



## News

### News & Events

The COST Action closed on 24<sup>th</sup> November 2013. Read here the summary of our final report:

The COST Action ES0806 SIBAE successfully addressed all objectives of its four and a half years. 22 European countries as well as one non-COST country (Australia) participated. In total, 15 workshops, six MC meetings, two international conferences and two training schools took place, providing ample opportunity to exchange knowledge, coordinate European research and to support early-career researchers. 90 applications for STSMs were received, 72 carried out (on average, 42% female awardees). In addition, information exchange within and beyond the Action was done via the SIBAE listserver and web (<http://www.sibae.ethz.ch/cost-sibae/>). Among many joint experiments, ring-tests and cross-calibration activities, questionnaires, workshop reports and best practice recommendations are available freely on the web. The clear highlights of the Action were the launch of a new webpage, called **Isonumb3r5**, providing relevant numbers within the stable isotope field; two international conferences (2010: with 68 participants from 14 countries organized in Switzerland; 2013: with 80 participants from 23 countries organized in Poland), a special issue in the open-access journal *Biogeosciences*, with an editorial highlighting SIBAE; as well as the establishment of the Nordic network for Stable Isotope Research (NordSIR). During these four and a half years, more than 38 proposals using stable isotopes have been submitted to European and national funding agencies, 37 new projects received funding, and almost 100 papers have been written jointly by MC members, Action experts, STSM awardees or originated from Action activities. Altogether, the COST Action ES0806 was a full success.

The **final conference of SIBAE** took place in Wroclaw, Poland, during 14-19 May, 2013. For more info, see [here](#)

Within SIBAE, we launched a new webpage called **Isonumb3r5**. Here you can find (and provide) many numbers related to stable isotopes. [Check it out!](#)

**Short term scientific missions (STSMs)** could be proposed using the on-line tool provided in the [Activities section](#). Applications were evaluated by the selection committee within a week.

Working Groups 1, 3 and 4 posted a [user directory](#) and several questionnaires at their pages to collect relevant information on [compound specific facilities](#), [laser facilities](#), [teaching materials to be shared and technical/scientific staff affiliations](#).

### SIBAE Newsletter

A mailing list was available for interested users and reported on SIBAE activities.

Here you find the **SIBAE logo**.

Here you find the **4<sup>th</sup> year SIBAE poster**.



## COST Action ES0806

# Stable Isotopes in Biosphere- Atmosphere-Earth System Research (SIBAE)

2009 | 2013

## Objectives

- to use stable isotopes on C, N, O and water cycles as a critical research tool in Biosphere-Atmosphere-Earth System research across scales and disciplines
- synthesize isolated experiments and assess current state-of-the-art models
- benchmark and advance innovative cutting-edge technologies
- create a training/teaching network across Europe

## Main Achievements

- Further development and support of „daughter“ network NordSIR (Nordic network for Stable Isotope Research)
- Joint experiments, best practise recommendations, laboratory inter-comparisons
- New webpage called Isonumb3r5 (<http://isonumbers.weizmann.ac.il/>) maintained
- Special Issue in the open-access journal *Biogeosciences* finished, editorial highlighting SIBAE, providing international visibility
- >35 papers/manuscripts, >11 proposals submitted, > 16 projects funded in 4<sup>th</sup> year
- Twenty-eight STSM applications funded within the fourth reporting year (46% female awardees), second training school carried out

## Working Group 1

- Standard used in compound-specific stable isotope analysis created
- Joint workshop WG1/WG3 held in Innsbruck on “IsoFlux”, which also included colleagues from the US and our former sister network BASIN in the USA
- Meta-database on existing (ongoing and finished) experiments

## Working Group 2

- Workshop held in Gothenburg on “<sup>15</sup>N tracing modelling” (jointly with NordSIR), bringing experimentalists and modelers together
- Recommendations for new tracer experiments

## Working Group 3

- Workshop held in Ghent on “Compound-specific stable isotope analysis” with scientists and company representatives
- Best-practice guidelines for compound-specific isotope analysis
- Cross-laboratory intercomparison of concentration and isotope analysis of CO<sub>2</sub>, CH<sub>4</sub> and N<sub>2</sub>O, laboratory ring test
- Laboratory inter-comparison on N<sub>2</sub>O isotopomers

## Working Group 4

- Second SIBAE training school in Nancy, using format developed at a workshop earlier
- 35 applications received in 4<sup>th</sup> year, 28 STSMs granted until March 2013 (five more still pending due to budget constraints)
- Final conference held in mid May 2013 in Wroclaw, Poland with >100 participants

## Earth System Science and Environmental Management (ESSEM)

### Participating countries

AT, BE, CH, DE, DK, ES, FI, FR, GR, HR, IS, IT, LU, NL, NO, PL, PT, RS, SI, SK, SE, UK

Australia

### Contact details

#### Chair of the Action

Nina Buchmann,  
Professor of Grassland Sciences  
ETH Zurich, Switzerland  
Nbuchmann@ethz.ch

#### Dr Basak Kisakurek

Science Officer Earth System Science  
and Environmental Management  
COST Office  
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#### Website

<http://www.sibae.ethz.ch/cost-sibae/>



<sup>13</sup>CO<sub>2</sub> labeling in the field, laser spectrometer



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## About us

### About us

There is a long tradition of environmental research in Europe, particularly within the framework of Environmental and Global Change, which has been supported by several national and European programs over the last decades. Although stable isotopes provide an indispensable and powerful research tool, they had not yet been well integrated into European Biosphere-Atmosphere-Earth System research across scales and disciplines.

COST Action ES0806 SIBAE (25<sup>th</sup> May, 2009 to 24<sup>th</sup> November, 2013) specifically focused on addressing this shortcoming with a multi-disciplinary, integrated approach with scientists from a range of disciplines, addressing highly relevant topics such as the turnover of soil carbon, the effect of global change on greenhouse gas exchange between ecosystems and the atmosphere, and the role of biodiversity for ecosystem processes in a changing environment. For more details, please refer to the Memorandum of Understanding to the right.

COST Action ES0806 SIBAE built on the experiences of past international collaborations, in particular the [ESF programme SIBAE](#). If you have questions please don't hesitate to contact us. Please refer to the [People](#) section for a list contact persons.

The COST Action ES0806 SIBAE started in May 2009 and ran until November 2013 (with half a year extension). It was launched during the kick-off meeting in Brussels on 25. May 2009. As of May 2011, 22 European countries participate in this Action (Austria, Belgium, Croatia, Denmark, Finland, France, Germany, Greece, Israel, Italy, Luxembourg, Netherlands, Norway, Poland, Portugal, Serbia, Slovak Republic, Slovenia, Spain, Sweden, Switzerland, United Kingdom) as well as one non-COST country (Australia).

**Memorandum of  
Understanding  
COST ES0806**

**Official COST page  
COST Domain ESSEM**

## People

### People

#### Management Committee (MC)

The Action was coordinated by a Management Committee (MC), including all national MC delegates.

Within the MC, official roles were carried out by the persons listed below. Please click on the names to find contact information (the links open new browser windows).

Chair:	<a href="#">Nina Buchmann (CH)</a>
Vice-Chair:	<a href="#">Dan Yakir (IL)</a>
Financial rapporteurs:	<a href="#">Jaleh Ghashghaie (F)</a> , <a href="#">Rolf Neubert (NL)</a>
Grant holder:	<a href="#">Nina Buchmann (CH)</a>
Webmaster:	<a href="#">Nina Buchmann (CH)</a>

#### Working Group (WG) Leaders

The Action was structured along four Working Groups, for which the positions of Chair and Vice-Chair were held by the persons listed below. More information about the Working Groups can be found in the [Working Groups](#) section.

WG 1 Experiments:	<a href="#">Cristina Maguas (P)</a> , <a href="#">Roland Bol (DE)</a>
WG 2 Modelling:	<a href="#">Daniel Epron (F)</a> , <a href="#">Thomas Wutzler (DE)</a>
WG 3 Technology:	<a href="#">Nicolas Brüggemann (DE)</a> , <a href="#">Pascal Boeckx (BE)</a>
WG 4 Outreach/Training:	<a href="#">Jens-Arne Subke (UK)</a> , <a href="#">Christina Biasi (FL)</a>

#### STSM Selection Committee

Proposals for Short Term Scientific Missions (STSMs) were evaluated by the Selection Committee, composed of the Chair, the Vice-Chair and the Working Group Leaders.

#### MC Members

The table below lists the members participating in the MC. More information about the Working Groups can be found in the [Working Groups](#) section.

Name, First Name	Represents	WG1	WG2	WG3	WG4
Ambus, Per	DK	x			x
Angert, Alon	IL	x	x		
Andresen, Louise	SE		x		x
Argiriou, Athanasios	GR	x			x
Bahn, Michael	AT	x	x	x	
Barancikova, Gabriela	SK		x		
Bardgett, Richard	UK	x			
Biasi, Christina	FL		x	x	
Boeckx, Pascal	BE	x	x	x	
Bol, Roland	(DE)	x	x	x	
Bruggemann, Nicolas	DE	x		x	
Brugnoli, Enrico	IT	x	x		x
Buchmann, Nina	CH	x		x	x
Curjel Yuste, Jorge	ES	x	x		
Doersch, Peter	NO	x	x	x	
Dotsika, Elissavet	GR				
Ekblad, Alf	SE	x		x	
Emmenegger, Lukas	CH			x	
Epron, Daniel	FR	x	x		x
Fulajtar, Emil	SK	x			x
Ghashghaie, Jaleh	FR	x		x	x
Gorka, Maciej	PL	x		x	
Hansen, Erik	DK		x	x	
Hissler, Christophe	LU	x	x		
Hogberg, Peter	SE		x		x
Janssens, Ivan	BE		x		
Knohl, Alexander	DE		x	x	
Lojen, Sonja	SI	x			x
Maguas Hanson, Cristina	PT	x	x		x
Miljevic, Nada	RS	x		x	x

Mohn, Joachim	CH			x	
Neubert, Rolf	NL	x			x
Nogues, Salvador	ES	x		x	
Ogrinc, Nives	SI	x			x
Oinonen, Markku	FI	x			
Pfister, Laurent	LU	x	x		
Pumpanen, Jukka	FI	x		x	
Reichstein, Markus	DE		x		
Richter, Andreas	AT	x	x		x
Robins, Richard	FR	x		x	
Roller Lutz, Zvezdana	HR			x	x
Rüttig, Tobias	SE	x	x		
Sebastia, Maria Teresa	ES	x	x		
Silvennoinen, Hanna	NO	x	x	x	
Skrzypek, Grzegorz	AUS	x		x	
Subke, Jens-Arne	UK	x		x	x
Tirola, Marja	FI	x			
Vadillo, Inaki	ES	x			x
Van Groenigen, Jan-Willem	NL	x	x		
Wachniew, Przemyslaw	PL	x			x
Wutzler, Thomas	DE		x		
Yakir, Dan	IL		x	x	x
Zisi, Nikoleta	GR	x			x



## Research

### Research

The main objective of this Action was to coordinate and integrate the diverse and isolated stable isotope activities in biosphere-atmosphere- Earth system research (including atmospheric chemistry, hydrology, soil sciences and plant ecophysiology) in Europe to provide a scientific impact that is greater than that of its individual components.

The COST Action reviewed and synthesized existing data, stimulated and initiated new joint research activities, and created standardized methods and protocols for stable isotope measurements. The COST Action brought together scientists from multidisciplinary backgrounds and various scientific communities to work jointly towards these goals and to train the next generation of scientists. For more details, check out the [Working Groups section](#).

#### Success stories

- A new webpage has been launched, called [Isonumb3r5](#). The aim is provide relevant numbers within the stable isotope field, references to the original literature, useful comments, and related numbers.
- A new network has formed in 2010 among the nordic countries, called Nordic network for Stable Isotope Research (NordSIR). For more info, please read [here](#). Close collaborations are on-going.
- The joint SIBAE/BASIN/ETH Zurich conference on [Stable Isotopes and Biogeochemical Cycles in Terrestrial Ecosystems](#) took place in Ascona, Switzerland, March 2010. 68 participants from 14 countries discussed topics addressing stable isotope applications, planned joint papers and initiated new proposal/project ideas.
- A Special Issue on "[Stable isotopes and biogeochemical cycles in terrestrial ecosystems](#)" in the open-access journal *Biogeosciences* has been published. Guest editors are Michael Bahn, Nina Buchmann and Alexander Knohl, all members of SIBAE.
- The second international SIBAE conference on [CHALLENGES IN THE APPLICATIONS OF STABLE ISOTOPES ACROSS DISCIPLINES AND SCALES](#) took place in Wroclaw, Polen, May 2013. About 80 participants from 23 countries discussed topics addressing stable isotope applications, planned joint papers and initiated new proposal/project ideas.
- Two training schools took place within the last four year, for more info, read [here](#).
- Close contacts and exchange with the manufacturers of equipment used to analyze stable isotopes.



## Activities

### Activities

Short Term Scientific  
Missions STSM  
Workshops  
Training Schools  
Conferences

Within Cost Action ES0806, the following activities took place and products are available:

- Management Committee meetings: Kick-Off in Brussels in May 2009; 2<sup>nd</sup> MC meeting in Lisbon in Oct. 2009, 3<sup>rd</sup> MC meeting in Exeter in April 2010, 4<sup>th</sup> MC meeting in Malaga in May 2011, 5<sup>th</sup> MC meeting in Leipzig in September 2012, 6<sup>th</sup> MC meeting in Wroclaw in May 2013.
- Workshops: WG1 in Ghent (Belgium) in June 2012; WG 2 in Innsbruck (Austria) in September 2009, in Nancy (France) in March 2010, in Gothenburg (Sweden) in May 2011, in Nancy (France) in October 2011, in Gothenburg (Sweden) in November 2012, in Storrs Hall (UK) end of April/beginning of May 2013, in Ski (Norway) during end of April 2013 as well as Copenhagen in September 2013; WG3 in Zurich (Switzerland) in June 2010 and in Nantes (France) in October 2013, WG1-3 in Barcelona (Spain) in October 2010; WG4 in Zurich (Switzerland) in September 2011. A cross-cutting meeting among WGs took place in Ascona (Switzerland) in March 2010 as well as in Innsbruck (Austria) in November 2012. For details see [Workshops](#).
- Conferences: [Joint SIBAE/BASIN/ETH Zurich conference "Stable Isotopes and Biogeochemical Cycles in Terrestrial Ecosystems"](#) in Ascona (Switzerland), March 2010 as well as [Final SIBAE conference "CHALLENGES IN THE APPLICATIONS OF STABLE ISOTOPES ACROSS DISCIPLINES AND SCALES"](#) in Wroclaw (Poland) during May 2013.
- Short Term Scientific Missions (STSMs). There were four deadlines per year for applications (31 March, 30 June, 30 September, 31 December) that were evaluated by the selection committee within four weeks after submission. For details see [STSMs](#).  
Who can apply: [COST Info on STSM](#).  
How to apply: [STSMs](#)
- Meta-databases: [Isotope User Directory](#)
- Guidelines: For details see [Workshops](#).
- Recommendations: [What to measure in <sup>13</sup>C labeling experiments](#)
- Protocols: For details see [Workshops](#).
- [Publications](#)
- Training schools: SIBAE has provided funds via STSMs to attend training schools organized by members of SIBAE. Furthermore, a stable isotope training school took place in Portugal end of October/beginning of November 2011 and in France mid of September 2012.

## Short Term Scientific Missions STSM

### Activities

[Short Term Scientific Missions STSM](#)
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There were four **deadlines** per year for applications (31 March, 30 June, 30 September, 31 December) that were evaluated by the selection committee within four weeks after submission.

**Who** could apply: [COST Info on STSM](#).

**How** to apply: [STSMs](#)

**Successful applications:** In total, we received 72 successful applications of early- (and later) stage researchers for STSMs of the Action (42% female applicants).

### 1<sup>st</sup> year of Action (May 2009 to May 2010)

- Tamir Klein, from Israel to Italy: Tree-ring  $d_{13}C$  as a marker for hydraulic adaptation of *Pinus halepensis*
- Carla Rodrigues, from Portugal to Austria: Strontium isotope abundance determination in green coffee beans
- Michael Schmitt, from Austria to Switzerland: Application of a quantum cascade laser for measuring stable isotopes in ecosystem  $CO_2$  fluxes
- Matthias Barthel, from Switzerland to Spain: Understanding the carbon cycle on the leaf scale: Can aquaporins explain the  $CO_2$  diffusion inside leaves

### 2<sup>nd</sup> year of Action (May 2010 to May 2011)

- Paul Gauthier, from France to Spain: Scientific linkage for isotopic GC-C-IRMS measurements
- Mariela Yevenes, from the Netherlands to Spain: Environmental isotopes applied for groundwater pollution studies
- Dr Illa Tea-Dugue, from France to Spain: Isotopic profiling of amino acids during the trophic steps in the foetal nutritional environment
- Dr Jordi Palau, from Spain to Switzerland: Compound-specific hydrogen isotope analysis of volatile organic compounds
- Jan Reent Köster, from Germany to Switzerland: Identification of  $N_2O$  source and sink processes in fertilised soils
- Dominika Lewicka-Szczebak, from Poland to Sweden: Summer School on Stable Isotopes
- Merjo Laine, from Finland to Sweden: Stable Isotopes in Earth System Sciences
- Steve Granger, from the UK to Sweden: Summer School on Stable Isotopes
- Sami Taipale, from Finland to Israel: The Isonumb3r5 project
- Paul Carrey, from Spain to Belgium: Isotopic analysis by denitrifier method
- Lydia Gentsch, from Switzerland to the UK: Exploring analysis methods for  $CO_2$  isotope data
- Emmi Hiltasvuori from Finland to France: Preparation of high resolution tree ring cellulose samples for isotopic analysis
- Dr. Daniel Said Pullicino from Italy to Germany: Evaluating N immobilisation as a function of soil redox conditions by  $^{15}N$  CS-IRMS
- Dr. Maria Teresa Dominguez from United Kingdom to Belgium: Effects of climate change (warming and drought) on the flow of plant-derived C into rhizosphere microorganisms in an upland heathland
- Dr. Gai Petit from Italy to Switzerland: Hydraulic limitations on height growth of treeline trees based  $d_{13}C$
- Sanni Turunen from Finland to Austria:  $^{13}CO_2$  labeling experiment in alpine grassland

### 3<sup>rd</sup> year of Action (May 2011 to May 2012)

- Adriana Rossi from Italy to Spain: Compound-specific isotope analysis applied to groundwater contamination
- Salvador Lopez from Spain to Belgium: Analysis of amino sugars and free sugars by LC-c-IRMS to know ecophysiological responses of wheat
- Dr. Youri Rothfuss from France to Israel: Improving and finalizing a search-engine on useful stable isotopes numbers: IsoNumbers
- Dr. Daniele Pittalis from Italy to Spain: Use of environmental isotopes for groundwater pollution studies
- Carola Blessing from Switzerland to Germany: Using isotope specific laser instruments to study the oxygen isotope exchange between carbon dioxide
- Marco Lehmann from Switzerland to Germany: In-Tube Incubation: A method to measure  $d_{13}C$  of respired  $CO_2$  during dark respiration
- Prof. Inaki Vadillo from Spain to Austria: Fundamentals on Isotope Laser Spectroscopy (IAEA)
- Athanasios Katsogiannis, from the UK to Spain: Use of stable-isotopes techniques for source identification of environmental pollutants
- Olga Gavrikova from Italy to Germany: Stable isotopes in studying mechanisms behind aboveground-belowground coupling
- Jorge del Castillo Diaz from Spain to Germany: Combining stable isotopes in tree-rings and NDVI derived from satellites
- Bernd Hoffmann from Germany to Switzerland: Stable Isotopes of Xylem Water
- Susanne Burri from Switzerland to Germany: Impacts of Summer Drought on the Short-Term Carbon Cycle of Managed Grasslands in Switzerland
- Simo Jokinen from Finland to Austria: Inter-Comparison for isotopic analysis of dissolved, inorganic and organic N fractions
- Lukasz Plesniak from Poland to Slovenia: Isotopic analysis as an indicator of migration paths in natural gas deposit
- Bruno Gamarra from Switzerland to Germany: Extracting and quantifying leaf wax n-alkanes for stable hydrogen isotope analysis

### 4<sup>th</sup> year of Action (May 2012 to May 2013)

- Cristina Antunes from Portugal to Switzerland: Stable isotopes as tracers of long-term groundwater stress
- Dürte Bachmann from Switzerland to France: Determining plant water uptake in grassland communities under controlled conditions
- Florian Ulm from Germany to Portugal:  $^{15}N$  as a tracer of the impact of invasive species on the nitrogen cycle of dune systems
- Johannes Ingrisch from Germany to Austria: Application of a quantum cascade laser in  $^{13}C$  tracer experiments in alpine grasslands
- Friederike Gnädiger from Germany to Switzerland: Diurnal transport and storage of recently fixed carbohydrates in beans (*Phaseolus vulgaris*)
- Koen Hufkens from Belgium to Germany: Multi-decadal carbon and water relations of tropical forests: a tree-ring stable isotope analysis
- Lorenzo Menichetti from Sweden to Finland: SIER 2012 NordSIR PhD course - Participation
- Anna Mikusinska from Sweden to Finland: SIER2012- NordSIR, PhD Course - Participation
- Gesche Blume-Werry from Sweden to Finland: SIER 2012 NordSIR PhD Course - Participation
- Anna Sterngren from Sweden to Finland: SIER 2012 NordSIR, PhD course - Participation
- Anna-Karin Björnsjö from Sweden to Finland : SIER 2012 NordSIR PhD Course - Participation
- Alessandra D'Annibale from Denmark to Finland: SIER 2012 NordSIR PhD Course - Participation
- Carla Minguet from Spain to the UK: Using synthetic biology to increase assimilation of  $^{13}C$
- Florian Ulm from Germany to Switzerland: Change of nutrient availability,  $\delta^{15}N$  and soil microbiota underneath the invasive *Acacia longifolia*
- Mario Thurn from Germany to Switzerland: Modeling  $d_{18}O$  of hemicellulose biomarkers along an Argentinean climate gradient
- Konstantin Gavazov from Switzerland to Austria:  $^{13}C$  substrate induced respiration and PLFA profiles of microbial communities from snow covered soils
- Sylwia Gorka from Poland to Portugal: Plant functional groups water use in a groundwater limitation situation
- Fabiana Saporiti from Spain to the UK: Changing topology in trophic webs: the use of SIBER as quantitative tool
- Dr. Michael Zech from Germany to Switzerland: Compound-specific  $\delta^{18}O$  analyses of sugars as novel tool in plant physiology



- Dr. Francesca Scandellari from Italy to Germany: Stable isotopes as a tool for studying the ecological role of ectomycorrhizal fungi

▪ **Last half year of the Action (May 2013 to November 2013)**

- Carla Minguet from Spain to the UK: Using synthetic biology to increase assimilation of C
- Konstantin Gavazov from Switzerland to Austria: Winter climate extremes and their role in biogeochemical processes in the soil
- Dr. Juan Pedro Ferrio from Spain to Germany: Data analysis and discussion of double labelling experiments
- Jannis Heil from Germany to Switzerland: Site-specific analysis of isotopic signatures of N<sub>2</sub>O produced abiotically in soils
- Dr. Michael Schmitt from Austria to Switzerland: Rapid Isotope Measurements During Plant Gas Exchange Under Controlled Environmental Conditions
- Dr. Sara Palacio from Spain to Germany: What do d<sup>15</sup>N signals in plant species tell us about plant-soil nitrogen dynamics?
- Thomas Wieloch from Sweden to Austria: Is there a metabolic explanation for the intra-annual d<sup>13</sup>C variations in tree-rings of broadleaves?
- Dr. Ioannis Matiatos from Greece to Belgium: Stable isotopes for contaminant source identification in groundwater
- Joan Gimenez from Spain to the UK: Elucidating the food web and the diet of small dolphins in the Gulf of Cadiz through stable isotopes
- Prof. Dr. Teresa Sebastia from Spain to France: Using isotopic signatures to understand the biodiversity-function relationships
- Jasper Bloemen from Belgium to Austria: Testing a quantum cascade laser system to trace stable isotopes in tree CO<sub>2</sub> efflux
- Yakir Preisler from Israel to Switzerland: Do trees under various levels of drought stress differ in their isotopic composition?
- Carla Minguet from Spain to the UK: Using synthetic biology to increase assimilation of C
- Marco Lehmann from Switzerland to Germany: Investigation of the respiratory mechanism of malate in plants using carbon stable isotopes
- Alba Grau from Spain to the UK: Isotopic analysis N<sub>2</sub>O
- Dr. Thomas Wutzler from Germany to Austria: Model data integration of soil microbial substrate use efficiency
- Konstantin Gavazov from Switzerland to Austria: Winter climate extremes and their role in biogeochemical processes in the soil



## Workshops

### Activities

Short Term Scientific  
Missions STSM

Workshops

Training Schools

Conferences

Several workshops have taken place so far within COST Action ES0806 SIBAE addressing various aspects of stable isotope research.

- "Using stable isotopes in the development of models of soil respiration and soil organic matter cycling" in Innsbruck, September 2009 (more details [here](#))
- "Analysing post labelling experiments" in Nancy, March 2010 (more details [here](#))
- "Stable Isotopes and Biogeochemical Cycles in Terrestrial Ecosystems" in Ascona, March 2010, cross-cutting workshop (more details [here](#))
- "Isotope-specific laser-based instruments in biosphere-atmosphere-Earth system research: Latest instrument developments, applications and best-practice guidelines" in Zurich, June 2010 (more details [here](#))
- "Compound specific isotope analyses" in Barcelona, October 2010 (more details [here](#))
- "Advances in N tracer experiments and <sup>15</sup>N methods" in Gothenburg, May 2011 (more details [here](#))
- "Meeting to discuss SIBAE training schools" in Zurich, September 2011 (more details [here](#))
- "Integrating vertical dimension in soil carbon studies/models using natural abundance of stable isotopes" in Nancy, October 2011 (more details [here](#))
- "Intercomparison CSIA" in Ghent, June 2012 (more details [here](#))
- "Stable isotopes in flux measurements, IsoFlux" in Innsbruck, November 2012 (more details [here](#))
- "<sup>15</sup>N tracing modelling" in Gothenburg, November 2012 (more details [here](#))
- "Nitrogen cycle and stable isotopes - Focus on nitrification and denitrification" in Ski, April 2013 (more details [here](#))
- "Understanding the movement and significance of DON in terrestrial ecosystems" in Storrs Hall, Windermere, April/May 2013 (more details [here](#))
- "Priming of soil organic carbon" in Copenhagen, September 2013 (more details [here](#))
- "Intramolecular Isotope Compositions" in Nantes, November 2013 (more details [here](#))



## Activities

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## Training Schools

### **Stable Isotopes and Sampling Issues**

Lisbon, Portugal, 30 October-6 November 2011

Stable isotopes of carbon, nitrogen, oxygen and hydrogen are built into organic substances by different physical and biological processes. These processes leave distinct isotopic fingerprints in organic matter, organisms, biosphere and atmosphere, that integrate in time and space. Therefore, stable isotope techniques are increasingly used in ecology, biogeochemistry and environmental sciences.

This course addressed graduate students and postdoctoral researchers that want to get a thorough introduction into the application of stable isotopes in ecology. The course focused on the use of stable isotopes of C, N, and O at the natural abundance level and as tracers, particularly on sampling issues. It was based on lectures and practical experiments.

### **SIBAE Introductory Training School**

Nancy, France, 23 to 29 September 2012

The school addresses various applications of stable isotopes in ecosystem research, including the use of major stable isotopes (carbon, nitrogen, water and metals) in ecosystem studies, fractionation processes that affect isotope distribution in ecosystem pools, their use as tracers using either natural abundance distribution or labelling experiments, and modelling at ecosystem and global scales. Questions related to instrumentation, technology and measurement quality have also been addressed.



## Activities

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## Conferences

The joint SIBAE/BASIN/ETH Zurich conference "Stable Isotopes and Biogeochemical Cycles in Terrestrial Ecosystems" has been taking place in Ascona, Switzerland, with 68 participants from 14 countries in March 2010. For detailed infos and the book of abstracts, please see [here](#). For a summary, please see [here](#).

The final SIBAE conference "Challenges in the Applications of Stable Isotopes Across Disciplines and Scales" has been taking place in Wroclaw, Poland, with 80 participants from 23 countries in March 2013. For detailed infos, please see [here](#). For abstracts, please see [here](#).

Conference, 21 to 26 March 2010

## **Stable Isotopes and Biogeochemical Cycles in Terrestrial Ecosystems - Coupling Plant and Soil Processes Across Spatial and Temporal Scales –**

### **Scientific Report**

#### **Topic and structure**

The conference brought together an interdisciplinary group of scientists applying stable isotopes of C, O, H, and N as well as radiocarbon for tracing fluxes of carbon, water and nitrogen in terrestrial ecosystems, unraveling plant and soil interactions, including species effects, and their coupling at various temporal and spatial scales.

The conference was organized with morning lectures and evening poster sessions by senior and early-career scientists as well as three topic-based parallel workshops in the afternoons. The lecture sessions were organized along four thematic topics: (a) Carbon cycle and plant-soil interactions, (b) Linking the carbon and water cycles, (c) Linking the carbon and nitrogen cycles, and (d) Modeling and Integration. The three parallel workshops focused on topics (a), (b), and (c) and aimed at producing outlines for review/opinion papers.

The conference was attended by 68 scientists from 14 countries in and outside Europe, ranging from early stage PhD students to world class leaders of their fields. Among the participants, there were 28 female and 21 early-career scientists.

#### **Most important results**

The flux of carbon dioxide between the terrestrial biosphere and the atmosphere is an order of magnitude larger than the anthropogenic release of CO<sub>2</sub> and thus has a significant imprint on atmospheric CO<sub>2</sub> concentration and its carbon isotope ratio. Over the recent years a body of evidence from field studies including <sup>13</sup>C pulse labeling experiments has shown that not only environmental drivers such as temperature, radiation, and humidity drive the exchange of CO<sub>2</sub> with the atmosphere, but that plant-soil interactions play a key role in controlling these fluxes. Presentations at this conference provided insights into latest research results on mechanisms explaining plant-soil coupling, i.e. the age of respired CO<sub>2</sub> (Trumbore et al.) and root lifetimes (Keel et al.), short-term variations of isotopic signatures in organic matter and respired CO<sub>2</sub> (Gessler et al., Grams et al.), the role of biochemistry in driving isotopic signatures of plant, soil or ecosystem respired CO<sub>2</sub> (Barbour et al. and Ghashgahaie et al.) and controls on the time lag between plant carbon uptake and soil respiration (Salmon et al., Bahn et al., Barthel et al., Gavrichkova et al.). The working group on plant-soil interactions synthesized the current knowledge, prepared a list of recommendations for designing new pulse labeling experiments and is preparing a comprehensive review paper. A short comment paper on a recent study published in *New Phytologist*, a top journal in this field, has already been submitted. The working group also identified the importance of environmental extremes on plant-soil interactions as an important but

missing gap in current published literature. Also here, a perspectives paper is planned for a top multi-disciplinary journal.

Plant-soil interactions are also closely linked to nutrient and water availabilities. Presentations showed new results on the importance of nitrogen for root-soil microbe interactions (Firestone et al., Bird et al.), the link between root or soil derived carbon for denitrification (Baggs et al.) and the role of nitrogen-carbon interactions for biodiversity-ecosystem functioning relationships (Buchmann et al.). The linkage of carbon and water cycles was also highlighted by presentations by Dawson et al., Zeeman et al., Schnyder et al. and others. A number of presentations showed very innovative approaches using new atmospheric components as tracers in biogeochemical cycles, e.g. COS (Yakir et al.) and  $^{17}\text{O}$  (Wright et al.). The presentations on modeling provided new conceptual insights on soil carbon models (Reichstein et al.) as well as global cycle carbon isotope modeling (Miller et al. and Cuntz et al.).

Allyson Wright, a PhD student from Purdue University/USA, was awarded the CSF Award for her outstanding oral presentation on *Analysis of atmospheric nitrogen inputs to the forest through isotope ratio mass spectrometry*.

## Dissemination

All three parallel workshops are currently working on opinion or review papers in their respective fields. We are currently preparing a special issue on the topic of the conference in the international peer-reviewed ISI-journal *Biogeosciences* (<http://www.biogeosciences.net/>). Based on the interaction with Dr. Armstrong, an editor of *Nature Geosciences* who participated in the conference, a couple of proposals for review/opinion papers are in preparation for *Nature Geosciences*. One manuscript has already been submitted.

## Acknowledgements

We gratefully acknowledge the administrative and financial support of the Centro Stefano Franscini, the Swiss National Science Foundation (SNF) and the Stable Isotope Networks BASIN (funded by the National Science Foundation, USA) and SIBAE (COST Action ES0806 SIBAE).



Stable Isotopes in Biosphere-Atmosphere-Earth System Research



## **COST-SIBAE 2013 MEETING**

# **CHALLENGES IN THE APPLICATIONS OF STABLE ISOTOPES ACROSS DISCIPLINES AND SCALES**

**WROCLAW POLAND | MAY 14-17, 2013**



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## TUESDAY, MAY 14, 2013

16:30 **Ice breaker**

## WEDNESDAY, MAY 15, 2013

08:15-08:30 Opening session: **Greetings by SIBAE chair, local organizer**

08:30-13:05 Session 1: **Linking biogeochemical cycles with stable isotopes**  
Chair: **Jens-Arne Subke, Dan Yakir**

08:30 **Prof. Xuhui Lee** - Yale University  
*Ecosystem isotopic fluxes of water vapor and carbon dioxide: measurement techniques, processes and applications*

09:05 **Prof. Boaz Luz** - Hebrew University  
*The ratios  $^{17}\text{O}/^{16}\text{O}$  and  $^{18}\text{O}/^{16}\text{O}$  in atmospheric  $\text{CO}_2$ : Modeling and observational constraints*

09:40 **Dr. Ilya Yevdokimov** - Russian Academy of Sciences  
*Stability of soil organic matter pools under C3-C4 transition*

10:05 **Prof. Dan Yakir** - Weizmann Institute  
*The case for isoflux: Integrating stable isotopes and flux studies*

10:30 **Coffee Break**

11:00 **Prof. Nina Buchmann** - ETH Zurich  
*Short-term carbon flow on temperate grassland: The impact of simulated summer drought*

11:25 **Dr. Marja Tirola** - University of Jyvaskyla  
*From whole plants to single cells - isotopes reveal connections between methanotrophy and diazotrophy in boreal mosses*

11:50 **Ms. Lydia Gentsch** - INRA  
*Natural variations of the  $^{13}\text{C}/^{12}\text{C}$  signature of leaf nocturnal respiration in *fagus sylvatica* branches related to previous day photosynthesis*

12:15 **Dr. Dominika Lewicka Szczebak** - Thünen Institute  
*Stable isotopic signatures of  $\text{N}_2\text{O}$  produced by denitrification: experimental determination of fractionation factors*

12:40 **Prof. Alexander Knohl** - University Goettingen  
*Aa field based quantification of leaf oxygen isotope exchange in water vapor and carbon dioxide of *fagus sylvatica* l.*

13:05 **Lunch break and poster viewing**

14:30-18:15	<b>Session 2: Integrating isofluxes across spatial and temporal scales</b> Chair: <b>Cristina Maguas and Nina Buchmann</b>
14:30	<b>Prof. David Bowling</b> - University of Utah <i>Stomatal response to humidity dominates the <sup>13</sup>C land disequilibrium in a Rocky Mountain subalpine forest</i>
15:05	<b>Prof. Gabriel Bowen</b> - University of Utah <i>Multi-scale partitioning of the water cycle with water isoscapes</i>
15:40	<b>Dr. Rolf Siegwolf</b> - Paul Scherrer Institute <i>Climate driven variations in needle extracted sugars from Larix trees at the latitudinal and altitudinal tree line</i>
16:15	<b>Dr. Koen Hufkens</b> - Ghent University <i>Multi-decadal carbon and water relations of african tropical humid forests: a tree-ring stable isotope analys</i>
16:40	Coffee Break
17:00	<b>Prof. Jaleh Ghashghaie</b> - University of Paris-Sud <i>Opposite carbon isotope discrimination during dark respiration in roots and leaves - a review</i>
17:25	<b>Prof. Christiane Werner</b> - University Bayreuth <i>Isotope fractionation during respiration and carbon allocation into primary and secondary metabolism</i>
17:50	<b>Dr. Nyncke Hoekstra</b> - Teagasc, Environment Research Centre, Johnstown Castle, Wexford, Ireland <i>Depth of water uptake of grassland species as determined by natural <sup>18</sup>O isotope abundance is affected by drought and sward type</i>
18:15	City Tour
19:30	Dinner
20:30-22:30	<b>Posters and Drinks 1</b>

## THURSDAY, MAY 16, 2013

08:30-13:05 Session 3: **Advances in stable isotope methodology**  
Chair: **Nicolas Bruggemann, Pascal Boeckx**

08:30 **Prof. Karl-Heinz Gericke** - University Braunschweig  
*Ultra-sensitive detection of nitric oxide isotopologues in exhaled air*

09:05 **Prof. Nicolas Brueggemann** - Forschungszentrum Juelich  
*Monitoring water stable isotope composition in soils using gas-permeable tubing and infrared laser absorption spectroscopy*

09:40 **Prof. Karsten Kalbitz** - University of Amsterdam  
*Simultaneous analysis of dissolved organic carbon (DOC) concentration and  $^{13}\text{C}$  by TOC-IRMS – assessment of analytical performance*

10:05 **Dr. Joachim Mohn** - Empa  
*Recent advances in laser spectroscopic analysis of GHG stable isotopes*

10:30 **Coffee Break**

11:00 **Dr. Grzegorz Skrzypek** - The University of Western Australia  
*Uncertainties in stable isotope analyses due to normalization method and standards selection*

11:25 **Prof. Andreas Richter** - University of Vienna  
*How can we measure microbial process rates in soil?*

11:50 **Dr. Douglas Baer** - Los Gatos Research  
*Advances in instrumentation based on cavity enhanced laser absorption spectroscopy*

12:15 **Dr. Kate Dennis** - Picarro  
*Quantifying the relative contribution of natural gas fugitive emissions to total methane emissions in Colorado, Utah, and Texas using mobile  $\delta^{13}\text{CH}_4$  analysis*

12:40 **Dr. David Nelson** - Aerodyne Research  
*Recent progress in development of infrared laser based instruments for real-time ambient measurements of isotopologues of carbon dioxide, water, methane, nitrous oxide and carbon monoxide*

13:05 **Lunch break**

14:30-18:25 Session 4: **Stable isotopes in ecosystem to global modeling**  
Chair: **Thomas Wutzler and Daniel Epron**

14:30 **Dr. John Miller** - NOAA  
*Progress in interpretation and modeling of atmospheric  $\delta^{13}\text{CO}_2$*

15:05 **Dr. Jerome Ogee** - INRA  
*A process-based interpretation of the stable C and O isotope signals in the soil-plant-atmosphere continuum using the ecosystem model MuSICA*

15:40 **Dr. Miroslaw Zimnoch** - AGH University of Science and Technology  
*Diurnal changes of carbon budget in the urban atmosphere of Krakow, southern Poland*

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<b>16:05</b>	<b>Prof. Ansgar Kahmen</b> - ETH Zurich <i>Leaf wax lipid <math>\delta D</math> values as proxies for past climatic change and tracers of large-scale biogeochemical cycles</i>
<b>16:30</b>	<b>Coffee Break</b>
<b>17:00</b>	<b>Dr. Lisa Wingate</b> - INRA <i>Investigating high-resolution stable isotope signals of annual tree rings across a moisture gradient with the musica model</i>
<b>17:35</b>	<b>Dr. Matthias Cuntz</b> - Helmholtz Centre for Environmental Research - UFZ, Leipzig, Germany <i>Incomplete information in isotopic interpretations</i>
<b>18:00</b>	<b>Dr. Thomas Wutzler</b> - Max Planck Institute for Biogeochemistry <i>The use of radiocarbon to constrain organic matter turnover and vertical transport in a deciduous forest under current and future conditions</i>
<b>18:25</b>	<b>Posters and Drinks 2</b> <b>Thomas Wutzler</b> , Max Planck Institute for Biogeochemistry
<b>20:00</b>	<b>Conference Dinner</b>

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## FRIDAY, MAY 17, 2013

08:30-13:05	<b>Session 5: Tracers and manipulations with stable isotopes</b> Chair: <b>Alexander Knohl and Michael Bahn</b>
08:30	<b>Prof. Susan Trumbore</b> - MPI-Biogeochemistry <i>Tracing the bomb <math>^{14}\text{C}</math> pulse label in plant respiration</i>
09:05	<b>Prof. Daniel Epron</b> - University of Lorraine <i>The fate of carbon in trees: transfer rates and residence times</i>
09:40	<b>Mr. Jasper Bloemen</b> - Ghent University <i><math>^{13}\text{C}</math> as a tracer for respired <math>\text{CO}_2</math> transport in the xylem at leaf, branch and tree level</i>
10:05	<b>Dr. Fernando Lattanzi</b> - Technische Universitaet Muenchen <i>Using <math>^{13}\text{C}</math> labeling to analyze carbon fluxes in situ</i>
10:30	<b>Coffee Break</b>
11:00	<b>Dr. Salvador Nogues</b> - Universitat de Barcelona <i>Elevated and low <math>\text{CO}_2</math> concentration effects on old and modern durum wheat varieties using <math>^{13}\text{C}</math> and <math>^{15}\text{N}</math> labelling</i>
11:25	<b>Prof. Per Ambus</b> - Technical University of Denmark <i>In-situ <math>^{13}\text{CO}_2</math> pulse-labeling in a temperate heathland - development of a mobile multi-plot field setup</i>
11:50	<b>Prof. Hans Schnyder</b> - Technische Universitaet Muenchen <i>Carbon residence time in the above-ground biomass of a temperate pasture as related to leaf life span</i>
12:15	<b>Dr. Tobias Rutting</b> - University of Gothenburg <i>Effect of ammonium adsorption in <math>^{15}\text{N}</math> tracing experiments</i>
12:40	<b>Dr. Horacio E. Bown</b> - Universidad de Chile <i>The influence of N and P supply and genotype on N remobilization in containerized <i>Pinus radiata</i> plants</i>
14:00-15:30	<b>SIBAE MC meeting</b>







**LECTURE ABSTRACTS**



## Ecosystem isotopic fluxes of water vapor and carbon dioxide: measurement techniques, processes and applications

Xuhui Lee<sup>1,2</sup>, Tim Griffis<sup>3</sup>, Wei Xiao<sup>2</sup>, Xuefa Wen<sup>4</sup>

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**Abstract:** This paper is an overview of the research to date carried out by our research groups on measuring, interpreting and modeling the surface-air exchange of  $\text{H}_2^{18}\text{O}$ , HDO,  $\text{C}^{18}\text{OO}$  and  $^{13}\text{CO}_2$  in terrestrial and aquatic environments. We will discuss in-situ measurement techniques, flux partitioning in cropland, performance of the Craig-Gordon model for lake evaporation, and large eddy simulation of isotope processes in the atmospheric boundary layer. We show that at the ecosystem scale, the isotopic exchange can be expressed as flux ratio (ratio of the molar flux density of the minor to the major isotopologue) or as isoforcing (the covariance between the vertical velocity and the delta value), each requiring a different measurement strategy and being driven by different biophysical processes. We find that in the presence of a plant canopy, (1) the kinetic fractionation factors are variable quantities dependent on stomatal resistance and air turbulence, (2) the mean isotopic composition of canopy foliage water is a well-behaved property predictable according to the principles established by leaf-scale studies, (3)  $^{18}\text{O}$  appears to be a more robust tracer than D for partitioning evapotranspiration of cropland ecosystems, (4) isoforcing is a more logical choice than other isotopic flux formulations for atmospheric models but does not improve our ability to partition ecosystem net flux of carbon dioxide. We also find that the  $^{18}\text{O}$  content of evaporation from water surfaces is higher than that predicted by the Craig-Gordon model, suggesting the formation of an enriched interfacial layer at the water surface.

## THE RATIOS $^{17}\text{O}/^{16}\text{O}$ AND $^{18}\text{O}/^{16}\text{O}$ IN ATMOSPHERIC $\text{CO}_2$ : MODELING AND OBSERVATIONAL CONSTRAINTS

**Boaz Luz, Institute of Earth Sciences, Hebrew University, Jerusalem, Israel**

For over a decade, it has been suggested that the triple oxygen isotope composition ( $^{17}\text{O}/^{16}\text{O}$  and  $^{18}\text{O}/^{16}\text{O}$ ) of tropospheric  $\text{CO}_2$  can be used as a tracer of global gross carbon fluxes and rate of air exchange between the troposphere and the stratosphere. Despite the potential, application of this tracer has been quite limited, mainly so due to inadequate precision in measurements of  $\delta^{17}\text{O}$  of carbon dioxide. Over the past two years we have developed a new approach involving  $\text{CO}_2$ - $\text{H}_2\text{O}$  equilibration,  $\text{H}_2\text{O}$  fluorination and mass spectrometric measurements of the evolved  $\text{O}_2$  gas. We then started measurements of atmospheric  $\text{CO}_2$  in uncontaminated air as well as triple oxygen isotope signatures of the main global  $\text{CO}_2$  sources and sinks and simple global modeling.

Oxygen has three naturally occurring isotopes, of mass numbers 16, 17 and 18. In most chemical reactions these isotopes fractionate in a mass dependent way such that variations in  $\delta^{17}\text{O}$  are smaller by a factor of about 0.52 from the corresponding variations in  $\delta^{18}\text{O}$ . In contrast, mass independent fractionation (MIF) is evident in stratospheric ozone and  $\text{CO}_2$  and the ratio of changes in  $\delta^{17}\text{O}$  to  $\delta^{18}\text{O}$  is greater than one. This behavior is the result of photochemical reactions in the stratosphere in which  $^{17}\text{O}$  and  $^{18}\text{O}$  are preferentially transferred from  $\text{O}_2$  to ozone and then to  $\text{CO}_2$ . As a result, stratospheric  $\text{CO}_2$  has MIF signature. The corresponding MIF enrichment or excess  $^{17}\text{O}$  ( $^{17}\Delta$ ) is defined as  $^{17}\Delta = \ln(\delta^{17}\text{O}+1) - 0.528 \ln(\delta^{18}\text{O}+1)$  with  $\text{CO}_2$  in equilibrium with VSMOW as the standard. The magnitude of  $^{17}\Delta$  of stratospheric  $\text{CO}_2$  with respect to that of tropospheric  $\text{CO}_2$  is of great interest as a tracer of the exchange rate of oxygen between the atmosphere, hydrosphere and biosphere.

Using a simple  $^{16}\text{O}$ ,  $^{17}\text{O}$  and  $^{18}\text{O}$  global budget, showed that the  $^{17}\Delta$  which originates from stratospheric photochemistry should not be completely removed by exchange with water in the troposphere. Indeed, our new measurements show that a small, but significant fraction remains and the magnitude of this fraction depends on rates of isotopic exchange in leaves, soils and the ocean. Our new measurements and modeling demonstrate that measurements of  $^{17}\Delta$  in tropospheric  $\text{CO}_2$  provide new means for estimating gross  $\text{CO}_2$  exchange by the terrestrial biosphere and also between the troposphere and stratosphere.

## STABILITY OF SOIL ORGANIC MATTER POOLS UNDER C3-C4 TRANSITION

Ilya Yevdokimov<sup>1</sup>, Alla Larionova<sup>1</sup>, Bertha Zolotareva<sup>1</sup>, Yakov Kuzyakov<sup>2</sup>

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Carbon sequestration during plant litter decomposition and accumulation of soil organic matter (SOM) in stable pools is known to be one of the key processes mitigating greenhouse effect. Among other approaches used, combining physical and chemical fractionation with <sup>13</sup>C natural abundance method in soil sampled from C3 - C4 transition is promising in separating heterogeneous SOM into the pools with turnover times ranging from years to centuries. We used this approach on soils: 1) in 5-yr field manipulation experiment where maize litter was added at the rate of 1160 g C m<sup>-2</sup> to Phaeozem (Pushchino, Moscow Region, 54°50'N, 37°35'E) developed solely under C3 vegetation, and 2) in 46-yr continuous maize cropping on Chernozem (Orlovka, Voronezh Region, 51°41'N, 39°15'E). The separation of pools was done by acid hydrolysis particle size and density fractionation. Decomposability of isolated fractions was estimated by CO<sub>2</sub> emission in incubation experiments. Microbial biomass was determined by fumigation-extraction method, and the structure of microbial community was estimated by the phospholipid fatty acids (PLFA) profiling procedure.

In Phaeozem, the most labile SOM pool with mean residence time (MRT) of 4 yrs was organic C immobilized in microbial biomass (1.5% of total SOM). The pool with highest recalcitrance (MRTs of 70-100 yrs) was represented by SOM resistant to acid hydrolysis (approximately 50-60% of total SOM).

The decomposition of size fractions was inversely proportional to the size of particles. Labile organic carbon associated with sand and coarse silt turned over with mean residence time (MRT) shorter than that of non-fractionated bulk soil, while organic matter bound on fine fractions (clay, medium and fine silt) decayed slower than non-fractionated SOM. Higher recalcitrance of fine silt and clay fractions determined by δ<sup>13</sup>C was consistent with lower CO<sub>2</sub> emission of these fractions in comparison with coarse fractions during incubation experiment. The recalcitrance of organic matter of density fractions estimated by δ<sup>13</sup>C signature was directly related to the fraction density, i.e. light fractions belong to labile pool, while heavy fractions contribute to recalcitrant organic C. In contrast with particle size, density fractions did not show close correlation between CO<sub>2</sub> emission and SOM recalcitrance determined by δ<sup>13</sup>C. The CO<sub>2</sub> released from heavy fractions was higher than that for the light fraction.

The isotopic signature of PLFAs evidence that microorganisms decomposed old carbon derived from C3 organic matter, along with maize-derived organic matter, and used it as a growth substrate as well. The order of C4 percentage in the groups of soil microorganisms was as follows: protozoa < saprotrophic fungi < Actinomycetes < Gram-positive bacteria < Gram-negative bacteria < mycorrhiza fungi.

In general, partitioning of maize-derived organic C followed the similar pattern both in Phaeozem and Chernozem, while turnover rates of both C4- and C3-derived SOM in Chernozem were substantially lower than in Phaeozem.

## THE CASE FOR ISOFLUX: INTEGRATING STABLE ISOTOPES AND FLUX STUDIES

Yakir Dan<sup>1</sup>, Bahn Michael<sup>2</sup>, Reichstein Markus<sup>3</sup>

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Major technological developments and the transition from magnetic-sector lab-based mass spectrometers to laser spectroscopy and field deployable isotope analyzers are transforming the field of stable isotope research. This is an unusual opportunity to push for better utilization and integration of stable isotope measurements (and other tracers) in ecosystem studies, including eddy flux studies and other flux components. This push requires re-visiting the overall motivation to, and benefits from, using stable isotopes and other tracers in ecosystem and flux studies: When trying to understand ecosystem functioning and its response to environmental change, stable isotopes and other tracers provide strong constraints on hypotheses and interpretations. There are many examples of critical information obtained by adding stable isotopes to our measurements, such as: partitioning the ocean/land CO<sub>2</sub> sink/source (<sup>13</sup>C); partitioning biotic vs. abiotic and anthropogenic components of biogeochemical cycles (O<sub>2</sub>/N<sub>2</sub>; CO<sub>2</sub>/O<sub>2</sub>); large scale biosphere response to El-Nino/RH (<sup>18</sup>O-CO<sub>2</sub>); canopy vs. soil CO<sub>2</sub> flux partitioning (<sup>13</sup>C, <sup>18</sup>O); <sup>18</sup>O in linking CO<sub>2</sub>, water, and O<sub>2</sub> fluxes and biogeochemical cycles; C<sub>3</sub>/C<sub>4</sub>, partitioning; tracer experiments to study allocation, turnover, transport rate, spatial distribution; plant soil water uptake profiles, hydraulic lift; water turnover/recycling in plants, soil, and water bodies; sources of precipitation moisture; tree rings—paleo-environmental aspects. The potential of stable isotopes for identifying, tracing and integrating processes across spatial and temporal scales has been strongly enhanced by recent technological developments and rapidly growing datasets of high time resolution fluxes, which calls for the development of a new generation of experimental and synthesis activities across disciplines.

## SHORT-TERM CARBON FLOW ON TEMPERATE GRASSLAND: THE IMPACT OF SIMULATED SUMMER DROUGHT

Nina Buchmann<sup>1</sup>, Susanne Burri<sup>1</sup>, Patrick Sturm<sup>2</sup>, Thomas Baur<sup>1</sup>, Ulrich Prechsl<sup>1</sup>, Alexander Knohl<sup>3</sup>

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Mean summer precipitation in Switzerland is projected to decrease by the end of the 21<sup>st</sup> century, with a tendency towards more frequent dry spells during summer. However, large uncertainties exist in terms of how extreme events like heat waves or drought periods influence the short-term carbon cycle.

We will present results from an experiment performed in June 2011 where we investigated the effect of drought on the short-term carbon transport within the plant-soil system at an intensively managed lowland grassland in Switzerland (ETH research station Chamau) using rainout shelters. A pulse labeling experiment with <sup>13</sup>CO<sub>2</sub> allowed tracing the flow of freshly assimilated carbon from above-ground biomass to the roots and to soil respiration. Continuous measurements of soil respiration and its isotopic composition were performed with a laser spectrometer (QCLAS-ISO, Aerodyne Research Inc., MA, USA) coupled to a custom-built chambers. The measurements were complemented by sampling community above- and below-ground biomass and subsequent stable isotope analysis.

Drought-stressed plots showed reduced <sup>13</sup>CO<sub>2</sub> tracer uptake. However, proportional below-ground allocation was higher under drought stress, thus, residence time in shoots did not increase. Soil CO<sub>2</sub> efflux as well as the use of fresh assimilates in root respiration were reduced under drought stress. Our results suggest that during a dry spell, less carbon was entering the plant-soil system, but proportionally more was allocated below-ground. This carbon was more likely to be incorporated into below-ground biomass instead of being used in fast respiratory processes. Since grasslands are widespread agroecosystems in Switzerland and beyond, these results have important implications for carbon sequestration under future climate conditions.

## FROM WHOLE PLANTS TO SINGLE CELLS - ISOTOPES REVEAL CONNECTIONS BETWEEN METHANOTROPHY AND DIAZOTROPHY IN BOREAL MOSSES

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Cyanobacteria have been considered the main nitrogen (N<sub>2</sub>) fixing bacteria (diazotrophs) in mosses, but heterotrophs and methane (CH<sub>4</sub>) oxidizing bacteria (methanotrophs) may also contribute to N<sub>2</sub> fixation. Moreover, as oxygen conditions affect both mineralization and CH<sub>4</sub> oxidation, water table level may have a significant impact on the N<sub>2</sub> fixation. To test these hypotheses we studied diazotrophy and methanotrophy in forest mosses, wetland mosses and stream mosses by double tracing (<sup>15</sup>N<sub>2</sub> and <sup>13</sup>CH<sub>4</sub>) and stable isotope analysis of whole plants and plant parts. Characterization of the microbial diversity was done by the *nifH* gene, which codes for the dinitrogenase reductase, and localization of methane oxidizing and nitrogen fixing bacteria was performed by halogen *in situ* hybridization coupled to secondary ion mass spectrometry (HISH-SIMS). Direct stable isotope method (<sup>15</sup>N<sub>2</sub> incorporation) was slightly more sensitive than the traditional acetylene reduction method in studying N<sub>2</sub> fixation in feather mosses (*Hylocomium splendens* and *Pleurozium schreberi*) in southern sites, where the process suffered from the higher anthropogenic N deposition (≥ 3 kg/ha/yr). In *Sphagnum* mosses both nitrogen fixation and methane oxidation were observed with a noticeable temporal and spatial variation in the moss stems and stem parts, showing response to the water table level in the wetland environment, where *Bradyrhizobium* sp. and methanotrophs were the most abundant diazotrophs of mosses. HISH-SIMS proved the connection between methanotrophy and diazotrophy within the same bacterial cells inside the *Sphagnum* mosses. In stream mosses of the genus *Fontinalis* fixed nitrogen was quickly transferred from bacteria to the moss, while methane derived carbon was localized in the bacterial cells. The studies demonstrated the advantages and difficulties of the single-cell isotope technique and established the role of non-cyanobacterial nitrogen fixation especially in wetland mosses.



## NATURAL VARIATIONS OF THE $^{13}\text{C}/^{12}\text{C}$ SIGNATURE OF LEAF NOCTURNAL RESPIRATION IN *FAGUS SYLVATICA* BRANCHES RELATED TO PREVIOUS DAY PHOTOSYNTHESIS

Gentsch Lydia<sup>1,2</sup>, Sturm Patrick<sup>2</sup>, Wingate Lisa<sup>1</sup>, Ogée Jérôme<sup>1</sup>, Siegwolf Rolf<sup>3</sup>, Hammerle Albin<sup>4,2</sup>, Plüss Peter<sup>2</sup>, Baur Thomas<sup>2</sup>, Barthel Matthias<sup>5,2</sup>, Buchmann Nina<sup>2</sup> and Knohl Alexander<sup>6,2</sup>

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Leaf respiration represents about a quarter of total plant carbon loss. Understanding the dynamics of this flux and identifying its substrates is therefore a prerequisite for an accurate description of the carbon budget of terrestrial ecosystems. The  $^{13}\text{C}/^{12}\text{C}$  ratio of nocturnal leaf respiration ( $\delta^{13}\text{C}_{\text{noc}}$ ) is one of the few signals that can provide clues on the dynamics of leaf non-structural carbohydrate pools in the field and their use for leaf respiration. However, the physiological mechanisms driving long- and short-term dynamics of  $\delta^{13}\text{C}_{\text{noc}}$  are not straightforward, given likely impacts of post-photosynthetic fractionations, temporal dynamics in putative respiratory substrates and the modulation of the latter two by environmental drivers, such as temperature. Progress in laser spectrometry technology now enables high-frequency measurements of  $\delta^{13}\text{C}_{\text{noc}}$  in the field and hence supports the pursuit for a better mechanistic understanding of nocturnal leaf respiration. Here, we present continuous, hourly measurements of nocturnal respiration ( $R_{\text{noc}}$ ) and  $\delta^{13}\text{C}_{\text{noc}}$  on leafy branches of three mature *Fagus sylvatica* trees from a temperate mixed-deciduous forest in Switzerland. These measurements were conducted with open branch chambers and a laser spectrometer for  $\text{CO}_2$  isotopologue measurements (QCLAS-ISO, Aerodyne Research Inc.) in August and September during two consecutive years (in total 38 and 60 days). The data was then analysed for trends occurring over the course of individual nights and for trends from one night to the next. Night-to-night variation in flux-weighted means of  $\delta^{13}\text{C}_{\text{noc}}$  was clearly linked to previous day photosynthetic  $^{13}\text{C}$  discrimination, measured by the same chambers. This link was, however, modified by the strength of previous-day photosynthesis. For a large fraction of measured nights,  $\delta^{13}\text{C}_{\text{noc}}$  decreased progressively over the course of the night (up to  $0.7\text{‰ hour}^{-1}$ ). This trend was analysed with regard to concurrent dynamics of  $R_{\text{noc}}$ , leaf temperature and previous-day environmental conditions. Collectively, these results allowed us to draw a clearer picture of leaf respiratory processes of *F. sylvatica* branches in the field.

## STABLE ISOTOPIC SIGNATURES OF N<sub>2</sub>O PRODUCED BY DENITRIFICATION: EXPERIMENTAL DETERMINATION OF FRACTIONATION FACTORS

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Quantifying denitrification in arable soils is crucial in predicting the microbial consumption of nitrogen fertilizers as well as N<sub>2</sub>O emissions. Stable isotopologue analyses of denitrification substrates ( $\delta^{15}\text{N}_{\text{NO}_3}$ ,  $\delta^{18}\text{O}_{\text{NO}_3}$ ) and products ( $\delta^{15}\text{N}_{\text{N}_2\text{O}}$ ,  $\delta^{18}\text{O}_{\text{N}_2\text{O}}$  and SP<sub>N<sub>2</sub>O</sub> = Site Preference, i.e. difference in  $\delta^{15}\text{N}$  between the central and peripheral N positions of the asymmetric N<sub>2</sub>O molecule) can help to distinguish production pathways and to identify N<sub>2</sub>O reduction to N<sub>2</sub>. However, such interpretations are often ambiguous due to insufficient knowledge on isotopic fractionation mechanisms and wide differences in isotope fractionation factors determined by various studies for N<sub>2</sub>O production and reduction.

Here we present an original approach to determine fractionation factors associated with denitrification. This determination is based on simultaneous modeling of both reaction steps (N<sub>2</sub>O production and reduction) and comparison of the results with experimental data from a laboratory incubation experiment carried out under N<sub>2</sub>-free atmosphere. During the incubations N<sub>2</sub>O and N<sub>2</sub> concentrations were measured continuously, hence the reduced fraction (N<sub>2</sub>/(N<sub>2</sub>+N<sub>2</sub>O)) was calculated directly from measured gas fluxes. Various modeling approaches have been applied to estimate the ranges of isotopic fractionation factors controlling the isotopic signatures of soil-emitted N<sub>2</sub>O. Initially, assumed isotope fractionation factors and the Rayleigh equations describing isotopic fractionation were used to calculate the theoretical  $\delta^{15}\text{N}$ ,  $\delta^{18}\text{O}$  and SP values for emitted N<sub>2</sub>O. Afterwards, the best fit fractionation factors for N<sub>2</sub>O production and reduction were determined by comparing modeled and measured values.

In different experimental setups with an arable soil (sandy loam) the isotopic fractionation factors were very consistent. For N<sub>2</sub>O production mean net isotope effects of  $\eta^{15}\text{N}_{\text{NO}_3\text{-N}_2\text{O}} \sim -37\text{‰}$ ,  $\eta\text{SP}_{\text{NO}_3\text{-N}_2\text{O}} \sim -1\text{‰}$  and  $\eta^{18}\text{O}_{\text{H}_2\text{O-N}_2\text{O}} \sim +36\text{‰}$  have been found. For N<sub>2</sub>O reduction mean net isotope effects of  $\eta^{15}\text{N}_{\text{N}_2\text{O-N}_2} \sim +1\text{‰}$ ,  $\eta\text{SP}_{\text{N}_2\text{O-N}_2} \sim -7\text{‰}$  and  $\eta^{18}\text{O}_{\text{N}_2\text{O-N}_2} \sim -6\text{‰}$  have been found. When compared to previous reports these results show significantly lower fractionation for  $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$  values during N<sub>2</sub>O reduction, which is most likely due to enhanced experimental approach that largely eliminates laboratory artifacts.

## A FIELD BASED QUANTIFICATION OF LEAF OXYGEN ISOTOPE EXCHANGE IN WATER VAPOR AND CARBON DIOXIDE OF *FAGUS SYLVATICA* L.

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The stable isotope <sup>18</sup>O in water and carbon dioxide is a powerful tracer for investigating ecological processes, such as the coupling of the carbon and water cycles. Furthermore, it can provide a constraint on the contribution of terrestrial ecosystems to the global carbon and water cycles. The mechanisms of oxygen isotope exchange in CO<sub>2</sub> and H<sub>2</sub>O have been investigated primarily under laboratory conditions, whereas field measurements are still scarce. Especially the availability of combined datasets of <sup>18</sup>O in both carbon dioxide and water vapor fluxes is, so far, very limited. Recent developments of laser spectroscopic techniques for isotopologue measurements now allow for field deployable, high accuracy and high frequency measurements of these isotopic gas-exchanges with the atmosphere.

Here, we present a unique dataset of <sup>18</sup>O in water vapor as well as carbon dioxide fluxes during leaf gas-exchange, measured simultaneously with two laser spectrometers and open branch chambers under field conditions. The study was conducted on mature beech trees (n=3) in a mixed-deciduous forest in Switzerland in 2010.

Frequently-taken leaf water samples from outside the branch chambers were used to determine the effective path-length (L), an important parameter in models of leaf water enrichment. L was constrained by using a Bayesian inversion scheme in combination with the Péclet-modified Craig Gordon model and was estimated to be roughly 0.02 m. Using the determined value of L, modeled <sup>18</sup>O leaf water enrichment inside the chambers was compared with leaf water enrichment derived from the branch chamber transpiration measurements. The good agreement between these two approaches implied a very good performance of the chamber system in measuring isotopic gas-exchange of water. As an application, we estimated the coefficient of CO<sub>2</sub> hydration (Θ) for the enzyme carbonic anhydrase using the leaf water enrichment values and the measured apparent discrimination of C<sup>18</sup>OO. Our results support the few recent findings of other groups that carbonic anhydrase efficiency measured under field conditions may be lower compared to lab-derived values.

In summary, this study provides (i) an estimate of L in beech leaves derived with a Bayesian inversion scheme, (ii) an extended dataset of parallel measurements of <sup>18</sup>O in water vapor and carbon dioxide gas exchange fluxes measured at the branch level under field conditions, and (iii) a first field estimate of the carbonic anhydrase efficiency of mature beech trees.

# STOMATAL RESPONSE TO HUMIDITY DOMINATES THE $^{13}\text{C}$ LAND DISEQUILIBRIUM IN A ROCKY MOUNTAIN SUBALPINE FOREST

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## Abstract

Stable isotopes are used as a tracer to investigate carbon cycle processes at a variety of spatial and temporal scales. Fossil fuel combustion has increased atmospheric  $\text{CO}_2$ , with a concomitant decrease in the carbon isotope composition ( $\delta^{13}\text{C}$ ) of the air of  $\sim 1.6\text{‰}$  (the  $^{13}\text{C}$  Suess effect). Because carbon is stored in the terrestrial biosphere for tens to many hundreds of years, the  $\delta^{13}\text{C}$  of the  $\text{CO}_2$  released by terrestrial ecosystems should differ from that removed from the air by photosynthesis of land plants. The difference in the  $\delta^{13}\text{C}$  of the land-atmosphere respiration ( $\delta_{\text{R}}$ ) and photosynthetic assimilation ( $\delta_{\text{A}}$ ) fluxes is referred to as the  $^{13}\text{C}$  land disequilibrium. Biogeochemical models based on bomb radiocarbon residence times suggest that this disequilibrium is small and positive ( $\delta_{\text{R}} > \delta_{\text{A}}$ ).

In this study a 7-year record of biosphere-atmosphere carbon exchange was used to examine the  $^{13}\text{C}$  land disequilibrium. A novel isotopic mixing model was employed to evaluate of the  $\delta^{13}\text{C}$  of net land-atmosphere exchange during the daytime, and combined with tower-based flux partitioning methods to assess the isotopic composition of the photosynthesis flux ( $\delta_{\text{A}}$ ). The  $^{13}\text{C}$  land disequilibrium varied seasonally, and in midsummer was opposite in sign and larger than expectations from the Suess effect. This unexpected result is a consequence of the response of plant stomata to humidity, and associated change in photosynthetic carbon isotope discrimination. If these results are representative of forests on a large land area, these results suggest that a re-evaluation of the land and ocean carbon sinks is needed

## MULTI-SCALE PARTITIONING OF THE WATER CYCLE WITH WATER ISOSCAPES

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Models and maps of the spatial distribution of isotopes in the water cycle have been developed for decades and applied to studies of hydrological processes, animal migration, and forensics. Most emphasis has been placed on large-scale patterns expressed in continental precipitation and fresh waters. Recently, reduced analytical cost and enhanced access have accelerated the pace of local and regional-scale water isotope data collection. I will introduce a series of statistical and process-based data analysis tools applicable to interrogating spatiotemporal water isotope data at multiple spatial scales, and demonstrate how these tools can be used to understand structure and connectivity within hydrological systems. I will also make the case for data sharing and open data access, which if supported by robust, flexible, and easy-to-use cyberinfrastructure could promote multi-scale, multi-system analyses within the hydrological systems and beyond.

## CLIMATE DRIVEN VARIATIONS IN NEEDLE EXTRACTED SUGARS FROM LARIX TREES AT THE LATITUDINAL AND ALTITUDINAL TREE LINE.

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High-latitude and high altitude tree-line ecosystems are very sensitive to temperature variations and are strongly affected by the recent warming trends. Extensive forest ecosystems in continental Siberia on permanently frozen ground and in mountainous forests are particularly vulnerable. The vegetation periods are short, temperature is limiting and nutrient and water (in Siberia) availabilities are low. If the temperature increases in the future, with an expected decrease of the summer precipitation, the enhanced evaporative demand and water loss for the plants will reduce productivity and carbon sequestration of these forests.

Using compound specific stable isotope ratio mass spectrometry, we study the isotopic variations of different sugars extracted from needles and different plant tissues from Siberian and Swiss mountainous larch forest. Needle samples were collected on a seasonal scale during the vegetation period in 2011 and 2012, representing two contrasting meteorological constellations. In a tracer study we studied the fate of carbon from needles to the wood. The main goal of our studies was to identify specific responses to changes in water availability and temperatures, in the isotopic composition and concentrations of the leaf sugars. The analysis of the fractionation in compound-specific isotope ratios of needle the content of carbohydrates is a means to evaluate to what degree the thawing of permafrost and gradually increasing summer drought conditions are key factors influencing forest growth.

### Results:

- The growing seasons 2011 and 2012, which were climatically different (length of snow cover, period of maximum T, length of the dry period) facilitated the exploration of physiological responses of the trees to changing environmental conditions:
- These responses were strongly seen in the  $\delta^{13}\text{C}$  values and the concentrations of the main sugars (sucrose, glucose and fructose) containing a strong signal of stomatal conductance (the latter related to  $\delta^{18}\text{O}$  of bulk sugars).
- Periods with differing relationships between the different sugar proxies themselves and their relationship with climate parameters were detected, and were more prominent than in tree rings.
- Water limitations were well visible in the periods, when precipitation was low. The impact of the following increase in precipitation could be seen in the  $\delta^{13}\text{C}$  (after 14/8 in 2012) and concentration (see 2<sup>nd</sup> period in 2011) data.
- Tree ring-width measurements (width of the annual ring and rate of growth for 2011) corresponded very well with the isotope data.
- The relative concentration of the three individual sugars showed high variability from one to the other season and within the two sampling seasons.

## MULTI-DECADAL CARBON AND WATER RELATIONS OF AFRICAN TROPICAL HUMID FORESTS: A TREE-RING STABLE ISOTOPE ANALYSIS

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Little is known about the temporal dynamics of the carbon sequestering capacity and dynamics of African tropical humid forest ecosystems in response to various environmental drivers. This lack of knowledge is mainly due to the absence of ecosystem scale flux measurements of gas exchange. However, tree growth often displays itself as alternating pattern of visible rings due to the seasonally varying growth speed of the vascular cambium. Consequently, analysis of tree growth through tree-ring analysis provides us with insights into past responses of the carbon sequestering capacity of key species to abrupt ecosystem disturbances and, while slower, a changing climate.

Not only does the width and density of growth rings reflect annual growth but their isotopic composition of  $^{13}\text{C}/^{12}\text{C}$  and  $^{18}\text{O}/^{16}\text{O}$  isotopes also reveal the environmental conditions in which the trees were growing. In particular, stable isotope ratios in tree-rings of carbon are influenced by fractionation through carboxylation during photosynthesis and changes in leaf stomatal conductance. Similarly, fractionation of oxygen isotopes of soil water occurs at the leaf level through evapotranspiration. As a consequence,  $^{18}\text{O}/^{16}\text{O}$  ( $\delta^{18}\text{O}$ ) values in wood cores will reflect both the signal of the source water as well as that of for example summer humidity. Therefore, both C and O stable isotopes might not only be valuable as proxy data for past climatic conditions but they also serve as an important tool in understanding carbon and water relations within a tropical forest ecosystems.

To this end we correlate long term climate records (1961 – present) with tree ring measurement of incremental growth and high resolution analysis of tree-core stable isotope composition ( $\delta^{13}\text{C}$ ,  $\delta^{18}\text{O}$ ) at a tropical humid forests in the DR Congo. The Yangambi Man And Biosphere (MAB) reserve is located in the north-eastern part of DR Congo, with a distinct tropical rainforest climate. In addition to the tree-core data records and extensive meteorological records collected at both sites, observations on green leaf phenology of key species will provide us with additional information on potential carbon sequestration dynamics. Because, phenology is a first order control on plant productivity. In this unique study, using detailed tree-ring analyses together with auxiliary data, we explore the temporal dynamics of carbon and water relations and the influence on carbon sequestration of key tree species in African tropical humid forests.

## OPPOSITE CARBON ISOTOPE DISCRIMINATION DURING DARK RESPIRATION IN ROOTS AND LEAVES - A REVIEW

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Leaves are in general <sup>13</sup>C-depleted compared to all other organs (e.g. roots, stem/trunk and fruits) suggesting that post-photosynthetic discriminations do likely occur leading to the observed isotopic difference between autotrophic and heterotrophic tissues/organs. Different hypotheses are formulated in the literature to explain this difference. One of these states that CO<sub>2</sub> respired by leaves in the dark is <sup>13</sup>C-enriched compared to leaf organic matter, while it is <sup>13</sup>C-depleted in the case of root respiration. The opposite respiratory fractionation between leaves and roots was invoked as explanation for the wide-spread between-organ isotopic differences. We analysed recent findings on the carbon isotope composition of respired CO<sub>2</sub> ( $\delta^{13}C_R$ ) by both leaves and roots compared with respective organic matter. Our analysis cumulates evidences for opposite apparent carbon isotope discrimination during respiration in roots as compared to leaves in herbaceous species. However, significant differences between functional groups (C<sub>3</sub> herbs vs C<sub>3</sub> woody species, and C<sub>3</sub> vs C<sub>4</sub> herbs) are evidenced mainly for roots. While leaf- $\delta^{13}C_R$  is <sup>13</sup>C-enriched in both C<sub>3</sub> herbs and C<sub>3</sub> woody species (and also in some C<sub>4</sub> herbs) roots of C<sub>3</sub> herbs show opposite respiratory fractionation compared with roots of C<sub>3</sub> woody plants; root- $\delta^{13}C_R$  being <sup>13</sup>C-depleted in C<sub>3</sub> herbs and <sup>13</sup>C-enriched in C<sub>3</sub> woody species compared to root material. The respiration of mycorrhiza associated with tree roots could explain the opposite respiratory fractionation observed between C<sub>3</sub> herb roots and C<sub>3</sub> woody roots. Different activity of PEPc between heterotrophic vs autotrophic organs could be a potential candidate for explaining the observed differences between leaves and roots.



## Isotope fractionation during respiration and carbon allocation into primary and secondary metabolism

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Our understanding of post-photosynthetic carbon isotope fractionation during dark and light respiration has much advanced. Nevertheless we still lack a clear picture of the origin of short-term variability in  $\delta^{13}\text{C}$  of respired  $\text{CO}_2$  ( $\delta^{13}\text{C}_{\text{res}}$ ) on a diel basis, which can amount to a maximum variation of 14.8‰ in leaves<sup>1</sup>. There are striking differences in diurnal variations of  $\delta^{13}\text{C}_{\text{res}}$  between plant functional groups, which have been proposed to be related to carbon partitioning in the metabolic branching points of the respiratory pathways and secondary metabolism. Plant primary and secondary metabolism is closely linked via a number of interfaces including the central metabolite pyruvate. Notably, it is a known substrate in a large array of secondary pathways leading to the biosynthesis of many volatile organic compounds (VOCs), such as volatile isoprenoids, oxygenated VOCs, aromatics, fatty acid oxidation products, which can be emitted by plants.

**On hypothesis is that carbon isotope fractionation in light and dark respired  $\text{CO}_2$  is associated with carbon allocation into primary and secondary metabolism.** Specifically, a high carbon flux through the pyruvate dehydrogenase bypass, mevalonic, MEP/DOXP, shikimic, and fatty acid pathways for investment in secondary metabolites (such as VOCs) may be associated with a pronounced  $^{13}\text{C}$ -enrichment of respired  $\text{CO}_2$  above the putative substrate, as it involves the decarboxylation of the  $^{13}\text{C}$ -enriched C-1 from pyruvate.

Based on simultaneous real-time measurements of stable carbon isotope composition of branch respired  $\text{CO}_2$  (CRDS) and VOC fluxes (PTR-MS with compound identification via GC-MS) we traced carbon flow into these pathways by pyruvate positional labeling.

In *Halimium halimifolium* the  $^{13}\text{C}$ -enriched C-1 from pyruvate was released in substantial amounts as  $\text{CO}_2$  in the light. Simultaneously, naturally  $^{13}\text{C}$  depleted C-2 and C-3 carbon atoms of the acetyl-moiety are emitted as a variety of VOCs including volatile isoprenoids, oxygenated VOCs, green leaf volatiles, aromatic, sulfide and nitrogen containing VOCs. A high carbon allocation into VOC production was not visible in *Oxalis triangularis*.

Moreover, during light-dark transitions leaf emission bursts of the oxygenated metabolite acetaldehyde were observed as part of the PDH bypass pathway in the cytosol<sup>2</sup>. This is hypothesized to be due to the “pyruvate overflow” mechanism in which pyruvate is rapidly decarboxylated to acetaldehyde upon darkening, thereby releasing  $^{13}\text{C}$ -enriched  $\text{CO}_2$  from C1 position of pyruvate. This may be a new piece of evidence for the origin of  $^{13}\text{C}$ -enriched  $\delta^{13}\text{CO}_2$  which is released during Light-Enhanced Dark Respiration (LEDR).

This study provides the first evidence that the isotopic signature of respired  $\text{CO}_2$  is closely linked to carbon partitioning between anabolic and catabolic pathways and plants strategies of carbon investment into secondary compound synthesis.

### References

1. Werner C. & Gessler A. (2011) Diel variations in the carbon isotope composition of respired  $\text{CO}_2$  and associated carbon sources: a review of dynamics and mechanisms. *Biogeosciences* 8, 2437–2459, [www.biogeosciences.net/8/2437/2011/](http://www.biogeosciences.net/8/2437/2011/)

## DEPTH OF WATER UPTAKE OF GRASSLAND SPECIES AS DETERMINED BY NATURAL <sup>18</sup>O ISOTOPE ABUNDANCE IS AFFECTED BY DROUGHT AND SWARD TYPE

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Benefits of grassland mixtures over monocultures are believed to be due to facilitation and niche complementarity among plant species. One potential mechanism, for which there is little evidence in agronomic grassland systems, is soil niche complementarity between deep-rooting and shallow-rooting species. This mechanism may be of particular importance under drought stress, when water and nutrients may be less available in dry top layers compared to relatively moist deep layers.

The objective of this study was to assess the depth of water uptake of shallow-rooting and deep-rooting species in grassland mixtures and monocultures under benign and drought conditions by using a natural abundance <sup>18</sup>O isotope method.

In 2011, a field experiment was established in Zürich-Reckenholz, Switzerland, with plots containing monocultures and mixtures of *Lolium perenne* (Lp), *Cichorium intibus* (Ci), *Trifolium repens* (Tr) and *Trifolium pratense* (Tp) sown according to a simplex design. In 2012, half of the plots were subjected to a drought treatment of 10 weeks summer rain exclusion, using rainout shelters. Plots were cut 6 times per year and received 200 kg N ha<sup>-1</sup> yr<sup>-1</sup>. At the end of the drought period, stem bases (up to 2 cm above soil level) were collected for five to eight tillers of all four sown species. Samples were taken from two replicate plots of all the monocultures (Mono) and the 4-species mixture (Mix) of both control (Ctr) and drought (Drt) plots. At the same time, three 2 cm diameter soil cores were taken to 40 cm depth per plot and divided into 5 segments (0-5, 5-10, 10-20, 20-30 and 30-40 cm). All samples were stored frozen in airtight glass vials. Water from the soil and plant samples was extracted using cryogenic water distillation and analysed for <sup>18</sup>O.

The IsoSource stable isotope mixing model was applied to quantitatively determine the proportional contribution of each of the 5 soil depth increments to the plant stem water δ<sup>18</sup>O signature. The 0-5 and 5-10 cm layers were combined a posteriori. The mean proportional contribution to water uptake of each soil layer was analysed using ANOVA (R version 2.12.2).

The mean proportional contribution to water uptake of the 0-10 cm soil layer (PC<sub>0-10</sub>) was on average 0.48 and varied from 0.07 (Ci-Drt-Mono) to 0.88 (Tr-Ctr-Mix). The PC<sub>0-10</sub> was significantly (p<0.01) greater for the shallow-rooting species Tr and Lp. Drought significantly (p<0.05) reduced the PC<sub>0-10</sub> from 0.56 to 0.42 on average, confirming that the species shifted their water uptake to deeper layers under drought conditions. The PC<sub>0-10</sub> of Tp was lower in mixtures compared to monocultures, whereas the opposite was true for the other species, resulting in a significant (p<0.01) species × mixture interaction. The changes in the 10-20 and 20-30 cm layer were relatively small (average PC of 0.16 and 0.17, respectively), and therefore the proportional contribution of the 30-40 cm layer mirrored that of the 0-10 cm layer, and ranged from 0.02 (Tr-Ctr-Mix) to 0.45 (Ci-Drt-Mono).

This confirms that the grassland species in this experiment occupied distinct niches in relation to the depth of soil water uptake. These niches appear to be flexible both in reaction to drought stress (move to deeper soil layers) and depending on whether species were grown in mixture or monoculture.

The next step will be to determine whether these findings translate into i) yield advantages of deep rooting species under drought stress and ii) yield advantages of combining deep and shallow rooting species in mixtures.

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## ULTRA-SENSITIVE DETECTION OF NITRIC OXIDE ISOTOPOLOGUES IN EXHALED AIR

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The detection of nitric oxide (NO) is of considerable medical interest. NO is involved in a multitude of mammal physiological processes and, therefore, labeling and isotope-specific detection are important tools for elucidating chemical pathways. Both laser-induced fluorescence (LIF) and mass selective REMPI spectroscopy are extremely sensitive techniques for the determination of low nitric oxide concentrations. These techniques are used for monitoring the most abundant isotopologues such as  $^{14}\text{N}^{16}\text{O}$ ,  $^{15}\text{N}^{16}\text{O}$  and  $^{14}\text{N}^{18}\text{O}$  without these species interfering spectroscopically with other molecules in human breath. This has been realized by using the  $A^2\Sigma^+(v'=0)\leftarrow X^2\Pi_Q(v''=0)$  transition (NO  $\gamma$ -band) in the ultraviolet region around 226 nm. In case of LIF, the fluorescence is monitored around 247 nm from the  $A^2\Sigma^+(v'=0)\rightarrow X^2\Pi_Q(v''=2)$  transition.

Selected rovibrational transitions are employed for the ultra-sensitive and isotope-specific detection of exhaled NO by LIF. The detection limit is in the lower ppt range, f.e. for  $^{14}\text{N}^{18}\text{O}$  it is 0.8 parts per trillion.

For an online measurement of human exhaled air, a respiratory mask has been constructed and integrated into our system. An overview is given of the applied spectroscopic devices for non-invasive detection of NO originated from exhaled human air. The main advantages of these techniques compared to established methods are as follows: high sensitivity for NO concentrations in the ppt region, a high time resolution of 20 ms and isotopic selectivity to distinguish between the different nitric oxide isotopologues. Visualizations of single-exhalation profiles and long-time online measurements including the determination of absolute NO concentrations are presented and the influence of quenching gases present during the experiment is discussed.

# Monitoring water stable isotope composition in soils using gas-permeable tubing and infrared laser absorption spectroscopy

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The water stable isotopologues  $^1\text{H}^2\text{H}^{16}\text{O}$  and  $^1\text{H}_2^{18}\text{O}$  are powerful tracers of processes occurring in nature. Their slightly different masses as compared to the most abundant water isotopologue ( $^1\text{H}_2^{16}\text{O}$ ) affect their thermodynamic (e.g. during chemical equilibrium reactions or physical phase transitions with equilibration) and kinetic (liquid and vapor phases transport processes and chemical reactions without equilibration) properties. This results in measurable differences of the isotopic composition of water within or between the different terrestrial ecosystem compartments (i.e. sub-soil, soil, surface waters, plant, and atmosphere). These differences can help addressing a number of issues, among them water balance closure and flux partitioning from the soil-plant-atmosphere continuum at the field to regional scales. In soils particularly, the isotopic composition of water ( $\delta^2\text{H}$  and  $\delta^{18}\text{O}$ ) provides qualitative information about whether water has only infiltrated or already been re-evaporated since the last rainfall event or about the location of the evaporation front. From water stable isotope composition profiles measured in soils, it is also possible, under certain hypotheses, to derive quantitative information such as soil evaporation flux and the identification of root water uptake depths. In addition, water stable isotopologues have been well implemented into physically based Soil–Vegetation–Atmosphere Transfer models (e.g. SiSPAT-Isotope; Soil–Litter iso; TOUGHREACT) and have demonstrated their potential. However, the main disadvantage of the isotope methodology is that, contrary to other soil state variables that can be monitored over long time periods,  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  are typically analyzed following destructive sampling. Here, we present a non-destructive method for monitoring soil liquid water  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  over a wide range of water availability conditions and temperatures by sampling and measuring water vapor equilibrated with soil water using gas-permeable polypropylene tubing and a cavity ring-down laser absorption spectrometer. By analyzing water vapor  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  sampled with the tubing from a fine sand for temperatures ranging between 8–24°C, we demonstrate that (i) our new method is capable of monitoring  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  in soils online with high precision and, after calibration, also with high accuracy, (ii) our sampling protocol enabled detecting changes of  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  following non-fractionating addition and removal of liquid water and water vapor of different isotopic compositions, and (iii) the time needed for the tubing to monitor these changes is compatible with the observed variations of  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  in soils under natural conditions.

## **SIMULTANEOUS ANALYSIS OF DISSOLVED ORGANIC CARBON (DOC) CONCENTRATION AND <sup>13</sup>C BY TOC-IRMS – ASSESSMENT OF ANALYTICAL PERFORMANCE**

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Stable carbon isotopes provide a powerful tool to assess carbon pools and their dynamics. Dissolved organic carbon (DOC) has been recognized to play an important role in ecosystem functioning and carbon cycling and has therefore gained increased research interest. However, direct measurement of <sup>13</sup>C isotopic signature of carbon in the dissolved phase is technically challenging particularly using high temperature combustion. Until recently, mainly custom-made systems existed which were modified for coupling of TOC instruments with IRMS for simultaneous assessment of C content and isotopic signature. The variety of coupled systems showed differences in their analytical performances. For analysis of DOC high temperature combustion is recognized as best performing method, owing to its high efficiency of conversion to CO<sub>2</sub> also for highly refractory components (e.g. humic, fulvic acids) present in DOC and soil extracts. Therefore, we tested high temperature combustion TOC coupled to IRMS (developed by Elementar Group) for bulk measurements of DOC concentration and <sup>13</sup>C signature. The instruments are coupled via an Interface to exchange the carrier gas from O<sub>2</sub> to He and to concentrate the derived CO<sub>2</sub> for the isotope measurement.

Analytical performance of the system was assessed for a variety of organic compounds characterized by different stability and complexity, including humic acid and DOM. We tested injection volumes between 0.2-3 ml, thereby enabling measurement of broad concentration ranges. With an injection volume of 0.5 ml (n=3, preceded by 1 discarded injection), DOC and <sup>13</sup>C signatures for concentrations between 5-150 mg C/L were analysed with high precision (standard deviation (SD) predominantly <0.1‰), good accuracy and linearity (overall SD <0.9‰). For the same settings, slightly higher variation in precision was observed among the lower concentration range and depending upon specific system conditions. Differences in <sup>13</sup>C signatures of about 50‰ among samples did not affect the precision of the analysis of natural abundance and labelled samples. Natural DOM, derived from different soils and assessed at various concentrations, was measured with similar good analytical performance, and also tested for the effect of freezing and re-dissolving.

We found good performance of TOC-IRMS in comparison with other systems capable of determining C concentration and isotopic signatures. We recognize the advantages of this system providing:

- High sample throughput, short measurement time (15 minutes), flexible sample volume
- Easy maintenance, handling, rapid sample preparation (no pre-treatment)

This preliminary assessment highlights wide-ranging opportunities for further research on concentrations and isotopic signatures by TOC-IRMS to elucidate the role of dissolved carbon in terrestrial and aquatic systems.

## RECENT ADVANCES IN LASER SPECTROSCOPIC ANALYSIS OF GHG STABLE ISOTOPES

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Isotope ratios of greenhouse gases (GHG) are excellent tools to study their sources and sinks, because the involved chemical and biochemical processes lead to characteristic isotopic fractionations. For example, the distinct CO<sub>2</sub> isotopic signatures of respiration, photosynthesis and fossil fuel burning can be used for the apportionment of CO<sub>2</sub> fluxes. Similarly, the analysis of CH<sub>4</sub> and CH<sub>3</sub>D allows to distinguish between natural gas and methane from landfills. Finally, the intramolecular distribution of <sup>15</sup>N in N<sub>2</sub>O is a valuable tracer to study bacterial nitrification and denitrification processes as well as abiotic N<sub>2</sub>O production in soils and aquatic systems, which are the main sources of atmospheric N<sub>2</sub>O.

The development of QCL based spectrometers and their ongoing improvement have led to high-precision instruments, suitable for environmental studies based on isotopic ratios, such as  $\delta^{13}\text{C-CO}_2$ ,  $\delta^{18}\text{O-CO}_2$ ,  $\delta^{15}\text{N}^\alpha\text{-N}_2\text{O}$ ,  $\delta^{15}\text{N}^\beta\text{-N}_2\text{O}$ ,  $\delta^{18}\text{O-N}_2\text{O}$  and  $\delta^{13}\text{C-CH}_4$ . To detect relevant signatures in ambient air, it is often necessary to reach a precision of 0.1 ‰. Although this is feasible, it requires great care in the procedures for sampling and calibration. Furthermore, trace gases with low atmospheric abundance, such as nitrous oxide, must be preconcentrated before their spectroscopic analysis. This has been achieved by developing an automated, liquid nitrogen free preconcentration unit and coupling it to the spectrometer. While instrumental improvements (including calibration and validation procedures) are ongoing, several studies already illustrate the real benefit of high precision, laser based isotopic GHG analysis in a range of environmentally and technically relevant topics.

## UNCERTAINTIES IN STABLE ISOTOPE ANALYSES DUE TO NORMALIZATION METHOD AND STANDARDS SELECTION

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The uncertainty inherent in stable isotope analyses depends not only on the technical aspects during the measurement process, but also on how “raw” data obtained from mass spectrometers are normalized to one of the international isotope scales (e.g., VSMOW, VPDB or VCDT). Stable isotope ratio mass spectrometers are highly precise but not accurate instruments; hence few calculation schemes for normalization have been developed world-wide. These normalization schemes encompass the selection of reference materials (and hence the  $\delta$ -values of these reference materials), the numbers of replicates, and the type of normalization technique applied (e.g., linear regression). Significantly, inconsistencies between laboratories can result simply from different normalization schemes, with the scheme employed critical in determining the final level of uncertainty in the results.

The accuracy of these various normalization methods has been compared by using both analytical laboratory data sets and Monte Carlo simulations (Skrzypek, 2013). Normalization methods using linear regression based on two or more certified reference standards have been identified as introducing the lowest uncertainty. This method produces a smaller normalization induced uncertainty whenever the reference materials are bracketing the whole range of isotopic composition of unknown samples. However, even when normalization based linear multi-point regression is used, the final normalization error still depends highly on the selection of reference materials. Furthermore, the uncertainties associated with the calibration of each of the reference materials influences the overall normalization uncertainty. We argue that the optimal selection of reference materials and an increase in the numbers of their replicates will significantly improve the overall uncertainty in analysed samples. The uncertainty can be reduced by 50% if measurements of two different standards, bracketing the whole range of  $\delta$ -values of natural variability, are performed four times, or measurements of four standards are performed twice, with each batch of samples (Skrzypek, 2013).

It is crucially important that the same set of standards should always be employed in a stable isotope analysis. A world-wide unified protocol for normalization procedures, including a clearly defined optimal set of reference materials, would improve significantly the inter-laboratory comparison of results from different laboratories. A research paper that presents stable isotope results should clearly report the normalisation technique that was used, what standards were used for normalization and the  $\delta$ -values that were obtained for those standards to ensure that all published data can be recalculated, if  $\delta$ -values of standards were to be updated.

Skrzypek G., 2013, Normalization procedures and reference material selection in stable HCNOS isotope analyses – an overview. *Analytical and Bioanalytical Chemistry* (DOI: 10.1007/s00216-012-6517-2).



## HOW CAN WE MEASURE MICROBIAL PROCESS RATES IN SOIL?

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Today, it is widely accepted, that depolymerisation of high molecular weight compounds in soils and sediments constitutes the rate-limiting step in the mineralization of organic carbon and nitrogen. Traditionally, these depolymerisation processes are estimated by measuring enzyme activities. While numerous methods exist to measure enzyme activities, they all have in common that only potential transformation rates are measured (artificial substrates, substrate saturation, optimal conditions, etc), not the actual rates that would be needed. I will argue here that such measurements are therefore reflecting the microbial community structure rather than microbial process rates. It is therefore clear that other methods and techniques are needed to estimate microbial transformation rates of organic matter in situ.

I will demonstrate that such methods already exist. Isotope pool dilution (IPD) methods can be used to measure actual (sometimes even in-situ) process rates. IPDs have been developed in the 1950s, but due to limitations in compound-specific isotope analysis and sensitivity they have largely been applied to mineralization processes, such as pool dilution of ammonium (N mineralization), nitrate (nitrification) and phosphate (P mineralization). Recently, however, IPDs have also been developed and applied for organic substances, such as amino acids, allowing to estimate protein depolymerisation rates or for glucose, allowing to determine glucan depolymerisation rates (Wanek et al. 2010; Leitner et al. 2012). I will review these methods and their advantages and disadvantages and discuss their potential application. Finally, I will try to make a strong case for developing more such methods that would allow entering a new area of soil process research.

Leitner, S., Wanek, W., Wild, B., Haemmerle, I., Kohl, L., Keiblinger, K.M., et al. (2012). Influence of litter chemistry and stoichiometry on glucan depolymerization during decomposition of beech (*Fagus sylvatica* L.) litter. *Soil Biology and Biochemistry*, 50, 174–187.

Wanek, W., Mooshammer, M., Blöchl, A., Hanreich, A. & Richter, A. (2010). Determination of gross rates of amino acid production and immobilization in decomposing leaf litter by a novel <sup>15</sup>N isotope pool dilution technique. *Soil Biology and Biochemistry*, 42, 1293–1302.



## ADVANCES IN INSTRUMENTATION BASED ON CAVITY ENHANCED LASER ABSORPTION SPECTROSCOPY

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Novel instrumentation based on high-resolution laser absorption spectroscopy now allows high precision measurements of isotopic ratios continuously in real time and without preconcentration. These analyzers from Los Gatos Research employ tunable lasers that operate in the near-infrared or the mid-infrared spectral regions and an optical cavity as a measurement cell. The basic operation of these instruments is quite simple. The laser wavelength is continuously and repetitively scanned over selected absorption features of target isotopologues to record high-resolution absorption lineshapes at data rates of 1 Hz or faster. The integrated areas of the measured lineshapes enable determination of the respective concentrations of the isotopologues from which the appropriate isotopic ratios are determined. No longer constrained to operate in a laboratory, these analyzers offer opportunities to record measurements in remote sites in less-developed areas. This presentation will summarize recent developments (in both hardware and software analysis) that enable measurements of isotopic ratios in carbon dioxide ( $\delta^{13}\text{C}$ ,  $\delta^{18}\text{O}$ ,  $\text{CO}_2$ ), methane ( $\delta^{13}\text{C}$ ,  $\text{CH}_4$ ), nitrous oxide ( $\delta^{15}\text{N}^\alpha$ ,  $\delta^{15}\text{N}^\beta$ ,  $\delta^{18}\text{O}$ ,  $\text{N}_2\text{O}$ ) and water (liquid and vapor;  $\delta^2\text{H}$ ,  $\delta^{17}\text{O}$ ,  $\delta^{18}\text{O}$ ,  $\text{H}_2\text{O}$ ) in ambient air and in complex sample streams. An overview of the current performance of LGR instruments and perspectives on future developments will be presented.

## QUANTIFYING THE RELATIVE CONTRIBUTION OF NATURAL GAS FUGITIVE EMISSIONS TO TOTAL METHANE EMISSIONS IN COLORADO, UTAH, AND TEXAS USING MOBILE $\delta^{13}\text{CH}_4$ ANALYSIS

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Fugitive emissions of methane into the atmosphere are a major concern facing the natural gas production industry. Because methane is more energy-rich than coal per kg of  $\text{CO}_2$  emitted into the atmosphere, it represents an attractive alternative to coal for electricity generation, provided that the fugitive emissions of methane are kept under control. A key step in assessing these emissions is partitioning the observed methane emissions between natural gas fugitive emissions and other sources of methane, such as from landfills or agricultural activities. One effective method for assessing the contribution of these different sources is stable isotope analysis, using the  $\delta^{13}\text{CH}_4$  signature to distinguish between natural gas and landfills or ruminants. Cavity Ringdown Spectroscopy (CRDS) has the potential to be a very useful technique for these measurements. Fast and rugged enough to be used on a mobile platform, yet sensitive enough to provide discriminating isotopic information.

We present measurements of mobile field  $\delta^{13}\text{CH}_4$  using a spectroscopic stable isotope analyzer based on CRDS, in three intense natural gas producing regions of the United States: the Denver-Julesburg basin in Colorado, the Uintah basin in Utah, and the Barnett Shale in Texas.

Mobile isotope measurements of individual sources and in the nocturnal boundary layer have been combined to establish the fraction of the observed methane emissions that can be attributed to natural gas activities. The fraction of total methane emissions in the Denver-Julesburg basin attributed to natural gas emissions is 78 +/- 13%. In the Uinta basin, which has no other significant sources of methane, the fraction is 96% +/- 15%. In addition, preliminary results in the Barnett shale, which includes a major urban center (Dallas), are presented.

## RECENT PROGRESS IN DEVELOPMENT OF INFRARED LASER BASED INSTRUMENTS FOR REAL-TIME AMBIENT MEASUREMENTS OF ISOTOPOLOGUES OF CARBON DIOXIDE, WATER, METHANE, NITROUS OXIDE AND CARBON MONOXIDE

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The capacity for real-time precise in-situ measurements of isotopic ratios of a variety of trace gases at ambient concentrations continues to create new opportunities for the study of the exchanges and fluxes of gases in the environment. Aerodyne Research, Inc. has made rapid progress in laser based instruments since our introduction in 2007 of the first truly field worthy instrument for real-time measurements of isotopologues of carbon dioxide. We have focused on two instrument design platforms, with either one or two lasers. Absorption cells with more than 200 meters path-length allow precise measurements of trace gases with low ambient concentrations. Thermoelectrically cooled detectors allow completely non-cryogenic operation. Most of our systems employ mid-infrared quantum cascade lasers. The recently available 3 micron antimonide-based diode lasers also are proving useful for isotopic measurements. By substituting different lasers and detectors, we can simultaneously measure the isotopic composition of a variety of gases, including: H<sub>2</sub>O, CO<sub>2</sub>, CH<sub>4</sub>, N<sub>2</sub>O and CO.

Our newest instrument for true-simultaneous measurement of isotopologues of CO<sub>2</sub> (<sup>12</sup>CO<sub>2</sub>, <sup>13</sup>CO<sub>2</sub>, <sup>12</sup>C<sup>18</sup>O<sup>16</sup>O) has (1 s) precision of 0.1 ‰ or better for both ratios. The availability of 10 Hz measurements allows measurement of isotopic fluxes via eddy correlation. The single-laser instrument fits in a 19 inch rack and is only 25 cm tall. A two-laser instrument is larger, but with that instrument we can also measure clumped isotopes of CO<sub>2</sub>, with 1 second precisions of: 2.3 ‰ for <sup>13</sup>C<sup>18</sup>O<sup>16</sup>O, and 6.7 ‰ for <sup>13</sup>C<sup>17</sup>O<sup>16</sup>O. The sample size for such a measurement corresponds to 0.2 μmole of pure CO<sub>2</sub>. Another variation on the two-laser instrument simultaneously measures isotopologues of CO<sub>2</sub> (<sup>12</sup>CO<sub>2</sub>, <sup>13</sup>CO<sub>2</sub>, <sup>12</sup>C<sup>18</sup>O<sup>16</sup>O) and H<sub>2</sub>O (H<sub>2</sub><sup>16</sup>O, H<sub>2</sub><sup>18</sup>O, HD<sup>16</sup>O). Preliminary results for water ratio precisions (in 1s) are 0.1 ‰ for H<sub>2</sub><sup>18</sup>O and 0.3 ‰ for HD<sup>16</sup>O, simultaneous (1 s) precisions for isotopologues of CO<sub>2</sub> of ~0.1 ‰.

Several trace gases, such as methane, nitrous oxide and carbon monoxide, have such low ambient concentrations that real-time isotopologue measurements are a serious challenge. For these gases, we typically use our 200 m absorption cell. One of these instruments has already been used for long-term field measurements of isotopologues of methane, (<sup>12</sup>CH<sub>4</sub>, <sup>13</sup>CH<sub>4</sub>), with a demonstrated (1 s) precision of 1.5 ‰. A similar instrument platform configured for measuring ambient <sup>13</sup>CO demonstrated (1 s) precision of 1.9 ‰. We also have configured this platform for measurement of isotopologues of N<sub>2</sub>O (<sup>14</sup>N<sub>2</sub><sup>16</sup>O, <sup>14</sup>N<sup>15</sup>N<sup>16</sup>O, <sup>15</sup>N<sup>14</sup>N<sup>16</sup>O, <sup>14</sup>N<sub>2</sub><sup>18</sup>O), with demonstrated (1 s) precision at ambient levels (320 ppb) of ~3 ‰. For N<sub>2</sub>O, a quasi-continuous pre-concentrator has been used to give even better precisions (<0.1‰).

## Progress in interpretation and modeling of atmospheric $\delta^{13}\text{CO}_2$

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The promise of utilizing large-scale atmospheric  $\delta^{13}\text{CO}_2$  measurements to understand terrestrial processes has not been fully realized. Here, we will present recent progress in the use of measurements and simulations of atmospheric  $\delta^{13}\text{C}$  to better understand the signals of the biosphere contained within atmospheric data. The motivation for atmospheric  $\delta^{13}\text{C}$  data over the past 20 years has been as tracer that would help partition global surface carbon fluxes into terrestrial and oceanic components. While this is still possible, it is probably not the most effective use of  $\delta^{13}\text{C}$ . First, the uncertainty in isotopic disequilibrium and its inter-annual variability makes most land/ocean partitioning uncertain. Second, the presence of a greatly increased density of terrestrial  $\text{CO}_2$  observations in the Northern Hemisphere obviates this application of  $\delta^{13}\text{C}$  to a large extent. What this suggests, however, is that in a system in which land/ocean uptake is already well constrained,  $\delta^{13}\text{C}$  can constrain terrestrial biosphere parameters.

At the global scale, mass balance of total C and  $^{13}\text{C}$  isofluxes (as constrained by atmospheric growth rates) has shown that interannual variability in terrestrial isofluxes may be underestimated. We will present these results and contrasting results from a state-of-the-art bottom-up biosphere model that can not produce the variability required by the atmosphere. At regional scales ( $10^5 - 10^6 \text{ km}^2$ ), analysis of atmospheric  $\text{CO}_2$  and  $\delta^{13}\text{C}$  correlations suggests seasonality in photosynthetic discrimination and stomatal conductance that is different than in models. Other regional analyses of  $\text{CO}_2$  and  $\delta^{13}\text{C}$  correlations show changes in C4 and C3 productivity. The success of these data analyses have led to an effort to use atmospheric  $\delta^{13}\text{C}$  and  $\text{CO}_2$  data as formal constraints in an atmospheric inversion, the goal of which is to calculate photosynthetic discrimination on a regional scale in North America. We will present preliminary results of this study that show the ability of a dense network of  $\text{CO}_2$  and  $\delta^{13}\text{C}$  observations to constrain C3:C4 distribution and conductance-driven variations in C3 discrimination.

# A process-based interpretation of the stable C and O isotope signals in the soil-plant-atmosphere continuum using the ecosystem model MuSICA

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## Abstract

Carbon and oxygen stable isotope measurements are often performed in the field and at natural abundance in order to bring additional information on key variables regulating the CO<sub>2</sub> and water cycles within terrestrial ecosystems (e.g. plant water-use efficiency, root water uptake depth...). For some applications, simple two-pool mixing models are enough to interpret such measurements. However, when more than two pools are considered, process-based isotope-enabled soil-plant-atmosphere transfer models are often required for unambiguous data interpretation. Here we will present several examples of such cases using the multi-layer, multi-leaf isotope-enabled model MuSICA (Ogée *et al.* 2003a; 2003b). We will illustrate how such a model can be used (1) to infer the representativeness of field chamber measurements on single branches (e.g. Wingate *et al.* 2010b), (2) to quantify the CO<sub>2</sub>-H<sub>2</sub>O oxygen isotope exchange rates below soil chambers (e.g. Wingate *et al.* 2010a) or (3) to interpret evaporation lines in the field.

## References

- Ogée J, Brunet Y, Loustau D, Berbigier P, Delzon S (2003a) MuSICA, a CO<sub>2</sub>, water and energy multilayer, multileaf pine forest model: evaluation from hourly to yearly time scales and sensitivity analysis. *Global Change Biology*, **9**, 697–717.
- Ogée J, Peylin P, Ciais P, et al. (2003b) Partitioning net ecosystem carbon exchange into net assimilation and respiration using <sup>13</sup>CO<sub>2</sub> measurements: a cost-effective sampling strategy. *Global Biogeochemical Cycles*, **17**, 1070.
- Wingate L, Ogée J, Burlett R, Bosc A (2010a) Strong seasonal disequilibrium measured between the oxygen isotope signals of leaf and soil CO<sub>2</sub> exchange. *Global Change Biology*, **16**, 3048–3064.
- Wingate L, Ogée J, Burlett R, et al. (2010b) Photosynthetic carbon isotope discrimination and its relationship to the carbon isotope signals of stem, soil and ecosystem respiration. *The New phytologist*, **188**, 576–589.

## DIURNAL CHANGES OF CARBON BUDGET IN THE URBAN ATMOSPHERE OF KRAKOW, SOUTHERN POLAND

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Emissions of carbon dioxide related to burning of fossil fuels constitute an important component of atmospheric carbon budget. For heavily industrialized and populated regions such as western and central Europe, a large proportion of the total CO<sub>2</sub> flux entering the atmosphere is attributed to this source. However, the measurements of atmospheric CO<sub>2</sub> mixing ratios alone are not sufficient for apportionment of fossil-fuel related and biogenic contributions to the total CO<sub>2</sub> burden of the regional or local atmosphere. Such apportionment is required for independent verification of anthropogenic fluxes of carbon dioxide assessed on the basis of CO<sub>2</sub> emission statistics.

Krakow (50°04'N, 19°55'E, 220 m a.s.l.) is a large urban agglomeration located in the southern Poland, with about 1 million inhabitants, rapidly growing car traffic and significant industrial activities. Consumption of coal, gas and oil for communal and transport purposes generates substantial fluxes of anthropogenic carbon dioxide and carbon monoxide within the region. In addition, due to prevailing westerly air circulation, the Krakow region is under substantial influence of a large coal mining and industrial centre (Upper Silesia) located approximately 60 km west of the city.

Here we present four examples of CO<sub>2</sub> budgeting in the urban atmosphere of Krakow, performed on daily time scale. Combined measurements of CO<sub>2</sub> concentration and its isotopic composition ( $\Delta^{14}\text{C}$ ,  $\delta^{13}\text{C}$ ,  $\delta^{18}\text{O}$ ) were used as a tool allowing quantification of the local CO<sub>2</sub> budget. In addition, two potential proxies of fossil-fuel CO<sub>2</sub> emissions (carbon monoxide and polycyclic aromatic hydrocarbons) were tested as substitutes of radiocarbon in carbon balance calculations. The following measurements were performed during each analysed pollution event: (i) quasi-continuous measurements of CO<sub>2</sub> and CO mixing ratios, supplemented by continuous measurements of PAH (polycyclic aromatic hydrocarbons), (ii) CO<sub>2</sub> and CO concentration as well as  $\Delta^{14}\text{C}$ ,  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  of CO<sub>2</sub> in several samples of air collected during each analysed event, (iii) meteorological parameters (air temperature, pressure, wind speed and wind direction). With the mass and isotope balance of CO<sub>2</sub> solved for each sampling interval it was possible to assess diurnal variations of anthropogenic and biogenic CO<sub>2</sub> components in the local atmospheric CO<sub>2</sub> and to separate the anthropogenic component into the fraction originating from burning of coal and oil and that originating from burning of methane. The analysed events have demonstrated the importance of the biogenic CO<sub>2</sub> emissions for the local carbon budget during summer. The tested fossil-fuel CO<sub>2</sub> proxies (CO, PAH) allowed apportionment of CO<sub>2</sub> load in the local atmosphere with sub-hourly resolution.

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# Leaf wax lipid $\delta D$ values as proxies for past climatic change and tracers of large-scale biogeochemical cycles

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The cuticle of leaves is a waxy layer that contains lipids that can accumulate and persist in sediments and serve as biomarkers for past climates or biogeochemical processes. Most importantly, the hydrogen isotope composition ( $\delta D$ ) of leaf wax lipids has been suggested to contain powerful paleohydrological and biogeochemical information that could be highly useful for applications in paleoclimatology, earth-and ecosystem sciences and plant ecophysiology. While original research has suggested that leaf wax lipid  $\delta D$  values reflect the  $\delta D$  values of precipitation, we were able to show over the past years that additional plant physiological processes, such as leaf water evaporative enrichment in deuterium, timing of leaf wax synthesis and species-specific biosynthetic fractionation also influence the  $\delta D$  values of leaf wax lipids. By resolving these additional plant physiological drivers of leaf wax lipid  $\delta D$  values it now possible to interpret the  $\delta D$  values of leaf wax lipids with increased accuracy and to employ the  $\delta D$  values of leaf wax lipids to address new paleohydrological and large-scale biogeochemical research questions.

In our presentation, we will provide a brief overview over the key plant physiological processes that determine the  $\delta D$  values of leaf wax lipids by highlighting some of our latest research. We will then give an example how the improved mechanistic understanding of leaf wax lipid  $\delta D$  values can be used to accurately resolve past abrupt changes in the European hydrological cycle. Finally, we will give an outlook on new applications, where we will use leaf wax lipid  $\delta D$  values to trace the lateral flow of organic carbon across the landscape and from terrestrial ecosystems to the ocean.

# INVESTIGATING HIGH-RESOLUTION STABLE ISOTOPE SIGNALS OF ANNUAL TREE RINGS ACROSS A MOISTURE GRADIENT WITH THE MUSICA MODEL

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## Abstract

Seasonal variations in annual precipitation have recently been shown to dominate the mean intra-annual variation in the carbon isotope composition of evergreen wood ( $\delta^{13}\text{C}_{\text{cellulose}}$ ) across a range of biomes (Schubert & Jahren, 2011). When such strong relationships are preserved in plant cellulose it may also be possible to spatially reconstruct rainfall patterns in detail over large areas and back in time using empirical relationships (del Castillo *et al.*, 2013). Using a new high-resolution carbon and oxygen isotope dataset of cellulose, we investigated this relationship further. At five FLUXNET sites spanning a significant gradient of moisture (MAP range of 280 to 930mm) and temperature (MAT range of -1 to 18°C), we collected tree core samples and extracted cellulose at high resolution. For each site, we explored whether the inter-annual variability of seasonal precipitation over the past 15 years dominated the inter-annual variations in the amplitude of  $\delta^{13}\text{C}_{\text{cellulose}}$ . Using the process-based model MuSICA (Ogée *et al.*, 2009), that links  $\delta^{13}\text{C}_{\text{cellulose}}$  and  $\delta^{18}\text{O}_{\text{cellulose}}$  signals in tree rings to environmental conditions, we explored the sensitivity of cellulose isotope signals to light, temperature and growing season length as these drivers may gain importance when trees grow without moisture limitations across large temperate regions.

## References

- Ogée, J., Barbour, M.M., Wingate, L., Bert, D., Bosc, A., Stievenard, M., Lambrot, C., Pierre, M., Berbigier, P., Bariac, T., Loustau, D. and Dewar, R. (2009). A single-substrate model to interpret intra-annual stable isotope signals in tree ring cellulose. *Plant, Cell and Environment*, Vol. 32, 8, 1071-1090, doi:10.1111/j.1365-3040.2009.01989.x
- Schubert, B.A. & Jahren, A.H., 2011. Quantifying seasonal precipitation using high-resolution carbon isotope analyses in evergreen wood. *Geochimica et Cosmochimica Acta* 75, 7291-7303.
- Del Castillo J, Aguilera M, Voltas J, Ferrio JP (2013) Isoscapes of tree-ring carbon-13 perform like meteorological networks in predicting regional precipitation patterns. *Journal of Geophysical Research - Biogeosciences*, doi: 10.1002/jgrg.20036.



## THE USE OF RADIOCARBON TO CONSTRAIN ORGANIC MATTER TURNOVER AND VERTICAL TRANSPORT IN A DECIDUOUS FOREST UNDER CURRENT AND FUTURE CONDITIONS

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Since soil organic matter (SOM) comprises a mixture of fractions of different qualities, it is usually represented in soil carbon models as several pools with different decomposition rates. Since each additional pool requires at least two extra parameters, measurements of carbon stocks and fractions are in general not sufficient to estimate all turnover rates of a multi-pool SOC model. Radiocarbon measurements have been proven valuable to constrain SOM turnover rates and parameters defining the flow between pools. When such measurements are performed for several levels within the vertical profile, they usually show that average turnover rates decrease with depth. This may be explained by the combined effects of vertical SOM transport and selective preservation of recalcitrant fractions. This suggests that, when combined with a vertically explicit model, the information contained by multiple measurements along the profile may provide a better constraint on decomposition parameters. On the other hand, since vertical SOM transport rates are poorly known, they need to be included in the parameter estimation, partially negating improved constraint on decomposition rates. We studied the use of radiocarbon measurements of SOM and heterotrophic respiration, in addition to other data, to constrain turnover and transport rates for a temperate deciduous forest in Germany. Eleven model parameters were estimated for the soil carbon model SOMPROF, which includes descriptions of vertical transport by bioturbation and leaching. The calibration was performed in a Bayesian framework, allowing inclusion of prior knowledge and quantification of posterior uncertainty.

Addition of radiocarbon in the calibration reduced uncertainty for most, but not all, model parameters. Furthermore, radiocarbon had strong effects on the parameters of the slowest organic matter pool, for which the turnover rate was reduced by an order of magnitude compared to a calibration without this data. We additionally studied how the parameter values and their uncertainty propagate to predictions of future soil carbon cycling under conditions of climate change. This was done by running an ensemble of simulations based on litter fluxes and soil climate calculated by a land surface model. Surprisingly, the results show that the spread of predicted carbon stocks *increases* when radiocarbon is included in the calibration. However, the estimated turnover rates in the deep soil, as well as organic matter leaching rates are improved compared to observations. Clearly, a more realistic description of soil carbon cycling has come at the cost of increased predictive uncertainty.

## INCOMPLETE INFORMATION IN ISOTOPIC INTERPRETATIONS

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Carbon dioxide CO<sub>2</sub> exhibits its lowest concentrations in atmospheric background air in August on the Northern hemisphere. This coincides with the warmest month, the largest plant productivity, and the greatest vertical mixing on land. But longitudinal mixing is lowest in summer because of low wind speeds. The oxygen isotope composition of atmospheric carbon dioxide <sup>18</sup>O, on the other hand, displays its lowest values about two months later. This shift is not understood. All existing models of <sup>18</sup>O in atmospheric CO<sub>2</sub> have both minima at the same time, i.e. CO<sub>2</sub> and <sup>18</sup>O show similar seasonal cycles. It was argued that the timing of soil water isotopes was ill-defined in the models. It turns out that not only <sup>18</sup>O in background atmospheric CO<sub>2</sub> has its minimum in October on the Northern hemisphere but also carbonyl sulphide COS as well as molecular hydrogen H<sub>2</sub>. COS is strongly related to stomatal conductance and hence plant production. Earlier studies had to at least double the assumed biospheric sink in the models in order to match observations. But H<sub>2</sub> on the other side is almost independent of plant productivity. Its largest sink is soil uptake and its largest production is by photochemistry. Atmospheric inversion studies had to change their a priori soil sink by a factor of two in order to match observations. So three atmospheric tracers show a similar seasonal behaviour but three contrasting explanations are given to solve the model-data mismatch.

The seasonal cycle of atmospheric <sup>18</sup>O in CO<sub>2</sub> will be just one of the examples highlighting deficiencies in our understanding of isotopes in the water and carbon cycles. Some of the shortcomings in the isotopic descriptions are real conundrums and will need brand new ideas to solve them. Others may be solved simply by more complex modelling. However, I will try to estimate potential errors that are introduced, for example in estimated carbon and water fluxes, if such inadequacies are ignored.

Abstract for SIBAE

Tracing the bomb  $^{14}\text{C}$  pulse label in plant respiration

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The radiocarbon signature of atmospheric  $\text{CO}_2$  in recent decades has been dominated by the distribution of  $^{14}\text{C}$  produced in atmospheric weapons testing, which peaked in the early 1960s. The long-term and well documented decline in  $^{14}\text{C}$  since that time allows for tracing of photosynthetic products that can reside in trees from years to decades. Nonstructural carbohydrates in mature trees demonstrate radiocarbon signatures indicating the C can persist over these timescales. This is demonstrated by its use as a source of C for resprouts, and further as a contributor to  $\text{CO}_2$  respired from tree stems and roots. While normally it is thought that older C reserves would be a source of 'emergency' carbon, our results indicate that this C is also used to fuel respiration under unstressed conditions. Further research to link radiocarbon signatures of sugars and starch with tissue respiration is needed.

## THE FATE OF CARBON IN TREES: TRANSFER RATES AND RESIDENCE TIMES

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Pulse-labelling of trees with stable carbon isotope (<sup>13</sup>C) offers the opportunity to trace the fate of labelled CO<sub>2</sub> into the tree and its release to the soil and the atmosphere. Based on several studies we conducted in France, Japan and Brazil we summarized results from pulse-labelling experiments on several tree species (oak, beech, pine and eucalypt). We will specifically address how fast the assimilated C is transferred belowground, how the rate of C transfer differs among species, how this transfer rate responds to environmental factors and how it changes seasonally. Our current knowledge on residence times in several tree and forest compartments will also be investigated.

Labelled CO<sub>2</sub> recovery was delayed relative to the labelling pulse from a few hours or days, depending on the distance between the source leaves and the observed sink, and on the rate of transport of C within the plant and into the soil. The rate of C transfer was calculated from the time lag between labelling time and the recovery of the label in the CO<sub>2</sub> efflux, or from differences in time lags between the start of the labelling and the appearance of <sup>13</sup>C in CO<sub>2</sub> efflux measured at different positions along the trunk. It differed between broadleaved (0.2-1.2 m/h in oak, beech and eucalypt) and coniferous species (0.10-0.2m/h in pine), reflecting difference in phloem anatomy between angiosperm and gymnosperm, and decreased as temperature and soil water content decreased. Labelled C was transferred belowground within a few days or less and this transfer was slowed down by drought, potassium deficiency and low temperature.

Half-lives of labelled C in phloem sap (transfer pool) and in foliage (source organs) changed with season but were short compared to those of sink organs such as growing leaves or storage compartments. <sup>13</sup>C measurements in respiratory efflux at high temporal resolution enabled us to estimate of the mean residence times of C in respiratory substrate pools, using compartmental modelling. The recovery patterns of labelled C in trunk and soil CO<sub>2</sub> efflux highlights the existence of a rapid cycling, metabolically active C pool with half-lives of a few days and much slower cycling pools of stored C with half-lives of several weeks.

## **<sup>13</sup>C AS A TRACER FOR RESPIRED CO<sub>2</sub> TRANSPORT IN THE XYLEM AT LEAF, BRANCH AND TREE LEVEL**

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Stable-isotope-labeling techniques have successfully been applied in the context of tree carbon cycling in order to trace the flow of photosynthates from leaves to sinks via the phloem. For instance, pulse-labeling of the tree canopy and analyzing soil CO<sub>2</sub> efflux have revealed the fast coupling between leaf-level and belowground processes. However, important knowledge gaps still exist on carbon cycling at the tree level regarding the upward transfer of CO<sub>2</sub> with the transpiration stream. In trees, respired CO<sub>2</sub> can dissolve in xylem water and diffuse to the atmosphere or be assimilated within tissues remote from the site of respiration. Therefore this internal carbon flux compels us to rethink long-standing assumptions regarding tree physiology (Trumbore et al., 2013) in which the use of stable isotopes might lead the way.

In this study, we summarize the findings from our recent studies at leaf, branch and tree level to illustrate the use of dissolved <sup>13</sup>CO<sub>2</sub> as a tracer for xylem-transported CO<sub>2</sub> within trees. A <sup>13</sup>C label was dissolved in water and either infused in the stem base of poplar trees (*Populus deltoides* Bartr. Ex. Marsh) in the field or supplied at detached poplar branches and leaves under controlled conditions, allowing manipulation of the transpiration rate by altering the vapor pressure deficit (VPD). The experiment on the field-grown trees showed that the label was transported from the base of the stem throughout the tree and assimilated in the different woody and leaf tissues. The largest fraction of the label (up to 94%) diffused to the atmosphere during upward transport. In the branch and the leaf experiments, higher tissue enrichment was observed under high VPD compared to low VPD, illustrating the importance of transpiration rate on assimilation of xylem-transported CO<sub>2</sub>. The amount of xylem-transported CO<sub>2</sub> assimilated in the different tissues was low compared to atmospheric fixation (up to 1.9%), however this “recycling” of respired CO<sub>2</sub> might gain importance at higher VPD levels when atmospheric CO<sub>2</sub> assimilation might be limited and transpiration rate maximizes to a plateau level.

Our findings indicate that labeling the transpiration stream with carbon isotopes can provide novel insights on the impact of xylem-transported CO<sub>2</sub> on tree assimilation and respiration. Accurate quantification of tree metabolism is crucial, in particular with regard to understanding dynamics in forest carbon budgets due to climate change. In addition, infusing stable isotopes in the transpiration stream of trees could be an alternative for canopy labeling experiments of field-grown trees, which are often more cumbersome and expensive.

### Reference:

Trumbore SE, Angert A, Kunert N, Muhr J, Chambers JQ. 2013. What's the flux? Unraveling how CO<sub>2</sub> fluxes from trees reflect underlying physiological processes. *New Phytologist* 197: 353-355.

## USING $^{13}\text{C}$ LABELING TO ANALYZE CARBON FLUXES *IN SITU*

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The controls operating on carbon fluxes belowground are most relevant for understanding the long-term functioning of agroecosystems. Yet there is a dearth of data on actual measurements under field conditions. Here, we present a system for continuous, steady-state  $^{13}\text{C}$ -labeling of  $\text{CO}_2$  assimilation, and demonstrate its use in grasslands to quantify (i) gross assimilation, (ii) carbon allocation, and (iii) above- and belowground primary productivity. The labeling apparatus generates  $\text{CO}_2$ -free air, mixes it with pure  $\text{CO}_2$  of known isotopic composition ( $\delta^{13}\text{C}$ ), and distributes it to transparent chambers enclosing the stands to be labeled. The facility is able to provide a labeling atmosphere with constant  $\text{CO}_2$  partial pressure and constant  $^{13}\text{CO}_2$ -enrichment, while at the same time preventing condensation and keeping the temperature inside chambers within  $-1^\circ\text{C}/+3^\circ\text{C}$  of ambient air. In principle, it is possible to label a stand continuously over a complete growing season. The whole facility is mobile, as it is mounted on a trailer towed by a truck. Gross daily carbon assimilation is estimated as the amount of tracer present in individual plants immediately after 6 to 7 hours of continuous labeling. The allocation of carbon to aboveground growth is estimated from the proportion of tracer remaining in shoots 100 growing degree days after the pulse. Above- and belowground primary productivity is estimated as the rate of tracer accumulation in these compartments over a two-weeks period of continuous labeling. In different experiments, we found that (i) daily gross assimilation is strongly influenced by the size of individuals in tall canopies, (ii) the fraction of assimilated carbon allocated to aboveground growth shows substantial plasticity, depending largely on plant nitrogen status, and also important interspecific differences, and (iii) the partitioning of carbon between above- and belowground growth is affected by season (autumn vs. spring). We believe that these results show that analyses of plant competition and species coexistence in heterogeneous stands could benefit from *in situ* measurements of carbon capture and allocation. Further, the possibility of measuring *in situ* the rate at which roots grow in a field community, and thus of estimating the rate of turnover of root systems, would benefit the study of the link between vegetation dynamics and soil organic matter.

# ELEVATED AND LOW CO<sub>2</sub> CONCENTRATION EFFECTS ON OLD AND MODERN DURUM WHEAT VARIETIES USING <sup>13</sup>C AND <sup>15</sup>N LABELLING

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Responses of plants to elevated CO<sub>2</sub> remain a critical uncertainty in global changes studies. For this reason, researchers are guiding efforts to study how plants are adapted to elevated CO<sub>2</sub>. Moreover, an increased understanding of plant responses to low CO<sub>2</sub> contributes to our knowledge of how natural global change factor in the past may continue to influence plant responses to future anthropogenic changes.

This study will help us to understand how the plants have been behaved in the past and how they will adapt to future environmental changes. It was conducted during 2011-2012 at the Serveis de Camps Experimentals, Universitat de Barcelona, using two varieties of wheat, one of them modern (*Triticum turgidum* var. Sula) and the other one old (*Triticum turgidum* var. Blanqueta) The plants were grown in three different CO<sub>2</sub> levels: future climatic conditions, high CO<sub>2</sub> (ca. 731,7 ± 6,9 ppm), or preindustrial climatic conditions, low CO<sub>2</sub> (ca. 249,4 ± 13,6 ppm), compared with actual conditions (ca. 409,3 ± 2,5 ppm). In this study, we conducted a simultaneous double labelling with <sup>13</sup>CO<sub>2</sub> and <sup>15</sup>NH<sub>4</sub>-<sup>15</sup>NO<sub>3</sub> (5% enriched in solution) in order to characterize C and N management during anthesis of the plant. Samples of different part of the plant were collected to study the δ<sup>13</sup>C and δ<sup>15</sup>N in Total Organic Matter (TOM) by EA-IRMS. Samples of air respired CO<sub>2</sub> of different tissues of plants (flag leaf, spike, leaves, stem and root) were collected after the labelling and CO<sub>2</sub> concentration and δ<sup>13</sup>C were analysed by GC-C-IRMS.

Blanqueta variety have similar photosynthetic assimilation rate (23,8±2,1) than Sula variety (23,3±14,3). Blanqueta has higher total biomass, more length and number of stems and spikes than Sula, but Sula has bigger spike numbers, and is more productive than Blanqueta. Blanqueta show more δ<sup>13</sup>C in TOM in all the treatments than Sula before and after the labelling and in all the different organs of the plant. The δ<sup>13</sup>C in TOM between CO<sub>2</sub> treatments was higher in low CO<sub>2</sub> than in Current or Future CO<sub>2</sub> conditions. Differences between organs were founded and the new <sup>13</sup>C is sent from photosynthetic organs to reproductive one and other sink organs. The δ<sup>13</sup>CO<sub>2</sub>\_Respired by plants is higher in Blanqueta than in Sula, but also it is higher in past CO<sub>2</sub> condition than in current and future condition. No big differences in δ<sup>15</sup>N for total organic matter (TOM) between different treatments of CO<sub>2</sub> were found. However Blanqueta was more <sup>15</sup>N enriched than Sula.

Our results showed that the plants in past conditions have less discrimination against <sup>13</sup>C than in future conditions because the availability of CO<sub>2</sub> in the environment was lower. The old variety is more <sup>13</sup>C enrich than actual variety.

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## ***IN-SITU* <sup>13</sup>CO<sub>2</sub> PULSE-LABELING IN A TEMPERATE HEATHLAND – DEVELOPMENT OF A MOBILE MULTI-PLOT FIELD SETUP**

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Pulse-labeling with <sup>13</sup>CO<sub>2</sub> and the subsequent analysis of <sup>13</sup>C-carbon via isotope ratio mass spectrometry (IRMS) has been shown to be an excellent method to investigate the terrestrial carbon cycle. The improvement of <sup>13</sup>CO<sub>2</sub> manipulation experiments will facilitate our understanding of carbon cycling processes.

A mobile field setup for *in-situ* <sup>13</sup>CO<sub>2</sub> pulse-labeling was developed for low vegetation field experiments. Two pulse-labeling experiments were conducted in September 2010 (Exp1) and May 2011 (Exp2) in a Danish heathland. A flow-through system was developed where labeling chambers were supplied with <sup>13</sup>CO<sub>2</sub>-enriched air from a gas reservoir. Reservoir and chamber air was sampled over the course of the experiments and analyzed for CO<sub>2</sub> concentration and isotopic composition on a GasBench II interfaced with an IRMS. Soil CO<sub>2</sub> efflux and the atom% excess in soil respiration was assessed after the <sup>13</sup>CO<sub>2</sub>-pulse to verify the setup performance.

Carbon dioxide concentrations and <sup>13</sup>CO<sub>2</sub> enrichments were stable during the experiments. The CO<sub>2</sub> concentrations conformed to the aimed values, whereas the <sup>13</sup>CO<sub>2</sub> enrichments were lower than expected. We discuss sources of error for the deviation in observed atom% <sup>13</sup>CO<sub>2</sub> values, and suggest a measurement procedure for samples highly enriched in <sup>13</sup>C by using adjusted resistor settings of the IRMS. However, more work has to be done. Enrichment patterns in soil respiration agree with published observations indicating satisfactory performance of the developed system. We can conclude that our developed mobile flow-through system was suitable for continuous *in-situ* <sup>13</sup>CO<sub>2</sub> pulse-labeling that is easily applicable in remote natural ecosystems.



## CARBON RESIDENCE TIME IN THE ABOVE-GROUND BIOMASS OF A TEMPERATE PASTURE AS RELATED TO LEAF LIFE SPAN

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The residence time of carbon in biomass of grassland ecosystems is an important factor in the terrestrial carbon cycle. Here we present a study of the mean residence time of carbon ( $MRT$ ) in bulk, structural and non-structural biomass ( $MRT_{\text{bulk}}$ ,  $MRT_{\text{structural}}$ ,  $MRT_{\text{non-structural}}$ ), and the relationship between  $MRT_{\text{structural}}$  and leaf life span. The work was performed on a pasture at the Grünschwaige Grassland Research Station and comprised three 16 days-long  $^{13}\text{CO}_2/^{12}\text{CO}_2$  labelling experiments. These took place in autumn 2006, spring 2007 and autumn 2007, and used the open top chamber system described by Gamnitzer et al. (2009, 2011). Above-ground biomass of four dominant species, two grasses (*Lolium perenne* and *Poa pratensis*) and two dicots (*Taraxacum officinale* and *Trifolium repens*), and bulk community was sampled at intervals. Leaf life span data were taken from Schleip et al. (2013). Species and community biomass samples were analysed for  $^{13}\text{C}$ .  $MRT_{\text{non-structural}}$  and  $MRT_{\text{structural}}$  were estimated by fitting a two-pool model to the time-course of  $^{13}\text{C}$  tracer content. In general, the  $MRT_{\text{structural}}$  of a species (21 to 38 days) was  $\sim 10$  days shorter than its leaf life span.  $MRT_{\text{non-structural}}$  of species' and bulk above-ground biomass was 4 to 6 days.  $MRT_{\text{structural}}$  did not differ systematically between species, between species and community, or among seasons or years. In conclusion, C cycled rapidly through the living aboveground biomass of this intensively grazed grassland ecosystem. Rapid cycling was related to the short leaf life span of this strongly disturbed biome.

### References

- Schleip I, Lattanzi FA, Schnyder H (2013) Common leaf life span of co-dominant species in a continuously grazed temperate pasture. *Basic and Applied Ecology* 14: 54–63
- Gamnitzer U, Schäufele R, Schnyder H (2009) Observing  $^{13}\text{C}$  labelling kinetics in  $\text{CO}_2$  respired by a temperate grassland ecosystem. *New Phytologist* 184: 376–386
- Gamnitzer U, Moyes AB, Bowling DR, Schnyder H (2011) Measuring and modelling the isotopic composition of soil respiration: insights from a grassland tracer experiment. *Biogeosciences* 8: 1333–1350

## EFFECT OF AMMONIUM ADSORPTION IN $^{15}\text{N}$ TRACING EXPERIMENTS

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A common observation in  $^{15}\text{N}$  tracing experiments is a rather low recovery of  $^{15}\text{N}$  after application of  $^{15}\text{NH}_4^+$ , even immediately (<1 hours) following the tracer application. This is often explained by a fast, abiotic adsorption of  $\text{NH}_4^+$  on clay particles and/or soil organic matter. Some of the adsorbed  $^{15}\text{N}$  may become bioavailable again during the course of an incubation experiment. The  $^{15}\text{N}$  pool dilution and  $^{15}\text{N}$  tracing techniques are widely applied for the quantification of gross N transformation rates. However, a release of initially adsorbed  $^{15}\text{NH}_4^+$  (i.e. desorption) may violate the assumption of the pool dilution that no “remineralization” of  $^{15}\text{N}$  occurs, leading to biased gross transformations. Little work has focused on the potential effects of  $\text{NH}_4^+$  adsorption on the quantification of gross N transformation. I will discuss some approaches to account for the  $\text{NH}_4^+$  adsorption when applying the pool dilution techniques and the potential of numerical tracing models to overcome the limitations by specifically considering the processes of  $\text{NH}_4^+$  adsorption/desorption. However, there are still severe limits in our understanding of  $\text{NH}_4^+$  adsorption that needs to be addressed in order to have a more reliable quantification of gross N transformation rates, particularly in soils with a high capacity for cation exchange (i.e. clay or organic soils). These limitations will be highlighted.

The influence of N and P supply and genotype on N remobilization in containerized *Pinus radiata* plants

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A large proportion of the nitrogen (N) used in the current-year growth of the widely grown plantation species *Pinus radiata* D. Don is N that was stored in plant tissues the previous year. However, the extent to which an imbalance between levels of phosphorus (P) and N may change the capacity of plants to remobilize N is unknown. In this study, the N remobilization responses of four *P. radiata* genotypes to a factorial combination of N and P additions were assessed in containerized plants in a two-year greenhouse experiment. N supply was enriched with <sup>15</sup>N at 2.5 ‰ (labeled N) during the first year. Plants were then transferred to clean sand and grown for another year with <sup>15</sup>N at levels close to natural levels (0.3664899 atom percent <sup>15</sup>N, δ<sup>15</sup>N 0.5115 ‰). Calculations of N storage and remobilization were based on the recovery of labeled N from new tissues during the second year of growth. Over the second year, N remobilization for the high-N high-P supply regime (953 mg N plant<sup>-1</sup>) was five-fold the N remobilization for the low-N low-P supply

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**POSTER ABSTRACTS**



## DYNAMICS OF THE NATURAL ABUNDANCE OF $^{13}\text{C}$ AT PYRENEAN PASTURES

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The annual net ecosystem exchange (NEE) at a certain ecosystem is driven by the processes involved in the carbon (C) cycle of the plant-soil system. The inherent capacity/functionality of the ecosystem is not constant in time and space. It changes under the natural seasonality and it varies amongst its components. Thus, the “instantaneous” exchange rate not only depends on the prevailing environmental factors but it is also constrained by the state of the plants. As different plant functional types follow different functional strategies for optimization of the resources, they also present different patterns of change in their capacities such as photosynthetic fixation, belowground C allocation, and C loss via respiration. This work looks into these patterns with the use of stable isotopic techniques, to determine the general variation of these key processes and whether different temporal patterns arise between different guilds.

In this context, we are examining several semi-natural grazed grasslands in the Pyrenees. Particularly, at the sites of La Bertolina and Castellar (1300 and 1900 m a.s.l. respectively) we are monitoring ecosystem-scale flux measurements and the state of vegetation including below- and above-ground biomass and leaf area. In addition, we have been monitoring the natural abundance of  $^{13}\text{C}$ , differencing amongst plant functional types.

We have used  $^{13}\text{C}$  isotopic discrimination as a convenient tracer of integrated plant physiology, understanding that leaf C isotope ratio ( $\delta^{13}\text{C}$ ) and C isotope discrimination ( $\Delta$ ) integrate information about the regulation of C fluxes.  $\Delta$  is used as a proxy for whole-plant constraints, more stable over time than instantaneous fluxes, and considered as a long-term indicator of plant metabolism. Further information on the allocation of resources can be obtained with the measure of  $\delta^{13}\text{C}$  from other compartment and components. Furthermore, the C isotope ratio of ambient  $\text{CO}_2$  ( $\delta^{13}\text{C}$ ) reflects the C cycle as a whole integrating processes of assimilation, respiration and decomposition

The sites were sampled every 3 weeks during the growing season from July 2011 to November 2012. We obtained samples of air, litter, soil and vegetation. Vegetation was sorted in the lab in order to obtain leaves and roots of the different functional groups present.

This poster presents the results of the study.

## Pitfalls and promises: a guide to field site assessment of soil carbon

### dynamics (INCREASE): a synthesis of isotope techniques

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*In a 12 year long field experiment, manipulating shrubland ecosystems with elevated temperature and summer droughts, we urged to avoid sequential destructive sampling. In this review we evaluate the difficulty of non-destructive sampling and compare methods used at the INCREASE field sites for determination of soil carbon dynamics. We present experiences and recommendations for future studies using in situ and in vitro isotope labelling techniques with <sup>13</sup>C and <sup>14</sup>C and natural abundance study. It is unique that so many methods have been applied to the same type of soil and ecosystem, undergoing the same manipulation treatments.*

*Out of urge to follow and identify separable carbon flows through all ecosystem compartments, the technique of pulse labelling was developed. During pulse labelling, a concentrated input of <sup>13</sup>C enriched CO<sub>2</sub> is amended to the undisturbed living ecosystem in a restricted period of time. We aimed to study the patterns of microbial utilisation of plant root exudation, by applying a <sup>13</sup>C pulse labelling in the growing-season. The analysis of <sup>13</sup>C in specific compounds such as PLFA, allows determining which functional groups actively assimilate C-labelled substrates (of autotroph origin). We used transparent domes of 50 cm of diameter and 1m of height enclosing *Calluna vulgaris* plants to apply a pulse of <sup>13</sup>CO<sub>2</sub> during 8 hours.*

*A method based on substrate-induced respiration (SIR) amends carbohydrate rich substrates to intact soil in the lab. The evolved headspace CO<sub>2</sub> is sampled and measured (MicroResp<sup>TM</sup>). We determined the catabolic utilisation profile, turnover and pool allocation of LMW carbon by utilising a selection of <sup>14</sup>C organic C substrates in a multiple SIR assay on whole soil. This has the advantage of enabling the attribution of the respired carbon dioxide to metabolism of the radiolabelled substrates. It enables the study of microbial uptake kinetics and turnover in absence of plants (no autotrophic input), using substrates varying in structural complexity and recalcitrance.*

*The use of 'bomb-C' technique has a great advantage, which is the ability to determine the age of C stored in or lost from the soil, with a minimal disturbance of the soil-plant subsystem. Soil efflux samples were collected in the late experimental drought period and analysed by AMS. The results revealed a high heterogeneity in the <sup>14</sup>C signature of the soil efflux with a trend towards the release of older carbon from the drought plots. On average, the carbon being released from the plots was fixed between six and eight years ago; this suggests that the autotrophic respiration may be the dominant source of CO<sub>2</sub> in the soil efflux.*



## The isotopic $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ signatures of shrub-land vegetation subject to climate manipulations

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The study was conducted at shrubland field sites along climatic gradients across Europe, i.e. Spain (SP, 41°18'N 1°49'E), Wales (UK, 53°03'N 3°28'W), Hungary (HU, 46°53'N 19°23'E), Netherlands (NL, 52°24'N 5°55'E), Denmark (DK, 56°23'N 10°57'E), and Italy (IT 40°36'N 8°9'E) (Beier *et al.* 2004). The sites comprise a gradient in mean annual precipitation SP < IT < HU < DK < NL < UK and in mean temperatures UK < DK < NL < HU < SP < IT. Increased summer droughts (D) and passive night-time warming (W) was obtained using transparent polyethylene plastic curtains that unfold when activated by daylight and rain sensors. The experimental sites consist of three plots of 4×5m receiving the warming treatment, three plots receiving the drought treatment, and three controls. The shrubs were all C3 plants: *Erica multiflora*, *Globularium alypum*, *Calluna vulgaris*, *Populus alba*, *Cistus monspeliensis*, *Helichrysum italicum* and *Dorycnium pentaphyllum*. Current year leaves were sampled each year (2001 – 2004). The nutrient poor soils were sandy, podzol or peaty soils.

During CO<sub>2</sub> assimilation in the C3 plant leaf, fractionation processes discriminate heavy <sup>13</sup>C and builds a <sup>13</sup>C depleted leaf. This is the case if the available CO<sub>2</sub> pool is constantly refreshed (open system). However, if the plant is in a drought situation the stomata close and create a limited pool of available CO<sub>2</sub> (closed system). Hereby fractionation is overruled and <sup>13</sup>C-CO<sub>2</sub> is no longer discriminated, and a leaf less depleted in <sup>13</sup>C is built. Plant uptake of nitrogen consists of a mixture of inorganic and organic sources. **Nitrate** in soil represents the most **<sup>15</sup>N depleted** nitrogen source for plants. Hereby, the  $\delta^{15}\text{N}$  signature of the plant leaves represents the importance of nitrate for the plant nutrition and the availability of nitrate in the soil. Net nitrification rate in the soil hereby determines the  $\delta^{15}\text{N}$  signature of the plant.

**Water availability**, measured as precipitation accumulated through the past year and soil moisture through the past three months, controlled the  $\delta^{13}\text{C}$  of plant leaves (both P<0.001) across sites and treatments: the <sup>13</sup>C depletion of the plant was less in drought treatments compared to control and warming treatments. Response to drought treatment was the same as moving to drier site. The high **nitrification rate** ( $2.2 \pm 0.8 \text{ gN} \cdot \text{m}^{-2} \cdot \text{yr}^{-1}$ ) at the Hungarian field site probably caused the relatively highly depleted  $\delta^{15}\text{N}$  of *Populus* (-11.7‰), when compared to ericaceous shrubs (*Erica m.* -7.2‰ and *Globularium a.* -5.8‰) at sites with small net nitrification rates ( $0.1 \pm 0.2 \text{ gN} \cdot \text{m}^{-2} \cdot \text{yr}^{-1}$ ). Nitrate availability is coupled with water availability; we hypothesized that drought treatment would decrease nitrate acquisition of the plant hereby the  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  can be coupled: with higher  $\delta^{13}\text{C}$  reflecting smaller water availability and larger  $\delta^{15}\text{N}$  reflecting smaller use of nitrate.

COASTAL DUNE FORESTS UNDER SCENARIOS OF GROUNDWATER LIMITATION: FROM TROPICS TO MEDITERRANEAN - *GWTROPIMED PROJECT*

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Groundwater (GW) drawdown is important to vegetation as can produce dramatic changes in plant communities, on physiological performance or survival of plant species. GW lowering and surface water diversions will affect vulnerable coastal dune forests, ecosystems particularly sensitive to GW limitation and inevitably affect GW-dependent species. Sand dune plant communities encompass a diverse number of species that differ widely in root depth, tolerance to drought and fluctuations of the water table, and capacity to shift between seasonal varying water sources. The high ecological diversity of sand dune forests, characterized by sandy soils, well or poorly drained, poor in nutrients and with different levels of salinity and GW, can occur in different climatic regions of the globe. Such is the case of Tropical, Meso-mediterranean and Mediterranean areas, where future climate change is predicted to change water availability. Accordingly, the core idea of this project is to evaluate, along a climatic gradient, the capacity of different plant communities to adapt to future scenarios of changing GW by an integrative spatial approach of GW stress indicators.

This large-climatic-scale study, covering Brazil, Portugal and Spain, will provide an excellent experimental condition to study the GW dynamics and community functioning in natural ecosystems of high ecological value. To fulfill the main objective, suitable short- and long-term GW limitation stress indicators will be integrated in spatio-temporal water dynamics. In a global consortium partnership, 4 main tasks will be developed in this project: (i) Characterize and understand plant functional groups water use in a GW limitation situation in a climatic gradient (Task 1); (ii) Understand ecophysiological responses of functional groups in a GW gradient and define suitable short-term stress indicators in GW limitation scenarios, using stable isotopes (leaf <sup>13</sup>C and xylem <sup>18</sup>O) as the main approach (Task 2); (iii) Estimate important factors that could function as GW long-term stress tracers and evaluate long-term stress sensitivity of the functional groups to temporal/seasonal changes in water availability, through the use of tree-rings isotopic signal (<sup>13</sup>C and <sup>18</sup>O) as an archive tool (Task 3); (iv) Develop a model to evaluate community water use and response under future groundwater change scenarios through ecophysiological parameters (Task 4).

Ultimately, this approach will contribute to trace GW stress in vegetation in an early stage and help to manage vulnerable communities.

## LINKING THE DEGRADATION OF SIMPLE AND COMPLEX CARBON SUBSTRATES WITH SPECIFIC MICROBIAL GROUPS IN SOIL

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The degradation of organic substrates with different stability in soils is thought to be accomplished by variable microbial groups. However, linking the utilization of single to complex compounds to specific microbes has proven to be a difficult task. On top of that, variable soils with different characteristics exhibit different microbial utilization patterns increasing the complexity of tracing C flow through microbial groups.

Here, we amended mineral and organic (peat) soils sampled from central Finland with <sup>13</sup>C-labelled substrates of different stability (Glucose, Cellulose, Phenol, plant litter) and followed the label in PLFAs as well as respired CO<sub>2</sub>. Substrate utilization among microbial groups was relatively similar for easily available substrates, e.g. glucose, but differed substantially with increasing complexity. While phenol was e.g. largely utilized by fungi in mineral soils, the ability to degrade this stable organic compound was widely distributed among microbial groups in organic (peat) soils, where phenol is more abundant.

The data also allowed us to calculate the priming effect (the increase or decrease in soil organic matter decomposition rate after fresh organic matter input) of variable substrates in different soils. While large priming effect was detected in mineral soils, organic soils exhibited only small or negative priming effect. No priming effect was observed for labelled litter, the only compound containing also nitrogen, in both mineral and organic soils, suggesting that the availability of nitrogen plays an important role in activation of SOM decomposition through priming.

Together, the data show distinct substrate utilization patterns by microbes in variable soils, especially for stable substrates. The addition of fresh substrates triggers variable responses in soil organic matter decomposition rates dependent on chemical composition of substrates and nature of soils.

**SEASONAL VARIATION OF SOIL DEPTH WHERE TWO COMMON SWISS TREE SPECIES FAGUS SYLVATICA AND PICEA ABIES GET THEIR WATER FROM**

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The vulnerability of trees to global change-induced drought events depends, among others, on where trees can access water in the soil. Here we investigate the seasonal variation of soil depth, where the two most common European tree species get their water from in two sites in Switzerland. For the purpose of our study, we used stable oxygen isotope profiles of soil water that occur along soil depth profiles.

Since no isotopic fractionation occurs during water uptake via roots, the  $\delta^{18}\text{O}$  values of xylem water can thus be related to the soil depth where trees take up their water from.

The objectives of our study were (1) to determine the origin of source water for *Fagus sylvatica* and *Picea abies* and identify differences in the source water depth. (2) Determine potential seasonal and annual variation in the soil depth where the two investigated tree species take up their water from. We conducted the study in a highly diverse mixed mountain forest (Lägeren) and a subalpine mountain forest (Davos) in Switzerland. Additional data collection with same sampling technique is planned for the current year 2013. The poster will show first results of our study.

Key words: stable isotopes, soil water,  $\delta^{18}\text{O}$ , xylem water

## ALLOCATION OF RECENTLY ASSIMILATED CARBON IN BEECH LEAVES

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Investigating the short-term dynamics of trees' carbon allocation is critical for understanding the mechanisms underlying annual patterns of carbon allocation in forest ecosystems, influenced by tree phenology and environmental conditions. Pulse-labelling with stable carbon isotope ( $^{13}\text{C}$ ) enables to trace the fate of labelled carbon into tree and its release to the atmosphere by respiration. This study aimed at characterizing the short-term allocation pattern of the recently assimilated carbon in beech leaves, from its assimilation to its partitioning among the several carbon-containing metabolic pools, and its evolution across seasons.

*In situ* whole-tree crown  $^{13}\text{CO}_2$ -pulse labelling experiments were performed on 10 adult beeches (20-year-old *Fagus sylvatica* L.) in a natural regeneration mixed stand located in the state forest of Hesse in Northeastern France. Pulse-labelling campaigns were conducted four times during the growing season, in May, July, August, and September, in order to cover the different beech growing phases. For each labelled tree, leaf samples were harvested over a 6-day chase period. Purification and quantification of C-containing metabolic pools - including the isolation of soluble carbohydrates (including soluble sugars, amino and organic acids), starch and structural compounds - were performed. Total leaf organic matter (bulk) and metabolic pools  $\delta^{13}\text{C}$  were determined by IRMS. In addition, the isotope composition of leaf  $\text{CO}_2$  efflux was monitored by incubating leaves and analysing respired  $\text{CO}_2$  by IRMS.

The labelled  $^{13}\text{C}$  was rapidly assimilated in leaves of each tree and recovered in starch, soluble carbohydrates, structural compounds, and in leaf  $\text{CO}_2$  efflux. Variations among the different labelling periods were observed and the implication of season vs environmental factors will be discussed. Finally, these results will also be used for feeding a leaf-scale compartmental model, which describes the fate of the recently-assimilated carbon among leaf respiration, growth, storage and export, giving the opportunity to isolates kinetics parameters of carbon allocation in leaves.

## TO BASELINE OR NOT TO BASELINE? APPLICATION OF A BASELINE CORRECTION TO STUDY THE GROUND BEETLES INHABITING LAKE ISLANDS IN NORTHEASTERN POLAND.

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Trophic position and trophic niche width estimates are perhaps the most widely reported metrics in food web studies employing natural stable isotopes analyses. The  $\delta^{13}\text{C}$  values of animal tissues differ by less than 1 ‰ between the animal and its diet. When the potential food sources have a variety of  $\delta^{13}\text{C}$  signatures, the method provides the degree of dependence of a given animal on its food sources. In addition  $\delta^{15}\text{N}$  exhibits stepwise enrichment with trophic transfers, and allows for estimating the trophic position of organisms. The ideal situation is when we know the list of potential food sources, or when we are able to collect the good baseline samples characterizing well the study site.

The substantive goal of a baseline is to reflect the isotopic signature of the primary source of production for the food web. However, finding and choosing an appropriate baseline depends on the spatial and temporal context of the ecological question under consideration. For scientists, studying animal migrations, the differences in baseline isotopic values of certain regions allow to track their origin. On the other hand, when dealing with organisms originating from study sites which differed bio-geochemically, observed patterns in isotopic niche may be a function of baseline variability and not reflect true trophic differences between species.

The study were conducted on ground beetles which were collected in 27 sites on 18 lake islands and two adjacent mainland sites within two regions in northeastern Poland. Humid Alder (*Alnion glutinosae*) and Lime-Oak forests (*Tilio-Carpinetum betuli*) dominated on islands. Additionally, there were abandoned pastures (*Arrhenatherion* and *Cynosurion alliances*) on three islands. Stable isotopes ratios ( $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ ) were measured for 1156 beetles from 59 species inhabiting 20 island and mainland sites of the Masuria Lakeland in northern Poland. Baseline (litter, soil, dominant plants) were collected during trapping sessions of carabids.

Due to isotopic baseline variation among islands and habitats, we implemented a baseline correction to allow for interspecific isotopic niche space comparison between ground beetles collected in different sites.

## DO CHANGES IN FOLIAR $^{15}\text{N}$ COMPOSITION WITH STAND AGE IN TROPICAL *EUCALYPTUS* AND *ACACIA* PLANTATIONS REFLECT CHANGE IN NITROGEN NUTRITION?

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Nitrogen (N) is the main nutrient limiting factor for tree growth in costal Congo where tropical trees have been planted on a former savannah. Foliage composition in  $^{15}\text{N}$  depends on the N sources used by the trees (e.g. soil N versus N derived from the atmosphere through symbiotic N fixation) and is affected by fractionation occurring during mycorrhizal transfer of N from the fungi to the trees.

Changes in foliar  $\delta^{15}\text{N}$  composition were studied in both a *Eucalyptus urophylla*  $\times$  *grandis* clonal plantation and an *Acacia mangium* plantation along a full rotation (7 years).  $\delta^{15}\text{N}$  was higher in eucalypt leaves than in acacia phyllodes at 18 months of age (2.1 versus 0.1‰), which indicate a high contribution of nitrogen derived from the atmosphere (Ndfa%) to foliar N of acacia at this age (85%) (considering that the two species used the same source of soil N).  $\delta^{15}\text{N}$  of acacia phyllodes increased to 1.1‰ at 96 months of age suggesting a decline in Ndfa% with stand age while leaf N content remained high (3.1%).

In the meantime,  $\delta^{15}\text{N}$  of eucalypt leaves decreased to -2.5‰ while leaf N content decreased from 2.0 to 1.6%. This decrease in  $\delta^{15}\text{N}$  might be related to the isotope fractionation during the transfer of N from the mycorrhizal fungi to eucalypt trees, suggesting that more N is obtained from the mycorrhizal network and/or that more N is retained in the mycorrhizal network with stand ageing. This is consistent with a low ratio of aboveground production to total belowground carbon flux (ANPP:TBCF = 0.54) suggesting that a large amount of net primary production is diverted to ectomycorrhizal fungi. As litter N is depleted in  $^{15}\text{N}$  compared to soil N, these results also suggest a change in N source for the nutrition of eucalypt trees from soil N to litter N along the rotation.

When grown in mixture (50% acacia – 50% eucalypt),  $\delta^{15}\text{N}$  of acacia phyllodes was lower (-0.7‰) while the  $\delta^{15}\text{N}$  of eucalypt leaves was higher (-1.7‰) than in a pure stands of the corresponding species at 96 months of age. This may indicate bidirectional N transfer between the two species that exhibited a less pronounced difference in leaf N content (2.7 and 2.2%, respectively).



## AUTOMATED ANALYSIS OF $^{15}\text{N}$ SIGNATURES OF $\text{N}_2$ , $(\text{N}_2 + \text{N}_2\text{O})$ AND $\text{N}_2\text{O}$ IN GAS SAMPLES – ADVANCES IN MEASUREMENT TECHNIQUE

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Quantification of nitrous oxide ( $\text{N}_2\text{O}$ ) and dinitrogen ( $\text{N}_2$ ) fluxes from the entire microbial pathway of denitrification is challenging. Especially determining the amount of molecular  $\text{N}_2$ , the final product of the denitrification reaction chain, still bears difficulties, mainly due to the high  $\text{N}_2$  background in atmospheric samples.

One way to overcome these difficulties is to conduct tracer experiments where  $^{15}\text{N}$  labeled nitrate is added to the soil. Hitherto  $\text{N}_2$  and  $\text{N}_2\text{O}$  emissions were analyzed as  $^{15}\text{N}_2$ ,  $^{15}(\text{N}_2 + \text{N}_2\text{O})$  and  $^{15}\text{N}_2\text{O}$  in separate runs, eventually using even different instrumentations.

We developed an enhanced analytical approach for analyzing  $\text{N}_2\text{O}$  and  $\text{N}_2$  originating from  $^{15}\text{N}$  enriched nitrogen pools. The system is based on a GasBench II (Thermo Scientific, Bremen, Germany) coupled to a MAT 253 – IRMS (Thermo Scientific, Bremen, Germany). The sample transfer paths, valves, liquid nitrogen traps, the GC column and the open split were modified. Furthermore, a reduction oven was added in order to eliminate oxygen and convert  $\text{N}_2\text{O-N}$  to  $\text{N}_2$  so that finally all N is measured as  $\text{N}_2$ .

By these means we are now able to rapidly determine the stable isotope ratios  $^{29}\text{N}_2/^{28}\text{N}_2$  and  $^{30}\text{N}_2/^{28}\text{N}_2$  of dinitrogen alone ( $\text{N}_2$ ), of the sum of the denitrification products ( $\text{N}_2 + \text{N}_2\text{O}$ ), as well as of  $\text{N}_2\text{O}$ , simultaneously. This allows for the calculation of the  $^{15}\text{N}$  atom fraction of the soil N pools ( $^{15}\text{X}_\text{N}$ ) which underwent  $\text{N}_2$  and  $\text{N}_2\text{O}$  formation. Moreover, the quantities of  $\text{N}_2$  and  $\text{N}_2\text{O}$  emission from the  $^{15}\text{N}$  labelled pool (d) can be determined. The accuracy of the method was tested with standards of known  $^{15}\text{X}_\text{N}$  in the range from 0.10 to 0.99 and d between 24 and 290ppm. Depending on the magnitude of  $^{15}\text{N}$  labelling the obtained accuracy for  $^{15}\text{X}_\text{N}$  was between 0.2 and 3.5% and for d between 3.2 and 3.5%. Both the precision and accuracy we achieved with this methodological approach described here is at least comparable if not better than values reported previously for similar measurements conducted on two separate samples though.

The ability to analyse all quantities ( $\text{N}_2$  as well as  $\text{N}_2\text{O}$  originating from the denitrification) within one run is the main advantage of the method we suggest. Furthermore, sample preparation time and measurement time are reduced while the reliability of the results increases.

Finally, the presented method shows a wide application potential in traced gas-flux experiments. It provides not only the precise quantification of gas emissions due to denitrification, but may also help in distinguishing different  $\text{N}_2$  and  $\text{N}_2\text{O}$  forming processes, like denitrification, nitrification, anaerobic ammonium oxidation or co-denitrification.



## STATIC CHAMBER SYSTEM FOR PARALLEL MEASUREMENTS OF SOIL CARBON ISOFLUXES

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The static chamber method, besides the eddy covariance technique, belongs to the most widely used tools in studying the gas exchange between soil and the atmosphere. There are numerous literature examples of using the static chamber method to quantify the surface fluxes of  $^{222}\text{Rn}$ ,  $\text{CO}_2$ ,  $\text{CH}_4$ ,  $\text{N}_2\text{O}$  and other gases. Only point measurements of fluxes are possible with this technique. Therefore, always the question arises to which extent the given measurement is representative for the larger area given spatial variability of soil fluxes of the investigated gases. In case of relatively simple measurements (e.g. determination of soil  $\text{CO}_2$  flux) one can overcome this problem by repeating the measurements at several locations inside the area of interest. However, in other cases such as analyses of isotopic composition of the investigated gases using mass spectrometry, multiple measurements at the given area are troublesome due to cost and time limits. Currently available new optical methods of determining the isotopic composition of carbon helped to reduce these limits through on-site isotopic analysis. Still, the time of and representativeness of measurements, especially in case of very small signals, remains a problem in some cases.

Here we present a static chamber system designed for quasi-simultaneous measurement of soil  $\text{CO}_2$  and  $\text{CH}_4$  fluxes as well as the  $^{13}\text{C}$  isotopic signature of the respired  $\text{CO}_2$  in three locations. The system consists of 3 static chambers, the module for controlling air circulation through the chambers and monitoring the environmental parameters inside the chambers (pressure, temperature, relative humidity) and the Picarro G2101-i CRDS analyser for  $\text{CO}_2$ ,  $\text{CH}_4$  mixing ratios and  $\delta^{13}\text{C}$  in  $\text{CO}_2$ . In the presented system, three chambers are connected to the module equipped with pumps and mass flow meters, which circulates air with the constant flow rate in each chamber independently and connect sequentially each chamber to the analyser for a specified time interval using multi-position valve. In addition, the module is equipped with a 1 l glass flask filling system allowing to sample air from the chambers for mass spectrometric determination of isotopic composition of  $\text{CO}_2$ . The system allows parallel measurements of  $\text{CO}_2$  and  $\text{CH}_4$  fluxes at three points inside the area of 10 m radius, giving the information about degree of spatial variability of the fluxes and their isotopic composition.

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## SITE-SPECIFIC ISOTOPIC SIGNATURES OF ABIOTICALLY PRODUCED N<sub>2</sub>O

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Nitrous oxide (N<sub>2</sub>O) is an important anthropogenic greenhouse gas and today's single most ozone depleting substance. Soils, predominantly agricultural soils, have been identified as the major source of N<sub>2</sub>O. The microbial processes nitrification and denitrification are considered as the main N<sub>2</sub>O emissions sources. However, N<sub>2</sub>O production, especially during nitrification, is far from being completely understood. Several abiotic reactions involving the nitrification intermediates hydroxylamine (NH<sub>2</sub>OH) and nitrite (NO<sub>2</sub><sup>-</sup>) have been identified leading to N<sub>2</sub>O emissions, but are still being neglected in most current studies. For efficient mitigation strategies, the identification of the main source and sink processes and their contribution to total soil N<sub>2</sub>O turnover is required. Here, the intramolecular distribution of <sup>15</sup>N in the asymmetric N<sub>2</sub>O molecule is a promising tool to give more insight into these mechanisms.

In this study, we investigated the <sup>15</sup>N site-specific isotopic signature of N<sub>2</sub>O produced by different abiotic reactions in a laboratory study. All reactions involved the nitrification intermediate NH<sub>2</sub>OH in combination with different soil constituents (NO<sub>2</sub><sup>-</sup>, Fe<sup>3+</sup>, Fe<sup>2+</sup>, Cu<sup>2+</sup>). The experiments were conducted in aqueous solution placed in flow-through reaction chambers. N<sub>2</sub>O production and its four main isotopic species (<sup>14</sup>N<sup>14</sup>N<sup>16</sup>O, <sup>15</sup>N<sup>14</sup>N<sup>16</sup>O, <sup>14</sup>N<sup>15</sup>N<sup>16</sup>O, <sup>14</sup>N<sup>14</sup>N<sup>18</sup>O) were quantified simultaneously, online, at a temporal resolution of 1 Hz, using quantum cascade laser absorption spectroscopy. Thereby, our study presents the first continuous analysis of δ<sup>18</sup>O in N<sub>2</sub>O.

The experiments revealed the possibility of purely abiotic reactions over a wide range of acidities (pH 3-8) by different mechanisms: the reaction of NH<sub>2</sub>OH with NO<sub>2</sub><sup>-</sup> at low pH, the oxidation of NH<sub>2</sub>OH by Fe<sup>3+</sup>, and the Cu<sup>2+</sup> catalyzed autoxidation of NH<sub>2</sub>OH at higher pH. The δ<sup>15</sup>N and δ<sup>18</sup>O of N<sub>2</sub>O produced by these abiotic pathways were significantly different between reaction mechanisms and reaction conditions. However, the δ<sup>15</sup>N of N<sub>2</sub>O reflected the δ<sup>15</sup>N of the precursors NH<sub>2</sub>OH (-1.93‰) and NO<sub>2</sub><sup>-</sup> (-27.0‰) only partially. Therefore, reactions with the same nitrogen substrate resulted in significantly different δ<sup>15</sup>N values under different reaction conditions, which constricts the identification of the nitrogen source. For δ<sup>18</sup>O in N<sub>2</sub>O, a similar picture emerges, caused by complex oxygen exchange processes between water and dissolved nitrogen species. All abiotic pathways showed a characteristic site preference of δ<sup>15</sup>N in N<sub>2</sub>O of about 35‰. The site preference was unaffected by process conditions such as pH and remained constant during the experimental run.

These new findings reflect the real benefit of continuous N<sub>2</sub>O isotopic analysis by laser spectroscopy, contribute new information to the challenge of source partitioning of N<sub>2</sub>O emissions from soils, and emphasize the potentially significant role of abiotic reactions in soils. Finally, the role of abiotic reactions in the terrestrial N-cycle will be discussed based on the results of this study and the isotopomer data of N<sub>2</sub>O from recent studies investigating pure and mixed microbial cultures.

## NITROFUNGI AND PYROFUNGI PROJECTS: LINKAGE BETWEEN PLANT'S C UPTAKE, ALLOCATION, AND SOIL ORGANIC N CYCLING

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Biosphere-atmosphere interactions have been actively and successfully studied in Finland for few decades, the oldest continuous monitoring site has been in function since 1991 in north-eastern Lapland in Väriö. Despite many advances made in gas flux measurements, the functioning of forest soil is still not properly understood. In addition to the methodological challenges that are intimate part of soil science, relatively little research effort has been put in understanding soil and microbial processes and their effects on ecosystem-level responses. To fill this gap, the projects 'The interactions between trees and ground vegetation for organic nitrogen uptake via ericoid and ectomycorrhizal fungi' (NITROFUNGI) and 'The interactions between soil fungal communities and soil organic nitrogen (SON) transformation in boreal forests- the effects of season, geographical location and natural disturbances (PYROFUNGI-N)' will be conducted at University of Helsinki between 2012-2017.

The aim of NITROFUNGI project is to investigate how changes in climatic conditions affect carbon (C) storage ability of the boreal forest soils. It has been shown that nitrogen (N) has a key role in the decomposition of soil organic matter (SOM) because the need for N partly controls SOM decomposition. Fungi are important decomposers of SOM and some fungi form symbiosis with forest trees and shrubs. The interaction between SOM decomposition, soil organic N uptake, symbiotic fungi and forest plants will be studied using modern methods from stable isotopes and radiocarbon dating to molecular biology. The results improve our knowledge on the effect of climate change on soil C and N storage pools, and provide data to improve ecosystem-scale models used to predict forest growth in the changing climate.

The main aim of the PYROFUNGI-N project is to identify for the first time the fungal community structure and some of their functions in three main ecosystem stations in Finland. We will connect pyrosequence data to organic nitrogen decomposition from several aspects: spatio-temporal dynamics and disturbances in forest ecosystem (fire and reindeer husbandry). The recently developed, extremely powerful sequencing technology (454-pyrosequencing) allows detailed community analysis with relatively low labour costs.

## **BIOGENIC CO<sub>2</sub> EMISSIONS IN URBAN ENVIRONMENT: A CASE STUDY FROM KRAKOW, SOUTHERN POLAND**

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Most of studies focusing on carbon balance in urban environment deal with emissions of carbon dioxide related to combustion of fossil fuels. However, urbanization processes modify also the soil and river systems, leading to changes of biogenic CO<sub>2</sub> flux and its isotopic composition.

An attempt to quantify biogenic CO<sub>2</sub> fluxes into the urban atmosphere of the city of Krakow, is presented. Two main biogenic sources of CO<sub>2</sub> within the city can be identified: (i) urban soils, and (ii) the Vistula river. To investigate spatial variability of CO<sub>2</sub> emissions from these sources, several sites were chosen for regular measurements of CO<sub>2</sub> flux and its <sup>13</sup>C isotope composition within and outside the city borders. The sites were characterized by different level of anthropogenic influence, such as intensity of car traffic or varying degree of river bed alteration. The chamber method was used to quantify both soil and riverine emissions of CO<sub>2</sub>. Flux measurements were performed at approximately monthly intervals, from June 2009 to March 2013 (soil sites) and from October 2011 to October 2012 (river sites). Appropriate parameters (soil temperature and soil moisture content; temperature, pH, alkalinity, CO<sub>2</sub> partial pressure and conductivity of river water) were recorded at each site during measurements. Source δ<sup>13</sup>CO<sub>2</sub> signature was determined for each measurement run using the Keeling plot approach.

The measurements of soil CO<sub>2</sub> flux revealed distinct seasonal fluctuations, following the natural seasonal variations of the biospheric activity. The minimum fluxes of soil CO<sub>2</sub> were recorded during winter months, when low soil temperature was limiting the respiration activity. In contrast, no distinct seasonal variability could be detected for the riverine CO<sub>2</sub> flux. However, its spatial variability along the river was large: from the site located at the river inflow to the city, to the site at the river outflow, a distinct increase of the CO<sub>2</sub> flux was observed.

As isotopically light CO<sub>2</sub> from fossil fuels combustion is present in the urban atmosphere, its imprint should be in principle detectable in the <sup>13</sup>C signal of the CO<sub>2</sub> respired by urban biosphere. However, no such imprint could be detected; only small differences of δ<sup>13</sup>CO<sub>2</sub> signature of soil CO<sub>2</sub> flux between individual sites were observed. One possible explanation for the observed absence of fossil-fuel imprint in the local urban biosphere could be linked to the fact that strongest assimilation occurs during midday, when the city atmosphere is mixed well enough to mask spatial variability of CO<sub>2</sub> concentration and its isotopic signature. No distinct seasonal variability of δ<sup>13</sup>C in the soil CO<sub>2</sub> flux was observed.

δ<sup>13</sup>C value of riverine CO<sub>2</sub> flux was approximately constant throughout the year at the given observation site. However, significant variations of this parameter were observed between the sites: the most negative δ<sup>13</sup>CO<sub>2</sub> values were observed at the river inflow to the city, with progressive increase of this value along the river flow.

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## STABLE WATER AND CARBON ISOTOPES IN THE DANUBE RIVER

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Stable isotope techniques can be applied for investigation of the sources and cycling of water and solutes in river systems. The River Danube, as the second largest river in Europe, drains an area of 817,000 km<sup>2</sup> of which about 16% falls within the Serbian territory. On this section, the Danube water discharge increases by about 2.5 times from its principal tributaries the Tisza (157,174 km<sup>2</sup> drainage area) and the Sava (95,719 km<sup>2</sup>) and complex of two hydropower dams (Iron Gate Dam I and II) exist.

The river water samples were collected during the longitudinal survey of the Danube, in the Serbian territory (1260 to 851 rkm) at 12 locations, during four daylight surveys (April, Jun, September, and November) in the period 2009–2011. All grab samples were collected by boat from mid-stream 0.5 m below the water surface and analyzed for a range of physicochemical parameters and stable isotopes (<sup>2</sup>H, <sup>13</sup>C, and <sup>18</sup>O). The stable isotope values exhibited significant changes in the Danube (from –11.1 to –9.7‰ for  $\delta^{18}\text{O}$  and from –77.3 to –69.2‰ for  $\delta^2\text{H}$ ) depending on the time of survey. The significantly heavier values in respect to those measured for the Danube in Vienna (from –12.5 to –10.6‰ for  $\delta^{18}\text{O}$  and from –90.6 to –73.7‰ for  $\delta^2\text{H}$ ) for the period 2002–2005 can be explained by the input of isotopically heavy tributaries, controlled by their catchments. The measured isotope composition of dissolved inorganic carbon (DIC),  $\delta^{13}\text{C}_{\text{DIC}}$ , for the main stem of the Danube waters were pretty constant (–9.7 to –14.0‰) along the river and seasons, but significantly correlated to discharge ( $r = -0.78$ ) with two extremes of –3.4‰ in April 2009 and –7.2‰ in November 2010. Lower  $\delta^{13}\text{C}_{\text{DIC}}$  values indicated higher influence of degradation of organic matter. Dissolved major element concentrations ( $\text{HCO}_3^-$ ,  $\text{SO}_4^{2-}$ ,  $\text{Cl}^-$ ,  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{K}^+$ ,  $\text{Na}^+$ ) and  $\delta^{13}\text{C}_{\text{DIC}}$  are used to discuss chemical weathering rates and exchanges of CO<sub>2</sub> between the atmosphere, hydrosphere, and lithosphere.

## ENVIRONMENTAL INFLUENCES ON CARBON ISOTOPE RATIOS OF DARK-RESPIRED CO<sub>2</sub> AND RESPIRATORY COMPOUNDS IN POTATO

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The regulation of respiratory processes is a primary step for all green plants to adapt to changing environmental influences. During the breakdown of respiratory compounds via glycolysis and the Krebs cycle plants gain energy and intermediates for all essential metabolic reactions, producing CO<sub>2</sub> by enzymatic decarboxylation reactions. Plant dark-respired CO<sub>2</sub> ( $\delta^{13}\text{C}_{\text{res}}$ ) shows a diel cycle with <sup>13</sup>C-enrichment under illumination and <sup>13</sup>C-depletion during darkness. The magnitude of the  $\delta^{13}\text{C}$  variation depends on environmental factors like temperature and water status. Under drought,  $\delta^{13}\text{C}_{\text{res}}$  and the  $\delta^{13}\text{C}$  values of the respiratory compounds were shown to be more positive in long-term treatments. However, not much is known about the isotopic variation during diel respiratory mechanisms and the involved compounds when more than just one environmental factor is varied simultaneously, i.e. temperature and water availability. Thus, to observe variations in diel isotopic cycles under different environmental conditions, we carried out climate chamber experiments with potato plants grown under two different temperature and two water regimes. The diel cycle of  $\delta^{13}\text{C}_{\text{res}}$  was sampled by the in-tube incubation technique. In parallel,  $\delta^{13}\text{C}$  and concentrations of several carbohydrates (soluble mono- and disaccharides; transitory starch) as well as organic acids (malic and citric acid) were measured by EA- and LC-IRMS over 32h. We found significantly more negative  $\delta^{13}\text{C}_{\text{res}}$  and  $\delta^{13}\text{C}$  values of respiratory compounds at higher temperatures, while photosynthetic assimilation rate decreased. As expected,  $\delta^{13}\text{C}$  values were significantly more positive under drought, since stomatal conductance was lower in dry plants. In contrast,  $\delta^{13}\text{C}_{\text{res}}$  and  $\delta^{13}\text{C}$  values of all carbohydrates under simultaneous influence of higher temperature and drought were similar to  $\delta^{13}\text{C}$  values the control plants, indicating that both factors can compensate each other. Moreover, strongest relationships were found between  $\delta^{13}\text{C}_{\text{res}}$  and  $\delta^{13}\text{C}$  values of the organic acids fraction. Our results suggest that temperature and drought alter the isotopic composition of respiratory compounds in opposite ways and consequently the  $\delta^{13}\text{C}_{\text{res}}$ .

## The application of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ to plant seeds in semi-arid environments

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The subject of this work, plant seeds and their natural products, have become an important object of study in the past decade, with the isotopic composition of certain bio and geo-elements yielding relevant information on plant ecophysiology (e.g. water use efficiency). Most seeds have the remarkable ability to survive in dry conditions. In the later steps of maturation, seeds of many species acquire the capacity to withstand removal of the majority of their water (i.e. they become desiccation-tolerant). Seeds become important material of study; however, one must bear in mind that they always refer to a specific “temporal window”, i.e. the seed developmental period, which varies according to plant species and biology, and depends on the climate conditions where the plant is grown. Isotope analysis of chemical elements of plant seeds or of specific organic compounds extracted from those seeds may help to understand how different ecological processes influence plant development and physiology during the seed developmental period. Because this is intrinsically related with local climatic conditions (i.e., temperature, precipitation, air humidity), results generally potentiate the development of analytical tools towards traceability of the plant material. Economically important plant species were selected as case-studies, namely the argan tree (*Argania spinosa*) and the cork oak (*Quercus suber*). The geographical distribution of the areas where these plants grow ranges from southwest of Morocco to south of western Iberia, crossing the Mediterranean Sea and enclosing regions under extreme climatic conditions. The first task was to select producing regions of oak acorn and argan fruit, according to soil use, aridity and desertification indices and community sustainability importance. Next, isotope ratio mass spectrometry was applied to measure the plants' seeds and oil products' isotope delta values of carbon and nitrogen. Preliminary results indicate a possible differentiation of producing areas of the argan almonds and paste. These isotopic composition values were enriched in relation to the correspondent argan oils. In what refers to N, argan almond and paste  $\delta^{15}\text{N}$  values ranged from +3.1‰ up to enriched values as high as +10.5‰ possibly indicating animal fertilization. In the case of acorns, a wide range of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values has been observed in acorns sampled across a desertification gradient previously defined in the region of Alentejo (Portugal) in South Iberia. Acorn  $\delta^{13}\text{C}$  values varied from -26.9 to -21.9‰, with average value of -24‰ ( $\pm 1.4\%$ ) suggesting differences in water availability to plants. In the case of N, acorn  $\delta^{15}\text{N}$  values varied from -1‰ to 4.8‰, possibly indicating different silvo-agricultural practices. This work reveals the importance of isotope analysis to the differentiation of argan forests' and *montado* products. If so, this would support the use of stable isotopes as a tool for the verification of products geographical origin. Moreover, isotopes may contribute to trace the environmental impacts occurring in (semi)-arid regions, in particular if related with pasture activity, distance to the coast, anthropogenic activity and altitude.



Mapping nitrogen deposition and fingerprinting its sources in complex landscapes

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Changes in the nitrogen cycle to human activities may have already crossed the safety boundaries for safe human activities. This excessive nitrogen has impacts of biodiversity, ecosystems functioning and human health. Among human activities the major sources of nitrogen is agriculture, which emits mostly reduced nitrogen (ammonia) and industries, sources of both reduced but also oxidized nitrogen (NO<sub>x</sub>). In the very patchy Mediterranean landscapes, numerous sources of nitrogen are scattered over large regions. Thus, it is important to know where nitrogen is deposited after being emitted. Besides knowing where it is being deposited we must also be able to know the sources of nitrogen for correct management of their impacts. In order to know this, in a complex Mediterranean landscape we used lichens as accumulators. More specifically, we assess nitrogen concentration and nitrogen isotopic composition in lichens collected in a region in south-western Europe, in an area with multiple industrial and agricultural nitrogen sources. This data was analyzed using geostatistics to characterize spatial characteristics and mapping.

We observed that nitrogen concentration in lichens was mostly associated to agriculture activities, including intensive agriculture and animal farming units. Nitrogen concentration showed low spatial continuity (range < 5 km), which is probably related to the short dispersion of ammonia from agriculture activities. Mapping of nitrogen isotopic composition showed a different scenario. This variable presented much higher spatial continuity (> 12 km) which is probably related to the dispersion of the nitrogen from industrial areas as NO<sub>x</sub>, with a dispersion much longer than ammonia. Moreover, we observed that both agriculture activities and water treatment plants were associated to higher <sup>15</sup>N discrimination, probably because both emit ammonia. Thus, we could use nitrogen concentration and nitrogen isotopic composition analyzed using a spatial explicit approach to map and fingerprinting nitrogen deposition and sources in a complex region.



## FACTORS INFLUENCING THE OXYGEN ISOTOPIC COMPOSITION OF PHOSPHATE EXTRACTED FROM SOYBEAN LEAVES

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The oxygen isotopic composition of phosphate ( $\delta^{18}\text{O-PO}_4$ ) is used to study phosphorus (P) cycling in sediments, water bodies and also in the soil-plant system. It is based on the fact that in nature, P is mostly associated to oxygen (O) and that biological processes favour the oxygen exchange between phosphate and ambient water. Among these processes, inorganic pyrophosphatase promotes a temperature-dependent equilibrium between oxygen in phosphate and in water, and it is shown now that phosphate in plant leaves is close to equilibrium with leaf water.

To test whether and to which extent the  $\delta^{18}\text{O}$  of phosphate extracted from plants is affected by changes in  $^{18}\text{O}$  of leaf water (e.g. diurnal cycle), we set up the following experiment. *Glycine max* cv. Toliman (soybean) was grown in hydroponic systems for three weeks with an optimal nutrient supply. Over a period of 48 hours fully developed trifoliolate leaves from soybeans were harvested every three hours (16 time points in total). Phosphate was afterwards extracted from soybean leaves using 0.3 M trichloroacetic acid (TCA P). After purifying the extracts in several steps, samples were analysed for their oxygen isotopic composition using a thermal conversion elemental analyser coupled to an isotope ratio mass spectrometer (TC/EA-IRMS). The  $\delta^{18}\text{O}$  of the leaf water, relative air humidity, light intensity, leaf temperature, and the activity of inorganic pyrophosphatase were also determined.

Our results indicate that the  $\delta^{18}\text{O-PO}_4$  of TCA P extracted from soybean leaves show a diurnal cycle similar to the  $\delta^{18}\text{O}$  of leaf water, but that the  $\delta^{18}\text{O}$  of leaf water is not the only factor influencing the  $\delta^{18}\text{O-PO}_4$  of TCA P. Relative air humidity and to a lesser degree also light intensity and leaf temperature seem to influence the  $\delta^{18}\text{O-PO}_4$  of TCA P in soybean leaves. For this reason, the  $\delta^{18}\text{O-PO}_4$  of TCA P extracted from fresh plant material could be used as a proxy for changes in e.g. relative air humidity. To verify this, further studies using different plant species grown under different environmental conditions and measurements of plant litter have to be conducted.

## EFFECT OF FAST CYCLING CARBON ON THE DECOMPOSITION OF OLD SOIL ORGANIC MATTER

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The response of plant photosynthesis to changing environmental factors is of crucial importance when trying to understand and quantify changes in ecosystem processes determining the carbon balance of soil. Increased atmospheric CO<sub>2</sub> concentration and elevated temperature are assumed to increase net primary production (NPP) in boreal forest zone. Plant photosynthesis is the main source of labile, easily utilizable carbon in soil through litter production but especially through root exudation. Input of fast-cycling C has also been shown to induce so called priming effect, i.e. the accelerated decomposition of more recalcitrant C compounds. In this project, the aim was to study the effect of external energy input and root exudation on soil organic matter (SOM) decomposition by adding glucose and/or the plant (Scots pine, *Pinus sylvestris*) to the microcosms containing boreal humus.

We measured the <sup>14</sup>C and <sup>13</sup>C isotopic composition of the respired CO<sub>2</sub> from the microcosms, the mass loss of soil organic matter and other biological factors affecting the SOM decomposition such as microbial biomass and protease enzyme activities. The presence of plant seemed to have a substantial effect on the decomposition of SOM. The addition of glucose as such did not induce increased decomposition activity unlike the presence of plants, which induced a significant decrease in SOM pool during the incubation period of 6 months. The plants also affected the isotopic composition of the respired CO<sub>2</sub> from the soil compared to the glucose addition. Both the <sup>13</sup>C and <sup>14</sup>C measured from the respired CO<sub>2</sub> indicated that the source of decomposition could have been shifted towards more stable SOM pool in the presence of plants, but the glucose addition did not result in similar change. Our findings stress the importance of including the soil plant interaction in the SOM decomposition studies.

## SELECTIVE INHIBITION AND ISOTOPOMER ANALYSIS OF N<sub>2</sub>O TO ESTIMATE THE N<sub>2</sub>O FORMATION BY FUNGAL DENITRIFICATION IN SOIL

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N<sub>2</sub>O predominantly results from microbial communities in soil. However, the contribution of microbial groups (e.g. bacteria or fungi) to N<sub>2</sub>O formation during denitrification is not sufficiently investigated yet.

The site preference (SP= difference between  $\delta^{15}\text{N}$  of the central and terminal N-position of the asymmetric N<sub>2</sub>O molecule) of <sup>15</sup>N in N<sub>2</sub>O is a clue as to whether fungal or bacterial metabolism was involved in its production. Bacteria and fungi produced different SP of N<sub>2</sub>O in pure culture studies (0 to -11 ‰ for bacterial N<sub>2</sub>O and ~-37 ‰ for fungal N<sub>2</sub>O). Additionally it became apparent that most fungi lack N<sub>2</sub>O reductase, resulting in N<sub>2</sub>O as the end product of denitrification. This enables fungi to potentially produce more N<sub>2</sub>O than bacteria and might influence SP in N<sub>2</sub>O. N<sub>2</sub>O reduction leads to a preferred cleavage of N-O bonds including lighter isotopes which results in remaining N<sub>2</sub>O with <sup>15</sup>N enrichment at the central N position as well as <sup>18</sup>O enrichment. Studies combining the analysis of SP and N<sub>2</sub>O production from selective groups of the microbial community to distinguish between fungal and bacterial N<sub>2</sub>O are lacking so far.

The method of substrate induced respiration with selective inhibition was modified to

- (i) determine the fungal contribution to N<sub>2</sub>O production in soil,
- (ii) determine the effect of N<sub>2</sub>O reduction on SP and
- (iii) to verify, if the contribution of bacteria and fungi to N<sub>2</sub>O emission can be assessed by analyzing SP.

We conducted two incubation experiments with a sandy arable soil (sampling time summer 2011 and winter 2012) under denitrifying conditions. Four treatments were established to quantify the N<sub>2</sub>O production by bacteria or fungi: a) control without growth inhibition, b) inhibition of bacterial growth, c) inhibition of fungal growth and d) inhibition of bacterial and fungal growth. Additionally all treatments were analyzed with and without blocking the N<sub>2</sub>O reduction by acetylene. Treatments without N<sub>2</sub>O reduction show the net production of N<sub>2</sub>O and its isotopic signature, due to avoided isotope effects by N<sub>2</sub>O reduction to N<sub>2</sub>. <sup>15</sup>N-labelled NO<sub>3</sub><sup>-</sup>-fertilizer was supplied to check the amount of N<sub>2</sub>O reduction, whereas non-labelled parallel treatments were conducted to measure SP of N<sub>2</sub>O.

The expected inhibition effect was visible for each experiment, where N<sub>2</sub>O production was highest in control (a) and lowest with both growth inhibitors (d). In the experiment with soil samples from summer, bacterial growth inhibition (b) resulted in higher N<sub>2</sub>O production than fungal growth inhibition (c), whereas treatments with soil samples from winter with fungal growth inhibition (c) produced more N<sub>2</sub>O than bacterial growth inhibition (b).

Acetylene amendment resulted in almost complete blockage of N<sub>2</sub>O reduction in all treatments (a, b, c, d): In all treatments with soil samples from summer and winter, SP was negative (-1.0 to -4.9 ‰ in summer and -0.4 to -2.3 ‰ in winter samples). Growth inhibition of bacteria even resulted in a more negative SP compared to fungal growth inhibition treatments. SP results of pure culture studies could thus not be verified for the fungal or bacterial community under soil conditions.

**EXTERNAL SOURCES OF C AND N TO DECOMPOSING STUMP-COARSE ROOT SYSTEMS USING NATURAL ABUNDANCE OF <sup>13</sup>C AND <sup>15</sup>N ISOTOPES**

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**ABSTRACT**

Nitrogen (N) content increases with advancing wood decay; however, the sources contributing to such increase have not been investigated. Similarly, C-sources (external to the decomposing wood) during wood decay remain unexplored. We investigated changes in the sources of C and N during the advancing decay of stump-coarse root systems. Two managed Sitka spruce (*Picea sitchensis* (Bong.) Carr.) stands from the Irish temperate forest were selected and a dual isotope technique was applied to measure  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  in stump and coarse root wood. Both  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  in the decaying stumps and roots changed significantly from their initial (DC 0) decay stage to those in the advanced decay class (DC 4), indicating multiple N sources. In addition, stumps and roots also differed in their <sup>15</sup>N pattern at the different decay stages, indicating a different pattern of N utilization in the decomposition of stump and root material. We conclude that the sources of C and N immobilized in decaying wood originate not only in the substrate itself, but also from external sources. We suggest that the likely source of external C and N at the advanced decay stage is through fungal transfer from forest soil and the direct wood absorption of N from soil .

**Key-words:** Carbon, Nitrogen, Dead wood, <sup>15</sup>N, <sup>13</sup>C, Stump, Coarse root, Spruce

## RELATION OF NUTRITIONAL STRATEGIES OF MACROMYCETES AND SULFUR ISOTOPE PATTERN

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Although the role of macromycetes in nitrogen and carbon cycling within ecosystems is well established, very little is known regarding the uptake and translocation of sulfur. Sulfur isotope signature has already been used to track food origin and to study sulfur cycle in several ecosystems, but, to our knowledge, it has never been applied to fungal dynamics. Macromycetes can be divided in saprotrophic fungi obtaining carbon and nutrients from the organic matter and ectomycorrhizal fungi (ECM) obtaining carbon mainly from the symbiotic plant and nutrients from the soil. Due to these different sources, the two fungal groups present distinct carbon and nitrogen isotopic signatures. This feature is widely used in ecological studies. With the present research we aimed to determine if the source of nutrients could affect also the sulfur isotopic signature and its potential as tool in ecological studies.

Fungal sporocarps were collected in different forest locations of NE Bavaria (Germany) in the region around the city of Bayreuth. We collected 54 individuals divided in 4 fungal species of saprotrophs and 13 of ECM. Samples were prepared and analyzed for carbon, nitrogen and sulfur concentration and isotopic signature using an Elemental Analyzer coupled with an Isotopic Mass Spectrometer. Fungal samples for sulfur isotope analyses were precipitated as barium sulfate. We used an unpaired t-test for comparing the concentration and isotopic signature of saprotrophic versus ECM fungi and a simple linear model to identify correlations between variables. Data were analyzed with the statistical package Statgraphics.

Nitrogen concentration was lower in ECM fungi than in saprotrophs while carbon concentration was lower in saprotrophs than in ECM. Sulfur concentration was similar in the two groups (overall average  $0.25 \pm 0.09\%$ ). ECM had a lower  $\delta^{13}\text{C}$  and a higher  $\delta^{15}\text{N}$  than saprotrophs.  $\delta^{34}\text{S}$  was higher in ECM ( $3.37 \pm 0.96\%$ ) than in saprotrophs ( $1.69 \pm 0.91\%$ ). We found a significant relationship between  $\delta^{15}\text{N}$  and  $\delta^{34}\text{S}$  in ECM but not in saprotrophs. On the contrary, we found a significant relationship between  $\delta^{13}\text{C}$  and  $\delta^{34}\text{S}$  in saprotrophs but not in ECM. These correlations suggest the potential for sulfur isotopes to become a new, powerful tool in environmental studies in particular to entangle the role of macromycetes in the nutrient fluxes within ecosystems.

This work was planned as a preliminary survey to explore the isotopic pattern of sulfur macromycetes. The different isotopic signature of sulfur of the two fungal groups corresponds to different nutritional strategies indicating an interesting pattern that could be exploited in ecological studies. While some research has been done using carbon and nitrogen isotopes, to our knowledge this is the first work reporting on the sulfur isotopic pattern of macromycetes. We are aware that more research is needed to understand the biochemical patterns and the enzymatic processes involving sulfur compounds differing between ECM and saprotroph mushroom, but our data suggest that the isotopic signature of this element could be a useful tool in ecological studies.

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## DIVERSITY EFFECTS ON WATER AND NITROGEN USE EFFICIENCY IN MIXED FORAGE SWARDS

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The carbon (C) isotopic signal  $d^{13}C$  can give an estimation of water use efficiency. Plant interactions in mixed communities may modify water use efficiency through different mechanisms: changing soil moisture, modifying evapotranspiration and environmental conditions within the canopy, hydraulic lift, etc. Atmospheric nitrogen (N) fixation has been found to increase in mixtures above the expected value according to the relative contribution of legumes and non-legumes. This increase in the atmospheric N source with diversity results in a decrease in the isotopic signal ( $d^{15}N$ ). On the other hand, because of isotopic discrimination against  $^{15}N$  in N loss processes (leaching and emission), if diversity affects these processes, the isotopic N signal picked up by plant tissues could reflect changes in N loss processes. We manipulated the diversity of 3-species forage mixtures following a simplex design. Between 2008 and 2010, we studied the diversity effects on several ecosystem functions, including plant productivity, drainage and leaching. We analysed the C and N isotopic signals ( $d^{13}C$  and  $d^{15}N$ ) through periodic harvests, 2 in 2008 (establishment year) and 4 in the subsequent two years. Using the diversity-interaction modelling for data analysis, we found: 1) a different isotopic C and N signal for each of the 3 sown species; 2) a decrease in the  $d^{13}C$  and  $d^{15}N$  with diversity. On the other hand, drainage and leaching were also negatively affected by diversity. This could indicate reduced water stress and increased N fixation, as well as decreased N losses. We conclude that diversity could increase water and N use efficiency.

## REFERENCE MATERIAL SELECTION VERSUS ANALYTICAL ACCURACY IN $\delta^{13}\text{C}$ OF DOC ANALYSES USING LC-ISOLINK

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The development of laboratory standards for compound-specific stable isotope analyses can be challenging owing to the analytical limitations of instruments used to process primary reference materials. Several studies have demonstrated that chemical matrix matching between standards and analysed samples is needed for reducing analytical uncertainty, particularly where sample matrix is very different to the primary reference. This can be the case for dissolved organic carbon compounds (DOC) directly analysed in liquid phase (e.g. using IsoLink, Thermo Fisher Scientific). Raw isotope data are frequently normalised against calibrated  $\text{CO}_2$  gas from a high pressure cylinder; however, single-point normalization versus tank gas introduces the highest uncertainty among normalization methods (Skrzypek, 2013). The major anchors for updated VPDB for referencing the stable carbon isotope composition are LSVEC and NBS19. However, neither of these is an organic standard and therefore do not match the chemical matrix of DOC. Alternatively, the preparation of standard solutions of carbonates is also challenging and may introduce additional high uncertainty of results.

In our study we evaluated reproducibility, linearity, memory effect, detection limit and efficiency for  $\delta^{13}\text{C}$  DOC analysis on IsoLink. We also evaluated which of the international secondary reference materials normally used for Elemental Analyser technique (IAEA600, USGS41, USGS40, CH6, IAEA601) are suitable for direct use in water solution for  $\delta^{13}\text{C}$  DOC on IsoLink analyses.

We found that the general reproducibility of IsoLink is high ( $<0.20$ , 1 st.dev) for simple compounds such as benzoic acid, glutamic acids and even carbonates. In contrast the reproducibility was low for compounds containing aromatic rings such as caffeine. The linearity effect is also small if the sample has signal  $>1.5\text{V}$ , which is the equivalent of  $10\text{ mg C L}^{-1}$ . This concentration of  $10\text{ mg C L}^{-1}$  seems to be a reasonable minimum for reliable analysis, avoiding linearity and background effects. Memory effect was negligible (below the level of reproducibility) even after consecutively analysing samples with highly contrasting  $\delta^{13}\text{C}$  values e.g.,  $-29\text{‰}$  and  $+37\text{‰}$ .

We conclude that solutions of USGS41, USGS40, CH6, IAEA 601 can be used for direct normalization of DOC results to VPDB scale but not IAEA600. The linearity and memory effects are negligible if the obtained signal varies in the range  $1.5\text{-}9\text{V}$  (equivalent of approximately  $10\text{-}55\text{ mg C L}^{-1}$ ).

Skrzypek G., 2013, Normalization procedures and reference material selection in stable HCNOS isotope analyses – an overview. *Analytical and Bioanalytical Chemistry* 405: 2815-2823.



## INVESTIGATION OF STABLE ISOTOPES ( $\delta^2\text{H}$ , $\delta^{18}\text{O}$ , $\delta^{13}\text{C-DIC}$ ) AND $^{222}\text{Rn}$ TO ASSIST IN THE SURFACE WATER/GROUNDWATER INTERACTIONS STUDIES (VELEZ RIVER BASIN, SOUTH OF SPAIN)

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The in-depth knowledge of the interaction between surface water and groundwater is one of the challenges of the Water Framework Directive (WFD) in order to achieve good quantitative status of water bodies. However, despite the progress made in other aspects introduced by Directive as they are; the chemical quality assessment or identification of pressures and impacts, as regards the interaction surface water / ground water remains one of the information gaps more important in most hydrological plans.

This fact makes necessary to improve the currently held knowledge about the relationship between groundwater and surface water in recharge and discharge areas of aquifers (wetlands, rivers, lakes, etc.), the dependence by each other and the importance they have for the associated ecosystems. In many cases, this task involves a number of uncertainties that prevent a real understanding of the operation of the study area.

The application of isotopic studies of the water molecule ( $\delta^{18}\text{O}$  and  $\delta^2\text{H}$ ) provides very relevant information about the origin of the water, type of groundwater recharge and the calculation of the period in it was made (in short term due to seasonal variation suffering isotopic signal and long term to differentiate water from different ages). Furthermore, the study of the isotopes of carbon ( $\delta^{13}\text{C-DIC}$ ) allow to quantify the water-rock interactions and determine the initial geological environments of the groundwater recharge.

In this paper we have chosen the Velez River alluvial aquifer, located in the province of Malaga (southern Spain), as experimental aquifer to meet river-aquifer relationships by studying the content of  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  of water molecule and  $\delta^{13}\text{C}$  of dissolved inorganic carbon. As reinforcement it was also performed an analysis of the activity of dissolved radon ( $^{222}\text{Rn}$ ). This radioactive isotope is a strong signal of the origin of surface or ground water flows. Velez River basin has an area of approximately 610 km<sup>2</sup> and the alluvial aquifer about 20 km<sup>2</sup> and it is situated a distance of 10 km to the coastline. This aquifer has a saturated thickness of up to 70 m and a hydraulic head that, under normal conditions, is very close to the surface and strongly influenced by the regime of the river. These circumstances make the Vélez River basin be a suitable framework for the implementation of these isotope techniques in understanding the interactions between surface water and groundwater.



# EVALUATING CALIBRATION STRATEGIES FOR ISOTOPE RATIO INFRARED SPECTROSCOPY FOR ATMOSPHERIC MEASUREMENT

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Isotope ratio infrared spectroscopy (IRIS) provides an in-situ technique for measuring  $\delta^{13}\text{C}$  in atmospheric  $\text{CO}_2$ . A number of methods have been proposed for calibrating the IRIS measurements, but few studies have systematically evaluated their accuracy for atmospheric applications. In this study, we carried out laboratory and ambient measurements with two commercial IRIS analyzers and compared the accuracy of four calibration strategies. We found that calibration based on the  $^{12}\text{C}$  and  $^{13}\text{C}$  mixing ratios ([Bowling et al. 2003](#)) and that based on linear interpolation of the measured delta using the mixing ratio of the major isotopologue ([Lee et al. 2005](#)) yielded

accuracy better than 0.06‰. Over a 7-day atmospheric measurement in Beijing, the two analyzers differed by  $9.44 \pm 1.65\%$  (mean  $\pm 1$  standard deviation of hourly values) before calibration and agreed to within  $-0.02 \pm 0.18\%$  after properly calibration. However, even after calibration the difference between the two analyzers showed a slight correlation with concentration, and this concentration dependence propagated through the Keeling analysis resulting in a much larger difference of 2.44‰ for the Keeling intercept. The high sensitivity of the Keeling analysis to the concentration dependence underscores the challenge of IRIS for atmospheric research.

## Tracing spatial impact of ecosystem functioning after plant invasion by $^{15}\text{N}$ isoscapes

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Isoscapes, i.e. spatially continuous observations of variations in stable isotope ratios, have greatly improved our ability to understand biogeochemical processes on continental to global scales. Additionally, isoscapes may be a valuable tool for resolving the spatial component of plant-plant-interactions within communities. For example, exotic plant invaders often strongly impact native species performance by altering ecosystem functioning of the systems they invade, particularly regarding water-, carbon- and nutrient-cycles. However, the spatial extent of such alterations is largely unknown.

Here we show that substantial N input by the  $\text{N}_2$ -fixing exotic invasive *Acacia longifolia* to a nutrient poor Portuguese dune system can be traced using spatially resolved information on native plants' leaf  $\delta^{15}\text{N}$ . N isotopic signatures of the native system ( $\delta^{15}\text{N} \approx -10\text{‰}$ ) differed strongly from the atmospherically derived N in *A. longifolia* phyllodes ( $\delta^{15}\text{N} \approx 0\text{‰}$ )<sup>a</sup>. Thus, sources of N for native plants could be readily distinguished. Leaf  $\delta^{15}\text{N}$  of a native, non-fixing species was increasingly enriched the closer the plant grew to the invader, indicating uptake of N derived from decaying *A. longifolia* litter. The enrichment was evident far beyond the stands of the invader, demonstrating that *A. longifolia* affected N budgets of native species up to a distance of 8 m exceeding the margin of the canopy. Furthermore, using the isoscapes approach, we were able to quantify the total area of N enrichment and could thus show that the area affected by invasion was at least 3.5 times larger than the area actually occupied by the invader<sup>b</sup>. However, a native  $\text{N}_2$ -fixing species had no such effects.

Thus, downscaling isoscapes to the community level opens new frontiers in quantifying the spatial dimension of functional changes associated with plant invasions. Moreover, considering the feasibility and applicability of this approach, it may provide a promising tool to identify, quantify and monitor different types of functional plant-plant interactions within communities at a spatially explicit scale.

### References

<sup>a</sup> Hellmann C, Sutter R, Rascher KG, Máguas C, Correia O, Werner C (2011) Impact of an exotic  $\text{N}_2$ -fixing *Acacia* on composition and N status of a native Mediterranean community. *Acta Oecologica* 37: 43-50, doi:10.1016/j.actao.2010.11.005.

<sup>b</sup> Rascher KG, Hellmann C, Máguas C, Werner C (2012) Community scale  $^{15}\text{N}$  isoscapes: tracing the spatial impact of an exotic  $\text{N}_2$ -fixing invader. *Ecology Letters* 15: 484-491, doi: 10.1111/j.1461-0248.2012.01761.x.

## Partitioning evapotranspiration a Mediterranean oak savannah – Testing the Craig and Gordon model with field measurements of oxygen isotope ratios of evaporative fluxes

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Stable oxygen isotopes of water provide a valuable tracer for water movements within ecosystems and are used to estimate the contribution of transpiration to total ecosystem evapotranspiration (ft). We tested the Craig and Gordon equation against continuous field measurements of isotopic composition of evaporation and assessed the impact for partitioning evapotranspiration. Therefore, evaporation (E) and its isotopic signature ( $^{18}\text{O}_E$ ) on bare soil plots, as well as evapotranspiration (ET) and its corresponding isotopic composition of ( $^{18}\text{O}_{ET}$ ) of an herbaceous understory layer was measured with a cavity ring-down spectrometer connected to a soil chamber throughout 2011 on a field site in central Portugal. We quantified the variation in  $^{18}\text{O}_E$  arising from uncertainties in the determination of environmental input variables to the Craig and Gordon equation: the isotope signature at the evaporating site ( $^{18}\text{O}_e$ ), the temperature at the evaporating site ( $T_e$ ), and the kinetic fractionation factor ( $\alpha_k$ ). Eddy covariance technique was used to partition between understory and total ecosystem ET. We could hence quantify the impact of oak transpiration, understory transpiration and soil evaporation.

Our results demonstrate that predicting  $^{18}\text{O}_E$  using the Craig and Gordon equation leads to good agreement with measured  $^{18}\text{O}_E$  given that the temperature and isotope profiles of the soil are thoroughly characterized. However, modeled  $^{18}\text{O}_E$  is highly sensitive to changes in  $T_e$  and  $^{18}\text{O}_e$  as well as  $\alpha_k$ . The relative contribution of understory ET was up to 50 % both in spring and fall; however, whereas understory transpiration was mainly driven by VPD and played a dominant role during the peak growth phase in spring, soil evaporation was mainly driven by top-soil water content and dominated the understory flux in fall. Due to their access to groundwater oak trees maintained a relative stable transpiration rate throughout the year.

These findings provide a first comparison of laser-based and modeled isotopic compositions of evaporation based on the Craig and Gordon equation under field conditions. This is of special interest for studies using stable isotopes to separate soil evaporation and plant transpiration fluxes and highlights the need for a thorough characterization of the micrometeorological and isotopic constitution of the upper soil layer to locate the evaporating front with a resolution of a few cm soil depths. We also call on a better characterization of the kinetic fractionation factor of soil evaporation.

## METABOLIC ORIGIN OF CARBON ISOTOPE COMPOSITION OF CO<sub>2</sub> RESPIRED BY DARKENED LIGHT ACCLIMATED LEAVES IN THREE DIFFERENT PLANT SPECIES

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Significant diel variations of leaf dark-respired CO<sub>2</sub> ( $\delta^{13}\text{C}_{\text{Res}}$ ) were observed in many plant species with largest diel variations of up to 14.8‰ (for a review see Werner & Gessler 2011).  $\delta^{13}\text{C}_{\text{Res}}$  values are increasing under illumination and decreasing during darkness. The degree of diel variability and <sup>13</sup>C enrichment of the respired CO<sub>2</sub> was shown to be species-dependent, with evergreens, shrubs and some herbaceous species showing the largest variations (Werner *et al.* 2009) in contrast to fast-growing herbs and grasses (Priault *et al.* 2009). These large variations in  $\delta^{13}\text{C}_{\text{Res}}$  and the extent of <sup>13</sup>C enrichment in respired CO<sub>2</sub> cannot be explained by the carbon isotopic composition of putative respiratory substrates alone (Werner & Gessler 2011). The PEPc in leaves of C<sub>3</sub> plants could be responsible for the extent of <sup>13</sup>C enrichment of  $\delta^{13}\text{C}_{\text{Res}}$ . A connection between PEPc activity in leaves of C<sub>3</sub>-plants and <sup>13</sup>C enrichment of respired CO<sub>2</sub> (during LEDR) was already discussed by Gessler *et al.* (2009), Werner *et al.* (2009) and Werner *et al.* (2011). In this project, we aim to identify the origin of the sometimes exceptionally high diel variation of  $\delta^{13}\text{C}_{\text{Res}}$  of *Halimium halimifolium* and *Arbutus unedo* in comparison to *Oxalis triangularis*, previously shown to have a more moderate diel cycle and diurnal <sup>13</sup>C enrichment of  $\delta^{13}\text{C}_{\text{Res}}$ . The  $\delta^{13}\text{C}_{\text{Res}}$  values and the amount of respired CO<sub>2</sub> were measured by the in-tube-incubation technique (Werner *et al.* 2007). In parallel, we determined the  $\delta^{13}\text{C}$  value and concentration of malate with compound-specific LC-IRMS. *Halimium* showed the highest <sup>13</sup>C enrichment in respired CO<sub>2</sub> and the highest respiratory CO<sub>2</sub> flux. In contrast to recent findings,  $\delta^{13}\text{C}_{\text{Res}}$  of *Oxalis triangularis* was similar to those of *Arbutus*; both species showed similar CO<sub>2</sub> production. *Halimium* showed the lowest malate concentration and the most negative  $\delta^{13}\text{C}$  value of malate. *Oxalis* showed the most positive  $\delta^{13}\text{C}$  values of malate (up to -11.2‰) and a concentration of malate at a medium level, whereas *Arbutus* has the highest concentration of malate and a  $\delta^{13}\text{C}$  value at the medium level. To summarize: High amount of respired CO<sub>2</sub> with high <sup>13</sup>C enrichment seems to be connected to low concentration and negative  $\delta^{13}\text{C}$  values of malate (*Halimium*). Light-activation of PEPc in C<sub>3</sub>-plant leaves is known to replenish the Krebs cycle with carbon in case C is removed in form of CO<sub>2</sub> and/or carbon skeletons for biosynthesis (Bowsher *et al.* 2008). Surplus malate (and/or citrate) can be stored temporarily (Bowsher *et al.* 2008) and metabolized later on. The results will be discussed on the basis of partitioning of malate (around the Krebs cycle) into storage, energy-gaining processes and biosynthesis of amino acids.

### References:

- C. Bowsher *et al.* (2008): Plant Biochemistry. Garland Science, NY, USA  
 A. Gessler *et al.* (2009): New Phytol **181**, 374–386  
 P. Priault *et al.* (2009): New Phytol **181**, 400–412  
 C. Werner, A. Gessler (2011): Biogeosci, **8**: 2437–2459  
 C. Werner *et al.* (2007): RCMS **21**, 1352–1360  
 C. Werner *et al.* (2009): RCMS **23**, 2428–2438  
 R.A. Werner *et al.* (2011): New Phytol **191**, 10–15

**VALIDATION OF THE CRAIG-GORDON ISOTOPIC MODEL FOR LAKE EVAPORATION**

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The theory on isotopic composition of evaporation ( $\delta_E$ ) of open water was first proposed by Craig and Gordon in 1965 (C-G model) and has since been widely employed in studies of isotope hydrology and ecology. In a typical application, the interfacial surface water layer is assumed to be well-mixed so that the isotopic composition of the evaporating surface is equal to that of the bulk water. To date, the C-G model and the associated well-mixed assumption have not been validated against field measurements over natural water bodies. In this study, in-situ measurement of  $\delta_E$  was made on a near-continuous basis using the flux-gradient approach over Lake Taihu, a large shallow lake in East China. The key instrument was a water vapor analyzer based on the off-axis integrated cavity output spectroscopy. The analyzer switched between the two intakes at the 1.1 and 3.5 m height above water surface every 30s, measuring the water vapor mixing ratio and the  $H_2^{16}O$ , HDO and  $H_2^{18}O$  water vapor mixing ratios. It was calibrated every 3 h against a vapor standard at 5 concentrations that bracketed the ambient humidity. Hourly  $\delta_E$  was calculated using the gradient data. Supporting measurements included the isotopic compositions of the lake water and meteorological variables to provide inputs to the C-G model. The preliminary results indicate that the model captured the temporal variations in  $\delta_E$ , the model showed better agreement with observation for D than for  $^{18}O$ , and the modeled  $\delta_E$  for D and  $^{18}O$  were much closer to GMWL than the observation.

## DOES THE ADDITION OF LABILE SUBSTRATE DESTABILISE OLD SOIL ORGANIC MATTER?

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Input of fresh organic matter to soil can stimulate microbial activity and modify the mineralization of native soil organic matter (SOM) through a phenomenon called priming effect (PE). Assessing the age of SOM affected by PE is a major challenge because the destabilisation of long-term preserved SOM would be much more damageable for the overall carbon budget than the destabilisation of fresh SOM. We took advantage of Congolese eucalypt plantations established on a former savannah to investigate the age of SOM primed after glucose addition. In these plantations, old SOM exhibited a C4 signature while recent SOM exhibited a C3 signature.

Over one week incubation, we partitioned the relative contributions of glucose, recent SOM and old SOM to CO<sub>2</sub> production by recording emission of <sup>12</sup>CO<sub>2</sub> and <sup>13</sup>CO<sub>2</sub> using a tuneable diode laser spectrometer (TDLS). This was combined with measurements of phospholipids fatty acid (PLFA) biomarkers and with analyses of the microbial potential for various C- substrate using Biolog<sup>TM</sup> tests.

Glucose mineralization peaked one day after its application. Notably a transient emission of C3-CO<sub>2</sub> before the peak of glucose consumption indicate that not all soil microbes but only some specific communities exhibiting a strong feeding preference for recent SOM were triggered by the addition of glucose. A few days after the glucose peak, significant over-mineralisation of recent SOM was detected. A significant positive PE response was observed on old C4 SOM after one week of incubation.

PLFA and Biolog tests showed evidences that various microbial communities were successively responsible for SOM mineralization. Microbes responsible for SOM mineralization just after the peak of glucose consumption exhibited great abilities to rapidly consume a large panel of substrates, whereas microbes extracted from soil one week after glucose addition better utilise SOM-like substrates than the other tested substrates.







## Publications

### Publications

- Memorandum of Understanding (MoU) of COST Action ES0806 SIBAE » [\(PDF\)](#)

The following papers/manuscripts originated from Action activities and/or have authorships with multiple MC members:

- Bahn M, Buchmann N, Knohl A (2012) Stable Isotopes and Biogeochemical Cycles in Terrestrial Ecosystems. *Biogeosciences* 9: 3979–3981
- Bahn M, Lattanzi FA, Wild B, Koranda M, Danese V, Hasibeder R, Brüggemann N, Schmitt M, Siegwolf RTW, Richter A (2013) Responses of belowground carbon allocation dynamics to extended shading in mountain grassland. *New Phytologist* 198: 116–126
- Biasi C, Tavi NM, Jokinen S, Shurpali N, Hämäläinen K, Jungner H, Oinonen M, Martikainen P (2011). Differentiating sources of CO<sub>2</sub> from organic soil under bioenergy crop cultivation: A field-based approach using <sup>14</sup>C. *Soil Biology & Biochemistry* 43: 2406–2409.
- Björnsen A-K, T Rütting, P Ambus (2013) Combined climate factors alleviate changes in gross soil nitrogen dynamics in heathlands. *PNAS* (submitted)
- Brüggemann N, Gessler A, Kayler Z, Keel SG, Badeck F, Barthel M, Boeckx P, Buchmann N, Brugnoli E, Esperschütz J, Gavrichkova O, Ghashghaie J, Gomez-Casanovas N, Keitel C, Knohl A, Kuptz D, Palacios S, Salmon Y, Uchida Y, Bahn M (2011) Carbon allocation and carbon isotope fluxes in the plant–soil–atmosphere continuum: a review. *Biogeosciences Discussion* 8: 3619–3695.
- Brüggemann N, Gessler A, Kayler Z, Keel SG, Badeck F, Barthel M, Boeckx P, Buchmann N, Brugnoli E, Esperschütz J, Gavrichkova O, Ghashghaie J, Gomez-Casanovas N, Keitel C, Knohl A, Kuptz D, Palacios S, Salmon Y, Uchida Y, Bahn M (2011) Carbon allocation and carbon isotope fluxes in the plant–soil–atmosphere continuum: a review. *Biogeosciences* 8: 3457–3489
- Callesen I, Nilsson LO, Schmidt IK, Vesterdal L, Ambus P, Christiansen JR, Högberg P, Gundersen P (2013) The natural abundance of <sup>15</sup>N in litter and soil profiles under six temperate tree species: N cycling traits depend on tree species and site fertility. *Plant and Soil* available on-line
- de Bello F, Buchmann N, Casals P, Lepš J, Sebastià MT (2009) Relating plant species and functional diversity to community  $\delta^{13}\text{C}$  in NE Spain pastures. *Agriculture Ecosystems and Environment* 131: 303–307
- Epron D, Bahn M, Derrien D, Lattanzi F, Pumpanen J, Gessler A, Högberg P, Dannon M, Gerant D, Maillard P, Buchmann N (2012) Pulse-labelling trees to study carbon allocation patterns: a review of methods, current knowledge and future prospects. *Tree Physiology* 32: 776–798
- Gauthier P, Molero G, Mahé A, Hodges M, Nogués S, Tcherkez G (2012) Metabolic origin of <sup>15</sup>N in nitrogenous compounds from *Brassica napus* leaves. *Plant, Cell and Environment* 36: 128–137
- Gavrichkova O, Proietti S, Moscatello S, Portarena S, Battistelli A, Matteucci G, Brugnoli E (2011) Short-term natural  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  variations in pools and fluxes in a beech forest: the transfer of isotopic signal from recent photosynthates to soil respired CO<sub>2</sub>. *Biogeosciences* 8: 2833–2846
- Gentsch L, Hammerle A, Sturm P, Wingate L, Ogeé J, Siegwolf R, Barthel M, Plüss P, Baur T, Buchmann N, Knohl A. Carbon isotope discrimination during branch photosynthesis of *Fagus sylvatica*: A Bayesian modeling approach. *Plant, Cell & Environment* (submitted April 2013)
- Gessler A, Brandes E, Buchmann N, Helle G, Rennenberg H, Barnard R (2009) Tracing the carbon and oxygen isotope signals from newly assimilated sugars in the leaves to the tree ring archive. *Plant Cell and Environment* 32: 780–795.
- Gilbert A, Silvestre V, Segebarth N, Guillou C, Robins RJ, Akoka S, Remaud GS Impact of environmental factors on the intramolecular <sup>13</sup>C distribution in glucose from European grapevines accessed by the site-specific <sup>13</sup>C/<sup>12</sup>C ratio of ethanol determined by isotopic <sup>13</sup>C NMR spectrometry. *Plant Cell and Environment* (submitted February 2010)
- Hellmann C, Sutter R, Rascher K, Máguas C, Correia O, Werner C (2011) Impact of an exotic N<sub>2</sub>-fixing *Acacia* on composition and N status of a native Mediterranean community. *Acta Oecologica* 37: 43–50.
- Kayler K, Gessler G, Buchmann N (2010) What is the speed of link between above- and belowground processes? A comment on the article “The significance of phloem transport for the speed with which canopy photosynthesis and belowground respiration are linked” by Maurizio Mencuccini and Teemu Hölttä. *New Phytologist* 187: 885–888.
- Keel SG, Campbell CD, Högberg MN, Richter A, Wild B, Zhou X, Hurry V, Linder S, Näsholm T, Högberg P (2012) Allocation of carbon to fine root compounds and their residence times in a boreal pine forest depend on root size class and season. *New Phytologist* 194: 972–981
- Keidel A, Von Stetten D, Rodrigues C, Máguas C, Hildebrandt P (2010) Discrimination of green arabica and robusta coffee beans by Raman spectroscopy. *Journal of Agricultural and Food Chemistry* 58: 11187–11192.
- Kodama N, Ferrio JP, Brüggemann N, Gessler A (2011) Short-term dynamics of the carbon isotope composition of CO<sub>2</sub> emitted from a wheat agroecosystem – physiological and environmental controls. *Plant Biology* 13, 115–125.
- Köster J R, Well R, Tuzson B, Bol R, Dittler K, Giesemann A, Emmenegger L, Manninen A, Cárdenas L, Mohn J (2013) Novel laser spectroscopic technique for continuous analysis of N<sub>2</sub>O isotopomers - application and intercomparison with isotope ratio mass spectrometry. *Rapid Commun. Mass Spectrom.* 27(1): 216–222
- Kornfeld A, Horton T, Yakir D, Searle SY, Griffin KL, Atkin OK, Subke J-A, Turnbull MH (2012) A field-compatible method for measuring alternative respiratory pathway activities in vivo using stable O<sub>2</sub> isotopes. *Plant, Cell and Environment* 35:1518–1532
- Lindén A, Heinonsalo J, Buchmann N, Oinonen M, Sonninen E, Hiltavuori E, Pumpanen J (2013) Contrasting effects of increased carbon input on SOM decomposition in boreal forest soil with and without living root system of *P. sylvestris*. *Tree Physiology*, in rev.
- Máguas C, Rascher KG, Martins-Loução MA, Carvalho P, Pinho P, Ramos M, Correia O, Werner C (2011). Responses of woody species to spatial and temporal ground water changes in coastal sand dunes systems. *Biogeosciences Discussion* 8: 1591–1616.
- Máguas C, Rascher KG, Martins-Loução MA, Carvalho P, Pinho P, Ramos M, Correia O, Werner C (2011). Responses of woody species to spatial and temporal ground water changes in coastal sand dunes systems. *Biogeosciences* 8: 3823–3832.
- Milcu A, Lukac, M, Subke J-A, Manning P, Heinemeyer A, Wildman D, Anderson R, Ineson P (2012) Biotic carbon feedbacks in a materially closed soil-vegetation-atmosphere system. *Nature Climate Change* 2: 281–284.
- Mohn J, Tuzson B, Manninen A, Yoshida N, Toyoda S, Brand WA, Emmenegger L (2012) Site selective real-time measurements of atmospheric N<sub>2</sub>O isotopomers by laser spectroscopy. *Atmospheric Measurement Techniques Discussion* 5: 813–838.
- Mohn J, Tuzson B, Manninen A, Yoshida N, Toyoda S, Brand A, Emmenegger L (2012) Site selective real-time measurements of atmospheric N<sub>2</sub>O isotopomers by laser spectroscopy. *Atmos. Meas. Tech.* 5: 1601–1609
- Mohn J, Guggenheim C, Tuzson B, Vollmer M, Emmenegger L. A liquid nitrogen-free preconcentration unit for measurements of ambient N<sub>2</sub>O isotopomers by QCLAS. *Atmospheric Measurement Techniques* (in review)
- Moyano FE, Vasilyeva N, Bouckaert L, Cook F, Craine J, Curiel Yuste J, Don A, Epron D, Formanek P, Franzluebbers A, Iltstedt U, Kätterer T, Orchard V, Reichstein M, Rey A, Ruamps L, Subke J-A, Thomsen IK, Chenu C (2012) The moisture response of soil heterotrophic respiration: interaction with soil properties. *Biogeosciences* 9: 1173–1182
- Nelissen V, Rütting T, Huygens D, Staelens J, Ruyschaert G, Boeckx P (2012) Maize biochars accelerate short-term soil nitrogen dynamics in a loamy sand soil. *Soil Biology & Biochemistry* 55: 20–27
- Rascher C, Máguas C, Werner C (2010) On the use of phloem sap  $\delta^{13}\text{C}$  as an indicator of canopy carbon discrimination. *Tree Physiology* 30 (12): 1499–1514.
- Rascher KG, Große-Stoltenberg A, Máguas C, Meira J, Werner C (2011). *Acacia* invasion impacts vegetation structure and regeneration dynamics in open dunes and pine forests. *Biological Invasions* 13: 1099–1113
- Rascher KG, Hellmann C, Máguas C, Werner C (2012) Community scale <sup>15</sup>N isoscapes: tracing the spatial impact of an exotic N<sub>2</sub>-fixing invader. *Ecology Letters* 15: 484–491.
- Richter A, Wanek W, Werner RA, Ghashghaie J, Jäggi M, Gessler A, Brugnoli E, Hettmann E, Göttlicher SG, Salmon Y, Bathellier C, Kodama N, Nogués S, Søe A, Volders F, Sörgel K, Blöchl A, Siegwolf RTW, Buchmann N, Gleixner G (2009) Preparation of starch and soluble sugars of plant material for analysis of carbon isotope composition: a comparison of methods. *Rapid Communications in Mass Spectrometry* 23: 2476–2488.
- Rodrigues C, Máguas C, Prohaska T (2011) Strontium and oxygen isotope fingerprinting of green coffee beans and its potential to proof authenticity of coffee. *Food Chemistry* 232: 361–373.
- Rodrigues C, Brunner M, Steinman S, Bowen G, Nogueira JMF, Gautz L, Prohaska T, Máguas C, (2011) Isotopes as tracers of the Hawaiian coffee-producing regions. *Journal of Agricultural and Food Chemistry* 59: 10239–10246.
- Rodrigues C, Máguas C, Nogueira JMF (2012) Coffees and industrial blends aroma profile discrimination according to the chromatic value. *Coffee Science* 7(2): 167–176

- Rodrigues C, Maia R, Máguas C (2013) The application of isotope ratio mass spectrometry to the study of the ecophysiology of plant seeds. *Spectroscopy Europe* 25(1): 12-18
- Rütting T, Boeckx P, Müller C, Klemetsson L (2011) Assessment of the importance of dissimilatory nitrate reduction to ammonium for the terrestrial nitrogen cycle. *Biogeosciences Discussions* 8: 1169-1196.
  - Rütting T, Boeckx P, Müller C, Klemetsson L (2011) Assessment of the importance of dissimilatory nitrate reduction to ammonium for the terrestrial nitrogen cycle. *Biogeosciences* 8: 1779-1791
  - Rütting T, Huygens D, Staelens J, Müller C, Boeckx P (2011) Advances in <sup>15</sup>N tracing experiments: new labelling and data analysis approaches. *Biochemical Society Transactions* 39: 279-283.
  - Rütting T, D Huygens, P Boeckx, J Staelens, L Klemetsson (2013) Increased fungal dominance in N2O emission hotspots along a natural pH gradient in organic forest soil. *Biology and Fertility of Soils* DOI 10.1007/s00374-012-0762-6
  - Rühr NK, Offermann CA, Gessler A, Winkler BJ, Ferrio JP, Buchmann N, Barnard RL (2009) Drought effects on allocation of recent carbon: From beech leaves to soil CO<sub>2</sub> efflux. *New Phytologist* 184: 950-961.
  - Sachse D, Billault I, Bowen GJ Chikaraishi Y, Dawson TE, Feakins SJ, Freeman KH, Magill CR, McInerney FA, van der Meer MTJ, Polissar P, Robins RJ, Sachs JP, Schmidt HL, Sessions AL, White JWC, West JB, Kahmen A (2012) Molecular paleohydrology: interpreting the hydrogen isotopic composition of lipid biomarkers from photosynthetic organisms. *Annu. Rev. Earth Planet. Sci.* 40: 221-249
  - Seeber J, Rief A, Richter A, Traugott M, Bahn M (2012) Drought-induced reduction in uptake of recently photosynthesized carbon by springtails and mites in alpine grassland. *Soil Biology & Biochemistry* 55: 37-39.
  - Serrano H, Antunes C, Branquinho C, Martins-Loução MA, Pinto MJ, Máguas C (2013) Ecological niche of a rare Al hyperaccumulator plant. *Plant Ecology & Diversity* (in press)
  - Spiegel JK, Aemisegger F, Scholl M, Winhold FG, Collett J, Lee T, van Pinxteren D, Mertes S, Tilgner A, Herrmann H, Werner RA, Buchmann N, Eugster W (2012) Stable water isotopologue ratios in fog and cloud droplets are not size-dependent. *Atmos. Chem. Phys. Discuss.* 12, 12663-12684
  - Spiegel JK, Aemisegger F, Scholl M, Winhold FG, Collett J, Lee T, van Pinxteren D, Mertes S, Tilgner A, Herrmann H, Werner RA, Buchmann N, Eugster W (2012) Stable water isotopologue ratios in fog and cloud droplets are not size-dependent. *Atmos. Chem. Phys.* 12: 9855-9863
  - Spiegel JK, Aemisegger F, Scholl M, Winhold FG, Collett J, Lee T, van Pinxteren D, Mertes S, Tilgner A, Herrmann H, Werner RA, Buchmann N, Eugster W (2012) Temporal evolution of stable water isotopologues in cloud droplets during HCCT-2010. *Atmos. Chem. Phys. Discuss.* 12: 15139-15175
  - Spiegel JK, Aemisegger F, Scholl M, Winhold FG, Collett J, Lee T, van Pinxteren D, Mertes S, Tilgner A, Herrmann H, Werner RA, Buchmann N, Eugster W (2012) Temporal evolution of stable water isotopologues in cloud droplets during HCCT-2010. *Atmos. Chem. Phys.* 12: 11679-11694
  - Staelens J, Rütting T, Huygens D, De Schrijver A, Müller C, Verheyen K, Boeckx P (2012) In situ gross nitrogen transformations differ between temperate deciduous and coniferous forest soils. *Biogeochemistry* 108: 259-277.
  - Streit K, Rinne KT, Hagedorn F, Dawes MA, Saurer M, Hoch G, Werner RA, Buchmann N, Siegwolf RTW (2013) Tracing carbon allocation in Larix decidua exposed to elevated CO<sub>2</sub> and soil warming at the alpine tree line using compound-specific stable isotope analysis. *New Phytologist* 197: 838-849
  - Sturm P, Tuzson B, Henne S, Emmenegger L (2013) Tracking isotopic signatures of CO<sub>2</sub> at Jungfraujoch with laser spectroscopy: analytical improvements and exemplary results. *Atmos. Meas. Tech. Discuss.* 6: 423-459
  - Subke J-A, Voke N, Leronni V, Garnett M, Ineson P (2011) Dynamics and pathways of autotrophic and heterotrophic soil CO<sub>2</sub> efflux revealed by forest girdling. *Journal of Ecology* 99: 186-193.
  - Subke J-A, Carbone MS, Khomik M, Stoy P, Bahn M (2012) Biotic interactions and biogeochemical processes in the soil environment. *Biogeosciences* 9:1823-1825
  - Subke J-A, Heinemeyer A, Vallack HW, Leronni V, Baxter, R, Ineson, P (2012) Fast assimilate turnover revealed by in situ <sup>13</sup>C<sub>2</sub> pulse labelling in Subarctic tundra. *Polar Biology* DOI: 10.1007/s00300-012-1167-6
  - Tcherkez G, Schäufele R, Nogués S, Piel C, Boom A, Lanigan G, Barbaroux C, Mata C, Elhani S, Hemming D, Maguas C, Yakir D, Badeck Fw, Griffiths H, Schnyder H, Ghashghaie J (2010) On the <sup>13</sup>C/<sup>12</sup>C isotopic signal of day and night respiration at the mesocosm level. *Plant Cell And Environment* 33: 900-913.
  - Tuzson B, Henne S, Brunner D, Steinbacher M, Mohn J, Buchmann B, Emmenegger L (2011) Continuous isotopic composition measurements of tropospheric CO<sub>2</sub> at Jungfraujoch (3580m.a.s.l.), Switzerland: real-time observation of regional pollution events. *Atmospheric Chemistry and Physics* 11: 1685-1696.
  - Unger S, Máguas C, Pereira JS, Aires L, David T, Werner C (2010) Disentangling drought-induced variation in ecosystem and soil respiration by stable isotope partitioning. *Oecologia* 163: 1043-1057.
  - Unger S, Máguas C, Pereira JS, David T, Werner C (2010) The influence of precipitation pulses on soil respiration – Assessing the “Birch effect” by stable carbon isotopes. *Soil Biology and Biochemistry* 42: 1800-1810.
  - Unger S, Máguas C, Pereira JS, David TS, Werner C (2012) Interpreting post-drought rewetting effects on soil and ecosystem carbon dynamics in a Mediterranean oak savannah. *Agricultural and Forest Meteorology* 154-155: 9-18
  - Vallack HW, Leronni V, Metcalfe DB, Högberg P, Ineson P, Subke J-A (2012) Application of nitrogen fertilizer to a boreal pine forest has a negative impact on the respiration of ectomycorrhizal hyphae. *Plant and Soil* 352: 405-417.
  - Vargas R, Baldocchi D, Allen MF, Bahn M, Black TA, Collins SL, Curiel Yuste J, Hirano T, Jassal RS, Pumpanen J, Tang J (2010) Looking deeper into the soil: biophysical controls and seasonal lags of soil CO<sub>2</sub> production and efflux across multiple vegetation types". *Ecological Applications* 20(6) 1569-1582.
  - Vinagre C, Maguas C, Cabral HN, Costa MJ (2012) Effect of body size and body mass on delta C-13 and delta N-15 in coastal fishes and cephalopods. *Estuarine Coastal And Shelf Science* 95: 264-267.
  - Vinagre C, Máguas C, Cabral HC, Costa MJ (2012) Food web structure of the coastal area adjacent to the Tagus estuary revealed by stable isotope analysis. *Journal of Sea Research* 67(1): 21-26
  - von Felten S, Niklaus PA, Scherer-Lorenzen M, Hector A, Buchmann N (2012) Do grassland plant communities profit from N partitioning by soil depth? *Ecology* 93: 2386-2396
  - Werner C, Zumkier U, Beyschlag W, Máguas C (2010) High competitiveness of a resource demanding invasive Acacia under low resource supply. *Plant Ecology* 206: 83-96
  - Werner C, Máguas C (2010) Carbon isotope discrimination as a tracer of functional traits in a Mediterranean macchia plant community. *Functional Plant Biology* 37 (5): 467-477.
  - Werner C, A Gessler (2011) Diel variations in the carbon isotope composition of respired CO<sub>2</sub> and associated carbon sources: a review of dynamics and mechanisms. *Biogeosciences Discussion* 8: 2183-2233.
  - Werner C, A Gessler (2011) Diel variations in the carbon isotope composition of respired CO<sub>2</sub> and associated carbon sources: a review of dynamics and mechanisms. *Biogeosciences* 8: 2437-2459.
  - Werner C, F Badeck, E Brugnoli, B Cohn, M Cuntz, T Dawson, A Gessler, J Ghashghaie, TEE Grams, Z Kayler, C Keitel, M Lakatos, X Lee, C Máguas, J Ogée, KG Rascher, H Schnyder, R Siegwolf, S Unger, J Welker, L Wingate, MJ Zeeman (2011) Linking carbon and water cycles using stable isotopes across scales: progress and challenges. *Biogeosciences Discussion* 8: 2659-2719.
  - Werner RA, Buchmann N, Siegwolf RTW, Kornel BE, Gessler A (2011) Metabolic fluxes, carbon isotope fractionation and respiration – lessons to be learned from biochemistry. *New Phytologist* 191: 10-15
  - Werner C, F Badeck, E Brugnoli, B Cohn, M Cuntz, T Dawson, A Gessler, J Ghashghaie, TEE Grams, Z Kayler, C Keitel, M Lakatos, X Lee, C Máguas, J Ogée, KG Rascher, H Schnyder, R Siegwolf, S Unger, J Welker, L Wingate, MJ Zeeman (2012) Linking carbon and water cycles using stable isotopes across scales: progress and challenges. *Biogeosciences* 9: 3083-3111
  - Wolf B, Chen W, Brüggemann N, Zheng X, Pumpanen J, Butterbach-Bahl K (2011) Applicability of the soil gradient method for estimating soil-atmosphere CO<sub>2</sub>, CH<sub>4</sub> and N<sub>2</sub>O fluxes for steppe soils in Inner Mongolia. *Journal of Plant Nutrition and Soil Science*. DOI: 10.1002/jpln.201000150
  - Wunderlin P, Lehmann MF, Siegrist H, Tuzson B, Joss A, Emmenegger L, Mohn J (2013), Isotope signatures of N<sub>2</sub>O in a mixed microbial population system: Constraints on N<sub>2</sub>O producing pathways in wastewater treatment. *Environ. Sci. Technol.* 47 (3): 1339-1348
  - Wunderlin P, Mohn J, Joss A, Emmenegger L, Siegrist H-R (2012), Mechanisms of N<sub>2</sub>O production in biological wastewater treatment under nitrifying and denitrifying conditions. *Water Res.* 46 (4): 1027-1037



## Working Groups

- Working Group 1
- Working Group 2
- Working Group 3
- Working Group 4

## Working Groups

### **WG1:**

Review and synthesis of existing field experiments using stable isotope analyses as quantitative tools to understand, trace and partition C, N, O and water cycles within the biosphere- atmosphere-Earth system and to identify innovative process- and system-oriented research areas,

### **WG2:**

Assessment of current state-of-the-art soil models to improve soil process representation based on stable isotope studies and to closer link experimental and modelling communities,

### **WG3:**

Harmonizing and benchmarking the development of innovative cutting-edge technologies for stable isotope analyses,

### **WG4:**

Training and capacity building in stable isotope applications across Europe.



## Working Groups

[Working Group 1](#)

[Working Group 2](#)

[Working Group 3](#)

[Working Group 4](#)

## Working Group 1

Leaders: Cristina Maguas (P), Roland Bol (DE)

### Objective

Review and synthesis of existing field experiments using stable isotope analyses as quantitative tools to understand, trace and partition C, N, O and water cycles within the biosphere- atmosphere-Earth system and to identify innovative process- and system-oriented research areas.

### Workshops

WG 1 met with WGs 2 and 3 for a workshop on “**Compound specific isotope analyses**” in Barcelona in October 2010 (14 and 15 October 2010). Coordinators: Salvador Nogues (E), Jaleh Ghashghaie (F), Pascal Boeckx (BE)

The aims of this WG1-3 workshop were to collect information about the latest research on compound-specific stable isotope analysis (CSIA) and post-photosynthetic isotope fractionations. It brought together researchers working with NMR as well as IRMS on mainly carbon and nitrogen containing compounds. The program and the outcomes of these discussions can be found [here](#).

WG 1 met for a workshop on “**Intercomparison of Compound specific isotope analyses**” in Ghent in June 2012 (22 and 23 June 2012). Coordinator: Pascal Boeckx (BE)

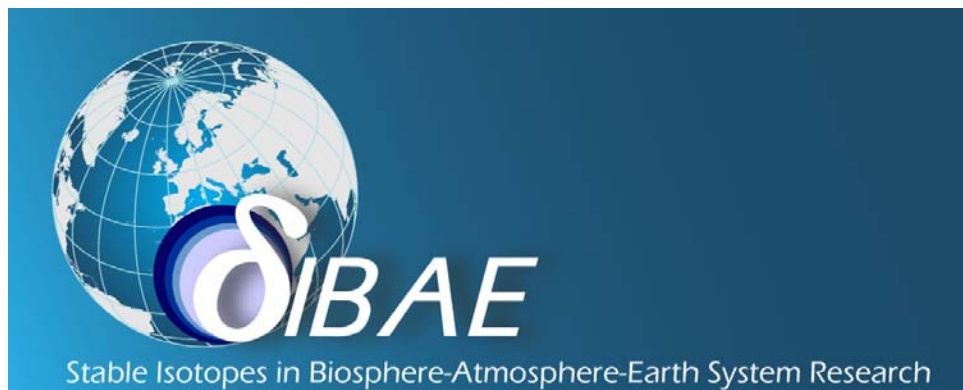
The aims of this WG1 workshop were to identify technical problems and solutions with GC- and LC-IRMS equipment - including harmonization of analytical protocols, to share and compare sample preparation protocols for GC- and LC-IRMS analyses (i.e., extraction, storage and sample preparation; target compounds: amino sugars, sugars, amino acids, organic acids, n-alkanes) and to discuss the need for standards for CSSIA work. The outcomes of these discussions can be found [here](#).

### Isotope user directory

Please register [here](#)

**COST-SIBAE meeting**  
**14<sup>th</sup> – 15<sup>th</sup> October 2010**  
**Barcelona, Catalonia, Spain**

**WG1/2/3 meeting on specific  
compounds and post-photosynthesis**





**Program of the COST-SIBAE meeting**  
**14<sup>th</sup>-15<sup>th</sup> October 2010 – Barcelona, Catalonia, Spain**

**Scientific Organising Committee:**

- **Salvador Nogués** (University of Barcelona, Spain)
- **Jaleh Ghashghaie** (University Paris Sud XI, France)
- **Pascal Boeckx** (University of Ghent, Belgium)

**Local Organisers:**

- **Salvador Nogués** (University of Barcelona, Spain)
- **Salvador Aljazairi** (University of Barcelona, Spain)

**Program:**

▪ **Day 1 (14/10/10)**

**13:00 – 14:00 Lunch (optional)**

**14:00 – 14:15 Welcome and coordinator presentation**

**14:15 – 17:30 Session 1: Specific compounds (25' presentations and plenary discussion)**

**Chair: Jaleh Ghashghaie**

14:15 *Andreas Richter – Methods to measure carbohydrates*

14:45 *Richard Robins – Compound specific in plant sugars at natural abundance obtained by NMR*

15:15 *Pascal Boeckx – Amino sugars by LC-IRMS: method development and turnover assessment*

15:45 *Coffee break*

16:15 *Gemma Molero –  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  of aminoacids by GC-C-IRMS*

16:45 *Discussion*

▪ **Day 2 (15/10/10)**

**9h00 – 12:15 Session 2: Post-photosynthesis and respiration (25' presentations and plenary discussion)**

**Chair: Pascal Boeckx**

9:00 *Dan Yakir – Can we obtain quantitative physiological information from tree rings  $^{13}\text{C}$ ?*

9:30 *Rolf Siegwolf – Is it worth the effort to extract cellulose from plant bulk material*



*to link environmental changes with stable isotopes?*

10:00 Jaleh Ghashghaie – *Metabolic origin of respired <sup>13</sup>CO<sub>2</sub>: autotrophic vs heterotrophic organs*

10:30 *Coffee break*

11:00 Christiane Werner – *Short-term dynamics of isotopic fractionation during dark respiration at the leaf, plant and ecosystem scales*

11:30 Discussion

**12:45 – 14:00 Lunch**

**14:00 – 17:15 Session 3: Carbohydrates in soil and modelling (25' presentations and plenary discussion)**

**Chair: Nina Buchmann**

14:00 Michael Bahn – *Effects of photosynthesis on the transfer and respiratory use of carbon in the plant-soil system*

14:30 Jan Willem van Groenigen – *Are biofuel rest products suitable for soil amendment? An isotope tracing study*

15:00 Pitter Ferrio – *Differential water uptake patterns among tree species: tracking water isotopes from soil to xylem*

15:30 *Coffee break*

16:00 Fabrice Elegbédé – *A compound specific model of soil organic carbon dynamics : application to carbohydrates*

16:30 Discussion

**17:15 End of the meeting**

**Time schedules:**

Timing	Day 1 – 14/10/10	Day 2 – 15/10/10
9:00 – 13:00		Plenary session 2 <i>Residència</i>
13:00 – 14:15	<i>Lunch (optional)</i>	<i>Lunch</i>
14:15 – 18:00	Plenary session 1 <i>Residència</i>	Plenary session 3 <i>Residència</i>
Evening	<i>Visit of the Gothic quarter Tapas y Cerveza at Restaurant "Origens"</i>	



## Logistical information

### ▪ Accommodation :

#### CSIC-Residència Investigadors

c/ Hospital, 64

Barcelona

Tel. : 93 443 86 10

fax. : 93 442 82 02

Web link : [http://www.resa.es/eng/residencias/investigadors/\(reservas\)/Curso](http://www.resa.es/eng/residencias/investigadors/(reservas)/Curso)

The Investigadors Residence Hall is in the centre of Barcelona, next to *Plaça Catalunya*.



### ▪ Meeting

The meeting will take place also at the Residència d'Investigadors (Room **Sala d'exposicions**)

### ▪ Contact

#### Dr Salvador Nogués :

Office: +34 93 403 72 43 (direct) - Secretary: +34 93 40214 65

[salvador.nogues@ub.edu](mailto:salvador.nogues@ub.edu)

#### Mr Salvador Aljazairi

Secretary: +34 93 40214 65

[sal337@hotmail.com](mailto:sal337@hotmail.com)

### ➤ How to get from the Prat airport to the Investigadors Residence Hall?

The airport, which is 13 km from Barcelona, is well communicated with the city centre by several means of transport. Please note that there are two terminals (T1 and T2). Most fly arrives at the T1 terminal.



- **Taxi** (T1 and T2): it usually costs between 25 and 30 €.

- **Train and underground** (train station located at T2, free buses between T1 and T2 for train station): there are trains to Barcelona every 30 minutes. You must get off at the *Passeig de Gràcia* station and catch the underground (L3) until you get to *Liceu*. The residence hall is a 2-minute walk away. It is next to the CSIC building, on the corner of *Hospital* Street with *Egipcíaques* Street (see map above).

- **Bus** (Aerobus T1 and T2) (**best option**): they stop at the *Plaça Catalunya* station, which is very near the residence hall (an approximate 10 minutes walking down *La Ramblas*, see map above).



#### ▪ Restaurants

##### ➤ Restaurant for Dinner – 14<sup>th</sup> of February (20h00):

Restaurant « Origen»

**Address:** Carrer Enric Granados, 9.

**How to get there?** : underground (L3), station “Catalunya”

**Menu price:** 27,5 Euros (including wine)

##### ➤ Restaurant for Lunch – 15<sup>th</sup> of February (13h00):

Restaurant «Antic Forn»

**Address:** Carrer Pintor Fortuny, 28.

**How to get there?** : underground (L3), station “Liceu” (near Residència d’Investigadors)

**Menu price:** 13,5 Euros (all included)

#### List of participants:

1. Salvador Nogués (University of Barcelona, Spain)
2. Nina Buchmann (ETH Zurich, Switzerland)
3. Jaleh Ghashghaie (University Paris Sud XI, France)
4. Pascal Boeckx (University of Ghent, Belgium)
5. Roland Bold (BBRS, UK)
6. Richard Robins (University of Nantes, France)
7. Enrico Brugnoli (CNR, Italy)
8. Per Ambus (Risø National Laboratory for Sustainable Energy, Denmark)
9. Andreas Richter (University of Vienna, Austria)
10. Poul Erik (Roskilde University, Denmark)
11. Dan Yakir (Weizmann Institute of Science, Israel)
12. Jan Willem van Groenigen (Wageningen University, The Netherlands)
13. Cristina Maguas (Institute for Science and Applied Technology, Portugal)
14. Rolf Siegwolf (Paul Scherrer Institute, Switzerland)



15. Jussi Heinonsalo (University of Helsinki, Finland)
16. Maria Teresa Sebastià (CTFC, Spain)
17. Rosa Llurba (CTFC, Spain)
18. Àngela Ribas (CTFC, Spain)
19. Fabrice Gouriveau (CTFC, Spain)
20. Iker Aranjuelo (University of Barcelona, Spain)
21. Gemma Molero (University of Barcelona, Spain)
22. Salvador Aljazairi (University of Barcelona, Spain)
23. Luis López-Sangil (University of Barcelona, Spain)
24. Neus Otero (University of Barcelona, Spain)
25. Jordi Palau (University of Barcelona, Spain)
26. Monica Aguilera (University of Barcelona, Spain)
27. Pilar Teixidor (SCT-UB, Spain)
28. Juan Pedro Ferrio (University of Lledia, Spain)
29. Laura Llorens (University of Girona, Spain)
30. Maria Paz Esquivias Segura (University of Sevilla, Spain)
31. Victor Resco (University of Castilla-La Mancha, Spain)
32. Joan Romanyà (University of Barcelona, Spain)
33. Mireia Martí (University of Barcelona, Spain)
34. Miriam Burriel (University of Barcelona, Spain)
35. Jorge Curiel (Autonomous University of Barcelona, Spain)
36. Roland A. Werner (ETH Zurich, Switzerland)



## Summary of the COST-SIBAE meeting of WG1/2/3 on specific compounds and post-photosynthesis, held in Barcelona, Catalonia, Spain, during 14th and 15th October 2010

The meeting had three major sessions:

- Session 1 on Specific compounds, chair by Jaleh Ghashghaie
- Session 2 on Post-photosynthesis and respiration, chair by Pascal Boeckx
- Session 3 on Carbohydrates in soil and modelling, chair by Nina Buchmann

In the first session (afternoon 14/10/10), Andreas Richter presented three methods for measuring carbohydrates (GC-IRMS, GC-MS and LC-IRMS). Regarding the GC-IRMS he proposed to use the PA20 column, which turned out to be better than Ca or Pb based columns. Richard Robins presented  $^{13}\text{C}$  positional data of starch (glucose) using NMR. He showed that C1, C3 and C4 on glucose were clearly  $^{13}\text{C}$  enriched

Pascal Boeckx showed results of amino-sugars cell wall of soil microorganisms by LC-IRMS. Gemma Molero presented a new method for  $\text{d}^{13}\text{C}$  and  $\text{d}^{15}\text{N}$  determination of aminoacids in plants by GC-IRMS. She showed a fluxome between the nodule and the root in *Medicago sativa* plants.

During the discussion of this session, a methodological group was established and agreed to meet during the meeting (see below).

In the second session (morning 15/02/10), Dan Yakir informed about the information that can be obtained from the  $\text{d}^{13}\text{C}$  of tree rings.

Rolf Siegwolf showed a method to measure  $\text{d}^{13}\text{C}$  of cellulose (published in Sidorova et al 2008 JGRB).

Jaleh Ghashghaie presented an overview of more than 10 years of research on the isotopic fractionation during dark respiration. She also showed the metabolic origin of the  $\text{d}^{13}\text{C}$  signal in autotrophic and heterotrophic plant organs.

Christiane Werner presented also data on  $\text{d}^{13}\text{C}$  of respired  $\text{CO}_2$ , and concluded that large diurnal differences in  $\text{d}^{13}\text{C}$  of respired  $\text{CO}_2$  depend on plant functional groups and environmental conditions.

The discussion was mainly focused on understanding why we lose the  $\text{d}^{13}\text{C}$  signal of leaf-respired  $\text{CO}_2$  at the ecosystem level.

In the third session (afternoon 15/02/10), Michael Bahn combined  $^{13}\text{CO}_2$  pulse-labelling and shading experiments in order to study the transfer of C in the plant-soil system.

Jan Willem van Groenigen presented a potential use of isotopes to study bionergy and its residual products (RPs).

Pitter Ferrio presented data on  $^{18}\text{O}$  obtained in Freiburg (SW Germany) and Oliola (WE Spain).

Fabrice Elegbédé presented a new model on compound specific of soil organic carbon dynamics.

The discussion was mainly focused on modeling and WP2 links to soil modeling.

The following announcements were also made during the meeting:

- Dan Yakir presented the isonumbers as part of bionumbers.org website.
- Richard Robins presented the meeting Isotopes 2011 to be held from 20 to 25 June 2011 in France.



### Mean conclusions of the ‘methodological group’:

1. Andreas Richter will prepare "laboratory standards" for the purpose of interlaboratory comparisons. These standards are not “real” standards as we will not be able to know the real isotopic values for target compounds.
2. The target compounds are:
  - + low-molecular-weight carbohydrates (glucose, fructose, sucrose), starch and polyols (myo-inositol and methylated inositols, if present)
  - + organic acids (malate, citrate, and others if present)
  - + free amino acids (second priority)
3. We will use 2 species, soybean shoots (*Glycine max*) and either a grass or spruce, labelled and unlabelled (4 samples altogether). We need to prepare at least 500 g of each material. Distribution of material will be in 0.5g batches. Each laboratory will only get 2 samples.
4. The standard material will be labelled ( $^{13}\text{CO}_2$  pulse for a couple of hours, no pulse chase; exact conditions will be determined later, the aim is to get the target compounds enriched compared to the bulk material. Sugars should be labelled at least at  $\delta^{13}\text{C}$  of +500 ‰ to ensure that the other target compounds will be also slightly labelled.
5. The samples will be finely ground and thoroughly mixed to ensure homogenous material and designated a unique sample name (e.g., SIBAE Glycine-2). For each of the samples, a complete GC-MS spectrum will be recorded and the concentration of all target compounds will be determined. This information will be supplied with all standards.
6. A group of labs (5-10) that already have experience with compound specific analysis of sugars, organic acids, starch and amino acid will analyse the samples prior to distribution to get baseline values for  $^{13}\text{C}$ .
7. A website will be set up and samples will be distributed to all interested labs. When ordering the standards, users will have to agree to provide isotope values for the target compounds and to publish these values together with their own CSI values.
8. At the Malaga MC meeting, a workshop shall be proposed to compare CSI methods for sugars and organic acids (maybe also amino acids), but there are currently not too many labs actually analyse AA yet.
9. Andreas Richter will try to look into possibilities to measure the C isotope composition in starch directly with a 1280 SIMS. It is not clear if this will be possible at natural abundance level, but if so, it would be a breakthrough to get a starch standard material.
10. The ETH Zürich (Nina Buchmann & Roland Werner) agreed to grow the two plant species and perform the labelling. The University of Vienna (Andreas Richter) agreed to determine concentrations (GC-MS, GC, HPLC) of sugars, organic acids and amino acids. The website and distribution of material can be done by the Andi's lab, provided that some money (a few thousand Euro) would come from outside the University of Vienna. Volunteers for a SIBAE workshop (preferably with experience in HPLC- and GC-IRMS) are highly welcome.

**WG 1 met with WG 3 for a workshop on “Intercomparison of Compound specific isotope analyses” in Ghent in June 2012 (22 and 23 June 2012). Coordinator: Pascal Boeckx (BE)**

GC-C working group. Filley Notes.

GC-C.  
Thematic Areas.

Urgent Need: designate worker bees to mine ISOGEOCHEM threads for best management practices. Specific High priority issues discussed below.

1. Sample collection and storage (analyte specific). Recommend general guidelines for possible isotope fractionation impacts (e.g. storage time and conditions, what kind of vials and archives are useful; prepping prior to storage including grinding).
2. Sample preparation before analysis (analyte specific). Flow chart of sample preparation for purification and derivatization of compounds for GC-C. Highlight separation of labeled and unlabeled material. Convene AGU/EGU BMP session for sample prep etc for GC-C with goal of developing manual for community. Announce on ISOGEOCHEM. With respect to derivatization--compile isotope effects and recommendations for correction.
3. Understand range of analytes applicable for GC-C and recommended column/phases and diverter methods (flow chart).
4. Develop and test analysis method specific to analytes. GC programming, column, injection type, flow diverter type---know limitations of GC-C kit and method. Focus on low molecular fractionation with diverters or injector type.
5. Develop working lab manual that highlights specific common technical difficulties related to: injector, column, combustion/pyrolysis/reduction column, source inlet IRMS response, gas purity, leaks, maintenance.
6. Develop working lab trouble shooting manual that highlights specific common technical difficulties related to: injector, column, combustion/pyrolysis/reduction column, source inlet IRMS response, gas purity, leaks, maintenance.
7. Suggestions for data processing and data storage
  - determine protocols for peak integration and acceptable peak overlap
  - make sure to store raw data also methods and events.
  - make note of internal std values (make sure to add internal stds)
  - constantly update std results in active file excel type file.
  - insist on good instrument notebook documentation.
  - active backup protocols of raw data accessible by group manager for 10+ years.
8. Choose range of standards and their proper use.
  - establish QA/QC for stds (extraction IS, ARS, Isotope std).
  - establish lab working std for specific extractions (plant, soils)
9. Recommended training steps and background knowledge of instrumentation-lab protocol and behavior
  - basic instrument configuration
  - sample preparation
    - screening
    - storage on/chemistry.

10. NOTE-When you process samples establish separate regions in lab for your stable isotope labeled and unlabeled materials. Sample, glassware, lab bench handling.

11. Determine acceptable range in stable isotope labels that can be processed in your lab.

## LC-IRMS brainstorming

Separate systems for LC- and GC-IRMS recommended

Water quality – pre-cleaner in front of Millipore device

Rather LC than IRMS specialist, with technical skills and calm hands

Reduction furnace: increasing filament lifetime, reducing variability

Sample purification: purify as far as possible to increase column lifetime and improve separation

“It’s worth the suffering.”

Put slides of Pierre-Joseph in presentation for comparison

Is the LC Isolink unnecessarily complicated? – separation simpler in the Isoprime system

Why does it always have to stay open? – visual inspection necessary (filter clogging etc.)

Design too delicate for high-maintenance

Liquid lines above electronics

Start a blog?

DOC – carbonate issues

Standards and reference material:

Do we need a referenced standard compound for comparison?

### Ten Golden Rules

- Purify your samples as far as possible (and make sure you don’t have a fractionation)
- Purify eluent and reagents
- Use referenced standards of all compounds in the sample at different concentrations and  $\delta^{13}\text{C}$  values to calibrate your system
- Make sure the peak area per C is the same for each compound -> make direct comparisons by direct injections with reference material of the compound to be analyzed
- Keep a logbook of essential system parameters and of modifications
- Do not be shy to ask experienced experts
- HPLC experience

### Major issues for Thermo

- Reduction reactor

-





## Working Groups

Working Group 1

Working Group 2

Working Group 3

Working Group 4

## Working Group 2

Leaders: Daniel Epron (F), Thomas Wutzler (DE)

### Objective

Assessment of current state-of-the-art soil models to improve soil process representation based on stable isotope studies and to closer link experimental and modelling communities.

### Workshops

WG 2 met for a workshop close to Innsbruck in September 2009 (23 September 2009 - Friday 25 September 2009). Coordinators: Michael Bahn (A), Daniel Epron (F), Thomas Wutzler (DE)

The aim of the workshop was to assess the current state of **using stable isotopes in the development of models of soil respiration and soil organic matter cycling**. Further, the workshop aimed at exchanging experience and at synergy effects. The main question was which processes require a better representation and how stable isotope studies can help to improve this representation. A publication is anticipated which reviews current work and suggest further paths of development. Improving process-based models will indeed require strengthening the link between experimentalists (both ecosystem-based and plant-based studies) and modelers. Increasing scientific interactions between these two communities will help to answer the questions posed during the workshop. Thus, the major part of the workshop was devoted to discussions. As a result, new activities [new activities](#) were proposed

WG 2 met for a workshop on "**Analysing post labelling experiments**" in Nancy in March 2010 (2./3. March 2010). Coordinator: Daniel Epron (F)

This meeting brought together researchers from Europe that are involved or have recently been involved in research projects in which labelling experiments were conducted. It aimed at exchanging experiences and at synergy effects among groups. The objective was to discuss and compare existing methods for analysing post labelling, and to prepare a methodological paper about advantages and disadvantages of the different approaches and about recommendations on how to analyse such experiments in the future. The major part of the workshop was devoted to discussions and short presentations highlighting hot spots of ongoing work in this field. The program and the summary of the outcome can be found [here](#).

WG 2 met for a workshop on "**Advances in N tracer experiments and <sup>15</sup>N methods**" in Gothenburg in March 2011 (9.-11. May 2011). Coordinator: Tobias Rütting (SE)

This meeting brought together researchers from Europe that use <sup>15</sup>N at tracer or natural abundance levels to understand various processes relevant in biogeochemical cycles as well as plant competition. Short presentations as well as intense discussion sections enabled a fruitful exchange and lead to the identification of important, but still open research questions. The summary of these open questions can be found [here](#).

WG 2 met for a workshop on "**Integrating vertical dimension in soil carbon studies/models using natural abundance of stable isotopes**" in Nancy in October 2011 (17.-20. October 2011).

Coordinators: Bernard Longdoz (F), Delphine Derrien (F), Daniel Epron (F)

The objectives of this meeting were to identify causes of vertical and temporal fluctuations in soil CO<sub>2</sub> and soil carbon isotopic signature and to distinguish knowledge and gaps about processes causing carbon isotope discrimination in the soil profile. The workshop gathered 30 participants from both CO<sub>2</sub> fluxes and soil organic matter communities, including experimentalists and modellers (to investigate how models and new experiments could improve our understanding). It started with presentations providing an overview on isotope discrimination processes within the soil vertical profile, of current methodological limitations and of various ongoing modelling approaches. A large part of the workshop was devoted to discussions about how models and new experiments could improve our understanding. These discussions lead to the formulation of a roadmap for future activities. These activities should join forces of the two scientific communities with the goal to make progress on the understanding of subsoil processes by making use of carbon isotope discrimination. A synopsis of a meta-analysis paper (about the stable carbon isotopes composition of soil organic matter and its vertical distribution) was also produced.

WG 2 met for a workshop on "**<sup>15</sup>N tracing modelling**" in Gothenburg in November 2012 (12 to 14 November 2012 ). Coordinator: Tobias Rütting (SE)

Ten participants (of which half were post-graduate students) discussed issue on how to best use nitrogen isotopes to learn about plant-soil processes. As a start, several presentations were given as stimulus for subsequent discussions. Topics ranged from "Long-term tracing of whole catchment <sup>15</sup>N additions: measurements and simulations with the TRACE model", "Modelling of triplet <sup>15</sup>N tracer experiments - results and challenges", "Complex <sup>15</sup>N tracing models - new insights into N dynamics" to "Gross rates of protein depolymerization and amino acid consumption measured by <sup>15</sup>N pool dilution technique". But most of the time was used to discuss relevant issues. The summary of these open questions can be found [here](#).

WG 2 met for a workshop on "**Understanding the movement and significance of DON in terrestrial ecosystems**" in Storrs Hall, Windermere (UK) in April/May 2013 (29 April to 1 May 2013 ).

Coordinators: Jens Subke (UK), Franciska de Vries (UK), Richard Bardgett (UK) & Roland Bol (DE)

The general scope of the workshop was to generate a synthesis of current knowledge of the behavior and ecological significance of DON in terrestrial ecosystems. Key questions addressed were: (1) What are key sources & sinks, and what determines turnover of DON? (2) What do we know about the fate and ecological significance for community dynamics (productivity and community structure) of DON in contrasting ecosystems? (3) What techniques and new technologies are available to address these questions? The summary can be found [here](#).

WG 2 met for a workshop on "**Nitrogen cycle and stable isotopes - Focus on nitrification and denitrification**" in Ski (Norway) in April 2013 (22 to 24 May 2013 ). Coordinators: Hanna Silvennoinen and Peter Dörsch (both NO)

The participants focussed on intensive discussions about various issue of nitrification/denitrification research, using nitrogen isotopes as well as nitrogen isotopologues. Talks and round table discussions resulted in new insight and new plans. The summary can be found [here](#).

WG 2 met for a workshop on "**Priming of soil organic carbon**" in Copenhagen (Denmark) in September 2013 (9 to 10 September 2013 ). Coordinator: Per Ambus (DK)

The general topic of the workshop was on new developments and observations on the application of stable isotopes to study the extent of soil carbon priming and important drivers for this process. The summary can be found [here](#).

- Planned activities
    - Mathematical tools for analysing pulse labelling data
      - Meeting spring 2010
      - including both field and greenhouse
    - Modelling vertical soil profile of SOM and CO<sub>2</sub> isotope
      - Selection of the dataset 2010, Workshop in 2011
      - including <sup>18</sup>O, including fractionation during decomposition
      - open to N<sub>2</sub>O (and N in SOM)
  - Possible activities (have to be planned)
    - hydrological modelling, watershed to regional scale
    - Saturated/Unsaturated zone (deep zones, peat, anoxic processes, methane, ...)
    - Modelling above ground processes
      - Post-photosynthetic fractionation, phloem transport, storage ...
  - Joined activities with COST action “Belowground carbon turnover in European forests (FP0803) I. Brunner”
    - meeting in January
    - Alf Ekblad, Daniel Epron
-

## **Mathematical tools for analyzing transfer of labeled compounds in pulse labeling experiments**

- The objectives of this working subgroup will be to :
  - To federate people involved in labelling experiments in the COST action (6 countries, 8 groups).
  - To address the question of the different ways of expressing results and agree of the best ones (depending on context and questions)
  - To list equations used to analyse post labelling signals, to identify their weaknesses, and to propose new tools that will be better adapted to high temporal resolution of post labelling data
  - To make recommendations to experimentalists that are planning labelling experiments for the different aspects of the experiment :
    - labelling, tracing, analysing data => publication
- Spring 2010 (D. Epron, M. Bahn, C. Biasi...)
- 12 persons => 10 k€(+ organising cost)

### **Model for Organic Carbon and CO<sub>2</sub> Isotopes in Soil Profiles (MOCCISP)**

- The objectives of this working subgroup will be to :
  - To initiate the collaboration between few representative modellers working on distinct but strongly related fields: the biochemical production processes, and the physical transport and fluxes. Such collaboration will really enhance the progresses in soil carbon dynamics knowledge.
  - To built the coupling between biochemical and physical models and comes with MOCCISP offering different levels of complexities depending on specific applications;
  - To produce some lists of the measurements needed in view of the parameterisation, calibration and validation of the MOCCISP;
  - Find the site(s) with the more appropriate data set in view to parameterise, calibrate and validate MOCCISP
- Summer 2011 (B. Longdoz, D. Derrien, L. Wingate, ...)
- 16-20 persons => 13-16 k€(+ organising cost)

**COST Action ES806**  
**Stable Isotopes in Biosphere-Atmosphere-Earth System Research (SIBAE)**

WG2 Workshop: "Analysing post labelling experiments"  
Tuesday 2 March 2010 (9 am) - Wednesday 3 March 2010 (5 pm)

**Aims and outline of the workshop**

Stable isotopes are widely used in ecology as tracers in ecosystem fluxes and trophic webs. The tracing of carbon, nitrogen or water in the soil-plant-atmosphere system can be done by pulse-labelling the plants with labelled substrate for a short period of time. The heavy isotope (<sup>13</sup>C, <sup>15</sup>N, <sup>2</sup>H, <sup>18</sup>O) is then tracked in the plant, in the soil and in the effluxes (respiration, transpiration...) during the following days and weeks. This approach was mostly used under controlled conditions but during recent years, several field experiments have been implemented in both grassland and forest ecosystems. In addition, the development of laser diode spectrometry tools allows tracing isotope in gaseous efflux or water samples at high frequency increasing our ability to estimate residence and transfer times in the S-P-A system. This meeting will bring together researchers from Europe that are involved or have recently been involved in research projects in which labelling experiments were conducted. It aims at exchanging experiences and at synergy effects among groups. The objective is to discuss and compare existing methods for analysing post labelling, and to prepare a methodological paper about advantages and disadvantages of the different approaches and about recommendations on how to analyse such experiments in the future.

The major part of the workshop will be devoted to discussion but to fulfil the discussion, all participants are invited to propose a short communications highlighting hot spots of their ongoing work in this field.

**List of participants**

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## Scientific programme

Tuesday , 2 March 2010

9:00-12:30 Oral presentations

### **Overview on recent labelling experiments: How do we analyse our data?**

- Carbon balance and allocation of assimilated CO<sub>2</sub> in Scots pine, Norway spruce and silver birch seedlings determined with gas exchange measurement and pulse labelling (Jukka Pumpanen).
- Dual 15N and 13C labelling for tracing storage and remobilisation of carbon and nitrogen in trees (Dominique Gérant).
- Labelling and post-labelling dynamics of soil emitted CO<sub>2</sub> in mountain grassland (Michael Bahn)
- D and O labelling via roots and via leaves and NO<sub>2</sub> labelling via leaves (Rolf Siegwolf)
- Tracing <sup>13</sup>C in respiration at high frequency (Caroline Plain).

14:00– 17:30: Discussion

Wednesday , 3 March 2010

9:00-12:30 Oral presentations

### **Overview on recent labelling experiments: Can we gain with a more mechanistic approach?**

- Quantifying the velocity of carbon transfer from canopy to the soil (Masako Dannoura).
- A comparative analysis of pulse-chase vs. steady-state (ie. dynamic) labelling (Fernando A. Lattanzi).
- New approaches to assess phloem transport of organic matter and transfer from the root to the soil (Arthur Gessler).
- Pathways of N<sub>2</sub>O fluxes investigated by <sup>15</sup>N tracing technique (Tobias Rütting).
- The use of compartmental modelling to analyze tracer time-courses (Fernando A. Lattanzi).

14:00– 17:00. Discussion

### **Open questions for discussion**

Labelling experiments and post labelling data analyses

- Crown chamber versus canopy chamber?
- <sup>13</sup>C versus <sup>14</sup>C?
- How to calculate the amount of label delivered to the plant?
- How to calculate the amount of label recovered in a compartment or a flux?
- Draft outline of a methodological paper: who do what?

Modelling the fate of the labelled compound in complex metabolic pathways

- Short term versus long term labelling?
- How to characterise temporal patterns that depart from classical exponential decay?
- What can we gain with a more mechanistic approach?
- Draft outline of a methodological paper: who do what?

## Short report

Despite a wider subject of the workshop, the discussion was mainly focused of carbon labelling because most of participants were involved in this kind of experiments these last years and because the development of laser spectrometry has strongly increased the frequency of measurement and the ability of tracing carbon in respiratory efflux. Organisation of another workshop on soil nitrogen is needed within the WG2. Water might be considered by WG1. Several points have been discussed and will be addressed in planned papers that will be the output of this workshop. Among these points are:

- The different kind of information that might be gained from labelling experiment compared to time series analysis. Labelling experiments do not get information on signal transfer that might be mediated by pressure concentration waves thought the phloem but to transfer of molecules, and especially carbohydrates. Both aspects should be considered together in future experiment because changes in signal transported by pressure concentration waves might affect the sink strength of organs and therefore the flow of carbohydrates. We therefore disagree with the conclusion of Mencuccini and Hölltä (2010, *New Phytologist* 185: 189–203). The redaction of an **opinion paper** will be supervised by Arthur Gessler and Nina Buchman on those points.
- High frequency measurements of the isotope composition of CO<sub>2</sub> in respiratory efflux have highlighted that the kinetic is complex and cannot be fully described with simple function like exponential decay. It requires more mechanistic approaches that might account for the existence of different pools of <sup>13</sup>C labelled compound with different mean residence time and different rates of exchange among them. The **compartmental modelling** approach (Lattanzi et al. 2005, *Plant Physiology* 137: 383–395) seems promising. In the frame of this workshop, the already published data of <sup>13</sup>C recovery in soil respiration in beech forest (Plain et al. 2009, *Tree Physiology* 29:1433-1445) and in alpine grassland (Bahn et al. 2009, *New Phytologist* 182: 451–460) will be revisited in a methodological paper that will put emphasis on the powerful of compartmental analysis for identifying underlying pools of labelled compounds, their half-life, and the extent of cycling between them. This might give new information to explain why the mean residence time differs between grass and trees. This will be supervised by Fernando Lattanzi with a direct input from Michael Bahn and Daniel Epron. Fernando is now waiting for our data. Another paper will be encouraged using Nadine Ruehr dataset in which several pools of metabolites have been measured after pulse labelling.
- Several C labelling experiments have been conducted these last years and there is an open slot for a **review paper** about both **methodological aspects** (<sup>13</sup>C/<sup>14</sup>C ; branch/crown/ ecosystem' labelling with back diffusion issue ; pulse/continuous labelling), **computation aspects** (amount of label delivered to the plant), **terminology**, data **interpretation** aspects (uneven labelling of starch substrate, mean residence time, heterotrophic carbon refixation and back transport of C by transpiration stream, ...) and some emerging properties that might come from comparing different dataset, both on **allocation** (plant type, phenology, stress effects...) and on **kinetics of recovery** (relationship between peak time and MRT, meaning of diurnal kinetic patterns, differences between beginning of recovery and peak time,...) and **future direction** (new tools to analyse data; dual labelling, high frequency metabolite tracing; back to more controlled conditions to get more on control of C transport (physiology/environment). Daniel Epron will circulate a first outline in a couple of weeks.

During the course of the joint SIBAE /NordSIR workshop “**Advances in labelling and analysis approaches in <sup>15</sup>N enrichment studies**” (Gothenburg, 09-11 May 2011) the participants identified several problems that hinder the application of <sup>15</sup>N and <sup>18</sup>O as well as several important open research questions, which need urgently to be addressed:

- 1) How much of added isotope label is actually available for the process of interest?
  - a) Is (immediate) immobilisation abiotic or biotic? How can we distinguish/ quantify abiotic and biotic immobilisation? What is the influence of the SOM content?
  - b) Isotopic exchange between free and adsorbed N pools: how long does it take until isotopic equilibrium is reached?
  - c) Does added and native N behave the same?
  - d) NanoSIMS: can we upscale the results?
  - e) Investigating label addition with “artificial soil”, other minerals, water, soil porosity, surface area, physical and chemical properties, etc.: How do these influence the isotope label distribution?
- 2) Organic nitrogen turnover
  - a) What is the chemical composition of dissolved organic nitrogen (DON)?
  - b) What is the importance of DON turnover for the N cycle and how do various DON compounds differ in turnover?
  - c) How much of the DON is biologically available or recalcitrant?
  - d) Is organic N mineralised via a direct or indirect route?
  - e) Does non-monomer DON turnover to NH<sub>4</sub><sup>+</sup> directly or via (free) monomers?
- 3) Soil sampling and handling of samples
  - a) To what extent does extraction represent soil solution N?
  - b) What soil extracting agent should be used for which application (e.g. water, KCl or CaCl<sub>2</sub>)?
- 4) Influence of DON degradation in soil extracts on mineral <sup>15</sup>N measurement
  - a) How widespread is the problem (so far only investigated for phenylalanine) and what is the extent of bias?
  - b) Can we correct for this effect?
  - c) Comparison (planned) of N analysis methods for isotopic composition of mineral N and DON (diffusion, chemical and biological)
  - d) Do we have to develop a new protocol for <sup>15</sup>N measurement in soil extracts?



- 5) Fumigation techniques to measure microbial biomass N?
  - a) What fraction of microbial biomass is measured?
  - b) How reliable are conversion factors to up-scale to total biomass?
  - c) Does measured “biomass N” also include some fraction of organic (non microbial) N and/or faunal biomass N?
- 6) Accurate  $^{15}\text{N}$ - $\text{N}_2\text{O}$  measurements
  - a) Inter-comparison of labs is needed
  - b) Standard gas: no IAEA standard and one-point calibration
  - c) Compare lasers with IRMS
  - d) Dual-inlet needed for reference gas calibration
- 7) How can we analyze reliably  $^{15}\text{N}$ - $\text{N}_2$ ? Identified problems are:
  - a) Natural-abundance: high background
  - b) Enriched: not high enough enrichment achieved and “mass 30” problematic to measure
- 8) Label distribution testing:
  - a) Inert gas (Radon, Argon,  $\text{SF}_6$ ) to monitor the gradient, distribution and transport of a gas in soil to ensure homogeneous gaseous tracer distribution and to correlate to transport of GHG
  - b) Recommended to test homogenous label distribution in soil after addition of labelled solution (e.g. extract various soil layers separately)
  - c) On-field application ( $^{15}\text{N}$  fertilizer): monitor distribution by suction cups, resins etc.
- 9) Possibilities and problems of with Oxygen stable isotope tracer
  - a) Can we account for O-exchange with water?
  - b) Is  $^{18}\text{O}_2$  a useful tracer?
  - c) Can we develop a chemical method to measure  $^{18}\text{O}$  in  $\text{NO}_3^-$ ?

The SIBAE workshop “**<sup>15</sup>N tracing modelling**” was held the 12 to 14 November 2012 at the University of Gothenburg with a total of 10 participants (of which half were post-graduate students). As a start, several presentations were given as stimulus for subsequent discussions.

- Long-term tracing of whole catchment <sup>15</sup>N additions: measurements and simulations with the TRACE model
- Modelling of triplet <sup>15</sup>N tracer experiments - results and challenges
- Complex <sup>15</sup>N tracing models – new insights into N dynamics
- Gross rates of protein depolymerization and amino acid consumption measured by <sup>15</sup>N pool dilution technique

The remaining of the workshop was dedicated to discussions. For that, participants were asked to formulate questions and points for discussion beforehand. Some topics that were discussed included:

- Should we measure microbial <sup>15</sup>N and how to represent in tracing models?
- How to represent organic N (different pools, sequential mineralization, kinetics)?
- Is there an isotopic equilibrium between free and adsorbed/fixed ammonium and how is the exchange regulated?
- Which information can <sup>15</sup>N provide on N<sub>2</sub>O production pathways and how are these represented in current tracing models?
- When to conduct the first soil extraction in <sup>15</sup>N enrichment studies?

As several practical aspects of <sup>15</sup>N enrichment studies have been discussed, the workshop will likely lead to somewhat improved future experiments by the participants, but also stimulated new ideas for <sup>15</sup>N tracing models that can be tested in the future. Currently, the possibilities for a comparative study of <sup>15</sup>N tracing models, applying the models to a common data set, are explored, which would lead to a scientific publication.

# Scientific Report for SIBAE workshop (COST action ES0806)

29<sup>th</sup> April to 1<sup>st</sup> May 2013 at Storrs Hall, Windermere

## Understanding the movement and significance of DON in terrestrial ecosystems

### Objective and rationale

The purpose of the workshop was to assemble members of the COST action as well as external experts working on different aspects of dynamics of dissolved organic nitrogen (DON) in terrestrial ecosystems. Despite many advances in DON research over recent years, including novel stable isotope methods, there is a need for approaches cutting across disciplines and spatial scales. The aim of this workshop was to identify research needs and new approaches to improve our understanding of DON dynamics.

### Workshop structure

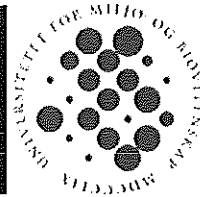
A keynote presentation by Prof. Davey Jones (University of Bangor) marked the starting point for this workshop. Professor Jones identified a range of areas where recent advances have been made. He also highlighted a range of key processes where the scientific community lacks appropriate data for interpreting dynamics of DON, and areas where there is no consensus within the community regarding the relative significance of particular processes and fluxes for DON dynamics between plants and soil. Brief presentations by all participants introduced the respective areas of expertise. Sessions in smaller groups were used repeatedly, tasked with identifying current knowledge gaps and “bottlenecks” of research, or compiling areas relevant for synthesising current understanding of DON cycles.

### Scientific outcomes

- The relationship between pool size of different DON forms (as well as precursors and products) and rates of turnover and transformation is poorly characterised. While isotopic pool-dilution approaches are useful for addressing this issue, current methods of extracting dissolved organic matter further compound our lack of understanding of actual pools and processes. There is a need for new tools to measure actual rates of DON formation, transformation and decomposition more directly
- There is a perceived contradiction between DON being a soil pool with the fastest turnover rate, but many DON species often being regarded as recalcitrant
- Many aspects have been tackled in disparate areas, this is first attempt to bring together these disciplines to provide a unified view of DON in terrestrial ecosystems
- Impact of climatic change and potential feedbacks (via GHG emissions) not currently clear
- There is a lack of understanding of the significance of small-scale processes (molecular, rhizosphere, or individual plant species scale) for plant community dynamics and field-level and catchment fluxes
- The use of current stable isotope techniques and new methods (such as Compound Specific IRMS and nanoSIMS) increases our understanding of DON dynamics on small scales, but up-scaling these methods to larger scales remains challenging

### Review paper

The publication of a synthesis paper (targeted at *Nature Geoscience*) addressing these points and proposing a more unified approach on DON research will be led by Prof. D Jones with all participants of the workshop having been assigned tasks for writing specific sections of the manuscript.



SIBAE WG 2/3 & NordSIR workshop 22.-24.4.2013, Ski, Norway

**NITROGEN CYCLE AND STABLE ISOTOPES - FOCUS ON NITRIFICATION AND DENITRIFICATION**

## **FINAL REPORT**

### **1. Participation**

The workshop took place at Ski, Norway 22.-24.4.2013, as planned. 16 researchers/PhD students participated from seven different countries. Three of the participants, Prof. Elizabeth Baggs, Dr. Reinhard Well and Dr. Tibisay Perez were present as invited experts. From those Prof. Baggs and Dr. Well are fully reimbursed by Sibae and Dr. Perez by NordSIR. Four of the participants (Dr. Biasi, Dr. Dörsch, Dr. Biasi and Dr. Silvennoinen) are MC members of SIBAE. In addition, 6 students (Kang, Juusela, Liimatainen, Reunamo, Yu, Gill) reimbursed by NordSIR, from 4 different Nordic Universities, and three other participants (Dr. Carrey, Dr. Okuka, and Dr. Mooshammer) participated the meeting.

### **2. Program**

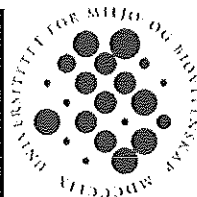
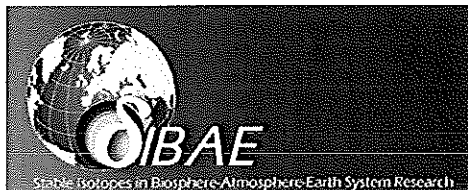
The workshop consisted of three scientific sessions, concentrating on  $^{15}\text{N}$  approaches in general, on  $^{15}\text{N}$  as a tool for measuring total in situ denitrification and on general use of  $\text{N}_2\text{O}$  isotopomers. In addition, a specific session was dedicated for short presentation of the NordSIR students and for the posters. Each of the scientific sessions consisted of invited talks and round table discussions, which were designed according to the ideas and questions, sent by the participants before the workshop. Please, see the detailed program attached.

### **3. Implementation**

The workshop started in the evening of 22<sup>nd</sup> with a dinner (not marked in the program). There are not fixed times given in the program as each of the talks and round table discussions were given as long time as needed. Sessions 1 and 2 took place on 23<sup>rd</sup> of April, sessions 3 and 4 on 24<sup>th</sup>.

### **4. Scientific outcome**

Need for review papers was generally recognized in more than one of the topics covered. Most importantly, a need for a review on existing  $^{15}\text{N}$  methods regarding in situ denitrification was recognized and the participants agreed on writing a review with running title of "Pitfalls and possibilities in measuring total denitrification with  $^{15}\text{N}$  labeling techniques" coordinated by Dr. Silvennoinen.



The workshop was also very useful for establishing new networks and collaboration. As an example of this, Braunschweig and Vienna will collaborate in using isotopomers for detecting archeal N<sub>2</sub>O production, Norwegian University of Life Sciences, University of Eastern Finland and University of Jyväskylä will collaborate in N<sub>2</sub> and N<sub>2</sub>O production in lake sediments.

#### 5. Educational outcome

The organizers were very pleased with the input of the students and the students got an excellent opportunity to present their work and discuss about their work with the specialists.

#### 6. Overall assessment

Participants found the workshop very useful, and the loose program accompanied with open discussions in designed topics were considered as a very fruitful also providing a nice platform and open atmosphere for the students to discuss with the specialists. The workshop provided the first opportunity for the meeting of the two leading world specialist on N<sub>2</sub>O isotopomers.

The participants also hope to establish a future network concentrating on nitrogen, once a suitable source for funding is found.

On behalf of organizers,

Ås, 21.5.2013

Hanna Silvennoinen

Peter Dörsch



30 September 2013

## Report on joint NordSIR and SIBAE workshop on priming of soil organic carbon.

The workshop took place in Copenhagen, Denmark from 9 – 10 September 2013. The workshop was hosted by The Technical University of Denmark, Chemical and Biochemical Engineering Department, and organized by Prof. Per Ambus and postdoc Sabine Reinsch,

The general topic of the workshop was on new developments and observations on the application of stable isotopes to study the extent of soil carbon priming and important drivers for this process. Presentations and discussions were focused on three main areas, but adjacent topics were also touched upon:

- The use of stable isotopes to investigate soil organic matter priming (Key-note Dr Eric Paterson, James Hutton Institute, UK: *Stable-isotope labelling to quantify priming of SOM mineralisation*)
- Soil carbon priming in relation to change in climate and land-use (Key-note Prof. Bent Tolstrup Christensen, DK: *Using changes in the natural <sup>13</sup>C abundance to study C turnover in arable soils*)
- Mechanisms and controls of priming processes

The workshop was attended by 24 scientists of which 10 were associated to the Sibae community, 7 were associated to the NordSIR community, 5 were independent (self paying), and two invited speakers. A total of 13 oral presentations and four poster presentations (5 min oral) were given during the workshop.

### Program for the workshop:

#### MONDAY 09.09.2013

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**13:00-14:00 Lunch at the Badehotel (Dragør, Denmark)**

**14:00-14:10 Welcome**

Per and Sabine

**14:10-17:00 Session: Soil organic carbon priming across ecosystems (Chair: Per Ambus)**

**14:10-14:55 Bent Christensen**

Using changes in natural <sup>13</sup>C abundance to study carbon turnover in arable soils

**14:55-15:15 Christina Biasi**

How susceptible are highly organic soils to priming? Results from a comparative study between mineral and peat soils

**15:15-15:30 Discussion**

**15:30-15:50 Coffee Break**

**15:50-16:10 Andreas Richter**

Stimulation of organic matter decomposition in tundra soils by increased availability of labile or easily decomposable organic compounds

**16:10-16:30 Frida Keuper**

Priming in permafrost project

**16:30-16:50 Jens-Arne Subke**

Carbon turnover at the boreal/tundra ecoton, addressing priming by ECM

**16:50-17:10 Discussion**

**17:15-18:00 Poster presentations (Chair: Sabine Reinsch)**

**17:15-17:25 Jussi Heinonsalo:** Linking plant photosynthesis to soil processes

**17:25-17:35 Emmi Hilasvuori:** Temperature sensitivity of priming and SOC pools decomposed by priming in mineral layer of Boreal forest soil

**17:35-17:45 Paolo Di Lonardo:** The role of fungal ecology in the priming effects of soil organic matter

**17:45-17:55 Teresa Sebastia:** TBA

**18:00 Dinner at the Badehotel**

**TUESDAY 10.09.2013**

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**09:00-12:15 Mechanisms and applications to quantify soil organic carbon priming (Chair: Andreas Richter)**

**09:00-9:45 Eric Paterson**

Stable-isotope labelling to quantify priming of SOM mineralisation

**10:55-11:15 Marina Bleken**

Priming effect and mineralization of shoot- versus root-derived litter

**10:20-10:35 Discussion**

**10:35-10:55 Coffee break**

**11:15-11:35 Per Bengtson**

The influence of elevated CO<sub>2</sub>, soil depth and (ecto)mycorrhizal fungi on rhizosphere priming effects

**11:35-11:55 Jukka Pumpanen**

Longer term pattern in runoff DOC fluxes in two boreal upland forested catchments: does the increasing NEE affect DOC fluxes?

**11:55-12:15 Dan Yakir**

O<sub>2</sub>/CO<sub>2</sub> ratios in soil profits indicate large losses of CO<sub>2</sub> that may compromise respiration measurements

**11:55-12:35 Discussion****12:35-13:00 Walk around Dragør harbor****13:00-14:00 Lunch at the Badehotel****14:00-15:20 Soil organic matter priming under changing climatic conditions & modeling approaches (Chair: Eric Paterson)****14:00-14:20 Mette Vestergård**

Long- and short-term effects of elevated CO<sub>2</sub> on lignolytic enzyme activity

**14:20-14:40: Sabine Reinsch**

Effects of climate change on soil organic matter priming

**14:40-15:00 Thomas Wutzler**

Priming and substrate quality interactions in soil organic matter models

**15:00-15:20 Discussion****15:20-16:00 Final discussions on new directions and ways to move forward (Chair: Per Ambus)****16:00-16:10 Final words**

Per and Sabine

Six key-scientific questions were addressed during the workshop, which were the topic for the final wrapping-up discussion taking place in three working groups:

- Q1. Are mechanisms and controls of soil C priming of purely academic interest since priming of SOM has no quantitative significance?
- Q2. Scale of timing. Do we need to reconsider priming effects to include also long-term assessments?  
– processes in nature are not discontinuous.
- Q3. We need to scale up, and move to field/undisturbed studies?
- Q4. What are the fundamental mechanisms and do chemical, physical and biological processes interact?
- Q5. Is priming mediated by specific components of the microbial community, and does priming act only on specific SOM pools?
- Q6. What are the controls of priming: theories of 'nutrient mining' and 'co-metabolism/ stoichiometry'?  
(N, P availability, other nutrients/elements)?

Some main conclusions from the working groups:

Q1: Regarding the "relevance" of priming (1st question), we thought that whilst priming per se may be quite an academic question, understanding the underlying processes and feedbacks are fundamental to our understanding of soil C and nutrient dynamic, and definitely relevant to wider applications (modelling!)

Q2: Nature is continuous, but we need to define manageable units for modelling concepts. Ultimately, each experiment may address a different scale or act over different time scales



Q3: Moving to natural systems is definitely desirable. Using jar experiments or other highly disturbed set-ups is good for detailed understanding, but we have to be mindful of how undisturbed systems function, particularly with regard to physical arrangement of components, and biochemical iterations. More studies in situ are needed, even if other compromises are necessary (less control of complexity in soils).

Q6: Not only the controls of priming are poorly known, but also our knowledge about the process per se is inadequate. What is the actual mechanism(s) is not very well known, and the responsible organisms (Q5) are certainly not well identified.

Feedbacks from the participants to this workshop have been very positive, leaving the impression that the arrangement has been very successful in several aspects. Below, some quotes copy-pasted from subsequent e-mails by several participants:

...Thanks for organising the workshop I heard a lot of new stuff and it's great to interact with people working on similar systems...

...thanks for the organization of this workshop. It was a wonderful place and there was time for discussions in the breaks...

..Many thanks again for a very enjoyable couple of days. It was very interesting to hear the different views on priming- not least what it actually is. It would be great if we can keep these mutual interests going in some way or another...

...Thank you once again for organizing a nice workshop...

...I enjoyed the workshop and learned quite a bit !...

...Thanks again for organizing a great workshop...

...Thanks for organizing the meeting, it was very nice and usefull for me...

...Thank you for organizing the workshop. It was a good meeting in a very pleasant environment...

...Thanks again for the workshop !...



Per Ambus, 30.09.2013

## Working Groups

Working Group 1

Working Group 2

Working Group 3

Working Group 4

## Working Group 3

Leaders: Nicolas Brüggemann (DE), Pascal Boeckx (BE)

### Objective

Harmonizing and benchmarking the development of innovative cutting-edge technologies for stable isotope analyses.

### Workshop

WG 3 met for a workshop on “**Isotope-specific laser-based instruments in biosphere-atmosphere-Earth system research: Latest instrument developments, applications and best-practice guidelines**” close to Zurich in June 2010 (15 and 16 June 2010). Coordinators: Nicolas Brüggemann (CH), Lukas Emmenegger (CH)

The aims of this WG3 workshop were to gather information on the latest research with isotope-specific laser instruments in biosphere-atmosphere-Earth system research and on the latest instrument developments in this field, to identify future research and development needs, and to communicate this knowledge to the scientific community. It brought together manufacturers and researchers developing and applying the innovative technology of stable isotope measurements with laser spectroscopy, identified potential sources of uncertainty as well as technological requirements. It stimulated further development of isotope-specific laser instruments in collaboration with the manufactures and discussed guidelines for best practice calibration routines and data quality assurance. The program and the outcomes of these discussions can be found [here](#).

Members of WG 3 contributed 3 out of 7 key talks at a pre-AGU workshop on **Routine application of novel stable isotope spectroscopy instruments** held in Berkeley, USA, in December 2010, organized by our sister network **BASIN**.

This meeting brought together researchers from the USA and Europe as well as from manufacturing companies who were interested in solutions how to better utilize the new spectroscopy technologies in continuous operation at field sites and to prepare for future collaborative opportunities, e.g. within new observational networks. The goal was to discuss what works best and what did not work well, to document these outcomes and to report to the community. The program and the talks of the speakers can be found [here](#).

Members of WG 3 met for a workshop on **Stable isotopes in flux measurements, IsoFlux** in Innsbruck, Austria in November 2012 (8-9 November 2012). Coordinator: Michael Bahn (AT)

This meeting again brought together researchers from the USA and Europe. Since major technological developments and the transition from magnetic-sector lab-based mass spectrometers to laser spectroscopy and field deployable isotope analyzers transform the field of stable isotope research, the unusual opportunity to push for better utilization and integration of stable isotope measurements (and other tracers) in ecosystem studies, including eddy flux studies and other flux components, was discussed. The goal was to discuss what works best and what did not work well, to document these outcomes and to report to the community. The outcome can be found [here](#).

Members of WG 3 met for the INTRA2013 Workshop on **Exploitation of Intramolecular Isotope Compositions and Emerging Techniques for their Measurement**“, held under the auspices of the COST-SIBAE Network and the University of Nantes (CEISAM Laboratory) in Nantes, France, in October 2013 (21-23 October 2013). Coordinator: Richard Robins (F)

The overall aim of the workshop was to review the state of the art in determining intramolecular isotope fractionation. The programme covered the ways of measuring position-specific isotopic distributions in small inorganic (e.g. N<sub>2</sub>O, N<sub>2</sub>, CO<sub>2</sub>) and organic molecules, what we aim to discover by making these measurements, and what needs we envisage required for the research area to advance in the future. Further information can be found [here](#).

### Questionnaire for existing compound-specific isotope analyses facilities and/or experiments

Working Group 3 of SIBAE wants to facilitate the development, application, harmonization and benchmarking of compound-specific isotope analyses among interested users across Europe. As a first step, we want to collect relevant information on such issues. Please forward [this questionnaire](#) to persons you think might be interested to join in such activities.

A first compilation you can find [here](#).

### Questionnaire for existing laser facilities and/or experiments

Working Group 3 of SIBAE wants to facilitate the development, application, harmonization and benchmarking of laser facilities and/or experiments among interested users across Europe. As a first step, we want to collect relevant information on such issues. Please forward [this questionnaire](#) to persons you think might be interested to join in such activities.

A first compilation you can find [here](#).

## **COST Action ES0806**

### **Stable Isotopes in Biosphere-Atmosphere-Earth System Research (SIBAE)**

#### Working Group 3

Joint user and manufacturer workshop

#### **“Isotope-specific laser-based instruments in biosphere-atmosphere-Earth system research: Latest instrument developments, applications and best-practice guidelines”**

Tuesday, 15 June 2010 – Wednesday, 16 June 2010

EMPA, Dübendorf, Switzerland

#### **Aims of the workshop**

The aims of this SIBAE WG3 workshop are to gather information on the latest research with isotope-specific laser instruments in biosphere-atmosphere-Earth system research and on the latest instrument developments in this field, to identify future research and development needs, and to communicate this knowledge to the scientific community. It will bring together manufacturers and researchers developing and applying the innovative technology of stable isotope measurements with laser spectroscopy, identify potential sources of uncertainty as well as technological requirements. It should stimulate further development of isotope-specific laser instruments in collaboration with the manufacturers and define guidelines for best practice calibration routines and data quality assurance.

The workshop will be arranged around three major focal points:

- 1) Scientific presentations (talks and posters) for an overview of the current state of isotope-specific measurements with laser-based instruments in biosphere-atmosphere-Earth system research.
- 2) Update by the manufacturers on their latest developments.
- 3) Identification and definition of future research and development needs and challenges.

The workshop will take place at the EMPA Academy in Dübendorf near Zürich, Switzerland.

General information and “how to find us” can be found at [www.empa-academy.ch](http://www.empa-academy.ch) and in the attached map.

A list of hotels is available under “how to find us” at [www.empa-academy.ch](http://www.empa-academy.ch). We suggest staying within walking distance (ca. 10 minutes) at the Hotel Sonnental. A limited number of rooms is reserved until Mai 30, 2010. Price for accommodation is 125 CHF per night and includes wellness, internet, breakfast. Please make individual reservations immediately (if not done so already!) with the code «SIBAE». Hotel Sonnental can be reached by bus 759 within 29 minutes from the airport to “Ringstrasse”, followed by ca. 5 min walk (see map). Buses leave every 15 minutes.

See also [http://www.zuerich-hotels.ch/html/index.php?id=20&no\\_cache=1&L=1](http://www.zuerich-hotels.ch/html/index.php?id=20&no_cache=1&L=1) for further details.

## Programme

Tuesday, 15 June 2010

- 8:30-9:00 Welcome and coffee
- 9:00-9:20 **Alf Ekblad** – Determination of soil respiration rates and  $\delta^{13}\text{C}$  *in situ* using a spectroscopic Picarro G1101-i instrument
- 9:20-9:40 **Jukka Pumpanen** – Picarro isotopic  $\text{CO}_2$  analyser in analysing  $^{13}\text{CO}_2$  in soil microcosm incubations
- 9:40-10:00 **Bela Tuzson** – *In situ* investigation of  $\text{CO}_2$  dynamics of biosphere-atmosphere carbon exchange based on real-time measurement of  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  by QCLAS
- 10:00-10:20 **Dan Yakir** – Coupling IRMS and QCL measurements of  $\text{CO}_2$ ,  $^{18}\text{O}\text{-CO}_2$  and COS exchange by plants
- 10:20-10:40 Coffee break
- 10:40-11:00 **Patrick Sturm** – Tracing the carbon and water flux in tree saplings
- 11:00-11:20 **Nicolas Brüggemann** – Application of isotope-specific laser instruments in C and O tracer studies – scope and limits
- 11:20-11:40 **Joachim Mohn** – Site-selective on-site measurements of ambient  $\text{N}_2\text{O}$  isotopomers by laser spectroscopy
- 11:40-12:00 Discussion
- 12:00-13:00 Poster presentations with a short introduction at the poster
- Daniel Epron** – Tracing of recently assimilated carbon in respiration at high temporal resolution in the field with a tunable diode laser absorption spectrometer after *in situ*  $^{13}\text{CO}_2$  pulse labeling
- Pasi Kolari** – Using Picarro G1101-i isotopic  $\text{CO}_2$  analyser with dynamic gas exchange setup
- Roland Werner** – Comparing IRMS and laser spectroscopy techniques – measurement of water isotopes
- 13:00-14:30 Lunch
- 14:30-15:00 **Gregor Hsiao (Picarro)** – New Developments in Water Sample Preparation
- 15:00-15:30 **Mark Zahniser (Aerodyne)** – Development of a Continuous Wave Quantum Cascade Laser Spectrometer for Methane Isotopologues and Measurements at a New Hampshire Fen
- 15:30-16:00 **Doug Baer (Los Gatos)** – Recent Advances in Cavity Enhanced Laser Absorption Instrumentation for Measurements of Stable Isotopes

- 16:00-16:30 Coffee break
- 16:30-17:30 Identification and definition of future research and development needs and challenges
- 17:48 Departure of bus to city center
- 18:15-19:30 Guided city walk (to restaurant)
- 19:30 Dinner

Wednesday, 16 June 2010

- 9:00-10:30 Identification and definition of future research and development needs and challenges (continued)
- 10:30-11:00 Coffee break
- 11:00-12:30 Definition of guidelines for best practice calibration and measurement routines
- 12:30-13:30 Lunch
- 13:30-15:00 Visit of Laboratory for Air Pollution and Environmental Technology, including quantum cascade laser absorption spectrometers (Lukas Emmenegger)
- 15:00-15:30 Coffee break
- 15:30-16:30 Definition of guidelines for best practice calibration and measurement routines (continued)
- 16:30-18:00 Discussion of the outline of a review paper summarizing the results of this workshop
- 19:30 Dinner

Thursday, 17 June 2010

Departure after breakfast.

## SUMMARY OF DISCUSSION OUTCOME

### Identification and definition of future research and development needs and challenges

- (Further) development of multi-species, isotope-specific analyzers, e.g. for CO<sub>2</sub>/H<sub>2</sub>O or CO<sub>2</sub>/CH<sub>4</sub>/N<sub>2</sub>O.
- (Further) development of fast analyzers (at least 10 Hz) with low power consumption.
- Development of light-weight instruments for deployment on small aircraft, ideally multi-species instruments.
- More attention has to be paid to sampling systems and periphery, sharing knowledge between scientists & manufacturers and defining guidelines.
- More testing of precision, accuracy, cross-sensitivity, temperature dependence, improvement of response times is necessary. An agreement on standardized documentation should be reached.
- During calibration of instruments application of reference gas to the intake of the sampling lines is required for reliable testing of the whole experimental set-up.
- Research need for measuring isotopes in NO, NH<sub>3</sub>, O<sub>3</sub>.
- <sup>17</sup>O in CO<sub>2</sub>; difficult due to low abundance.
- CH<sub>3</sub>D; difficult due to low abundance, perhaps only possible with pre-concentration.

### Definition of guidelines for best practice calibration and measurement routines

- Application of laser instrument in isotope tracer studies: in principle the absorption lines of the single species (isotopomers, isotopologue) can be considered as independent. However, calibration needs to be performed over the whole expected concentration range for all isotopic species. As a rule of thumb one can assume that, as long as the maximum concentration of the enriched species does not exceed the upper concentration limit at natural abundance specified by the manufacturer, the instrument can be used in tracer studies with substances enriched in the rare isotope(s).
- Calibration: dilution of high concentration reference gases with known delta values over the whole concentration range of the instrument, natural zero air should be used for dilution, not synthetic air (no argon, effect on line broadening).
- Calibration of water vapor instruments in tracer studies: dilution of liquid water of known isotopic composition, again bracketing the whole concentration range.
- Memory effects to be tested with concentration or delta step changes.

- Temperature stability/drift of instruments has to be tested and documented, especially for field deployment.
- Thermal response time of instruments to inlet air temperature has to be tested, especially for fast analyzers with high throughput of air => solution for cold air: heating intake lines, but avoid simple pulsed heaters.
- Heating of lines, especially for H<sub>2</sub>O vapor measurements, is recommended; however, temperature should be stabilized (adsorption/desorption effects); Swagelok offers new, prepared heated lines.
- PTFE (“Teflon”) has proven to be the best tubing material for isotope analysis of water vapor; for isotopes in CO<sub>2</sub>: PE-Al-PE tubing (Synflex 1300 or Dekabon 1300) is fine
- Behavior of different instruments should be tested in an inter-comparison campaign, which is foreseen within WG3 for 2011.
- Possibility of analysis of reference gases by other laboratories (thanks to Roland Werner for the links):
  - IMECC (Infrastructure for Measuring the European Carbon Cycle):  
<http://imecc.ipsl.jussieu.fr/Transnational.html>
  - MPI Jena, Germany:  
Isotopes in CO<sub>2</sub>: [http://www.bgc-jena.mpg.de/service/iso\\_gas\\_lab/techniques\\_results/co2\\_in\\_air.shtml](http://www.bgc-jena.mpg.de/service/iso_gas_lab/techniques_results/co2_in_air.shtml)  
Gas concentrations: [http://www.bgc-jena.mpg.de/service/iso\\_gas\\_lab/gas\\_lab/techniques\\_results/index.shtml](http://www.bgc-jena.mpg.de/service/iso_gas_lab/gas_lab/techniques_results/index.shtml)
- Long-term stability of standards/reference gases (thanks to Roland Werner for the links):
  - Link to WMO-GAW meeting report 2007 # 186 (report from the last year meeting is still in preparation):  
[http://www.wmo.ch/pages/prog/arep/gaw/documents/revised\\_SEPT\\_2009\\_GAW\\_186\\_TD\\_No\\_1487\\_CD.pdf](http://www.wmo.ch/pages/prog/arep/gaw/documents/revised_SEPT_2009_GAW_186_TD_No_1487_CD.pdf)
  - Link to IAEA-WMO meeting 2009 abstracts and pdfs:  
[http://www.bgc.mpg.de/service/iso\\_gas\\_lab/IAEA-WMO2009/programm.shtml](http://www.bgc.mpg.de/service/iso_gas_lab/IAEA-WMO2009/programm.shtml)
  - Information on proper treatment of glass flasks for gas isotope analysis: [http://www.bgc-jena.mpg.de/service/iso\\_gas\\_lab/central\\_flask/index.shtml](http://www.bgc-jena.mpg.de/service/iso_gas_lab/central_flask/index.shtml)
  - Additional information from Roland Werner related to flask treatment: Flushing the flasks and the lines with ten times the volume of the “new” gas is usually sufficient for removing the “old” gas completely.

## Discussion on how to summarize the results of this workshop

- List of recommendations and specifications that have to be met especially for field measurements.
- EGU session on laser instrument technical issues/applications?
- Proposal for having meetings at a regular basis, e.g. every 2 years.



# BASIN

Biogeosphere - Atmosphere Stable Isotope Network

Meeting Summary:

## Routine application of novel stable isotope spectroscopy instruments

December 12, 2010

University of California, Berkeley

### • Presentations

- Continuous field observations of stable isotope abundance ("Getting the data") - *Knohl, Sturm, Siegwolf, Zeeman* ▶pdf
- Measuring  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  of  $\text{CO}_2$  at Harvard Forest using a Quantum Cascade Laser Spectrometer - *Wehr, Munger, Nelson, McManus, Zahniser, Saleska* ▶pdf
- Daily work with stable isotope spectroscopy instruments (and the things that DO not end up in papers) - *Wingate, Ogée, Burllett, Bosc* ▶pdf
- Daily work with stable isotope spectroscopy instruments in Hesse forest - *Plain, Parent, Longdoz, Marron, Epron* ▶pdf
- Experience with isotope-specific laser instrument - *Brüggemann* ▶pdf
- Calibration for DXS: Measurements from Mauna Loa 2008 - *Noone* ▶pdf
- Vertical profiles of water vapor isotopic composition from in-situ measurements - *Noone, Risi, Bailey, Brown, Buening, Nusbaumer, Sykes, Schneider, Vanderwende, Wong, Meillier, Wolf* ▶pdf

### • Meeting Notes ▶pdf

'Daily work with stable isotope spectroscopy instruments'

(...and the things that do not end up in papers)

2010-12-12, 13:00 - 17:00 hrs, UC Berkeley, Berkeley, USA  
-- Valley Life Science Building (VLSB) / Room 2063 --

#### MOTIVATION

We think it would be of importance to have our stable isotope community come up with solutions to better utilize the new spectroscopy technologies in continuous operation at field sites and prepare for future collaborative opportunities, e.g. within new observational networks.

#### GOAL

Discuss what works best and what did not work well, document these outcomes and report to the community.

#### MEETING SUMMARY

Introduction presentations, by the speakers listed below:

1. Matthias J. Zeeman, Oregon State University, Corvallis (OR), USA.
2. Rick Wehr, University of Arizona, Tucson (AZ), USA.
3. Lisa Wingate, University of Cambridge, Cambridge, UK / University of Edinburgh, Edinburgh, UK.
4. Daniel Epron, University of Nancy / Université Henri Poincaré, Nancy, France.
5. Nicolas Brueggemann, Forschungszentrum Jülich, Jülich, Germany.
6. David Noone, University of Colorado, Boulder (CO), USA.
7. Camille Risi, University of Colorado, Boulder (CO), USA.

The following topics were discussed:

#### *At lab level*

- Instrument characterization (lab, field) and calibration (field) are strongly application dependent.
- Instrument characterization and calibration information should be added to papers (as appendix)
- Instrument characterization
  - Instrument drift & precision (single gas, Allan variance statistics)
  - Response time & memory effects (step change, Allan variance statistics, (co) spectra. turbulence measures)
  - Cross sensitivity & interference (remove substance or correct, e.g. purging of the optical path, permeation drying of inlet gas)
  - Temperature sensitivity (analyze, stabilized ambient conditions, e.g. micro-dosing LN for detectors, water cooling for lasers, air conditioning)
  - Effect of your sampling system (tube heating, below ambient pressure, effect of different tubing materials)
- Calibration
  - Check if the calibration response is linear.
  - Generally, a 2-3 points calibration based on individual isotopologues. Delta values derived from isotopologue mixing ratios only in a final step.
  - Make sure that you bracket calibration well for the expected mixing and delta ratio range, as there are non-linear effects. Particularly important for labeling experiments.
  - Tie calibrations to mass spectrometer measurements.
- Keep spectra for long-term QC. Open questions: Data space considerations, info on how to calibrate or use the spectra needs to be made available from manufacturers.

#### *At community level*

What could be helpful to share/compare/standardize within the community and how:

- Standardization and comparison of H<sub>2</sub>O isotope measurement setups:
  - SMOW and Vostok standards do not cover the range found in the field; we need alternatives.
  - A performance test of currently used vaporizer systems and methods: through a ring exchange of liquid standards among labs, or the use of bottled Evian or Deep Blue as used at SIRFER (<http://sirfer.utah.edu>).
- Standardization of CO<sub>2</sub> isotope measurement setups
  - Tanks or flasks sources: IMECC, ICOS/NEON?
  - Or produce your own flasks/tanks and sent it to mass spectrometer labs.
- Comparison of data processing:
  - use of Golden files, similar to Ameriflux/CarboEuroflux, only for EC or just use existing CO<sub>2</sub> golden files
- Reporting and communicating progress and issues:
  - Need for a new journal. For example, RCM is no longer accepting spectroscopy work (without IRMS).
  - Exchange of information through BASIN (end of funding), NEON or SIBAE initiatives.
  - Peer-reviewed Wikis. For example, Prometheus (<http://prometheuswiki.publish.csiro.au>); A CSIRO managed, peer-reviewed wiki for 'Protocols in ecological & environmental plant physiology'
  - Manufacturer websites, e.g. Picarro forum.

#### *Reporting of this meeting*

- Online publication of the presentations and meeting notes.
  - BASIN: <http://basin.yolasite.com/>
  - SIBAE: <http://www.sibae.ethz.ch/cost-sibae/>
- Submit a meeting report to inform the broader community of our activities, e.g. to AGU's EOS.
- An international, collaborative effort for "best practice" guidelines (or tech note publication) is not considered at this time.

#### LIST OF ATTENDEES

The following (37) persons were present:

Mark Blonquist  
Dave Bowling  
Nicolas Brueggemann  
Matthias Cuntz  
Claudia Czimczik  
Masako Dannoura  
Todd Dawson  
Daniel Epron  
Jim Ehleringer  
Iain Green (Picarro)  
John Hunt  
Ali Ismail  
Larry Jacobsen (Campbell Sci)  
Hans-Joerg Jost (Thermo-Fisher/ Novawave)  
Alexander Knohl  
Xuhui Lee  
Hank Loescher  
Hongyan Luo  
Dayle McDermitt (LI-COR)

David Nelson (Aerodyne)  
David Noone  
Heath Powers  
Thom Rahn  
Camille Risi  
Adam Roddy  
Scott Saleska  
Greg Santoni  
Ulli Seibt  
Rolf Siegwolf  
Kevin Simonin  
Kevin Tu  
Aaron Van Pelt (Picarro)  
Bruce Vaughn  
Eric Wapelhorst (Thermo-Fisher/ Novawave)  
Rick Wehr  
Lisa Wingate  
Matthias Zeeman

#### **ORGANIZERS**

Matthias Zeeman (Oregon State University)  
Alexander Knohl (ETH Zurich/U Goettingen)  
Kevin Tu (UC Berkeley)

#### **ACKNOWLEDGEMENT**

This meeting was supported by BASIN. Many thanks to the speakers for presenting their work and making their presentations available.

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## **SIBAE ISOFLUX Workshop, Innsbruck, 8-9 November 2012**

### **I. List of participants**

Michael Bahn	Markus Reichstein
Nicolas Brüggemann	Hans Peter Schmid
Nina Buchmann	Marion Schruppf
Dave Bowling	Christopher Still (ill, presented by proxy)
Matthias Cuntz	Jens-Arne Subke
Alexander Knohl	Christiane Werner
Bernard Longdoz	Thomas Wutzler
John Miller	Dan Yakir
Jérôme Ogée	Matthias Zeeman
Dario Papale	Jukka Pumpanen

### **II. Motivation of the meeting**

Major technological developments and the transition from magnetic-sector lab-based mass spectrometers to laser spectroscopy and field deployable isotope analyzers are transforming the field of stable isotope research. This is an unusual opportunity to push for better utilization and integration of stable isotope measurements (and other tracers) in ecosystem studies, including eddy flux studies and other flux components. In this meeting we aimed to setup a framework for such discussion that will be further pursued in follow-up meetings with the wider community. The first such opportunity will be offered in the COST-IBAE workshop in Wroclaw, Poland during 14-16 May 2013 ([www.weizmann.ac.il/conferences/SIBAE2013](http://www.weizmann.ac.il/conferences/SIBAE2013)).

Beyond this ‘timeliness’ aspect, this push requires re-visiting the overall motivation to, and the benefits from the use stable isotopes in ecosystem and flux studies, which includes the following points:

- We try to understand ecosystem functioning and its response to change based on our ability to measure only a few of many of the system components: We have more unknowns than equations. Adding stable isotopes add more equations.
- Clearly, we cannot solve equations and budgets using only “concentration measurements”. What are the options?
  - Tracers (isotopes and others)

- Manipulations (changing one factor at a time)
- Separation (measuring each component separately)
- Modeling (need parameterization—go to step one...)
- There are many examples of critical information obtained by adding isotopes
  - Partition ocean/land CO<sub>2</sub> sink (<sup>13</sup>C)
  - Partitioning biotic vs. a-biotic and anthropogenic components (O<sub>2</sub>/N<sub>2</sub>; CO<sub>2</sub>/O<sub>2</sub>)
  - Large scale biosphere response to El-Nino/RH (<sup>18</sup>O-CO<sub>2</sub>)
  - Canopy vs soil CO<sub>2</sub> flux partitioning (<sup>13</sup>C, <sup>18</sup>O)
  - <sup>18</sup>O in CO<sub>2</sub>, water, O<sub>2</sub> cycles must agree and constrain interpretations
  - C<sub>3</sub>/C<sub>4</sub>, difficult without isotopes
  - Tracer experiments-turnover, transport rate, special distribution
  - Water uptake profiles, hydraulic lift
  - Water turnover (amazon ~50%)
  - Sources of precipitation moisture
  - Biotic-abiotic effects
  - Fossil fuel indicator (with CO, <sup>14</sup>C)
  - Tree rings—paleo-environmental aspects

### III. Presentations

N. Buchmann: Isofluxes in the context of the COST-Action SIBAE

D. Yakir: Framing the Isoflux meeting: background and perspectives

M. Reichstein: Isofluxes: a Fluxnet perspective

D. Bowling: Potential and limitations of carbon isotopes to constrain and interpret trace gas fluxes between ecosystems and the atmosphere

N. Brüggemann & A. Knohl: Recent developments and applications of infrared laser absorption analyzers for isotope-specific trace gas flux measurements

C. Still: Isotope mapping in terrestrial ecosystems: from empirical isoscapes to earth system model predictions

J. Miller: Modeling of atmospheric  $\delta^{13}\text{C}$ : progress and perspectives

In addition, all other participants briefly presented their own work related to the workshop topic. All presentations and their implications for moving ahead the field were then discussed in the plenary and breakup groups.

#### **IV. Summary from breakout group recommendations**

##### **Group 1: Isotopes and fluxes**

Nick Brüggemann, Alexander Knohl, Matthias Cuntz, UFZ, Matthias Zeeman, KIT, Bernard Longdoz, Nancy, Thomas Wutzler, MPI BGC

##### Questions/Topics

- Isotopic imprint (CO<sub>2</sub> and H<sub>2</sub>O) of different ecosystems to the atmosphere
- Ecosystem/component isotopic response to drivers e.g. drought
- Identify appropriate sampling approaches (chambers, EC, CS)
- Disentangle limitation on stomata (?), WUE
- Quantify continuous leaf water enrichment → link to tree rings (biological archives)

##### Scientific Steps

- Get processes at component level (identify variability, explain drivers, understand processes)
- Get ecosystem fluxes (link to atmospheric data, inversion, up-scaling, whole ecosystem response to drought, WUE)
- Develop community isotope ecosystem model (canopy-soil, allocation, water)

##### Proposal

- 2-4 Sites in Europe linked to ICOS in different biomes
- EC and conditional sampling, profile (?)
- mobile chamber system
- Both for <sup>13</sup>C and <sup>18</sup>O in CO<sub>2</sub> and <sup>18</sup>O and <sup>2</sup>H in H<sub>2</sub>O

##### Steps

- Identify funding possibilities
- Identify partners
- Move forward at SIBAE meeting in Polen

##### Work Packages

- WP1: isoflux measurement at 2-4 sites

- WP2: mobile chamber system
- WP3: community model development

### **Group 2: Flux partitioning/tracing**

Participants: Michael Bahn, Nina Buchmann, Jukka Pumpanen, HaPe Schmid, Jens Subke, Christiane Werner

#### What research questions are we after?

- Allocation in plants and ecosystems, using pulse labelling under different environmental conditions,
- Hydrological control of carbon allocation patterns, feedbacks to the environment
- Origin of C and H<sub>2</sub>O fluxes, e.g., partitioning of ET into E and T
- Effects of drought on allocation towards growth vs. storage vs respiration

#### How could we do this?

- Labelling experiments at different sites with the same objectives, probably starting with grasslands
- Continuous isotope measurements, preferentially at already existing eddy covariance sites
- Develop an isotope model, based on open-source/community model
- Link to new initiatives in other international projects, e.g. litter decomposition study of ExPeer
- Link to lysimeter studies
- Wishlist: isotope measurements on small, airborne vessels (quadrocop, ...)

#### Where to obtain funding?

- ESFRI, like ICOS or ANAE, but takes too long?
- FP8
- ERA-Net
- COST
- ERC synergy
- A collection of bilateral projects, national projects following the same objectives
- Letters of support from ICOS, iLeaps etc might be helpful

### **Group 3: Photosynthetic Discrimination and its Controls: Linkages from Ecosystem to Continental/Regional Scales**

Participants: John Miller, Markus Reichstein, Dave Bowling, Dan Yakir

- Leverage/link to ICOS Ecosystem (i.e. flux towers) and Atmosphere systems. Note that  $\delta^{13}\text{CO}_2$  measurements are already part of the planned suite of ICOS European ecosystem measurements. However there are no planned isotope observations at the flux tower sites.
- Add isotope measurements to ICOS eco-sites that will improve our understanding of (and thus ability to extrapolate)  $^{13}\text{C}$  discrimination/WUE/canopy conductance at various time scales (diurnally, annual, to inter-annually). These measurements could include intra-seasonal to inter-annual tree ring  $\delta^{13}\text{C}$ ; branch bags; soil chambers; time or vertical keeling plots.
- Need to address the question of whether discrimination could always yield WUE/conductance info because of uncertainty with respect to stomatal or mesophyll effects on conductance. Adding measurements of water vapor isotopes could help disentangle this.
- Using climate/weather data and remote sensing observations of the land surface, one would then extrapolate/upscale discrimination (and other isotopic signatures) to the European or at least ecosystem and regional scale.
- These regional to continental scale estimates could then be compared to regional scale calculations of delta-bio from Ballantyne/Miller/Tans style regressions of ICOS data and/or Discrimination from Alden/Miller style atmospheric inversions of  $\text{CO}_2$  and  $\delta^{13}\text{C}$ .
- Given the heterogeneity of the European landscape, it's unclear how many ICOS eco-sites would need to have good isotope measurements. However, to start with perhaps we could start with just a single eco-region with one or two eco-sites and then upscale from there and compare with  $\delta^{13}\text{CO}_2$  atm. obs sites that are largely sensitive to the targeted eco-region.



#### **IV. Some general recommendations**

- Cross-site technological consolidation of Isoflux measurements needed as basis for Isoflux network(s)
- Standardized protocols and data file templates for database (cf. e.g. BASIN; MIBA) needed to facilitate integration within Isoflux community and links to Fluxnet, ICOS etc.
- Open source type model code to be developed
- Cross-scale integration of tracer experiments, tree-ring data, ecosystem and regional flux and isotopic observations needed

#### **V. Plans for future activities**

- Mailing-list and blog for exchanging information, news, papers, etc.; website should also include talks of this meeting [implementation and maintenance to be discussed, linked to SIBAE webpage: <http://www.sibae.ethz.ch/cost-sibae>]
- Identify and dissemination relevant calls for proposals
- Follow-up Meeting at SIBAE conference in Poland (May 2013) to be used to implement and expand the basis laid out in the present workshop



# COST–SIBAE Workshop

## Exploitation of Intramolecular Isotope Compositions and Emerging Techniques for their Measurement

# INTRA2013

## 21 to 23 October 2013

### Summary

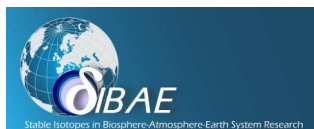
The INTRA2013 Workshop, held under the auspices of the COST-SIBAE Network and the University of Nantes (CEISAM Laboratory) took place the 21, 22 and 23 October 2013 in the CEISAM Laboratory building on the Campus of the Faculty of Sciences, University of Nantes, France.

The Workshop was attended by 31 people from 10 countries. Five laboratories member of the SIBAE Network were represented. Three industrial companies involved in the manufacture of instrumentation for isotope ratio measurement and one involved in its industrial application were present and sponsored the meeting.

The overall aim of the workshop was to review the state of the art in determining intramolecular isotope fractionation. The programme covered the ways of measuring position-specific isotopic distributions in small inorganic (e.g.  $N_2O$ ,  $N_2$ ,  $CO_2$ ) and organic molecules, what we aim to discover by making these measurements, and what needs we envisage required for the research area to advance in the future. The programme was covered in 4 sessions:

- (1) What can we gain by measuring intra-molecular isotope ratios?
- (2) Analysing intra-molecular isotope fractionation: technical issues
- (3) Analysing intra-molecular isotope fractionation: metabolic and environmental applications
- (4) Analysing intra-molecular isotope fractionation: future developments

The presentations stimulated extensive discussion. The need to improve and widely apply the determination of position-specific isotope fractionations was recognised. Methods were compared and discussed. The importance of having common reference compounds and a common terminology was emphasized. A notable focus of the Workshop was the cross-talk between the use of irm-MS and of NMR. Also, there was considerable interaction between researchers in different disciplines.



## Outcome

The final session of the Workshop focussed on future needs and developments in  $^{13}\text{C}$  determinations.

The following points that require action were recognised:

### Precision

This needs to be improved in relation to:

- The use of a Relative scale

- Profiling

- Classification

### Trueness

Three aspects of trueness were recognised for future development:

- Chemical degradation: what is the reference method?

  - Requirement that the fragments are identified

  - Requirement that the conversion yield is high enough

- Off-line Pyrolysis: what is the reference method?

  - Requirement that the fragments are identified

  - Requirement that the conversion yield is high enough

- Agreement between NMR and Off-line Py-GC-irm-MS

  - Trueness is established for small chemical ranges (e.g. ethanol)

  - Can trueness be established for On-line Pyrolysis?

  - Can trueness be established for NMR over a large chemical shift range? (e.g. acetic acid)

### What can we measure?

- By Chemical degradation:

  - Malic acid; Ascorbic acid; Tartaric acid; Vanillin

- By  $^{13}\text{C}$  isotopic NMR:

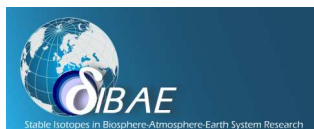
  - Glucose; Ethanol; Vanillin; Caffeine; Small molecules

- By Py-GC-irm-MS:

  - Acetone; Acetic acid; Ethanol; Alanine; Ethyl acetate

### What other targets do we want to be able to measure?

Different workers have different target interests. A list of these potential targets will be prepared for further discussion.



## Future interactions

Three working groups were established:

### Internal reference (chemical) for NMR

Action coordinators Gérald Remaud and Serge Akoka (Nantes, France)

The aim of this Workgroup is to identify potential internal reference materials for  $^{13}\text{C}$  NMR analysis. These are required for obtaining trueness over the full spectrum and for the calibration of distorted spectra acquired under rapid conditions (INEPT, 2D).

### Standards for irm-MS and NMR

Action coordinators Martin Elsner (Munich, Germany) and Alexis Gilbert (Tokyo, Japan)

The aim of this Workgroup is to identify potential molecules that can be used for absolute calibration between NMR and irm-MS. These are required to ensure that absolute values obtained by different techniques are referenced to the same basis. This requires a feasibility study (ring test, chemical and physical stability, transport, etc.).

### Terminology/harmonization

Action coordinators Richard Robins (Nantes, France) and Martin Elsner (Munich, Germany)

The aim of this Workgroup is to standardise the terminology used to describe isotopic distributions. Designation of the mesurand and designation on the method/instrument are required.

### Workshop

It was agreed to make this Workshop an annual event to be held within Europe.

Professor YOSHIDA cordially invited the participants to attend the next ISI meeting to be held in Tokyo, Japan, in July 2014.

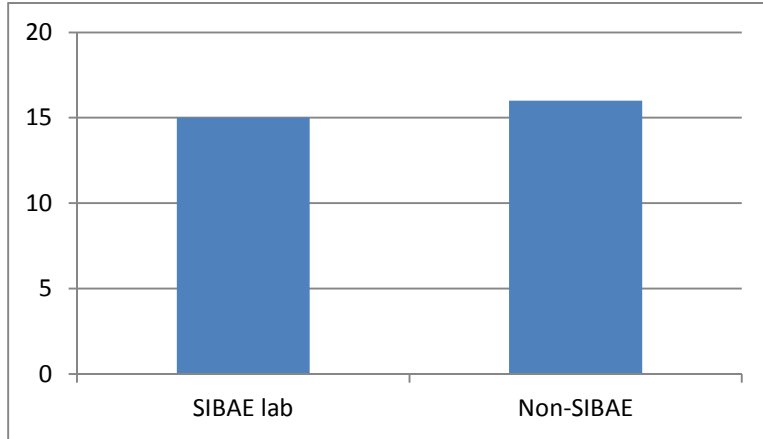
# Annexes

## Detailed Programme

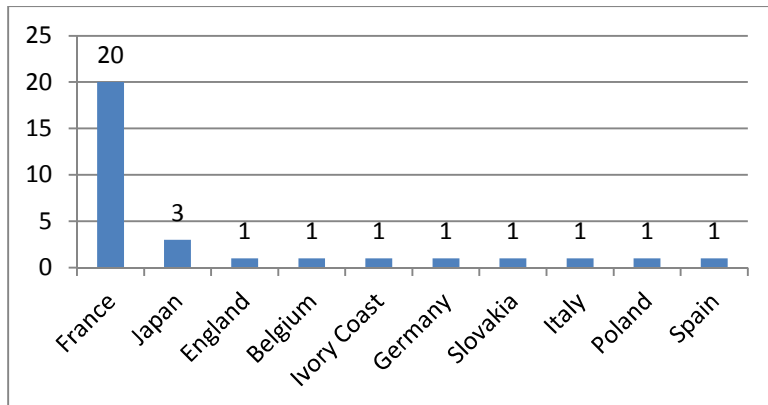
INTRA WORKSHOP 2013: PROGRAMME				
CEISAM Building		Conference Centre	Marie Curie Lecture Theatre	
<b>Monday 21 October</b>				
11:00 - 12:00	Registration			
12:00 - 13:00	Lunch			
13:00 - 14:00	Registration			
14:00 - 14:10	<b>Opening of the Workshop</b>	Richard Robins (Nantes)		
<b>Session 1: What can we gain by measuring intra-molecular isotope ratios?</b>				
14:10 - 14:20	Introduction	Gérard Remaud (Nantes)	<i>Intramolecular isotope composition: Why, what, how?</i>	
14:20 - 15:00	<b>Keynote speaker</b>	Naohiro Yoshida (Tokyo)	<i>Intra-molecular isotope distribution as a cutting edge aspect of the isotope substitutions</i>	
15:00 - 15:30	Short oral 1	Richard Robins (Nantes)	<i><sup>2</sup>H and <sup>13</sup>C during biotransformations - access to enzymatic isotope effects</i>	
15:30 - 16:00	Short oral 2	Patrick Höhener (Marseille)	<i>Tracking the fate of a pollutant in the environment by intramolecular isotope ratios and modelling</i>	
16:00 - 16:30	Refreshments			
16:30 - 17:00	Short oral 3	Philippe Lesot (Orsay)	<i>Contribution of Anisotropic NAD-NMR to the Measurement of Isotope Composition</i>	
17:00 - 18:00	Discussion			
<b>Tuesday 22 October</b>				
<b>Session 2: Analysing Intra-molecular isotope fractionation: technical issues</b>				
8:50 - 9:00	Chair's Introduction	Martin Elsner (Munich)		
9:00 - 9:40	<b>Keynote speaker</b>	Gérald Remaud (Nantes)	<i>Intramolecular isotope determination by NMR and Py-irm-MS: comparison, harmonization, profiling, standardization</i>	
9:40 - 10:10	Short oral 1	Alexis Gilbert (Tokyo)	<i>Presentation of the Py-GC-irm-MS system</i>	
10:10 - 10:40	Short oral 2	Serge Akoka (Nantes)	<i>Isotopic <sup>13</sup>C NMR</i>	
10:40 - 11:00	Refreshments			
11:00 - 11:30	Short oral 3	Pierrick Nun (Nantes)	<i>Sample preparation in <sup>13</sup>C qNMR</i>	
11:30 - 11:50	Discussion			
12:00 - 13:30	Lunch			
<b>Session 3: Analysing Intra-molecular isotope fractionation: application to the environment and authenticity</b>				
14:10 - 14:20	Chair's Introduction	Naohiro Yoshida (Tokyo)		
14:20 - 15:00	<b>Keynote speaker</b>	Martin Elsner (Munich)	<i>A Glimpse on Intramolecular Isotope Ratios in Organic Contaminants</i>	
15:00 - 15:30	Short oral 1	Kevin Bayle (Nantes)	<i>Measuring <sup>13</sup>C fractionation in organic acids</i>	
15:30 - 16:00	Short oral 2	Maxime Julien (Nantes)	<i>Best model for volatilization process using NMR measurements?</i>	
16:00 - 16:30	Refreshments			
16:30 - 17:00	Short oral 3	Julien Parinet (Marseille)	<i>Predicting Equilibrium Vapor Pressure Isotope Effect by a QSPR approach</i>	
17:00 - 17:25	Short oral 4	Freddy Thomas (Nantes)	<i>Application of site-specific isotope techniques in an accredited laboratory</i>	
17:30 - 18:30	Discussion			
<b>Wednesday 23 October</b>				
<b>Session 4: Analysing Intra-molecular isotope fractionation: future developments</b>				
8:50 - 9:00	Chair's Introduction	Richard Robins (Nantes)		
9:00 - 9:30	Short oral 1	Alexis Gilbert (Tokyo)	<i>Measurement of intramolecular isotope composition in lipids: preliminary results and future directions</i>	
9:30 - 10:00	Short oral 2	Claude Guillou (Ispra)	<i>Application of intra-molecular isotope measurements in the field of health and consumer protection</i>	
10:00 - 10:40	<b>Keynote speaker</b>	Serge Akoka (Nantes)	<i>Reducing experimental time in isotopic <sup>13</sup>C NMR</i>	
10:40 - 11:00	Refreshments			
11:00 - 11:30	Short oral 3	Gérald Remaud (Nantes)	<i>Future collaborative projects</i>	
11:30 - 11:50	Discussion		<i>Summary and closure</i>	
12:00 - 13:00	Lunch			
14:00 - 16:00	Visit	Richard Robins (Nantes)	<i>Visit to the CEISAM lab isotope measurement facility</i>	

## Participants

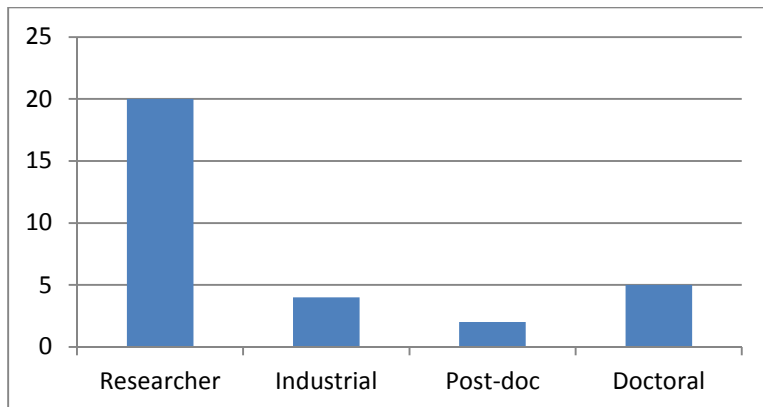
### Breakdown by SIBAE member laboratory/non-SIBAE laboratory



### Breakdown by country of origin

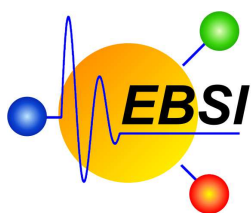


### Breakdown by Status of participant

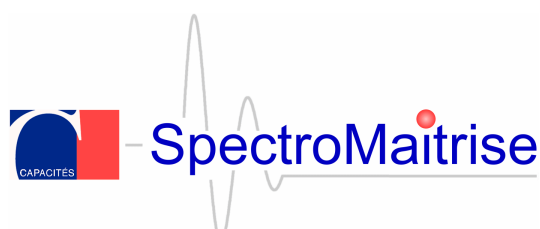


# INTRA Workshop - Sponsors

## Nantes 21-23 October 2013



UNIVERSITÉ DE NANTES



Institution	Compounds	Material	Isotope ratios
Barcelona U, Department of Plant Biology	Carbohydrates, amino acids, organic acids	N <sub>2</sub> -fixing and non- fixing plants	<sup>13</sup> / <sub>12</sub> C, <sup>15</sup> / <sub>14</sub> N, <sup>18</sup> / <sub>16</sub> O, <sup>34</sup> / <sub>32</sub> S
Gent U, Laboratory of Applied Physical Chemistry	PLFA, aminosugars, n- alkanes, amino acids	Soil, plants, micro- organisms, sediments, tissue	<sup>13</sup> / <sub>12</sub> C, <sup>15</sup> / <sub>14</sub> N, <sup>2</sup> / <sub>1</sub> H, <sup>18</sup> / <sub>16</sub> O
INRA Nancy	Total sugars, individual sugars, amino-sugars	Plants, soils, waters, gases, organic matter	<sup>13</sup> / <sub>12</sub> C, <sup>15</sup> / <sub>14</sub> N, <sup>2</sup> / <sub>1</sub> H, <sup>18</sup> / <sub>16</sub> O
Kuopio U	PLFA	Soil, soil fractions, microorganisms, plants, plankton	<sup>13</sup> / <sub>12</sub> C, <sup>15</sup> / <sub>14</sub> N
Paris-Sud U, Plant Biology	Carbohydrates, chlorophyll, DNA, amino acids, proteins	Plants	<sup>13</sup> / <sub>12</sub> C, <sup>15</sup> / <sub>14</sub> N
Paris-Sud U, Plant Ecophys.	Carbohydrates, proteins, lipids	Plants	<sup>13</sup> / <sub>12</sub> C
RISØ DTU	PLFA, NLFA	Plants, soil	<sup>13</sup> / <sub>12</sub> C, <sup>15</sup> / <sub>14</sub> N, <sup>2</sup> / <sub>1</sub> H, <sup>18</sup> / <sub>16</sub> O
Vienna U, SILVER	PLFA, sugars, amino acids, organic acids	Soil, plants, micro- organisms, sediments, tissue	<sup>13</sup> / <sub>12</sub> C, <sup>15</sup> / <sub>14</sub> N, <sup>2</sup> / <sub>1</sub> H, <sup>18</sup> / <sub>16</sub> O



Institution	Instrument	Species	Isotope ratios	Location
CRP	Los Gatos ICOS	H <sub>2</sub> O (g)	<sup>2</sup> / <sub>1</sub> H, <sup>18</sup> / <sub>16</sub> O	Laboratory
EMPA	QCLAS	N <sub>2</sub> O, CO <sub>2</sub>	<sup>13</sup> / <sub>12</sub> C, <sup>18</sup> / <sub>16</sub> O (CO <sub>2</sub> ), <sup>14</sup> N <sup>15</sup> NO, <sup>15</sup> N <sup>14</sup> NO	Laboratory
ETH Zurich	Aerodyne QCL	CO <sub>2</sub>	<sup>13</sup> / <sub>12</sub> C, <sup>18</sup> / <sub>16</sub> O	Field (forest)
ETH Zurich	Los Gatos ICOS	H <sub>2</sub> O (g)	<sup>2</sup> / <sub>1</sub> H, <sup>18</sup> / <sub>16</sub> O	Field (forest)
ETH Zurich	Los Gatos ICOS	H <sub>2</sub> O (l)	<sup>2</sup> / <sub>1</sub> H, <sup>18</sup> / <sub>16</sub> O	Laboratory
Groningen U	OFCEAS	H <sub>2</sub> O (g)	<sup>2</sup> / <sub>1</sub> H, <sup>17</sup> / <sub>16</sub> O, <sup>18</sup> / <sub>16</sub> O	Laboratory
Helsinki U	Picarro G1101-i	CO <sub>2</sub>	<sup>13</sup> / <sub>12</sub> C	Field (Hyytiälä)
Innsbruck U	Aerodyne QCL	CO <sub>2</sub>	<sup>13</sup> / <sub>12</sub> C, <sup>18</sup> / <sub>16</sub> O	Field (grassland)
Jaroslav Cerni Institute	Los Gatos ICOS	H <sub>2</sub> O (l)	<sup>2</sup> / <sub>1</sub> H, <sup>18</sup> / <sub>16</sub> O	Laboratory
KIT	Picarro L1102-i	H <sub>2</sub> O (l & g)	<sup>2</sup> / <sub>1</sub> H, <sup>18</sup> / <sub>16</sub> O	Laboratory
KIT	TGA100A	CO <sub>2</sub>	<sup>13</sup> / <sub>12</sub> C, <sup>18</sup> / <sub>16</sub> O	Field
KIT	TGA200	CO <sub>2</sub>	<sup>13</sup> / <sub>12</sub> C, <sup>18</sup> / <sub>16</sub> O	Laboratory
Nancy U	TGA100A	CO <sub>2</sub>	<sup>13</sup> / <sub>12</sub> C	Field (forest)
Öröbrö U	Picarro G1101-i	CO <sub>2</sub>	<sup>13</sup> / <sub>12</sub> C	Laboratory
Paris-Sud U	TGA100A	CO <sub>2</sub>	<sup>13</sup> / <sub>12</sub> C	Field (forest)

**Working Groups**[Working Group 1](#)[Working Group 2](#)[Working Group 3](#)[Working Group 4](#)**Working Group 4**

Leaders: Adriana Trojanowska (PL), Jens-Arne Subke (UK)

**Objective**

Training and capacity building in stable isotope applications across Europe.

**Isonumbers**

As a joint initiative, we launched a new webpage called **Isonumb3r5**, the database of useful isotope numbers. Here you can find (and provide) many numbers related to stable isotopes. Help us to compile YOUR most favorite and wanted number: [Check it out!](#)

**Template for Teaching Materials to be Shared**

One of the goals of Working Group 4 of SIBAE is to help lecturers to train and educate young scientists about stable isotope applications in various disciplines. This requires up-to-date teaching materials, successful lab and computer exercises, interesting on-line tools, animations, videos and much more. Therefore, we want to provide such teaching materials at one place. Please forward [this template](#) to persons you think might be interested to share their teaching materials with others.

**Questionnaire for Technical/Scientific Staff in Stable Isotope Labs**

Working Group 4 of SIBAE focuses on "Training and capacity building in stable isotope applications across Europe". Therefore, we also want to reach the technical and scientific staff working in or being responsible for stable isotope labs across Europe. Please forward [this file](#) to persons you think might be interested to join in such activities.

**Workshops**

A subgroup of WG4 met for a workshop in Zurich in September 2011 (19 September 2011). Coordinator: Nina Buchmann (CH) to discuss and plan a common structure of SIBAE training schools. Different formats and levels of training (introductory level vs. advanced level), funding opportunities sought and mandatory vs. nice-to-have contents were discussed. The first SIBAE training school in Portugal in 2011 was identified to test certain procedures and further develop contents and structures. Discussions will continue and reported on during a next meeting to the MC.