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# Soil Systems Ecology

Organic Matter, Energetics & Turnover

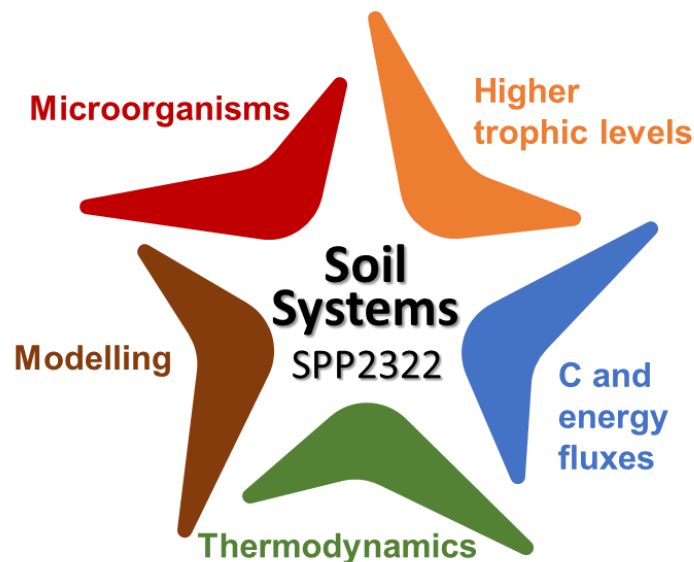
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## International Conference

9<sup>th</sup> – 11<sup>th</sup> September 2024

Humboldt Universität zu Berlin

## Program and Abstracts



# Conference Program and Abstracts



International Conference on

Soil Systems Ecology – Organic Matter, Energetics & Turnover

9–11 September 2024

Humboldt Universität zu Berlin

Germany



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## Organizers



Prof. Dr. **Sören Thiele-Bruhn**  
Trier University  
Soil Science  
[thiele@uni-trier.de](mailto:thiele@uni-trier.de)



Prof. Dr. **Liliane Ruess**  
Humboldt-Universität zu Berlin  
Institute of Biology  
[liliane.ruess@biologie.hu-berlin.de](mailto:liliane.ruess@biologie.hu-berlin.de)



Prof. Dr. **Matthias Kästner**  
Helmholtz Centre for Environmental Research – UFZ  
Dept. Molecular Environmental Biotechnology  
[matthias.kastner@ufz.de](mailto:matthias.kastner@ufz.de)



Dr. **Marcel Lorenz**  
Trier University, Soil Science  
UFZ, Molecular Environmental Biotechnology  
[lorenzma@uni-trier.de](mailto:lorenzma@uni-trier.de)

## Scientific Committee

Thomas Maskow (Helmholtz Centre for Environmental Research – UFZ)

Anja Miltner (Helmholtz Centre for Environmental Research – UFZ)

Liliane Ruess (Humboldt Universität zu Berlin)

Holger Pagel (Forschungszentrum Jülich/University of Bonn)

Christoph C. Tebbe (Thünen Institut für Biodiversität)

## Sponsors

We are grateful for the financial support by the Deutsche Forschungsgemeinschaft – DFG and Deutsche Bodenkundliche Gesellschaft – DBG.

## Editorial

The transformation and storage of organic matter in soils is essentially driven by soil biological processes, which in turn are determined by the supply of organic matter. A comprehensive assessment, and ultimately an in-depth understanding and modeling of carbon storage in soils, is only possible by taking these interacting aspects into account. It requires to consider not only mass fluxes of organic matter, but essentially also energy fluxes as determinants. This is because large fluxes of solar energy stored in organic matter pass through soil as a conduit from primary production of carbon towards mineralization. Soil organisms channel the flux, are fueled by the energy provided, and contribute to soil organic matter by their bio- and necromass. The lack of unified approaches resulted in previous research targeted either on biogeochemical turnover processes or on microbial coevolution and phylogeny, but rarely linked the two approaches, in particular not in light of the energy fluxes.

Therefore, we aim on raising awareness of a holistic approach, which is still in its infancy in soil research. With this conference, we want to offer a platform for new conceptual, experimental and empirical studies on the linking of mass and energy fluxes in soil ecosystems. It's time to get a systemic view on energy and matter fluxes and their interactions with living and non-living soil components. The conference is planned to review the state of knowledge and gaps in knowledge on this topic and to promote dialogue between researchers in the field. We will tackle the key questions:

- Which energetic properties and state variables can be determined in combination with information on the mass balance of organic matter in soil?
- How to apply the thermodynamic principles that link carbon and energy use efficiencies to microbial diversity, growth and activity in soil?
- How much energy is needed to maintain biotic soil functions?
- Does the microbiome, its structural and functional diversity and interacting trophic levels on the turnover and storage of SOM control the energy flux or is it actually converse?
- Do boundary conditions shape or even determine the energy use channels in soil?
- What causes the C-stabilization ('entombing effect') after conversion of microbial biomass to necromass in different soil types?

This conference was essentially conceived and developed from the activities and discussions of the scientific partners of the [SoilSystems](#) priority research program (funded by the German Research Foundation DFG). On the long run it is a continuation of the workshop "SOMmic - Microbial Contribution and Impact on Soil Organic Matter, Structure and Genesis" organized in Leipzig in November 2016, which initiated the activity for the joint research program.

Yours sincerely, Sören Thiele-Bruhn, Matthias Kästner, Marcel Lorenz, Liliane Ruess on behalf of the panel group of the research program SPP 2322 SoilSystems



## Keynote Speakers

Dr. **María Jesús Iglesias Briones**, Universidade de Vigo

Dr. Briones' main research interest is the functional role of soil fauna in terrestrial ecosystems. In particular, she aims to quantify soil biodiversity changes and their potential implications for the C sink/source function. Her research combines the use of stable isotopes ( $^{13}\text{C}$  and  $^{15}\text{N}$ ) at natural abundance levels as potential *in situ* tracers of the trophic structure of edaphic communities and their functional role in natural and agricultural systems. In addition, she is also taking advantage of developing radiocarbon ( $^{14}\text{C}$ ) technologies to investigate the interaction between soil fauna activities and the temperature sensitivity of soil organic matter decomposition in response to climate change.



Dr. **Stefano Manzoni**, Stockholm University



Dr. Manzoni is an associate professor in ecohydrology, with research interests spanning soil-vegetation-atmosphere interactions, hydro-climatic impacts on carbon and nutrient cycling, ecological stoichiometry, stochastic modeling of ecosystem processes, sustainable agriculture, and wetland dynamics. His approach is based on process-based, conceptual, and stochastic models of water, carbon, and nutrient fluxes, which are tested using local and global datasets. His recent projects focus on soil microorganisms and their adaptations to land use and climatic changes as drivers of carbon and nutrient cycles in different ecosystems.

Dr. **Andreas Richter**, University of Vienna

Dr. Richter investigates how growth and turnover of microbial communities control the deconstruction and mineralization of organic matter in terrestrial ecosystems under current and future climatic conditions. He and his group has redefined and expanded the concept of microbial carbon and nitrogen use efficiency, linking it to ecological stoichiometry theory. He also pioneered the development of methods to estimate microbial growth and carbon use efficiency based on stable oxygen and hydrogen isotopes. His recent research projects are focused on the interactive effects of future climate conditions and climate extremes on microbial processes, active community composition, and plant-microbe interactions.



Dr. **Noah W. Sokol**, Lawrence Livermore National Laboratory



Dr. Sokol is a Staff Scientist at Lawrence Livermore National Laboratory, and the Deputy Director of LLNL's new 'Terraforming Soil' Energy Earthshot Research Center. His research focuses largely on soil biogeochemistry and microbial ecology, particularly how living and dead soil microorganisms influence soil organic matter cycling under a range of environmental conditions. He also researches how plants, soils, microbes, and minerals can be harnessed as tools for carbon dioxide removal, including the synergies between the inorganic and organic carbon cycles. The overarching aims of his work are to improve our ability to understand and model the global carbon cycle, and to better manage soils to help mitigate climate change.

Dr. **Marie Spohn**, Oregon State University, Associate Professor

Dr. Spohn is a professor in biogeochemistry of forest soils. In her research, she explores cycling of carbon, phosphorus and nitrogen as well as their interactions in soils. In order to gain a better understanding of element cycling, she uses a large range of methods, including soil chemical analyses, isotopes as well as microbiological tools. Recent research activities are focused on stoichiometry of soil organic matter and microbial biomass stoichiometry as a driver of element cycling in soils, microbial carbon use efficiency and microbial biomass turnover, rhizosphere processes, microbial mobilization of inorganic phosphorus and N<sub>2</sub> fixation in soils.



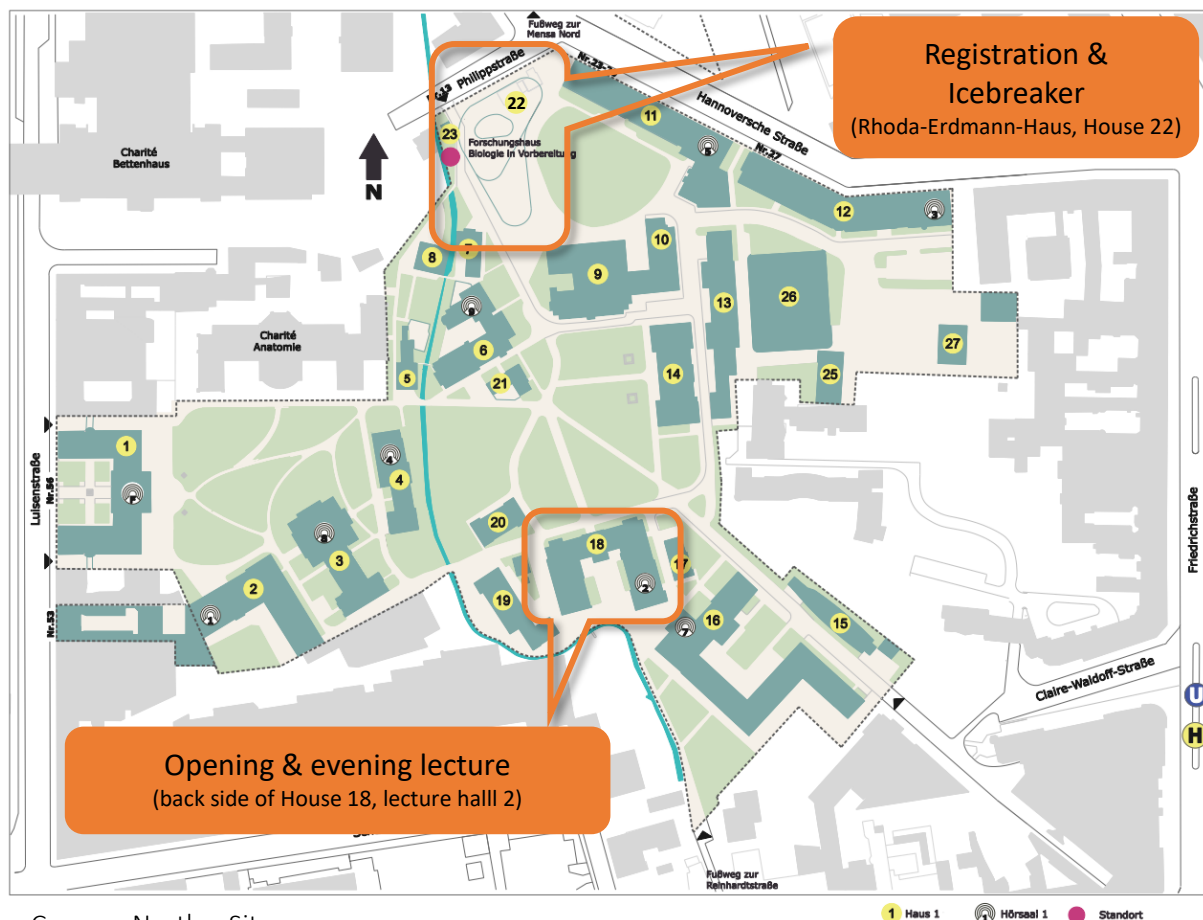
## Conference Venue

### General

The conference is held in Berlin-Mitte, the most central district of the German capital. No other part of the city attracts more visitors – here is the historic center. Most of Berlin's important sights are located in Mitte such as the Reichstag, the Brandenburg Gate and the Museum Island. Practically everything is within walking distance. The registration, evening lecture and icebreaker are located at the Campus North of the Humboldt-Universität zu Berlin. The following two conference days with talks and posters take place at the Heilig Geist Kapelle, also a property belonging to the university.

### Campus North of the Humboldt-Universität zu Berlin

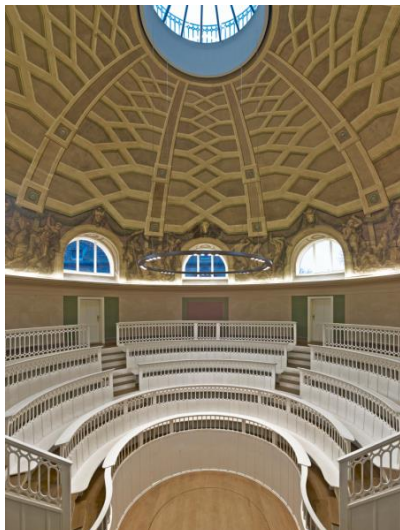
In the middle of Berlin's lively Mitte district, in the area between Invalidenstraße and Luisenstraße, is the **Campus North**, located at the **Philippstraße 13, 10115 Berlin**. The core of the campus is an 80,000 square meter park-like area with old trees and historic red brick buildings, which were built between the end of the 18th and the middle of the 20th century. The park, formerly called the Reuß Gardens, was designed by the garden artist and General Garden Director of the Royal Prussian Gardens, Peter Joseph Lenné.



Campus North – Site map



The most outstanding building in the park is the **Anatomical Theater** of the Royal Veterinary School designed by Carl Gotthard Langhans, the architect of the Brandenburg Gate, over two hundred years ago. It is the oldest academic facility in Berlin and its classical structure is reminiscent of an ancient amphitheatre. It is popularly known as the “Temple of Trichinosis”. For more information, please see: <https://tieranatomisches-theater.de/>. The [evening lecture](#) of the conference takes place in [House 18](#). It is the most imposing building in the ensemble of university buildings on the grounds of Campus North at the end of the Wilhelmine era. Dating from 1914, it was planned as a university laboratory and research building by Professors Abderhalden (physiology) and Schroeter (chemistry). Until 2009 it was dedicated to Emil Abderhalden, who was known for his work on “Abwehrfermenten”. As early as 1914 Leonor Michaelis showed that the so-called “Abderhalden Reaction” was based on artifactual observations. Today the house is named after this famous German biochemist and co-founder of the field of enzyme kinetics, who was associate professor at the University of Berlin from 1908 to 1922.



Tieranatomisches Theater  
(© Stefan Müller)



House 18  
(© HU Berlin, Dep. of Biology)

The modern life sciences campus is growing around these buildings, which includes the Institutes of Biology, Psychology, the Thier Institute for Agriculture and Horticulture, and the Charité Hospital Campus. Research alliances are e.g., the Bernstein Centre, the Mind and Brain Graduate School as well as the Neuroure, UniCat and “Image Knowledge Gestaltung” excellence clusters.

On Monday the 9<sup>th</sup> of September the [registration](#) is open from 4 and 6 p.m. in the [Rhoda-Erdmann-Haus \(House 22\)](#) at the Campus North, Philippstraße 13, 10115 Berlin. The Rhoda-Erdmann-Haus, also known as the “green amoeba”, is a modern green building surrounded by old red brick houses and is easily recognizable. Please see the campus site map. You will find the registration desk on the first floor in **room 1023**. If you are unable to register on Monday, you can do so on the other days at the Heilig Geist Kapelle.



Rhoda-Erdmann-Haus (© Silke Stutzke)

## Heilig-Geist-Kapelle (Chapel of the Holy Spirit)

Our conference house, the Heilig-Geist-Kapelle, is the former chapel of the Hospital of the Holy Spirit. The location is opposite to the Museum Island on the other side of the Spree in the **Spandauer Straße 1, 10178 Berlin**.



Heilig-Geist-Kapelle outside (© Stefan Müller) and inside (© Humboldt-Innovation GmbH)

The Heilig Geist Kapelle, built around 1300, is the oldest building in Berlin's historic city center that has been preserved in its entirety and is an architectural monument of exceptional importance. At the beginning of the 20th century, the chapel was initially to be demolished for the construction of the commercial college, but was saved by a campaign of civic engagement and integrated as a lecture hall in 1905. The building, with its roof truss dating back to 1476 and the three-bay star-ribbed vault, which was added around 1520, is a key architectural and art-historical testimony to medieval Berlin and was thoroughly restored in 2005.

The second and third conference day (10<sup>th</sup>/11<sup>th</sup> Sep) full with oral and poster presentations will take place in the Heilig-Geist-Kapelle. To get to the conference room, please enter the building from Spandauer Straße and go to the left side of the building.

## Events

### Campus Tour

The Campus North of the Humboldt-Universität zu Berlin is both a historically significant place and an idyllic natural area hidden in the middle of the city. Guided campus tours will take place on Monday the 9<sup>th</sup> of September between 4:30 and 6 p.m. Each tour lasts around 20 minutes and is conducted in either German or English. Meeting point is in the atrium of the **Rhoda-Erdmann-Haus** (House 22) at the **Campus North**.

### Opening and evening lecture

The evening lecture and opening keynote talk on Monday 9<sup>th</sup> of September will be given by Prof. Dr. Andreas Richter (University of Vienna). It takes place in **House 18, lecture hall 2** of the **Campus North**. Please go around house 18, the lecture hall is on the back side!

### Icebreaker

After the evening lecture we get together for an icebreaker with finger-food and barbeque. The event is located in the atrium of house 22 and the lawn between the houses 22, 11 and 9 of the **Campus North**. The costs are covered by the registration fee.

### Conference Dinner

On Tuesday 10<sup>th</sup> of September evening we have dinner together at the [“Brasserie am Gendarmenmarkt”](#) **Taubenstraße 30, 10117 Berlin**. The costs are covered by the registration fee. The restaurant is 20 min by foot from our conference house, the Heilig Geist Kapelle. If you walk, you pass by e.g., the Berlin Dome, the Berlin City Palace, the State Opera, and the Bebel square. You may also take the bus 200 starting at Spandauer Straße / Marienkirche. The bus runs every 10 min; take the stop “Jerusalemer Straße” and walk 5 min to the restaurant. The restaurant is located at the Gendarmenmarkt, which is arguably Berlin’s most beautiful square. The name Gendarmenmarkt derives from its former use as stables for the cuirassier regiment of the Gens d’armes, which the “Soldier King” Frederick William I. (German: Friedrich Wilhelm I.) had built here in 1736. The three monumental buildings German Dome, French Dome and the Konzerthaus beautifully frame the square.



Gendarmenmarkt (© Martin Lindberg)



## Conference Program at a Glance

### Monday 9 September 2024

|            |       |   |                               |              |
|------------|-------|---|-------------------------------|--------------|
| 16:00      | 18:00 | Registration  | Rhoda-Erdmann-Haus (House 22) | Campus North |
| 16:30      | 18:00 | guided campus tours   | Campus North                  |              |
| 18:00      | 18:15 | Opening   | House 18, lecture hall 2      | Campus North |
| 18:15      | 19:15 | <b>Evening Keynote - Andreas Richter</b> Beyond Carbon Use Efficiency: Microbial Growth as the Key Driver of Soil Processes | House 18, lecture hall 2      | Campus North |
| from 19:15 |       | <b>Icebreaker</b>   | Rhoda-Erdmann-Haus (House 22) | Campus North |

### Tuesday 10 September 2024

| Session 1: |       | Linking carbon and energy fluxes in soil systems  |                                    | Chair: Anja Miltner   | at Heilig-Geist-Kapelle   |
|------------|-------|---|------------------------------------|---|---|
| 8:30       | 9:10  | S1-K  | <b>Keynote: Marie Spohn</b>        | Microbial carbon cycling is related to the cycling of nitrogen and phosphorus |   |
| 9:10       | 9:30  | S1-1  | Herrmann                           | Anke  | Microbial heat dissipation in response to substrate diversity: Experimental and theoretical considerations                                  |
| 9:30       | 9:50  | S1-2  | Kaiser                             | Klaus   | Mineral-induced carbon stabilization in soil - A combined molecular and thermodynamic approach to unravel sorptive interactions             |
| 9:50       | 10:10 | S1-3  | Dehghani                           | Fatemeh   | Calorespirometry study of cellulose and its building blocks in soil: insights into the role of hydrolytic enzymes                           |
| 10:10      | 10:30 | S1-4  | Lorenzen                           | Christian   | Effects of temporal substrate supply variability on the activity of extracellular enzymes   |
| 10:30      | 11:00 | Coffee Break  |                                    |   |   |
| 11:00      | 11:20 | S1-5  | Shao                               | Guodong   | Linking soil metabolic activities and energy fluxes using diverse substrates with contrasting oxidation level                               |
| 11:20      | 11:40 | S1-6  | Yang                               | Shiyue  | Enhanced Isothermal Microcalorimetry: Simultaneous CO <sub>2</sub> and Heat Measurement with Non-Invasive Sensor Integration                |
| 11:40      | 12:00 | S1-7  | Di Lodovico                        | Eliana  | A macrocalorespirometer for soil science. What are the benefits?  |
| 12:00      | 12:20 | S1-8  | Leme Oliva                         | Rebeca  | The role of microbial residues on soil organic matter dynamics: EPS and necromass   |
| 12:20      | 12:40 | S1-9  | Simon                              | Carsten   | Long-term effects of farmyard manure addition on soil organic matter composition: C transformation as a major driver of energetic potential |
| 12:40      | 14:00 | Lunch Break   |                                    |   |   |
| Session 2: |       | Under the lens: soil carbon and energy channels across trophic levels                     |                                    | Chair: Liliane Rueß   | at Heilig-Geist-Kapelle   |
| 14:00      | 14:40 | S2-K  | <b>Keynote: María J.I. Briones</b> | Soil fauna: innocent or culpable of C emissions from soils?                   |   |
| 14:40      | 15:00 | S2-1  | Lu                                 | Jingzhong   | Reduced energy flux in soil food webs by introduced tree species: bottom-up control of multitrophic biodiversity across size compartments   |
| 15:00      | 15:20 | S2-2  | HU                                 | Junwei  | Do root phenotypic traits mediate the effects of bacterivorous and herbivorous nematodes on rhizosphere bacterial communities?              |
| 15:20      | 15:40 | S2-3  | van Bommel                         | Miriam  | Grazing pressure affects carbon flow and energy release in the soil micro-food web  |
| 15:40      | 16:00 | S2-4  | Wang                               | Yuxin   | Unveiling the Role of Protists: Species-Specific and Size-Dependent Regulation of Litter Decomposition                                      |
| 16:00      | 18:00 | Coffee & Poster (all posters)   |                                    |   | Seminar Room 22 & 23  |
| 19:00      |       | <b>Conference Dinner</b> at "Brasserie am Gendarmenmarkt" (Taubenstraße 30, 10117 Berlin) |                                    |   |   |



Wednesday 11 September 2024

| Session 3: |       |                           | Linking the composition of microbiomes to matter and energy fluxes        |           | Chair: Christoph Tebbe  | at Heilig-Geist-Kapelle |
|------------|-------|---------------------------|---|-----------|---|-------------------------|
| 8:30       | 9:10  | S3-K                      | <b>Keynote: Noah W. Sokol</b>   |           | Life, death, and decay in the soil microbiome: how soil microbes shape organic matter cycling   |                         |
| 9:10       | 9:30  | S3-1                      | Varsadiya   | Milan     | Exploring soil-free microbial cell extracts as a novel approach towards disentangling microbial carbon and energy utilization patterns in soil              |                         |
| 9:30       | 9:50  | S3-2                      | Poll  | Christian | Gradients of C flux and energy dissipation in the detritusphere   |                         |
| 9:50       | 10:10 | S3-3                      | Yang  | Xiaojing  | How do microbes mediate soil carbon dynamic in the deep rhizospheres of perennial crops?  |                         |
| 10:10      | 10:30 | S3-4                      | Urich   | Tim       | Kill'em all? Interactions of predatory Myxobacteria with soil microbes – an in vitro and microcosm perspective on their role in the soil microbial food-web |                         |
| 10:30      | 11:00 | Coffee Break              |   |           |   |                         |
| 11:00      | 11:20 | S3-5                      | Tamang  | Mandip    | Succession of bacteria and archaea within the soil micro-food web: from natural to synthetic communities  |                         |
| 11:20      | 11:40 | S3-6                      | Finn  | Damien    | Soil microbial metabolic quotient follows ecological successional patterns under one year of winter wheat cropping  |                         |
| 11:40      | 12:00 | S3-7                      | Camenzind   | Tessa     | Carbon-use efficiency 2.0? Insights from fungal growth dynamics in response to resource complexity and energy content                                       |                         |
| 12:00      | 12:20 | S3-8                      | Kucerik   | Jiri      | Biodegradable plastics in soils: benefits and threats   |                         |
| 12:20      | 12:40 | S3-9                      | Milesi  | Vincent   | Redox gradient across peat soils shapes the chemical and taxonomic composition of microbial communities   |                         |
| 12:40      | 14:00 | Lunch Break               |   |           |   |                         |
| Session 4: |       |                           | Modeling of matter and energy flows in soil systems                       |           | Chair: Holger Pagel   | at Heilig-Geist-Kapelle |
| 14:00      | 14:40 | S4-K                      | <b>Keynote: Stefano Manzoni</b>   |           | Disentangling organic matter stabilization pathways using dynamical models in phase space   |                         |
| 14:40      | 15:00 | S4-1                      | Blagodatsky   | Sergey    | Modeling of carbon and heat fluxes in soil with multiple limitations of microbial growth  |                         |
| 15:00      | 15:20 | S4-2                      | Ghersheen   | Samia     | Modelling nitrogen limitation of litter decomposing fungi   |                         |
| 15:20      | 15:40 | S4-3                      | Schwarz   | Erik      | When and why microbial-explicit SOC models can be unstable  |                         |
| 15:40      | 16:00 | S4-4                      | Wutzler   | Thomas    | Do thermodynamics and stoichiometry constrain soil carbon dynamics and yield optimum system behavior?   |                         |
| 16:00      | 16:30 | Coffee Break              |   |           |   |                         |
| Session 5: |       |                           | Calorimetry and thermodynamics: keys to unraveling complex soil processes |           | Chair: Thomas Maskow  | at Heilig-Geist-Kapelle |
| 16:30      | 16:50 | S5-1                      | Wadsö   | Lars      | Comments on the use of isothermal calorimetry in soil science   |                         |
| 16:50      | 17:10 | S5-2                      | Yildiz  | Cennet    | Interactions of N mineralisation from slurry-N with heat dissipation and organic matter dynamics  |                         |
| 17:10      | 17:30 | S5-3                      | DeFrang   | Emma      | Comparing Thermal Methods to Measure Changes in Thermal Stability of Organic Carbon in Amended Soils during Microbial Incubation                            |                         |
| 17:30      | 17:50 | S5-4                      | Miltner   | Anja      | Thermodynamic control of microbial turnover of organic substrates in soils  |                         |
| 17:50      | 18:10 | S5-5                      | Kästner   | Matthias  | The MTB model: thermodynamic predictions of microbial turnover to biomass   |                         |
| 18:10      | 18:30 | Wrap up/ Final discussion |   |           |   |                         |





## Poster

| Linking carbon and energy fluxes in soil systems                          |                 |                |  |
|---|-----------------|----------------|--|
| P1  | Siegenthaler    | Maja           | Oxygen exchange between water and phosphate can provide insights into carbon dynamics in soils   |
| P2  | Yang            | Shiyue         | Exploring Mass and Thermodynamic Energy Balances in Artificial Soil Under Diverse Environmental Settings   |
| P3  | Yousaf          | Ubaida         | Biotransformation of selected substrates and the consequent microbial growth — fate of 13 C – Carbon in soil.  |
| P4  | Lechtenfeld     | Oliver         | The life of a soil microbe is short but its MEMORIES remain: Production of stable Microbial ExoMetabOmites and ResIduES from simple and complex substrates.        |
| P5  | Rathnayake      | Dilani         | Molecular and bioenergetic signatures in mineral-associated and particulate organic matter fractions under long-term field experimental condition                  |
| P6  | Yuan            | Ye             | Mineral and substrate control on MOM formation efficiency, and feedbacks to microbial composition and function   |
| P7  | Attor           | Festus         | Soil microbial carbon use efficiency in a temperate cropland agroforestry system   |
| P8  | Xu              | Tianxing       | Effects of spatial heterogeneity on the fungal energy use channel  |
| P9  | Konrad          | Alexander      | Microbial carbon use efficiency of mineral-associated organic matter is related to its desorption  |
| P10   | Brtnicky        | Martin         | Benefits from biochar modified with various materials, used as soil amendment, on soil properties and plant biomass  |
| P11   | Holatko         | Jiri           | Evaluation of benefits from digestate amended with various materials, used as fertilizer, on soil properties and plant biomass                                     |
| P12   | Volikov         | Alexander      | From Biomass to Soil Carbon: Exploring the Fate of Artificial Humic Substances in Winogradsky column   |
| P13   | Rzepczynska     | Agnieszka      | Microbial Responses to Altered Resource Availability in a Changing Arctic: Implications for Nutrient Cycling and Climate Feedbacks                                 |
| P14   | Winterfeldt     | Sara           | Microbial growth resistance and resilience to drought across Europe  |
| P15   | Peter           | Anne           | Impact of Century-Scale Soil Warming n Soil Organic Matter Dynamics and Microbial Communities in Subarctic Ecosystems  |
| P16   | Montaño López   | Fernando       | Investigating energy fluxes and organic matter stability in permafrost through integrated physical, biological and chemical indices                                |
| P17   | Kuzyakov        | Yakov          | Activation energy of biochemical processes in soil   |
| Under the lens: soil carbon and energy channels across trophic levels     |                 |                |  |
| P18   | Gergócs-Winkler | Veronika       | How does soil food-web influence nutrient cycling after returning crop residues to agricultural fields? A mesocosm experiment                                      |
| Linking the composition of microbiomes to matter and energy fluxes        |                 |                |  |
| P19   | Elshal          | Abdelhady      | Impact of soil properties on microbiomes involved in the decomposition of cellulose  |
| P20   | Groven          | Anne-Catherine | The impact of nitrification inhibitors on the microbial community in German agricultural fields in mitigating nitrous oxide emissions.                             |
| P21   | Guasconi        | Daniela        | Effect of microbial diversity on soil responses to forest management   |
| P22   | Lumactud        | Rhea Amor      | Exploring the role of bacterial synthetic communities in alleviating drought stress by shaping trophic interactions and enhancing soil health and plant resilience |
| P23   | Mansour         | India          | Microhabitat spatial structure affects the establishment and persistence of necromass recycling-based model microbial systems                                      |
| P24   | Moreau          | Ulysse         | Effect of organic matter on the bioenergetic landscape of phytostabilized mine tailings  |
| P25   | Ruggaber        | Julian         | Metatranscriptomics for Studying Carbon Conversion in the Rhizosphere  |
| P26   | Müller          | Karin          | Land use change affects soil properties, microbial communities and carbon cycling  |
| Modeling of matter and energy flows in soil systems                       |                 |                |  |
| P27   | Collart         | Paul           | Hybrid Soil Microbiome Modeling - Combining process-based models with machine learning to predict microbial dynamics and organic matter turnover in soil           |
| P28   | Li              | Xiankun        | Are the mechanisms driving heterotrophic respiration after rewetting consistent in the lab and the field?  |
| P29   | Müller          | Markus         | Bioenergetic modeling and data-model integration to simulate coupled carbon and energy flows in artificial soils   |
| P30   | Li              | Jianwei        | Generalized or distinct microbial parameters in soil biogeochemical models? insights from a three-decade field experiment and a multi-site incubation experiment   |
| Calorimetry and thermodynamics: keys to unraveling complex soil processes |                 |                |  |
| P31   | Sibille         | Thomas         | Quantum Mechanics-Informed Refinement of Nominal Oxidation State of Carbon: Predictive Precision in Anaerobic Ecosystem Dynamics                                   |

## Abstracts of Oral Presentations

### Opening Keynote

#### **O-K Beyond Carbon Use Efficiency: Microbial Growth as the Key Driver of Soil Processes**

**Richter A.**

Centre for Microbiology and Environmental Systems Science, University of Vienna, Austria

[andreas.richter@univie.ac.at](mailto:andreas.richter@univie.ac.at)

Growth is the foundation of all life, essentially driving the dynamics of all ecosystems. Microbial communities tightly regulate their population growth to optimize survival in ever-changing environments. The growth of heterotrophic microorganisms involves deconstructing soil organic matter and assimilating organic carbon into biomass, and, upon death, their remains become part of soil organic matter. Without microbial growth, organic matter turnover would cease, effectively halting the fluxes of matter and energy. Thus, soil biogeochemistry is fundamentally driven by the growth and activity of microbial communities.

Despite its fundamental importance, microbial growth in soil remains poorly understood, particularly in the context of its control, regulation, and response to climate change and other anthropogenic pressures. Even the basic definition of growth in soil ecology lacks clarity, complicating our understanding of microbial contributions to soil processes.

In this talk, I will explore the processes encompassed by microbial growth and propose a theoretical framework for understanding growth dynamics in soil. I will share insights from a series of experiments investigating the impacts of climate change on growth, turnover, and activity of soil microorganisms. I will focus on different levels of resolution — from communities to individual populations — and examine different growth components, including cell division and the synthesis of storage compounds.

I will argue that to advance our understanding of soil functioning, we must move beyond traditional metrics like carbon use efficiency, microbial respiration, and nutrient mineralization rates. Instead, we should focus on microbial growth as the fundamental unit linking soil microbiomes to matter and energy fluxes.

### Session 1: Linking carbon and energy fluxes in soil systems

#### Keynote

#### **S1-K Microbial carbon cycling is related to the cycling of nitrogen and phosphorus**

**Spohn M.**

Department of Soil and Environment, Swedish University of Agricultural Sciences (SLU), Lennart Hjelms väg 9, P.O. Box 7014, 75007 Uppsala, Sweden

[marie.spohn@slu.se](mailto:marie.spohn@slu.se)

Carbon (C), nitrogen (N), and phosphorus (P) are covalently bound in organic matter. Therefore, microbial C cycling is closely related to the cycling of N and P. In this talk, I will explore and discuss three aspects of microbial C, N, and P cycling in soils.

The first part of the talk will tackle the questions: Why is there preferential stabilization of microbial-derived organic matter in soils?

Microbial biomass and microbial necromass are richer in N and P than plant biomass and plant detritus. Organic compounds containing N and P have a high affinity to adsorb to minerals, which likely slows down the decomposition of these compounds in soils. Thus, molecular composition (rather than origin) likely affects sorptive stabilization of organic matter derived from microbes and plants and leads to preferential stabilization of N- and P-rich microbial necromass in soils.

The second part of the talk will be about microbial C use efficiency, biomass turnover, and nutrient recycling.

The turnover time of C in the soil microbial biomass often varies more strongly than microbial C use efficiency. Thus, variation in the turnover time of C in the microbial biomass is likely key for microbial adjustment to substrate quality and availability. Furthermore, the adjustment of the turnover time of individual nutrients (N and P) in the microbial biomass might be an important mechanism that allows microorganisms to cope with unfavorable substrate stoichiometry.

The third part of the talk will deal with the question: What forms part of the soil system (and how large is the system)?

Even in the deep subsoil (>2 m), microbial activity depends on recent plant-derived C inputs to the soil. Furthermore, during the initial stages of decomposition of nutrient-poor plant litter, (fungal) transport of N and P into the litter occurs. Thus, transport processes are very likely crucial for microbial activity and tightly connect different soil horizons and the soil to plant activity. Hence, the system boundaries are likely much wider than many (incubation) studies indicate.

Taken together, the results show that for understanding soil microbial C dynamics in soils, it is necessary to explore how C cycling is related to the cycling of N and P at different temporal and spatial scales.

### S1-1 Microbial heat dissipation in response to substrate diversity: Experimental and theoretical considerations

Herrmann A.M.<sup>1</sup>, Dufour L.J.P.<sup>1</sup>, Nunan N.<sup>1,2</sup>

<sup>1</sup> Department of Soil & Environment, Swedish University of Agricultural Sciences, Sweden

<sup>2</sup> Sorbonne University, CNRS, EPHE, UR Environment, Transfers and Interactions in Soils and Water Bodies (METIS)

[anke.herrmann@slu.se](mailto:anke.herrmann@slu.se)

Recently, Lehmann et al. (2020. *Nature Geoscience* 13, 539-534) proposed a theoretical framework in which the persistence of carbon (C) in soil can be understood as the outcome of interactions between molecular variability of organic matter and spatio-temporal heterogeneities of the soil system. However, this theory has not yet been tested rigorously. In a pilot experiment, we explored the influence of substrate diversity on soils from a long-term field experiment, start 1987, on the south-west coast of Sweden (56°29'46.32"N, 13°2'22.56"E). Here, perennial ryegrass (*L. perenne* L.) was used as a cover crop undersown in spring cereals. In the laboratory, we amended soils with 50 µg C g<sup>-1</sup> soil of either 18 individual substrate additions (i.e. low molecular weight C substrates, substrate diversity = 1) or their combinations of 2, 4, 6, 9, 12 or 17 substrate diversity, and heat dissipation was determined in an isothermal calorimeter (TAM AIR, Sollentuna, Sweden) over 24 hrs at 15°C. Heat dissipation from individual substrates were used to calculate theoretical heat dissipation values of the various substrate diversity mixtures, and theoretical and experimental heat dissipation were compared. We expected a greater divergence between theoretical and experimental heat dissipation at high substrate diversity due to potential interactions in the microbial processing of the different substrates. Experimental heat dissipation, i.e. actual measured, was always higher and more variable than theoretical calculated heat dissipation. The increase in variability can be explained by differences in microbial communities not processing the substrates in the same way (more or less efficient), whereas theoretical heat dissipation are solely based on thermodynamic constraints. Higher experimental heat dissipation may be due to that the processing of substrates requires production of enzymes, membrane transport systems, removal of waste etc. that are not accounted for in theoretical heat dissipation calculations. Overall, the relation between heat dissipation and nominal oxidation state of carbon is bell-shaped with a maximum at a NOSC = 0. We will put the results from this pilot study into a thermodynamic framework and outline related research needs.

## S1-2 Mineral-induced carbon stabilization in soil – A combined molecular and thermodynamic approach to unravel sorptive interactions

Kaiser K.<sup>1</sup>, Konrad A.<sup>2</sup>, Lechtenfeld O.<sup>3</sup>, Mulder I.<sup>2,4</sup>, Siemens J.<sup>2</sup>, Simon C.<sup>3</sup>

<sup>1</sup> Martin Luther University Halle-Wittenberg, Halle (Saale), Germany

<sup>2</sup> Justus-Liebig-University Giessen, Giessen, Germany

<sup>3</sup> Helmholtz Centre for Environmental Research-UFZ, Leipzig, Germany

<sup>4</sup> Ruhr-Universität Bochum, Bochum, Germany

[klaus.kaiser@landw.uni-halle.de](mailto:klaus.kaiser@landw.uni-halle.de)

One way to study the turnover and persistence of soil organic matter (OM) is by tracking changes in its thermodynamics. Biological processes but also organic–mineral interactions need to be considered in the thermodynamics of soil OM. While it is possible to determine  $\Delta G$ ,  $\Delta H$ , and  $\Delta S$  for the sorption of specific small organic molecules to individual minerals, the quantification of thermodynamics of sorption processes of complex natural OM is challenging. Here, we tested the hypothesis that the thermodynamics of the sorption of OM to oxides is driven by exothermic surface complexation and the degree of the sorbing mineral's loading. Citrate served as reference for simple organic molecules and water-soluble OM from either fresh (OM<sup>fresh</sup>) or partially decomposed maize litter (OM<sup>degraded</sup>) were used as examples of complex natural mixtures. The sorbent was a high-surface goethite (120 m<sup>2</sup> g<sup>-1</sup>). We combined quantification of organic carbon sorption, isothermal titration calorimetry (ITC) for the determination of  $\Delta G$ ,  $\Delta H$ , and  $\Delta S$ , and solution and solid-state molecular analyses by Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR-MS) for detection of sorption-related fractionation. On carbon base, the extent of sorption was citrate < OM<sup>fresh</sup> < OM<sup>degraded</sup>. Sorption of citrate revealed a clear saturation of the mineral's sorption capacity, while the sorption of complex maize litter-derived OM featured gradually increasing carbon retention even at high loadings. Strongest heat flows were detected for all test compounds at initial stages of the sorption but became smaller at later stages, indicating an increasing degree of complexation of the hydroxyl groups at the mineral's surface. Changes in molecular composition suggested initial selection for lower-mass molecules and increasing selection of higher-mass molecules with increasing mineral loading for the two complex OM samples, which explains the slight increases in carbon retention even at high loadings. Our results suggest that the large heat fluxes during initial sorption stages reflect interactions at available binding sites, while the smaller fluxes at later stages may be due to successive replacement of smaller by larger molecules at occupied binding sites. In summary, the study showed the great potential of combined molecular and thermodynamic measurements to unravel the processes underlying the sorptive stabilization of soil OM.

## S1-3 Calorespirometry study of cellulose and its building blocks in soil: insights into the role of hydrolytic enzymes

Dehghani F.<sup>1</sup>, Reitz T.<sup>1</sup>, Schlüter S.<sup>2</sup>, Blagodatskaya E.<sup>1</sup>

<sup>1</sup> Department of Soil Ecology, Helmholtz-Centre for Environmental Research – UFZ, Theodor-Lieser-Str. 4, 06120 Halle (Saale), Germany

<sup>2</sup> Department of Soil System Science, Helmholtz-Centre for Environmental Research – UFZ, Theodor-Lieser-Str. 4, 06120 Halle (Saale), Germany

[fatemeh.dehghani@ufz.de](mailto:fatemeh.dehghani@ufz.de)

Carbon fixation in soil, facilitated by microbial processing of plant residues, plays a central role in carbon cycling. Plant residues serve as a source of C, nutrients, and energy for soil microorganisms and their transformation into stabilized soil organic matter or CO<sub>2</sub> involves a range of microbe-driven processes including enzymatic hydrolysis. For example, the degradation of cellulose, which is the most abundant C component of plant cell walls, entails steps of depolymerization to glucose catalyzed by hydrolytic enzymes as well as further mineralization to CO<sub>2</sub>. To comprehensively understand these

processes and the bottleneck step during cellulose degradation in soil, we employed isothermal microcalorimetry and respirometry techniques and monitored the dynamics of heat and CO<sub>2</sub> release during the degradation of cellulose and its oligomeric and monomeric building blocks, cellobiose and glucose, respectively. Using fluorogenically labeled substrates, we also assessed the dynamics of activities of cellobiohydrolase and  $\beta$ -glucosidase and heat release associated with extracellular degradation of cellulose.

Our results revealed a 38-hour temporal decoupling between heat and CO<sub>2</sub> release for cellulose, suggesting distinct stages in its degradation pathway, in contrast, synchronous heat and CO<sub>2</sub> curves were observed for cellobiose and glucose. Enzyme activity assays highlighted cellobiohydrolase, but not  $\beta$ -glucosidase as the rate-limiting step over cellulose breakdown as cellobiohydrolase activity increased after cellulose amendment by a factor of 2 to 5 but that of  $\beta$ -glucosidase only increased by a factor of 1.5.

Our study reveals differences in temporal dynamics of heat and CO<sub>2</sub> release between cellulose and its building blocks cellobiose, and glucose. For the first time, we estimated the heat associated with extracellular cleavage of cellulose to cellobiose emphasizing the role of enzymatic hydrolysis in the degradation of polymeric substrates. Understanding these processes is crucial for modeling carbon cycling in terrestrial ecosystems and assessing their response to environmental changes.

#### **S1-4 Effects of temporal substrate supply variability on the activity of extracellular enzymes**

**Lorenzen C.<sup>1</sup>, Zimmermann I.<sup>1</sup>, Razavi B.<sup>2</sup>, Spielvogel S.<sup>1</sup>**

<sup>1</sup> Institute of Plant Nutrition and Soil Science CAU Kiel, Department of Soil Science, Germany

<sup>2</sup> Institute of Phytopathology CAU Kiel, Department of Soil and Plant Microbiome, Germany

[c.lorenzen@soils.uni-kiel.de](mailto:c.lorenzen@soils.uni-kiel.de)

The nutrient and energy acquisition from organic substrates by microorganisms are energy-demanding processes, and therefore a sufficient energy yield from the substrate is needed to provide an energetic basis for cellular maintenance and microbial growth. However, the amount of energy return on investment is strongly linked the functional complexity of the soil system. One aspect of this functional complexity is the temporal variability of substrate availability, which is driven by: i) varying release rates of substrates, ii) changes in soil moisture, iii) substrate transport processes.

We conducted an incubation experiment to analyze how the microbial community of an arable soil from a long-term fertilization experiment reacts to fluctuations in substrate supply from a maize litter based detritusphere. For this purpose, xylooligosaccharides (XOS) were solved in water and provided to soil microcosms (4.74 g of soil with a gravimetric water content of 100 mg g<sup>-1</sup> soil) in three different time patterns: i) one day XOS solution (53.2 g/l) and three days pure water, ii) two days of XOS solution (26.6 g/l) and pure water each (alternating), iii) XOS solution (13.3 g/l) on four days. The total amount of XOS was constant throughout the treatments, and corresponded to four times microbial biomass carbon. Each XOS solution and pure water addition was carried out with a volume of 64.7  $\mu$ l. During the incubation heat dissipation and CO<sub>2</sub> were measured to monitor substrate utilization related energy flows. Enzyme activities of  $\beta$ -xylosidase and leucine-aminopeptidase were determined at heat flow peaks and contextualized by an analysis of the microbial community composition.

Multiple applications of the lower concentrated substrate solution increased the amount of heat that was dissipated per unit of substrate and altered the shape of the substrate-induced heat flow peaks. Carbon cycle related enzymes displayed an increased activity and substrate affinity after multiple substrate pulses and frequent substrate additions stimulated the production of nitrogen cycle related enzymes, thus indicating a link between the changes in heat flow dynamics and enzyme activity.

Our results show that the addition of a fixed amount of substrate with varying time patterns induces different substrate utilization strategies. Therefore, temporal variability of substrate supply affects the fate of chemically bound energy in the soil.



### S1-5 Linking soil metabolic activities and energy fluxes using diverse substrates with contrasting oxidation level

Shao G.<sup>1</sup>, Xu X.<sup>2</sup>, Banfield C.C.<sup>1</sup>, Shi L.<sup>1</sup>, Wu W.<sup>3</sup>, Mason-Jones K.<sup>1</sup>, Dippold M.A.<sup>1</sup>

<sup>1</sup> Geo-Biosphere Interactions, University of Tübingen, Tübingen, Germany

<sup>2</sup> Institute of Applied Ecology, Chinese Academy of Sciences, Shenyang, China

<sup>3</sup> College of Marine Science, Shanghai Ocean University, Shanghai, China

[guodong.shao@uni-tuebingen.de](mailto:guodong.shao@uni-tuebingen.de)

Microorganisms metabolize soil organic carbon (C) as a source of energy and biosynthetic precursors, while releasing carbon dioxide (CO<sub>2</sub>) to the atmosphere and contributing to long-term soil C storage. Mass and energy flows through soil microbial metabolism are subject to the laws of thermodynamics. C allocation through central metabolic can be reconstructed by <sup>13</sup>C-labelling coupled to metabolic flux analysis. However, mass flow calculated via metabolic flux analysis alone cannot fully characterize microbial C use. Here, we coupled energetics and mass flow on a metabolic level by selecting optimal sets of isotopomer tracers. Fifteen position-specific or uniformly <sup>13</sup>C-labelled isotopomers - four alanine, seven glucose, and four glutamic acid ones - were added to a Luvisol, and we quantified substrate-derived <sup>13</sup>CO<sub>2</sub> fluxes along with microbial physiological traits (e.g. CUE) as well as heat dissipation via isothermal microcalorimetry.

Our results demonstrate that the temporal dynamics of catabolic CO<sub>2</sub> release resembles that of the heat dissipation, i.e. peak respiration and peak heat dissipation were reached approximately 18 h after substrate addition, irrespective of whether the substance entered the central metabolic pathway at the monosaccharide level (glucose), at the pyruvate level (alanine) or in the citric acid cycle (glutamic acid). This indicates that heat dissipation in the initial growth period was strongly dominated by catabolic processes. However, whereas <sup>13</sup>CO<sub>2</sub> release leveled off during the 36 hours of incubation, the heat dissipation remained above its original level, suggesting that anabolic processes increasingly contribute to the heat dissipation in the later phases of incubation. Calorespirometric ratio dropped after the exponential growth phase indicates heat dissipation reduces over proportionally compared to catabolic C oxidation after multiplicative growth. Glucose isotopomer utilization indicated dominance of the pentose phosphate and Entner Doudoroff pathways over glycolysis, suggesting a high activity of fast-growing organisms with considerable C allocation to anabolism. The dominance of this anabolic C use in the later stage of the incubation was confirmed by the isotopomer utilization of alanine and glutamic acid. This study shows that the heat dissipation of growing microbial communities under high C supply is closely linked to their catabolic CO<sub>2</sub> release, whereas slow, potentially recycling-based growth after resource depletion releases energy more via anabolic reactions. We furthermore demonstrated that coupled metabolic flux analysis and calorespirometry provides a powerful tool to understand microbial response in C and energy use in soils *in-situ*.

### S1-6 Enhanced Isothermal Microcalorimetry: Simultaneous CO<sub>2</sub> and Heat Measurement with Non-Invasive Sensor Integration

Yang S.<sup>1</sup>, Paufler S.<sup>1</sup>, Miltner A.<sup>2</sup>, Kästner M.<sup>2</sup>, Maskow T.<sup>1</sup>

<sup>1</sup> Department of Microbial Biotechnology, Helmholtz-Centre for Environmental Research – UFZ, Permoserstraße 15, 04318 Leipzig, Germany

<sup>2</sup> Department of Molecular Environmental Biotechnology, Helmholtz-Centre for Environmental Research – UFZ, Permoserstraße 15, 04318 Leipzig, Germany

[shiyue.yang@ufz.de](mailto:shiyue.yang@ufz.de)

Soil is the most pivotal carbon stock pool in terrestrial ecosystems. Respiration serves as a vital parameter for characterizing microbial activity in soil systems, a fundamental concept universally applied in soil science. All microbial activities involve the release of heat, with the energy generated by catabolic reactions fueling anabolic reactions for biomass production and cellular activities. The ratio between

heat and CO<sub>2</sub>, termed the calorespirometric ratio, serves as a crucial indicator of carbon and energy use efficiency in microbial activities.

Various conventional methods exist for measuring respiration. Gas sampling, typically analyzed via gas chromatography (GC), ensures accurate CO<sub>2</sub> measurement but sacrifices continuous data collection. CO<sub>2</sub> traps, employing solutions such as NaOH, are also widely used. Here, absorbed CO<sub>2</sub> is quantified through titration or inorganic C determination. However, the diffusion time from soil into NaOH solution presents an unavoidable variable leading to delays in CO<sub>2</sub> flux measurements. Isothermal microcalorimeters (IMCs) often measure heat production rates at user-defined intervals to enable continuous monitoring. Yet, these parameters are typically measured on different timescales and within separate vials, complicating their integration.

To address these challenges, we propose the further development of an isothermal calorimeter equipped with a non-invasive infrared CO<sub>2</sub> sensor, enabling simultaneous measurement of heat and respiration. In our approach, the CO<sub>2</sub> sensor is fixed at the cap of a calorimetric ampoule (20 mL, glass), while heat production is monitored by a Peltier element. For our incubation setup, we utilized soil samples from Dikopshof, from plots which were fertilized with farmyard manure. The soil samples were amended with glucose and incubated at 16% water content and a temperature of 20 °C. Our incubation setup allowed for continuous measurement of both heat and CO<sub>2</sub> in the same incubation vial.

To validate the accuracy of CO<sub>2</sub> measurements, we employed the BlueSens gas sensor as a reference outside the calorimeter, maintaining the same air-to-soil ratio and coupling it with O<sub>2</sub> consumption measurements. We will present the setup of our IMC development and discuss the results with respect to heat and CO<sub>2</sub> production rates.

### S1-7 A macrocalorespirometer for soil science. What are the benefits?

Di Lodovico E.<sup>1,2</sup>, Fricke C.<sup>1</sup>, Meyer M.<sup>1</sup>, Maskow T.<sup>2</sup>, Schaumann G.E.<sup>1</sup>

<sup>1</sup> RPTU Kaiserslautern-Landau, Fortstraße 7, 76829 Landau in der Pfalz

<sup>2</sup> Helmholtz Zentrum für Umweltforschung - UFZ, Permoserstraße 15, 04318 Leipzig

[eliana.di-lodovico@ufz.de](mailto:eliana.di-lodovico@ufz.de)

The carbon cycle is currently receiving significant attention due to its critical connection with climate change. Soil, as one of the largest carbon sinks, plays a central role in the carbon cycle through the activity of microorganisms. These soil microorganisms regulate carbon fluxes and the associated energy fluxes. They use the Gibbs energy content of soil organic matter (SOM) for growth. The change in Gibbs energy during growth reactions contains an enthalpic and entropic part (von Stockar, 2010). This enthalpic part can be measured as heat using calorimetry. Calorespirometry, which combines calorimetric and respirometric measurements, provides valuable insights into SOM and microbial degradation pathways (Hansen et al., 2004). Despite its versatility and non-destructive nature, conventional calorespirometric methods have limitations, such as oxygen constraints, low sample throughput, indirect CO<sub>2</sub> measurements, and small sample size. The macrocalorespirometer developed in this study overcomes these weaknesses. It combines a modern respirometer with a calorimetric unit in one unique channel (Fricke et al., 2024), for a total of 24 channels. The application of this device to soil is demonstrated with glucose-amended agricultural soil with low organic matter (Dikopshof, Luvisol). The simultaneous heat production rate, and CO<sub>2</sub> evolution rate are presented. The resulting calorespirometric ratios are evaluated and discussed. Further design and technical improvements are needed to enhance thermal stability and reduce result variability.

Fricke, C., Di Lodovico, E., Meyer, M., Maskow, T., Schaumann, G.E., 2024. Design, calibration and testing of a novel isothermal calorespirometer prototype. *Thermochim. Acta* 179785. <https://doi.org/10.1016/j.tca.2024.179785>

Hansen, L.D., Macfarlane, C., McKinnon, N., Smith, B.N., Criddle, R.S., 2004. Use of calorespirometric ratios, heat per CO<sub>2</sub> and heat per O<sub>2</sub>, to quantify metabolic paths and energetics of growing cells. *Thermochim. Acta* 422, 55–61.

von Stockar, U., 2010. Biothermodynamics of live cells: A tool for biotechnology and biochemical engineering. *J. Non-Equilib. Thermodyn.* - J NON-EQUIL THERMODYN 35, 415–475. <https://doi.org/10.1515/JNETDY.2010.024>

**S1-8 The role of microbial residues on soil organic matter dynamics: EPS and necromass****Oliva R.L.<sup>1</sup>, Dyckmans J.<sup>2</sup>, Joergensen R.G.<sup>1</sup>**<sup>1</sup> Soil Biology and Plant Nutrition, University of Kassel, Witzenhausen, Germany<sup>2</sup> Centre for Stable Isotope Research and Analysis, University of Göttingen, Germany[rebeca.oliva@uni-kassel.de](mailto:rebeca.oliva@uni-kassel.de)

There is no doubt that microorganisms are key contributors to (SOM) dynamics. In addition to their role on the breakdown of complex OM, soil microorganisms are also vital for the management of carbon stocks as they generate SOM, which is stable for long periods of time. Furthermore, even though microbial biomass is recognized as an important component of matter and energy fluxes in soil systems, little is known about how the microbial residual fraction (MRF) contributes to this scenario. The MRF is composed mainly by necromass (dead microbial cells) and EPS (extracellular polymeric substances, building blocks of microbial biofilms). Here, in an attempt to further understand the contribution of EPS to SOM, we carried out a series of experiments assessing microbial EPS production and composition. Our analyses focused especially on amino sugars (AS), thoroughly used as indicators for soil microbial necromass as they are not produced by plants. We hypothesized that particularly galactosamine (GalN), is an EPS component, as this is the second most common AS present in soils and its origin has not yet been identified. Firstly, we cultured fungal and bacterial species and analyzed the AS composition both in the cell cultures and the extracted EPS fraction. We found that not only GalN, but also mannosamine (ManN) were integral parts of microbial EPS. Subsequently, EPS composition (including AS, proteins, carbohydrates and DNA) was analyzed from ten bacterial and ten fungal species incubated with contrasting substrate and surface treatments. We observed EPS composition shifted according to the microbial growth conditions, suggesting that each biofilm component may have distinct functions within the matrix. Lastly, we conducted an incubation experiment with two different agricultural soils, each with contrasting fertilization treatments. Soils were amended with <sup>13</sup>C and <sup>15</sup>N labelled substrates and we traced the added C and N into multiple SOM fractions: total SOM, microbial biomass, soil AS, and EPS (and its composition). As results, we found that the MRF (EPS and necromass) is stable even long after substrate amendment (70 days). Also, there was supporting evidence indicating that not all AS are necromass markers in soils. While muramic acid and glucosamine certainly can be used to indicate accumulation of soil necromass, GalN is a good representative for microbial EPS production. All AS together can indicate the contribution of the MRF to SOM and therefore carbon stocks in soils. Overall, our results reinforce that AS are great MRF indicators and studying their dynamic can provide insights on how microbes contribute to stable SOM, not only as cell wall components, but also as integral parts of biofilms; calling attention to the importance of biofilms for SOM stocks.

**S1-9 Long-term effects of farmyard manure addition on soil organic matter molecular composition: C transformation as a major driver of energetic potential****Simon C.<sup>1</sup>, Miltner A.<sup>1</sup>, Mulder I.<sup>2,3</sup>, Kaiser K.<sup>4</sup>, Lorenz M.<sup>1,5</sup>, Thiele-Bruhn S.<sup>5</sup>, Lechtenfeld O.<sup>1</sup>**

(1) Helmholtz Centre for Environmental Research-UFZ, Leipzig, Germany

(2) Justus-Liebig-University Giessen, Giessen, Germany

(3) Ruhr-Universität Bochum, Bochum, Germany

(4) Martin Luther University Halle-Wittenberg, Halle (Saale), Germany

(5) Trier University, Trier, Germany

[carsten.simon@ufz.de](mailto:carsten.simon@ufz.de)

Long-term addition of farmyard manure (FYM) to arable soils supports the accumulation of soil organic carbon (SOC), higher microbial activity, and the presence of distinct extractable microbial metabolites. Yet, FYM-induced effects on energy storage, as opposed to SOC storage, remain unknown. In particular, it remains unresolved whether the increased microbial imprint relates solely to the FYM employed

(which is microbially active) or also relates to the stimulation of microbial transformation of soil organic matter (SOM). The latter would suggest that the accumulation of SOM transformation products controls energy storage, rather than FYM directly, and that immediate recycling of building blocks may be favoured over oxidation. We employed solid-state laser desorption ionization Fourier transform mass spectrometry (LDI-FT-ICR-MS) to study the molecular signatures of a selection of FYM samples and topsoils from four long-term field experiments (24 – 118 years) receiving FYM, and control plots receiving no fertilizer. We hypothesized that soils would be molecularly distinguishable and that the overlap with original FYM signatures could be used as a measure of long-term SOM transformation processes and their effect on SOM energetic potential as estimated by nominal oxidation state of C (NOSC) and standard molal Gibbs energies of oxidation half reaction (ion abundance weighted  $\Delta G^0_{C_{ox}}$ ). LDI-FT-ICR-MS suggests that FYM addition led to a significant increase in energetic potential across the four sites (0.7 – 1.2 kJ/mol C). FYM addition changed SOM composition by 3 – 16% of ion abundance as compared to control soil mass spectra, being largest in longest-running field experiments and smallest in the youngest experiment. Although FYM addition led to a uniform response in terms of molecular composition, site differences persisted. Markers unrelated to original FYM signatures (i.e., indirect treatment effects) explained 67 – 84% of molecular changes while markers directly associated to FYM explained only 2 – 12%, and were more relevant in the youngest experiment. FYM addition shifted molecular composition to higher H/C, O/C and m/z, and lower aromaticity. Accumulated molecules were more energy-rich and chemically similar to original FYM, but elevated in mass. Together, our results indicate a common shift in SOM properties upon FYM addition but also indicated site-specific trajectories of SOM compositional change. We demonstrated that FYM-induced microbial transformations and their impact on energy storage in SOM may have relevant implications for the long-term stability of SOM and C sequestration in soils in general.

## Session 2: Under the lens: soil carbon and energy channels across trophic levels

### Keynote

#### S2-K Soil fauna: innocent or culpable of C emissions from soils?

Briones M.J.I.

Department of Ecology and Animal Biology, University of Vigo, Spain

[mbriones@uvigo.es](mailto:mbriones@uvigo.es)

Soil fauna plays a critical role in soil organic matter (SOM) transformations, with important implications for the sequestration/emissions of carbon (C) from soils. This is the result of a wide array of activities, including feeding, casting, burrowing, and interacting with other above- and below-ground organisms. The soil mixing, the incorporation of fresh organic residues into the soil, the formation of micro and macroaggregates, and the changes in soil structure promote both SOM mineralization and stabilization. Several studies have tried to elucidate whether their positive effects on decomposition processes would make them responsible for increased greenhouse gas emissions (namely CO<sub>2</sub>) or the mediators for helping to enhance the C sink function of soils. However, resolving this dilemma will require to disentangle their “direct” contribution to the dynamic transformations (mobilization vs. stabilization) of SOM from their “indirect” effects (via plant and microbial interactions) as well as a more complete understanding of their functional dissimilarity and the modulating effects of the soil abiotic conditions. Here, I will provide an overview on field and laboratory experiments to determine the role of macrofauna (earthworms) and mesofauna (enchytraeids) on C turnover and explore how their activities could act either as potential hotspots for C retention or C losses. By using different manipulation approaches (warming and upturning soils) and tracer techniques (<sup>13</sup>C and <sup>15</sup>N stable isotopes and <sup>14</sup>C dating), I will show whether substrate quality or microclimatic gradients drive these responses. I will also argue that “biological accessibility and “feeding flexibility” rather than “substrate availability” are

crucial for understanding the magnitude and direction of their effect on C dynamics and that they need to be adequately incorporated into soil C models to make accurate predictions of the climatic sensitivity of C turnover.

## **S2-1 Reduced energy flux in soil food webs by introduced tree species: bottom-up control of multitrophic biodiversity across size compartments**

Lu J.-Z.<sup>1</sup>, Wenglein R.<sup>1</sup>, Potapov A.M.<sup>2</sup>, Ammer C.<sup>1</sup>, Scheu S.<sup>1</sup>

<sup>1</sup> University of Göttingen, Germany

<sup>2</sup> Senckenberg Museum for Natural History Görlitz, Germany

[jlu@gwdg.de](mailto:jlu@gwdg.de)

Introduced tree species have become a global issue. Tree species planted outside of their native range provide a range of economic values but may threaten local ecosystems. Evaluating consequence on soil invertebrates is challenging due to their multitrophic structure and wide range in body size. Here, we employed an energetic food web approach, and estimated energy flux in soil food webs using a four-node model including soil meso- and macrofauna decomposers and predators. We included pure and mixed stands of native European beech, non-native Douglas fir and range-expanding Norway spruce across a range of site conditions. Douglas fir reduced total mass of macrofauna predators at sandy sites but not that of soil decomposers, suggesting trophic downgrading of soil food webs in Douglas fir forests. Further, the results indicate soil macrofauna to be more sensitive than soil mesofauna to changes in forest type and environmental conditions. In decomposers, total mass of mesofauna was lower than that of macrofauna, but the energy flux through mesofauna outweighs that through macrofauna when considering energy loss to predators, highlighting the importance of mesofauna in forest soil food webs. Total energy flux positively correlated with species richness, pointing to the importance of soil biodiversity for trophic functionality. Further, energy flux in mixed forests of Douglas fir was intermediate between the respective monocultures, supporting the potential of tree mixtures in mitigating negative impacts of introduced tree species on energy fluxes. Overall, the findings highlight the importance of tree species composition, site condition and soil biodiversity in driving energy flux in soil food webs and maintaining forest ecosystem functions.

## **S2-2 Do root phenotypic traits mediate the effects of bacterivorous and herbivorous nematodes on rhizosphere bacterial communities?**

Hu J.<sup>1</sup>, Méndez S.G.<sup>2,3</sup>, Hassi U.<sup>1</sup>, De Tender C.<sup>4</sup>, Radisras N.<sup>5</sup>, Gebremikael M.T.<sup>6</sup>, Sleutel S.<sup>1</sup>, Goormachtig S.<sup>2,3</sup>, De Neve S.<sup>1</sup>

<sup>1</sup> Department of Environment, Ghent University, Gent, Belgium

<sup>2</sup> Department of Plant Biotechnology and Bioinformatics, Ghent University, Gent, Belgium

<sup>3</sup> Center for Plant Systems Biology, Vlaams Instituut voor Biotechnologie (VIB), Gent, Belgium

<sup>4</sup> Department of Biochemistry and Microbiology, Ghent University, Gent, Belgium

<sup>5</sup> Department of Plants and Crops, Ghent University, Gent, Belgium

<sup>6</sup> Department of Food Science, Aarhus University, Aarhus, Denmark

[junwei.hu@ugent.be](mailto:junwei.hu@ugent.be)

The rhizosphere bacterial community is mainly dependent on the plant species and soil types. It is also known that nematodes, the most abundant fauna in the rhizosphere, may impact rhizosphere bacteria either via top-down (bacterivores) or bottom-up (herbivores and bacterivores) regulation. However, the complex trophic control of herbivorous and bacterivorous nematodes on the rhizosphere bacterial community remains largely unexplored. Here, we investigated the separate and combined influence of bacterivorous and herbivorous nematodes (*Poikilolaimus oxycercus* and *Pratylenchus zeae*) on the abundance, diversity and activity of the rhizosphere bacterial community of Italian ryegrass (*Lolium*



*multiflorum*), and whether root phenotypic plasticity mediated these effects. Our results show that both bacterivorous and herbivorous nematodes changed root traits, particularly root mass density and root C:N ratio, which in turn mediated their effect on rhizosphere chemistry (e.g. pH and DOC). Bacterivorous nematodes had both a direct effect, which reduced bacterial abundance, and an indirect effect, which increased bacterial abundance via increase in root mass density and root C:N ratio, resulting in a negative overall effect. The presence of bacterivorous nematodes, either alone or in combination with herbivorous nematodes, led to different compositions of the rhizosphere bacterial community. Both root traits and rhizosphere chemistry contributed to explaining variations in community composition, with rhizosphere chemistry accounting for a larger portion of the variation, though the majority remained unexplained. We conclude that mass density and C:N ratio of the root system are key factors mediating the trophic control of bacterivorous and herbivorous nematodes on rhizosphere bacterial community, especially their abundance and activity. Given the high variation of bacterial community composition and the heterogeneous nature of root systems, our results suggest the need for investigations at finer scales to understand the effects of root traits on rhizosphere bacteria community and trophic interactions mediated by root plasticity.

### S2-3 Grazing pressure affects carbon flow and energy release in the soil micro-food web

van Bommel M.<sup>1</sup>, Dehghani F.<sup>2</sup>, Blagodatskaya E.<sup>2</sup>, Russ L.<sup>1</sup>

<sup>1</sup> Humboldt-Universität zu Berlin, Institute of Biology, Ecology, Philippstraße 13, 10115 Berlin, Germany

<sup>2</sup> Helmholtz-Zentrum für Umweltforschung (UFZ), Theodor-Lieser-Straße 4, 06120 Halle (Saale), Germany

[miriam.van.bommel.1@hu-berlin.de](mailto:miriam.van.bommel.1@hu-berlin.de)

Carbon and energy from soil organic matter is released by bacterial and fungal decomposers. These microorganisms are regulated by grazers such as nematodes. However, the interactions within this microbiome remain largely unexplored. In a microcosm experiment with arable soil, <sup>13</sup>C labelled maize was used as resource. The micro-food web was manipulated by amendment with nematode grazers (no inoculation, bacterial feeders, fungal feeders) and the effects on carbon flow were assessed in compartments. Analyzed were the microbial carbon ( $C_{mic}$ ,  $\delta^{13}C_{mic}$ ), nematode metabolic footprints (as measure of nematode C metabolism and ecosystem services), nematode bulk tissue ( $\delta^{13}C_{nematode}$ ), and soil respiration ( $CO_2$ ,  $\delta^{13}CO_2$ ). This was related to the heat released by the entire micro-food web. The impact of grazers on carbon turnover was followed over a period of 32 days, i.e. on day 0, 4, 8, 16 and 32.

Substrate addition shifted the isotopic signal of microbial biomass and strongly enriched  $\delta^{13}C_{mic}$ . In contrast, microbial biomass ( $C_{mic}$ ) was largely unaffected by substrate addition or grazer manipulation. The nematode metabolic footprints generally mirrored the grazer treatments, i.e. inoculation of a trophic group increased its contribution to soil carbon turnover. Substrate amendment increased the metabolic footprints, especially in fungal feeder dominated soils. Although the respiration of maize-residues ( $\delta^{13}CO_2$ ) declined over the span of 32 days, it was not affected by nematode grazing pressure. Yet, both trophic groups increased and accelerated substrate induced microbial respiration ( $CO_2$ ) as well as heat release, again with strongest responses in the fungal decomposition channel. Overall, nematode grazing pressure affected carbon flow and energy release in the soil micro-food web, indicating a positive impact of microfaunal grazers on soil organic matter turnover.

## **S2-4 Unveiling the Role of Protists: Species-Specific and Size-Dependent Regulation of Litter Decomposition**

**Wang Y.**<sup>1</sup>, Jacob J.K.S.<sup>2</sup>, dela Cruz T.E.E.<sup>2</sup>, Lejoly J.<sup>3</sup>, Veen G.F.C.<sup>3</sup>, Geisen S.<sup>1</sup>

<sup>1</sup> Laboratory of Nematology, Wageningen University, Wageningen, The Netherlands

<sup>2</sup> Department of Biological Sciences, College of Science, University of Santo Tomas, Manila, The Philippines

<sup>3</sup> Department of Terrestrial Ecology, Netherlands Institute of Ecology (NIOO-KNAW), Wageningen, The Netherlands

[yuxin1.wang@wur.nl](mailto:yuxin1.wang@wur.nl)

Litter decomposition is an important process for carbon and nutrient cycling. Litter decomposition is mainly driven by bacterial and fungal decomposers that are subsequently controlled by their predators, particularly the highly diverse protists. However, the role of different protist species individually and in combinations, as well as the potential protist trait differences, in determining microbial-mediated litter decomposition, remain largely unknown. To study the role of protists as top-down regulators in litter decomposition and carbon cycling, we first investigated the role of three phylogenetically distant protist species and their combinations in litter decomposition. Our findings revealed that protists generally did not affect litter decomposition except for one protist species that decreased litter decomposition. Furthermore, we tested whether the body size determines protists' role in litter decomposition. We found that larger protists decreased litter decomposition by 29% and also increased cumulative carbon respiration in the later phases of litter decomposition by altering bacterial composition and abundance of specific bacterial taxa. Overall, our results highlight that protists affect litter decomposition in a species-specific and size-dependent manner by shaping the microbiome. Studies on litter decomposition and the overall carbon cycle should include protists to obtain a comprehensive understanding of the biodiversity underlying these processes.

## **Session 3: Linking the composition of microbiomes to matter and energy fluxes**

### **Keynote**

## **S3-K Life, death, and decay in the soil microbiome: how soil microbes shape organic matter cycling**

**Sokol N.W.**<sup>1</sup>, Foley M.<sup>2</sup>, Blazewicz S.<sup>1</sup>, Hungate B.<sup>2</sup>, Estera-Molina K.<sup>1</sup>, Pett-Ridge J.<sup>1</sup>

<sup>1</sup> Lawrence Livermore National Laboratory, California, USA

<sup>2</sup> Northern Arizona University, California, USA

[sokol1@llnl.gov](mailto:sokol1@llnl.gov)

Soil microorganisms are frontline managers of the terrestrial carbon cycle. They shape the cycling of soil organic matter both in life and after death. Living soil microorganisms drive the formation and turnover of soil organic matter, and their metabolic functions are influenced by ecological interactions with other soil microbial populations, soil fauna and plants, and the surrounding soil environment. Remnants of dead microbial cells serve as fuel for these biogeochemical engines because their chemical constituents persist as microbial necromass, accreting over time to form one of the largest pools of organic matter on the planet. Here, I discuss how the structural and functional diversity of the soil microbiome can influence soil organic matter dynamics. Since microbial traits are one tractable means of distilling the vast complexity of microbial communities, I primarily focus on what we do and do not know about how the functional traits of microbes affect the formation and persistence of mineral-associated soil carbon. One key insight from several recent studies in semi-arid grassland soils is that the microbial traits that affect soil carbon are likely not universal, but rather vary in distinct microbial

habitats of soil, as well as from different sources of plant input and under different environmental conditions. Distinct pathways of mineral-associated organic matter may thus be linked to different microbial traits, and different chemical compositions of the soil organic matter formed. Findings from quantitative stable isotope probing (qSIP) also reveal that a relatively small number of taxa may be driving a disproportionate amount of the C transformations in soil, which means it may be increasingly possible to link taxon-specific activity with soil carbon dynamics. I conclude by discussing key next steps for linking microbial traits with soil carbon cycling, and how a trait-based understanding of microbial life and death within improved biogeochemical models may better predict ecosystem functioning under new climate regimes.

### **S3-1 Exploring soil-free microbial cell extracts as a novel approach towards disentangling microbial carbon and energy utilization patterns in soil**

**Varsadiya M.<sup>1</sup>, Dehghani F.<sup>2</sup>, Blagodatskaya E.<sup>2</sup>, Lueders T.<sup>1</sup>**

<sup>1</sup> Chair of Ecological Microbiology, Bayreuth Center of Ecology and Environmental Research (BayCEER), University of Bayreuth, Dr-Hans-Frisch-Str. 1-3, 95448 Bayreuth, Germany

<sup>2</sup> Department of Soil Ecology, UFZ-Helmholtz Centre for Environmental Research, Halle (Saale), Germany

[milankumar.varsadiya@uni-bayreuth.de](mailto:milankumar.varsadiya@uni-bayreuth.de)

Microbial carbon use efficiency (CUE), the ratio of C retained in biomass versus that assimilated, helps us to understand organic matter transformation in soil. A precise quantification of CUE can be challenging, given considerable analytical uncertainties of biotic and abiotic organic carbon pools in soil. As a proxy between laboratory cultures and complex soil microbiomes, we present the use of soil-free microbial cell extract (SFCE) as a promising approach to unravel patterns of C utilization and metabolic activity in the microcosm of reduced complexity. We propose that by isolating viable microbial cells from the soil, substrate-specific activities and population-level C utilization can be more precisely quantified than while facing the complex background of the soil matrix.

For this, we have revisited and optimized established protocols to extract microbial cells from agricultural soil via Nycodenz density gradients. The total extracted cells were counted via fluorescent staining and accounted for up to ~25% of the original soil biomass. We then used calorimetric measurements (metabolic heat and respiration) to compare CUE values of SFCE and intact soil under the provision of different substrates (glucose, glutamine, glycerol, and citric acid). Respiration data was collected for 24 and 48 hours, whereas metabolic heat release was continuously measured during incubation. Our results indicated that SFCE exhibited higher per-cell CUE than intact soil during the initial 24-hour incubation, with clear substrate-specific distinction in metabolic heat production. Amplicon sequencing of prokaryotic communities in soil and SFCE before and after incubation revealed significant shifts in microbial taxa in response to substrate addition. Differentially proportionated families identified through DESeq2 analysis demonstrated that individual substrates promoted the growth of specific microbial taxa, particularly members of the *Alpha*- and *Gammaproteobacteria*, as well as the *Actinomycetota* were involved in SFCE substrate utilization, while also abundant in the original soil. This shows that after ridding off the complex soil matrix, population-level activities in substrate and C usage are becoming more apparent in SFCE. Thus, SFCE are substantiated as a valuable novel handle to query specific contributions to microbial CUE and C cycling in soils.

### S3-2 Gradients of C flux and energy dissipation in the detritusphere

Poll C.<sup>1</sup>, Schwarz K.<sup>1</sup>, Schwormstede J.<sup>1</sup>, Finn D.<sup>2</sup>, Elshal A.<sup>2</sup>, Tebbe C.C.<sup>2</sup>, Kandeler E.<sup>1</sup>, Marhan S.<sup>1</sup>

<sup>1</sup> Institute of Soil Science and Land Evaluation, University of Hohenheim, Germany

<sup>2</sup> Thünen-Institute of Biodiversity, Germany

[christian.poll@uni-hohenheim.de](mailto:christian.poll@uni-hohenheim.de)

Plant litter entering the soil is the means by which C and energy initially stored by plants via photosynthesis enters the C and energy dissipation system in soils. While C dissipation in the detritusphere has been studied previously, the role of the detritusphere in energy turnover and dissipation in soil has not yet well understood. We, therefore, aimed to study microbial C and energy use of litter-derived DOC in the detritusphere. To address this research aim, we performed a 42 days microcosm experiment simulating the detritusphere in soil cores of increasing depths (2, 4, and 8 mm). We studied the microbial filter function along the developing gradient of microbial activity by using differences in the natural <sup>13</sup>C abundance between maize litter and SOC, calorimetry, ultra-high resolution FT-ICR mass spectrometry of leachates as well as network analysis of microbial communities. Preliminary results show a fast development of gradients of substrate availability and microbial activity with pronounced activity in the 0-2 mm layer. The peak in heat production during the initial phase of the experiment was much more pronounced and declined much stronger than that of substrate availability (measured as extractable organic C). In comparison, the microbial biomass increased immediately after litter addition, but peaked towards the end of the experiment. This was linked to an initial increase in bacterial abundance and fungal growth 14 days after litter addition, which may be related to shifts in substrate quality. The microbial filter function increased with increasing distance to the litter layer as indicated by decreasing DOC concentrations in leachates. Nitrogen leaching from soil cores strongly increased during the experiment, again pointing to a shift in quality of the leachates. The <sup>13</sup>C isotopic data are currently evaluated, quality of the leachates as well as amplicon sequencing of universal prokaryote and fungal taxonomic marker genes are currently being analyzed. Overall, the preliminary results indicate a temporal decoupling of substrate availability and use from microbial abundance in the detritusphere, which might be explained by a shift in substrate quality.

### S3-3 How do microbes mediate soil carbon dynamic in the deep rhizospheres of perennial crops?

Yang X.<sup>1</sup>, Niu G.<sup>2</sup>, Brangarí A.<sup>1</sup>, Hicks L.<sup>1</sup>, Rousk J.<sup>1</sup>

(1) Department of Biology, Lund University, Lund, Sweden

(2) Lushan Botanical Garden, Chinese Academy of Sciences, Jiujiang, China

[xiaojing.yang@biol.lu.se](mailto:xiaojing.yang@biol.lu.se)

Amid the effort to address global warming, agriculture, as one of the most artificially disturbed ecosystems, could be managed to store more carbon, which should be paid enough attention. Kernza, a perennial grain crop, with a deeper root system than annual wheat, is considered to have the possibility to introduce more carbon into the deep soil. Microorganisms play a pivotal role in carbon sequestration, by using labile plant-derived carbon for growth and forming microbial tissue and subsequent necromass, that ultimately can adhere to the surface of minerals and thereby become stabile (namely “microbial carbon pump (MCP)”). With the help of MCP, perennial crops may have a huge potential in carbon sequestration in deep soil. However, experimental studies remain scarce, and very few relate soil profiles below 30 cm depth, raising several interesting questions: How does the microbial community respond to different crops in depths gradient? What’s the role of the microbes in mediating different soil organic matter (SOM) fractions?

To address this, we collected soil samples from an eight-year Kernza crop along soil profile (0-90cm, once every 10cm) in South Sweden, and compared these profiles with samples from a conventional

crop rotation growing wheat. We found that Kernza significantly increased microbial biomass throughout the 90 cm profile, with higher rates of bacterial growth for the whole soil profile and higher rates of fungal growth at depths >10 cm. This provides evidence for a stronger MCP working under perennial crops, pumping more carbon into microbial growth and subsequent storage. To obtain a clear understanding of the carbon dynamic, we conducted  $^{13}\text{C}$  label treatment in Kernza in June 2023 and tracked the pulse of C into the Kernza rhizosphere down to 90 cm depth. We will determine the  $^{13}\text{C}$  content in both microbial biomass markers (PLFAs), necromass (chitin and muramic acid), and mineral-associated organic matter (MAOM) and particulate organic matter (POM) fractions of SOM to quantify the strength of the MCP, and the turnover times of the intermediate steps of the MCP. The results will show a direct comparison of MCP strength in perennial vs conventional agricultural practices and provide guidance in agricultural management to achieve carbon sequestration and sustainable development.

### S3-4 Kill'em all? Interactions of predatory Myxobacteria with soil microbes – an in vitro and microcosm perspective on their role in the soil microbial food-web

Piecha M.<sup>1</sup>, Tamang M.<sup>2</sup>, Pester M.<sup>2</sup>, van Bommel M.<sup>3</sup>, Ruess L.<sup>3</sup>, Borg Dahl M.<sup>1</sup>, Groß V.<sup>1</sup>, Reinhard A.<sup>1</sup>, Streblow M.<sup>1</sup>, Woyde J.<sup>1</sup>, Wang H.<sup>1</sup>, **Urich T.**<sup>1</sup>

<sup>1</sup> Greifswald University, Institute for Microbiology, Molecular Biology and Physiology, Germany

<sup>2</sup> Leibniz Institute DSMZ - German Collection of Microorganisms and Cell Cultures GmbH, Braunschweig, Germany

<sup>3</sup> Institut für Biologie, Humboldt Universität zu Berlin, Berlin, Germany

[tim.urich@uni-greifswald.de](mailto:tim.urich@uni-greifswald.de)

Soil represents one of the most intricate ecosystems and a crucial source of food and resources for humanity. Despite their critical role in shaping and transforming the soil ecosystem, the microbial food web remains poorly understood, particularly regarding matter and energy flows to higher trophic levels. Myxobacteria, a group of microbiome predators that have recently garnered more attention, are notable for their predatory lifestyle and their potential impact on microbial lifestyles and communities through predation and a complex array of secondary metabolites. To elucidate their role in the soil food web, we examined their predatory behavior and interactions with potential prey and predators using both in vitro and in situ approaches.

*In vitro* binary interaction assays were conducted with four different Myxobacteria (*Haliangium ochraceum*, *Myxococcus virescens*, *Myxococcus fulvus*, *Coralloccoccus coralloides*) and 16 different prey bacteria isolated from soils. Results indicated that each Myxobacterium exhibited a species-specific prey spectrum. *H. ochraceum* and *M. virescens* demonstrated the strongest predation effects on prey bacteria, while *C. coralloides* lysed the fewest. Remarkably, none of the tested bacteria, including members of the phyla *Verrucomicrobiota* and *Gemmatimonadota*, were resistant to lysis by at least one Myxobacterium.

The interactions of Myxobacteria with other microbiome members *in situ* were investigated through a 32-day microcosm study using agricultural soil. The study manipulated the carbon source and the grazing pressure from higher trophic levels by adding fungal- and bacterial-feeding nematodes. Quantitative PCR and quantitative metatranscriptomics microbiome profiling of 80 samples were employed. Three-Domain SSU rRNA profiling revealed that Myxobacteria were highly abundant (up to 20%) in the agricultural soil, while fungal abundance was much lower (1.5%). Protozoa, mainly Amoebozoa and Cercozoa, were more abundant than fungi (5%), indicating a dominance of the bacterial carbon and energy channel in the micro-food-web. Contrary to expectations, abundance and composition of Myxobacteria were unaffected by addition of fungal-feeding nematodes and, surprisingly, also by bacterial feeders. This suggests that Myxobacteria may reduce nematode predation pressure through secondary metabolites while killing prey bacteria for their own needs. These findings suggest a significant role for their predatory activity in soil matter and energy fluxes.

In summary, the *in vitro* assays showed that Myxobacteria lysed all prey bacteria. Alongside their high



abundance and resistance to predation in *in situ* microcosm, this positions Myxobacteria as crucial players in the micro-food web of arable soils. Future steps will integrate metatranscriptomics results with respiration rates and energy fluxes through flux-web modeling.

### S3-5 Succession of bacteria and archaea within the soil micro-food web: from natural to synthetic communities

**Tamang M.**<sup>1</sup>, Sikorski J.<sup>1</sup>, van Bommel M.<sup>2</sup>, Piecha M.<sup>3</sup>, Urich T.<sup>3</sup>, Ruess L.<sup>2</sup>, Huber K.<sup>1</sup>, Neumann-Schaal M.<sup>1,4</sup>, Pester M.<sup>1,4,5</sup>

<sup>1</sup> Leibniz Institute DSMZ – German Collection of Microorganisms and Cell Cultures, Inhoffenstr. 7B, D-38124 Braunschweig, Germany

<sup>2</sup> Humboldt – Universität zu Berlin, Institute of Biology, Ecology Group, Philippstraße 13, 10115, Berlin, Germany

<sup>3</sup> University of Greifswald, Institute of Microbiology, Felix – Hausdorff – Str. 8, D – 17487 Greifswald, Germany

<sup>4</sup> Braunschweig Integrated Centre of Systems Biology (BRICS), Rebenring 56, D-38106 Braunschweig

<sup>5</sup> Technical University of Braunschweig, Institute for Microbiology, Spielmannstrasse 7, D-38106 Braunschweig, Germany

[mandip.tamang@dsmz.de](mailto:mandip.tamang@dsmz.de)

Microbial communities in soil ecosystems are modulated by trophic interactions. However, this impact is not well investigated and quantified so far. The present study investigated how trophic interactions modulate the bacterial and archaeal microbiome within a natural and synthetic soil community. For the natural soil community, a microcosm system with circulating airflow was filled with haplic cambisol soil, provided with <sup>13</sup>C-labelled maize litter, a bacterivorous nematode (*Acrobeloides buetschlii*), and incubated for 32 days. Soil respiration and microbial communities were determined at days 0, 4, 8, 16 and 32. Bacterial and archaeal communities were compared through 16S rRNA gene amplicon sequencing and qPCR analysis. Nematode treatment resulted in maximum soil respiration at day 6 as compared to the day 12 of the no nematode treatment. Meanwhile, maize litter addition significantly ( $p < 0.05$ ) increased soil respiration rates. Furthermore, nematode and maize litter treatments had a pronounced effect on the bacterial and archaeal compositions during the first 16 days. In total, 221 dominant amplicon sequence variants (ASVs) were involved in such community dynamics. These dominant ASVs could be grouped into five different response types, which changed with different treatment and substrate conditions. Subsequent analysis of these response types revealed feeding preferential of *A. buetschlii* on gram-negative bacteria (*Acidobacteriota*, *Bacteriodota*, *Gemmatimonadota*, *Pseudomonadota*) and ammonia-oxidizing archaea (*Nitrososphaerota*) as compared to the gram-positive bacteria (*Actinobacteriota*, *Bacillota*). Moreover, addition of nematode and maize litter resulted in a succession of soil microbiota which was driven by population changes first in the *Bacteroidota*, then in the *Pseudomonadota*, and last in the *Acidobacteriota* and *Nitrososphaerota*. In a parallel synthetic soil community approach, a similar microcosm system was used. Here, artificial soil was inoculated with eight typical soil bacterial species (*Pseudomonadota*, *Actinomycetota*, and *Bacteriodota*) with starch as substrate and a myxobacterium (*Myxococcus virescens*) as predator. Treatments with starch as bottom-up control and *M. virescens* as top-down control resulted in elevated soil respiration in the first eight days of incubation. Changes in the synthetic community will be analyzed as done before in the natural soil experiment. Our results will be an important step forward to understand how the trophic interactions modulate the microbial community in both a natural and synthetic model soil system.

### S3-6 Soil microbial metabolic quotient follows ecological successional patterns under one year of winter wheat cropping

Finn D.<sup>1,2</sup>, Schroeder J.<sup>3</sup>, Poeplau C.<sup>3</sup>, Tebbe C.C.<sup>2</sup>

<sup>1</sup> Institute for Geoecology, Technical University of Braunschweig, Germany

<sup>2</sup> Thünen Institute for Biodiversity, Germany

<sup>3</sup> Thünen Institute for Climate-Smart Agriculture, Germany

[damien.finn@tu-bs.de](mailto:damien.finn@tu-bs.de)

The main energy and carbon source inputs for cropping soil microbiomes comes from root exudates and plant residues, which change in quantity and chemical structure over crop developmental cycles. Here we hypothesized that the annual change in input carbon was the driving factor of microbiome successional states, with different states having distinct microbial metabolic quotients (MMQ). In this study we followed soil microbiomes over six developmental stages of winter wheat, bordered by fallow management at initial seeding and two months after harvest, from Oct 2020 to Sept 2021. Paired analyses for microbial composition (via shotgun metagenomic sequencing) and MMQ were conducted. Communities underwent ecological succession from 'fallow' to 'rhizosphere' states during intensive plant growth phases of stem elongation, flower development and ripening. Rhizosphere communities were marked by a 14% loss in alpha-diversity, due to decreased relative abundance of saprotrophic (*Acidobacteriota*, *Actinobacteriota*, *Planctomycetota*), bacteriovore (SAR and Amorphea protists, *Myxococcota*) and ammonia oxidizing archaeal phyla. A concomitant increase in Archaeplastida and *Cyanobacteriota* (likely chloroplasts from plant DNA) and proteobacterial taxa was observed. In vitro incubation identified rhizosphere communities as having significantly poorer MMQ relative to earlier successional states. As wheat underwent senescence, successional patterns shifted to a new state that continued through the fallow period. The MMQ associated with this new state was highly variable, perhaps reflecting that community compositions were still transitioning, but improved in that MMQ was more reflective of activity under fallow. Thus, the composition and activity of the microbiome were primarily affected when wheat was undergoing intensive plant growth stages, with rhizosphere-associated microbiomes demonstrating relatively poor MMQ, and relative decreases in saprotrophic, bacteriovore and ammonia oxidising groups. On-going work seeks to link MMQ with microbial growth efficiency, *i.e.* carbon use efficiency, and provide a metabolic explanation for observed shifts in growth efficiency based on assembled protein-encoding genes.

### S3-7 Carbon-use efficiency 2.0? Insights from fungal growth dynamics in response to resource complexity and energy content

Camenzind T.<sup>1,2</sup>, Finn D.<sup>3</sup>, Tebbe C.C.<sup>2</sup>, Oliva R.L.<sup>4</sup>, Joergensen R.G.<sup>4</sup>, Rillig M.C.<sup>1,2</sup>

<sup>1</sup> Institute of Biology, Freie Universität Berlin, Germany

<sup>2</sup> Berlin-Brandenburg Institute of Advanced Biodiversity Research (BBIB), Berlin, Germany

<sup>3</sup> Thünen Institute of Biodiversity, Braunschweig, Germany

<sup>4</sup> Soil Biology and Plant Nutrition, University of Kassel, Germany

[tessa.camenzind@fu-berlin.de](mailto:tessa.camenzind@fu-berlin.de)

Microbial carbon-use efficiency (CUE) is applied as an important predictor for soil carbon (C) inputs via microbial activity. Understanding the ecological significance of this metric in more detail is crucial to improve its implementation in biogeochemical models. CUE is determined by a variety of factors: Beside environmental conditions, microbial community composition as well as resource types are key drivers of C being accumulated in microbial biomass versus C respired. Using saprobic fungal isolates as a model system, we tested the hypotheses that (i) C resource complexity and energy content affect fungal CUE via enzyme costs and biomass formation efficiency and (ii) CUE and its responses to substrate type strongly vary among isolates with different life-history strategies. We used fungal isolates from agricultural soils tested in the *SoilSystems* priority program, and analyzed fungal CUE parameters

in artificial soils with defined resource availability.

The results revealed that saprobic fungal species strongly vary in their ability to use complex C substrates like cellulose, starch or lignin, with a predictable variation along the previously described fungal economics spectrum. However, the CUE of fungal growth was consistently affected by the complexity of C substrates. In respect to C substrates, energetic costs of enzymatic degradation appear more relevant for the fate of C than its energy content, which only showed minor effects on fungal growth efficiencies. Based on time-dependent aspects of these results we further tested the impact of growth dynamics over time on measured energy and C-use efficiency. First insights highlight the importance to integrate not only the fungal growth but also degrowth/death phase into calculations of soil C inputs.

In conclusion, fungal growth dynamics and C turnover under controlled conditions provide important mechanistic insights for soil C cycling, which now can be tested and modelled in real soil systems. It remains to be tested how isolate-specific variation affects CUE in diverse fungal communities, whereas complexity of C sources may be a strong predictor for microbial CUE. Regarding microbial growth and death dynamics, it will be interesting to discuss CUE and alternatives as an appropriate predictor of soil C storage.

### S3-8 Biodegradable plastics in soils: benefits and threats

Kucerik J.<sup>1</sup>, Brtnicky M.<sup>1,2,4</sup>, Hammerschmiedt T.<sup>1</sup>, Kintl A.<sup>1,3</sup>, Latal O.<sup>1,4</sup>, Holatko J.<sup>1,4</sup>

<sup>1</sup> Department of Agrochemistry, Soil Science, Microbiology and Plant Nutrition, Faculty of AgriSciences, Mendel University in Brno, 613 00 Brno, Czech Republic

<sup>2</sup> Institute of Chemistry and Technology of Environmental Protection, Faculty of Chemistry, Brno University of Technology, Purkynova 118, 612 00 Brno, Czech Republic

<sup>3</sup> Agricultural Research, Ltd., 664 41 Troubsko, Czech Republic

<sup>4</sup> Agrovyzkum Rapotin, Ltd., Vyzkumníku 267, 788 13 Rapotin, Czech Republic

[jiri.kucerik@mendelu.cz](mailto:jiri.kucerik@mendelu.cz)

Biodegradable plastics are becoming increasingly popular in various industries, including agriculture, where they are used for packaging, fertilizer coatings, mulching, and delivering active compounds. Although the cost of biobased bioplastics is currently higher than non-biodegradable plastics, their usage is expected to grow significantly in the future. However, there is still a lack of understanding regarding the environmental impact of biodegradable plastics, both during production and at the end of their life cycle.

It is speculated that the increased use of biodegradable plastics in soil could disrupt nutrient levels and alter microbial communities. Additionally, rapid erosion could lead to the fast production of microplastics, which may affect soil functions by interacting with organic matter and minerals. Our research group is addressing these concerns by studying the effects of poly-3-hydroxybutyrate bioplastics on soil health and plant growth.

Preliminary studies have shown that bioplastics can negatively impact plant growth by causing nutrient imbalances. Plant growth-promoting bacteria and compost did not alleviate these effects, but the addition of digestate improved growth. Increased microbial activity resulted in changes to the soil microbial community and enhanced the production of enzymes responsible for breaking down organic matter, resembling a priming effect.

Despite the negative effects of bioplastics on soil desiccation and plant growth, they may still have potential benefits. Bioplastics could serve as a short-term substrate in soils lacking organic matter and the formation of a biofilm on their surface could promote soil aggregation, protecting the substrate from quick degradation. We recommend further research to better understand the influence of biodegradation on chemical, physical, and microbiological properties of soil.

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### S3-9 Redox gradient across peat soils shapes the chemical and taxonomic composition of microbial communities

**Milesi V.**

University of Orléans, France

[vincent.milesi@univ-orleans.fr](mailto:vincent.milesi@univ-orleans.fr)

Peatlands represent only 3% of the terrestrial surface but store up to 30% of the world's soil carbon. How environmental and anthropic forcing will impact the microbial community controlling peatland carbon balance is poorly understood. Multivariate analyses are commonly used to relate environmental variables to microbial communities but lack mechanistic understanding. Here, a thermodynamic model of the chemical activity of community-level proteins derived from metagenomic data was developed to investigate the relation between microbial community composition and redox gradient across peat soils. The model relies on the database of the SPRUCE experiment, a climate change manipulation conducted in an ombrotrophic peatland in the Marcell Experimental Forest (Minnesota, USA), which includes shotgun metagenomic sequencing and porewater chemical composition.

The average carbon oxidation state and the redox potential of maximum chemical activities of in-silico proteins decrease with depth following a linear and logarithmic trend, respectively. The protein-inferred Eh values are (1) above equilibrium with the  $\text{Fe}^{2+}$ -goethite equilibrium at 10 cm depth, (2) cross equilibrium with the  $\text{SO}_4^{2-}$ - $\text{H}_2\text{S}$  and  $\text{CO}_2$ - $\text{CH}_4$  equiactivity at 40 cm depth, (3) and keep decreasing down to 150 cm depth. The evolution of protein-inferred Eh values with depth are consistent with the water table fluctuation zone and the evolution of gene abundance, from methanotrophy and sulfur oxidation in the shallow horizons to methanogenesis and sulfate reduction at depth. A principal component analysis reveals that the evolution of taxonomic abundance with depth partly encodes the chemical variability of in-silico proteins. Based on this correlation, a model of taxonomic evolution as a function of redox potential is proposed that allows quantifying the redox dependency of anaerobes habitats playing a key role in peatland carbon cycling.

Showing how thermodynamic forcing shapes the chemical and taxonomic composition of peatland microbial communities, this work offers prospects for the predictive modelling of microbial community composition and redox conditions in a variety of environments, including disturbed peatlands.

## Session 4: Modeling of matter and energy flows in soil systems

### Keynote

### S4-K Disentangling organic matter stabilization pathways using dynamical models in phase space

**Manzoni S.**<sup>1</sup>, Cotrufo F.<sup>2</sup>

<sup>1</sup> Department of Physical Geography and Bolin Centre for Climate Research, Stockholm University, Sweden

<sup>2</sup> Department of Soil and Crop Sciences, Colorado State University, USA

[stefano.manzoni@natgeo.su.se](mailto:stefano.manzoni@natgeo.su.se)

Organic carbon and nitrogen are stabilized in soils via microbial assimilation and stabilization of necromass (in vivo pathway) or via adsorption of the products of extra-cellular decomposition (ex vivo pathway). How to quantify the contributions of these two pathways is still an open question, framing the scope of this contribution. Here we use a diagnostic model to quantify which stabilization pathway is dominant, using data on residue-derived carbon and nitrogen incorporation in mineral associated organic matter from ~40 published studies. Our model differs from previous approaches as it is solved analytically in the phase space—i.e., expressing one soil state variable as a function of another variable instead of time. By focusing on the relations among state variables, this approach allows comparing

widely different experimental systems that would be difficult to compare due to their variability across a range of time scales. Using this modelling approach, we find that the *in vivo* pathway is the dominant stabilization pathway for both organic carbon and nitrogen. *In vivo* stabilization is particularly important in fine-textured soils with low organic matter content, in which mineral surfaces are relatively more available compared to coarse-textured or organic matter rich soils. Thus, our results highlight the role of microbial necromass and its interactions with mineral surfaces in the organic matter stabilization process.

#### **S4-1 Modeling of carbon and heat fluxes in soil with multiple limitations of microbial growth**

**Blagodatsky S.<sup>1</sup>, Endress M.-G.<sup>1</sup>, Dehghani F.<sup>2</sup>, Schlüter S.<sup>2</sup>, Reitz T.<sup>2</sup>, Blagodatskaya E.<sup>2</sup>**

<sup>1</sup> University of Cologne, Institute of Zoology, Terrestrial Ecology Group, Cologne, Germany

<sup>2</sup> Department of Soil Ecology, Helmholtz Centre for Environmental Research – UFZ, Halle(Saale), Germany

[sblagoda@uni-koeln.de](mailto:sblagoda@uni-koeln.de)

Modeling of microbial growth and the transformation of organic substrates entering the soil is challenging due to the heterogeneity of the medium and the complex interplay of physicochemical and biological processes in time and space. Measuring heat production by soil microorganisms during the decomposition of soluble carbonaceous substrates like glucose can help to constrain dominant metabolic pathways and to determine growth-limiting factors when combined with quantitative simulation schemes. For example, the availability of nutrients and oxygen as well as spatial separation of microorganisms from their food could affect the temporal patterns of heat and CO<sub>2</sub> production in typical soil incubation experiments. The aim of our investigation was to disentangle the potential reasons for the delay in microbial decomposition of glucose in differently structured soil samples. To that end, we monitored heat and CO<sub>2</sub> release from soil after the addition of glucose, which was either mixed homogeneously into the soil or applied drop by drop to create spatial hotspots. To identify potential limiting factors, we also measured O<sub>2</sub> consumption and further added nutrient solution to a subset of the samples. Finally, we used a simple microbial-explicit model to interpret our experimental results. We observed lower maximum rates of CO<sub>2</sub> and heat release as well as a delayed peak after spatially heterogeneous amendment compared to the well-mixed treatment. However, this difference between spatially homogeneous and heterogeneous treatments disappeared when nutrients were added along with glucose, which suggests local nutrient limitation as the underlying mechanism. We found no evidence of anaerobiosis. The carbon and energy balances indicated highly efficient aerobic growth and were in good agreement with each other and with theoretical predictions. Notably, spatial heterogeneity had no effect on apparent growth efficiency after 50 h of incubation, despite pronounced differences in temporal dynamics. Model simulations accurately captured the observed dynamics, with aerobic growth and maintenance as the dominant model processes. Our simple formulation of nutrient limitation was sufficient to represent both the temporal pattern and the efficiency of microbial growth in all treatments. Overall, the model predicted a smaller active fraction of microorganisms throughout the incubation under spatially heterogeneous conditions, thereby capturing the effect of reduced co-location of microorganisms and substrate. In summary, the integration of complex experimental results and mechanistic modeling enabled us to quantify the effect of spatial substrate heterogeneity on the dynamics and the limitations of microbial growth in soil.



## S4-2 Modelling nitrogen limitation of litter decomposing fungi

Ghersheen S.<sup>1</sup>, Manzoni S.<sup>2</sup>, Spohn M.<sup>1</sup>, Lindahl B.<sup>1</sup>

<sup>1</sup> Department of Soil and Environment, Swedish University of Agricultural sciences, Uppsala, Sweden

<sup>2</sup> Department of Physical Geography and Bolin Centre for Climate Research, Stockholm University, Stockholm, Sweden

[samia.ghersheen@slu.se](mailto:samia.ghersheen@slu.se)

Most of the widely used soil organic matter models have been developed with a focus on carbon dynamics rather than on element interactions. However, in many regions of the world, particularly at high latitudes, soil organic matter decomposition is constrained by low nitrogen availability, but this phenomenon is usually not explicitly incorporated in the decomposition models. Nitrogen dynamics is pivotal in regulating aboveground productivity, and retention of nitrogen in soil may hamper availability to trees. Yet, the mechanisms behind belowground retention and release of nitrogen are poorly understood.

Here we focus on plant litter decomposition—the first step of transformation of plant residues into mineralized products or soil organic matter. We formulated a process-based model of litter decomposition to investigate the effect of nitrogen limitation on decomposition. The model describes the dynamics of a single litter cohort over time, as it is decomposed. Unlike most other decomposition models, our model reflects the fungal mycelial dynamics explicitly. Fungal biomass is divided into three different reactions describing the different states of fungal cells, 1) cytoplasmic cells active in decomposition, 2) vacuolised cells with a lower nitrogen content and without decomposition capacity, and 3) dead cells (necromass), which are treated as a separate pool.

The model is capable of predicting mass loss trajectories of a variety of litter types with different nitrogen content and similar lignin content based on a single parameter space. The fungal mycelium adapts to nitrogen limitation by increasing the proportion of vacuolised, inactive cells, reducing decomposition rates. Under nitrogen limitation, nitrogen accumulates in the necromass pool. To predict the observed patterns of nitrogen retention/release, decomposition of fungal necromass had to be slow, with decomposition rates close to those of recalcitrant lignin but always lower than cellulose decomposition. Moreover, we found an optimum for carbon use efficiency (CUE) with respect to cumulative fungal biomass accumulation; high CUE intensified nitrogen limitation and retarded decomposition, leading to slower biomass accumulation compared to lower values of CUE. Our results disentangled the interplay between nitrogen availability, mycelial dynamics and decomposition, pointing towards the potentials of more explicit incorporation of fungi in models of nitrogen limited ecosystems

## S4-3 When and why microbial-explicit SOC models can be unstable

Schwarz E.<sup>1</sup>, Ghersheen S.<sup>2</sup>, Belyazid S.<sup>1</sup>, Manzoni S.<sup>1</sup>

<sup>1</sup> Department of Physical Geography and Bolin Centre for Climate Research, Stockholm University, Stockholm, Sweden

<sup>2</sup> Department of Soil and Environment, Swedish University of Agricultural Sciences, Uppsala, Sweden

[erik.schwarz@natgeo.su.se](mailto:erik.schwarz@natgeo.su.se)

Microbial-explicit models aim to describe soil organic carbon (SOC) cycling more realistically than traditional models, by explicitly describing the biotic control on the relevant processes. These models can express rich dynamics, including oscillations around and instability of their equilibrium points (EPs – i.e. when all state variables are at steady state). At small spatial and temporal scales these complex behaviors can represent relevant ecosystem dynamics, but if applied at large scales as in Earth-system models, instability of EPs can lead to unrealistic and unexpected simulation outcomes. Here we analyzed the stability behavior of an archetypal microbial-explicit SOC model (explicitly describing microbial biomass growth, production of extracellular enzymes, enzymatic degradation of SOC, and microbial uptake of dissolved organic carbon [DOC]) and some simplified versions of it. We found that these

models can be unstable if the resupply of a growth substrate (i.e. DOC) is dependent on the quantity of the available growth substrate itself (DOC). Such a positive feedback can allow for extinction of the modelled microbial community. We identify a conservative sufficient condition that ensures stability of these models. Three major approaches to avoid instability emerge from our analysis: 1) avoiding the explicit representation of DOC dynamics, 2) reducing the sensitivity of uptake to changes in microbial biomass, and 3) introducing explicit correlations between parameters describing e.g. changing environmental controls and microbial physiology. While the first two approaches aim at simplifying the system to reduce positive feedbacks, the third approach allows for a more realistic representation of microbial ecology in these models. Our findings have implications for further development of SOC models and potential upscaling approaches, primarily calling for a more consistent representation of microbial ecology.

#### **S4-4 When and why microbial-explicit SOC models can be unstable**

**Wutzler T.<sup>1</sup>, Kleidon A.<sup>1</sup>**

<sup>1</sup> MPI for Biogeochemistry Jena, Germany

[twutz@bgc-jena.mpg.de](mailto:twutz@bgc-jena.mpg.de)

Thermodynamic modeling has gained insight into microbial processing of fresh substrates in soil and nutrient-rich conditions. Similarly, stoichiometric modeling generates hypotheses about microbial growth, derived enzyme activities, and associated turnover of soil organic matter (SOM). However, explaining observed differences in heat dissipation at experiments that modified the nutrient status cannot be explained by either of those two modeling approaches in isolation. Microbially mediated SOM transformations can be constrained by either energy limitation, carbon limitation, nutrient limitation, a co-limitation, or by transport of educts and products depending on the boundary conditions of the system.

Consequently, we aim at a quantitative understanding of the combined energy, carbon, nutrient limitations and potential adaptation strategies of the microbial community. Therefore, we combine thermodynamic and stoichiometric model developments guided by the data of the SPP2322 priority program. We develop a consistent description of the soil as a dissipative system, quantifying potentials at every pool and transformation and quantify the degradation of energy at every step. While this contribution presents preliminary results at daily timescale, we hypothesize that thermodynamic optimality principles emerge at larger spatial and temporal scales by interactions with the boundary conditions, similar as with other components of the earth system. Hence, we will explore modeling the system at wider boundary conditions.

### **Session 5: Calorimetry and thermodynamics: keys to unraveling complex soil processes**

#### **S5-1 Comments on the use of isothermal calorimetry in soil science**

**Wadsö L.**

Building Materials, Lund University, Sweden

[lars.wadso@byggtek.lth.se](mailto:lars.wadso@byggtek.lth.se)

Measuring the heat production rate and heat on soil can give interesting information that is difficult to obtain by other means. However, to do good calorimetric measurements, a couple of prerequisites discussed in the first part of this presentation are needed: a sensitive and temperature stable heat conduction calorimeter, calibration coefficients, baselines and proper reference balancing. If that is in place, a large number of interesting types of measurements – discussed in the second part – can be performed, such as:

- Amendment studies, in which a known amount of an easily digestible carbon substrate (typically glucose) is added to a soil so that it is possible to quantify the heat that this produces and compare that with the theoretical heat that would be produced if all added substrate was oxidized.
- Measurements on undisturbed soils, for example as a function of temperature and depth above ground, to quantify the natural turnaround of biomass.
- Studies of the effect of pollutants, biocides etc by adding them to soils in a calorimeter and using the change in heat production rate as a measure of their effect.
- Calorespirometry, in which both heat and the exchange of oxygen and carbon dioxide are assessed, making it possible to use metabolic models to calculate such parameters as the carbon conversion efficiency.

The presentation ends with a discussion of calorimetry versus measurements of oxygen concentration, as these two measurement techniques both essentially measure the oxygen consumption rate for aerobic systems; the connection is the heat produced per oxygen molecule consumed, the so called Thornton constant.

## **S5-2 Interactions of N mineralisation from slurry-N with heat dissipation and organic matter dynamics**

**Yildiz C.<sup>1,2</sup>, Minceva M.<sup>2</sup>, Heuwickel H.<sup>3</sup>, Wiesmeier M.<sup>4</sup>, Schweizer S.<sup>1</sup>**

<sup>1</sup> Technical University of Munich, TUM School of Life Sciences, Chair of Soil Science, Freising

<sup>2</sup> Technical University of Munich, TUM School of Life Sciences, Professorship of Biothermodynamics, Freising

<sup>3</sup> University of Applied Science Weihenstephan-Triesdorf, Faculty of Sustainable Agro-and Energy Systems, Freising

<sup>4</sup> Bavarian State Research Center for Agriculture (LfL), Institute for Agroecology and Organic Farming, Freising

[cennet.yildiz@tum.de](mailto:cennet.yildiz@tum.de)

Nitrogen (N) is an essential element for biomass production of crops, while N applied in excessive amounts of can harm the environment. While plants usually take up N in mineral forms, soils store most N in organic form. Soil microorganisms play a crucial role in the turnover of organic N in the soil organic matter pool into crop-available N. During this process, energy is converted, partially leading to heat release. The prediction of heat dissipation driving microbial-derived N transformations enables a better understanding of N transformation. In this study, we set up an experiment of cattle slurry added to agricultural soils with different organic matter contents. We use isothermal calorimetry to measure the heat flow over time at 25°C in combination with an indirect method to determine the CO<sub>2</sub> production and NH<sub>3</sub> volatilisation. In addition, we calculate the calorespirometric ratio over the course of our incubation experiment. We will discuss the integration of time-dependent heat flow data for monitoring microbial activity and potential bioenergetic constraints involved in N transformation. We expect shifts in the microbial N turnover pathways as modulated by different temperatures and soil moisture content. Energy fluxes and entropy generation rates in the soil system will be compared with the biological N transformation. Our approach aims to disentangle the complex interplay between organic N sources such as slurry and soil organic matter pools by developing a novel integration of a microcalorimetric approach in order to shed light on the mechanisms governing soil N transformation.

### S5-3 Comparing Thermal Methods to Measure Changes in Thermal Stability of Organic Carbon in Amended Soils during Microbial Incubation

DeFrang E.L.<sup>1</sup>, Marmello G.<sup>1</sup>, Trabuco J.<sup>3</sup>, Aeppli M.<sup>1</sup>

<sup>1</sup> Swiss Federal Institute of Technology, Lausanne (EPFL), Switzerland

<sup>2</sup> YpHen SAS, France

[emma.defrang@epfl.ch](mailto:emma.defrang@epfl.ch)

Soil organic matter (SOM) undergoes biotic transformation from labile, easily degradable forms to more stable, energy-dense forms. This stabilization limits microbial degradation due to energetic constraints on microbial metabolism. Understanding the energetic quality of SOM is crucial for modeling the terrestrial carbon (C) cycle and assessing soil health. To investigate this, we conducted proof-of-concept experiments using two thermal analysis techniques—Rockeval and Differential Scanning Calorimetry-Thermogravimetry (DSC-TG)—to capture changes in SOM thermal signatures during microbial incubation in amended artificial soils.

Our experiments aimed to: (1) determine the sensitivity of these methods to small fluctuations in SOM stability, (2) compare their effectiveness in assessing SOM stabilization, and (3) evaluate thermal SOM stability under different conditions post microbial transformation.

In the preliminary experiment, Rockeval was used to monitor changes in SOM thermal signatures during the transformation of glucose (a labile C substrate) into more complex organic matter. We tested two microbial communities typical of soil environments across varying clay contents (5%, 10%, and 20%). Results indicated significant thermal stabilization during microbial incubation, with the highest stabilization observed in the 20% clay content samples and combined microbial treatment samples, highlighting the importance of soil matrix and biodiversity in thermal SOM stabilization.

The follow-up experiment utilized DSC-TG on the same system, but also included a complex substrate (sterile compost) and biochar amendments which were used in conjunction microbial consortia. While TG data suggested increased thermal stability post incubation, DSC results were inconclusive. These samples will also be analyzed with Rockeval for direct method comparison.

In my presentation, I will assess the capabilities of Rockeval and DSC-TG in detecting SOM stabilization in both simple and complex substrate systems. I will address the limitations of each method, including experimental design challenges and data interpretation issues such as clay interference. Finally, I will discuss our findings on thermal C stability under various conditions.

### S5-4 Thermodynamic control of microbial turnover of organic substrates in soils

Miltner A.<sup>1</sup>, Kästner M.<sup>1</sup>, Maskow T.<sup>2</sup>, Lorenz M.<sup>1,3</sup>, Thiele-Bruhn S.<sup>3</sup>

<sup>1</sup> Helmholtz Centre for Environmental Research - UFZ, Department of Molecular Environmental Biotechnology, Permoserstr. 15, 04318 Leipzig

<sup>2</sup> Helmholtz Centre for Environmental Research - UFZ, Department of Microbial Biotechnology, Permoserstr. 15, 04318 Leipzig

<sup>3</sup> Trier University, Faculty of Spatial and Environmental Sciences, Department of Soil Science, Behringstr. 21, 54296 Trier, Germany

[anja.miltner@ufz.de](mailto:anja.miltner@ufz.de)

Microbial turnover of organic substrates is a key process in soil organic matter formation and turnover. As microorganisms require both carbon and energy for growth and maintenance, carbon and energy fluxes in soils are tightly coupled. At the level of cellular metabolism, the substrates are allocated to catabolism and anabolism according to the requirements of the cells. In the soil system, additional processes have to be considered such as multiple substrate use, recycling of biomass components, interaction between different organisms and abiotic processes. As most of the energy flux in catabolism is created by the reduction of the terminal electron acceptors, the availability of the electron ac-

ceptors strongly affects carbon use efficiency and energy use efficiency. Here, we present a thermodynamic concept that combines experimental approaches of calorimetry and turnover mass balances, paving the way for a better understanding of microbially mediated organic matter turnover and stabilization in soil.

Mass balances in soil systems need to be set up for exemplary substrates using isotope labelled compounds. They should be combined with information on energy fluxes, which can be obtained using calorimetric methods and thermodynamic calculations. Recently, calorimetric methods have been introduced into soil studies, e.g. differential scanning calorimetry or isothermal reaction calorimetry. Alternatively, enthalpies of combustion or formation must be known or estimated, e.g. based on the nominal oxidation state or the degree of reduction of the substrates and the reaction products. All of these methods have their strengths and weaknesses, which need to be considered when interpreting the results. From a thermodynamic perspective, it is crucial to define the system boundaries and to use thermodynamic state variables such as reaction enthalpy, entropy, and Gibbs free energy. If applied properly, the predictive power of thermodynamics can be fully utilized for process evaluation. In particular, this approach will enable us to identify whether or not a particular process is thermodynamically feasible under the given conditions.

In summary, linking mass balances and thermodynamics will allow us to better understand and predict soil organic matter turnover and sequestration. Finally, it will also enable us to determine the minimum energy fluxes through the soil system needed to maintain SOM.

## S5-5 The MTB model: thermodynamic predictions of microbial turnover to biomass

Kästner M.<sup>1,2</sup>, Trapp S.<sup>2</sup>

<sup>1</sup> Helmholtz-Centre for Environmental Research – UFZ, Department Molecular Environmental Biotechnology, Permoserstr. 15, 04316 Leipzig, Germany

<sup>2</sup> DTU Sustain, Technical University of Denmark, Bygningstorvet building 115, 2800 Kongens Lyngby, Denmark

[matthias.kaestner@ufz.de](mailto:matthias.kaestner@ufz.de)

In biodegradation studies with isotope-labelled pesticides (OECD test for soil, 307), fractions of so-called non-extractable residues (NER) or microbial residues remain; however, their nature and composition are rarely known, leading to uncertainty in assessment in particular about the risk from environmental chemicals. Microbial degradation of the compound with growth of the microorganisms leads to incorporation of labelled carbon into the microbial mass, resulting in biogenic residues. The formation of microbial biomass mass can be estimated from the theoretical growth yield, in particular if experimental data is rare.

We developed a general prediction method for the theoretical (max.) yield based on the thermodynamic balancing of the Gibbs energy release of the overall turnover reaction including the related electron acceptor (Trapp et al. 2018; Brock et al. 2017). The Microbial Turnover to Biomass (MTB) calculation model needs a minimum of input data. Growth yields of more than 40 organic chemicals (31 pesticides) using MTB were evaluated in comparison to two more elaborate existing methods in biotechnology that need degradation pathway information. For the tested xenobiotic compounds MTB performed best in determination of the potential amounts of microbial residues. MTB was originally developed for the regulatory risk assessment of non-extractable residues from pesticides but works also reliably and robust for the assessment of the C transfer from organic natural compound to microbial biomass. The current state of the calculation model provides the min. and max. range of non-extractable C residues as well as the highest potential microbial yield for the productive turnover reactions including the potential carbon use efficiency (CUE) and energy use efficiency (EUE), which is always related to the C use. In addition, with knowing the CO<sub>2</sub> formed from the turnover of the test compounds, the related formation of biomass can be estimated.

The MTB calculation sheet is simply based on excel®, which can be applied also by thermodynamically less well-trained users, in order to allow universal application in environmental and soil science. The



sheet and the underlying rules will be presented in this lecture with a demonstration for application of the yield assessment from cellulose degradation.

OECD, 2002. Test No. 307: Aerobic and Anaerobic Transformation in Soil, OECD Guidelines for the Testing of Chemicals, Section 3, OECD Publishing, Paris, <https://doi.org/10.1787/9789264070509-en>

Brock, A.L., Kästner, M., Trapp, S., 2017. Microbial growth yield estimates from thermodynamics and its importance for degradation of pesticides and formation of biogenic non-extractable residues. SAR/QSAR 28(8):629-650. <https://doi.org/10.1080/1062936X.2017.1365762>

Trapp, S., Brock, A.L., Nowak, K., Kästner, M. 2018. Prediction of the formation of biogenic nonextractable residues during degradation of environmental chemicals from biomass yields. Environ Sci Technol 52:663–672. <https://doi.org/10.1021/acs.est.7b04275>

## Abstracts of Poster Presentations

### Session 1: Linking carbon and energy fluxes in soil systems

#### **P1 Oxygen exchange between water and phosphate can provide insights into carbon dynamics in soils**

**Siegenthaler M.**<sup>1</sup>, Tamburini F.<sup>2</sup>, Pistocchi C.<sup>3</sup>

<sup>1</sup> Department of Physical Geography, Stockholm University, 10691 Stockholm, Sweden

<sup>2</sup> Group of Plant Nutrition, IAS, D-USYS, ETH Zurich, 8315 Lindau, Switzerland

<sup>3</sup> Eco&Sols, CIRAD, INRAE, Institut Agro, IRD, 34060 Montpellier, France

[mbsiegenthaler@outlook.com](mailto:mbsiegenthaler@outlook.com)

Carbon (C) cycling in soil is driven by microorganisms using organic matter as C and energy source. But phosphorus (P) is essential for cellular metabolism as many metabolic pathways and processes depend on it. In marine primary producers, the stoichiometric ratio between C and P is relatively constant and thus often used as a constraint in modeling. In soil microorganisms and in soil, the C:P ratio is more variable. Soil microorganisms exhibit a wide range of metabolic adaptations to environmental pressure, and the physical and mineralogical properties of the soil play a significant role in controlling P availability. Due to these complexities, we were interested in an alternative way of representing the tight link between C and P in cellular metabolism.

By examining the oxygen (O) isotope composition in inorganic phosphate ( $\delta^{18}\text{O}\text{-Pi}$ ), we can determine the extent of O exchange between water and phosphate, which is controlled by biological processes. During the last 10 years, we conducted a series of incubation experiments with soils characterized by different mineralogy, P content, age, and from different climates. We measured  $\text{CO}_2$  respiration and  $\delta^{18}\text{O}$  in microbial cytosolic phosphate. By labelling with  $^{18}\text{O}$ -enriched water and analyzing  $\delta^{18}\text{O}$  in cytosolic phosphate at the beginning and end of the incubation, we determined the extent of O exchange between water and phosphate.

Across these incubations, we observed a significant correlation between the percentage of O exchanged and the cumulative  $\text{CO}_2$  respired during the incubation. When comparing the moles of O exchanged in the microbial cytosol to the moles of C respired, it appears that for every mole of O exchanged due to phosphoryl transfer, there is a nearly fixed amount of C respired. This suggests that the moles of O exchanged through phosphoryl transfer recorded in soil microbial cytosolic phosphate can provide information about metabolic C expenditure. If this link between P and C in soil microbial biomass can be confirmed in field-based experiments, this information could potentially improve our understanding of C dynamics and be used for further modeling purposes.

#### **P2 Exploring Mass and Thermodynamic Energy Balances in Artificial Soil under Diverse Environmental Settings**

**Yang S.**<sup>1</sup>, Rupp A.<sup>2</sup>, Miltner A.<sup>2</sup>, Kästner M.<sup>2</sup>, Maskow T.<sup>1</sup>

<sup>1</sup> Department of Microbial Biotechnology, Helmholtz-Centre for Environmental Research – UFZ, Permoserstraße 15, 04318 Leipzig, Germany

<sup>2</sup> Department of Molecular Environmental Biotechnology, Helmholtz-Centre for Environmental Research – UFZ, Permoserstraße 15, 04318 Leipzig, Germany

[shiyue.yang@ufz.de](mailto:shiyue.yang@ufz.de)

In natural soil ecosystems, the stock of soil organic matter (SOM) serves as a critical indicator of soil quality, playing a fundamental role in sustaining ecosystem health and feeding microbial activity. In particular against the backdrop of climate change, the greenhouse gas balance of SOM degradation versus sequestration plays a key role in the terrestrial carbon cycle. Therefore, the measurement of

respiration emerges as a crucial parameter directly reflecting microbial activity and soil quality. In addition, understanding SOM as an outcome of a steady state of carbon and energy fluxes as well as identifying and quantifying SOM degradation pathways under various conditions requires comprehensive analyses of these fluxes. To address this challenge, isotope methods utilizing  $^{13}\text{C}$  are commonly employed to trace carbon dynamics. Moreover, the release of heat serves as a universal principle for all microbial activities, prompting the utilization of isothermal microcalorimetry (IMC) as an emerging tool to elucidate thermodynamic energy balances and enhance our understanding of SOM dynamics, complementing carbon balances.

In this study, we used simplified soil systems to minimize the interference of different processes and of natural  $^{13}\text{C}$  background. This artificial soil consisted of sand, silt, clay, trace elements, nutrient solutions, and soil extracts as inoculum. The artificial soil was amended with cellulose as a substrate and incubated under five distinct environmental conditions, including three water content levels, two temperature levels, and two nitrogen supply levels. Respiration rates were measured using gas chromatography coupled with thermal conductivity detection, while residual substrate was quantified through phenol sulfuric acid assays. Experimentally derived carbon use efficiency (CUE) was calculated under the assumption that carbon from degraded substrates is either released as  $\text{CO}_2$  or utilized for biomass production.

For thermodynamic energy balance calculations, the heat production rate of microbial turnover in artificial soil was measured using an isothermal microcalorimeter. Treating substrate degradation as a chemical reaction, the reaction enthalpy was computed from total heat release and substrate degradation amounts. Similarly, the calculation of reaction Gibbs energy was conducted based on known Gibbs energy values of each reactant. Entropy was determined using the measured reaction enthalpy and Gibbs energy, establishing a thermodynamic energy balance to calculate energy use efficiency (EUE) and CUE. A comparison between thermodynamically and experimentally derived CUE values will also be presented.

### **P3     Biotransformation of selected substrates and the consequent microbial growth — fate of $^{13}\text{C}$ -Carbon in soil**

**Yousaf U.<sup>1</sup>, Vonhoege D.<sup>1</sup>, Thiele-Bruhn S.<sup>1</sup>**

<sup>1</sup> Soil Science, Faculty of Spatial and Environmental Sciences (FB VI), University Trier, Germany

[yousaf@uni-trier.de](mailto:yousaf@uni-trier.de)

Microbial carbon use efficiency (CUE) is crucial in comprehending the soil carbon cycle and its impact on the environment and climate change. CUE refers to the allocation of absorbed carbon into either growth (biomass) or respiration ( $\text{CO}_2$ ). Inputs of substrate-C into the soil can have a considerable impact on the breakdown of soil organic matter (priming effect), resulting in substrate-specific carbon use efficiency and energy use efficiency (EUE). This makes it challenging to accurately predict the environmental impacts on soil organic matter dynamics and the balance of carbon in the soil. The present study provides a conceptual overview of microorganisms as agents that facilitate the synthesis of soil organic matter (SOM). We examine the impact of selected substrates, such as Glucose (180 Da),  $\alpha$ -1,4-maltotetraose (666.6 Da), starch (325 kDa), and cellulose ( $\beta$ -1,4), on the development of microorganisms, their respiration, and the consequent CUE. We specifically analyze how the size and rigidity of these substrates affect these factors. The soil used (farmyard manure fertilized Luvisol) was taken from a long-term fertilization experiment Dikopshof, Bonn, Germany. Regarding substrate size, we hypothesize that exoenzymes are necessary to break down any substrate larger than 600 Da. This would result in a different carbon use efficiency (CUE) and energy use efficiency, as the process type shifts from growth-oriented processes (which have a fast turnover and low CUE for glucose degradation) to adaptation-oriented processes (which involve interlinkage of energy flux networks within the system) for larger substrates such as maltotetraose and starch. Regarding substrate rigidity, our hypothesis suggests that the chemical stability of the substrate affects both the rate at which it degrades and its carbon use efficiency (CUE), hence promoting processes that are directed towards adaptability. The

substrates were labelled with  $^{13}\text{C}$  to balance the turnover, identify the carbon in the functional pools, and determine kinetics. Incubation experiments were time-resolved samples and gas flux sampling and isotope selective  $\text{CO}_2$  analysis were done. Elemental analysis of C, H, N, S, O, and P was done to calculate the stoichiometry of OM. Chloroform fumigation extraction was performed to determine microbial biomass carbon and nitrogen. In combination with further data the microbial quotient ( $C_{\text{mic}}/\text{OC}$ ), the respiratory quotient ( $q\text{CO}_2 = \text{resp.}/C_{\text{mic}}$ ), and CUE were calculated. Aminosugars were used as markers of microbial biomass/necromass. This enabled the estimation of carbon and energy accumulation in the form of additional biomass, necromass, and metabolites. The first results align with our hypotheses, adding a simple sugar (Glucose) triggered a faster turnover leading to a decline in the microbial CUE and fast biomass growth which normalized after the glucose was completely consumed (4 and 8 days). For larger substrates, a peak in the production of extracellular enzymes was seen, and the effect of substrate rigidity was seen as the turnover rates were mediocre (a long flat peak) and this medium efficiency resulted in the maximum formation of biomass and SOM.

#### **P4 The life of a soil microbe is short but its MEMORIES remain: Production of stable Microbial ExoMetabOlitEs and ResiduES from simple and complex substrates**

Stumpf K.<sup>1</sup>, Simon C.<sup>1</sup>, Lechtenfeld O.<sup>1</sup>

<sup>1</sup> Department of Environmental Analytical Chemistry, Helmholtz Centre for Environmental Research - UFZ, Leipzig, Germany .

[oliver.lechtenfeld@ufz.de](mailto:oliver.lechtenfeld@ufz.de)

Organic matter (OM) is an important driver of the global carbon cycle and plays a central role in soil protection (e.g., fertility, biodiversity, erosion). Our understanding of soil organic matter (SOM) is hampered by its chemical complexity and various sources (i.e., plant, fungal and bacterial metabolites and their decomposition products). Ultimately, this limits our insight into the mechanisms of SOM formation and stabilization.

We conducted incubations with typical soil substrates - from simple mono- and polymeric substrates (glucose, glucosamine, chitin, cellulose) to complex microbial and fungal necromass, as well as plant litter amended with an inoculum from arable topsoil and maintained as suspensions over one year. Incubations were conducted at contrasting redox regimes: aerobic, anaerobic and changing after 180 days from aerobic to anaerobic and vice versa to study the long-term turnover of the substrates and the production and release of MEMORIES (microbial exometabolites and residues).

Substrate turnover and dissolved organic matter (DOM) production was analyzed by direct injection liquid chromatography coupled to ultrahigh resolution mass spectrometry (LC-FT-ICR-MS) and complementary data (e.g., pH, redox potential,  $\text{O}_2$  saturation, DOC concentrations and optical density). Using contrasting boundary conditions and differently complex substrates, we can explore potential mechanisms of C sequestration in soils on a molecular and structural level.

Microbial turnover and production of complex DOM was independent from initial substrate composition and explained by the contribution of MEMORIES. Redox regime controlled the rate of substrate turnover and the production of DOM with distinct nominal oxidation states.

The detailed molecular-level information of stable compounds produced by microbes was then linked to signatures of extractable SOM from the soil used to obtain the inoculum. This comparison allowed us to estimate the contribution of MEMORIES and substrate-related decomposition products to SOM stabilization under field conditions. Our findings have important implications for the formation of complex SOM and the role of the microbial carbon pump for the long-term stabilization of organic matter in arable soils.

**P5 Molecular and bioenergetic signatures in mineral-associated and particulate organic matter fractions under long-term field experimental condition****Rathnayake D.<sup>1</sup>, Steiner S.K.<sup>2</sup>, Guillaume T.<sup>2</sup>, Leifeld J.<sup>1</sup>**<sup>1</sup> Agroscope, Climate and Agriculture Group, Reckenholzstraße 191, CH-8046 Zürich, Switzerland<sup>2</sup> Agroscope, Field-Crop Systems and Plant Nutrition, Route de Duillier 50, P.O. Box 1012, CH-1260 Nyon, Switzerland[dilani.rathnayake@agroscope.admin.ch](mailto:dilani.rathnayake@agroscope.admin.ch)

Understanding the impact of different nutrient management practices on soil organic matter stabilization pathways is essential to optimize carbon and nutrient use efficiencies. In this context, molecular composition and bioenergetic signatures of soil organic matter could provide important information about their thermal and chemical stability and turnover rates. Here, we analyzed the soil sampled from a 48-year-old long-term field experiment in Switzerland, which contains treatments with varying levels of mineral N fertilizer (recommended N application dose  $\pm$  40 N units) and organic amendment (70 t/ha manure with and without crop residue) application. The bulk soils and fractionated soil samples were analyzed for their elemental composition (C, H, N, O), molecular composition using pyrolysis gas chromatography-mass spectrometry, and activation energy and energy density using simultaneous thermal analysis. The molecular composition of different soil fractions was further categorized into plant, microbial, and mixed-originated compounds to understand the preferential carbon stabilization pathways under different nutrient management conditions. The bioenergetic signatures and stoichiometric indices were used as complementary approaches to understand the quality and stability of particulate and mineral-associated soil organic matter fractions.

**P6 Mineral and substrate control on MOM formation efficiency, and feedbacks to microbial composition and function****Yuan Y.<sup>1,2</sup>, Kölbl A.<sup>2</sup>, Kaiser K.<sup>2</sup>, Mikkuta R.<sup>2</sup>, Goldmann K.<sup>3</sup>, Schrumpf M.<sup>1</sup>**<sup>1</sup> Max Planck Institute for Biogeochemistry, Germany<sup>2</sup> Martin Luther University, Germany<sup>3</sup> Helmholtz Centre for Environmental Research, Germany[eyuan@bgc-jena.mpg.de](mailto:eyuan@bgc-jena.mpg.de)

Mineral-associated organic matter (MOM) plays an important role for organic matter persistence in soils with turnover times of decades to millennia. It is currently assumed that microbial necromass contributes significantly to MOM formation. Differences in the processing and stabilization of microbial versus plant residues are still unresolved. We studied on how substrate quality (bacterial necromass, *Bacillus subtilis*, C:N=3.7; fungal necromass, *Aspergillus niger*, C:N=12.5; maize litter, *Zea mays*, variety Yucon Chief, C:N=15.4) and mineral type (iron oxide goethite (SSA=15.4 m<sup>2</sup> g<sup>-1</sup>), clay mineral illite (SSA=34.6 m<sup>2</sup> g<sup>-1</sup>), and quartz sand (SSA=0.2 m<sup>2</sup> g<sup>-1</sup>)) influence substrate decomposition and MOM formation, and how mineral-OM interactions feedback to microbial community composition, their carbon use efficiency (CUE), and enzyme activities in a one month incubation.

Our results show that substrates and minerals both influence decomposition by changing the microbial community composition and its functioning. Minerals with higher sorption capacity reduced decomposition rates and microbial biomass, increased activities of extracellular enzymes involved in C-, N-, and P-cycling, and shifted microbial community composition. Among the added substrates, bacterial necromass, being nutrient rich, decomposed fastest. At the end of the incubation, mineral-substrate mixtures with bacterial necromass had the smallest microbial biomass, CUE, activities of extracellular enzymes involved in C- and P-cycling, and a less diverse microbial community than samples with maize litter or fungal necromass addition. We find no evidence for preferential stabilization of bacterial necromass by mineral types so far, though results of the heavy density fractionation of the samples to determine MOM formation are still pending. Our results highlight that fresh surfaces of secondary



minerals in soils are no inert background, but modifying decomposition rates of substrates with different qualities by shifting microbial substrate availability, community composition and functioning.

## **P7 Soil microbial carbon use efficiency in a temperate cropland agroforestry system**

**Attor F.<sup>1</sup>, Wachendorf C.<sup>1</sup>**

<sup>1</sup> Department of Soil Science, University of Kassel, 37213 Witzenhausen, Germany

[ftattor@uni-kassel.de](mailto:ftattor@uni-kassel.de)

Tree incorporation through temperate alley-cropping systems (ACS) serves as a C sink by promoting soil organic carbon (SOC) sequestration in agroecosystems. In view of this, temperate ACS are gaining enormous surge as sustainable land-use systems for increasing SOC stocks in meeting both climate change mitigation and adaptation. This can be achieved through enhancing resource-use efficiency between trees and crops within the same land unit. ACS are a suitable alternative to conventional agricultural practices through a combination of unique ecological and economic benefits. Thus, ACS arguably have larger potential for carbon sequestration in plant biomass and soil. Greater net SOC contents are observed within close proximities to trees in comparison to open croplands without trees which may be explained by the promotion of microbial communities that lead to high carbon use efficiencies. Numerous parameters including microbial traits and metabolic quotient have critically been used as indirect indicators for measuring the partitioning of SOC between respiratory energy production and substrate assimilated into microbial biomass and stabilized in SOM; commonly referred to as microbial use efficiency (CUE). In this study we explore the microbial CUE of a Vertic Cambisol soil in tree and crop alleys of a silvo-arable system using labile carbon substrate (glucose) in top and subsoil during a short-term laboratory incubation. We aim to verify if: (i) lower C accumulation in subsoils is explained by lower CUE efficiency and higher priming in top than subsoils (ii) there is a significant effect of imbalanced stoichiometry of C and N in SOM and microbial biomass in subsoils.

## **P8 Effects of spatial heterogeneity on the fungal energy use channel**

**Xu T.<sup>1</sup>, Lorenzen C.<sup>1</sup>, Zimmermann I.<sup>1</sup>, Spielvogel S.<sup>1</sup>**

<sup>1</sup> Institute of Plant Nutrition and Soil Science, Christian-Albrecht-University of Kiel, Kiel, Germany

[t.xu@soils.uni-kiel.de](mailto:t.xu@soils.uni-kiel.de)

For insoluble, high-molecular C sources typical for plant detritus, fungal communities are likely better adapted to a patchy distribution of the substrate compared to bacteria. We conducted experiments designed to simulate the modulating effect of C translocation through fungal hyphae on fluxes of energy and C through the soil microbial community in a spatially heterogeneous environment. The hypotheses were: a) Fungal transport among hot spots and cold spots allows for optimized resource allocation and thus further increases microbial efficiency of substrate utilization. b) Spatial heterogeneity favors the fungal energy use channel due to hyphal transport outcompeting diffusion for insoluble substrates.

To evaluate the effect of fungal C transport from a spatially heterogeneously distributed substrate, we used custom-made cylinders with four compartments divided by perforated walls. We added 1.25 g of arable soil from a long-term fertilization experiment to each compartment and a total amount of 4 x MBC hemicellulose C was added either to a single compartment (100 %), or to two compartments (50 %), or to all four compartments (25 %). The prepared cylinders were then incubated for 3 days and the heat release as well as the CO<sub>2</sub> production were determined.

The first results reveal that, although the same total amount of substrate was added to all treatments, the heat release from the 100 % treatment was lower than from the 50 % and the 25 % treatment, most likely due to the high substrate concentration in a small soil volume leading stoichiometric limitation of the microbial growth.

## **P9 Microbial carbon use efficiency of mineral-associated organic matter is related to its desorption**

Konrad A.<sup>1</sup>, Hofmann D.<sup>2</sup>, Siemens J.<sup>1</sup>, Stutz K.<sup>3</sup>, Lang F.<sup>3</sup>, Mulder I.<sup>1</sup>

<sup>1</sup> Institute of Soil Science and Soil Conservation, iFZ Research Center for Biosystems, Land Use and Nutrition, Justus Liebig University Giessen, Heinrich Buff Ring 26-32, 35390 Giessen, Germany

<sup>2</sup> Institute of Bio- and Geosciences, Agrosphere (IBG-3), Forschungszentrum Jülich, Wilhelm-Johnen-Str., 52425 Jülich, Germany

<sup>3</sup> Chair of Soil Ecology, University of Freiburg, Bertoldstraße 17, 79098 Freiburg, Germany

[alexander.konrad-1@umwelt.uni-giessen.de](mailto:alexander.konrad-1@umwelt.uni-giessen.de)

Interactions between small organic molecules, mineral phases, and microorganisms play a pivotal role in the accumulation and stabilization of organic carbon in soil. In our study we used model soil minerals as well as organic model substrates, i.e. small organic monomers, in order to gain a mechanistic understanding. We hypothesized that sorption affinity and desorption of monomers control microbial processing with a threshold of sorption determining the extent of microbial processing, with ligand exchange reactions providing the most effective stabilization.

Sorption and desorption of uniformly <sup>14</sup>C-labeled glucose, acetylglucosamine, phenylalanine, salicylic acid, and citric acid to goethite, kaolinite and illite were studied in batch experiments. Monomers adsorbed to either goethite, kaolinite, or illite were subsequently mixed into a loamy and a sandy arable topsoil and incubated at 25°C. Mineralization of mineral-adsorbed monomers was observed over a three-week period, after which the assimilation of <sup>14</sup>C into microbial biomass, and the <sup>14</sup>C remaining in soil were quantified.

Assimilation of monomers into microbial biomass and the microbial carbon use efficiency (CUE) of mineral-adsorbed monomers in both soils increased linearly with the amount of monomer-C desorbable from mineral phases. Furthermore, the CUEs of monomers adsorbed to goethite were observed to be lower than those of the same monomers adsorbed to clay minerals. The very low CUE of 6% of goethite-adsorbed glucose incubated in loamy soil can at least partially be explained by phosphate limitations. Mass balances indicated that carboxylic acids were mainly stabilized against mineralization by sorption to goethite, while <sup>14</sup>C of sugars sorbed to clay minerals was mainly retained in soil through assimilation into the microbial biomass. Most monomer C was retained in the soil as carboxylic acids adsorbed on goethite, thereby emphasizing the significance of oxides for the stabilization of C within soil.

We conclude that of sorption and desorption processes of organic molecules to minerals shape the microbial use of these compounds.

## **P10 Benefits from biochar modified with various materials, used as soil amendment, on soil properties and plant biomass**

Brtnicky M.<sup>1,2</sup>, Holatko J.<sup>1,2</sup>, Hammerschmidt T.<sup>1</sup>, Kintl A.<sup>1,3</sup>, Latal O.<sup>1,2</sup>, Kucerik J.<sup>1</sup>

<sup>1</sup> Department of Agrochemistry, Soil Science, Microbiology and Plant Nutrition, Faculty of AgriSciences, Mendel University in Brno, 613 00 Brno, Czech Republic

<sup>2</sup> Agrovyzkum Rapotín, Ltd., Vyzkumníku 267, 788 13 Rapotín, Czech Republic

<sup>3</sup> Agricultural Research, Ltd., 664 41 Troubsko, Czech Republic

[martin.brtnicky@mendelu.cz](mailto:martin.brtnicky@mendelu.cz)

Biochar is a secondary material produced from various types of organic matter (OM), used as a soil amendment. It exerts many positive effects on bulk density, water retention, carbon sequestration, OM, microbial enrichment and nutrient stabilization. Its properties vary according to the feedstock and conditions for pyrolysis, they can be further modified by blending with other types of materials, by process of activation or prior to soil application. Modification of biochar by co-composting with manure, incubation with mineral fertilizer, and incubation or co-application with Leonardite based humic

product (HUMAC) was carried out. The impact of final mixed amendments on the soil properties and plant biomass was tested in pot experiments. The beneficial effect of variably modified biochar on the soil traits and plant yield was proven: (i) soil amended with biochar co-composted with elemental sulfur + manure led to increased dry barley biomass yield, compared to effect of unamended manure (ii) soil amended with biochar co-composted with manure increased total carbon, C:N, sulfur, respiration and dry barley biomass yield, compared to unamended manure (iii) activated biochar with HUMAC decreased C:N ratio compared to inactivated biochar in soil, while co-application of biochar + HUMAC increased microbial biomass carbon and respiration (iv) activated biochar with mineral fertilizer increased enzyme activities in soil, microbial respiration and N, P, K concentrations in soil pore water. This research was supported by the Ministry of Agriculture of the Czech Republic, institutional support MZE-RO1224, MZE-RO1724.

### **P11 Evaluation of benefits from digestate amended with various materials, used as fertilizer, on soil properties and plant biomass**

**Holatko J.<sup>1,2</sup>, Kucerik J.<sup>1</sup>, Hammerschmiedt T.<sup>1</sup>, Kintl A.<sup>1,3</sup>, Latal O.<sup>1,2</sup>, Brtnicky M.<sup>1,2</sup>**

<sup>1</sup> Department of Agrochemistry, Soil Science, Microbiology and Plant Nutrition, Faculty of AgriSciences, Mendel University in Brno, 613 00 Brno, Czech Republic

<sup>2</sup> Agrovyzkum Rapotin, Ltd., Vyzkumniku 267, 788 13 Rapotin, Czech Republic

<sup>3</sup> Agricultural Research, Ltd., 664 41 Troubsko, Czech Republic

[jiri.holatko@mendelu.cz](mailto:jiri.holatko@mendelu.cz)

Digestate, a by-product of biogas production via anaerobic fermentation, has become a surplus material due to the recent increase in green energy initiatives, necessitating further utilization. The use of digestate as a fertilizer offers both benefits and disadvantages as nitrogen loss by volatilization (NH<sub>3</sub> and N<sub>2</sub>O) and carbon emissions. Amendment of digestate with additives, which prevent nitrogen and carbon losses via sorption, and change both its chemistry and a ratio of labile and recalcitrant carbon, could overcome these adverse impacts. Amending digestate with limiting nutrients (e.g. elemental sulfur) could benefit nutrient-demanding crops. In several studies (pot experiments with lettuce and maize), we tested the effect of biochar, elemental sulfur or leonardite-based product (HUMAC) co-incubated with digestate on soil traits and plant yield. The positive effect of digestate co-incubation with amendments on its beneficial impact on soil properties has been demonstrated: (i) HUMAC-enriched digestate increased nutrient (C, N and P) transforming enzyme activities and maize biomass production, (ii) elemental sulfur + biochar-enriched digestate increased total content of C, N, S, arylsulfatase, urease, N-acetyl-D-glucosaminidase and phosphatase values in soil with maize, (iii) biochar-enriched digestate increased soil microbial biomass, soil C:N, fresh and dry plant biomass in soil with lettuce, and (iv) elemental sulfur-enriched digestate increased arylsulfatase, phosphatase, urease in soil with lettuce.

This research was supported the Ministry of Agriculture of the Czech Republic, institutional support MZE-RO1224, MZE-RO1724.

### **P12 From Biomass to Soil Carbon: Exploring the Fate of Artificial Humic Substances in Winogradsky column**

**Volikov A.<sup>1</sup>, Filonenko S.<sup>1</sup>, Antonietti M.<sup>1</sup>**

<sup>1</sup> Max Planck Institute of Colloids and Interfaces, Potsdam Science Park, Am Mühlenberg 1, 14476, Potsdam, Germany

[alexander.volikov@mpikg.mpg.de](mailto:alexander.volikov@mpikg.mpg.de)

Natural humic substances (NHS) are essential components of soil, playing a pivotal role in structure and fertility. Their complex structure, characterized by extreme structural heterogeneity, renders them

resistant to biodegradation, allowing them to function as long-term soil carbon pools. This research investigates the transformation of artificial humic substances (AHS), synthesized through hydrothermal treatment of biomass, to mimic the properties of NHS. While AHS share similar structural heterogeneity with NHS, they also have different properties. The presence of readily degradable components such as low molecular weight acids, phenols, aliphatic fragments and sugar residues in AHS can stimulate rapid bacterial growth and subsequent carbon cycling upon incorporation into soil. This raises a critical question: can carbon introduced via AHS persist in the soil for long periods of time and replicate the functionality of NHS?

This study used Winogradsky columns, a microcosm system that promotes natural stratification of the soil microbiome due to a gradient of redox conditions. These columns, filled with soil and nutrient media, facilitated the monitoring of organic matter transformation over time. Columns modified with AHS were compared with controls: without organic carbon addition, original biomass and with NHS. After six weeks, soil samples were taken from different depths within the columns and the microbiome and extracted humic matter were analyzed using various methods.

The Winogradsky column experiment revealed a dynamic transformation of organic carbon within both AHS and NHS influenced by the soil microbiome. Interestingly, a process of organic matter unification was observed. Despite initial structural differences, AHS and NHS converged to a remarkably similar composition. This suggests selective degradation by the microbiome, targeting easily degradable components while leaving a core biostable fraction shared by both AHS and NHS. This finding holds significant potential for the practical application of AHS. Selective degradation by soil microbes suggests that the introduction of thermodynamically unstable AHS results in the immobilization of carbon in a stable form, mirroring the function of NHS. This has the potential to contribute to carbon sequestration and soil fertility, paving the way for a more sustainable approach to soil management.

### **P13 Microbial Responses to Altered Resource Availability in a Changing Arctic: Implications for Nutrient Cycling and Climate Feedbacks**

Rzepczynska A.<sup>1</sup>, Rousk J.<sup>1</sup>, Hicks L.<sup>1</sup>

<sup>1</sup> Section of Microbial Ecology, Department of Biology, Lund University, Lund, Sweden

[agnieszka.rzepczynska@biol.lu.se](mailto:agnieszka.rzepczynska@biol.lu.se)

Arctic climate warming will affect microbially-controlled carbon (C) and nutrients cycling through elevated nutrient availability in the soil and changes in vegetation productivity and composition. Microbes can sequester and stabilize C and nutrients in soil organic matter (SOM) through growth and necromass formation, termed “the microbial pump”. The strength of microbial pump can be estimated by assessing microbial growth along with microbial resource retention time, elucidating how long a resource will be retained in the microbial biomass. In addition, microbial resource use efficiency can describe the resource partitioning between anabolic and catabolic processes, thus revealing whether a resource will be liberated through decomposition or sequestered via the microbial pump. In this study, we used litter and inorganic nitrogen (N) additions to mimic the effects of elevated nutrient availability and shrubification on microbial responses in a subarctic tundra heath. We applied the resources in the field either annually for 6 consecutive years or every third year at triple concentrations, simulating the effects of gradual climate change and extreme weather events, respectively. We measured microbial growth rates (radio-isotope tracing), C and gross N mineralization rates (gas chromatography and <sup>15</sup>N pool dilution methods, respectively), and microbial community size was estimated with phospholipid fatty acids analysis. We found that the microbial C pump tended to get stronger and more efficient across the treatments, suggesting a potential for C uptake and storage through microbial pathways in the future climate. Contrastingly, the microbial N pump became less efficient under field N-fertilization, where pronounced microbial growth was counterbalanced by enhanced gross N-mineralization. In addition, we found no consistent differences between gradual and extreme resource change. Our findings highlight that altered nutrient availability independently impacted microbial C and N turnover and indicate that different fates of C and N cycling - retention and loss - may be expected in the future.

## **P14 Microbial growth resistance and resilience to drought across Europe**

**Winterfeldt S.<sup>1</sup>, Paredes C.C.<sup>2</sup>, Rousk J.<sup>1</sup>, Leizeaga A.<sup>3</sup>**

<sup>1</sup> Microbial Ecology, Department of Biology, Lund University, Sweden

<sup>2</sup> Environmental Dynamics Section, Department of Science and Environment, Roskilde University, Denmark

<sup>3</sup> Soil Ecology and Ecosystem lab, Department of Earth and Environmental Sciences, The University of Manchester, United Kingdom

[sara.winterfeldt@biol.lu.se](mailto:sara.winterfeldt@biol.lu.se)

Drought and rainfall events are becoming more frequent and intense. At the same time, soil moisture is one of the main factors controlling soil microbial processes. Microorganisms release carbon into the atmosphere via respiration, and can accumulate carbon in the soil via microbial growth. When challenged with drought, microbial communities are thought to have two different responses; they can maintain growth rates during drought (i.e., resistance) and their growth rates can recover faster when the drought ends (i.e., resilience). Higher resistance and resilience to drought disturbances might lead to a proportionally higher carbon storage via higher growth rates. Microbial communities are also shaped by many other aspects in the soil environment, however how those interact with drought remains unclear.

Here we investigated how differences in climate and soil properties determine microbial drought resistance and resilience. To do so, we used a climate gradient across Europe ranging from the Arctic to Southern Mediterranean. Sites were also selected to represent a wide range of soil properties that were likely drivers of microbial responses, including soil organic matter, pH, and soil texture.

We found that bacterial alpha diversity was the strongest driver of both bacterial resistance and resilience. This was most probably due to changes in pH, where higher pH coincided with higher diversity. This suggests that high diversity helps maintain bacterial functions during drought. Climate was the second most important driver, where bacterial communities from arid climates showed higher resistance and resilience than those from humid climates. Moreover, the bacterial community composition was linked to resilience and could be associated with shifts in the relative abundance of specific taxa. We also found that fungal communities were both more resistant and resilient compared to bacteria. The fungal resistance and resilience were unaffected by the climate and measured soil properties. These findings also show that bacteria are more sensitive to drought and rainfall events than fungi. Our results can be used to predict bacterial responses to global change. For example, if the climate becomes drier or are managed to promote bacterial diversity, bacterial growth will be higher during drought perturbations which can promote soil carbon storage.

## **P15 Impact of Century-Scale Soil Warming on Soil Organic Matter Dynamics and Microbial Communities in Subarctic Ecosystems**

**Peter A.<sup>1</sup>, Kehr J.<sup>2</sup>, Finn D.<sup>1</sup>, Poeplau C.<sup>2</sup>, Tebbe C.C.<sup>1</sup>**

<sup>1</sup> Thuenen Institute of Biodiversity, Bundesallee 50, 38116 Braunschweig, Germany

<sup>2</sup> Thuenen Institute of Climate-Smart Agriculture, Bundesallee 50, 38116 Braunschweig, Germany

[anne.peter@thuenen.de](mailto:anne.peter@thuenen.de)

Soil organic matter (SOM) dynamics under long-term warming are critical to understanding how climate change may impact carbon cycling. This study investigates the effects of century scale soil warming on SOM dynamics and microbial communities in subarctic deciduous forest near the Takhini Hot Springs in Yukon Territory, Canada. Utilizing a natural geothermal gradient, we examine changes in soil microbial community composition and functional potential as carbon use efficiency. Initial findings indicate that warming increases microbial decomposition of litter and native SOM, with significant shifts in substrate preference from plant-derived particulate organic matter to microbially-derived compounds, particularly in deeper soil layers. We hypothesize that warming enhances microbial activity,



leading to increased decomposition and altered SOM composition. As a result, microbial communities adapt to relatively oligotrophic conditions, observable as an increase in traits associated with high carbon use efficiency (CUE), like higher codon use bias, as it enhances translational efficiency and reduces metabolic costs.

Our methodology incorporates the  $^{18}\text{O}$ -CUE method to measure microbial CUE by tracking microbial growth using  $^{18}\text{O}$ -labeled water under steady-state conditions. Incubation experiments will quantify CUE across different temperatures, while 16S rRNA and ITS gene amplicon sequencing analyses will link microbial community composition with functional potential. Additionally, exoenzyme analysis, of enzymes involved in SOM decomposition, e.g. chitinase or  $\beta$ -glucosidase will be performed to connect genomic features to soil functions.

This research should enhance our understanding of the link between SOM dynamics under climate change and microbial adaption, providing a framework for predicting long-term ecological responses in subarctic ecosystems. The outcomes will inform broader ecological models and potential mitigation strategies for climate change impacts on soil health and carbon cycling.

## **P16 Investigating energy fluxes and organic matter stability in permafrost through integrated physical, biological and chemical indices**

**Montaño-López F.<sup>1</sup>, Chávez-Vergara B.<sup>2</sup>, Biswas A.<sup>3</sup>, Pries C.H.<sup>1</sup>**

<sup>1</sup> Department of Biological Sciences, Dartmouth College, NH, USA

<sup>2</sup> Instituto de Geología, Universidad Nacional Autónoma de México, CDMX, México

<sup>3</sup> School of Environmental Sciences, University of Guelph, ON, Canada

[fernando.gr@dartmouth.edu](mailto:fernando.gr@dartmouth.edu)

Permafrost-affected landscapes are experiencing drastic changes in response to climate change, resulting in potential destabilization of vast pools of carbon. Permafrost carbon dynamics are balanced by gains and losses of energy and matter from plants, soil minerals and microbes. However, further studies are needed to better elucidate soil organic matter (SOM) persistence, transformation and their implications for Arctic carbon balance. Emerging applications of thermodynamic theory on SOM dynamics contribute to better understand the state and transitions that soil systems undergo. Here, we propose a preliminary experimental approach to link how changes in energy control matter turnover in permafrost soil. We sampled permafrost soil on the North Slope, Alaska, USA across different glacial drifts where we hypothesized the degree of soil weathering and pedogenesis, and therefore capacity to protect SOM, would differ by landscape age. We plan to couple calorimetry, incubation and chemical extractions to provide an overview of the energy requirements for degradation processes. Firstly, thermal analysis will provide the total exothermic energy content necessary for various processes in soil, including (de)sorption, dissolution, and microbial decomposition. We hope to gain individual understanding of such processes by compartmentalizing the total energy from thermal decomposition via selective dissolution extractions of mineral phases and available organic matter through incubations. Pre and post-incubation analysis of mineral phases, available organic matter and microbial communities will give us insights into separate energy consumption requirements in physical, chemical and biological soil processes. We plan to compare energy and mass balances of minerals, microbes and OM in pre and post-incubation analyses to better understand how these fluxes shape SOM stability. The findings of this study hope to provide greater clarity on the links between microbial decomposition, chemical changes of soil minerals and OM and the thermal stability differences in permafrost soil.

**P17 Activation energy of biochemical processes in soil****Kuzyakov Y.<sup>1</sup>, Filimonenko E.<sup>2</sup>**<sup>1</sup> Dept. of Soil Science of Temperate Ecosystems, University of Goettingen, 37077 Göttingen, Germany<sup>2</sup> Tyumen State University, 6 Volodarskogo Street, 625003 Tyumen, Russia[kuzyakov@gwdg.de](mailto:kuzyakov@gwdg.de)

Activation energy ( $E_a$ ) is a fundamental energetic threshold defining the possibility and the rate of all reactions, but its level for most biochemical processes in soil is nearly unknown. The  $E_a$  of biochemical processes in soil can be measured by four approaches, all of which are based on the Arrhenius equation regardless the conversion mechanisms of organic compounds to  $\text{CO}_2$  or intermediate decomposition products: i) change of the reaction rate by microbial decomposition with increasing temperature, ii) kinetics of hydrolyzing and oxidizing exoenzymes, iii) heat release by calorimetric incubation, and iv) heat release by combustion of organic matter at continuously increasing temperature (chemical process). After short explanation of the background of each approach, we specify the groups of biochemical processes, for which the approaches are useful. Based on the collected database of  $E_a$  values, we present the average  $E_a$  for following reactions: i) microbial decomposition of soil organic matter (SOM) ( $E_a$  from  $67 \text{ kJ mol}^{-1}$ ), ii) enzymatic hydrolysis of polymers involved in C, N, P and S cycling ( $29 - 39 \text{ kJ mol}^{-1}$ ), iii) enzymatic oxidation of SOM ( $40 \text{ kJ mol}^{-1}$ ), lignin ( $44 \text{ kJ mol}^{-1}$  for enzymatic oxidation and  $80 \text{ kJ mol}^{-1}$  for chemical oxidation), iv) thermal decomposition of SOM ( $79 \text{ kJ mol}^{-1}$ ), lipids ( $60 \text{ kJ mol}^{-1}$ ), and proteins ( $120 \text{ kJ mol}^{-1}$ ).

The highest  $E_a$  was common by the thermal analysis – solely chemical reactions, reflecting the absence of enzymatic reactions common in soil. Nevertheless, this  $E_a$  decrease by enzyme driven reactions compared to chemical process groups was not overwhelming. We explain this by specificity of hydrolysis reactions compared to the complete mineralization by oxidation. Despite the  $E_a$  of chemical oxidation increases from  $65 \text{ kJ mol}^{-1}$  for carbohydrates to  $138 \text{ kJ mol}^{-1}$  for non-hydrolysable SOM residue, this  $E_a$  increase is not sufficient to explain high stability and very long turnover of SOM. Therefore, the long SOM turnover is explained not only by the intrinsic SOM stability (reflected by  $E_a$ ), but also by the spatial separation of microorganisms and enzymes from the substrates (e.g. encapsulation). Concluding,  $E_a$  is one of the fundamental parameters defining the rates of chemical and biochemical process in soil, and is crucial to understand C stabilization. The fixed  $E_a$  of  $50 \text{ kJ mol}^{-1}$  commonly used in C cycle models do not reflect the broad range of reactions of organic matter in soils and consequently, does not reflect its stability.

**Session 2: Under the lens: soil carbon and energy channels across trophic levels****P18 How does soil food-web influence nutrient cycling after returning crop residues to agricultural fields? A mesocosm experiment****Gergocs-Winkler V.<sup>1</sup>, Flórián N.<sup>1</sup>, Takács T.<sup>1</sup>, Dombos M.<sup>1</sup>**<sup>1</sup> Institute for Soil Sciences (HUN-REN Centre for Agricultural Research), Hungary[gergocs.veronika@atk.hun-ren.hu](mailto:gergocs.veronika@atk.hun-ren.hu)

Preserving soil carbon content and optimizing nutrient cycling in soil are two important activities in sustainable agriculture. After crop harvest, leaving crop residues on the fields reduces soil nutrient loss and increases soil organic material. Soil-dwelling microorganisms have an important role in nutrient cycling during decomposing crop residues. The nitrogen content of cereal straw is low. Therefore, during its decomposition process, soil microbiota removes nitrogen from the soil which causes nitrate depression hindering plant development. In the complex soil food-web, not only soil microbiota are present in the decomposition processes. Soil fauna is usually neglected from agro-ecological studies

but they significantly influence the activities of soil microorganisms as nematodes and soil microarthropods mainly feed on soil microbiota.

In a field experiment, we investigated the role of soil mesofauna in nutrient cycling. 40 pieces of semi-closed mesocosms, including a soil-plant (barley) system, were built in the spring of 2024 in two soil types (chernozem and sandy soils). The main treatment was to eliminate soil-dwelling mesofauna from half of the mesocosms and so we could compare the soil-plant systems with and without soil fauna. So far, such experiments were only successful in laboratory microcosm experiments and it is important to support these achievements in the field. We have been developing our mesocosms for years to reach fauna-free soil-plant systems in the field. In addition, plant residues were also added to half of the mesocosms and carbon and nitrogen cycling were investigated through measuring exchangeable and soluble ammonium and nitrate ions, and organic carbon content of the soil. Plant decomposition was measured with litterbags and plant nitrogen content was also determined. Finally, soil mesofauna density and community-level physiological profile of soil microbiome were determined.

We expect that crop residue returning causes higher carbon content but lower nitrate content in those mesocosms where crop residues were added. We also expect that nitrate depression is lower and mineral nitrogen content of soil is higher with soil fauna compared to the absence of soil fauna. We also expect that soil microbiota differ in natural and fauna-free soil.

In the conference, we present the whole experimental setup and the initial results which were gained in July 2024, when the experiment was terminated. This open-field-experiment type provides a new method for *in situ* investigation of the relationship between soil mesofauna and nutrient cycling and may support the nutrient cycling models in the future.

### Session 3: Linking the composition of microbiomes to matter and energy fluxes

#### P19 Impact of soil properties on microbiomes involved in the decomposition of cellulose

Elshal A.<sup>1</sup>, Wirsching J.<sup>2</sup>, Finn D.<sup>1</sup>, Kandeler E.<sup>2</sup>, Marhan S.<sup>2</sup>, Poll C.<sup>2</sup>, Tebbe C.C.<sup>1</sup>

<sup>1</sup> Thuenen-Institute of Biodiversity, Germany

<sup>2</sup> Institute of Soil Science and Land Evaluation, Soil Biology Department, University of Hohenheim, Germany

[abdelhady.elshal@thuenen.de](mailto:abdelhady.elshal@thuenen.de)

Soil microbiomes are dependent on the supply of carbon, other nutrients and energy sources. Here we analyzed the effect of cellulose addition on the microbiome, which is the most abundant macromolecule in terrestrial ecosystems. More specifically we asked: (i) Which members of the soil microbiome utilize cellulose (as carbon and energy source) for growth? (ii) How strong is the influence of different soils on the identity of the microbial taxa growing with cellulose? (iii) Does the addition of farm-yard manure (FYM) as an organic fertilizer (and additional nutrient and energy source) affect the organisms growing with cellulose? Growth was measured by quantifying PLFAs and with soil DNA by qPCR of 16S rRNA genes and ITS sequences for prokaryotes and fungi, respectively. The same target DNA sequences were selected to analyze the microbial community compositions. The study was conducted in microcosms over a period of 64 days and a total of four agricultural soils, each with an unfertilized and a FYM-fertilized variant. Cellulose-C was added at a rate of 4-fold of the microbial biomass C of the soil. Irrespective of the soil and their FYM treatments, cellulose was predominantly used by fungi, specifically taxa within the phylum Ascomycota. This was reflected by increased production of fungal PLFAs in response to cellulose addition, particularly evident after 16 days. No cellulose-stimulated prokaryotic phyla were detected. Rather, gram-positive bacterial PLFAs increased primarily in response to fertilization, with a lesser sensitivity to cellulose addition. In contrast, gram-negative bacterial PLFAs exhibited distinct responses to both fertilization and cellulose addition among the four soil types. Cellulose addition did not alter the overall community of prokaryotes or fungi. Rather, a small subset

of specific taxa was enriched in the presence of cellulose e.g., preliminary, with two of four soils analyzed, the fungi growing with cellulose belonged to the class Leotiomycetes. Our preliminary data suggest that fungi are more efficient in utilizing cellulose than prokaryotes. More insights are expected with current analyses on the taxonomic identity of the microorganisms' growth with cellulose.

## **P20 The impact of nitrification inhibitors on the microbial community in German agricultural fields in mitigating nitrous oxide emissions.**

**Groven A.-C.<sup>1</sup>, Wonneberger A.<sup>2</sup>, Schulte auf'm Erley G.<sup>2</sup>, Binder I.<sup>3</sup>, Ruser R.<sup>3</sup>, Pacholski A.<sup>2</sup>, Tebbe C.C.<sup>1</sup>**

<sup>1</sup> Thünen Institute of Biodiversity, Germany

<sup>2</sup> Thünen Institute of Climate-Smart Agriculture, Germany

<sup>3</sup> Department of Fertilization and Soil Matter dynamics, University Hohenheim, Germany

[anne-catherine.groven@thuenen.de](mailto:anne-catherine.groven@thuenen.de)

Agricultural soils in Germany contribute as the second largest polluters of N<sub>2</sub>O emissions in Europe. As part of reaching the Sustainable Developmental Goals the reduction of N<sub>2</sub>O emission, which are mainly a result of microbial nitrification, is of great importance. Because of their potential of mitigating N<sub>2</sub>O emissions, the use of nitrification inhibitors (NI) on agricultural fields have gained attention. They decrease emissions and also leaching of nitrate, thereby benefitting the uptake of N. However, concerns have raised about the impact of NI on the soil microbiome, including target organisms (microbial nitrifiers) and non-target organisms, which may provide important ecosystem functions.

This work investigates the influence of continuous addition of different NI at the scale of microbial communities but also individual members. More specifically, we ask whether specific nitrifying organisms are resilient on a short term and/or develop resistance to the NI over longer periods of time. For the study different experimental agricultural field sites in Germany were selected.

Questions will be approached with both molecular and soil chemistry techniques, including amplicon and metagenomic soil DNA sequencing, especially targeting functional genes related to the N cycle for a better understanding of the nitrogen processes. Soil chemistry characteristics include gas measurements, N mineralization, pH, C and N contents will be measured simultaneously.

This work is part of the NitriKlim project and funded by the Federal Ministry of Food and Agriculture by decision of the German Bundestag

## **P21 Effect of microbial diversity on soil responses to forest management**

**Guasconi D.<sup>1</sup> & Pallandt M.<sup>1</sup>, Adamczyk B.<sup>2</sup>, Lehtonen A.<sup>2</sup>, Martinovic T.<sup>3</sup>, Mäkipää R.<sup>2</sup>, Tupek B.<sup>2</sup>, Manzoni S.<sup>1</sup>**

<sup>1</sup> Department of Physical Geography, Stockholm University, Sweden

<sup>2</sup> Natural Resources Institute Finland (LUKE), Finland

<sup>3</sup> Institute of Microbiology of the Czech Academy of Sciences, Czech Republic

[daniela.guasconi@natgeo.su.se](mailto:daniela.guasconi@natgeo.su.se) , [marleen.pallandt@natgeo.su.se](mailto:marleen.pallandt@natgeo.su.se)

Forest management and disturbance can change soil microbial community composition and activity, which in turn may affect C turnover by altering microbial ecosystem functions such as decomposition. A previous study theorized that microbial functional diversity would contribute to accelerating decomposition rate (Khurana et al., 2023), but this hypothesis needs to be tested with empirical data. In particular, our aim is to test: 1) if it is possible to disentangle the effects of climate, management and microbial diversity on C turnover and 2) if and how microbial diversity affects C dynamics in soils with different management regimes. For this purpose, we will use the GHG fluxes and microbial diversity data collected from the long-term forest N-fertilization experiment in Karstula (Finland). In particular, we investigate the relationships between heterotrophic respiration, soil temperature, soil moisture, management, and microbial diversity at that site. Previous studies showed that N addition

leads to higher SOC stocks, but initial analysis focused on microbial diversity suggests that the relationship between and respiration might differ between microbial functional groups. The study is carried out within the HoliSoils project (Holistic management practices, modelling and monitoring for European forest soils), a consortium aimed at identifying and testing soil management practices for climate change mitigation and to optimize ecosystem service provision.

Khurana, S., Abramoff, R., Bruni, E., Dondini, M., Tupek, B., Guenet, B., Lehtonen A., Manzoni, S., 2023. Interactive effects of microbial functional diversity and carbon availability on decomposition – A theoretical exploration. *Ecological Modelling* 110507. <https://doi.org/10.1016/j.ecolmodel.2023.110507>

## **P22 Exploring the role of bacterial synthetic communities in alleviating drought stress by shaping trophic interactions and enhancing soil health and plant resilience**

**Lumactud R.A.**<sup>1</sup>, Austin T.<sup>2</sup>

<sup>1</sup> Department of Plant, Food, and Environmental Sciences, Dalhousie University, Nova Scotia, Canada

<sup>2</sup> Department of Animal Science and Aquaculture, Dalhousie University, Nova Scotia, Canada

[rheaamor@dal.ca](mailto:rheaamor@dal.ca)

As Canada aims to meet stringent emission and carbon targets by 2030, sustainable agricultural practices are essential. This research explores the potential of bacterial synthetic communities (SynComs), isolated from the rhizosphere of forage plants in drought-prone fields, to alleviate drought stress in forage plants under greenhouse conditions. The study investigates how these SynComs shape trophic interactions among nematodes, bacteria, and fungi and whether these interactions can be linked to improvements in soil health and plant resilience. By inoculating forage plants with SynComs, we aim to observe changes in plant stress responses and identify patterns in microbial community interactions. This study includes higher trophic-level organisms, such as nematodes, which are highly sensitive to soil moisture levels due to their reliance on water films in soil for mobility and survival. The impact of drought on nematodes and their subsequent effect on bacteria, archaea, and fungi is largely unknown. Microscopic and molecular techniques will be employed to identify nematode communities. Soil samples will be analyzed for physicochemical properties, including soil organic matter turnover, nutrient cycling, microbial diversity, and community structure. The expected outcomes include identifying key microbial interactions that enhance plant growth and stress tolerance and providing a scientific basis for sustainable agricultural practices that mitigate the impacts of climate change. This approach aims to validate the efficacy of SynComs in promoting soil health and plant resilience, ultimately supporting the development of innovative ecosystem management and conservation strategies. By focusing on the resilience and stability of these microbial communities, the research seeks to uncover critical insights into how SynComs contribute to soil health, plant growth, and ecosystem sustainability.

## **P23 Microhabitat spatial structure affects the establishment and persistence of necromass recycling-based model microbial systems**

**Mansour I.**<sup>1</sup>, Hähnlein M.<sup>2</sup>, Rillig M.C.<sup>3</sup>

<sup>1</sup> Institut für Biologie, Freie Universität Berlin, D-14195 Berlin, Germany

<sup>2</sup> Berlin-Brandenburg Institute of Advanced Biodiversity Research, D-14195 Berlin, Germany

[india.mansour@gmail.com](mailto:india.mansour@gmail.com)

Microbial necromass production and cycling play key roles in soil carbon cycling and carbon storage. A diverse community of decomposer microorganisms in soils participate in necromass recycling, and eventually via death, necromass production. While a soil may host many species of microbial decomposers and receive a variety of carbon substrate inputs through time, the typical state experienced by a microscale soil microbial community is one of limited local diversity and limited carbon/energy availability. Here, necromass recycling is critical, as it may be the only resource available to sustain the



community during some periods of time. In this research, we investigated the boundary condition parameter space in which carbon cycling could persist (or not) over time, in systems where microorganisms were forced to recycle their own necromass to survive. Our initial experiments asked the question: can a two-species producer-decomposer (*Chlamydomonas-E. coli*) community maintain carbon cycling without any initial carbon aside from their own biomass in a closed system, i.e. microbial biosphere? Upon confirming this, we delved further into exploring the influence of spatial boundary conditions on persistence in this system. Specifically, we looked at the effects of microhabitat physical structure and then necromass spatial distribution on persistence. We observed strong effects of microhabitat physical structure, including sand particle size and moisture level, on persistence at both the population and ecosystem levels. Systems containing the smallest sand particles failed quickly and often could not support decomposer populations. Persistence was promoted by larger sand particles, likely due to larger pore sizes resulting in shorter movement distances and better accessibility to resource patches (i.e. necromass). Building on these findings, we then manipulated microbial necromass patch distribution and observed that while *Chlamydomonas* clustered around necromass patches when present, necromass patch distribution did not have a strong effect on persistence time in systems with large sand grain size, i.e. when necromass was fairly accessible. Together these findings indicate a limit to the spatial/physical parameter space in which producer-decomposer communities can establish and self-sustain via self-recycling of necromass.

## P24 Effect of organic matter on the bioenergetic landscape of phytostabilized mine tailings

Moreau U.<sup>1</sup>, Milesi V.<sup>1</sup>, Devau N.<sup>2</sup>, Thouin H.<sup>2</sup>, Le Forestier L.<sup>1</sup>, Battaglia-Brunet F.<sup>2</sup>

<sup>1</sup> UMR 7327 Institut des Sciences de la Terre d'Orléans, Université d'Orléans-CNRS/INSU-BRGM, 45071 Orléans, France

<sup>2</sup> BRGM, Water, Environment, Processes and analytics Division, 3 av. Claude Guillemin, 45060 Orléans, France

[ulyse.moreau@univ-orleans.fr](mailto:ulyse.moreau@univ-orleans.fr)

Total mining waste in Europe amounts to 6Gt, most of which contains iron (Fe), sulfur (S), and other toxic pollutants including metalloids and metals such as lead (Pb), arsenic (As), and zinc (Zn). To reduce pollutant fluxes from mine tailings, phytostabilization based on growing metal-tolerant plants is a common practice, notably to reduce erosive fluxes. Usually, organic amendments are also added in the mine tailing to promote plant growth. This practice as well as root growth impacts the biogeochemical properties of the tailings. They are suspected to drastically modify the microbially-mediated redox reactions controlling metal fluxes but this impact still remains unclear. Combining mesocosm and microcosm experiments with a geochemical model based on simulations of microbially-mediated redox reactions thermodynamically constraints, our study evaluates the impact of assisted phytostabilization on the bioenergetic landscape of mine tailings. In mesocosm (i.e. an instrumented metric column containing homogenized mine tailings), the 6-month pre-amendment period showed a decrease of  $\text{NO}_3^-$  concentrations followed by an increase of Fe(II) and As concentrations. Simulations suggest that Fe(II) concentrations, pH values, and redox potentials are controlled by Fe(II) reductive reactions, promoting the dissolution of hematite.  $\text{NO}_3^-$  and As(V) reduction reactions also occur. The presence of heterotrophic anaerobic bacteria suggests that traces of organic matter in the mine tailings (i.e. roots and barks) are sufficient to act as an electron donor in order to induce the aforementioned redox reactions. Results provided by microcosm-scale experiments (mine tailings batch experiments where increasing concentrations of sodium acetate are used) confirm this hypothesis. A decrease in redox potential and acetate concentration was indeed measured after 27 days of experiments. Our results highlight the key role of organic carbon (either naturally occurring or added as an amendment) as an electron donor regulating the bioenergetics landscape. A Comprehensive study of the bioenergetics landscape seems a suitable framework to improve the management of phytostabilization practices by assessing the interplay between organic carbon and the Fe, S, and As redox couples.

**P25 Metatranscriptomics for Studying Carbon Conversion in the Rhizosphere****Ruggaber J.<sup>1</sup>, Wende S.<sup>1</sup>, Kolb S.<sup>1,2</sup>**<sup>1</sup> Leibniz Center for Agricultural Landscape Research (ZALF e.V.), Microbial Biogeochemistry, Germany<sup>2</sup> Humboldt University, Thae Institute - Microbial Biogeochemistry, Germany[julian.ruggaber@zalf.de](mailto:julian.ruggaber@zalf.de)

Soil organic carbon (SOC) is a chemically heterogeneous pool that is composed of both plant litter-derived necromass and microbial debris. Arable soils have a large potential to store SOC, while sequestration efforts in these soils would also help to improve their fertility and resilience towards climate stress. The rhizosphere is a highly dynamic hotspot of C transformations. Organic C compounds exuded by the host plant shape the functional and taxonomic composition of the rhizosphere microbiome. We used metatranscriptomics - an RNA-based approach - to resolve the rhizosphere microbial processes that form and degrade SOC in an eroded soil cultivated with rapeseed (*Brassica napus* L.). We extracted RNA from the rhizosphere at two different growth stages and two simulated soil erosion states. The soil originates from an eroded site in northeast Germany. mRNA reads were used to investigate the metabolic processes of the rhizosphere microbiome. CAZy and KEGG orthologues (KO) databases were used to investigate active catabolic and anabolic enzymes of rhizosphere microbiomes as well as cellular transporters for saccharides. CAZy classifications showed about 50% of the identified transcripts were glycoside hydrolases (GHs), of which enzyme family GH13 was most abundant. Our tailored analysis using KO further revealed chitin synthase transcripts were elevated at flowering, while chitinase transcripts were elevated at the rosette growth stage. Likewise, transcripts of enzymes genes involved in lignin degradation were elevated in the rosette growth stage. The saccharide transporters of rhamnose, cellobiose and fructose were significantly affected by growth stage and erosion state. Statistical analysis revealed, as expected, that growth stage had the largest effect on the functional microbiome composition. This confirms the well-known control of plant host on its root and rhizosphere microbiome by signaling and growth substrate exudation. However, microbiome functional patterns based on the metatranscriptome were modulated by the erosion state. Our experimental study demonstrates the utility and potential of metatranscriptomics for the elucidation of temporal dynamics of microbial metabolic processes including degradation and synthesis of SOC in the rhizosphere. The developed tailored gene analysis may be useful for other soil environments and may help to understand the specific routes C takes when being transformed in the rhizosphere.

**P26 Land use change affects soil properties, microbial communities and carbon cycling****Müller K.<sup>1</sup>, Simpson R.<sup>1</sup>, Anderson C.<sup>1</sup>, Sharp J.<sup>1</sup>, Cummins M.<sup>1</sup>, Eyre V.<sup>1</sup>**<sup>1</sup> The New Zealand Institute for Plant & Food Research, New Zealand[karin.mueller@plantandfood.co.nz](mailto:karin.mueller@plantandfood.co.nz)

Land use significantly impacts the physical, chemical and biological properties of soil, which in turn affect its ability to provide essential ecosystem services like water regulation, nutrient and carbon cycling. In particular, the type of vegetation cover — whether indigenous vegetation, pasture, annual or perennial crops — plays a crucial role in determining soil health and its functionality. We studied the early effects of land use change on soil properties, microbial community composition and carbon cycling. Our study site, a 137-ha dairy farm with permanent pasture and a gully of remnant indigenous bush, located in Northland, New Zealand, was transitioned to a kiwifruit orchard between 2019 and 2021. Along with planting 69 ha with *Actinidia chinensis* (kiwifruit gold), 43,000 indigenous shrubs and trees were established on 17 ha of steep slopes, banks close to waterways, and gullies. The island of remnant bush was preserved. In January 2021, we established nine transects across the boundaries between indigenous vegetation (newly established or existing), permanent pasture and kiwifruit blocks (established in 2019) with 5 sampling points spread along 50 m extending from the boundaries

into each land use. We took 90 topsoil samples along these transects in May 2021, May 2022, November 2022 and March 2023, and analyzed them for the following parameters: (i) the composition of the soil microbial communities using next generation sequencing, (ii) a range of extracellular enzymes as indicators of microbial activities related to carbon and nutrient cycling, and (iii) concentrations of bio-available nutrients and labile carbon. Proxy measures of seasonal soil organic carbon decomposition and stabilization were established by burying commercially available tea bags of two types with contrasting decomposability, green (*Camellia sinensis*) and rooibos (*Aspalathus linearis*) (teabag index). In this poster, we discuss our preliminary findings, which overall indicate clear differences in most soil properties and functions between the four land uses and highlight the interactions between plants and microbial communities on carbon cycling.

## Session 4: Modeling of matter and energy flows in soil systems

### P27 Hybrid Soil Microbiome Modeling - Combining process-based models with machine learning to predict microbial dynamics and organic matter turnover in soil systems.

Collart P.<sup>1</sup>, Pagel H.<sup>1,2</sup>

<sup>1</sup> IBG-3, Forschungszentrum Jülich, Germany

<sup>2</sup> INRES, University of Bonn, Germany

[p.collart@fz-juelich.de](mailto:p.collart@fz-juelich.de)

Soil microorganisms control organic matter cycling and, to a large extent, determine how soil systems can cope with and mitigate climate change and environmental threats. Leveraging microbial genome and DNA sequencing data to infer functional microbial traits enables to better understand the complexity of the soil microbiome. Integrating trait information with process-based microbially explicit models is required for reliable predictions. We present the concept of a hybrid modeling framework that uses a data-driven machine learning approach to leverage the information in metagenomic and DNA sequencing data for improving the parameterization of process-based models. We invite you to discuss i) what data is informative for parameterizing process-based models, ii) how to reflect life-strategies in process-based models and iii) what is the best approach of linking process-based and data-driven modeling.

### P28 Are the mechanisms driving heterotrophic respiration after rewetting consistent in the lab and the field?

Li X.<sup>1</sup>, Rousk J.<sup>2</sup>, Manzoni S.<sup>3</sup>

<sup>1</sup> Department of Physical Geography, Stockholm University, Stockholm, Sweden; Bolin Centre for Climate Research, Stockholm University, Stockholm, Sweden

<sup>2</sup> Microbial Ecology, Department of Biology, Lund University, Lund, Sweden

<sup>3</sup> Department of Physical Geography, Stockholm University, Stockholm, Sweden; Bolin Centre for Climate Research, Stockholm University, Stockholm, Sweden

[xiankun.li@natgeo.su.se](mailto:xiankun.li@natgeo.su.se)

Understanding the mechanisms driving CO<sub>2</sub> emissions from soils upon rewetting of a dry soil is important to develop models to predict soil carbon fate under climate change. While laboratory drying and rewetting (DRW) experiments have revealed many drivers of CO<sub>2</sub> emissions after rewetting, it is still unknown whether mechanisms inferred from these laboratory studies are consistent with mechanisms dominant in the field. Both the number of putative mechanisms and the challenge of scaling up from laboratory to field conditions have hindered the development of accurate models for CO<sub>2</sub> emissions. Here we collected mean CO<sub>2</sub> emission rates over 48 hours after rewetting from 38 laboratory studies with more than three DRW cycles, and from six field datasets recording hourly resolution

soil moisture and respiration fluctuations. Laboratory and field respiration rates after rewetting were predicted by six predictors using random forest algorithms and partial dependence plots. In the laboratory studies, soil organic carbon content and temperature had positive effects on respiration that were similar to the effects observed in the field. Laboratory results were partly consistent with the positive effects of dryness before the rewetting and the negative (and surprising) effect of soil moisture increment on respiration in the field. Both laboratory and field studies highlighted the importance of climate background on respiration—a possible indication of microbial legacy effects. We concluded that the mechanisms driving CO<sub>2</sub> emissions after rewetting in the lab and the field were generally consistent, but some of the observed responses would be difficult to capture in classical soil carbon cycling models that do not include specific mechanisms to describe rewetting pulses and microbial adaptations.

## **P29 Bioenergetic modeling and data-model integration to simulate coupled carbon and energy flows in artificial soils**

**Müller M.**<sup>1</sup>, Yang S.<sup>2</sup>, Maskow T.<sup>2</sup>, Streck T.<sup>3</sup>, Pagel H.<sup>1,4</sup>

<sup>1</sup> Soil Systems Modeling, Agrosphere (IBG-3), Forschungszentrum Jülich GmbH, Jülich, Germany

<sup>2</sup> Department of Microbial Biotechnology, UFZ - Helmholtz Centre for Environmental Research, Leipzig, Germany

<sup>3</sup> Biogeophysics, Institute of Soil Science and Land Evaluation, University of Hohenheim, Stuttgart, Germany

<sup>4</sup> Soil Systems Modeling, Institute of Crop Science and Resource Conservation, University of Bonn, Bonn, Germany

[mark.mueller@fz-juelich.de](mailto:mark.mueller@fz-juelich.de)

Artificial soils are a well-defined and clean system that consists of defined mixtures of minerals, substrates, nutrients, and microorganisms. They enable the calculation of complete mass and energy balances allowing for rigorous testing of bioenergetic modeling approaches to predict coupled carbon and energy flows in soil systems. This work uses artificial soil to improve the understanding of coupled carbon and energy flows in soil systems with a huge soil organic matter (SOM) background. We extended the biogeochemical model database *bgc\_md2* to reflect microbial growth and energy flows in soil models. *bgc\_md2* is an open computational framework of Python libraries to formulate, collect, analyze and compare element cycling models generically. Different model structures were implemented to test thermodynamically controlled mechanisms of microbial carbon turnover using measurements of carbon dioxide, residual substrate concentration and heat production from incubation experiments with glucose and cellulose. For data-model integration, a likelihood-free inverse surrogate modeling method based on neural networks (simulation-based inference) is compared to a standard Bayesian inference method based on Markov Chain Monte Carlo parameter sampling. In our analysis we show how data density affects model parameterization and prediction uncertainty. Our suitability analysis of the tested modeling approaches for predicting glucose and cellulose decomposition in artificial soils provides process formulations for more complex bioenergetic soil system models.

**P30 Generalized or distinct microbial parameters in soil biogeochemical models? Insights from a three-decade field experiment and a multi-site incubation experiment**

Li J.<sup>1</sup>, Wang X.<sup>1</sup>, Jian S.<sup>2</sup>, Wang G.<sup>3</sup>, Mayes M.<sup>4</sup>, Liang C.<sup>5</sup>, Reddy K.C.<sup>1</sup>

<sup>1</sup> College of Agriculture, Tennessee State University, Nashville TN, 37209, USA

<sup>2</sup> Institute for Environmental Genomics and Department of Microbiology & Plant Biology, University of Oklahoma, Norman, OK, USA

<sup>3</sup> Institute for Water-Carbon Cycles and Carbon Neutrality, State Key Laboratory of Water Resources Engineering and Management, Wuhan University, Wuhan, China

<sup>4</sup> Environmental Sciences Division & Climate Change Science Institute, Oak Ridge National Laboratory, Oak Ridge, TN, USA

<sup>5</sup> Institute of Applied Ecology, Chinese Academy of Sciences, Shenyang 110016, China

[jli2@tnstate.edu](mailto:jli2@tnstate.edu)

Unified and common parameters are sought to achieve a simple and workable soil model. The baseline of microbial carbon use efficiency and microbial biomass turnover rate and their temperature sensitivities received attention due to their key control of soil organic carbon quantity and quality, and consequently on soil health. Our recent studies, however, showed that distinct baseline values of the two parameters can be achieved in heated and unheated conditions and at different time scales based on a three-decade-long soil warming experiment. On the other hand, our recently published work also supported a common set of parameters controlling microbial growth and maintenance as well as extracellular enzyme production and turnover that could be generalized at the soil series level. This raised concerns about how the soil model development shall advance at the prospect of integrating microbial functions into global soil and climate predictions. Nevertheless, the uncertainties of these data-model fusions are also high partly driven by the data imbalances between the high frequency of soil respiration data and the scarcity of microbial biomass, extracellular enzyme activities, and soil organic carbon pool. The need for robust experiment designs and sampling strategies is imperative to achieve intensive and balanced measurements and collections. The newly established University Center of Soil Health at Tennessee State University (TSU) will improve soil energetics research centered around carbon turnover and accretion, train a new generation of soil scientists, particularly from less represented and minority groups, and advance soil conservation and agricultural sustainability in Southeastern US and beyond in collaboration with local NGOs and governmental agencies.

**Session 5: Calorimetry and thermodynamics: keys to unraveling complex soil processes****P31 Quantum Mechanics-Informed Refinement of Nominal Oxidation State of Carbon: Predictive Precision in Anaerobic Ecosystem Dynamics**

Sibille T.<sup>1</sup>, Knorr K.H.<sup>2</sup>, Lechtenfeld O.<sup>3</sup>, Lau M.P.<sup>1</sup>

<sup>1</sup> Institute of Mineralogy; TUBAF IOEZ, Freiberg, Germany

<sup>2</sup> Ecohydrology & Biogeochemistry Group, Institute of Landscape Ecology, University of Münster

<sup>3</sup> BioGeoOmics, Department of Environmental Analytical Chemistry, Helmholtz Centre for Environmental Research – UFZ, 04318 Leipzig, Germany

[thomas-peter.sibille@mineral.tu-freiberg.de](mailto:thomas-peter.sibille@mineral.tu-freiberg.de)

The assessment of the Nominal Oxidation State of Carbon (NOSC) within organic matter (OM) is instrumental in discerning energy dynamics within ecosystems, offering insights into biochemical selectivity and microbial metabolism's energy yields. NOSC serves as a predictive metric for the stabilization and selective preservation of chemically reduced organic matter in anoxic environments, thus aiding in the understanding of complex biogeochemical cycles and energy transformations in oxygen-deficient eco-



systems. Despite its significance, accurately predicting Gibbs free energy changes ( $\Delta G$ ) presents challenges, partly due to the intricate structural nature of dissolved organic matter (DOM) and historically limited sample sizes. The complexity of DOM poses a notable hindrance, complicating energy yield calculations and impacting  $\Delta G$  deductions' precision.

To confront these challenges, our study employs a quantum chemistry modelling framework based on Density Functional Theory, enabling precise geometry optimization and frequency calculations. By leveraging this approach, we have constructed an extensive thermochemistry database comprising 2000 molecules sourced from the PubChem database, aligning molecular formulas with those referenced in seminal work (LaRowe and Van Cappellen, 2011). This database surpasses previous efforts in scale and comprehensiveness. Through this database, we have developed a refined model enhancing NOSC's precision as an indicator of energy availability potential.

This refined model holds promise for broad applications in thermodynamic analyses of environmental processes, offering a deeper understanding of natural chemical reactions' energetic landscapes and microbial processing of OM. Our findings contribute to advancing energy proxies' comprehension in ecosystem dynamics, emphasizing theory-based predictions' potential in environmental research. We present preliminary results and discuss the implications of our refined model, particularly its role in elucidating OM selectivity during anaerobic metabolism.

LaRowe, D.E., Van Cappellen, P., 2011. Degradation of natural organic matter: A thermodynamic analysis. *Geochimica et Cosmochimica Acta* 75:2030-2042.

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