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A novel fluxomics approach to decipher the flux partitioning between anabolic and catabolic processes in soil microbial communities

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ter:labs TERRESTRIAL ECOSYSTEM RESEARCH

EGU General Assembly 2020

universität wien

Introduction

Soils represent the biggest terrestrial carbon (C) pool. Thereof, roughly two thirds are present in the form of soil organic C (SOC), for the most part derived from phytomass (Scharlemann et al. 2014). Microorganisms take up and transform SOC and thereby control C cycling in the soil environment (Gougoulias et al. 2014).

Generally, the microbial metabolism can be distinguished into two main processes:

- **Anabolism** leads to the incorporation of C into biomass, which can further lead to the stabilization of C.
- **Catabolism** breaks down molecules to release energy and ultimately results in the release of CO_2 to the atmosphere.

The distinction, and moreover the quantification of anabolic and catabolic processes in soil microorganisms is crucial for our understanding of the C cycle (Liang et al. 2017).

In the soil environment different microbial species interact (e.g. via extracellular enzymes) with one another and interdepend regarding their metabolic functions (Jansson & Hofmockel 2018). Therefore we speak of the **soil microbial community metabolism**.

This, and the circumstance that 99% of soil microbes are not cultivable, highlights the importance of unraveling the metabolic network of natural soil microbial communities, which has rarely been applied via soil metabolomics or never by soil fluxomics.

We decided on a **targeted metabolomics** approach, in which we preselected a set of 46 metabolites, each being a unique **biomarker** for a specific metabolic pathway and covering the microbial metabolism on a broad scale. Furthermore, we wanted to test our method by measuring metabolite concentrations in the framework of a soil warming experiment.

The aim of this study was to develop a novel technique that allows us to trace the fate and to quantify the flux of ^{13}C through the metabolic network of soil microorganisms.

Methods

Detecting metabolites in soil samples using a stable isotope tracing approach confronts us with several problems:

- **Sorption** of metabolites to mineral and organic surfaces (Dijkstra et al. 2011)
- **Limited accessability** of intracellular compounds
- **High sensitivity** of high-resolution electrospray ionization - MS to salts
- **Rapid mineralization** of glucose
- **Chemical heterogeneity** of metabolites

Experimental setup The soil used in this study was collected from a temperate forest dominated by European beech (*Fagus sylvatica L.*) in Lower Austria in April 2019. Only top soil (0-5 cm) was sampled. Samples were pre-incubated at **ambient** (8 °C measured in situ) or **elevated temperature** (18 °C). One part of the soil was **autoclaved**.

All samples, blanks, internal as well as external standards were prepared in triplicates. At timepoint $t_{(0)}$ ^{13}C-Glucose (equivalent to 1% of SOC) was added to the samples followed by their respective incubation period {0 min, 15 min, 30 min, 60 min, 120 min, 24 h}.

Immediately before the termination of the reaction by 1 M KCl - $CHCl_3$ extraction, gas was sampled and further analyzed by a headspace analyzer (Gasbench) coupled to a Delta V Advantage IRMS. Salts were removed by various purification steps including freeze-drying and methanol dissolution.

Chromatography The first set of samples was prepared for measurement with **IC & Orbitrap** → amino sugars, sugar phosphates and organic acids. The second set for **HILIC & Orbitrap** measurement → amino acids, (poly-) amines, nucleosides and nucleobases.

Data processing & statistical analysis Chromatograms were processed with Xcalibur™ software. Graphs were created and regressions calculated using SigmaPlot (SYSTAT Software, Inc.).

Results (1)

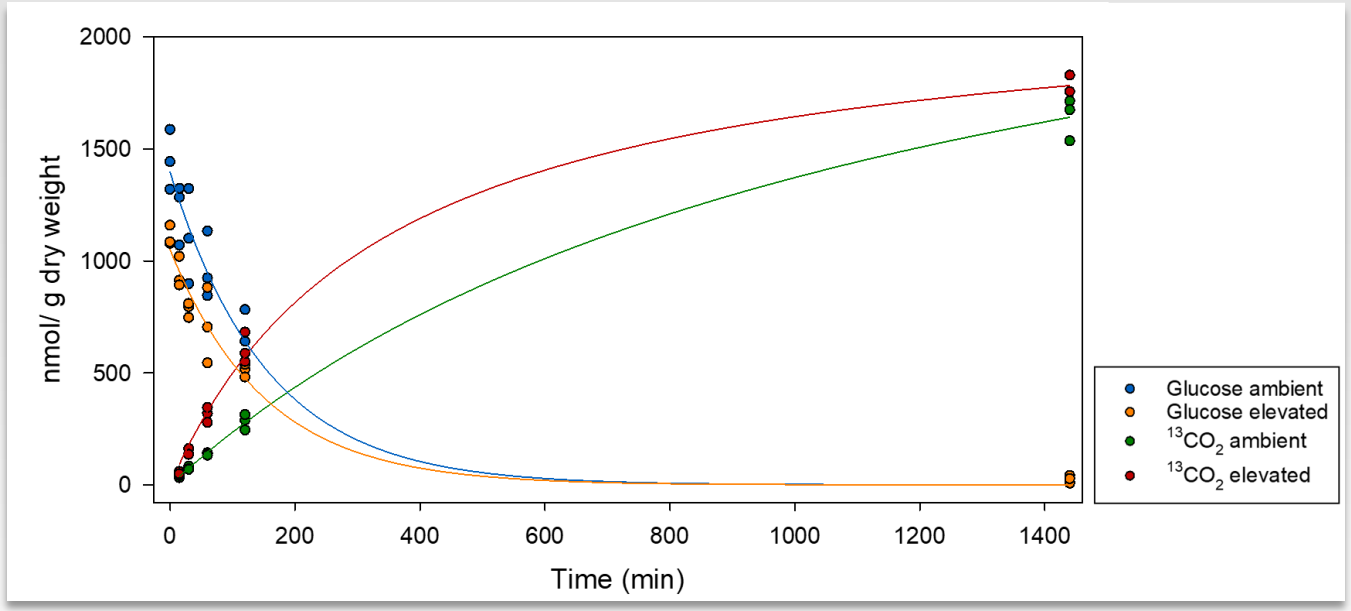


Figure 1. $^{13}CO_2$ versus Glucose (nmol/ g dry weight)

Glucose metabolism was extremely rapid as can be seen by the exponential decay of both ambient and warmed soils. Calculation of the **half-life** ($t_{(1/2)}$) of Glucose showed similar results for both treatments:

$t_{(1/2)}$ glucose elevated = 105 min

$t_{(1/2)}$ glucose ambient = 107 min

$$t_{(1/2)} = \frac{\ln(2)}{rate\ of\ decay}$$

A supporting one-way ANOVA confirmed **no significant difference** in glucose concentrations among treatments.

In contrast, **$^{13}CO_2$ concentrations** in ambient as well as in elevated treatment samples increased hyperbolically in a 24 h time frame. Soils exposed to elevated temperature showed a significant higher increase (one-way ANOVA, P = 0,026) in $^{13}CO_2$ concentrations compared to soils incubated at ambient temperature.

During the first 120 min after ^{13}C-glucose addition we calculated an **increase of $^{13}CO_2$ concentration** per g dry weight per hour of **139 nmol/ g dry weight/ h** in control soils, whereas warmed soils showed a significantly (one-way ANOVA, P≤ 0,001) faster increase of **315 nmol/ g dry weight/ h**.

Results (2)

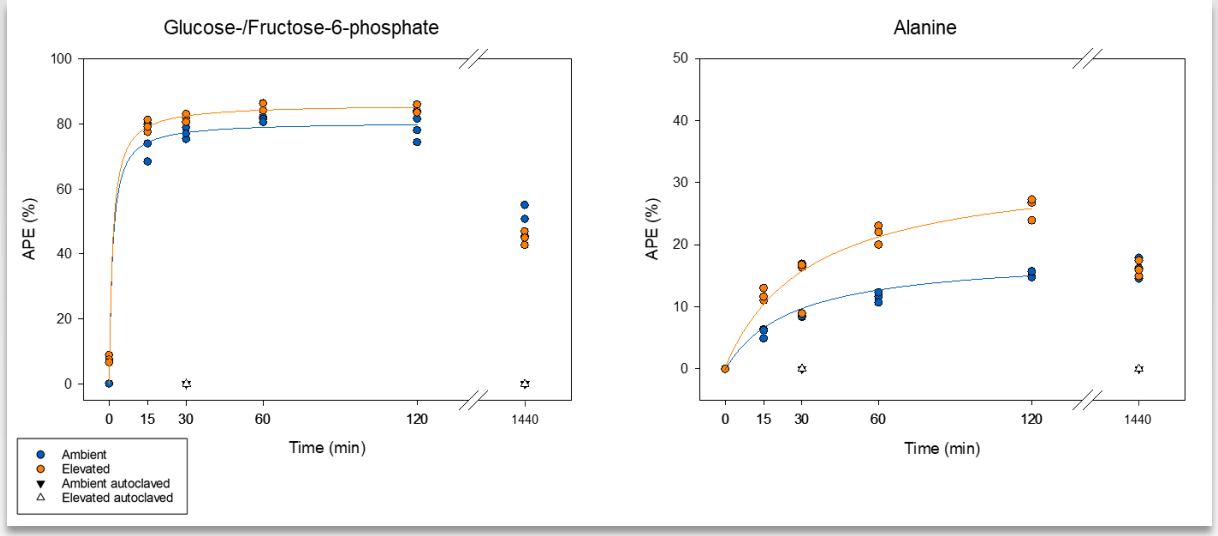


Figure 2. ^{13}C atom percent enrichment (APE) over time in live and autoclaved soils for ambient and warmed soil samples

The isomers glucose-/fructose-6-phosphate showed a **steep increase** together with a **high level of APE** for both treatments in the first 120 min. APE dropped after 1440 min (=24 h) incubation.

In comparison, APE in alanine rose **slower and lower** (note: y-axes differ in magnitude), but showed a **significant difference** between ambient and warmed soils (one way ANOVA, P= 0,025) regarding their maximum APE.

Autoclaved soils in general did not show any remarkable ^{13}C enrichment in metabolites.

Metabolites fitting a hyperbolic curve allowed to calculate the rates of **metabolic flux (nmol/ g dry weight/ h)** using an equation from Farell et al. (2014), with k as the rate constant (min) and Q as the size of a pool of a substrate (average metabolite concentrations in nmol/ g dry weight).*

$$Flux = Q * k$$

Flux (nmol/ g dry weight/ h)	Glucose-/ Fructose-6-phosphate	Ribose-/ Xylulose-5-phosphate	α-Ketoglutaric acid	Alanine
ambient	17,24	0,15	7,14	7,97
elevated	23,77	0,91	12,66	3,81

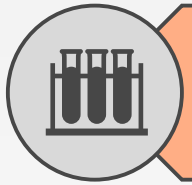
The rate of metabolic flux needed to be adapted for metabolites following a linear increase in APE over 24 h: $k = \Delta APE/h = \text{slope} * 60$).

Flux (nmol/ g dry weight/ h)	Glutamine	Asparagine	UMP	Glycine	Serine
ambient	0,06641	0,00625	0,00555	0,00026	0,00101
elevated	0,05216	0,00854	0,01230	0,00044	0,00000

*Here, calculations were based on data in the 120 min time frame after tracer application.

Discussion & Conclusion

- Evaluating gas-sampling data confirmed that the application of different temperature treatments to soil was successful. Soils exposed to a higher temperature showed faster increases in $^{13}CO_2$ and therefore accelerated glucose mineralization.
- Autoclaved soil samples remained unaltered regarding their ^{13}C fluxes, which indicates that autoclaving was an effective way of sterilizing soil and that there was no extracellular glucose metabolism.
- The fast and high increase in APE of metabolites, like glucose-/fructose-6-phosphate (glycolysis), ribose-/xylulose-5-phosphate (pentose phosphate cycle) and α-ketoglutarate (citric acid cycle), displays that ^{13}C fluxes can be sensitively traced through catabolic and anabolic processes, and thereby through the central metabolic pathways.
- In general, microbial metabolism was most active in the first 2 hours after ^{13}C-Glucose addition, as was anticipated.



We demonstrated that with our method it is possible to work with natural soil microbial communities in a metabolomics and fluxomics framework.

We detected 33 out of 46 targeted metabolites and are thereby able to cover the soil community metabolism on a broad scale.

Merging data derived from gas measurements with metabolomics/fluxomics data allows sensitive tracking of fluxes through major metabolic pathways.

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