Metaproteomics to investigate the impact of sampling-site biogeochemistry on structure and functionality of leaf-litter degrading microbial communities

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Jessica Toller

Alexander Grunau
**Global Warming & Microbial Biofilms in Rivers**

Which effects have drying and re-wetting on structure and function of microbial biofilms in European rivers?

Future cooperation with universities in Vienna (T. Battin), Rome (S. Fazio) and Giessen (J. Marxsen)

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**Microbial Symbiosis in Lichens**

How are lichens protected against desiccation, UV-light, and coldness? What is the molecular basis of the symbiosis?

Cooperation with the TU Graz (G. Berg) and the University of Graz (M. Grube)
OTHER RESEARCH INTERESTS

PATHOGENS

Pseudomonas aeruginosa
Staphylococcus aureus

in presence / absence of drug candidates

INFECTION MODELS

Caenorhabditis elegans
Murine model

COMPARATIVE QUANTITATIVE PROTEOME ANALYSES

- of infected hosts in the absence and presence of drug candidates

- global changes in protein expression in both the host and the pathogen in response to the drug treatment

„METAPROTEOMICS“

- Advantage: genomic sequence of hosts and pathogens is available.
MOTIVATION & HYPOTHESES

• Yearly litter production:
  \[ \sim 10^{11} \text{ t (Drymass)} \]
  Large pool of organic carbon, nitrogen
  & phosphorous

• Global mean steady state turnover time:
  \[ \sim 1.4 \text{ to } 3.4 \text{ years} \]

HYPOTHESES

⇒ in most aerobic habitats fungi are the main degraders of litter
⇒ in anaerobic habitats or in environmental niches with increased temperature, bacteria contribute to litter decomposition
⇒ community structure and function is influenced by leaf-litter biogeochemistry
GOALS & EXPERIMENTAL DESIGN

LEAF LITTER FROM DIFFERENT SAMPLING SITES
(February & May)

Protein separation and identification
1D-PAGE LC-MS/MS Orbitrap

Affiliation of proteins to different phylogenetic & functional groups
- Bacteria
- Fungi
- Protozoa
- Plants
- Insects
- Animals

Contribution of metabolically active organisms to the respective habitat
# LITTER BIOGEOCHEMISTRY (FEBRUARY)

![Map of Austria with Schottenwald, Achenkirch, and Klausen-Leopoldsdorf locations](image)

Sophie Zechmeister-Boltenstern
Katharina Keiblinger
BFW - Bundesamt für Wald, Vienna

<table>
<thead>
<tr>
<th></th>
<th>Ort</th>
<th>Achenkirch</th>
<th>Klausen-Leopoldsdorf</th>
<th>Schottenwald</th>
<th>Schottenwald</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water content [%]</td>
<td>57</td>
<td>73</td>
<td>79</td>
<td>77</td>
<td>68</td>
</tr>
<tr>
<td>C [g/kg]</td>
<td>472</td>
<td>502</td>
<td>468</td>
<td>455</td>
<td>470</td>
</tr>
<tr>
<td>N [g/kg]</td>
<td>8.2</td>
<td>12.4</td>
<td>10.0</td>
<td>12.5</td>
<td>10.0</td>
</tr>
<tr>
<td>C:N Ratio</td>
<td>57.8</td>
<td>40.3</td>
<td>46.8</td>
<td>36.4</td>
<td>47.0</td>
</tr>
<tr>
<td>P [g/kg]</td>
<td>0.35</td>
<td>0.03</td>
<td>0.04</td>
<td>0.64</td>
<td>0.22</td>
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<tr>
<td>C:P Ratio</td>
<td>1361</td>
<td>1439</td>
<td>1160</td>
<td>714</td>
<td>2175</td>
</tr>
<tr>
<td>Mn [ppm]</td>
<td>1041</td>
<td>88</td>
<td>832</td>
<td>1493</td>
<td>1306</td>
</tr>
</tbody>
</table>

- Beech litter
- Oak litter
1D-SDS-PAGE-LC-MS/MS

- Protein extraction (SDS-cont. buffer)
- Sonication / Centrifugation
- Concentration (Speedvac)

Prefractionation by 1-D PAGE

- Assignment of proteins to functional & phylogenetic groups
- Semi-quantitative analysis by spectral counting

MS and MS/MS data

Trypsin Digestion

Peptide mixture

Peptide Separation & Analysis
LC-MS/MS
(MS LTQ OrbiTrap)

Database: UniRef100

in total assignment of about ~ 2000 proteins/protein clusters

Leaf litter from different sampling sites and seasons
PROTEIN ASSIGNMENT & QUANTIFICATION

MS & MS/MS data

Database search (Mascot, X-tandem)
Data filtering and sorting (Scaffold: 95% peptide probability, 1 unique peptide)

Clusters of proteins identified by the same set of peptides

Quality control: cluster homology (> 50% homology)

Validated clusters of proteins identified by the same set of peptides

Data analysis pipeline using PERL based scripts

Assignment to phylogenetic groups according to UniPROT NEWT taxonomy
Quality control - cluster consistency

Validated clusters assigned to a certain phylogenetic group

Assignment to functional groups according to KEGG, KOG, COG, and SWISS PROT
Quality control - cluster consistency

Validated clusters assigned to a certain functional group

Quantification by counting of unique spectra assigned to taxonomic or functional groups
DRAWBACKS & CHALLENGES

- number of acquired MS/MS-spectra: \( \sim 300,000 \)
- number of proteins: \( \sim 1200 \)

\[\text{Percentage of assigned MS-MS spectra}\]

\[\text{Not-assigned} \quad \text{Assigned}\]

\[\text{Ort Beech} \quad \text{Schottenwald Beech} \quad \text{Schottenwald Oak}\]

\[\text{Sampling site}\]

\[\Rightarrow 90\% \text{ of the spectra do not match to any protein in the reference database}\]
\[\Rightarrow \text{database has to be improved (metagenomics & metatranscriptomics sequence data)}\]
COMMUNITY STRUCTURE - OVERVIEW

February

- Metazoa
- Viridiplantae
- Fungi
- Bacteria
- Archaea
- Viruses

May

- Schottenwald oak (422/1347)
- Schottenwald beech (649/1609)
- Klausen Leopoldsdorf beech (591/1375)
- Ort beech (673/1839)
- Achenkirch beech (955/2292)

Quantification is based on the numbers of unique spectra
COMMUNITY STRUCTURE - FUNGI

February
- Sordariomycetes
- Schizosaccharomycetes
- Saccharomycetes
- Pneumocystidomycetes
- Pezizomycetes
- Leotiomycetes
- Lecanoromycetes
- Eurotiomycetes
- Dothideomycetes

May

Relative abundance [%]

Ascomycota

Quantification is based on the numbers of unique spectra

- Schottenwald oak (50/524)
- Schottenwald beech (228/713)
- Klausen Leopoldsdorf beech (121/512)
- Ort beech (226/801)
- Achenkirch beech (259/505)
COMMUNITY STRUCTURE - BACTERIA

February

- Dictyoglomi
- Acidobacteria
- Verrucomicrobia
- Fusobacteria
- Firmicutes
- Cyanobacteria
- Actinobacteria
- Chlorobi
- Bacteroidetes
- Spirochaetes
- Chlamydiae
- Planctomycetes
- Proteobacteria
- Chloroflexi
- Nitrospirae
- Thermotogae

May

- Schottenwald oak (18/123)
- Schottenwald beech (74/356)
- Klausen Leopoldsdorf beech (42/262)
- Ort beech (33/213)
- Achenkirch beech (55/192)

Quantification is based on the numbers of unique spectra
COMMUNITY STRUCTURE - BACTERIA

February

δ-proteobacteria
γ-proteobacteria
β-proteobacteria
α-proteobacteria

Relative abundance [%]

Schottenwald oak (12/102)
Schottenwald beech (65/322)
Klausen Leopoldsdorf beech (33/223)
Ort beech (28/179)
Achenkirch beech (42/166)

May

Quantification is based on the numbers of unique spectra
COMMUNITY FUNCTION - FUNGI

KOG classification

Function unknown
General function prediction only
Secondary metabolites biosynthesis
Inorganic ion transport and metabolism
Lipid transport and metabolism
Coenzyme transport and metabolism
Nucleotide transport and metabolism
Amino acid transport and metabolism
Carbohydrate transport and metabolism
Energy production and conversion
PTM, protein turnover, chaperones
Intracellular trafficking
Cytoskeleton
Cell wall/membrane/envelope
Signal transduction mechanisms
Defense mechanisms
Chromatin structure and dynamics
Replication, recombination and repair
Transcription
RNA processing and modification
Translation

February

May

Relative abundance [%]

Schottenwald oak (52/531)
Schottenwald beech (173/708)
Klausen Leopoldsdorf beech (108/483)
Ort beech (175/859)
Achenkirch beech (218/518)

Quantification is based on the numbers of unique spectra
COMMUNITY FUNCTION – FUNGAL EXOENZYMES

February

Lipases
Proteases
Ligninases
Pectinases
Xylanases
Phosphatases
Exoglucanases
Endoglucanases

May

Number of unique spectra

Schottenwald oak
Schottenwald beech
Klausen Leopoldsdorf beech
Ort beech
Achenkirch beech

Hydrolytic enzymes are only of fungal origin

Quantification is based on the numbers of unique spectra
# Impact of Litter Biogeochemistry

<table>
<thead>
<tr>
<th>Sampling site</th>
<th>Litter type</th>
<th>N</th>
<th>P</th>
<th>Mn</th>
<th>Plant Proteins</th>
<th>Fungal Proteins</th>
<th>Bacteria Proteins</th>
<th>Fungal Exoenzymes</th>
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</thead>
<tbody>
<tr>
<td>Achenkirch</td>
<td>Feb</td>
<td>+++</td>
<td>+</td>
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<td>++</td>
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<td>Beech</td>
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<td>Klausen-Ld.</td>
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</table>

+, ++, and +++ refer to relative abundances between different sampling & litter types.
DATA VALIDATION

ENZYMATIC ACTIVITY MEASUREMENTS

<table>
<thead>
<tr>
<th>Sampling Site</th>
<th>Cellulase (Units / ml)</th>
<th>Protease (Units / ml)</th>
<th>Xylanase (Units / ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Feb</td>
<td>May</td>
<td>Feb</td>
</tr>
<tr>
<td>Achenk. Beech</td>
<td>0.007</td>
<td>0.01</td>
<td>0.12</td>
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<tr>
<td>Klau.-L. Beech</td>
<td>0.008</td>
<td>0.01</td>
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<tr>
<td>Ort Beech</td>
<td>0.013</td>
<td>0.018</td>
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<tr>
<td>Sch.W. Beech</td>
<td>0.009</td>
<td>0.016</td>
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<tr>
<td>Sch.W. Oak</td>
<td>0.007</td>
<td>0.012</td>
<td>0.04</td>
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</tbody>
</table>

△ increase in protease-, cellulase-and xylanase-activities from Feb to May confirm semi-quantitative proteome data

PHOSPHOLIPID FATTY ACIDS (PLFA) ANALYSES

△ increase of the fungal community from Feb to May at Ort and Schottenwald (high in Mn and P) corresponds well to the semi-quantitative proteome analysis

△ no temporal changes in the community at Achenkirch and even a decrease in the fungal/bacterial ratio at Klausen-Leopoldsdorf (low in P and Mn); similar trends were reflected in our unique spectral counts

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Katharina Keibling
BFW, Vienna
CONCLUSIONS – COMMUNITY STRUCTURE

- generally, the same taxonomic groups were found at all sampling sites & litter types
- the dominant taxa were plants, fungi, and bacteria
- while plant proteins decreased, fungal and bacterial proteins increased from Feb to May at sampling sites with high P and Mn
- a lower number of microbial spectral counts were observed in samples with low P and Mn concentration or of structurally inert leafs (oak); especially bacterial growth seems to be limited by phosphorus
- while the composition of the fungal community appeared unchanged between Feb and May, the proteobacterial community seemed to shift from δ- and γ- towards α- and β-proteobacteria
CONCLUSIONS – COMMUNITY FUNCTION

- under aerobic conditions fungi seem to be the main degraders of beech litter
- bacteria are present, but seem not to degrade the litter (cheaters); they might play a more important role in anaerobic microniches or at later time points of the degradation process
- less litter degradation enzymes were expressed when either P or Mn were limited (and thus fungal growth was limited)
- more fungal phosphatases were expressed at lower P concentrations

The presence of a certain taxonomic group does not necessarily mean that it participates in a particular functional process, i.e. litter decomposition