



18th Stable Isotope Network Austria (SINA) Meeting
11th & 12th November 2021

**University of Natural Resources and Life Sciences,
Vienna**

University and Research Center Tulln (UFT)
Konrad Lorenz Strasse 24
3430 Tulln an der Donau

PROGRAM AND ABSTRACTS

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2021 / R. Hood-Nowotny, A. Watzinger, E. Ziss (Ed.). University of Natural Resources
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ThermoFisher
SCIENTIFIC



PROGRAM

Thursday, 11th November 2021

09:30 - 10:15 Registration

10:15 - 10:30 Welcome & Introduction

SESSION 1 (Chair: Rebecca Hood-Nowotny, Christine Stumpp)

10:30 - 10:45

Micha Horacek | HBLFA Francisco-Josephinum, BLT Wieselburg

Control of declared geographic origin of Austrian and Slovak apricots: The IDARPO-Interreg-Project (AT-SK).

10:45 - 11:00

Kathrin Rosenthal | Elementar Analysensystem GmbH

New Frontiers in Compound-Specific $\delta^2\text{H}$ Analysis

11:00 - 11:30

Matthias Pilecky | Danube-University Krems and Wasser Cluster Lunz

New applications of compound specific ^2H and ^{13}C analysis of Fatty Acids in aquatic studies of animal movement, food-web dynamics and the analysis of metabolic processes.

11:30 - 11:45

Frederica Camin | International Atomic Energy Agency

$\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and $\delta^{34}\text{S}$ scales

11:45 - 12:45

Lunch in the Canteen at the UFT

Thursday, 11th November 2021

SESSION 2 (Chair: Rebecca Hood-Nowotny, Andrea Watzinger)

12:45 - 13:30

Keynote: Leonard I. Wassenaar | Wassercluster Biologische Station GmbH, Lunz am See

The new titanium method: a one-step, low-cost, reduction of aqueous NO₃⁻ to N₂O for analysis of δ¹⁵N, δ¹⁸O, and δ¹⁷O opens new possibilities for the analysis of diverse environmental samples.

13:30 - 13:45

Martin Kralik | University Vienna

Extensive mixing in deep thermal groundwaters in the Upper Austrian Alpine Foreland Basin indicated by triple isotope content (²H, ¹⁸O, ¹⁷O) and hydrochemistry data.

13:45 - 14:00

Sabrina Santos Pires | University of Natural Resources and Life Sciences, Vienna

Measurement of the stable isotopes of water in plant parts using the water-vapor equilibrium method.

14:00 - 14:15

Devakunjari Vadibeler | University of Natural Resources and Life Sciences, Vienna

Influence of equilibration time, soil texture, and saturation on the accuracy of porewater water isotope assays using the direct H₂O_(liquid)-H₂O_(vapor) equilibration method.

14:15 - 14:30

Christine Stumpp | University of Natural Resources and Life Sciences, Vienna

Introduction to the COST Action WATSON - Water Isotopes in the Critical Zone: from Groundwater Recharge to Plant Transpiration.

14:30 - 15:00

Coffee break

15:00 - 15:45

Poster Session - 3 min presentation in front of the poster

15:45 - 16:30

Open Discussion & Posters

Starting 18:30

Social Dinner - Gasthaus Rebhuhn, Berggasse 24, 1090 Vienna

Friday, 12th November 2021

SESSION 3 (Chair: Christine Stumpp, Andrea Watzinger)

09:00 - 09:45

Keynote: Eliza Harris | ETH Zürich and University of Innsbruck

Using isotopic measurements to understand N₂O emission pathways: Combining measurements, modelling, and data science approaches.

09:45 - 10:00

Kathiravan Meeran | University of Innsbruck and University of Natural Resources and Life Sciences, Vienna

Effect of soil warming and N availability on the fate of recent carbon in subarctic grassland.

10:00 - 10:15

Rebecca Hood-Nowotny | University of Natural Resources and Life Sciences, Vienna

Bark-BeAT Assessment of climate change induced drought stress and bark beetle susceptibility in Austrian forests with stable isotope approaches.

10:15 - 10:30

Andrea Watzinger | University of Natural Resources and Life Sciences, Vienna
New Ag₃PO₄ comparison material for stable oxygen isotope analysis.

10:30 - 11:00

Coffee Break

11:00 - 12:00

Lab tour

12:00 - 13:00

Lunch

Starting 13:30

General Assembly - Stable Isotope Network Austria

By participating in the event, you declare your consent to picture and sound recordings as well as to the publication of such recordings.

POSTERS

Name	Titel
Alba Canet-Marti	Water flux and hydrological processes quantification in agricultural fields under different tillage and irrigation systems using water stable isotopes.
Katharina Schott	Precipitation isotope time series and climate factors in the decade range in Austria.
Vesna Zupanc	Soil water dynamics in the upper vadose zone for two aquifers in Slovenia.
Dóra Cseresznyés	Petrographic and isotopic characteristics of carbonates from naturally CO ₂ -free and CO ₂ -flooded sandstones.
Raphael Müller	Effects of the addition of ¹³ C labeled artificial root exudates on carbon cycling in peat bog mesocosms.
Theresa Ukwamedua	Development of a physical-biological filter for groundwater remediation.
Mathilde Vantghem	Unraveling carbon dynamics in banana mats through a ¹³ C labelling experiment.
Corinna Eichinger	Impact of EDTA washing of heavy metal-contaminated soil on microbial life and ecosystem functions - a stable isotopes labelling approach using ¹³ C-PLFAs.
Christoph Noller	Assessing the effect of EDTA soil washing on mesofauna using stable carbon and nitrogen isotope labelling.
Rizki Maftukhah	Isotopic composition and nitrogen uptake in different soil amendment application on the ex-tin mining area.
Rebecca Hood-Nowotny	MaxRoot-C. Optimizing roots for sustainable crop production in Europe-pure cultures and cover crops.

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BOOK OF ABSTRACTS

Proceedings of the 18th Stable Isotope Network Austria Meeting 2021

Organizing Committee

Andrea Watzinger¹
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Katharina Schott¹
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Control of declared geographic origin of Austrian and Slovak apricots: The IDARPO-Interreg-Project (AT-SK)

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Consumer prefer food commodities of certain special origin over the same products of other provenance. Thus, it is possible to increase profit by just giving an incorrect geographic origin to a product. Therefore, the declaration of geographic origin has to be controlled to prevent fraud and consumer deception.

Apricot samples of the recent vintages are investigated to discriminate samples from different apricot-producing regions in Austria, Slovakia and other countries. In this project the isotope composition of the elements hydrogen (H), carbon (C), nitrogen (N) and oxygen (O) of fruit pulp (H, C, N, O), fruit stone (H, C, O) and fruit juice (O) is analysed, in combination with further analytical methods, to identify appropriate parameters (and combination of parameters) for the differentiation of geographic origin. In this project different sample tissues (pulp, stone, juice) are investigated to increase the number of parameters for the identification of differences in geographic origin. The different apricot fruit compartments are expected to be influenced by the respective environmental conditions during different seasonal intervals, creating more possibilities to establish spatially typical isotope patterns.

The results of the first vintages show notable variations between individual vintages, documenting the influence of weather varying between the years. These weather variations also result in differing success of differentiation between different regions of apricot production, together with sample numbers.

New Frontiers in Compound-Specific $\delta^2\text{H}$ Analysis

S. K. Lengger^{1,2,3}, S. Kelly⁴, K. W. R. Taylor⁵, Y. Weber⁶, S. K. Kopf⁷, R. Berstan⁵, M. Seed⁵,
I. D. Bull¹, J-P Mayser¹, W. D. Leavitt⁸, J. Blewett¹, A. Abraham⁴, A. Cannavan⁴, A.
Pearson⁶, R. D. Pancost¹

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The hydrogen isotopic composition ($\delta^2\text{H}$) of lipid “biomarker” compounds (molecules synthesized by and traceable to living organisms) have long been of interest to biogeochemists, with applications ranging from the investigation of food authenticity, to the reconstruction of ancient climate and environment. The preferred method of stable isotope analysis of such lipids employs gas chromatography-isotope ratio mass spectrometry (GC-IRMS), which effectively limits applications to those which measure compounds of relatively low molecular weight and polarity (i.e. compounds which elute from a typical capillary GC column at c. 320-350°C). As such, only very few compounds of molecular weight > c. 500 g/mol have been successfully analyzed intact by GC-IRMS to determine $\delta^2\text{H}$. However, the hydrogen isotopic composition of larger and/or polar compounds can be of significant interest.

Here we present two pioneering new techniques for the analysis of larger and/or more polar organic molecules of biogeochemical interest which are traditionally considered unsuitable for GC-IRMS analysis. This includes a rapid one-step derivatization procedure for the isotope analysis of the non-exchangeable hydrogen in mono and disaccharides and subsequent conversion to H_2 by chromium reduction (Abraham et al, 2020), and the development of a high-temperature GC-IRMS (HTGC-IRMS) methodology employed for the analysis of a suite of compounds of interest (Lengger et al., 2021). In particular we present the successful HTGC-IRMS analysis of triacylglycerides (TAGs), whose derivative fatty acids are often employed in studies of archaeological diet and modern food authenticity analysis, tetraether lipids including glycerol dialkyl glycerol tetraethers (GDGTs) which have been widely employed as proxies for paleoclimate and environmental analysis based on their relative distributions, and longer chain n-alkanes, the lower molecular weight homologues of which have been widely employed for paleohydrological analysis. We will present initial results demonstrating the performance and validation of the techniques, and their potential application to organic biogeochemistry.

References

Lengger S.K., Weber Y., Taylor K.W. R, Kopf S. H., Berstan R., Bull I.D., Mayser J., Leavitt W. D., Blewett J. & Pearson A. (2021). Determination of the $\delta^2\text{H}$ values of high molecular weight lipids by high temperature GC coupled to isotope ratio mass spectrometry. Rapid Communications in Mass Spectrometry 35:e8983. <https://doi.org/10.1002/rcm.8983>

Abraham, A., Cannavan, A., & Kelly, S. (2020). Stable isotope analysis of non-exchangeable hydrogen in carbohydrates derivatised with N-methyl-bis-trifluoroacetamide by gas chromatography-Chromium silver reduction/High temperature Conversion-isotope ratio mass spectrometry (GC-CrAg/HTC-IRMS). Food Chemistry 318 (126413) <https://doi.org/10.1016/j.foodchem.2020.126413>

New applications of compound specific ^2H and ^{13}C analysis of Fatty Acids in aquatic studies of animal movement, food-web dynamics and the analysis of metabolic processes

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Compound-specific stable ^2H and ^{13}C isotope analyses (CSIA) are a new analytical frontier with the potential to better understand trophic transfers of critical dietary molecules in and amongst ecosystems. Compound specific $\delta^2\text{H}$ and $\delta^{13}\text{C}$ values of fatty acids in consumer tissues promise to more definitively distinguish between dietary sources compared to use of bulk tissue ^{13}C or ^{15}N within and among habitats, but have only been attempted in ecological studies recently.

Here we present the results of new studies applying fatty acid CSIA of ^2H and ^{13}C for isotopically assigning fish to their catchment sites in a small regulated subalpine river catchment area (~30 km). We also show results on how combined ^2H and ^{13}C CSIA, help to untangle food-webs in the highly complex systems of the Austrian Waldviertler carp ponds, and reveal that metabolic processes can be detected by tracking isotopic changes during FA conversions.

By demonstrating successful applications of dual ^2H and ^{13}C CSIA as novel tools in ecological applications, we anticipate more researchers will be encouraged to use CSIA in their studies, to achieve better spatial, temporal and qualitative resolution, and a deeper understand of the underlying biochemical processes in their ecosystems.

$\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and $\delta^{34}\text{S}$ scales

Camin, F.¹, Groening, M.¹

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The presentation will focus on the outcomes from the Group of Experts participating in the Technical Meeting on the Development of IAEA Stable Isotope Reference Materials and Related Products held virtually from the 30 of August to the 3rd of September 2021.

An update on scale realisation and on the available reference materials for the $\delta^{13}\text{C}$ VPDB, $\delta^{15}\text{N}$ AIR-N₂ and $\delta^{34}\text{S}$ VCDT scales will be presented.

The new titanium method: a one-step, low-cost, reduction of aqueous NO_3^- to N_2O for analysis of $\delta^{15}\text{N}$, $\delta^{18}\text{O}$, and $\delta^{17}\text{O}$ opens new possibilities for the analysis of diverse environmental samples

Leonard I. Wassenaar

Guest Professor of Isotope Hydroecology, Wassercluster Biologische Station GmbH, Dr. Carl Kupelwieser Promenade 5, A-3293 Lunz am See, Austria

The nitrogen and triple oxygen ($\delta^{15}\text{N}$, $\delta^{18}\text{O}$, $\delta^{17}\text{O}$) isotopic composition of NO_3^- are crucial tracers of nutrient N sources and dynamics throughout the aquatic biosphere. Prevailing methods like the bacterial denitrifier or the Cd-Azide method utilize laborious multi-step conversion methods (and in the latter case highly toxic chemicals) to reduce nitrate to N_2O for stable N and O isotopic analyses by isotope ratio mass spectrometry. Accordingly, only few specialized laboratories undertake these complex assays. In both cases, $\delta^{17}\text{O}$ cannot be obtained using IRMS systems due to isobaric interferences between ^{15}N and ^{17}O in N_2O unless a further on-line conversion to N_2 and O_2 is conducted.

First, I will present a technical overview the method regarding the one-step Ti(III) chloride chemical conversion method, which employs off-the-shelf titanium (III) reagent to reduce nitrate to N_2O in IRMS-ready Exetainers or laser vials. Sample preparation takes only minutes (e.g., prepare 60+ samples per day in 1 hour), followed by a passive 24 h reaction producing N_2O gas which partitions into the vial headspace. Routine detection limits are ca. 60 ppb N-NO_3^- , depending on the IRMS. Typical analytical uncertainties for nitrate reference materials (i.e., USGS32, USGS34, IAEA NO_3) were 0.2 ‰ for $\delta^{15}\text{N}$, and ± 0.2 ‰ for $\delta^{18}\text{O}$ using IRMS, comparable or better than other methods. For laser-based N_2O triple isotope analyses the results were similar, and for direct measurement of N_2^{17}O average uncertainties for $\delta^{17}\text{O}$ were ± 0.9 ‰, without any need for isobaric corrections, but N_2O laser-based analyses remain hindered by a lack of automation.

Second, I will present exemplary results from two recent studies, illustrating how the low cost and speed of this new method facilitates possibilities previously hindered by the high cost and low throughput of conventional methods. The first is a study of nitrate in high frequency (5-15 min) rain events in Vienna covering a 1-year cycles, showing how high frequency nitrate isotopes sheds new light on the transience of local vs distal NO_x sources in an urban environment. The second example shows how the Ti method can be used to clearly differentiate certified organic from conventional grown fruits, with the ^{18}O of NO_3^- being an indisputable classification variable in methods for detecting food authenticity.

The Ti method is easily implemented in existing laboratories (i.e., Gas bench or Trace Gas Multiflow), or directly substituted into laboratories currently using multi-step bacterial or Cd methods. The ease of the Ti will facilitate the use of nitrate isotopes in studies of nutrient dynamics in a broad range of ecosystems.

Extensive mixing in deep thermal groundwaters in the Upper Austrian Alpine Foreland Basin indicated by triple isotope content (^2H , ^{18}O , ^{17}O) and hydrochemistry data

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A three-years project, funded by the ÖAW (Austrian Academy of Science), focused on the Upper Austrian part of the Alpine Foreland Basin (AFB) to delineate and characterise deep hydrostratigraphic groundwater units, to recognise water composition and to determine possible flow pathways. In addition, to hydrochemistry, stable isotopes, dissolved noble gases of groundwater and isotopic groundwater ages (^{14}C + ^{81}Kr ; Gross et al. 2021) were analysed. Triple isotope composition ($\delta^2\text{H}$, $\delta^{18}\text{O}$, $\delta^{17}\text{O}$) of selected groundwater samples was analysed at the AGH University of Science and Technology in Krakow using Picarro L2140-i CRDS laser spectrometer. Hydrothermal and oil field samples were run using Micro-Combustion Module to remove organics from the samples.

The cross-plot of ^{17}O -excess (in per meg) vs $\delta^{18}\text{O}$ support the general observation that old meteoric groundwater in the Upper Jurassic (Malm) aquifer mixes with oil field waters present in Eocene to Oligocene formations (Gross et al. 2021).

Gross, D., Heidinger, M., Götzl, G., Kriegl, C., Kralik, M., and Sachsenhofer, R. F. (2021): Dating (^{81}Kr) deep thermal groundwaters in the Upper Austrian part of the Alpine Foreland Basin, EGU General Assembly 2021, online, 19-30 Apr 2021, EGU21-14217, <https://doi.org/10.5194/egusphere-egu21-14217>, 2021.

Measurement of the stable isotopes of water in plant parts using the water-vapor equilibrium method

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Stable isotopes of hydrogen and oxygen ($\delta^2\text{H}$, $\delta^{18}\text{O}$) are widely used as effective tracers enabling the study of processes in the water cycle. Water is an important limiting factor for plant development, so, a better understanding of the ecosystem-water relations is crucial. Traditional methods for obtaining plant water sources information is laborious and challenging. The new water-vapor equilibrium method (WVEM) for measuring the stable isotopes of soil-water was adapted to plant-water in this study. Tomatoes and strawberries were grown in controlled conditions until fruiting, then plant material samples were analyzed by a laser spectrometer (Picarro L2130 -i). The project goals were: testing the use of this method to measure stable water isotopes of plants and establishing a protocol of best practice by analyzing different plant parts (roots, stem, leaves, and fruits), best preparation technique (cutting/grinding), ideal equilibrium time (24h, 48h, 72h), fruit parts isotopic difference, and the amount of material per sample to ensure accuracy. Biogenic gases and volatile organic compounds (VOCs) were analysed because their presence in the samples induces biased isotopic results. The most reliable results were: 24h of equilibrium time, cutting the material, 3g of roots/fruits, and 5g of leaves/stems per sample. Both plant species presented gradual isotopic enrichment of the irrigation water from roots to stem and leaves. Their fruits showed the inside isotopic composition more similar to the irrigation water than the outside. The intersection of the dual-isotope plot of the measured samples with the local meteoric water line plotted very close to the irrigation water enabling source water investigation. This project provides guidelines for measuring stable water isotopes in plants using the WVEM. These outcomes present a better understanding of the plant-water relations, enabling future efficient water management by knowing the plants source water with the isotopic analysis from a single leaf or fruit.

Influence of equilibration time, soil texture, and saturation on the accuracy of porewater water isotope assays using the direct $\text{H}_2\text{O}_{(\text{liquid})}$ - $\text{H}_2\text{O}_{(\text{vapor})}$ equilibration method

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The stable water isotopes of oxygen and hydrogen ($\delta^{18}\text{O}$ and $\delta^2\text{H}$) are used as tracers to study subsurface water flow processes. This requires the measurement of soil porewater isotopes, and various measurement approaches based on extracting the porewater from soil samples exist. However, differences were observed between measured isotopic composition values using the same or different approaches. As an alternative, direct liquid-vapor equilibration (DLVE) method was used to measure the isotopes in vapor headspace which is in equilibrium with porewater. Originally, it was suggested that samples are stored in inflated Ziploc freezer bags and equilibrated for three days at constant temperature before analysis using laser spectroscopy. However, different laboratories were using different equilibration times, and there is no standardized protocol for equilibration time for different soil textures and saturation levels. Hence, to study the influence of varying soil textures and saturation levels on equilibration time, different soil samples (sandy, silty loam with 2% and 4% organic carbon content, clay (kaolinite)) at four saturation levels (100%, 80%, 60%, and 40%) were equilibrated in Ziploc freezer bags for different time lengths under isothermal conditions, and the effect on measured porewater isotopes was analyzed. The experimental results showed that for loose sandy soil, 24 hours were sufficient for isotopic equilibration regardless of saturation levels. Similarly, 24 hours was also sufficient for loose kaolinite samples up to 60% saturation. But when saturation level was reduced to 40%, 48 hours was required. For silty soil with 2% organic carbon content, 96 hours and 168 hours were required for isotopic equilibration in 100% and 60% saturated condition respectively. As saturation level was reduced further, isotopic equilibrium was not reached within 168 hours. Loose silty soil with 4% organic carbon content on the other hand did not reach isotopic equilibration for any saturation level throughout 168 hours. Additionally, the ideal equilibration time depended on whether samples were broken down or kept aggregated in core cutters, with core cutters increasing the time to equilibrium. The results from this study show that more work is needed for silty and organic-rich soils to improve the application of the DLVE method.

Introduction to the COST Action WATSON - Water Isotopes in the Critical Zone: from Groundwater Recharge to Plant Transpiration

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Funded by the European Union, the COST Action WATSON brings together European experts in the field of water isotope hydrology and ecology. Its aim is to collect, integrate, and synthesize current interdisciplinary scientific knowledge on the partitioning and mixing of water in the critical zone. WATSON will provide a novel conceptual framework on the interactions between groundwater recharge, soil water storage, and vegetation transpiration useful for water resources management across a variety of climatic settings.

The COST Action is organized into four working groups focusing on (i) spatio-temporal patterns of groundwater recharge and soil water mixing processes, (ii) spatio-temporal patterns of water sources used by vegetation, (iii) catchment-scale water residence time and travel times, and (iv) networking and dissemination. Within those groups, protocols will be developed for standardized sampling procedures, and isotope-based methods combined with models will be reviewed for assessing groundwater recharge, water sources for transpiration and catchment-scale travel times. Further, an open-access database with water isotope-based studies in the critical zone will be developed. Among others, opportunities include the collaboration within the isotope community, for example through short term scientific missions and the interaction with stakeholders. Further information on the project can be found online (<https://watson-cost.eu/>) and on twitter (@ca19120).

**Using isotopic measurements to understand N₂O emission pathways:
Combining measurements, modelling, and data science approaches**

Harris, E.^{1,2} and many co-authors

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Anthropogenic greenhouse gas emissions are altering global climate, leading to increased temperature and altered precipitation patterns, with negative impacts on the environment and societies predicted worldwide. This presentation will focus on methods to understand sources, sinks, and biosphere-atmosphere interactions of trace gases, in particular nitrous oxide (N₂O). N₂O is a potent greenhouse gas and the strongest ozone-destroying substance emitted this century. A detailed mechanistic understanding of the biotic and abiotic sources and sinks of N₂O, as well as feedbacks to climate change, forms the basis for robust model predictions of future emissions to guide the development of targeted and efficient mitigation strategies. Isotopes fractionate during chemical reactions, thus isotopic composition can act as an fingerprint for sources, sinks and chemistry of trace gas and aerosol species like N₂O.

The development of laser spectroscopy for automated, in situ monitoring of N₂O isotopic composition has led to major scientific advances in recent years. Many challenges remain, including increasing precision and time resolution, coupling of instrumentation with other systems such as chambers, and the availability of calibration standards. Laser spectroscopy has been utilized in many applications, for example to understand how grassland N₂O emissions respond to changes in climate and management. The importance of denitrifying pathways for grassland N₂O emissions, even during severe drought, has been shown by several studies. Isotopic techniques have also been used to understand N₂O sources from the local to the global scale by implementing modelling approaches to interpret observed trends in isotopic composition at different timescales. For example, measurements made with preconcentration-laser spectroscopy delivered high precision measurements for the Jungfraujoch high alpine station (Switzerland) that were used to consider both local emissions and long-term changes in the global anthropogenic N₂O source. Data science and machine learning offer new potential to improve raw data calibration and analysis, interpret and extrapolate field observations, and develop and optimize modelling approaches. In combination with new methodological developments and innovative experimental approaches, this will drive many exciting discoveries in coming years.

Effect of soil warming and N availability on the fate of recent carbon in subarctic grassland

Kathiravan Meeran^{1,2}, Niel Verbrigghe³, Lucia Fuchslueger^{3,4}, Johannes Ingrisch¹, Sara Vicca³, Jennifer Soong⁵, Lena Müller¹, Bjarni D Sigurdsson⁶, Ivan Janssens³, Michael Bahn¹

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Climate warming has been suggested to impact high latitude grasslands severely, causing considerable carbon (C) losses from soil. Warming can also stimulate nitrogen (N) turnover, but it is largely unclear whether and how altered N availability impacts soil C dynamics. Even less is known about the individual and interactive effects of warming and N availability on the fate of recently photosynthesized C in soil. On a 10-year geothermal soil warming gradient in Iceland, we studied the effects of soil warming and N addition on the CO₂ fluxes and the fate of recently photosynthesized C through CO₂ flux measurements and a ¹³CO₂ pulse-labelling experiment. Under warming, ecosystem respiration exceeded maximum gross primary productivity causing increased net CO₂ emissions. N-addition treatments revealed that, surprisingly, the plants in the warmed soil were increasingly N-limited thus constrained primary productivity and decreased C allocation to shoots, while increasing C allocation to soil and microbes. Soil respiration was increased by warming and fuelled by increased C allocation and accelerated turnover of recently photosynthesized C. Our study highlights the importance of belowground C allocation and C-N interactions in C dynamics of subarctic ecosystems in a warmer world.

Bark-BeAT Assessment of climate change induced drought stress and bark beetle susceptibility in Austrian forests with stable isotope approaches.

Hood-Nowotny, R.¹, Scott, K.¹, Watzinger A.¹, Ziss E.¹, Leitner S¹, Grabner M.,² Wächter E.,² Hoch G.³, Kitzler B³

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This study aims to identify nationwide tree stand susceptibility to bark beetle (*Ips typographus*) infestation based on newly measured stable isotope data gleaned from archived and freshly collected tree rings from the Austrian National Forest Inventory (ANFI). Stable isotope data will be used to look, i) backwards to identify if and how drought stress influenced bark beetle infestation and ii) forwards to predict tree stands will respond to bark beetle under future climate change scenarios.

Tree ring stable isotope data provides an archive of information on past climate variability and physiological responses to environmental and geomorphological conditions specifically drought stress. This detailed knowledge can improve our understanding of the both legacy and future effects of climate change, particularly increasing temperatures and changes in precipitation patterns, such as increases in summer, conditions that could lead to higher tree stress and bark beetle infestation risks. Recent studies in the USA have shown that tree drought stress as measured by tree ring isotope chronology is a good predictor of susceptibility to subsequent bark beetle attack. The project Bark-BeAT will empirically identify the effects of climate change on the physiology (water use efficiency, stomatal conductance and photosynthetic capacity) of Norway spruce (*Picea abies* (L.) H. Karst.) in Austria and by flagging up areas susceptible to bark beetle infestation using stable isotope analysis of tree rings collected during the ANFI. The data on physiological tree response will be combined with site specific data of forest and soil properties, available from the ANFI and the Austrian Soil Inventory to test the relationship between soil water status, vapour pressure deficit and stable isotope chronologies.

We will seek to:

- 1) identify from pan-national archived core samples, forest susceptibility to bark beetle attack, by means of new stable isotope measurements.

- 2) ascertain climate driven changes in the physiological status of trees sampled at selected Austrian National Forest and Soil Inventory sites by stable isotope analysis of tree rings.
- 3) translate these isotope results into information on drought susceptibility by gaining information on intrinsic water use efficiency, stomatal conductance, temporal information on source water usage and photosynthetic capacity. Then using this information to create drought susceptibility maps.

New Ag₃PO₄ comparison material for stable oxygen isotope analysis

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A silver phosphate reference material (Ag₃PO₄) for the measurement of the stable oxygen isotope composition is much needed, however, not available from the authorities distributing reference materials. This study aims to fill this gap by calibrating a new Ag₃PO₄ stable isotope comparison material produced by the University of Natural Resources and Life Sciences (BOKU). Aliquots of Ag₃PO₄ were distributed to four laboratories frequently measuring the δ¹⁸O value in Ag₃PO₄; the University of Natural Resources and Life Sciences (BOKU), The University of Western Australia (UWA), the University of Helsinki (UH) and the Helmholtz Centre for Environmental Research (UFZ). The instruments used to perform the measurements were high-temperature conversion elemental analysers coupled with continuous flow isotope ratio mass spectrometers. The working gas δ¹⁸O value was set to 0 ‰ and the normalization was done by a three-point linear regression using the reference materials IAEA-601, IAEA-602, and NBS 127. Three major uncertainty sources were considered and combined into the uncertainty budget: (i) the uncertainty of the reference materials used for the normalization to the VSMOW-SLAP scale and their propagation during the normalisation procedure; (ii) the uncertainty associated with the homogeneity of the BOKU comparison material and analytical reproducibility; and (iii) the uncertainty associated with the analytical scatter between participating laboratories. The mean δ¹⁸O value of the new BOKU Ag₃PO₄ comparison material on the VSMOW-SLAP scale is 13.71 ‰ and the combined uncertainty is estimated as ± 0.34 ‰. This estimated uncertainty is within the range typical for comparison materials of phosphates and sulphates. Consistent results from the different laboratories likely derived from similar instrumentation, use of the same reference materials, and normalization procedure. The matrix effect of the different reference materials used in this study was deemed negligible. The BOKU Ag₃PO₄ can be used as an alternative comparison material for stable oxygen isotope analysis and is available for stable isotope research laboratories to facilitate calibration.

Water flux and hydrological processes quantification in agricultural fields under different tillage and irrigation systems using water stable isotopes

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In order to achieve sustainable agriculture, it is necessary to assess the impacts that management practices may have on the hydrological processes at the catchment level. The quantification of soil water fluxes would provide valuable information for understanding changes in hydrological processes. However, it is still difficult to directly quantify water fluxes in the soil which has been mainly indirectly quantified by complex numerical modelling methods. The aim of our study was to quantify average water fluxes in agricultural fields using a space for time concept, first employing oxygen and hydrogen isotope ($\delta^{18}\text{O}$, $\delta^2\text{H}$) measurements of soil pores to identify a period in soil depth profiles, and then volumetric water content to quantify soil water flux during that period, considered as mobile water. A water balance approach is used for quantifying the evapotranspiration. The bulk effect of management practices on water fluxes are analyzed across four irrigation systems (drip irrigation (DI), sprinkler irrigation (SI), hose reel boom irrigation with nozzles (BI), and no irrigation (NI)), and four tillage variants (conventional tillage (CT), reduced tillage (RT), minimal tillage (MT) and no tillage (NT)). Tracing the minimum in the pore water stable isotope profiles we observed a gradual change in water flow velocity on the order $\text{CT} > \text{RT} \approx \text{MT} > \text{NT}$. Considering both water content and differences in water flow velocities resulted in water fluxes ranging from 3.6 to 8.2 mm/month. Differences in soil mobile water were less pronounced in irrigation systems than in tillage variants, which followed an upward trend in mobile water as tillage intensity decreased (Fig 1). The resulting evapotranspiration within tillage and irrigation variants decreased in the order $\text{CT} > \text{RT} > \text{MT} > \text{NT}$, and $\text{SI} > \text{BI} > \text{DI} > \text{NI}$ (Fig 1). Irrigation water showed to contribute mostly to evapotranspiration, and drip irrigation showed the lowest evapotranspiration losses among irrigation systems. This study demonstrated that water stable isotopes can be used as indicators and are a promising method to quantify water fluxes in agricultural fields with great potential for evaluating management practices.

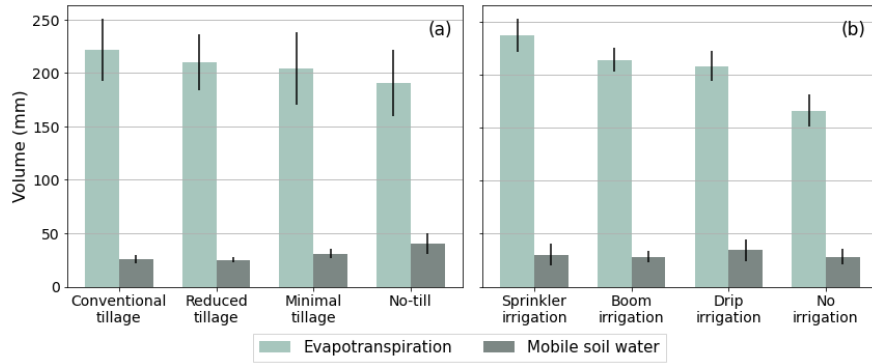


Fig. 1: Mean values of evapotranspiration and mobile soil water calculated for a period of six months for tillage variants and irrigation systems, in mm. The lines within the bars represent the standard deviation.

Precipitation isotope time series and climate factors in the decade range in Austria.

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The isotope data of the Austrian isotope monitoring network (ANIP) led to the conclusion that the deuterium or oxygen-18 time series of precipitation show a significant long-term trend in the decade range, even without extreme climate changes such as during the transition from the glacial to the post glacial age. Thus, for hydrological applications, for example, it is not sufficient to determine a long-term average value once for each station; the respective actual values must be taken into account.

The reason for these long-term trends in $\delta^{18}\text{O}$ values in precipitation is primarily the increase in air temperature in recent decades, but other climatic parameters also play a role. For example, changes in the seasonal distribution of precipitation (winter precipitation has lower $\delta^{18}\text{O}$ values, summer precipitation has higher $\delta^{18}\text{O}$ values) contributed to different trends in the $\delta^{18}\text{O}$ series of Austrian precipitation sampling stations. Changes in the origin of air moisture (Atlantic/Mediterranean influence) or air humidity can also influence the course of the isotope series in the decade range.

Knowledge of these sometimes local medium-term changes in the $\delta^{18}\text{O}$ series of precipitation helps in the interpretation of precipitation-influenced isotope time series in hydrological systems (lakes, rivers, groundwater), in biological (e.g., tree rings), and geological material (e.g., stalactites or lake sediments).

References:

ANIP, 2015. (Austrian Network of Isotopes in Precipitation and Surface water) database. Bundesministerium für Land- und Forstwirtschaft, Umwelt und Wasserwirtschaft. H2O Fachdatenbank, Vienna. Online: <https://secure.umweltbundesamt.at/h2odb/> (accessed on 1 Oktober 2021)

Soil water dynamics in the upper vadose zone for two aquifers in Slovenia

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To track the seasonal distribution of stable isotopes in soil water and use this information to identify hydrological processes, soil samples were collected seven times in 2019 and 2020 in three profiles in alluvial plains. Two profiles were sampled on the Krško polje aquifer in SE Slovenia (maize-wheat crop rotation, skeletal Eutric Cambisol, loamy texture and pepper-chili crop rotation, Eutric Cambisol, silty loam) and one on the Ljubljansko polje aquifer (maize-wheat crop rotation, skeletal Fluvisols) in central Slovenia. The isotopic composition of soil water was measured by the water vapor equilibration method. The isotopic composition of soil water was used to determine integrative information on water flux and transport as a function of time and soil depth. The isotopes of pore water were in a similar range to those of precipitation for all three profiles. The variable isotope ratios in the upper 60 cm at the different sampling times indicated dynamic water fluxes in this upper part of the vadose zone. The information from the stable isotopes will be important for further analysis of water fluxes in the vadose zone at the study sites.

This research was financed by the ARRS and WFW BIAT 20-21-32 and IAEA CRP 1.50.18 Multiple isotope fingerprints to identify sources and transport of agro-contaminants.

Petrographic and isotopic characteristics of carbonates from naturally CO₂-free and CO₂-flooded sandstones

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The study area, which includes one of the largest commercial CO₂ producing fields in Central Europe, is located in the Little Hungarian Plain (W-Hungary). In this region, we have the opportunity to study CO₂-free (not affected by natural CO₂-flooding) and CO₂-flooded sandstones in the same geological formation. Sandstone is one of the most potential reservoir-type to inject and store CO₂ (CCUS, Carbon-dioxide Capture, Utilization and Storage) on geological time scale worldwide. To better understand the processes taking place during and after CO₂ injection, petrographic observations, scanning electron microscopy and mineral chemistry analyses, X-ray diffraction and infrared spectroscopy, stable isotope analyses were performed on seven CO₂-free and six CO₂-flooded sandstone samples (from drill core).

Separated carbonate minerals' - such as dawsonite (an indicator mineral of large amount of CO₂ inflow in sandstones) and siderite - stable carbon and oxygen isotope compositions were determined. Based on these data the carbon isotope compositions of CO₂, which were in equilibrium with carbonates during their formation, were calculated. The $\delta^{13}\text{C}_{\text{CO}_2}$ from dawsonite are between -4.76 and 4.55‰. The calculated $\delta^{13}\text{C}_{\text{CO}_2}$ of siderite from CO₂-flooded sandstones can be divided into two different groups: Group-1 ranges from -6.04 to -5.98‰; while Group-2 from -10.88 to -8.37‰. In contrast, the calculated $\delta^{13}\text{C}_{\text{CO}_2}$ obtained from the siderite collected in CO₂-free sandstone define only one group which is similar to those of Group-2 from CO₂-flooded sandstones. These observations suggest that Group-1 were formed during the CO₂ inflow, similar to dawsonite, while the Group-2 likely precipitated before the CO₂-inflow.

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Effects of the addition of ^{13}C labeled artificial root exudates on carbon cycling in peat bog mesocosms

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Although covering only 3% of the world's land surface, peatlands store one third of all soil carbon (C). Peat-borne greenhouse gases as carbon dioxide (CO_2) and methane (CH_4) contribute substantially to global warming, especially as a result of peatland drainage and mining. Root exudates are a key driver of carbon cycling in peatlands. They have been found to influence peats substrate quality, methane release, decomposition and to cause priming effects. Investigating the fate of added root exudates in peatlands is challenging, as it requires the consideration of the gaseous, liquid (DOC), and solid phase, a traceable substrate, and as little disturbance as possible. We sampled six peat cores from "Pürgschachen Moor", stored them in daylight with intact vegetation at 22 °C. Ports for water sampling in 5, 15, and 25 cm depth were installed. Three cores were labeled with 140 mg ^{13}C labeled artificial root exudates at 15 cm depth. We monitored CO_2 , and CH_4 (including $^{13}\text{CO}_2$ and $^{13}\text{CH}_4$) efflux from the cores daily, sampled DOC weekly and collected drainage water after the experiment. Solid peat was sampled for all depths and ^{13}C content was determined. Results show that ca. 20% of labeled substrates were incorporated into peat, but this effect was restricted to 15 cm peat depth and ca. 30% were respired as CO_2 . We detected no priming effect and labeled cores did not release more CO_2 and CH_4 than the control cores. ^{13}C concentration in solid peat at 5 and 25 cm depth showed no increased ^{13}C concentration. These results indicate a low mobility of DOC and a limited effect of root exudate derived substrate in peat bogs with a low water table oscillation, explaining remarkably constant CH_4 release rates reported in other studies.

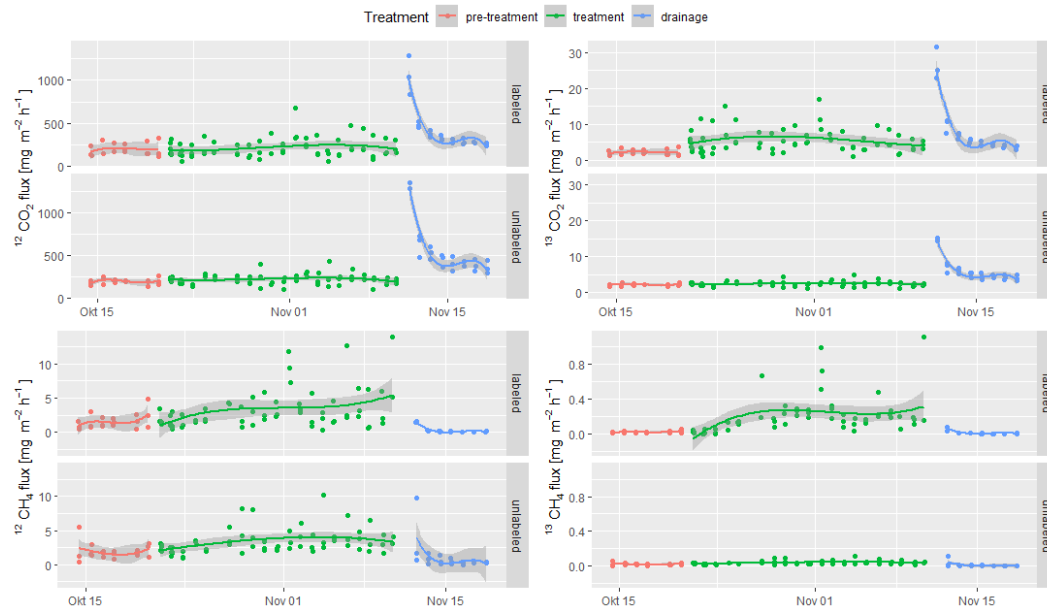


Fig. 1: CO₂ and CH₄ fluxes for ¹³C labeled and unlabeled peat mesocosms

Development of a physical-biological filter for groundwater remediation

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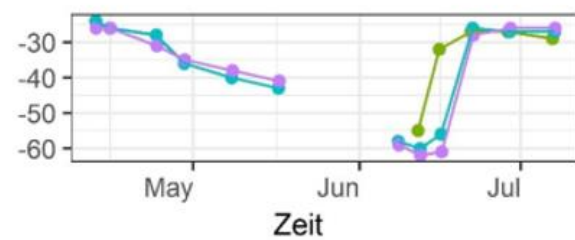
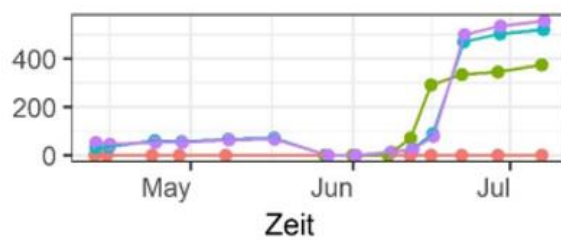
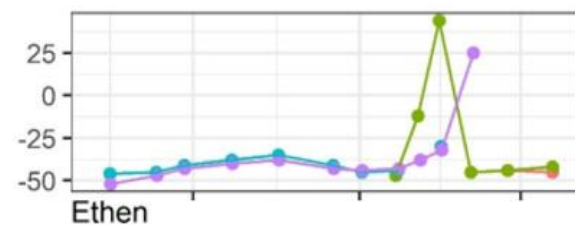
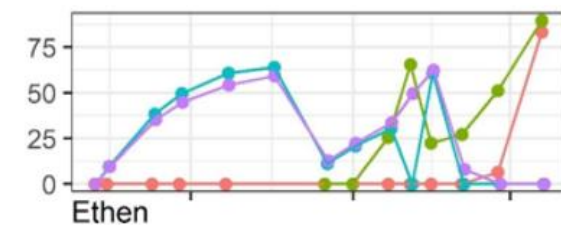
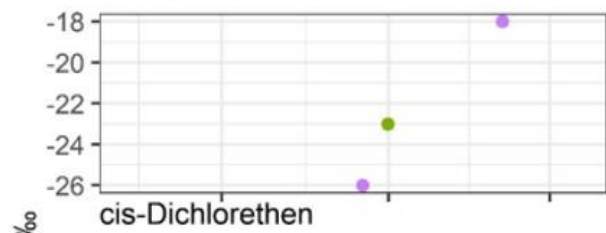
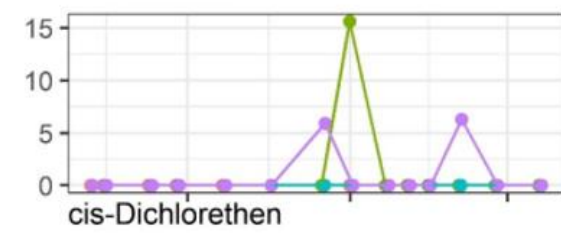
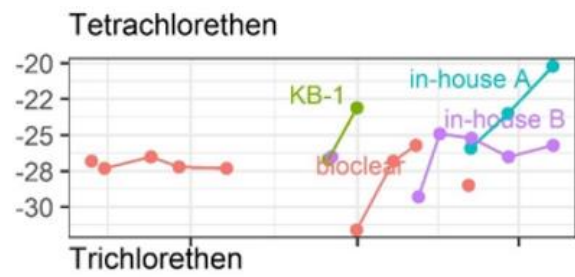
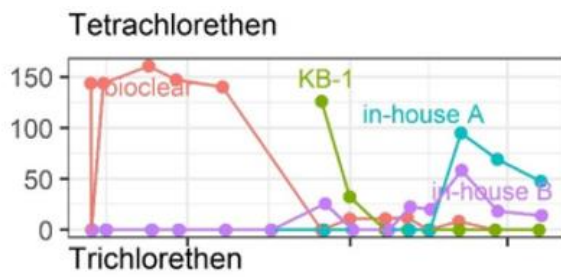
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Hydrophobic organic solvents such as tetrachloroethylene (PCE) and naphthalene are volatile organic compounds that account for a large number of soil and groundwater contaminated sites in Europe, as well as Austria. Hence, the overall goal of this study is the development of a physical-biological ex-situ filter for their effective removal. A combined approach of physical adsorption to charcoal and on-site degradation by microbial consortia was designed for that. Charcoals comprised of internally produced biochars and the gold standard, activated carbon. Microbial consortia were made available by in-house communities as well as cultures from industrial partner operating in bioaugmentation. Lab work comprised of various characterization methods including hydraulic, physico-chemical characterization, adsorption isotherms and kinetics were conducted by monitoring adsorption and dehalorespiration of chlorinated ethenes by purge&trap GC/MS-C/IRMS. Results so far revealed that PCE-sorption to biochars most significantly increased with their production temperature. Though, pursued biochars had to be selected due to their practical use. In addition, high adsorption capacity may influence bioavailability compromising the overall project goal of compound degradation. Microcosm experiments showed various degradation patterns of PCE facilitating the selection of most vital consortia (two in-house cultures and the consortia KB-1® from SiREM) which all showed complete dehalorespiration of PCE to ethene. Isotope enrichment in the carbon of PCE was observed for both, adsorption and dehalorespiration but with major differences ranging from $\delta^{13}\text{C}$ shifts of +0.5 ‰ for adsorption and up to +25 ‰ for dehalorespiration.

The effect of biochar on dehalorespiration of PCE is currently being investigated on a batch scale and is planned to be transformed to column experiments in the near future. Pending tasks also include repeating the work carried out with the second main compound naphthalene.



Unraveling carbon dynamics in banana mats through a ^{13}C labelling experiment

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Unavailability of water is probably the most important abiotic limitation for banana production worldwide and the incidence of drought spells is expected to increase with climate change. Field-applicable drought evaluation methods are missing. Our earlier findings indicate that $\delta^{13}\text{C}$ could be a useful proxy for drought stress in banana. However, the interpretation of the $\delta^{13}\text{C}$ value of a banana plant is complicated by its vegetative reproduction. Banana plants typically develop so-called suckers or daughter plants. Mother and daughter plants are both photosynthetically active, yet share a root system and exchange photo-assimilates. We found that daughter plants had significantly more negative $\delta^{13}\text{C}$ values than mother plants. This could be due to the different micro-climate below the canopy, but it could also indicate a preferential water and/or carbon allocation to the daughter plant. Moreover, drought stress increased this difference in $\delta^{13}\text{C}$ between mother and daughter plant.

In this experiment we aimed to understand and quantify carbon fluxes in banana mats under optimal and drought-stressed conditions. 24 banana mother plants, developed in the greenhouse, were pulse-labeled with enriched $^{13}\text{CO}_2$. Of half the plants, the daughter plant was removed prior to labeling. Of the other plants, the daughter plant was sealed off during labeling to prevent CO_2 uptake. A suboptimal and optimal water treatment were applied. After labeling, leaf, corm and phloem sap samples were taken at designated times over a period of seven days, after which destructive sampling took place. Different carbon fractions (sugar, starch, cellulose) of these samples will be analyzed in the Isotope Ratio Mass Spectrometer (IRMS) to determine their $\delta^{13}\text{C}$ value. This will allow us to trace the flux of photo-assimilates from the mother plant to all sinks present, as well as to understand the effect of drought on these dynamics. This would address a major gap in the understanding of banana mat functioning and will allow for a correct interpretation of $\delta^{13}\text{C}$ values of both mother and daughter plants.

Impact of EDTA washing of heavy metal-contaminated soil on microbial life and ecosystem functions - a stable isotopes labelling approach using ^{13}C -PLFAs

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Soil washing and removal of toxic metals with ethylenediamine tetraacetate (EDTA) is viewed as a soil preserving remediation option. However, EDTA has an impact on soil microbial life and ecosystem functions and amelioration by compost and biochar amendment is proposed. Using ^{13}C and ^{15}N labelled plant residues as tracer we aimed to better understand the impact of EDTA and amendments on the mineralisation of organic matter and nutrient cycling. We assessed nitrogen mineralisation and transformation by targeting available ^{15}N NH_4^+ and NO_3^- via microdiffusion extraction. Carbon mineralisation was monitored through ^{13}C CO_2 emissions and microbial community structures and its impact on the biodegradation was investigated by ^{13}C -phosphor-lipid-fatty-acid (PLFAs) analysis.

The results of the PLFA analysis showed that soil washed with EDTA shifted in microbial community composition with the most impact on the group of Actinobacteria. Overall, the removal of heavy metals had a positive effect on microbial activity and soil amendments seem to have a positive influence on revitalization of soil remediated with EDTA.

Assessing the effect of EDTA soil washing on mesofauna using stable carbon and nitrogen isotope labeling

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The importance of soil conservation and remediation is an important component in the fight against continuing loss of arable land and are needed to succeed in the aims of the new European Green Deal. Soil washing technologies can provide an efficient solution to reduce plant available heavy metal concentrations (e.g. Pd, Cd, Zn) in private gardens to background levels. The impact of such invasive technology on soil fauna is not documented yet but will be necessary for revitalization post treatments.

The investigations included a pot trial under greenhouse conditions using contaminated soil (total: 795 Pb, 4.47 Pb, 484 Zn mg kg⁻¹) and two washed variants (total: 189 Pb, 2.36 Cd, Zn 373 mg kg⁻¹), one amended with 2.6 %wt vermicompost and 2.4 %wt biochar. Half of the pots were frozen for one week to decimate the natural fauna community. All pots were subsequently inoculated with mesofauna and soil managed with crops for 6 months. 4 weeks before extracting the mesofauna, maize straw, highly labeled with ¹³C was applied to the pots. Stable ¹³C isotopes were measured in the fatty acids of single mesofauna groups (Collembola, Gamasida, Oribatida Astigmata) and microbial phospholipid fatty acids of the soil and the incorporation of label calculated. First results show differences in the microbial and mesofauna composition between the contaminated and washed soils. Incorporation of labeled material is higher in the washed soils pointing towards reduced metal toxicity or better soil physical conditions.

Isotopic composition and nitrogen uptake in different soil amendment application on the ex-tin mining area

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Tin mining has been part of the regional economy in Bangka Island, Indonesia. This activity had negative impact on ecosystems, disrupting both landscape and soils. Ex-tin mining areas often highly challenging for agricultural production due to low nutrient availability and water holding capacity. In this study we aim to improve nutrient supply for the plant, especially nitrogen. We hypothesize that soil amendments will improve nitrogen supply and uptake that were grown on different soil amendments in a tropical field experiment.

In the ex-tin mining area plots with five different soil amendments; (1) lime, (2) compost; (3) charcoal and combinations of (4) charcoal and compost; and (5) charcoal and sawdust and a control were established. The soil was amended with 10 t.ha⁻¹ for the single amendments and with rate 20 t.ha⁻¹ for combined amendments. Cassava and *Cetosema* grown as main and cover crops. The yield of main and cover crops was determined at harvest time. Nitrogen isotopic ($\delta^{15}\text{N}$) and elemental (% N) compositions were determined using an Elemental Analyser Isotope Ratio Mass spectrometer (EA-IRMS). The plant N uptake was calculated by multiplying the yield by the elemental N concentration.

The isotopic composition ($\delta^{15}\text{N}$) was the least negative in the cover crop, in the combined treatment of charcoal and compost suggesting highest nitrogen fixation rates of the *Centrocema*. However, the $\delta^{15}\text{N}$ values in cassava was positive, where the combined treatment of charcoal and compost had the highest values, reflecting uptake from the compost. Nitrogen uptake was crop specific and related to the yield. Soil amendments lead to higher N uptake in the crop. N uptake of cassava was not significantly different amongst soil amendments. Cumulative harvested nitrogen over the two growing seasons was highest in the combined treatment of charcoal and compost. Results from this study demonstrate that the cover crops may obtain their nitrogen both from soil or amendment nitrogen and also from atmospheric N₂. In general, soil amendments can improve nitrogen supply in the soil and improve yield as well as nitrogen uptake of crops on the ex-tin mining area.

MaxRoot-C

Optimizing roots for sustainable crop production in Europe-pure cultures and cover crops.

MaxRoot-C Team¹.

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To reduce the effect of climate change on food security, carbon farming is indispensable. Mobilizing crop producers to support this transformation requires promotion of cropping systems with equivalent profitability, but higher soil C sequestration. The most viable yet neglected option is through increased and deeper roots of main and cover crops. MaxRoot-C will pioneer assessment methods closing this knowledge gap by providing robust hard data on the root C inputs of main crop varieties and different cover crops across Europe.

We will use a combination of root traits, such as: root biomass, root stoichiometry, root architecture, and rhizodeposition, in conjunction with E-environmental- factors, like soil type, strength and fertility, to predict the effect of root systems on SOC stocks. Additionally, we will look at which soil-crop management practices can be implemented to optimise carbon sequestration. Using a combination of state of the art experimental and modelling approaches, we will quantify the belowground C allocation for the most prevalent cropping systems. Using a stable isotope labelling approach, we can estimate root carbon inputs and sequestration potential of different management practices, crop varieties and cover crop mixtures, in farmer's fields over significantly shorter times scales than traditional long-term experimental approaches, that usually require ten years of repeated measurements to detect significant impacts on soil carbon build-up. With isotopes we can determine net annual C inputs and their retention, moreover follow their biological fate and turnover, allowing us to maximise root derived carbon storage and persistence in relation to specific root traits and functions.

This approach to follow fate of root carbon in a robust replicated manner, allows us to quickly optimize and iterate management options based on either new incoming scientific evidence, farmer's recommendations, or even the adoption of new farming practices i.e. adoption of new bio-fertilizers. We will go beyond current knowledge, to evaluate the potential impact of promising C sequestering management interventions, such as: cover cropping, targeted-breeding, and soil management in these foremost cropping systems aiming at widespread adoption of more sustainable carbon sequestering and soil restorative practices.