critical zone

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Analyses of δ^2H and δ^{18}O composition of pore waters in saturated and unsaturated soil samples are routinely performed in (eco-)hydrological studies. Numerous in-situ and lab-based pore water extraction methods for the analysis of the stable isotopes of water now exist. While some have been used for decades (e.g. cryogenic vacuum extraction) others are relatively new, such as direct vapor equilibration or the microwave extraction technique. Despite their wide range of application, a formal and comprehensive intercomparison of soil water extraction methods for stable isotope analysis has been lacking and long overdue. Here we present results of an intercomparison of five commonly used lab-based soil water extraction techniques[1]: high pressure mechanical squeezing, centrifugation, direct vapor equilibration, microwave extraction, and cryogenic extraction. We applied these extraction methods to two physicochemically different soil types (silty sand and clayey loam) that were oven-dried and rewetted with water of known isotopic composition at three different gravimetric water contents (8, 20, and 30%). Our results showed that the extraction technique had a significant effect on the soil water isotopic composition. Each method exhibited deviations from spiked reference water. Soil type and water content exerted a secondarily effect. Most significantly, cryogenic water extraction showed the largest deviations from the spiked reference water, whereas mechanical squeezing and centrifugation were able to match most closely the spiked water reference for both soil types. We also compared results for each extraction technique where liquid water was run on both a laser spectroscope and a mass spectrometer. Differences between these two analytical instruments were negligible for our water extracts. We discuss these findings in the context of critical zone research and ecohydrological questions of water source, flowpath, and residence time.

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The Water – Isotope – Map (δ ^{18}O, δ ^{2}H, ^{3}H) of Austria

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The isotopic ratios of oxygen and hydrogen in water (^{2}H/^{1}H and ^{18}O/^{16}O) are important tools to characterise waters and their cycles. This starts in the atmosphere as rain or snow and continues in surface water and ends in shallow groundwater as well as in deep groundwater. Tritium formed by natural cosmic radiation in the upper atmosphere and in the last century by tests of thermonuclear bombs in the atmosphere, is characterised by its radioactive decay with a half-life of 12.32 years and is an ideal age-marker during the last 60 years.

To determine the origin and mean age of waters in many projects concerning water supply, engineering and scientific projects in the last 45 years on more than 1,350 sites, more than 40,000 isotope measurements were performed in Austria. The median value of all sites of oxygen-18 is δ ^{18}O -10.7 ‰ and for hydrogen-2 δ ^{2}H -75 ‰. As the fractionation is mainly temperature dependent the lowest negative values are observed in winter precipitation (oxygen-18 as low as δ ^{18}O -23 ‰) and in springs in the mountain regions (δ ^{18}O -15.1 ‰). In contrast the highest values were observed in summer precipitation (up to δ ^{18}O – 0.5 ‰) and in shallow lakes in the Seewinkel (up to δ ^{18}O + 5 ‰).

The isotopic ratios of the Austrian waters are also influenced by the origin of the evaporated water masses. Therefore the precipitation in the region south of the main Alpine crest (East-Tyrol, Carinthia and South-East Styria) is approximately 1 ‰ higher in δ ^{18}O-values than sites at the same altitude in the northern part. This is most probably caused by the stronger influence of precipitation from the mediterranean area.

The median value of all 1,120 sampling sites of decay corrected (2015) tritium measurements is 6.2 tritium units (TU). This is somewhat smaller than the median value of all precipitation stations with 7.2 TU. This can be explained by the fact that in most cases in groundwater the median value has been reduced by decay according to the residence time underground. The tritium concentration increases in the summer up to 10 – 11 TU and decreases in winter down to 3 – 4 TU. This is due to the better circulation in the atmosphere in spring which brings the tritium formed by cosmic radiation down to the lower atmosphere and precipitation.

A smaller mean tritium concentration in aquifers than approximately 3.5 TU indicates large amounts of water older than 60 years. Waters with approximately more than 12 TU contain still tritium from the 1960s and 1970s formed originally by thermonuclear bomb experiments. In Austria the highest Tritium values can be observed in the rivers Danube and March which show periodic or permanent tritium contamination up to 70 TU coming from nuclear power plants in the neighbouring countries.

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Rainfall isotopic features in South-West France: A comparison between the plain near Toulouse and the Pyrénées uphill of the Ariège river

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Southwest of France is characterized by a double climatic influence: the Nordwest Atlantic and the South-east Mediterranean moisture circulation. The mixing of the different air-masses depends on the main wind streams and orographic effects.

The isotopic rainfall feature in the area of Toulouse (Auzeville station: 154 m asl) is followed monthly since July 2011. The first results (Lambs et al. 2013) have shown the major influence of Atlantic rainfall and the absence of post-evaporation effect. The $\delta^{18}O$ / $\delta^{2}H$ relation gives value closed to the Global Meteorological Water Line ($y=7.70\ x + 9.86$).

Since 2013, extra samplings have been performed for rainfall occurring after the South-west Autan wind in view to get the signature of the Mediterranean moisture. And a second rain gauge (including snow) for isotope analyses have been installed 80 km more south in the Pyrenees mountains, up of the Ariège river upper basin (Bernadouze station 1400 m asl). We present here the first comparison of both stations between September 2013 to October 2014.

This comparison shows a different rainfall pattern, much higher water amount during winter in Bernadouze, but the temperature pattern remains similar with just a drop between 3.5 to 8.1 °C. The $\delta^{18}O$ values recorded in Bernadouze are until 5 units more depleted than in Auzeville. Interesting is the d-excess features difference for the $\delta^{18}O$ vs $\delta^{2}H$ diagram: higher d-excess values for less depleted values (relative to $\delta^{18}O$) in Bernadouze et reverse for Auzeville. This could show different Mediterranean air mass influence and different recycling processes.

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Soil water stable isotopes for quantitative studies of eco-hydrological processes in water limited environments (WLE): A synthesis of ^2H_2O labeling studies from northern central Namibia

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Stable water isotopes are considered ideal tracers for investigating eco-hydrological processes at the soil-vegetation-atmosphere interface. However, their use as environmental tracer for quantitative studies remains limited (Herczeg and Leaney, 2010). Deuterium (^2H_2O) based labeling techniques can support quantitative estimates of eco-hydrological processes.

Between 2013 and 2016, soil and plant water stable isotopes in the semiarid Cuvelai-Etosha Basin were investigated in the framework of the project SASSCAL (Southern African Science Service Centre for Climate Change and Adaptive Land Management) both in their natural abundances as well as by utilizing ^2H_2O as artificial tracer. We present a synthesis of these applications with focus on the additional information one can gain using ^2H_2O based labeling techniques. Applications include i) the quantification of groundwater recharge; ii) the determination of root water uptake depths and dynamics; and iii) the investigation of water vapor transport. Further, we discuss advantages and disadvantages compared to approaches using natural abundances.

For a forested site on deep Kalahari sand potential groundwater recharge rates were quantified as 29 mm y^{-1} and 0 mm y^{-1} in two subsequent years, respectively, which agrees with estimates based on the chloride mass balance. Tracer experiments at different times of the year further suggest immediate access of soil water by the local vegetation up to approximately 2.5 m and infrequent access of particular plant species (e.g. *Salacia luebertii*) to deeper reservoirs. Over the dry season, soil water isotope profiles revealed an advective water vapor flux towards the surface from soil layers labeled as deep as 4 m.

Labeling techniques are shown to be valuable for tracing depth-dependent root water uptake, water movement and water vapor transport in the deeper (> 2 m) unsaturated zone, where natural isotopic depth profiles approach a constant concentration. We encourage the use of labeling experiments in addition to natural profiles of soil water stable isotopes. Information derived from such studies can be used for approximating rooting depths and distributions, root zone storage capacity or for calibrating vapor phase and isotope transport of novel soil-vegetation-atmosphere models. Disadvantages of artificial applications of ^2H_2O include the disturbance of the natural isotopic composition on-site both for plants and soil, a generally more difficult handling of samples, higher standard deviation in comparison to environmental isotope analyses and the risk of losing the tracer through evaporation.

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Llandovery carbon isotope trend in Estonia – local versus global signatures and correlation

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Carbon and oxygen isotopes applied as environmental and chronological proxies have gained an eminent position in palaeoenvironmental studies and stratigraphy. Our main interest has been detailed investigations in Baltica and compilation of a complete general trend for the whole Ordovician and Silurian. The sequence of post-Tremadocian Ordovician and Silurian carbonate rocks is stratigraphically nearly complete in Baltoscandia. Complications caused by several local hiatuses, condensed sections and facies changes were mitigated by the study of overlapping drill core sections. Samples were analyzed without chemical pretreatment. Baltic sedimentary rocks are weakly altered diagenetically and the results of analyses are therefore trustworthy. The resulting carbon isotope curve (Ainsaar et al, 2010; Kaljo & Martma, 2006) is biostratigraphically well dated thanks to good cooperation with palaeontologists and stratigraphers of several institutions.

The Hirnantian and Sheinwoodian carbon isotope excursions are globally traced major events, whereas $\delta^{13}C$ trends through the Llandovery are less well documented and understood. In composite Silurian carbon isotope curve the Baltic data by Kaljo & Martma (2000) are often used as reference for Llandovery. In order to extend this data set, test the regional variability of Llandovery $\delta^{13}C_{carb}$ curve and further explore its potential for correlations and environmental interpretations, we collected new data from four sections in central Estonia (Viki, Paatsalu, Velise and Laeva-13 drill cores). Combined with biostratigraphy and previously studied isotope curves from deeper shelf settings (Ruhnu and Ikla cores) the main regional trends are summarized and compared with other high-resolution data sets.

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An improved online method for $\delta^{34}S$ measurements by EA-IRMS

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The isotopic composition of sedimentary sulfide and sulfate has provided incredible insights into the evolution of atmospheric oxygen over long geologic time scales (Farquhar et al., 2000). Sulfur isotopes have also provided new insights into state changes in water column redox conditions during OAEs. However, bulk isotope measurements conducted on outcrop samples, sediments, and aqueous samples require researchers to scale their sampling volume for the initial sulfur phase extraction (e.g. sulfides or sulfates), and then optimize yields of the purified sulfur phase (precipitated as silver sulfide or barium sulfate) for isotopic analysis. Reducing the amount of sample needed for the isotope ratio mass spectrometer (IRMS) could enhance data resolution, and more data would improve interpretations of secular changes in isotope composition as well as in modern systems such as water columns, pore waters, and microbial mats.

Continuous flow applications that use elemental analyzers (EA) to combust the sample into CO_2, N_2, or SO_2 gas and then transfer the separated gases into the IRMS by helium carrier have become the main method for bulk isotopic analysis (e.g. Fry 2007). We present a modified EA-IRMS method that highly improves isotopic sensitivity and precision for N, C and S isotopes. The modifications significantly shorten analysis time and enable precise N, C, and S isotope analysis on a single sample. Our results demonstrate that N, C, and S isotope analysis can be done on a single sample using the single combustion reactor set up in the EA.

For this presentation, we focus on the improvements for $\delta^{34}S$ analysis. Peak broadening of SO_2 gas is a common issue with EA-IRMS that affects isotopic precision and creates memory effects. Our optimized EA-IRMS system greatly improves SO_2 peak shapes, resulting in higher precision $\delta^{34}S$ values than current practice. In comparison to traditional EA, we observe a notable increase in sulfur sensitivity. For example, previous analyses would typically require 0.45 mg silver sulfide to produce a ~2V signal, however, with our new method, a 0.125 mg silver sulfide sample produces a peak height of 0.8V. This equates to a 40% increase in sensitivity for sulfur analyses. Overall, the precision on $\delta^{15}N$ is better than 0.1‰ for a sample size range of 20 to 250 µg N; the precision of $\delta^{13}C$ is better than 0.1‰ for 100 to 4000 µg C; and, the precision for $\delta^{34}S$ is better than 0.2‰ permil for 59 to 111 µg S. Further, we have successfully extended this approach and measured $\delta^{34}S$ on trace amounts of sulfur (5 µg S).

This novel approach offers a greatly improved method for $\delta^{34}S$ analysis of low-yield sulfur. In addition to recent and ancient sediments, there are many other applications that will benefit from this development such as measuring $\delta^{34}S$ of organic samples for food-web studies, or sulfur in materials such as shells/fossils, collagen, microbial cultures, and tree-rings.

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Continuous observation of tree interception effects on water stable isotopes

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Water stable isotope ratios ($^2H/^1H$ and $^{18}O/^{16}O$) have been employed successfully for several decades in various fields of hydrology. As stable isotopes are part of the water molecule itself, they can consequently be considered as ideal tracers as they are not subject to absorption, degradation or decay. They have been used to investigate hydrological processes in all parts of the water cycle and corresponding to that, throughout a wide range of scales. To predict the temporal dynamics of stable isotopes of water entering the soil, not only the temporal dynamics of precipitation but also the influences of the vegetation by the interception processes need to be considered. Interception effects on the isotopic signature of throughfall ultimately cascade through the entire hydrologic system, affecting soil water, groundwater, evapotranspiration and stream water isotopic signatures. In contrast to the quite common hydrometric field experiments to study interception and throughfall, until now only few field experiments have successfully extracted the potential of stable isotopes to study interception to understand canopy interception processes and its effect on the water cycle.

The isotopic composition of throughfall is affected by complex exchange and mixing processes in the canopy rather than simply evaporation and also by pre-event canopy storage. The differences in isotopic composition are driven by evaporation from the canopy during or between storms, isotopic exchange with ambient vapor in the canopy air space and selective canopy storage effects, where water is differentially retained by the canopy during rainfall events. These interception processes occur simultaneously in time and space generating a complex pattern of throughfall in amount and isotopic composition. However, most of the previous studies lack an appropriate temporal resolution of the stable isotope dynamics in gross precipitation and throughfall to test the different hypotheses of canopy interception processes and hence to adequately predict the isotopic signal of throughfall under a variety of precipitation and environmental conditions.

The more and more common laser-based isotope analysers (based on e.g. Cavity Ring-Down Spectroscopy (CRDS)) are capable of analyzing water vapor directly and continuously, which makes them ideal for the continuous observation of different processes. In combination with a CRDS-instrument we established an in-situ method to transfer liquid water to water vapor within seconds (Herbstritt et al., 2012). Core of the method is an off-the-shelf microporous hydrophobic membrane contactor, originally designed for degassing liquids. It is used in reverse mode with nitrogen as carrier gas in order to continuously transform small fractions of liquid water to water vapor which is then transferred directly to the analyser. Using this method and two CRDS-instruments in parallel throughfall below a decidious tree canopy and gross precipitation 15 m away were measured continuously (0.5 Hz) by the analysers. Additionally, rainfall and throughfall intensities were recorded every minute at both sites. The observed interception losses in precipitation amount as well as the observed differences at different temporal resolutions in the isotope composition between gross precipitation and throughfall will be shown, discussed and related to interception processes in the tree canopy.

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The H and O isotope geochemistry of interstitial fluids from the Baltic Sea (IODP Leg 347): A study of hydrographic changes since the Late Pleistocene

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The stable hydrogen and oxygen isotope ($^{2}H/^{1}H$, $^{18}O/^{16}O$) composition of interstitial waters extracted from long sediment cores retrieved during IODP Leg 347 in the Baltic Sea was analysed to constrain the main hydrographic changes of the Baltic Sea from the late Pleistocene to modern time. Prior to its reconnection with the North Sea (through the Skagerrak-Kattegat connection), the Baltic Sea had evolved into a lake, with a brief interruption during the Yoldia stage. Upon reconnection with the open ocean, hydrologic and bottom water compositional changes in the Baltic Sea were dramatic. Current pore water profiles reflect these changes, further impacted by internal transport processes like diffusion and advection. Investgations were carried out at Sites M0059 (Little Belt), M0060 (Kattegat), M0065 (Bornholm Basin), and M0063 (Landsort Deep). Associated with a steep vertical decrease in salinity a strong enrichment of the lighter stable isotopes in water with depth is observed. The resulting shape of the pore water gradients differ substantially from each others due to different modern and paleo bottom water and sedimentation rates conditions.

In order to reconstruct the evolution of the Baltic Sea hydrograhy since the late Pleistocene, it is planed to develop an integrated advection-diffusion model considering different scenarios for temporal changes in sedimentation and bottom water composition.

The use of stable isotopes of water to study groundwater dependent ecosystems in boreal environment

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Groundwater dependent ecosystems (GDEs) rely on availability of groundwater. Anthropogenic actions or climate change can however alter the groundwater levels and discharge patterns and have a negative impact on these ecosystems. For the water management perspective it is therefore important to classify GDEs. Stable isotopes of water have been proven to be especially practical tracers to study the groundwater dependence of different ecosystems. Here, we present case studies where stable isotopes of water are used to study lakes, springs and peatlands in Finland.

The kettle lakes on Rokua esker aquifer area in Finland were studied using stable isotopes of water (Isokangas et al. 2015). G index values were formed to describe the groundwater dependency of the lakes. The G index defines the percentage contribution of groundwater inflow to the total inflow of water, varying from 39 to 98 % in the 67 lakes studied. The stable isotope method was also used to verify the groundwater – surface water modelling results of the area (Ala-aho et al. 2015).

In Viinivaara esker aquifer area stable isotopes of water were used to study the groundwater discharge patterns in adjacent pristine peatland area. A stable isotope method was developed to quantify groundwater proportions in peatland surface water. It was found that groundwater dependent zones in peatland are scattered. All of them are not located near the esker aquifer and therefore are not covered by the current groundwater protection zones.

In total, 41 springs in Oulanka National Park were sampled for stable isotopes of water, nutrients, water quality parameters, geochemical parameters and major cations and anions (Rossi et al. 2015). Statistical methods were used to relate spring water quality and quantity to hydrogeology and land use of the spring capture zone and to cluster the springs based on their water chemistry. The best predictors of spring water chemistry were spring altitude and the stable hydrogen isotope ratio of the water. Thus stable water isotopes could be applicable for spring management and may also provide a rough estimate of groundwater flow route.

The case studies show that stable isotopes of water are efficient tools to determine groundwater dependency among lakes and peatlands and to predict spring water chemistry and groundwater flow routes to springs. They also demonstrate that groundwater – surface water interactions of lakes and peatlands are complicated. The current efforts e.g. in Finland to develop more accurate methods to determine GDEs are really needed for better water resources management.

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High-frequency isotopic analysis of liquid water samples using an automatic water sampling system at irrigation farming sites

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High-resolution spatial and temporal data are important to improve our understanding of environmental processes in ecosystems and will break the barrier to accurately model biogeochemical cycles across spatiotemporal scales. Stable water isotopes (^{2}H and ^{18}O) allow the tracing of relevant water exchange and transport processes in the soil-plant-atmosphere domain.

The ICON project (Introducing Non-Flooded Crops in Rice-Dominated Landscapes: Impact on Carbon, Nitrogen, and Water Cycles) focuses on the ecological impact of future changes in rice production in Southeast Asia. The project further aims to gain necessary process understanding that facilitates maintenance of fundamental ecosystem services, decreases environmental impacts, and increases the output of rice-based cropping systems. For this purpose, effects of altered flooding regimes (flooded *vs*. non-flooded) and crop diversification (wet rice *vs*. dry rice *vs*. maize) are studied using stable water isotopes in liquid water in the lowland farm of the International Rice Research Institute (IRRI) in the Philippines.

Here, we measure high-resolution water isotope data (30-minute sampling intervals) of groundwater, surface water, rainfall, and irrigation water. Our automatic sampling set-up[1] facilitates in-situ analysis of stable water isotopes and nitrate concentrations in spatially differentiated agricultural fields via a web-based control software. Our measuring network consists of 18 sampling points from different water sources. We use Wavelength-Scanned Cavity Ring Down Spectrometry system (WS-CRDS) (L2120-*i*, Picarro, Santa Clara, CA, USA) for δ^{2}H and δ^{18}O analyses connected to a diffusion sampler[2] which allows continuous water analysis using diffusion through porous polytetrafluorethylene (PTFE) tubing.

This data facilitate the understanding of system processes and help to investigate erratic events such as rain fall, irrigation and evapotranspiration effects on ground and surface water under flooded and non-flooded conditions with comparison to crop diversification. Since rice cropping systems show high variability in water and nutrient management within small scales, high spatial water monitoring with isotope and water quality analyses could be helpful in investigating multiple cropping systems with a single analytical system. This allows on-site monitoring of highly dynamic hydrochemical behaviour. Results of water isotopes during the wet season 2015 will be presented.

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The effect of environmental variables and leaf physiology on the isotopic signature of transpiration

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The oxygen isotopic signature of transpiration is widely applied in ecosystem studies. It is, for example, used to separate soil evaporation from transpiration in ecosystems. Environmental variables are important external drivers of the transpiration E and its oxygen isotopic signature $\delta_{E.}$ Important internal drivers of E and $\delta_{E.}$ are leaf physiological traits. Using a laser spectrometer coupled with a gas-exchange system, we investigated the effect of both environmental variables and leaf physiological traits on transpiration E and its oxygen isotopic signature δ_E. Seven plant species with distinct leaf physiological traits were exposed to step changes in relative humidity (*RH)*. The role of leaf water content (*W*) and stomatal traits on the temporal dynamics of E and δ_E following these changes in *RH* were analysed by models. The step changes in *RH* caused strong variations in δ_E in all species. A drop in *RH* led to isotopic depletion of transpired water by –7.20 ± 2.48 ‰ for all species, a rise in *RH* caused isotopic enrichment of transpiration by +12.17 ± 8.31 ‰. The studied species showed different behaviour in the subsequent speed of return to steady-state, three distinct groups could be identified with high (~3.2 to 4.5‰ h$^{-1)}$, medium (~1.5% to 2.2‰ h^{-1}) and low (<1.5‰ h^{-1}) speed of return to the isotopic steady state. Our results suggest that the turn-over time of leaf water, in which the studied species considerably differed, determined the speed of return to the isotopic steady state. Following changes in *RH*, the speed of return to isotopic steady-state was well described by current modelling approaches of leaf water turn-over time, but the relative influence of stomatal traits versus *W* on isotopic non-steady-state transpiration was strongly species-specific.

In this study, isofluxes of transpired vapour, i.e. isotopic signatures of transpiration weighted by the transpiration rate E (isoflux=$-E\Delta_E$), were strongly driven by *E*. Hence, the deviation of isofluxes from isotopic steady-state can be large, even though plants transpire near to isotopic steady-state. Furthermore, isofluxes of δ_E strongly differed between species and differed significantly from isotopic steady-state isofluxes after changes in *RH*, highlighting the forcing of isotopic non-steady-state transpiration on atmospheric vapour.

Temporal dynamics in dominant runoff sources and flow paths in the Andean Páramo

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The dynamics of runoff sources in headwater catchments are often still poorly understood, particularly in remote regions. It is known that the relative importance of sources and flow paths vary temporally and spatially, which complicates conceptualization of the hydrological catchment response. This is even more the case for remote areas such as the Páramo in the Andes. Despite a lack of understanding of runoff generation processes, this ecosystem acts as the water tower for much of the population in the region. Our aim was to improve our understanding of the dynamics in water provenances and flow paths using a set of hydrochemical tracers and water stable isotopes. Both have been broadly employed for identifying geographical sources for runoff generation by End Member Mixing Analysis (EMMA) and Mean Transit Time estimations. We assessed the temporal dynamics of different sources and flow paths in conjunction with measures of tracers damping by Inverse Transit Time Proxies as a simplified representations of transit times. The methodology was tested in a headwater catchment using data from the Zhurucay River Ecohydrological Observatory (7.53 km^2). The catchment is located in the Andes in southern Ecuador, and is characterized by a low seasonal variation with a rainier season from December to May and a drier period from June to November. We collected weekly stable water isotopes and infrequently solute samples at the outlet and from thirteen potential runoff sources during April 2012 to April 2014. Weekly pH and electrical conductivity were also measured in situ. Hydroclimatic data is available for the same period.

Preliminary results show that four End Members are the minimum required for a satisfactory representation of our system, i.e. rainfall, spring water and water from the bottom layers of two different soil types (Histosols, Andosols). The soil types determine the vertical permeability of the top soil layers and the lateral permeability of the bottom layer of the unsaturated zone. On a seasonal comparison, Histosols contributed less water to discharge during the drier season whereas spring waters increase its contribution under these conditions. On a day-to-day comparison, no clear relationships were found between the fraction of contribution to discharge and the specific discharge itself for rainfall and waters from the two soil End Members. Contrary, a clear trend can be observed for spring water contribution that clearly decreases with higher specific discharges. Results from the isotope based hydrograph separation suggested that most of the time stream water was dominated by pre-event water. Nevertheless, on few occasions, event water contribution exceeds 30% occurring mainly in the rainy season. The catchment Inverse Transit Time Proxies indicated similarities in ages between Andosols and stream water mainly during rainy season. We interpret this as an indication of connectivity between hillslopes and streams when soils have a higher saturation.

This study confirmed the usefulness of multiple tracer investigations to provide insights into the complex dynamics of water sources in Páramo catchments being essential for the hydrological understanding of mountain ecosystems.

Partitioning of water resources and xylem-leaf deuterium enrichment in seasonally dry Tanzania

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In this study, we measure $\delta^{18}O$ and δ^2H of lake water, precipitation, plant xylem and leaf water across different plant species, seasons and sites with varying distances to Lake Challa during 2013–2014. We assess spatial and temporal patterns of water utilization and provide insights in the factors affecting leaf water deuterium enrichment. Lake Challa is situated in equatorial East Africa on the foothills of Mt. Kilimanjaro. The local climate is tropical semi-arid and rainfall is concentrated in 2 rainy seasons. Southeasterly monsoon winds bring 'long rains' from February to June and the northeasterly monsoon winds bring 'short rains' from November to December.

In order to trace the average isotopic signature of the water source of xylem, the δ^2H intersection ($\delta^2H_{LMWL\text{-}int}$) of xylem water samples with the local meteoric water line was calculated. Although we expected that evergreen plants were adapted to tap water from deeper soil layers in order to survive the dry season, no statistical differences in δ^2H_{xylem} and $\delta^2H_{LMWL\text{-}int}$ between evergreen and deciduous species were observed. Plants at all locations showed very negative $\delta^2H_{LMWL\text{-}int}$ indicating that they relied mostly on depleted rains from the NE monsoon. $\delta^2H_{LMWL\text{-}int}$ of December approached the δ^2H of November rains. Probably this is because November followed after a distinct dry season of 4 months after which the soil is replenished during the onset of the monsoon rains. This is in accordance with Brooks et al. (2010), who described that precipitation fallen in the beginning of the rainy season gradually refilled an empty soil water pool. They described that this precipitation is tightly bound in small pores and retained in the soil throughout seasons despite other inputs. $\delta^2H_{LMWL\text{-}int}$ increased during the following months, indicating that the water pool of the plants was stepwise replenished by more enriched precipitation fallen after November. So our data seems to confirm the two water world hypothesis with one soil-bound pool that is used by the plants and another highly mobile precipitation pool that contributes to streams and groundwater (Brooks et al., 2010). According to our data, plant species and associated therewith phenology are the primary factors influencing the leaf water deuterium enrichment, while growth form and seasonality have negligible effects.

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How do soil types affect stable isotope ratios under evaporation: A post corrected evaporation fingerprint of the Cuvelai-Etosha-Basin, Namibia

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Stable isotopes are often utilized to improve knowledge about water flow paths and root water uptake, as well as for the partitioning of evapotranspiration. However, the extraction of soil water from soils with elevated clay content remains challenging. Especially at low water contents (~5%) the extraction procedure can produce significant errors. Therefore, isotope depth profiles of soils mainly differing in their clay content collected within the Cuvelai-Etoscha-Basin (CEB) in northern central Namibia are presented. Additionally, isotope depth profiles of x-ray fluorescents analysis, cation exchange capacity and grain sizes are illustrated.

Soil water is either extracted with the cryogenic vacuum extraction method using extraction temperatures of 105°C and 205°C to evaporate the soil water into a liquid nitrogen trap. In addition, isotope values extracted at 105°C are corrected using a post correction procedure that accounts for physicochemical soil properties. Further, isotope values from soil water extractions are compared to soil water isotope depth profiles that were measured directly in the field using an in-situ approach.

It can be shown that, if soil samples regarded as potentially dry are used for scale normalization following the identical treatment principle (i.e. for in-situ approaches), the added water of known isotopic composition will be enriched by the residual interlayer or adsorbed water. This shifts the anchor points that are used for scale normalization together with all sample results. Alternative standard preparation methods will be discussed. Finally the presented isotope depth profiles are used to determine actual soil evaporation rates.

Field scale variability of nitrification rate

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Nitrification is a key process in the soil nitrogen cycle and is an important biological source of N_2O emissions from soils. In addition, nitrification is known to promote nitrogen eluviation from soils, because the less mobile cation ammonium (NH_4^+) is oxidised by nitrifiers to the much more mobile anion nitrate (NO_3^-) (Abbasi and Adams, 1998). Nitrification is influenced by a number of environmental factors. The effects on nitrification rates of environmental factors e.g. substrate availability (ammonia and oxygen), temperature, soil moisture and pH are described. Most of these factors are investigated comprehensive and agreement was found that NH_3 availability is the most important.

It is well known that soils vary spatially even over short distances. Spatial variability is understood to have an effect on diverse processes in soils, e.g. on transport processes, biomass turnover rate and on nutrient cycling processes. In contrast to N_2O emission and ammonia oxidizer population (e.g. Webster et al. 2002), at my knowledge no study has addressed the spatial variability of gross nitrification rate in soils. Therefore, less is known about small scale variability. To close this gap, in the experiment variation of gross nitrification rates in the field was investigated. Cores of 4 cm diameter and 20 cm depth (app. 400 g soil) were taken at 172 points inside a 50 m *100 m area. Nitrification rates were determined in the laboratory by ^{15}N pool dilution technique (Kirkham & Bartholomew 1954). under constant temperature (21°C) and actual field moisture (between 10 an 15 % w/w). At time 1 and time 2 (120 or 135 h later) soil samples were extracted with 1M KCl at ratio of 1:5 and ^{15}N abundance of the nitrate in the extract was measured with the SpinMAS-technique (Stange et al. 2007).

Spatial variability of nitrification rate at the plot scale was very high (coefficient of variation 116 %) and the range of autocorrelation for nitrification was short (max. 10 cm) on this experimental plot. The determined nitrification rates were distributed lognormal and geometric mean nitrification rate for the sandy soil was 0.63 mg N kg^{-1} d^{-1}, (median 0.64 mg N kg^{-1} d^{-1} and arithmetic mean 0.72 mg N kg^{-1} d^{-1}).

The results of the experiment confirm a stochastic nature of nitrification rate at the plot scale and suggest nitrification is determinate at the microscale as proposed by others (e.g. Drury et al., 1991). These studies highlight the importance of the spatial-temporal distribution of substrates, microorganisms, plant roots and organic residues in soil in regulating the soil N cycle. Therefore representative soil sampling is a prerequisite for the quantification of nitrification rate for a field site.

The bottom lines are that

- spatial variability of nitrification rate is high in the field
- for representative rates a great number of samples or pooled samples are necessary
- arithmetic mean is inappropriate to calculate mean nitrification rates in soils.

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Tracing precipitation through soil-vegetation-atmosphere interfaces of a savannah understorey by stable isotope labelling

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Water availability is a major limiting factor for biomass productivity and governs long term vulnerability of many terrestrial ecosystems. Mediterranean ecosystems like savannahs suffer exceptional water stress by high atmospheric water demands and limited replenishment throughout the year, enforced by climate change impacts in the past (Piayda et al., 2014). Profound process knowledge of precipitation water transitions through pools and fluxes of savannahs is needed to understand interactions between soil, vegetation layers and atmosphere affecting ecosystem productivity in the future. Stable water isotopes are a powerful tool to trace water flows in ecosystems and disentangle different sources in space and time, water utilisation for productive and non-productive pathways (Dubbert et al., 2014) as well as residence time in different compartments.

In the present study, an artificial precipitation event with isotopically labelled water ($\delta^{18}O$ = -139‰) is traced through compartments and fluxes of a Portuguese cork-oak savannah understorey. Onsite CRDS measurements with open gas flux chambers were combined with IRMS analysis of water from ecosystem pools and microclimatic observations. Data analysis utilised fractionation modelling for flux partitioning and multi-source mixing models for event water partitioning and root water uptake allocation.

Results reveal higher infiltration amounts on bare soil plots compared to vegetated plots, reaching much deeper soil layers. Precipitation event water displaces up to 60% of the pre-event soil water downwards immediately after rain and is, despite the amount of 20 mm, completely consumed within nine days. Soil evaporation, plant transpiration as well as leaf water show immediate response only 1 hour after watering with a much stronger depletion of evaporation down to -150‰. However, maximum transpiration rates were recorded with a delay of ca. 24 h compared to evaporation. Water fluxes were strongly reduced under tree shading compared to plots in between trees as a result of limited radiative energy supply and water competition with tree roots in soil layers below -30 cm. Source water partitioning exposed much higher water losses of event water by unproductive evaporation compared with transpiration (62% and 18%, respectively) reasoned by major root water uptake located at -30 cm below the major infiltration depth.

In conclusion, application of stable water isotope tracers exposed crucial interactions between soil, understorey and trees governing ecosystem water uptake, release and distribution necessary for prediction of climate change impact on ecosystem productivity and vulnerability. Evaporation loss consuming event water at high rates was reduced by understorey vegetation raising ecosystem productivity while tree cover extended water residence time in upper soil layers. Still, reduced infiltration below vegetation cover and root water competition between vegetation layers leaves the ecosystem at fragile balance facing climate change progression.

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Three decades after afforestation are sufficient to yield decomposition-related vertical δ^{13}C depth profiles in soil

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Decomposition in soils is crucial in evaluating mitigation of rising atmospheric CO_2 levels with vertical carbon (C) stable isotope distribution in topsoils serving as easily applicable method to investigate C dynamics in soil. Since many parameters affecting δ^{13}C depth patterns can change fast and strongly during land-use changes, vegetation conversions provide valuable insights into C dynamics in soil.

We sampled 36 topsoil core profiles (0-10 cm) with sites under different land-use, i.e. arable sites and continuously forested sites as references for none or maximum development of vertical δ^{13}C distribution, respectively and reforested sites to test for the time of δ^{13}C depth profile formation in the Black Forest, Germany. Relations between the vertical decrease of C and the increase of δ^{13}C values served to approximate decomposition that were elucidated by measuring soil respiration in a laboratory incubation study.

We found an accumulation of C with increasing time of forest cover and a development of δ^{13}C depth profiles within decades. Carbon concentrations and decomposition were positively related, suggesting that C accumulation is not coupled with reduced decomposition. The Suess effect, increased belowground biomass production and related greater dissolved organic matter production as well as lower pH values may have accounted for greater isotopic differences in topsoil and increased soil respiration at reforested sites, leading to greater transport of ^{13}C enriched microbial products. These parameters became further amplified at continuously forested sites. In contrast, soils under agricultural use showed near zero vertical enrichment in ^{13}C but low measured soil respiration fits well to trends of increasing decomposition with time of forest cover.

Our results provide evidence that short timescales of < 50 yrs are sufficient to develop distinct δ^{13}C depth profiles in topsoils, underpin the contribution of respiration to shape vertical C stable isotope patterns and suggest a hampered applicability of vertical C stable isotope distribution to approximate low decomposition in soil.

Position specific labeling as a tracer of free lipid transformations in soils

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The assumed long-term preservation of soil lipids distinguishes them as important contributors to the stability of soil organic matter (SOM) and has led to their use as biomarkers of plant and microbial carbon (C) sources. While the turnover time of bulk lipids and individual lipid fractions have been extensively studied, little is known about their metabolic transformations by soil microorganisms. Potential transformations pathways include: 1.) Degradation followed by re-incorporation into microbial lipid compounds (resynthesis) or 2.) Intact incorporation of the alkyl chain with subsequent modification (recycling). Determining the prevalence of these transformation pathways in soils is essential for accurate interpretation of lipid biomarkers, as modification of original biomarker signatures by microbial processes may represent a significant source of error if not accounted for in biogeochemical models.

To determine the utilization and transformation of n-alkyl lipids in soil microbial metabolism, we applied position-specifically ^{13}C-labeled palmitate to an agricultural Luvisol and traced the C incorporation from individual positions into lipid fractions via compound specific stable isotope analysis. Using this novel approach, our group previously revealed a high rate of intact uptake and incorporation of palmitate into the fast-cycling pool of microbial phospholipid fatty acids (PLFAs) over a 10 day period, with subsequent modifications of the intact alkyl chain (e.g. branching, elongation) increasing with time (Dippold & Kuzyakov, 2016). Building on this research, the current study sought to identify ^{13}C incorporation from the labeled palmitate into various n-alkyl free lipid fractions (fatty acids, hydroxy acids, alkanes, ketones and alcohols) to determine the extent of lipid transformations over long time scales. Here, we detail the results of these analyses over a 4.5 year period and answer the question: Does recycling and subsequent modification of intact alkyl chains within microbial metabolism represent a significant source of free lipid transformations in soils?

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Novel results of stable carbon and hydrogen isotope measurements of chloromethane from several sources and sinks to better constrain the global budget of chloromethane

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Chloromethane (CH_3Cl) is the most abundant chlorinated organic compound in the atmosphere (currently ~550 pptv). It is considered to be responsible for over 15% of halogen-catalysed destruction of stratospheric ozone. Chloromethane is known to be produced by abiotic and biotic processes, and most predominantly from natural sources including vegetation. Nevertheless, the strength of sources and sinks contributing to the global chloromethane budget is not fully understood. Particularly the role of vegetation/phyllosphere in chloromethane cycling is of high interest (Bringel & Couée, 2015). A potentially powerful tool in studying the global CH_3Cl budget might be the use of stable isotope ratios (Bahlmann et al. 2011, Nadalig et al. 2014, Keppler et al. 2005).

In this context it is essential to know the carbon and hydrogen isotopic signature of CH_3Cl in the atmosphere, as well as the kinetic isotope effects of CH_3Cl with OH radical, the dominant sink for atmospheric CH_3Cl. We present first δ^2H measurements of atmospheric chloromethane.

Another focus was placed on the phyllosphere, i.e. the aerial part of plants, which might be a niche for methylotrophic bacteria, some of which might utilise CH_3Cl as a carbon source and may thereby contribute to limit CH_3Cl emissions to the atmosphere. Therefore, in the second part of this presentation we report first stable hydrogen isotope measurements of several plant species that might act as sources and sinks of atmospheric CH_3Cl.

The preliminary results confirm that plants may act as sources and phyllosphere bacteria may act as a microbial sink of CH_3Cl. The preliminary stable isotope measurements will be discussed in the context of their importance in constraining the global CH_3Cl budget.

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Short-term carbon dynamics in a temperate grassland ecosystem under 14 weeks of severe drought followed by irrigation

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Extreme weather events strongly influence carbon (C) cycling in the plant-soil system. However, the response of C cycling in the plant-soil system to severe drought still remains unclear.

We examined the effect of severe drought (14 weeks) followed by irrigation on the C uptake by plants and translocation of recently assimilated C using a $^{13}CO_2$ pulse–chase labelling experiment in the Event I experiment (Bayreuth, Germany). We applied $^{13}CO_2$ pulses three times during the experimental drought. For grassland, 4 plant species and for heathland 2 plant species were labelled in each campaign. First $^{13}CO_2$ labelling was performed on week 0 followed by second and third $^{13}CO_2$ pulse in weeks 5 and 9 of severe drought, respectively. Plant and soil samples were analysed for their organic C concentration and stable C isotope composition.

After the first $^{13}CO_2$ pulse, the highest ^{13}C tracer uptake was observed for shoots and decreased towards roots and soil. One day after the first labelling, heathland and grassland shoots revealed high ^{13}C tracer uptake (54 and 52 mg $^{13}C/m^2$, respectively). After the second isotope pulse (5 weeks of drought), all plants showed lower (25 – 70% less) ^{13}C uptake. No further ^{13}C uptake was observed after third pulse labelling (9 weeks of drought) for some species, while others still showed a slight uptake. Shoots of all investigated four plant species showed high tracer uptake in the beginning of the pulse labeling and decline of ^{13}C signal over time after each pulse labeling. Roots exhibited continuous ^{13}C enrichment within 2 weeks after each isotope pulse due to subsequent transfer of assimilated ^{13}C from shoots. Maximum 15% of ^{13}C enrichment in shoots was observed in roots. Similar like for shoots and roots, ^{13}C tracer uptake became lower for soil with increased drought duration. After the third $^{13}CO_2$ pulse, very low ^{13}C uptake in shoots led to negligible translocation of ^{13}C into roots and soil. Thus, during initial drought, C cycling in the investigated plant-soil system did not show a reasonable effect of drought, while already after 6 weeks of severe drought C uptake and translocation became limited. During the severe drought only selected plants (*Plantago lanceolata* and *Holcus lanatus*) still assimilated small amounts of CO_2, but C transportation towards the soil could not be observed. Furthermore, results of the irrigation after 14 weeks of drought suggested that even after long duration of drought, temperate plants can rapidly re-sprout leading to fast translocation of accumulated ^{13}C from shoots towards roots.

Hence, our study concludes that the severe drought led to reduced plant C assimilation and belowground C allocation into soil and rapid re-start up of C cycling in the plant-soil system after irrigation despite 14 weeks of drought.

Can Crops with Greater Rooting Systems Improve Nitrogen Retention and Mitigate Emissions of Nitrous Oxide?

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It has been suggested that crops with deeper root systems could improve agricultural sustainability, because scavenging of nitrogen (N) in the subsoil would increase overall N retention and use efficiency in the system. However, the effect of plant root depth and root architecture on N-leaching and emissions of the potent greenhouse N_2O remains largely unknown. We aimed to assess the effect of plant rooting depth on N-cycling and N_2O production and reduction within the plant-soil system and throughout the soil profile. We hypothesized that greater root depth and root biomass will (1) increase N use efficiency and decrease N losses in the form of N leaching and N_2O emissions; (2) increase N retention by shifting the fate of NH_4^+ from more nitrification toward more plant uptake and microbial immobilization; and (3) increase the depth of maximum N_2O production and decrease the ratio of N_2O:(N_2O+N_2) in denitrification end-products. To test these hypotheses, 4 winter wheat cultivars were grown in lysimeters (1.5 m tall, 0.5 m diameter, 3 replications per cultivar) under greenhouse conditions. Each lysimeter was equipped with an automated flux chamber for the determination of N_2O surface fluxes. At 7.5, 30, 60, 90 and 120 cm depth, sampling ports were installed for the determination of soil moisture contents, as well as the collection of soil pore air and soil pore water samples. We selected two older and two newer varieties from the Swiss winter wheat breeding program, spanning a 100-year breeding history. The selection was based on previous experiments indicating that the older varieties have deeper rooting systems than the newer varieties under well watered conditions. N_2O fluxes were determined twice per day on a quantum cascade laser absorption spectrometer interfaced with the automated flux chambers. Once per week, we determined concentrations of mineral N in pore water and of CO_2 and N_2O in the pore air. For mineral N and N_2O, also natural abundance isotope deltas were determined, to obtain *in situ* process-level information on N-cycling. Preliminary results show lower soil moisture content and higher subsurface N_2O and CO_2 concentrations for the old varieties compared to the new varieties. Currently, we are performing isotope analyses, surface flux analyses, and we are harvesting the plants for determination of root- and aboveground biomass, and C and N contents therein. Results from these analyses will be ready for presentation in September 2016, and will allow us to reconstruct the N budget and further explore to what extent our hypotheses are corroborated.

Priming effect in boreal forest soils along a fertility gradient

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Climate warming increases labile carbon (C) inputs to soil through increased photosynthesis and C allocation below ground. This could counterintuitively lead to losses of soil C via priming effects (PE): the stimulation of soil organic matter (SOM) decomposition caused by labile C addition. Positive PE is often reported in response to glucose addition, but there is a need for a more systematic quantification of PEs in relation to soil properties, before PEs can be explicitly represented in soil C models.

We measured PE in five boreal soils from a site fertility gradient. Sampling sites were chosen from the network of sites belonging to Natural Resources Institute Finland's long-term fertilisation experiments. We chose five sites, so that the gradient in C:N ratio of organic layers (reflecting site fertility) was as wide as possible, ranging from 24.1 to 46.2, while maintaining similar climate and soil type. Two of the sites were in Norway spruce (*Picea abies* (L.) Karst) stands, and three were in Scots pine (*Pinus sylvestris* L.) stands. On all of the sites we sampled soil from B1-layer (10-20 cm depth) of the control plot and an N-fertilised plot that had received N-amendments since the 1950's. In addition, we include results from an additional Norway spruce site, analysed with the exactly same methods before, to reach N=3 for both tree species. This additional site had no N-fertilised plot. We added ^{13}C labelled glucose (2.04 atom %) to sieved soil at a rate of 15 mg glucose C per g soil C. Soils were incubated at 14 °C for 11 days in the laboratory, during which CO_2 concentrations inside the bottles were measured every other day. The $\delta^{13}C$ of the respired CO_2 was measured at the end of the experiment, to calculate PE.

We hypothesised that, if positive PE results from microbes using the energy from added labile substrates to decompose recalcitrant materials to acquire N ("microbial N mining hypothesis"), 1) PE would be higher on the naturally less fertile sites, due to higher N limitation on microbial activity, 2) PE would be lower on the N-fertilised plots, due to higher N availability compared to control plots and 3) adding of N simultaneously with the labile C would cancel the observed positive PE, due to cancelling the need to decompose recalcitrant SOM to access N.

Preliminary results show that CO_2-C released by PE on the less fertile pine sites was greater than on the spruce sites, supporting hypothesis number 1). However, the effects of long-term N-fertilisation were not consistent, sometimes increasing and sometimes decreasing the magnitude of PE compared to control. Also N-additions (as NH_4^+Cl) in the lab had inconsistent effects on the magnitude of PE. Ongoing analysis of a ^{15}N labelling experiment to determine gross N mineralisation and immobilisation rates using ^{15}N pool dilution, will hopefully shed light on the reasons behind these variable responses. We will also measure phospholipid fatty acids (PLFA) to find out whether changes in microbial community composition were related to the magnitude of PE.

$\delta^{13}C$ signatures of algal fatty acids in the Fram Strait – A new tool for paleo-sea ice reconstruction?

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Arctic Ocean sea ice cover has decreased by ~ 480,000 km^2 per decade between 1997 and 2012 (IPCC, 2013), and models predict that the Actic Ocean may be ice free during late summer by 2100 (e.g. Boe et al., 2009). Arctic Ocean Sea ice influences global climate via its surface albedo, ocean-atmosphere heat exchange, and the meridional overturn circulation (Müller et al., 2011; and references therein). Furthermore, sea ice covered areas are an important habitat for considerable amounts of micro faunal biomass that forms the base of the food chain in the Arctic, and Nordic Seas. Due to its influence on the climate system, and its importance for the Arctic food web, palaeo-sea ice reconstruction is imperative to better understand sea ice/climate interactions.

Sea ice algae photosynthesize in ice-floe interstitial waters and brine channels, utilising a restricted dissolved inorganic carbon (DIC) pool. Thus the carbon isotopic signature of organic matter produced by ice algae is isotopically heavier than the organic matter produced by pelagic algae. Here we present preliminary results, assessing whether sedimentary C_{16}-fatty acid (C_{16}-FA) isotopic signatures can be used as a tool to elucidate, whether the FAs are predominantly produced by ice-, or pelagic algae and can be used to reconstruct changes in past sea ice extent.

Ice algae, ice core particulate organic matter (POM), water column POM, and sediment samples from the Fram Strait were collected aboard the *RRS* James Clark Ross during the ICECHASER2010 cruise. The ice algae samples have significantly more positive C_{16}-FA $\delta^{13}C$ values (average: -21.1 ‰) compard to POM C_{16}-FAs collected from the ice free sampling station (-32.2 ‰). The same is true for the sedimentary C_{16}-FA $\delta^{13}C$ values from sea ice influenced sampling areas, which are on average 2 ‰ more positive than the sedimentary C_{16}FA $\delta^{13}C$ values from the open ocean area. These results suggest that the influence of ice algal derived, isotopically heavy C_{16}-FAs is preserved in the surface sediments. Therefore we suggest that the isotopic signature of fatty acids may be useful for palaeo-sea ice extent reconstructions.

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Carbon isotope dynamics (DIC) in surface sediments of marginal seas

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Mineralization of organic matter in marine sediments by microbial activity leads to the liberation of dissolved inorganic carbon (DIC) and other metabolites into the sediment interstitial waters. Pore water profiles, therefore, sensitively reflect the zones of dominant biogeochemical processes, net transformation rates, and diffusive and advective transport of dissolved species across the sediment-water interface. They are controlled by different factors like sedimentology, bottom water currents and redox conditions, microbial activity, and the availability of electron acceptors/donors.

We present here the results of detailed biogeochemical investigations of interstitial waters from modern surface sediments in two brackish marginal seas: the Baltic Sea and the Black Sea. Biogeochemical processes and associated element fluxes at the sediment-water interface were studied using pore water and sediment samples retrieved from short sediment cores that were collected with multi-coring devices. Pore waters were taken in sufficient depth resolution and analyzed for metals, nutrients, and metabolites concentrations as well as stable carbon isotope composition of DIC to allow a modeling of steady-state transformation, volumetric transformation rates and element fluxes. Element fluxes across the sediment-water interface depend on bottom water redox conditions, sedimentology, organic contents. Advective fluxes induced by sedimentation events, macro zoobenthos and wave action can affect the top sections of the sediment, thereby modifying shallow concentration gradients. By means of (non)steady state modelling of pore waters we were able to identify the impact of mixing processes and sedimentation events in the oxic part of the Baltic Sea. In the Black Sea, on the other hand, anaerobic processes control the dynamics in DI13C under permanent euxinic conditions. The carbon isotope composition of DIC is found to be a highly sensitive tool for understanding carbon cycling in surface sediments.

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Using isotopologue analysis to elucidate processes of N_2O production and reduction in arable soils managed with climate-smart adaption strategies

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The study was performed to tackle the questions of how we can improve cropping systems, tillage and fertilization in order to reduce agricultural greenhouse gas emissions. The potential of optimized cropping rotations and farm structures to lower agricultural climate effects is investigated with three model farms (cash crop farm without livestock, livestock farm with biogas plant and climate-optimized farm) in two field studies in North Rhine-Westphalia (Soest) and Lower Saxony (Braunschweig) under the lead of the South Westphalia University of Applied Sciences.

Fertilizer treatments include mineral or organic plus mineral fertilization in varying amounts. Maize cropping in the climate optimized farm will also include a comparison between trailing hose fertilization and strip-till fertilization (below-root) at the Soest site. Our aim is to assess the impact of biogas digestate application on the emissions of ammonia (NH_3) and the greenhouse gases nitrous oxide (N_2O) and methane (CH_4) in maize. Moreover, organic fertilization with digestate is compared to mineral fertilization in winter wheat. Additional monitoring for soil parameters like soil temperature, water content and mineral nitrogen is done to elucidate regulation of gaseous N emissions.

Here we present our results from stable isotope analysis of N_2O and mineral N to assess processes of N_2O production and reduction. Soil N_2O fluxes originate from a multiple of mostly microbial processes where the currently known include production by nitrification (including hydroxylamine oxidation and nitrifier denitrification), fungal and bacterial denitrification, co-denitrification, dissimilatory nitrate reduction to ammonium as well as N_2O reduction to N_2 by bacterial denitrification. Isotopologue values of soil-emitted N_2O ($\delta^{15}N$, $\delta^{18}O$ and SP = difference in $\delta^{15}N$ between the central and the peripheral N positions of the asymetric N_2O molecule) may help to distinguish N_2O production pathways and to quantify N_2O reduction to N_2 in this field study. Different N_2O forming processes are characterised by distinct isotopic characteristics. For example, Bacterial denitrification shows significantly lower SP and $\delta^{18}O$ values when compared to fungal denitrification and nitrification processes. But SP and $\delta^{18}O$ values are also altered during N_2O reduction to N_2, when the residual N_2O is enriched in ^{18}O and centrally located ^{15}N, resulting in increased $\delta^{18}O$ and SP values. We will combine isotope mixing and fractionation modelling approaches to calculate the fraction of N_2O from bacterial denitrification as well as N_2O reduction to N_2. Results of the field studies in Soest and Braunschweig over two years will be presented.

Fraction-specific carbon isotope distribution pattern of oil from the Uzon valcano caldera (Kamchatka)

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The Uzon caldera is a unique natural laboratory of the present day oil formation from the organic matter of Pliocene-Quaternary sediments. The process of oil generation seems to take place in modern age. The goal of our study was to prove the existence of a genetic link between the Bogachevka oil, oil seeps and biota residing in hydrothermal lakes in the Uzon caldera. Oil was obtained from wells drilled in Bogachevka area adjacent to the Uzon caldera (70 km). Banded ash-pumice deposits up to 350 m thick were accumulated in lake basins in the Uzon caldera. The temperature can reach 250°C at a depth of 500 m.

All samples were divided into five fractions arranged in the order of increasing polarity: hexane hydrocarbon fraction – HC, hexane-benzene fraction – C_{HB}, benzene fraction - C_B, benzene-methanol fraction C_{BM} and asphaltene fraction – A. The polarity of these fractions correlate with β-factor (Galimov 1985). Samples of oil seeps and biota were also subjected to hydrothermolysis in a platinum capsule at 300°C for 24 h.The carbon isotope composition of fractions was measured on a DELTA Plus XP isotope ratio mass spectrometer (Thermo Fisher Scientific, Bremen, Germany) coupled to the Flash EA 1112 element analyzer. Biomarkers were analyzed in the saturated fraction by gas chromatography mass spectrometry using Trace GC Ultra (Thermo Fisher Scientific) coupled to a Polaris Q ion trap. They were identified on the basis of characteristic ions at m/z 191 (terpanes), m/z 217 (steranes), and m/z 218 (isosteranes) in hexane fraction extracted from the oil and biota.

The measured carbon isotope composition of oil seeps from the Uzon caldera ($\delta^{13}C$=-30.8 ‰) is typical for oils derived from biological materials of mostly bacterial origin. The low content of tricyclic terpanes, which is a qualitative oil maturity parameter, indicates that the oil maturity of the Uzon seeps is low. A difference was detected between the isotopic fractionation characteristics of naphtides from the Uzon caldera and products of the thermal hydrolysis of biota. In turn, the isotopic fractionation characteristics of the thermally hydrolyzed biota appeared to be similar to the isotopic compositions of the respective fractions of Bogachevka oil. The identical isotopic diagrams of the Bogachevka oil and the hydrothermally transformed biota confirm the biogenic nature of Bogachevka oil. Hydrocarbons from the Uzon caldera are mainly products of the hydrothermal transformation of the biomass of microorganisms and plants remains in the zone of thermal water circulation.

After the thermohydrolysis of oil from the Uzon caldera the $\delta^{13}C$ values of asphaltene and benzene-methanol fractions increased and approached the $\delta^{13}C$ values for the corresponding fractions of Bogachevka oil. Most probable the Bogachevka oil had been generated under more hard-thermobaric conditions as compared with oil seeps of the Uzon caldera.

The analysis have demonstrated that hydrothermal naphthides formed from living matter of different nature: bacterial communities, phytoplankton, arhaea, and remains of higher land plants and pollen. Hydrothermal naphthides are different in the degree of maturity, but in general they are less transformed than most of basin oils. The Uzon hydrothermal zone can now be considered to be an unique volcanic geological system with intense degradation, re-synthesis and redistribution of the both organic and inorganic matter.

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Gross N mineralization and nitrification in drained boreal peatlands with different land-use

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In natural peatlands nitrous oxide (N_2O) emissions are usually negligible or they may even act as sink for N_2O. When peatlands are drained, oxygen concentration in the peat increases causing acceleration in organic matter decomposition and associated nitrogen (N) mineralization and nitrification. Therefore N_2O emissions may increase substantially after peatland drainage for forestry or agricultural uses (Martikainen et al. 1993). Water table level is one of the main drivers for N_2O emissions in peatlands (Leppelt et al. 2014) but there are numerous other variables affecting spatial and temporal variation in N_2O emissions (Leppelt et al. 2014, Maljanen et al. 2010). Nitrate from nitrification can be consumed quickly in soil and net nitrification indicates poorly nitrification rate in soil. However, using ^{15}N pool dilution method gross N mineralization and nitrification rates can be determined. The aim of our study was to investigate gross N mineralization and nitrification in drained peatlands under different land-use to see how nitrate availability explains the variation in N_2O emissions.

We collected soil samples from 12 peatland sites in Finland, Sweden and Iceland. Sites represented 5 different land-use types including peatland forests (3 sites in Finland), cultivated fields (2 sites in Finland and one in Iceland), afforested cultivated fields (2 sites in Finland and one in Sweden), abandoned fields (2 sites in Finland) and one site which was drained but not used for agriculture, forestry or for other purposes (in Iceland). In 11 of the sites the C/N ratio in the peat was between 15 and 25 which is known to be the optimum range for high N_2O emissions (e.g. Maljanen et al. 2010). One forest site had a C/N ratio over 80 and it was included as a comparison. For the sites we used published N_2O emissions. Gross N mineralization and nitrification at field water content was determined using soil samples taken from the depth of 10-20 cm. Additionally, we made other laboratory analyses including incubation experiments to determine N_2O production rates, inhibition experiments with acetylene to estimate the contribution of nitrification and denitrification in the N_2O production and phospholipid fatty-acid (PLFA) analyses for microbial community structure.

According to the preliminary results there was a positive correlation between N_2O emission and gross N nitrification but N_2O emission did not correlate with gross N mineralization. There were no clear differences in gross N mineralization and gross N nitrification between different land-use types. An exception was the forest site with extremely high peat C/N ratio. This soil had the highest $^{13}C/^{12}C$ ratio and lowest $^{15}N/^{14}N$ ratio among the soils. In the forest sites where gross N mineralization and nitrification were determined several times, these processes had rather high variation during the study period. This highlights that sampling time is important when comparing mineralization and nitrification between various sites.

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Isotopomers reveal N_2O production and consumption zones along soil profiles in a Norway spruce forest (Fichtelgebirge, Germany)

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Since December 2015 nitrous oxide (N_2O) is not longer considered as one of the forgotten greenhouse gases. During the "United Nations Framework Convention on Climate Change, 21st Conference of the Parties" (COP 21) the focus from reducing the carbon dioxide emission was expanded, considering also the massive impact of the other greenhouse gas emissions e.g. methane and nitrous oxide.

N_2O is mainly produced as an intermediate in the stepwise denitrification by microorganisms in soils. To obtain information on these metabolic pathways the $\delta^{15}N^{bulk}$ value has frequently been used. However, this value is influenced by the isotopic composition of the denitrification substrate (Ostrom, 2011). To overcome this problem additional site preference measurement in the N_2O molecule reveals an ideal solution, because the site preference is independent of the substrates' isotopic composition. Site preference (SP) indicates the intramolecular distribution of the heavy N isotope in the asymmetric linear structure of nitrous oxide (N-N-O).

Currently very little information about the SP is available for soil air samples from depth profiles. It is expected that the SP increases with ongoing reduction of N_2O to atmospheric nitrogen (Park et al. 2011). The aim of the study was to ascertain SP values by analyzing the signature of isotopomers within the N_2O molecule along soil profiles (Haplic Podsol) of a Norway spruce forest in the Fichtelgebirge (Germany) and to deduce N_2O production and consumption zones.

N_2O in soil air and nitrate in soil water were collected along depth profiles in a nitrate fertilization study with five sampling dates on three nitrate application and control plots each. Nitrate was applied to stimulate microbial denitrification.

Nitrate concentrations and $\delta^{15}N$ values of nitrate were identified by anion exchange HPLC and UV detection and by manual preparation and subsequent EA-IRMS analysis, respectively. Soil air samples were analyzed by PreCon-GC-IRMS for $\delta^{15}N^{bulk}$, $\delta^{18}O$, $\delta^{15}N^{\alpha}$ and $\delta^{15}N^{\beta}$ values and for the N_2O concentration. The SP was calculated by subtracting $\delta^{15}N^{\beta}$ from $\delta^{15}N^{\alpha}$ values.

Highest SP values were found in soil air in 10 to 30 cm depth accompanied by $^{15}N^{bulk}$-enriched N_2O isotope signatures and by low N_2O concentrations. All of these parameters are indicative of denitrification in these soil layers that ends with atmospheric nitrogen. In contrary, the low SP values with high N_2O concentrations and negative $\delta^{15}N^{bulk}$ values as found in air from the subsoil show a production of N_2O without further reduction to N_2.

Thus, the topsoil in this forest serves as a sink for N_2O, which was produced in the subsoil. No significant correlations between nitrate and N_2O neither for concentrations nor for isotope signatures were found.

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Free amino acid dynamics in the soils of *Eucalyptus* FACE

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In an alluvial previous flood-plain northwest of Sydney, Australia, a native open *Eucalyptus* woodland with an understory vegetation of grasses and herbs, is since 2013 subjected to elevated CO_2 (550 ppm) in six 25 m high FACE rings (EucFAC, Drake *et al.* 2016). Free amino acids (FAA) in the soil are a very dynamic pool of organic nitrogen (N). They are produced from depolymerization of soil organic matter, and during plant root exudation. FAAs are likely important for nutrient cycling in this ecosystem, because the soil is nutrient poor (aeric podosol) and with low organic matter content (~3 %). Hence, N together with P (Crous et al. 2015) are likely limiting nutrients for primary production.

The FAA content in soils of FACE and non-FACE plots of the EucFACE research facility was analysed in early summer (November) and early fall (March) using GC-MS (at ISOFYS, Belgium). Total FAA quantity of ~0.8 µg FAA g^{-1} soil compared well to a nutrient poor forest soil (Podzol in Sweden; Andresen *et al.* 2016) analysed with same GC-MS method (Wanek *et al.* 2010), and a factor 5 lower than an *Eucalyptus* forest soil (ninhydrin method in: Paulding *et al.* 2010). Elevated CO_2 treatment only slightly affected the soil content of free AAs. Fresh soil samples from EucFACE plots were incubated with ^{15}N-enriched amino acids in order to determine: FAA mineralization and depolymerization rates, by analysis of content and ^{15}N enrichment of FAAs and of $^{15}N\text{-}NH_4$ (Andresen *et al.* 2016). Furthermore, FAA content in tension lysimeter water from two soil depths revealed slightly increased FAA by depth, and a dominance of serine, lysine and glycine, in amounts comparable to a forest soil in Sweden (Inselbacher *et al.* 2011).

Elevated CO_2 potentially increases the plant root exudation of labile carbohydrates, which could lead to rhizosphere priming. The soil priming in turn stimulates mineralization and SOM decomposition, hence tentatively stimulates depolymerization but also potentially enhances the microbial consumption of FAAs. Hence, availability of FAAs is only a result of a balancing of many fluxes. In this study we present ^{15}N tracing of the relevant fluxes.

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Field-scale in-situ analysis of ambient N_2O isotopic composition to trace source processes in an intensively managed grassland

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Nitrous oxide (N_2O), a strong greenhouse gas and an important ozone-depleting substance, is primarily emitted from pristine and fertilized soils. A multitude of biogenic and abiotic N_2O producing processes have been identified, but their relative contribution to total N_2O emission and relevance for different ecosystems is currently not known, as related processes are highly dispersed and variable. For the development of effective N_2O mitigation strategies, however, detailed knowledge of processes and their temporal and spatial variations is essential. Analysis of site-specific N_2O isotopic composition has proven potential to disentangle source processes based on their characteristic isotopic signatures (Wunderlin et al. 2013).

In recent years we developed an analytical technique for real-time high-precision analysis of N_2O site-specific isotopic composition, consisting of a quantum cascade laser absorption spectrometer (QCLAS) coupled to an automated preconcentration device, called TRace gas EXtractor (TREX) (Wächter et al. 2008, Eyer et al. 2016). In a pilot field study N_2O isotopic signatures could be interpreted in relation to management events and meteorological conditions (Wolf et al. 2015). Since then the field-applicability of the setup was significantly improved by advanced temperature control for both QCLAS and TREX, and installation of the instrumentation in a 19″ rack. In addition, the novel device offers the possibility for simultaneous analysis of $\delta^{17}O$-N_2O.

Here we present first results from a field study which will be carried out at an intensively managed grassland site in southern Bavaria, Germany (Fendt, 600 m.a.s.l.) between June and July 2016, as a sub-module of the ScaleX 2016 campaign organised by IMK-IFU. We will focus on the discussion of ambient N_2O isotopic composition measurements above the grassland site that are expected to shed light into different N_2O source processes based on their isotopic source signature. These results will be combined with atmospheric transport simulations and footprint analysis to interpret spatial variability. It is foreseen to evaluate N_2O isotopic information in conjunction with $\delta^{15}N$ values of nitrogen precursors (NH_4^+, NO_3^-), management events and additional supporting soil and meteorological parameters. Results from the field study will be discussed in relation to complementary approaches: A biogeochemical soil model (L-DNDC) with an isotope sub-modul currently developed at IMK-IFU, and a ^{15}N tracer approach applied by Thünen Institute.

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Silvicultural management of *Cupressus lusitanica* determines shifts in litter-derived C incorporation into microbial groups – a PLFA study

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In Ethiopia, about 87% of biodiversity-rich natural forests were deforested. To cope with the rising demand of wood, fast-growing exotic tree species such as *Eucalyptus saligna* or *Cupressus lusitanica* are grown in monoculture plantations. Concerns about the environmental sustainability of such land use practices led to the suggestion of silvicultural management of *Cupressus lusitanica* plantations in order to promote potential crop trees and to enable a transition towards higher biodiversity. Therefore, the objective of this study was to investigate whether such "more close to nature" management has benefits for the environment. As indicator for environmental sustainability, we used soil microbial biomass and its community structure, as it plays an important role in soil organic matter turnover and thus in sustaining ecosystem services. In order to specifically trace microbial activity and turnover upon silvicultural management, a ^{13}C *in situ* litter labelling experiment was established in the Munessa forest, Ethiopia to identify effects of (i) natural forest vs. *Cupressus* plantations, (ii) rotation ages of *Cupressus* plantations, and (iii) thinning intensities on litter-derived C turnover by soil microbial biomass.

Transformation of natural forest into young *Cupressus* plantations did not affect overall litter turnover. Obviously, litter turnover by protozoa and saprotrophic fungi was not different for the investigated treatments. In addition, different litter quality led to different microbes being responsible for litter degradation. When compared to natural forest, degradation of young *Cupressus* litter was enhanced by actinomycetes, while natural forest litter was preferably degraded by gram-negative bacteria. With increasing age of *Cupressus* plantations over all C turnover decreased. Gram-positive bacteria, gram-negative bacteria and actinomycetes were less involved in C turnover, while other microbial groups were not affected as the stand matures. Thinning of mature *Cupressus* monoculture plantations significantly increased microbial C turnover derived from ^{13}C-labelled litter. Thinning of young stands had no effect on overall C turnover, but significantly decreased C turnover by gram-positive bacteria, actinomycetes and arbuscular mycorrhiza. The opposite accounted for C turnover under mature stands, where gram-negative bacteria, gram-positive bacteria, actinomycetes and saprotrophic fungi incorporated more litter-derived C under thinned plots compared to non-thinned plots. However, C turnover by protozoa were always negatively affected by thinning.

Our results suggest that exotic tree plantations in the Munessa forest seem to be an option to balance the rising demand of wood and sustaining ecosystem functioning. The conversion of natural forests into plantations did not affect C turnover. Maturing of plantation stands always led to a decline in C turnover and seems not to be a useful practice to sustain ecosystem functioning. Thinning of young stands had no effect on C turnover but thinning of mature stands is a good practice to improve microbial C turnover and thus, nutrient cycling.

“CH4ScarabDetect” – Detecting and quantifying methane emissions from Scarab larvae using stable carbon isotopes

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The increase of the average global atmospheric methane (CH_4) concentration from ~700 ppb to ~1800 ppb since 1750 is the result of a global imbalance between CH_4 sources and sinks. Currently, the atmospheric CH_4 budget is in a near steady state condition, i.e. that slight changes in even small CH_4 sources and sinks can ultimately determine whether the Earth, as a whole ecosystem, is a net source or sink of atmospheric CH_4. Together with its short atmospheric lifetime of only nine years, this makes CH_4 an excellent candidate for short-term climate change mitigation strategies. However, this mitigation potential can only be accessed if we can accurately quantify all sinks and sources contributing to the global CH_4 budget. One of the poorly understood aspects of the global CH_4 budget is the effect of land-use change and management on the CH_4 budget of soils which themselves can be either sinks or sources of atmospheric CH_4 [1]. The project “CH_4ScarabDetect” aims to provide the first quantitative estimate of the importance of soil-dwelling larvae of the common cockchafer (*Melolontha melolontha*) and the forest cockchafer (*M. hippocastani*) for net soil CH_4 fluxes. These important European agricultural and forest pests are qualitatively known to emit CH_4, but have thus far been neglected in terrestrial CH_4 cycle research. To reach its aim, “CH4ScarabDetect” will develop a new non-invasive field monitoring method for separating gross CH_4 production and oxidation in soils in general, and for detecting larvae infestations in particular. Measurements of net soil CH_4 fluxes will be conducted with the well-known chamber flux method. For the in situ separation of gross CH_4 production and gross CH_4 oxidation, the chamber method will be combined with a $^{13}CH_4$ isotope pool dilution technique [2,3]. Methane concentrations and their isotopic signatures in the collected gas samples will be analysed with a state-of-the-art analyzer based on cavity ring-down spectroscopy (G2201-i analyzer equipped with the Small Sample Isotope Module 2 - A0314, Picarro Inc., USA). Different combinations of larvae age, abundance and depth distribution might yield the same net CH_4 flux rates. To account for this non-invasively, the chamber measurements will additionally be combined with acoustic measurements of larvae activity in the soil. This novel approach will not only further our understanding of the role of cockchafer larvae in the terrestrial CH_4 cycle and provide a new tool for soil CH_4 flux and soil insect studies, but promises to also improve the monitoring of cockchafer infestations, thus generating new knowledge of major relevance for both scientific and practical reasons.

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Tracing CO_2 emissions and microbial dynamics at the terrestrial-aquatic interface

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Worldwide, erosion on agricultural land plays an important role in carbon cycling. Deposition of eroded soil organic carbon (SOC) occurs on downslope soils or in adjacent inland waters such as streams or ponds, where SOC may be buried or be degraded and emitted as CO_2. The net effect on C exchange between soils, atmosphere and inland waters yet uncertain. We performed a 4-month incubation study, using a gradient of ten European agricultural soils to evaluate CO_2 emissions and the role of the microbial biomass at the interface of terrestrial and aquatic ecosystems.

Depositional conditions (terrestrial vs. aquatic) are crucial in determining CO_2 emissions, where transitions to inland waters induce a 2 – 3.5 fold increase in C turnover. Trends in CO_2 emission changed over time, and showed a lag phase for aquatic systems. A ^{13}C labelled tracer was used to gain insights in priming effects (i.e. changes in SOC degradation when a readily available C substrate is added). CO_2 emissions increased upon addition of a labile C substrate, but the effects differed for terrestrial and aquatic conditions. Microbial biomass dynamics were studied by tracing uptake of the ^{13}C labelled substrate. ^{13}C incorporation into the microbial biomass revealed different uptake and dynamics in terrestrial and aquatic ecosystems. Furthermore, we assessed physical and chemical soil properties as potential controls on CO_2 emissions.

The results of this study contributed to an empirical model that is able to predict CO_2 emissions from downslope soils and in inland waters. These quantitative relations are important to better integrate terrestrial and aquatic C fluxes.

The role of mycorrhizal fungi in plant carbon allocation under different nutrient availabilities

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Carbon taken up during photosynthesis is allocated to various processes and organs. This allocation determines its residence time in the ecosystem and ultimately the ecosystem carbon sink strength. Carbon allocation is poorly understood, mainly because allocation to the rhizosphere and especially to mycorrhizal symbionts remains unquantified. Earlier research has shown that some ecosystems invest only 30% of their photosynthates in growth, whereas others invest up to 70%. Evidence is growing that nutrient availability is behind this large variation, with a variable carbon cost of plant-mycorrhizal symbiosis as the hypothesized underlying mechanism.

In a mesocosm nutrient manipulation experiment, we want to test the hypothesis that the variation in C-allocation is driven by nutrient availability via its control on mycorrhizal carbon use. To this end, a full-factorial N and P fertilization experiment with *Zea mays* has been set up to quantify allocation of photosynthates to above- and belowground plant growth and respiration as well as to root exudates and mycorrhizal symbionts. A stable isotope approach is used to quantify belowground carbon allocation and partition its components. Specifically, we use the C_3/C_4 shift method, taking advantage of the difference in $\delta^{13}C$ between the C_4 plant maize and the C_3 soil, to partition the sources of the soil CO_2 efflux as well as mycorrhizal biomass production. The poster will present the methodologies as well as the first results from the experiment conducted in summer of 2016.

Depth differentiated N_2O production and surface emissions as a function of rice paddy water managment

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Increased crop nitrogen use efficiency and reduced nitrogen pollution, such as N_2O emissions, are essential to improving the sustainability of agricultural systems. Both aspects of nitrogen management should benefit from a better understanding of how nitrogen cycling is stratified within soil as function of management. A depth differentiated approach is particularly relevant in rice systems which are subject to oscillating redox conditions along a depth profile in relation to flood water management. This study focuses on N_2O production and emissions as a function of substrate availability and soil environment. A field trial in Northern Italy was established with two water regimes: continuously flooded (flooded) and alternate wetting and drying (AWD). Surface gas fluxes were complimented by depth profile measurements at five depths of soil gas, inorganic N, DOC, dissolved gas concentrations, redox potential and moisture. Sampling was concentrated around two periods during the 2015 growing season which were hypothesized to show significant variation in N dynamics; a fertilization event and final season drainage. Stable isotope measurements of ^{15}N and ^{15}N site-preference (SP, intermolecular distribution of ^{15}N) in $N_2O_{emitted}$ and $N_2O_{subsurface,}$ along with ^{15}N of pore water NO_3^-, and NH_4^+ were performed to obtain process-level information in situ.

High N_2O emissions were only observed in the AWD treatment at the beginning of the growing season. Emissions at this time corresponded to high N_2O production at 5 and 12.5 cm depths and to lower soil moisture and higher NH_4^+ availability. By conventional wisdom, these data together suggest nitrification as the source of N_2O. Isotopic analysis of bulk $\delta^{15}N$-$N_2O_{subsurface}$ and SP in the AWD treatment showed distinctly more depleted N_2O in upper layers and SP of~ 33‰ at times of highest emissions, therefore supporting the idea of nitrification as the source of emissions. $\delta^{15}N$-$N_2O_{subsurface}$ tended to become enriched as fluxes declined, this can be attributed to a declining contribution of N_2O from nitrification, it may also be in part attributable to progressive substrate utilization, enrichment of substrate due to ancillary processes such as NH_3 volatilization and/or increasing N_2O reduction. Reduction of N_2O to N_2 leads to an enrichment of residual N_2O, therefore if high rates of N_2O reduction occurred we would expect to see enrichment of N_2O in upper layers and $N_2O_{emitted}$, neither of which was observed. Analysis of ^{15}N-NO_3^- and ^{15}N-NH_4^+ are still on-going and are needed before we can better clarify the role of mineralization, nitrification and denitrification to N_2O emissions and draw more quantitative conclusions. Field work in 2016 will quantify NH_3 volatilization to help determine the effect on the size and signature of the NH_4^+ pool and a higher resolution dataset will be used to help develop a process rate model. In conclusion we found that high N_2O emissions could be linked to nitrification in upper layers at the beginning of the cropping season, therefore strategies that minimize mineralization and nitrification and/or promote N_2O reduction, may reduce N_2O emissions; unfortunately it may not be possible to combine such strategies with a reduction in water inputs.

Soil microorganisms overcome respiration inhibition toxics by coupling intra- and extracellular metabolism: ^{13}C metabolic tracing reveals the mechanisms

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The rate and amount of CO_2 evolved from soil are used as indicators of the effects of toxics e.g. heavy metals and other pollutants on overall heterotrophic microorganisms involved in SOM decomposition. However, mechanisms underlying persistent CO_2 release from soil exposed to toxics e.g. sodium azide (NaN_3) or contaminated with heavy metals e.g. Cadmium (Cd), Mercury (Hg), Copper (Cu) that inhibits microbial respiration is not well understood.

To unravel the mechanisms underlying substantial CO_2 emission after respiration inhibition, four isotopomeres of position-specifically ^{13}C labeled glucose were incubated with and without NaN_3. The ^{13}C from individual molecule position was quantifed in respired CO_2, bulk soil, extractable microbial biomass and in cell membranes of microbial groups classified by phospholipid fatty acids (PLFA). High ^{13}C recovery in CO_2 from C-1 position indicates that glucose was metabolized mainly by pentose phosphate pathway irrespective of inhibition. Although NaN_3 addition stopped ^{13}C incorporation into PLFA and strongly decreased total CO_2, ^{13}C recovery in CO_2 was 12% higher compared to uninhibited condition. ^{13}C recovery in CO_2 increased because glucose was used for energy production and synthesis of compounds needed to restore electron transport chain. Synthesis of compounds to transport electrons to extracellular environment was reflected by 5-fold higher ^{13}C recovery in bulk soil than microbial biomass after inhibition. Total fatty acid content doubled within 10 days because microbes recycle alkyl chains from soil, whose pool increased due to death of some microorganisms after NaN_3 addition. Preferential recycling of the cost-intensive alkyl chains under such conditions is in contrast to de-novo formation of other biomass compound classes and explains ^{13}C incorporation into microbial biomass but not PLFA after inhibition.

^{13}C incorporation into microbial biomass in soil under NaN_3 shows that glucose was metabolized intracellularly. Microorganisms circumvent the NaN_3 toxicity by extracellular electron transfer, which allows reconstruction of new electron transport chains and resumption of aerobic respiration. This bypass of intracellular toxicity by extracellular electron transport explains the unexpected high CO_2 efflux from soil after inhibition. We conclude that broad microbial communities in soil have high potential and various strategies to overcome toxicity of pollutants inhibiting metabolic functions. This is only possible in soil because it contains substances with redox properties unlike pure caltures. Therefore, the effects of toxics or pollutants on soil microorganisms are much less than pure caltures.

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A pilot study to investigate the pathway of deposited ^{15}N from root exudates into stable organo-mineral associations

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Long-term retention of nitrogen (N) in temperate forest soils after N deposition can follow several pathways. A possible pathway following after N immobilization by trees is that N containing root exudates will adsorb to soil minerals and contribute to the formation of stable organo-mineral associations. In order to investigate this pathway, a growing experiment with seedlings of two tree species (*Picea abies* and *Pinus sylvestris*) was conducted in a sandy soil for 6 weeks. A split-root technique was used to inject ^{15}N at one side of the root system and to trace it back at the other side of the root system in separate soil compartments. Highly enriched $^{15}(NH_4)_2SO_4$ (98 atom %) of high (41.8 mg N) and low (3.5 mg N) concentrations was injected in the soil at t0, after which enrichment of ^{15}N was analyzed in the rhizosphere, bulk soil, roots, needles and stem, 3 and 6 weeks after labeling. Most important results of this pilot experiment are: a fast ^{15}N enrichment of the organic and the rhizosphere samples, the minor enrichment of bulk soil samples, higher enrichment in *Pinus sylvestris* soil samples compared to *Picea abies* soil samples and higher enrichment in high labeled (41.8 mg N) soil samples compared to low labeled (3.5 mg N) soil samples. The results of this pilot study will be used for a long-term (24 months) plant growing experiment in which soil samples will be analyzed for ^{15}N concentrations in the light (<1.6 g cm^{-3}: particulate organic matter) and heavy (>1.6 g cm^{-3}: minerals and organo-mineral associations) density fractions, which will be indicative for the stability of retained N in the soil.

Microbial nitrogen recycling in agricultural soil revealed by ^{15}N tracing

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A large excess of mineral nitrogen (N) in European agriculture is causing harmful consequences to the environment (Sutton et al. 2011). The mineral N compound nitrate can be reduced by two dissimilatory soil processes: (i) denitrification, the stepwise reduction to atmospheric dinitrogen, and (ii) dissimilatory nitrate reduction to ammonium (DNRA). While denitrification is a major source of the greenhouse gas nitrous oxide, DNRA retains mineral N in the ecosystem in the form of ammonium. The aim of this study is to investigate the competition of these two processes in order to better understand the fate of nitrate in agricultural soil.

To compare the importance of denitrification and DNRA, we conducted a ^{15}N labelling experiment with two agricultural soils: an annual crop rotation with cereals and a grassland rotation system ('ley') with perennial forage crops and barley from a long-term trial field site near Uppsala (Sweden), both with different fertilisation levels. In laboratory incubations with fresh soil, subsamples of each treatment were enriched with ^{15}N labelled ammonium, nitrate or both moieties at about 25% of the native concentrations. For multiple time steps after labelling, we will analyse ^{15}N enrichment of mineral N species from soil extractions, and of nitrous oxide from headspace gas samples. The gross rates of denitrification and DNRA will be quantified using the *Ntrace* model (Müller et al. 2014). Furthermore, we analysed soil properties and quantified the 16S rRNA gene of soil bacteria, the ITS in fungi, the *NirS*+*NirK*- and *NosZ*I+*NosZ*II genes of denitrifying bacteria, and the *NrfA* gene of DNRA performing bacteria using qPCR in order to better understand, which microorganisms are involved and what conditions favour either process. Preliminary results indicate that increased denitrification produced larger amounts of nitrous oxide in the soil with annual crop, whereas more mineral N was conserved in the ley soil. In addition, analysis of the marker genes showed higher abundance of bacteria capable of denitrification than DNRA in the fertilised treatments indicating higher genetic potential for nitrous oxide emissions. These data agree with previous findings that N limited ecosystems, here represented by lower fertilisation levels and higher $C:NO_3^-$ ratios, conserve and re-cycle mineral nitrogen through DNRA (Rütting et al. 2011; Fernandes et al. 2012).

Our results suggest that different long-term agricultural management practices shift the importance of denitrification and DNRA due to altered soil conditions. However, it is still uncertain if and when DNRA can be an important N re-cycling mechanism in agricultural soil. For this, further work will be carried out for quantification of the gross N transformation rates and to evaluate the environmental factors that control both processes and the involved microbial communities.

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Carbon isotope fractionation during leaf and root respiration as a function of nitrogen nutrition

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The assimilation of nitrate and ammonium in plants follows different biochemical pathways. Thus, different enzymatic reactions, energy requirements and carbon skeletons are needed to assimilate nitrogen from soil. For instance, uptake and assimilation of NH_4^+ or NO_3^- influences the pH in the cytosol. This is regulated by a mechanism involving phosphoenolpyruvate carboxylase (PEPC) and malic enzyme (ME) which fix and release CO_2 depending on form and place of nitrogen assimilation. Thus, the chemical form of nitrogen fertilizer and the site of the nitrogen assimilation potentially influence the activity of the corresponding enzymes in roots and leaves. Accordingly, we hypothesize that applying different nitrogen inorganic ions (NO_3^- or NH_4^+) as the only source of nitrogen, will potentially influence respiration rate as well as the carbon isotopic composition of various biochemical intermediates and eventually of respired CO_2 ($\delta^{13}C_{res}$).

To address this hypothesis, we grew plants in a quartz sand medium along a nitrogen supply gradient ranging from 100% NO_3^- to 100% NH_4^+ under controlled environmental conditions. After six weeks, plants were harvested and analyzed for $\delta^{13}C$ of respired CO_2, sugars, amino acids, organic acids, starch, and bulk materials of roots and leaves, respectively. Accounting for photosynthetic discrimination (by using $\delta^{13}C_{sugar}$ as source of respiration) our results show that there is a stronger fractionation in leaves of NO_3^- treated plants than those of NH_4^+ plants, which resulted in more enriched $\delta^{13}C_{res}$ values in NO_3^- treated plants. In roots, on the other hand, such fractionation effect was not apparent although $\delta^{13}C_{res}$ showed a similar trend like observed in leaves.

Partitioning of CO_2 sources in an urban area

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Numerous studies report elevated CO_2 concentrations in urban areas due to presence of anthropogenic sources that increase local atmospheric CO_2 load and change its isotopic composition (e.g. Pataki et al. 2003; Zimnoch et al., 2014). These anthropogenic sources consist of three main components: industrial, residential and traffic. With respect to fuel that is being used they are characterized by distinct isotopic signatures ($\delta^{13}C$ and $\delta^{14}C$ values). Isotopic composition of atmospheric CO_2 in an urban area is also influenced by biogenic sources which isotopic signatures may be shifted due to assimilation of local ^{12}C-enriched CO_2 (Jasek et al., 2014).

High frequency CO_2 mixing ratio and its $\delta^{13}C$ measurements have been performed in Krakow, Poland from 2011 to 2014 with a Picarro G2101i analyzer. Mean source $\delta^{13}C$ value was calculated for each day using Keeling plot approach. Yearly mean $\delta^{13}C$ signature of CO_2 source in Krakow obtained from measurements is -31.8‰ with seasonal amplitude of ca. 4‰ (VPDB scale). During winter, mean source $\delta^{13}C$ values are significantly lower, associated with increased natural gas burning for heating purposes. During warm season, biospheric signal becomes apparent. Previous research characterized $\delta^{13}C$ signatures of biogenic (Jasek et al., 2014) and anthropogenic (Zimnoch, 2010) CO_2 sources in Krakow. Based on these results, a local bottom-up CO_2 emissions inventory have been performed to estimate mean $\delta^{13}C$ source composition and its seasonal variability. Estimation results were compared with a top-down assessment based on direct atmospheric observations.

Additionally, radiocarbon mass balance for Krakow (Zimnoch et al., 2012) have been utilized to calculate seasonal variability of fossil-fuel derived CO_2 in local atmosphere. $^{14}CO_2$ measurements were performed in Krakow and Kasprowy Wierch regional measurement station on biweekly basis; background CO_2 concentration was obtained from NOAA (GlobalView, 2016). Yearly mean value of fossil-fuel derived CO_2 in Krakow was calculated to 12 ppm with seasonal variability of 5-25 ppm.

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Effect of charcoalification on the carbon isotope signal of tree rings: Implication for paleoclimatic reconstructions

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Stable isotope composition of plants is known to be influenced by environmental conditions (Farquhar et al.,1982). In wood, growthing characteristics (i.e. ring width, latewood proportion) are also linked to environmental parameters. Applied at ring scale, stable isotopes combined to dendrology allow fine climatic reconstructions, not only on recent wood but also on ancient plant remains.

Charcoal fragments, which are the residues of firewood exploitation in archaeological contexts, are among the most frequent plant remains in the sediment record and their characterization combining dendrology and isotope geochemistry potentially constitutes a powerful tool to reconstruct paleoclimates. However, the effect of charcoalification on wood isotope composition is poorly documented. Previous studies have mainly monitored isotope signal during experimental charcoalification in conditions that are not representative of open domestic fires: often without O_2 and/or in muffle furnace. The aim of the present study was thus to investigate, at the growth ring scale, the effect of charcoalification in oxygenated conditions on the $\delta^{13}C$ signal of wood in order to better constrain the isotope signal of ancient charcoals.

A fully monitored open fire was designed to carbonize wood in reproducible condition. Growth rings were measured and sampled before and after charcoalification and analyzed for their stable carbon isotope composition. Experimental charcoalifications are under progress to investigate a temperature range varying from 300 to 800°C. Preliminary results obtained at 680°C show shift in $\delta^{13}C$ varying from -3.5 to +1.3‰ between uncharred and charred wood. This shift is higher than those previously reported that was mainly achieved in muffle fournace. Moreover, shift in $\delta^{13}C$ appears unconstant between each ring and it is possibly influenced by the chemistry of rings which will be studied in a second time. A second charcoalification obtained at 580°C shows spatial variation of $\delta^{13}C$ along a same ring, a result supported by Infra Red spectrometry and Raman microspectrometry analyses.

These results suggest that carbonization in open fires can potentially induce significant shift in tree rings $\delta^{13}C$ values. Further experimental charcoalifications are under progress to better document the observed shift in $\delta^{13}C$ values.

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Allocation of ^{13}C–labeled photoassimilates within the tropical tree species *Ceiba pentandra*

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Tropical forests are responsible for the largest fraction of global biospheric CO_2 uptake from the atmosphere. They store up to 285 ± 64 Pg C in aboveground biomass, which is estimated to be >30% of Earth`s terrestrial carbon stock. All carbon sinks of tropical forests are the balance between photosynthetic CO_2 uptake and ecosystem respiration that releases CO_2 back to the atmosphere. Tree roots greatly contribute to this respiratory flux, but the extent of total root respiration and its regulation by either the environment and/or allocation of photoassimilates from the canopy to the belowground are still largely unknown. This knowledge gap is mainly a result of the inaccessibility of the complex root systems of trees.

Here we report about a $^{13}CO_2$ pulse-chase experiment with *Ceiba pentandra*, also called Kapok or silk-cotton tree, grown in a unique large-scale aeroponic facility that can host trees up to 4m height and more than 6m long root systems. Here, we were able to fully access and manipulate tree shoots and roots and therefore study the short-term allocation of photoassimilates to the root system. A $^{13}CO_2$ pulse was applied to the top of the tree canopies and recovery of ^{13}C in different plant tissues and compounds were investigated for the same 6 young trees during a 118 hour chase period (sampled at 2, 4, 7, 22, 31, 46, 55, 70, 79, 94, 118 hours). We found a tight coupling between canopy photosynthetic CO_2 uptake and root respiration for this tropical tree species, supporting earlier results for temperate and boreal trees as well as herbs. Transfer of recently fixed photoassimilates from the leaves to the root system was detected in sugar already 7 hours after the labeling. Enrichment in non-woody root tips peaked in the first 22 hours after the labeling. Large differences in ^{13}C enrichment were found between woody and non-woody root tissues, with woody root tissues showing little to no ^{13}C enrichment, probably due to smaller sink strengths compared to those of non-woody roots.

Linking $\delta^{13}C$ and $\delta^{15}N$ of soil and sediment organic matter fractions to topography and mineral characteristics to better understand C and N dynamics in the aquatic-terrestrial transition zone

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Terrestrial and aquatic carbon (C) and nitrogen (N) dynamics are closely linked to each other not only due to C and N transfer between both systems but also due to spatio-temporal changes in the aquatic-terrestrial transition zone. Kettle holes are small (<1 ha), pond-like depressional wetlands interspersed in the moraine agricultural landscape of NE Germany that undergo pronounced wet-dry cycles. Kettle holes function as internal catchments for water and material, and therefore alter the soil moisture, plant productivity, and soil oxygencontent in their surroundings. Furthermore, topographical gradients together with land management cause soil translocation and change soil properties such as texture over the catchment, thus, kettle hole sediments originate from upland soils. All these biotic and abiotic processes impact soil organic matter stabilization and decomposition, while leaving an isotopic imprint on the composition of organic matter. We used the isotopic footprint of soil and sediment organic matter (OM) and their relationships to topography and mineral characteristics to better understand C and N dynamics in the aquatic-terrestrial transition zone. From one kettle hole, we sampled four transects that extend from the top soil of hilltops to surface sediments. We measured the $\delta^{13}C$, $\delta^{15}N$, C and N contents of the bulk soil and sediment and sequentially physically and chemically separated fractions that are hypothesized to represent OM pools under different stabilization mechanisms. We found bulk soils and OM fractions to decrease in $\delta^{13}C$ and $\delta^{15}N$ values and increase in C:N ratios closer to the kettle hole due to higher soil moisture and thus less oxygen availability that hampers microbial decomposition. In addition, we found that soil fractions that represent stabilized OM have higher $\delta^{13}C$ & $\delta^{15}N$ and lower C:N ratio in which we hypothesize proteins derived from microbes serve as a primary basis for organo-mineral/metal associations. Moreover, we further relate stable isotope ratios to mineralogical properties such as texture, exchangeable Ca^{2+} and Na-pyrophosphate extractable Al and Fe to examine possible mechanisms of OM stabilization via mineral and metal interaction. Finally, we correlate topographic indices like the topographical position index (TPI) and mineralogical properties with stable isotope ratios from OM fractions to fully characterize the kettle hole catchment on a functional basis and across spatial scales: from landscape to mineral. From these multiple lines of analyses, we will be able to better understand the mechanisms of C and N dynamics in the aquatic-terrestrial transition zone.

Isotope signatures to trace the origin and fate of nitrate in the Soyang Lake Watershed, South Korea

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The monsoon season in South Korea has a great influence on the biogeochemical and hydrological processes in the entire country, but is specifically of concern in the Soyang Lake Watershed, the main drinking water reservoir for the 20 million metropolis Seoul. Soyang Lake Watershed is composed of sub-catchments dominated by intensive agricultural management, and by pristine (semi-) natural broadleaf and coniferous forests. Therefore, nitrate leaching into surface waters may have different origins.

Stable isotopes are a useful tool to quantify and determinate the origin of nitrate inputs into the Soyang Lake. The $\delta^{15}N$ values of nitrate from different sources often show overlapping ranges but the additional measurement of the $\delta^{18}O$ values allows a more precise classification (Durka *et al.* 1994; Mayer *et al.* 2002; Deutsch *et al.* 2006). The $\delta^{18}O$ values of NO_3^- are especially useful for differentiating between NO_3^- deposited from the atmosphere and NO_3^- formed by microbial nitrification. The formation of NO_3^- in the atmosphere involves exchange of oxygen atoms with ozone, which has a high $\delta^{18}O$ value (Curtis et al 2011). In contrast NO_3^- formed by microbial nitrification derives most of its oxygen from water, which has a lower $\delta^{18}O$ value. (Curtis *et al.* 2011). According with this principle the nitrate derived from sewage or manure or fertilizes is isotopically distinct between each other and from the other sources.

With a sampling design made to determinate the influence of the precipitation regime and the land use in the nitrate discharge into the Soyang Lake Watershed, river water samples, soil water samples and rain samples were taken before and during the monsoon season to analyse the nitrate concentration, and ^{18}O and ^{15}N abundance in each phase of the nitrogen cycle. The sampling years 2013 and 2014 were particularly different among each other especially in terms of intensity and frequency of rain events. Data from amount of rain and river discharge were taken to quantify the total export of nitrate from these sub-catchments in this period. This data base together with the climate information shows us already how the monsoon season behaves and how differences in the monsoon intensity can influence in the isotope signatures in the Haean Valley and in the forests around it.

Preliminary results suggest the heavy nitrogen fertilization in the agriculture-dominated Haean basin as the major contributor to the nitrate output into the ground water systems and into the Mandae River and therefore, nitrate input into the Soyang Lake. Nitrate from atmospheric deposition and a surplus of microbial nitrification in the forest-dominated sub-catchments also contributes in smaller amount to nitrate output to the rivers and also suggest differences in nitrate assimilation capacities between the broadleaf and coniferous forest. The monsoon is the most important driver for the nitrate release between the different types of crops and also plays an important role in the nitrate delivery from forested sites being responsible as well for more than the 80% of input from the total nitrate transport within both seasons.

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Fate of organic carbon in paddy soils with contrasting mineralogy

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Submerged rice cultivation results in the formation of paddy soils. These soils often accumulate organic carbon (OC) during the initial phase of their development. However, it is unknown how the mineralogy and particularly the redistribution of Fe oxides affect OC storage. Two soil types with contrasting mineralogy (Alisol and Andosol) were exposed to 8 anoxic-oxic cycles over 1 year incubation experiment to mimic paddy soil development. Soils received rice straw labelled with ^{13}C (228 ‰) at the beginning of each cycle. A second set of samples without straw addition was used as control. Headspaces of the incubation vessels were regularly analyzed for CO_2 and CH_4 as well as their δ^{13}C. In soil solutions, redox potential, pH, dissolved organic C (DO^{13}C), and Fe^{2+} were measured after each anoxic and each oxic phase. Soils were fractionated by density at the end of the experiment and the different fractions were isotopically analyzed. Samples of genuine paddy soils that developed from the test soils were used as reference.

During anoxic cycles, soils receiving rice straw released large amounts of CO_2 and CH_4, indicating strong microbial activity. Consequently, Eh values dropped and pH as well as Fe^{2+} concentrations increased. As ^{13}C data show, more than 90% of the added straw was respired. Concentrations of DOC were relatively small, indicating either strong consumption and/or strong retention of dissolved organic compounds. During oxic cycles, concentrations of dissolved Fe dropped in both soils while DOC concentrations remained constant in the Alisol and decreased in the Andosol. Density fractionation revealed increased contents of mineral associated OC for the Andosol incubated with straw addition as compared to the parent soil. No changes were found for the Alisol. However, the mineral-associated OC fraction of both soil types contained ^{13}C of the added straw. Hence, fresh organic matter is incorporated while part of the older organic matter has been released or mineralized. The increase in the Andosol might be due to effective binding of fresh OC to minerals and/or stronger retention/preservation of older OC. Both could be explained by the more reactive mineralogy of the Andosol than of the Alisol. Although Fe oxide mobilization was stronger in soils rich in poorly crystalline Fe oxides (Andosol) during anoxic cycles, they stored additional OC.

Metabolism of exogenous testosterone in UGT2B17 del/del genotype – new targets for prolonged detection

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Testosterone (T) misuse still represents a major problem in sports drug testing.[1] Many strategies have been developed and applied to routine doping control samples within the recent years to both identify suspicious samples in routine screening and to clearly confirm the exogenous origin of urinary T afterwards. While the latter has successfully been addressed by determination of carbon isotope ratios of T itself and its urinary metabolites, the former is still subject to intensive research efforts.

One crucial factor is the fact that especially T-glucuronide is excreted into urine with a very high inter-individual variability even after T administration. Depending on the genotype of UGT2B17, significantly different amounts of T are glucuronidated and excreted which results in unaffected T/E (epitestosterone) ratios after T misuse in those subjects with the deletion/deletion polymorphism (del/del). The genotype does not only affect the phase II metabolism of T but also of many other steroids. But a diminished urinary excretion of T-glucuronide does not necessarily increase the amount of T metabolites found in urine.[2] As a consequence of these facts the question came up via which metabolic pathway the administered T is cleared in del/del persons.

Aim of this study was to investigate differences in metabolic pathways of orally administered T between del/del persons and persons with the insertion/insertion polymorphism (ins/ins). Therefore, the recently established method using hydrogen isotope ratios (HIR) together with high-resolution and high-accuracy mass spectrometry was applied after administration of deuterated T.[3] Participants collected urine specimens directly before and up to 7 days after the application. The aliquots were prepared to yield unconjugated, glucuronidated and sulphoconjugated fractions of excreted urinary steroids.

Besides the clear and significant difference in the excretion of T-glucuronide most of the other found metabolites vary rather on an individual basis than on a genotype based difference. No other 17β-hydroxylated metabolite showed a comparable suppression driven by the del/del polymorphism. None of the minor T-metabolites allowed for a clear differentiation between the genotypes as the amount of metabolites excreted as sulphoconjugates does.

Nevertheless, several important findings for sports drug testing were obtained:

- Deuterated metabolites could be detected throughout 7 days post administration with the clear over-all tendency to preserve the deuterium-label for a prolonged time in the sulphoconjugated metabolites. This allows for a prolonged detection of testosterone misuse employing carbon isotope ratios of the minor T metabolite epiandrosterone.
- New T-metabolites (both methylated and demethylated) could be identfied and might serve as potential targets for T screening.

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Diet reconstruction of monastic and secular individuals buried on the former cemetery of Herrenchiemsee (7th to 18th century)

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To elucidate differences in individual diets of the early medieval to the early modern period, bone collagen samples of 30 humans, buried on the former graveyard of Herrenchiemsee, and of some domestic animals of a kitchen waste pit were analysed for stable isotope ratios of H-C-N-S. According to prior antiquary analyses, the humans were special members of the monastic community, which belonged to the groups of monks/nuns (7th/8th century), Canons (10th/11th century), Augustinians (12th–17th centuries), abbots/provosts (7th–17th centuries), and secular persons (14th–18th centuries).

Stable isotope results indicate a change of individual diets in the course of time (7th to 17th century). In general, consumption of animal protein increased, this was particularly apparent within the group of abbots or provosts. In the early medieval, individual food of monks and nuns was diverse, whereas the canons had an ordinary and almost ascetic diet, including their abbot. In contrast, nutrition habits of the Augustinian monks and also their abbots became more diversified and exclusive (more animal protein) in the course of the centuries.

Over the entire period, the individuals of Herrenchiemsee ate water animals (fish, ducks, geese) from the Chiemsee. At the latest in the 14th century, additional fish from other lakes or fish ponds, most probably carp, was available for the community. The individuals did not have any lack of animal protein, particularly pronounced in the early modern period, when food became more diversified and of higher quality. The same has been found for the secular persons buried on Herrenchiemsee, which may have belonged to upper social classes.

Role of stable isotope tracers in a clinical trial: example of a clinical study for assessing health effects of whole grain versus refined grain diet in overweight/obese adults

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Over the last few decades stable isotope tracers have been deployed in many clinical studies to assess the rate of turnover of macronutrients (i.e. protein, carbohydrates and lipids). Understanding and measuring the dynamic aspect of these key components during a nutritional study can shed light on clinical outcomes that are not easily measurable based on concentration alone. Here, we present a crossover randomized clinical study in overweight/obese adults (n=33, 39.1±1.1 years, BMI: 32.5±0.7 kg/m^2) to look at the effect of whole grain (WG; 50g/1000 kcal) versus refined grain diets on metabolic health. All meals and snacks were provided for 8 weeks with a 10-week washout period between each diet. Metabolic adaptation to the whole grain diet was assessed using stable isotope tracers – [6,6-$^2H_2$] glucose and [1-^{13}C]glucose to measure hepatic glucose production and glucose oxidation, respectively; [^{15}N]glycine to assess whole body protein turnover; and ^2H_2O and [^{18}O]water to determine total energy expenditure (TEE). These approaches were combined with oral glucose ingestion and indirect calorimetry in order to assess insulin sensitivity and substrate oxidation, respectively. Although the WG intervention reduced diastolic blood pressure, peripheral insulin resistance and increased fasting fat oxidation, it did not alter body composition, TEE or whole body protein metabolism compared to a refined grain diet. We found that a protein intake of approximately 1.04 g/kg/day was sufficient to maintain protein turnover in overweight and obese adults. By measuring key metabolic pathways using stable isotopes, we were able to determine that replacing refined grains with WG positively influences glucose metabolism, but does not significantly alter protein metabolism or total energy expenditure, enabling future studies to focus on the most relevant outcomes. This work is an example of the utility of stable isotopes in nutrition research, and the importance of developing novel stable isotope methods for measuring *in vivo* fluxes in clinical studies.

Metabolism of beta-alanine in rat liver: degradation to acetyl-CoA and carboxylation to 2-(aminomethyl)-malonate

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Beta-alanine (BA) is a product of uracil and 3-hydroxypropionate catabolism. BA is a precursor of the dipeptides carnosine and anserine, which play an important role in the pH homeostasis of muscle during exercise. It is chronically used by athletes as a nutritional supplement to increase performance during strenuous exercise. An unwanted side-effect of the ingestion of BA is unpleasant paresthesia, the mechanism of which is unknown.

As part of a study of 3-hydroxypropionate metabolism, we perfused rat livers with 2 mM [^{15}N, $^{13}C_3$] BA (M + 4). A metabolomic study of the liver showed that acetyl-CoA was 12% M2 labeled, reflecting oxidation of BA in the citric acid cycle. Urea was ^{15}N-labeled further confirming the catabolism of BA. We also identified a new compound with a molecular weight equal to BA + 4 + 44. This suggested that BA was carboxylated (MW of CO_2 = 44). This was confirmed by perfusing livers with unlabeled BA + either unlabeled $NaHCO_3$ or $NaH^{13}CO_3$; the MW of the new compound was BA + 44 or BA + 45. This confirmed that BA was carboxylated. Based on the labeling and fragmentation patterns, we hypothesized that the compound was 2-(aminomethyl)-malonate (AMM). This was confirmed by synthesizing AMM which (after TMS or TBDMS derivatization) had the same mass spectrum and GC-MS retention time as AMM made from BA in perfused rat livers. Also, direct injection of synthetic AMM into a mass spectrometer source confirmed its MW. AMM was also formed in rat livers perfused with 3-hydroxypropionate. When 3-hydroxy-[$^{13}C_3$]propionate (M + 3) was perfused, AMM was M + 3, and M + 2 labeled, confirming the precursor to product relationship.

We showed that the carboxylation of BA is catalyzed by a side-reaction of pyruvate carboxylase. The enzyme uses acetyl-CoA or propionyl-CoA as an activator. The carboxylation of BA by pyruvate carboxylase is reversible in vitro, like the carboxylation of pyruvate.

AMM is an isomer of aspartate which is involved in neurotransmission. This raises the possibility that AMM formed after ingestion of BA influences neuronal metabolism. (Supported by the NIH Metabolomic RoadMap Initiative).

Hepatoblastoma in the light of isotope ratio mass spectrometry

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Hepatoblastoma is the most common primary liver tumor in children, however it occurs rarely with the incidence of 0.5-1.5 cases per million children. There is no clear explanation of the relationship between clinicopathologic features, therapy, and outcome in hepatoblastoma cases, so far. One of the most widely accepted prognostic factor in hepatoblastoma is histology of the tumor. The aim of the study was to determine the potential difference in biology of hepatoblastoma histological subtypes in atomic level with the use of the isotope ratio mass spectrometry. The prognosis in liver tumors cases in children may be challenging particularly because of the lack of versatile method of its evaluation. Our studies of ^{13}C and ^{15}N isotopic composition carried out to determine the difference between hepatoblastoma histological subtypes indicate the cases with better and worse outcome by analyzing the nitrogen isotope fractionation. Routine CF-irm-MS set up from Sercon has been used. The preparation of the biological samples has been described (Taran 2015a, Taran 2015b).

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Assessment of the authenticity of fruit spirits applying chemometric tools

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In recent years, the increasing consumer interest for superior quality of food and alcoholic or non-alcoholic beverages is becoming greater. Originality, authenticity and the technological process are essential for top quality products.

Serbian fruit brandy is the national, traditional alcoholic drink, which brings authenticity and distinctive feature of the region from which it originates. Fraud in the production of fruit brandies is based on the use of sugar non-fruit origin (made of sugar beet or cane sugar) during fermentation in order to increase the yield of ethanol.

In order to successfully detect counterfeits in the production of fruit brandies in Serbia in the past several years, used the method of determining the stable isotopes $\delta^{13}C$, $\delta^{2}H$ and $\delta^{18}O$. The determination of stable isotope ratios by Elemental Analyzer - Isotope Ratio Mass Spectrometry (EA-IRMS) is precise analytical method which can be used for checking the authenticity, botanical and geographical origin of fruit spirits.

In SP Laboratorija, for detecting the adulteration, we have made laboratory spirits from different kind of fruits, like plum, pear, apple, white and red grape, quince, from different geographical origin (South and North part of Serbia) and spirits from sugarcane and corn. At the same time, we have prepared all samples of fruit spirits with different amount of added beet sugar (5%, 10%, 20% and 50% sugar was added on the weight of fruit during fermentation). Also, we have prepared the laboratory control samples in which we added 3%, 5% and 10% sugar of sugar beet in the final products of alcoholic distillate. We have determined the ratio of stable isotopes after 24 hours and one month.

Application of Chemometrics, established a correlation between the concentration of sugar values (derived from sugar beet) and δD values. The value of percentage of added sugar was transformed in the negative logarithm of the molality (-log B) of added sugar to obtain a linear relationship between the variables. For the $\delta^{2}H$ values which are quite low, the use of the described equation will show a high content of added sugar derived from sugar beets. By applying this equation, it is possible to calculate the amount of added sugar in the unknown sample.

Combined results of δD and $\delta^{13}C$ values give important information about botanical origin of ethanol and possibility of distinguishing between fruit spirits and spirits with non-fruit origin (made from beet sugar, maize, cane sugar). In aim of geographical classification of brandy samples, originating from different locations in Serbia, brandy has been determined by isotope ratio δD and $\delta^{18}O$.

The results of the stable isotopes obtained by adding different percentage of sugar in the final product did not show a significant difference compared to the blank sample (without sugar), in both cases, after 24 hours, and after a month.

The obtained results allow database establishment and classification of commercial fruit spirits based on botanical and geographical origin.

Reference

Sanja O. Podunavac-Kuzmanović, Lidija R. Jevrić, Strahinja Z. Kovačević, Biljana V. Marošanović, Maja M. Lojović: Chemometric approach to determination of the authenticity of different types of Serbian and Macedonian brendy

A Compact and Real-Time Analyser for C-13 and O-18 Isotopic Ratios of Carbon Dioxide in Breath Air

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Breath air analysis provides a non-invasive alternative to traditional diagnosis methods in numerous clinical applications. In addition to the monitoring of the concentration of different chemical species, variations in isotopic composition can also be a reliable marker for diagnoses and monitoring purposes. For example, measurement of the $^{13}C/^{12}C$ ratio in exhaled CO_2 has been shown to have numerous applications (Braden 2007, Modak 2007), the most well known, perhaps, being the ^{13}C-labelled urea breath test (UBT) for Helicobacter pylori diagnosis. In addition to tests based on the ingestion of ^{13}C-labelled substrates, the latest isotope-selective breath analysis applications, such as early detection of sepsis (Butz 2014), diabetes (Ghosh 2015a), and non-invasive long-term glucose level monitoring in diabetes patients (Ghosh 2015b), employ long-term breath air monitoring or the ability to also monitor the ^{18}O-CO_2 isotopologue, an option currently not available with commercial analysers.

We present a compact, real-time, mid-infrared optical $\delta^{13}CO_2$ and $\delta^{18}O$-CO_2 isotope analyser for continuous breath monitoring. The instrument utilises an ultra-low volume multipass cell, allowing the sample gas to be exchanged at high rates with an embedded pump. This permits repeated measurement of the entire exhalation cycle. Real-time analysis of each breath cycle allows changes in the $\delta^{13}C$ and $\delta^{18}O$ isotope ratios within each exhalation to be studied and can thus provide information on potential isotopic fractionation effects within different parts of the lung volume.

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Isotope lipid tracers: a useful tool for in vitro and in vivo lipid metabolism studies

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At the time of transversal research ranging from in vitro to clinical studies, passing by animal experiments, we demonstrate here the big potential of isotope lipid tracers for lipid metabolism studies. Three applications will underline the facility to transfer a methodology from one biological matrix to another from different models.

An in vitro study[1] using [U-^{13}C]-oleate was performed with gastrointestinal cells (Caco-2) to investigate the role of mucins on the lipid absorption. Mucins are the principal component of mucus, which covers the gut lumen and are known to protect gut from inflammation. Their impact on lipid absorption was evaluated using synthetic mucins at different concentrations spread on Caco-2 cells. [U-^{13}C]-oleate was added in the form of ^{13}C-oleate-enriched mixed lipid micelles and the cellular uptake of [U-^{13}C]-oleate by Caco-2 cells was investigated by GC-MS in culture cell media corresponding to the apical- and basolateral side of the cells. We demonstrate in vitro that the higher the mucin concentration was, the less ^{13}C-oleate acid was taken up by Caco-2 cells after 2h of incubation ($p<0.001$). The mucus layer exerts a lipid micelle retention phenomenon and slows their uptake by Caco-2 cells.

Both following animal and human studies concerned the metabolic fate of lipids during postprandial phase. Few studies focus on tracer losses due to faecal elimination. The interest of this measurement is shown.

As for the animal study, [U-^{13}C]-oleate acid was used as a marker of intestinal absorption of lipids in a force-fed mouse model. Four mice were force-fed with a dose of [U-^{13}C]-oleate mixed with a specific diet and the faeces were collected during 3 days. ^{13}C-lipids excreted were determined by GC-MS and GC-C-IRMS analysis of FAMEs. Results were expressed as Cumulative Tracer Recovery (CTR% of administered dose). The ^{13}C-oleate excretion was observed primarily at day 1 (19.61±9.9%, 0.24±0.1% and 0.02±0.0% respectively at D1, D2, D3).

Regarding the clinical study, twenty subjects ingested a fat-rich meal containing [1,1,1-$^{13}C_3$]-tripalmitin and [1,1,1-$^{13}C_3$]-triolein. We examined the gastrointestinal handling of [1-^{13}C]-palmitate and oleate by determining tracer excretion in human faeces during 3 days. ^{13}C-lipids excreted were determined as for the animal study above[2]. The labelling was higher for C16 than for C18:1, showing a difference according if the fatty acid is saturated or mono-unsaturated. The CTR in TFA was 5.93±0.48% and 0.46±0.08% respectively for [1-^{13}C]-C16 and for [1-^{13}C]-C18:1(mean±sem).

These two studies confirm that the assessment of faecal elimination is of great importance when establishing the total lipid distribution after a test-meal[3].

A genuine interest was demonstrated in the use of lipid tracers in nutrition research with an important variety of models to study. In the context of metabolic diseases it is of utmost importance to study fate of dietary lipids for better understanding the impact of diet on metabolism.

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Natural isotopic signatures of variations in nitrogen metabolism during under- or over-nutrition

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Body tissues are naturally enriched in the heavier stable nitrogen isotope (^{15}N) over the diet and this ^{15}N bioaccumulation ($\Delta^{15}N$, also known as trophic step or discrimination factor) results from isotope effects associated with different metabolic pathways (Poupin *et al.*, 2014). Consequently, dietary-induced variations in nitrogen metabolic fluxes can result in $\Delta^{15}N$ variations and $\Delta^{15}N$ measurements could be used as new isotopic biomarkers for the metabolic impact of nutritional conditions. Since $\Delta^{15}N$ have been little investigated so far in controlled dietary conditions, our aim was to identify the tissue-specific $\Delta^{15}N$ signatures of the metabolic impact of both under- and over-nutrition in rats.

In a 1st study, we aimed at better characterizing the tissue-specific effects on nitrogen metabolism of an energy restriction inducing minimal protein loss. Two groups of rats were fed a similar high-fat diet ad libitun, either for 12 wks (T group, n=12) or for the first 4 wks with an important and progressive energy restriction from -25 to -50% for the following 8 wks (R groupe, n=12), while protein intake was kept constant. The rats were then euthanized and tissue proteins were precipitated and delipidated and their $\Delta^{15}N$ values were measured by EA-IRMS. Compared with T rats, R rats had (i) 20% less intestinal, hepatic and plasma proteins, 20% less cardiac proteins and 5% less muscle proteins, (ii) higher $\Delta^{15}N$ values in hepatic and plasma proteins and in the urine (+0.45‰, +0.25‰ and +0.35‰, respectively, $P<0.01$), (iii) 40% higher expression of ornithine aminotransferase and aminocarboxymuconate semialdehyde synthase and 70% lower expression of asparagine synthetase, NH2-transfering enzymes which may account for the ^{15}N-enrichment in hepatic proteins and the urine, and (iv) similar $\Delta^{15}N$ values in intestinal and muscle proteins, which were less affected than in other tissues and their amino acids less catabolized .

In a 2nd study, we aimed at better characterizing the dysregulations of protein metabolism during the onset of a metabolic syndrome (MS) with obesity and insulin resistance. We measured body proteins $\Delta^{15}N$ in one group of rats that, following 10 wks of high fat-feeding, stayed relatively lean and insulin-sensitive (MS resistant rats, n=10) and in another group of rats that became obese and insulin-resistant (MS prone rats, n=10) after having been fed the same diet. Compared with MS resistant rats, MS prone rats had (i) a higher food intake, body weight and nutrient retention efficiency, (ii) lower $\Delta^{15}N$ values in muscle (-0.13‰, $P<0.01$), cardiac (-0.17‰, $P<0.05$) and plasma proteins (-0.08‰, $P<0.05$) as a result of a higher dietary protein retention efficiency, (iii) higher $\Delta^{15}N$ values in intestinal (+0.22‰, $P<0.01$) and epidydimal adipose tissue proteins (+0.36‰, $P<0.05$) whose metabolim could be particularly altered during the MS onset, and (iv) similar $\Delta^{15}N$ values in hepatic, renal, red blood cells, spleen, skin and subcutaneous adipose tissue proteins.

Natural isotopic abundances promise interesting applications in nutrition and health for investigating how metabolism is reorganised or altered in response to nutritional or physiopathological conditions.

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Fully ^{13}C-labelled phytochemicals as internal standards in metabolomics studies in Life Sciences and Health

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The field of Inborn Errors of Metabolism is changing rapidly due to the advent of modern genetic techniques such as whole exome sequencing in patient diagnostics. Traditionally, biochemical diagnostics of rare metabolic diseases is carried out by targeted analysis of compound classes using classical analytical techniques but also increasingly using targeted Triple Quad mass spectrometry. At the Radboud University Medical Centre, Translational Metabolic Laboratory (TML) holistic approaches based on mass spectrometry, such as protein-glycomics and metabolomics, are additionally developed for introduction into patient diagnostics. Our progress is currently hampered by the availability of sufficient numbers of isotopically labelled compounds for use as internal standard. IsoLife offers unique fully ^{13}C labelled standards derived from plant materials that hold the potential of a rich source of different compounds which will provide a wider scope of available ^{13}C compounds. In addition, stable isotopes are crucial for metabolic flux studies in patient cells, which allow pathway mapping as a new approach in metabolic diagnostics. IsoLife develops new stable isotope enriched internal standards (SIIS) from plants for analytical and metabolomics-based markets in Life Sciences and Health. The Plant Metabolomics unit at Plant Research International Wageningen is at the forefront of metabolomics and has the biochemical and analytical expertise and equipment for characterizing SIIS from plants. This study aims at better characterizing and providing proof-of-principle for the suitability of ^{13}C-labelled plant extracts as SIIS in both targeted and untargeted metabolomics approaches in human urine and plasma samples from patients with a rare metabolic disease.

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^{13}C-labelled polysaccharides as tracers in nutrition and health research

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Accumulating evidence suggests that the gut microbiota play an important role in the harvest, storage, and expenditure of energy obtained from the diet. The composition of the gut microbiota has been shown to differ between lean and obese humans and mice; however, until recently, the specific roles that individual gut microbes play in energy harvest remain uncertain (Krajmalnik-Brown et al. 2012).

Here we will give an overview of the progress made by the use of ^{13}C labelled polysaccharides as in vivo tracers in gut microbial research in relation to nutrition and health.

Some plant polysaccharides, including resistant starches and inulins may pass undigested to the large bowel (colon) where they become carbon substrates for gut bacteria.

Identification of the bowel bacterial species involved in fermentation processes has led to a better insight of the functioning of the bowel in relation to effects of prebiotics or functional foods. Starch has been used in RNA-SIP in an vitro system (TIM) representing the human colon (Kovatcheva-Datchary et al. 2009; Venema et al 2010).

Investigations using natural bacterial communities inhabiting living animals have not been reported until early 2014 (Tannock et al.), studying decomposition of (^{13}C-) inulin and fructo-oligosaccharides in the bowel of rats. The latter study concluded that *Bacteroides uniformis, Blautia glucerasea, Clostridium indolis*, and *Bifidobacterium animalis* were the main users of the ^{13}C-inulin. *B. uniformis* utilized Fibruline-inulin for growth, whereas the other species used fructo oligosaccharide and monosaccharides. Thus RNA-SIP provided new information about the use of carbon from inulin in microbiota metabolism. Recently Butts et al. (2016) confirmed that the presence of inulin in the diet positively influences large bowel microbial fermentation.

In a study on the regulation of satiety, Frost et al. (2014) have demonstrated through ^{13}C high-resolution magic-angle-spinning NMR that ^{13}C acetate from fermentation of ^{13}C-labelled chicory inulin in the rat colon increases hypothalamic ^{13}C acetate above baseline levels.

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Deuterium oxide (^2H_2O) dosing to measure muscle protein synthesis rates *in vivo* in humans

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Skeletal muscle mass is a central factor dictating functional capacity and metabolic disposition. Assessment of muscle protein synthesis rates allows for investigation into the impact of various interventions (i.e., diet, physical activity) or disease on skeletal muscle reconditioning. Stable isotope-labeled amino acid methodologies are commonly utilized to assess muscle protein synthesis rates. For this method, stable isotope-labeled amino acids are typically infused to subsequently assess their incorporation in newly synthesized muscle proteins. Sequential muscle biopsies are collected to assess the incorporation rate of the stable isotope-labeled amino acid tracer into muscle protein, providing the means to calculate a fractional muscle protein synthetic rate. Stable isotope-labeled amino acid tracer infusions for assessing muscle protein synthesis rates are typically applied over short periods (less than 9 hours) to avoid tracer recycling. Therefore, this approach is typically applied to assess post-absorptive muscle protein synthesis rates or the muscle protein synthetic response to the ingestion of a single meal. Because muscle turnover is dynamic and responsive to numerous stimuli, there is a need to assess muscle protein synthesis rates over longer periods. Oral heavy water (deuterium oxide, ^2H_2O) administration has recently re-emerged as a viable isotope-based method for assessing muscle protein synthesis rates over days-to-weeks *in vivo* in humans. Oral ingestion of small amounts of heavy water enriches the body water pool with deuterium. With deuterium present in the body water pool, endogenously-synthesized amino acids such as alanine become deuterium-labelled. Deuterium-labelled alanine is incorporated in newly-synthesized muscle proteins which can be assessed following the collection of muscle biopsy samples. Assessment of muscle protein synthesis rates using heavy water dosing allows for assessment of muscle protein synthesis rates over a prolonged period with subjects in a free-living environment. Therefore, the impact of numerous factors such as habitual physical activity, exercise training, medication, diet, disease progression etc. on muscle protein synthesis rates can be assessed. In conclusion, the use of heavy water dosing may prove to be a practical and useful tool to study skeletal muscle conditioning in both health and disease *in vivo* in humans.

Simultaneous measurement of the concentration and enrichment of amino acids in human plasma using stable isotopes and LC/MS ion trap analysis

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Since amino acids plays a key role in many different pathways, as well as in protein synthesis and proteolysis, they are biologically important compounds. Stable isotope studies offer the opportunity to study the in-depth metabolic pathway of amino acids that are involved in these processes. The use of multiple stable isotopes can be used to elucidate the exact transformation of amino acids in metabolic pathways and protein synthesis.

Analytical techniques such as gas chromatography mass spectrometry (GC/MS), gas chromatography isotope ratio mass spectrometry (GC/C/IRMS), liquid chromatography mass spectrometry (LC/MS), and liquid chromatography isotope ratio mass spectrometry (LC/IRMS) have been used to measure isotope enrichments of amino acid tracers. Although the IRMS techniques are superior for precise isotopic measurement compared to MS, they have the disadvantages of being more expensive, being less sensitive and require a baseline separation of the compounds of interest and other co-eluting peaks for correct measurement. Furthermore, when using LC in combination with IRMS, gradient elution of compounds is challenging, since organic solvents cannot be used, because they contain carbon atoms which disturb the ^{13}C measurement in carbon dioxide. Therefore methods involving GC/MS and LC/MS analysis are used most frequently to measure the stable isotope enrichment of amino acids, since these techniques have not the disadvantages of the IRMS. In particular, LC/MS has gained more popularity in isotopic enrichment analysis due to better selectivity and sensitivity. Another commonly used method in amino acid analysis is the chemical modification of the amino acid by derivatization for increasing the volatility and polarity and obtaining more sensitivity and selectivity. In addition, derivatization of the amino acid decreases the matrix effects and increases the height of the isotopic signals measured due to the increase in mass which are beneficial features in isotopic enrichment studies.

Since LC/MS analysis is capable of measuring all the amino acids in a single run, a novel method was developed for the simultaneous measurement of the concentration and enrichment of amino acids in plasma using an LC/MS ion trap system. The method provides a fast and easy purification method using a protein precipitation and delipidation step in combination with a rapid and easy derivatization procedure. In contrary to most other derivatization procedures, this derivatization procedure is beneficial in terms of robustness, velocity, easiness, derivative stability and can be performed at room temperature, in an aqueous environment without incubation. Separation of the amino acid derivatives was performed using a Waters Acquity BEH C8 column (100 x 2.1 mm i.d., 1.7 µm particle size). The mobile phases consist of water (A) and methanol (B) with 0.2 mM tridecaheptafluoric acid (TDFHA) and 0.1% formic acid. The total run-to-run time (including equilibration prior to injection of the next sample) was 31 min. The amino acid derivatives were detected in the full-scan mode using a Linear Ion Trap Velos Pro mass spectrometer (LTQ Velos Pro^{TM}) equipped with a heated electrospray interface operating in the positive ion mode.

A combined stable isotope infusion method to assess therapeutic efficacy in primary hyperoxaluria patients

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To assess the efficacy of a new drug for the treatment of Primary Hyperoxaluria type 1 (PH1) patients, a novel stable isotope protocol has been set up. PH1 is a rare disease that is caused by a deficiency of the liver specific peroxisomal enzyme alanine:glyoxylate aminotransferase (AGT). Within the glyoxylate pathway, AGT catalyses the conversion of glyoxylate to glycine, which limits the oxidation of glyoxylate to oxalate. Malfunction of AGT leads to an excess formation of both oxalate and glycolate. Oxalate overproduction leads to supersaturation in the urine, resulting in development of urolithiasis, nephrocalcinosis and ultimately life-threatening renal failure. At hand, few supportive treatments with limited clinical benefit are available. Combined or sequential liver and kidney transplantation remains the only curative option. The new drug to be tested promises a less invasive treatment of this disease.

The recent development of this new therapeutic drug for PH1 stresses the necessity to develop a method that is an adequate marker for recovery of the enzyme function. Nowadays, urinary oxalate excretion or plasma oxalate concentration are used as marker. Plasma oxalate is not a good parameter, as washout of the systemic depositions of calcium oxalate crystals may take years, masking treatment effects. In addition, the variability of urinary oxalate excretion in these patients limits its use. Despite many decades of research into the metabolic pathways leading to oxalate synthesis, uncertainties still remain.

To obtain proof of principle for the new drug and to unravel the metabolic pathways leading to oxalate synthesis, we developed a novel mass spectrometric method making use of stable isotopes. Use of stable isotopes opens up new avenues to accurately assess the therapeutic efficacy of the new drug and offers the possibility to gain insight into the glycolate/glyoxylate pathway in PH1 patients, specifically into the dynamic processes of the metabolites oxalate, glycine and glycolate.

Endogenous oxalate production was determined before by infusion of stable isotope labelled U-^{13}C-oxalate at our hospital (Huidekoper, pilot study). The combination of U-^{13}C-oxalate, d_5-glycine and 1-^{13}C-glycolate in a primed continuous infusion is unique and enables quantification of glycine and oxalate production, measurement of the level of AGT function and the specific production of peroxisomal oxalate and glycine from glycolic acid are amongst the possibilities of this new method.

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Sudoku

Small surprizes wait for the first 3 JESIUM 2016 participants who return all 4 right solutions to the conference desk...

				8				9
			4	2	6	1	3	
			9		1	5		6
2			8	3		9	7	4
3		9		6			8	
			2	9	4			
	5	6	3	1				
						8		7
	8	4		5	2		1	

	2		5	1	9			
	5		7				1	
	6		2			9		
6	8	5		2	3			
						8		2
	7		1	4				
7		1					5	9
					1			7
	3		4	9			2	1

2						9	5	1
								7
8	7	6			5	2		
3		4		2	8			
						5		9
		5			1	3		4
	2			8	4		1	
				1			9	8

4	5							
		2		7		6	3	
							2	8
			9	5				
	8	6				2		
	2		6			7	5	
						4	7	6
	7			4	5			
		8			9			

How to play Sodoku

There are 9 rows in a traditional Sudoku puzzle as above. Every row must contain the numbers 1, 2, 3, 4, 5, 6, 7, 8, and 9. There may not be any duplicate numbers in any row. There are also 9 columns. Like the Sudoku rule for rows, every column must also contain the numbers 1, 2, 3, 4, 5, 6, 7, 8, and 9. Again, there may not be any duplicate numbers in any column. It's easy... or not? *Have fun!*

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