Rhizosphere, UE +VO 5 h, 7 ECTS-Points, 2018

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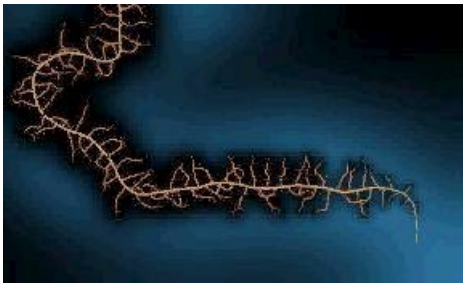

Aims

The practical class aims to provide system ecological knowledge about key organisms and their activities in the densely rooted soil environment - the rhizosphere. During this class, biochemichemical and soil zoological techniques of soil ecology (metabolic profiling, bioactivity monitoring) as well as their statistical evaluation are tought and practically applied.

Investigation

The Rhizosphere is the habitat of the intensively rooted soil system. Mucus (slime) are the main matrix of the biological interactions of this habitat. They cover the all living roots and are most abundant at the root tips. They consist of many different low molecular organic compouns in different stages of polymerisation. Slimes are colloidal matrices binding water and ions as well as enzymaticaly active proteins. We shall characterise different rhizospehres of specific plant physiotypes employing biochemical and electro-chemical measurements and we will determine typical soil organisms.

Modell systems- Plants

Lupine *Lupinus luteus*
(C3 legume, anual, pioneer)

Maize *Zea mais*
(C4-grass, anual)

Sugar cane *Saccharum officinale*
(C4-grass, annual)

Tomato *Solanum lycopersicum*
(C3, allelopathically active, low light resistant)

Collembola

The course will intruduce you into the determination of eu-edaphic Collembola in the rhizosphere and in the bulk soil of the model plants. Apart from the Abundance of the Organisms, the trophic preferences and the ecological functions of the determined taxa will be presented.

Root Exudates

These will be extracted from all four Plant. The Exudate will be separeted in a hydrophilic a lipophilic fraction. The first one comprises sugars, organic acids and amino acids, the latter the so called secondary metabolites. Hydrohilic mucilage slimes are mostly polymerized and need to be hydrolyzed prior to analysis. It is paramount to relate quality and quantity of the exudates to the other biotic and abiotic factors: SPAC (Soil Plant Athmosphere Contnuum)

Soil Respiration

As an expression of aerobic metabolic bioactivity, soil respiration will be measured in all soils by means of infra red gas analysis (IRGA), with and without additional substrates. (basic respiration BR, substrate induced respiration SIR).

BIOLOG

Preadaption of soils according their substrate utilization preference will be shown by means of a modified dehydrogenase activity assay with the addition of low molecular weight substrates well known for their common utilization by soil microorganisms in micro-plates.

Urease/Deaminase

A substantial part of mineralization in soils is facilitated by deaminases. These enzymes cleave ammonium groups from amides.amino acids and urea. Deaminase activity is a parameter for potential microbial soil activity.

pH-Value / Redoxpotential / Electric Conductivity / Voltammetry of Polyphenols

These important abiotic soil ecological factors we are going to measure in the soil water extracts as well as in the root exudates in two ways: actual pH, EC and RP and potential pH after addition of $CaCl_{2.}$. The difference between those two pH values depicts the actual cationic exchange capacity (CEC) also refered to as actual mineral nutrient retention capacity.

Voltammetry

5 ml of Root Extract is mixed with a pH 5.5 Acetate buffer and measured in a Polarograph (Metrohm 774VA) from 0.5V to 1.5V

Programme 2018 (11.06.-22.06.)

	Date	Programme
Mo	11/06/18	Sample preparation
Di	12/06/18	Exudate Fractination
Mi	13/06/18	Exudate Fractination , BIOLOG, Soil Respiration BR (IRGA)
Do	14/06/18	pH, Sample Preparation Chromatograpy, Urease
Fr	15/06/18	Zoology, Extraction of Organisms, C-Analysis
Mo	16/06/18	Soil Respiration SIR, Photosynthesis
Di	17/06/18	Voltammetry, BIOLOG evaluation
Mi	19/06/18	Zoology, Determination of Organisms, Data Processing
Do	20/06/18	Statistics, Data Mining
Fr	***21/06/18***	Result & Protokoll Discussion

Analysis Parameters and Sample Preparation

Analyte/Parameter	Sample	Prep	Analysis
Primary metabolites	Root Exudate	Wurzelwaschung	GC-MS
Secundary metabolite s	Root Exudate	Wurzelwaschung	HPLC-UV
PH, Redox Potential, Conductivity	Root Exudate, Rhizospere Soil	Water Extr., $CaCl_2$	E- Chemistry
BIOLOG	Rhizosphere Soil	Ringer-Extract	Microplate, Photometer
Soil resp (BR,SIR)	Rhizosphhere Soil	Soil FM 2mm	IRGA
Deaminase/Urease	Rhizosphere Soil	Soil FM 2mm	Photometer
C	Rhizosphere Soil	Soil DM 2mm	C-Analyses

Sample Preparation

All plants and soils are analyzed in triplicates (3 for chemistry, 3 for zoology)

Biomass

First, fresh weight (FM) of leaves, roots and stem will be measured. An aliquote of the biomass (5 -10g FM) will be dried at 80°C and weighed (do not forget the tara weight of the paper bags). Also, an aliquote of soil FM (5g) shall be dried in the oven at 100°C in order to estimate the fresh water content. The rest will be sieved (4-5mm) and stored at 4°C for soil respiraton analysis.

Root Exudates Extract for LMWOC Determinaton and Voltammery

According to size the roots of 1-3 Plants (for small plants: all of the together) will be cleaned from soil and the roots be incubated for at least 4h, better over night at 4-8°C in Aqua dest. In a Schott glass flask with the closing lid carefully tightened, with as few of supernatend air as possible. Die Exudates will be stored at 4°C and the remaining soil particles carefully removed with aquarium grade cotton wool. Separation in lipophilic an hydrophilic fractions is done in a separation funnel by means of ethyl- acetate.

Sieving and Storage of Soils

Soil samples are sieved with a mesh size of 5 mm. After sieving please remove remaining root material, store the soil in plastic bags at 4°C. In case of more than 4 weeks of storage prior to analysis, consider freezing, but as general rule, avoid it. In such cases, at least 3 days need to be allowed after thawing to avoid artifacts.

Soil extract for für pH/RP/EC

Approximately 3 g of air dried or naturally moist soil is shaken in falcon- tubes with 15 mL Aqua dest. or 15 mL 0,1 N $CaCl_2$ respectively, shaken vigorously , and allow to sediment for 12 h at 4°C. These extracts are measured directly, but avoid to immerse the electrodes into the soil slurry. Alternatively, root exudate extracts may be measured directly.

RINGER-Extrakt for BIOLOG

Shake 1 g of soil in 10 mL Ringer Solution (2,25 g NaCl, 0,105 g KCl, 0,12 g $CaCl_2$, 0,05 $NaHCO_3$ per L Aqua dest) and centrifuge 1 min at 500 U/min. Alternatively, root exudate extracts may be measured directly.

Chromatographic Analysis of Root Exudate

The root exudate extracts are prepared and fractionated as stated above.

Fractination

In order to analyze root exudates they must be separated into lypohilic and hydriphilic fractions. To that end, the extracts will be concentrated to an end volume of 100 mL and amended with a similar volume of ethyliacetate. To reach a quantitative separation of the phases, a minimum of 30min is required, but typically an extraction over night guarantees best results. The ethyl-acetate phase will subsequently be analyzed by means of liquid UV-HPLC chromatography employing a UV diode array detector. This will provide information about the phenolic compounds in the exudate extract. The hydrophilic phase will typically be analyzed with a GC-MS, a gas chromatography coupled to a mass detector (TOF, quadrupole).

Sample preparation for HPLC-UV:

The complete acetyl acetate phase will be concentrated until dry and transferred to auto-sampler vials. The next step is desiccation on a vacuum conceentrator, weighed and dissolved in an adequate concentration (10 mg mL*1) in a methanol:water:acetic acid (1:1:0,01, v/v/v) solution.

Sample Preparation for GC-MS:

The water phase will also be concentrated on a rotary vacuum conventator (rotavapor) transferred in auto-sampler vials and weighed. Aproximately. 10 mg will be transferred in a second vial, dissolved in in methanol and desiccated. The dried samples are going to be treated witht 50 µl of Hydroxylamin solution for 1 h at 70°C. At room temperature, the hydroxylic groups will be derivatized with trimethylsilylester) employing 50 µL MSTFA (N-Methyl-N-trimethylsilyltrifluoroacetamid) The resulting 100 µL will be transferred to GC vials and analyzed in the GC-MS.

Chemikals

Ethylic Acetate
Ethanol absolute
Hydroxylamine (20 mg/mL in Pyridine)
MSTFA (N-Methyl-N-trimethylsilyltrifluoroacetamid)
30% HCl
30% KOH
Methanol
Acetic Acid
Deionized Water (MilliQ)

BIOLOG

A modified Dehydrogenase reaction employing TTC (Triphenyltetrazoliumchlorid) as a substrate is utilized to test a microbial suspension for its preferances of labile organic substrates. The assay is conducted in micro-plates that already contain TTC and some mineral nutrients.
The ringer-extract of soils or root exudate extract (100 µl) and the C- substrates (60 µl) are combined in the wells of the micro-plate and the color change due to the TTC/TPF (Triphenylformazan) reaction quantified with an UV/VIS photometer.

The wells of the plate need to be covered whenever possible to minimize contamination. Previous to pipetting, a layout must be established in order to allow for documentation and later determination of values measured. Incubate at 25°C i(room temperature) measure immediately and every 12 h ati **542 nm until the maximum stain intensity has developed.**
Matrix controls: 160µL Aqua dest.

Layout der BIOLOG Platten

Platte 1	I	1	2	3	4	5	6	7	8	9	10	11	12
asn	A	Lup1	Lup2	Lup3	Mais1	Mais2	Mais3	Zu1	Zu2	Zu3	Tom1	Tom2	Tom3
ser	B	Lup1	Lup2	Lup3	Mais1	Mais2	Mais3	Zu1	Zu2	Zu3	Tom1	Tom2	Tom3
malat	C	Lup1	Lup2	Lup3	Mais1	Mais2	Mais3	Zu1	Zu2	Zu3	Tom1	Tom2	Tom3
citrat	D	Lup1	Lup2	Lup3	Mais1	Mais2	Mais3	Zu1	Zu2	Zu3	Tom1	Tom2	Tom3
gluc	E	Lup1	Lup2	Lup3	Mais1	Mais2	Mais3	Zu1	Zu2	Zu3	Tom1	Tom2	Tom3
sach	F	Lup1	Lup2	Lup3	Mais1	Mais2	Mais3	Zu1	Zu2	Zu3	Tom1	Tom2	Tom3
galac	G	Lup1	Lup2	Lup3	Mais1	Mais2	Mais3	Zu1	Zu2	Zu3	Tom1	Tom2	Tom3
zimtsre+mal	H	Lup1	Lup2	Lup3	Mais1	Mais2	Mais3	Zu1	Zu2	Zu3	Tom1	Tom2	Tom3

Platte 2	II	1	2	3	4	5	6	7	8	9	10	11	12
asn	A	Lup1	Lup2	Lup3	Mais1	Mais2	Mais3	Zu1	Zu2	Zu3	Tom1	Tom2	Tom3
ser	B	Lup1	Lup2	Lup3	Mais1	Mais2	Mais3	Zu1	Zu2	Zu3	Tom1	Tom2	Tom3
malat	C	Lup1	Lup2	Lup3	Mais1	Mais2	Mais3	Zu1	Zu2	Zu3	Tom1	Tom2	Tom3
citrat	D	Lup1	Lup2	Lup3	Mais1	Mais2	Mais3	Zu1	Zu2	Zu3	Tom1	Tom2	Tom3
gluc	E	Lup1	Lup2	Lup3	Mais1	Mais2	Mais3	Zu1	Zu2	Zu3	Tom1	Tom2	Tom3
sach	F	Lup1	Lup2	Lup3	Mais1	Mais2	Mais3	Zu1	Zu2	Zu3	Tom1	Tom2	Tom3
galac	G	Lup1	Lup2	Lup3	Mais1	Mais2	Mais3	Zu1	Zu2	Zu3	Tom1	Tom2	Tom3
zimtsre+mal	H	Lup1	Lup2	Lup3	Mais1	Mais2	Mais3	Zu1	Zu2	Zu3	Tom1	Tom2	Tom3

Layout der BIOLOG Platten

Platte 1	I	1	2	3	4	5	6	7	8	9	10	11	12
asn	A	Lup1	Lup2	Lup3	Mais1	Mais2	Mais3	Zu1	Zu2	Zu3	Tom1	Tom2	Tom3
ser	B	Lup1	Lup2	Lup3	Mais1	Mais2	Mais3	Zu1	Zu2	Zu3	Tom1	Tom2	Tom3
malat	C	Lup1	Lup2	Lup3	Mais1	Mais2	Mais3	Zu1	Zu2	Zu3	Tom1	Tom2	Tom3
citrat	D	Lup1	Lup2	Lup3	Mais1	Mais2	Mais3	Zu1	Zu2	Zu3	Tom1	Tom2	Tom3
gluc	E	Lup1	Lup2	Lup3	Mais1	Mais2	Mais3	Zu1	Zu2	Zu3	Tom1	Tom2	Tom3
sach	F	Lup1	Lup2	Lup3	Mais1	Mais2	Mais3	Zu1	Zu2	Zu3	Tom1	Tom2	Tom3
galac	G	Lup1	Lup2	Lup3	Mais1	Mais2	Mais3	Zu1	Zu2	Zu3	Tom1	Tom2	Tom3
zimtsre+mal	H	Lup1	Lup2	Lup3	Mais1	Mais2	Mais3	Zu1	Zu2	Zu3	Tom1	Tom2	Tom3

Platte 2	II	1	2	3	4	5	6	7	8	9	10	11	12
asn	A	Lup1	Lup2	Lup3	Mais1	Mais2	Mais3	Zu1	Zu2	Zu3	Tom1	Tom2	Tom3
ser	B	Lup1	Lup2	Lup3	Mais1	Mais2	Mais3	Zu1	Zu2	Zu3	Tom1	Tom2	Tom3
malat	C	Lup1	Lup2	Lup3	Mais1	Mais2	Mais3	Zu1	Zu2	Zu3	Tom1	Tom2	Tom3
citrat	D	Lup1	Lup2	Lup3	Mais1	Mais2	Mais3	Zu1	Zu2	Zu3	Tom1	Tom2	Tom3
gluc	E	Lup1	Lup2	Lup3	Mais1	Mais2	Mais3	Zu1	Zu2	Zu3	Tom1	Tom2	Tom3
sach	F	Lup1	Lup2	Lup3	Mais1	Mais2	Mais3	Zu1	Zu2	Zu3	Tom1	Tom2	Tom3
galac	G	Lup1	Lup2	Lup3	Mais1	Mais2	Mais3	Zu1	Zu2	Zu3	Tom1	Tom2	Tom3
zimtsre+mal	H	Lup1	Lup2	Lup3	Mais1	Mais2	Mais3	Zu1	Zu2	Zu3	Tom1	Tom2	Tom3

Chemicals

Ringer Solution (above)

Substrates (0,5 ww% solutions in Aqua dest.):

Asparagine, Serine, Malate, Citrate, Glucose, Sucrose, Galactose

Infra Red Gas Analysor IRGA -
Soil Respiration (BR und SIR), Plant CO2 Uptake

Heterogenic gases („greenhouse gases“) absorb infrar red radiation as the absorbed energy induces oscillations in all dimensions. The molecules involved are subject to an elevated temperature, and thatin turn induces elevated atmospheric pressure in a closed volume (law of Gay-Lussac). That effect is utilized in the IRGA as a CO_2- free reference gas as the gas samples to be measured are simultaneously subjected to the same amount of IR.

According to quality and amount of gases, part of that IR will be absorbed. The remaining transduced IR is quantified in a detector that is constructed from two adjacent closed CO2 filled compartments separated by a membrane. As a consequence, a pressure difference will ocurr between those two chambers, transferred into potential differences by a pressure transducer and displayed in a Volt Meter labeled with a CO2 concentration graduation.

The CO2 development of soils is measured with such a device (IRGA). The immediate CO2 amount set free by soils may be regarded as the microbial aerobic respiratory soil activity, also referred to as BR (basic soil respiration).

By adding any labile substrate, the measured CO2 development may be substancially higher and is subsequently defined als substrate induced potential aerobic soil respiration (SIR)

The substrates of choice are low molecular weight sugars, amino acids as found in soil solutions.

Procedure

SIR: 30 g of soil (2-5mm sieved, at lest two replicates each) are weighed in small plastic bags and ammended with 0,2 % FM of sugar powder or 0.6% of amino acid powder, mixed well (inflate and shake the plasic bag). The mixture is transferred to a special measuring cuvette after placing a small sheeet of cellulose tissue over the apperture where the gas will be comming in. For BR, no substrate is added.

All cuvettes are continuously purged with a speed of 6L/mih (100mL per min) in an open circuit and measured every 30 minutes . After the CO2 values have reached an asymptotic minimum, these values are taken or the resulting curves compared.

Calculation of Results

Results will be given in mg CO_2/h/100 g Soil Fresh weight utilizing this formula (taking into account the Avogadro-Constant of 24 L Gas per Mol at normal conditions:
Response: SIR–BR in % BR = RESP in % of BR

mg/L / 10e6 * flow *10e3
(c[ppM] / 10e6 * flow[L/h]] *10e3 / weight *10e3/*1,96

$$x = \frac{c * f * 1.000 * 1.000}{1.000.000 * w} * 1,96$$

mL CO2 in der Probe pro Stunde
mg CO2 /kg Boden

$$x = \frac{f * c}{w} * 1,96$$

x : mL CO2 /kg FW * h
f : air flow rate (L / h)
c : CO2 - concentration (mg/L)
w : weight of the soil sample (g)

Deaminase/Urease

Ammonium may be measured in KCl- extracts using a modified Berthelot-Reaktion. This Analysis is based on the reaktion of sodium salicylate mit NH_3 in the presence of Sodiumdichloro- isocyanuric acid. In an alkaline matrix a typical bluegreen stain will occur very reliably if Na- nitropusside is employed as a catalyser.

Procedure

Samples: put 2 g of fresh soil in two 50 ml Erlenmeyer-flöasks eingewogen, moisten with 1mL **Urea** solution (see below) , close with a corc stopperand incubate 2 hours at 37°C in an incubator

Controls: put 2 g of fresh soil in one 50 ml Erlenmeyer-flasks, moisten with 1mL **Aqua dest.** , close with a corc stopperand incubate 2 hours at 37°C in an incubator at the same time as the samples

During Incubation: prepare acidified KCl-Solution (below) and calibration solutions

After 2h: Add 20ml of adicified KCl and shake for 20 minutes, filtrate (only 1mL neded)

Filtration: 50 mL wide neck flasks and N-free folded filterd (no funnels needed)

Color Assay: in 2 mL Eppis:

Calibration: Use a stock solution (3,8207 g NH_4Cl in 1000 mL Aqua dest. (= 1000 mg/L N). Dilute employing the acidified KCl, in order to btain: 5, 10, 15, 20, 25 und 30 mg N/L. 100µl of these separate solution are used for the color reaction. Prepare two seperate calibration sets.

Color reaction : 100 µl Sample or calibration
900 µl Aqua dest.
500 µl mixed solution
200 µl oxidation reagent

The sequence of addition is of essence! Vortex the mixture allow for 30 min of reaction completion. Measure all Samples and controls simultaneously in a microplate at 600nm, 250 µl per well. Use the first two rows for the calibrations, place controls on top and samples below.

Chemikals:

***substrate*:** 2,4 g Urea in 500 mL

***extraction sulution*:** 1L 1N KCL + 10 ml 1N HCL

mixed reagent : 0,3 N NaOH/Natsalicylate/Aqua dest (1:1:1)

Na-Salicylat-Solution: 17 g Na-salicylate and 120 mg Natnitroprussid in 100 mL Aqua dest..

***oxidation reagent**:* 0,1 g Na- Dichlorisocyanuic acid in 100 mL Aqua dest. (dayly fresh!)

Data Evaluation:

$$\frac{(VP- BP)*22*100}{2* \%TS*2h} = x\ \mu g\ N \cdot g\ TS^{-1} \cdot h^{-1}$$

VP	Mittelwert Extinktionen der Vollprobe
LP	Mittelwert Extinktionen der Leerprobe
y = kx +d	Eichgerade
% TS	Trockensubstanzfaktor (TS in % FG)