Root exudates - What to measure?
How to measure!
Root functions

- Plant roots are responsible for water and nutrient uptake and for anchorage the plant in soil.

- Apart from these function plant roots are able to release a wide range of organic and inorganic compounds into the rhizosphere.
A considerable proportion of this carbon (up to 70%) can be released into the rhizosphere.

**RHIZODEPOSITION**
800 - 4500 kg C year\(^{-1}\) ha\(^{-1}\)
Organic Rhizodeposition
800 - 4500 kg C year\(^{-1}\) ha\(^{-1}\)

- **Lysates**: Passive release from damaged and sloughed-off root cells
- **Exudates**: Release from intact root cells
- **Diffusates**: Passive release by diffusion
  - Low molecular weight compounds
  - Phytosiderophores (Fe, Zn-Def.)
  - Carboxylates (P-Def., Al-Tox)
  - Phosphohydrolases (P-def)
- **Secretions**: Controlled release of compounds with specific functions
- **Excretions**: Controlled release of metabolic waste products
  - e.g. Lactic acid (O\(_2\)-def)
The rhizosphere

Soil influenced by the plant is defined as

Rhizosphere

Root exudates modulate the dialogue with soil microbes and are an important factors affecting microbial populations in the rhizosphere.
The fungal community differed significantly in the rhizosphere of potato from the community in the rhizosphere of lettuce.
COLLECTION TECHNIQUES

Whole root system
Collection Techniques with Trap Solutions

**Solution Culture Systems**

- Incubation of root systems in aerated Trap Solutions for a defined time.
  - Tap water or distilled water
  - Nutrient solution of the same composition as the culture media
  - Solutions of 0.5 – 2.5 mM CaSO$_4$ or CaCl$_2$ to provide Ca$^{2+}$ for membrane stabilization

- Collection of water soluble root exudates
  - The technique allow kinetic studies by repeated measurements using the same plants.
  - The technique gives a first impression about qualitative exudation patterns and even quantitative changes in response to different culture-conditions.

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Collection Techniques with Trap Solutions

- **Solution culture systems (non-sterile)**

  - Short incubation times (≤2 h) can minimize microbial degradation
  - **Concentration** (Speed-Vac, Lyophilisation) and/or solid phase extraction
    anion-exchange resins (carboxylates) or RP-18 phases (lipophilic compounds, phenolics)
  - Resolubilization ⇒ Analysis

  ![Graph showing temporal variation of citrate exudation in Lupinus albus](image)

  **Application**
  Temporal variation of citrate exudation in *Lupinus albus*

  *Neumann et al. 1999 Planta 208, 373*
Collection Techniques with Trap Solutions

- Solution Culture Systems -

**Advantages**
- Non-destructive
- Repeated measurements
- Applicable for sterile culture
- Whole root system integration

**Disadvantages**
- No spatial resolution
- Interpretation of data should be restricted to plants grown in solution culture
- Dilution of the exudates requires concentration and purification steps
- Quantitative recovery only for easily water soluble compounds

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v. Wirén et al. 1995, New Phytol 130:511
Wang et al. 2015, Physiol. Plant. 154:407
Collection Techniques with Trap Solutions

- **Solid Substrate Culture Systems** -

- Plants grown in solid substrates
  - sand
  - Vermiculite
  - Soil

- Percolation of culture vessels with trap solution for a defined time period (after removal of rhizosphere products by repeated washings with H₂O)

- Subsequent concentration (Speed-Vac, Lyophilisation) and/or solid phase extraction (e.g. on anion-exchange resins for carboxylates or RP-18 phases for lipophilic compounds and phenolics)

- Resolubilization ⇒ Analysis

*Neumann and Römheld 2007 in: The Rhizosphere, Eds. Pinton R et al. CRC Press*
**Collection Techniques with Trap Solutions**

- **Solid Substrate Culture systems** -

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**Application:** Collection of dioxin-mobilising compounds in root exudates of Zucchini

- Neumann et al. 1999 *Organohalogen Comp.* 41:331
- Johnson et al. 1996 *Plant Physiol.* 112:19
- Tang & Young 1982 *Plant Physiol.* 69:155

- **Advantages**
  - Non-destructive
  - Repeated measurements

- **Disadvantages:**
  - Limited recovery (adsorption effects)
  - No spatial resolution

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Extraction of dioxins by root exudates of tomato and Zucchini from contaminated soil

![Graph showing extraction of dioxins by root exudates of tomato and Zucchini from contaminated soil.](image-url)
COLLECTION TECHNIQUES
Localized Sampling
Spatial variation of root exudation needs to be considered to understand root exudate effects in the rhizosphere.

Neumann and Römheld 2002
In: Plant roots – The hidden half
Eds. Waisel et al. Marcel Dekker NY
Compartment systems to characterize radial rhizosphere gradients

- Compartment Rhizobox System -
  (vertical separation)

- Plants grown in compartment culture vessels (roots separated by nylon nets from attached soil compartments)
- Formation of a root mat along the net
- Removal of soil compartments
- Freezing and (microtome) slicing of soil compartments (increasing the distance from the root mat)
- Extraction of soil fractions

Gahoonia & Nielsen, 1992 Plant Soil 135:143
Kuchenbuch & Jungk 1982 Plant Soil 68:391
Compartment systems to characterize radial rhizosphere gradients

- **Compartment Pot System** – (horizontal separation)

**Advantages**
- Non-destructive
- Repeated measurements
- Spatial resolution (radial)

**Disadvantages**
- Limited recovery (adsorption effects)
- Co-extraction of damaged microbial cells
- Overestimation of rhizosphere effects (unrealistic root densities in root mats)
- No spatial resolution along single roots

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*Kuchenbuch and Jungk 1982 Plant Soil 68: 391*
Sampling techniques to characterize longitudinal gradients of root exudation

- Solution Culture Systems -

Different types of sorption media applied to the root surface

- Short collection periods (2.4 h) and matrix adsorption can minimize microbial degradation of exudate compounds.

Localized Sampling Techniques

- Solution Culture Systems -

Analysis of isoflavonoids in root exudates of soybean seedlings by Capillary Electrophoresis
- Spatial variation along the seedling root
- Collection with cellulose acetate filters

Kape et al. 1992 J.Plant Physiol. 141:54
Localized Sampling Techniques – Solution Culture Systems

Filter disks covering root tips

2 cm

Moist fleece layer

Extraction

Zentrifugation

HPLC-Analysis

Localized Sampling Techniques – Soil Culture Systems

Adantages
• Non-destructive
• Repeated measurements
• Spatial resolution
• applicable for rhizobox and field studies (root windows)

Disadvantages
• Adsorption effects
• Two-dimensional system
• Not applicable for fine roots
• Complex sample processing for ion-exchange membranes or agar sheets and limited purity of the absorption materials
• Small sample volumes
• No whole root system integration

Kamh et al. 1999 Plant Soi 211: 19
Göttlein et al. 1996 Geoderma 69: 147

**Applications:**
GC-MS profiling of root exudates collected from 2 cm-apical root zones of *Lactuca sativa* on three different soils.

<table>
<thead>
<tr>
<th>Chemical group</th>
<th>Compound</th>
<th>Loess Loam</th>
<th>Alluvial Loam</th>
<th>Dilluvial Sand</th>
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<tbody>
<tr>
<td>Amino acids and amines</td>
<td>Alanine</td>
<td>+</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td>beta-Alanine</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Aspartate</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Glutamate</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Glutamine</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Glycine</td>
<td>+++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td>Leucine</td>
<td>++</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Isoleucine</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<td>Proline</td>
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<td>+</td>
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<td>4-Hydroxyproline</td>
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<td></td>
<td>Pyroglutamate</td>
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<td>+</td>
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<tr>
<td></td>
<td>Serine</td>
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<td>++</td>
<td>+</td>
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<tr>
<td></td>
<td>Threonine</td>
<td>++</td>
<td>+</td>
<td>+</td>
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<tr>
<td></td>
<td>Valine</td>
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<tr>
<td></td>
<td>beta-Aminobutyric acid</td>
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<td>-</td>
<td>+</td>
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<td></td>
<td>4-Aminobutyric acid</td>
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<td>+</td>
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<td>Putrescine</td>
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<td>Sugars and sugar alcohols</td>
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<td>Fructose</td>
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<td>Mannose</td>
<td>+</td>
<td>-</td>
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</tr>
<tr>
<td></td>
<td>Maltose</td>
<td>+++</td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
<td></td>
<td>Trehalose</td>
<td>+++</td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
<td></td>
<td>Sucrose</td>
<td>+++</td>
<td>++</td>
<td>+</td>
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<tr>
<td></td>
<td>Glycerol</td>
<td>+++</td>
<td>+++</td>
<td>+</td>
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<tr>
<td></td>
<td>Inositol</td>
<td>+++</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Organic acids</td>
<td>Malate</td>
<td>+</td>
<td>+</td>
<td>-</td>
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<tr>
<td></td>
<td>Fumarate</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Succinate</td>
<td>++</td>
<td>+++</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td>Lauric acid</td>
<td>++</td>
<td>+</td>
<td>+</td>
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<tr>
<td></td>
<td>Benzoic acid</td>
<td>++</td>
<td>+</td>
<td>++</td>
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<tr>
<td>Others</td>
<td>Urea</td>
<td>+++</td>
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<tr>
<td></td>
<td>Phosphate</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Ornithine</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

Differences in profiles of exudate samples (rhizosphere soil solution) are rather quantitative than qualitative.

*Neumann et al. 2014 Front Microbiol.5: 2*
Localized Sampling Techniques – Soil Culture Systems

**Applications:**
Variation of citrate exudation during cluster root development of P-deficient white lupin as related to changes in microbial community structures (DGGE).

*Marschner et al. 2002 Plant Soil 246:167-174*
Localized Sampling Techniques – Soil Culture Systems

Applications: Detection of enzyme activities in the rhizosphere

Advantages
- Non-destructive
- Repeated measurements
- Applicable for soil and solution culture systems
- Spatial resolution

Disadvantages:
- Depending on availability of specific indicator reagents
- „Tricky“ adjustment of reaction conditions to ensure substrate saturation

Dinkelaker and Marschner 1992 Plant Soil 144: 199
Grierson et al. 2000 Plant Soil 218: 49
Localized Sampling Techniques – Up-scaling to field conditions

Root-Windows

Neumann et al. 2009
Plant Soil 321: 431
How to distinguish between root exudates and other rhizosphere products?

Use of Sterile culture systems

**But**: selective effects of microbial colonization on quality and quantity of rhizodeposits!

Inoculation with various microorganisms stimulates rhizodeposition in *Lolium perenne* after shoot labelling with $^{14}$CO$_2$


<table>
<thead>
<tr>
<th>inoculated Microorganisms</th>
<th>$^{14}$C [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Root exudates</td>
</tr>
<tr>
<td>Non- inoculated</td>
<td>1.0</td>
</tr>
<tr>
<td><em>Aspergillus niger</em></td>
<td>3.4</td>
</tr>
<tr>
<td><em>Penicillium rubium</em></td>
<td>9.5</td>
</tr>
<tr>
<td><em>Fusarium oxysporium</em></td>
<td>25.3</td>
</tr>
<tr>
<td><em>Penicillium notatum</em></td>
<td>33.8</td>
</tr>
</tbody>
</table>
How to distinguish between root exudates and other rhizosphere products?

Two step collection techniques:

**Step 1:** Collection of root exudates + other rhizosphere products (sample discarded)

**Step 2:** Short term collection of root exudates released during 2 h with minimized influence of microbial degradation

Estimated half life times of low molecular weight compounds in the rhizosphere soil solution: approx 3-6 h

Further stabilisation possible by adsorption to sorption media
How to distinguish between root exudates and other rhizosphere products?


Localized collection of $^{14}$C-labeled root exudates with sorption filters in 1 cm - apical root zones after $^{14}$CO$_2$ shoot pulse-labelling of bean depending on N supply and atmospheric CO$_2$ concentration.

Factors affecting recovery of root exudates

- **Microbial degradation**
  Minimized by:
  - sterile culture,
  - short-term collection (1-2 h),
  - use of antibiotics (testing for toxic effects)
  - sorption materials for exudate collection (immobilization)

- **Sorption effects** (metal chelating carboxylates, proteins, lipophilic compounds)
  - Recovery experiments with synthetic standards required

- **Retrieval mechanisms** (for amino acids, peptides, sugars)
  - Evaluation by up-take studies

- **Stress factors:** (mechanical impedance, root injury, nutritional stress, toxic compounds, physical stress)

- **Intra- and interspecific variability**
Concluding Remarks

- No general approaches for root exudate collection available
- Different methods for different scales frequently excluding each other (e.g., localised vs whole-root sampling)
- Detailed knowledge of methodological limitations essential for interpretation of results and design of control experiments
- Coming closer to natural growth conditions is associated with a reduced number of available methods
Thanks for your attention