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

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MICROBIAL PROTEOMICS GROUP ZURICH

FNSNF
Schweizerischer Nationalfonds zur Förderung der wissenschaftlichen Forschung

FWF
Der Wissenschaftsfonds.

FGCZ Functional Genomics Center Zurich
uni | eth | zürich

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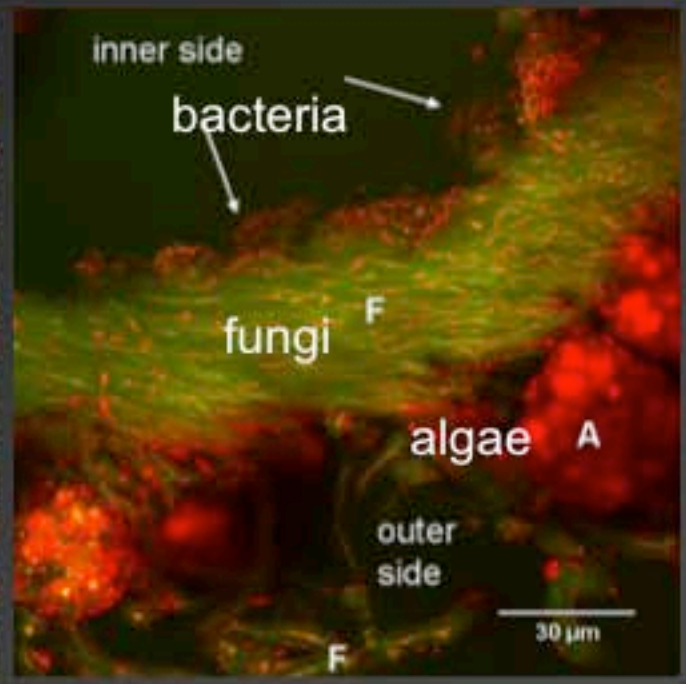
OTHER RESEARCH INTERESTS

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)

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)

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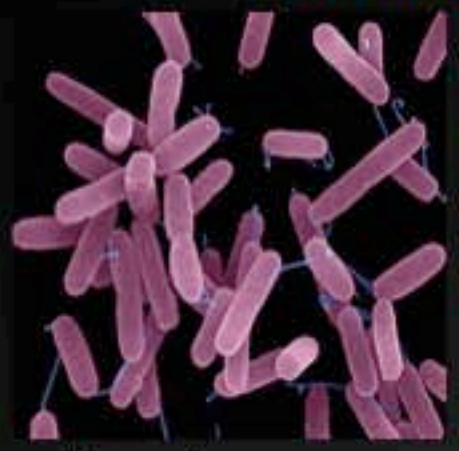
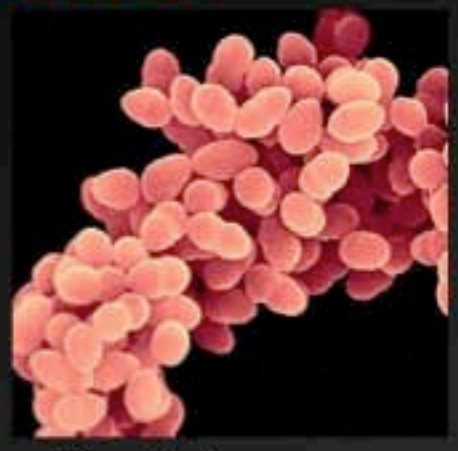
OTHER RESEARCH INTERESTS

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

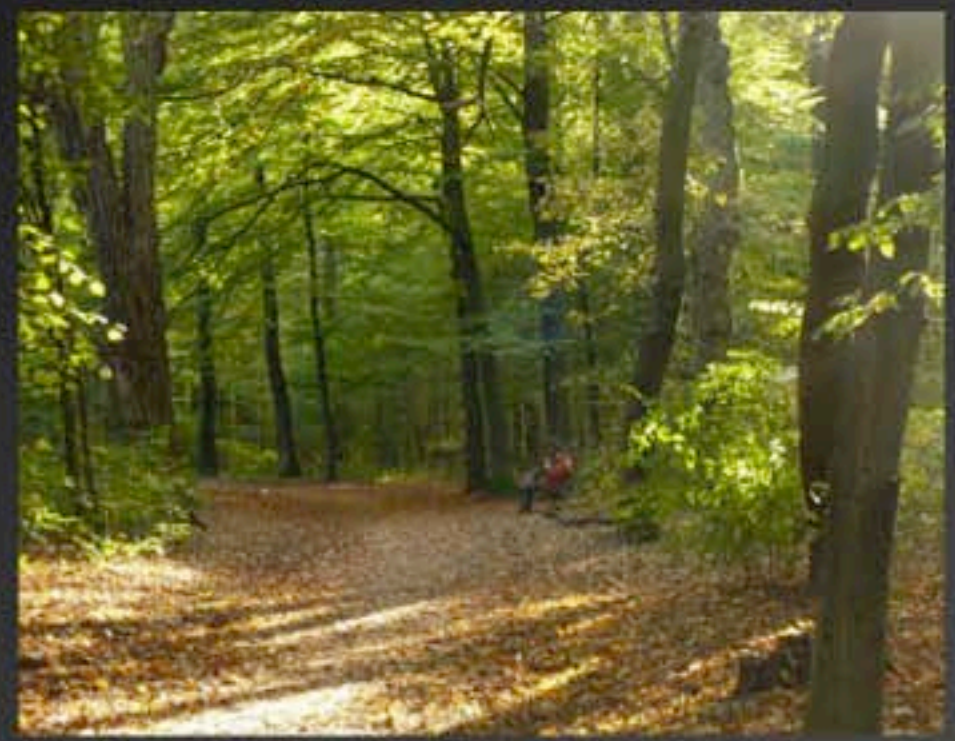
- **Yearly litter production:**

  ~ 10^{11} t (Drymass)

  Large pool of organic carbon, nitrogen & phosphorous

- **Global mean steady state turnover time:**

  ~ 1.4 to 3.4 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

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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

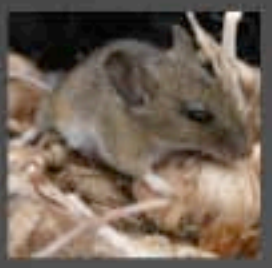

Bacteria


Fungi


Protozoa


Plants


Insects


Animals

Contribution of metabolically active organisms to the respective habitat

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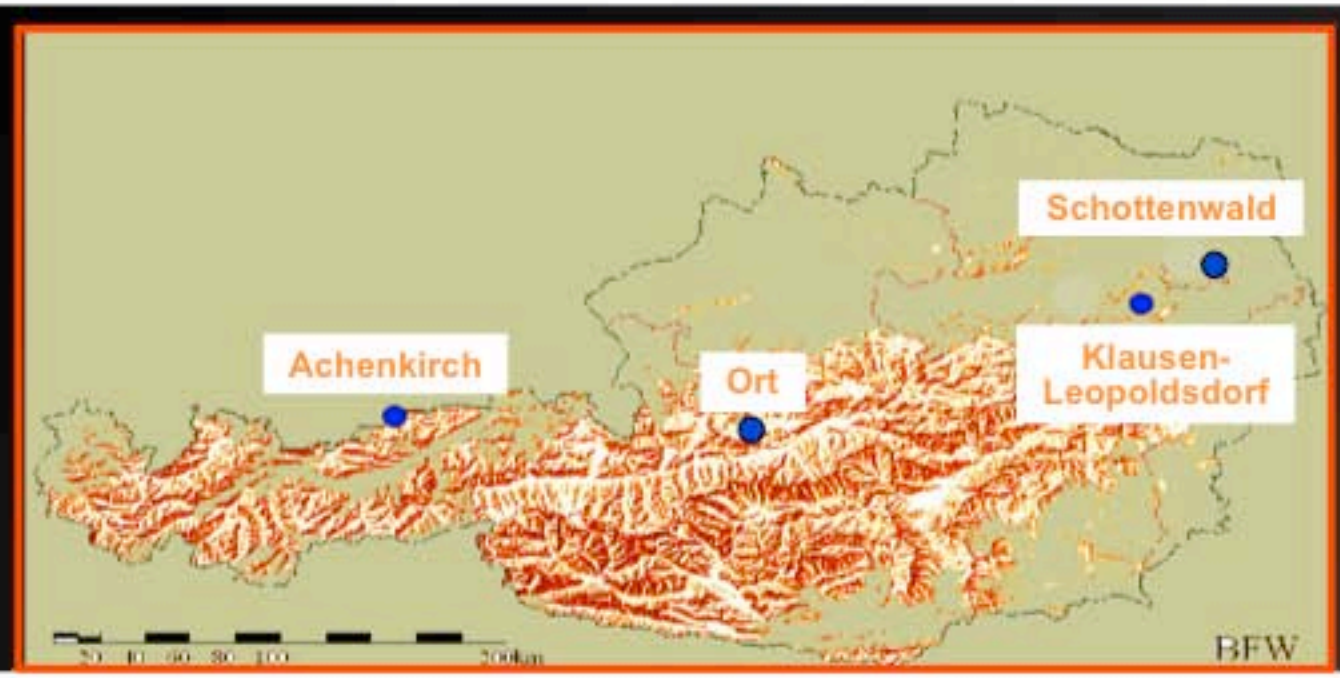
LITTER BIOGEOCHEMISTRY (FEBRUARY)



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

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

Beech litter

Oak litter

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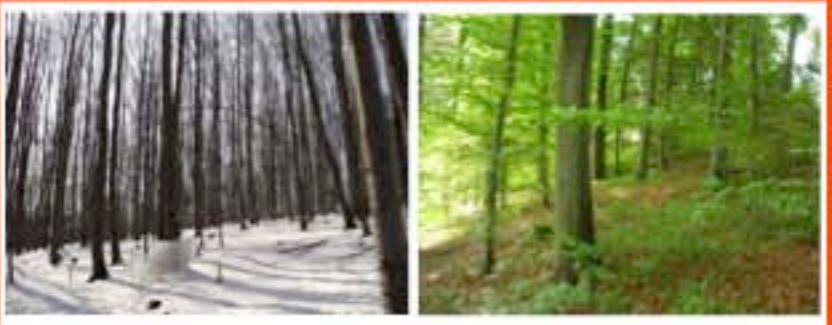
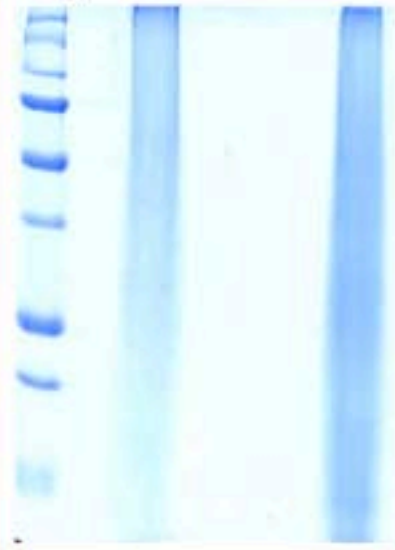
1D-SDS-PAGE-LC-MS/MS



Leaf litter from different sampling sites and seasons

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

Prefractionation by 1-D PAGE



Trypsin Digestion

Peptide mixture

Peptide Separation & Analysis

LC-MS/MS

(MS LTQ OrbiTrap)

MS and MS/MS data

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

Database: UniRef100

in total assignment of about
~ 2000 proteins/protein clusters

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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

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

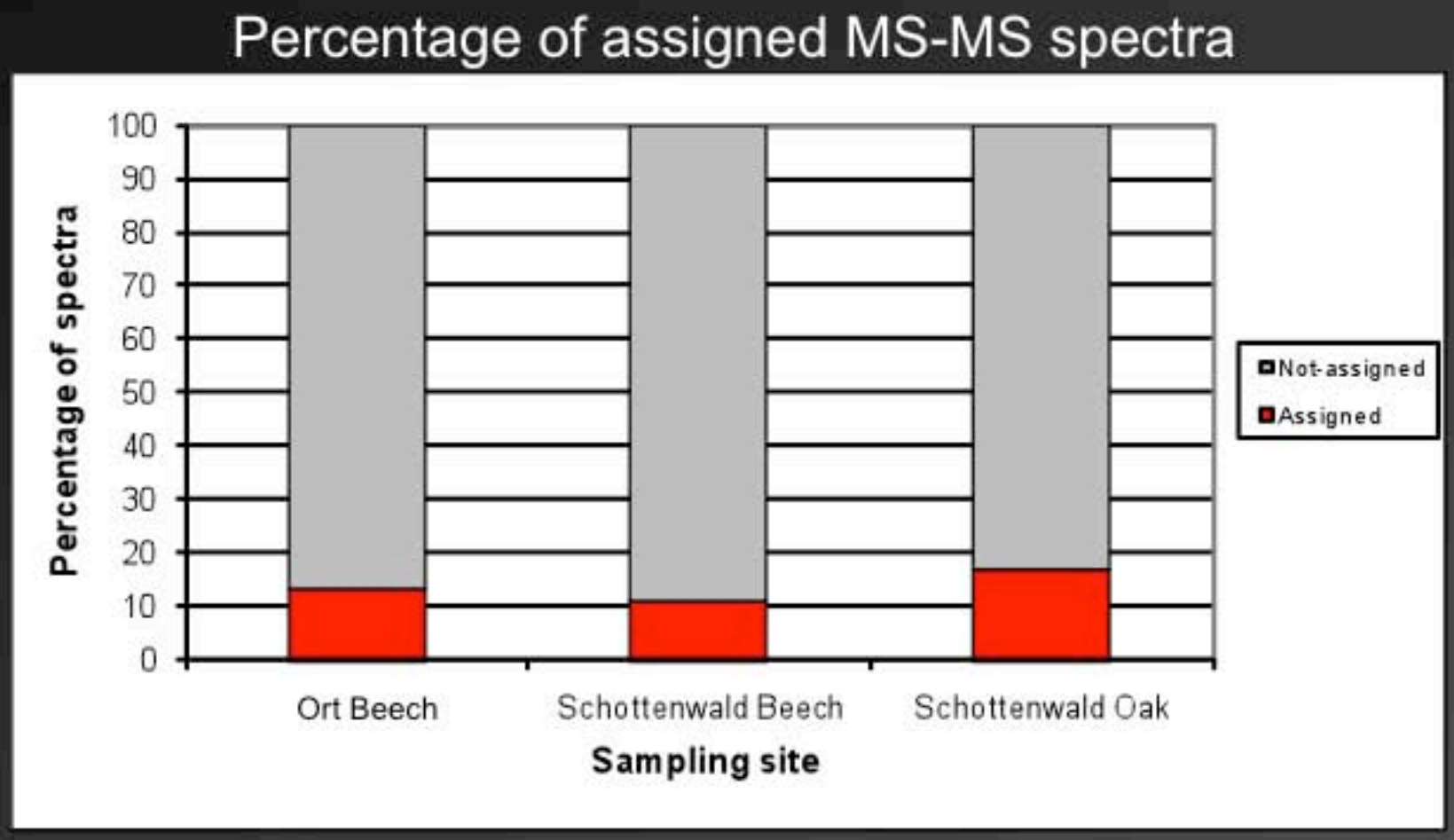
Quantification by counting of unique spectra assigned to taxonomic or functional groups

Emanuel Schmid

Data analysis pipeline using PERL based scripts

DRAWBACKS & CHALLENGES

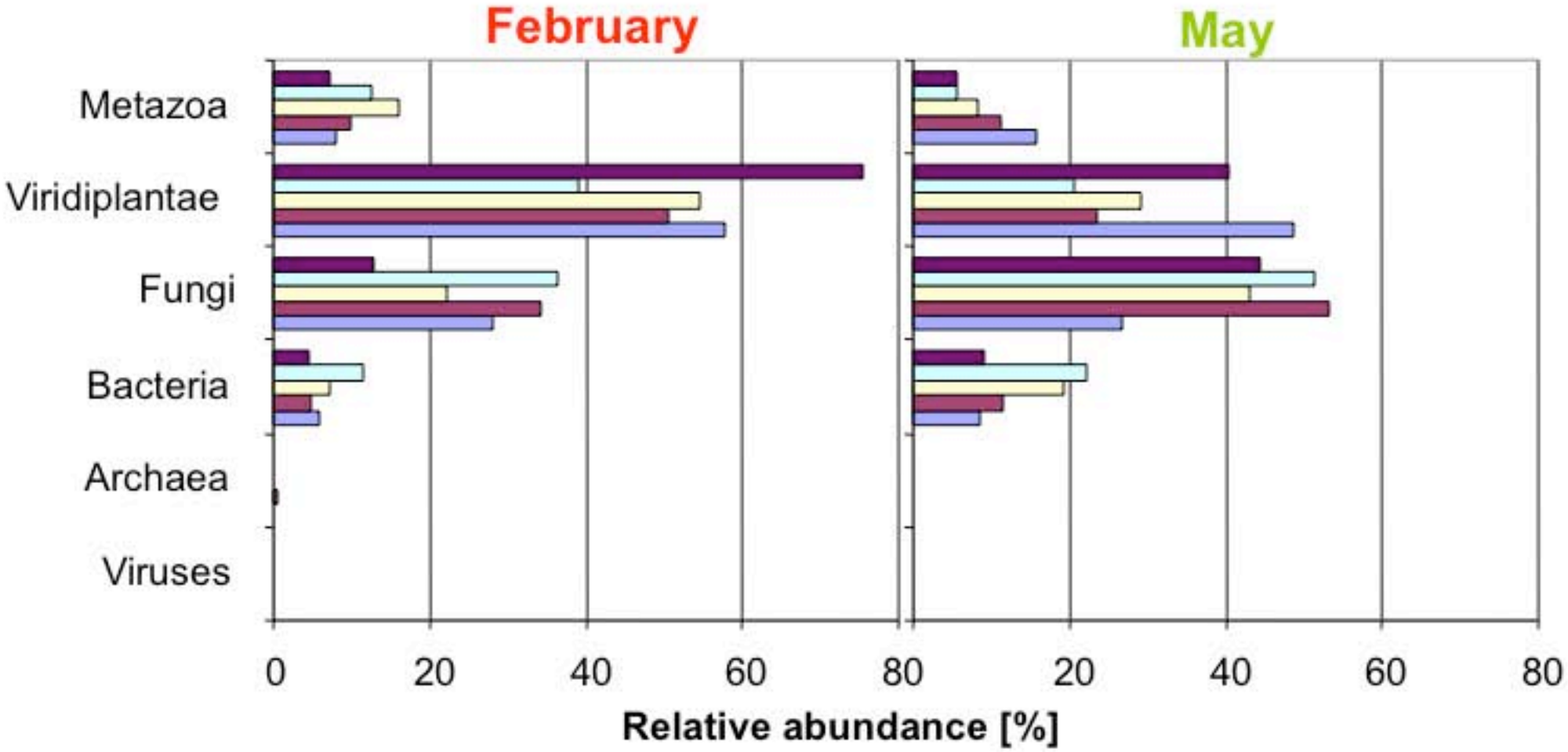
- number of acquired MS/MS-spectra: ~300,000
- number of proteins: ~1200



⇨ 90% of the spectra do not match to any protein in the reference database

⇨ database has to be improved (metagenomics & metatranscriptomics sequence data)

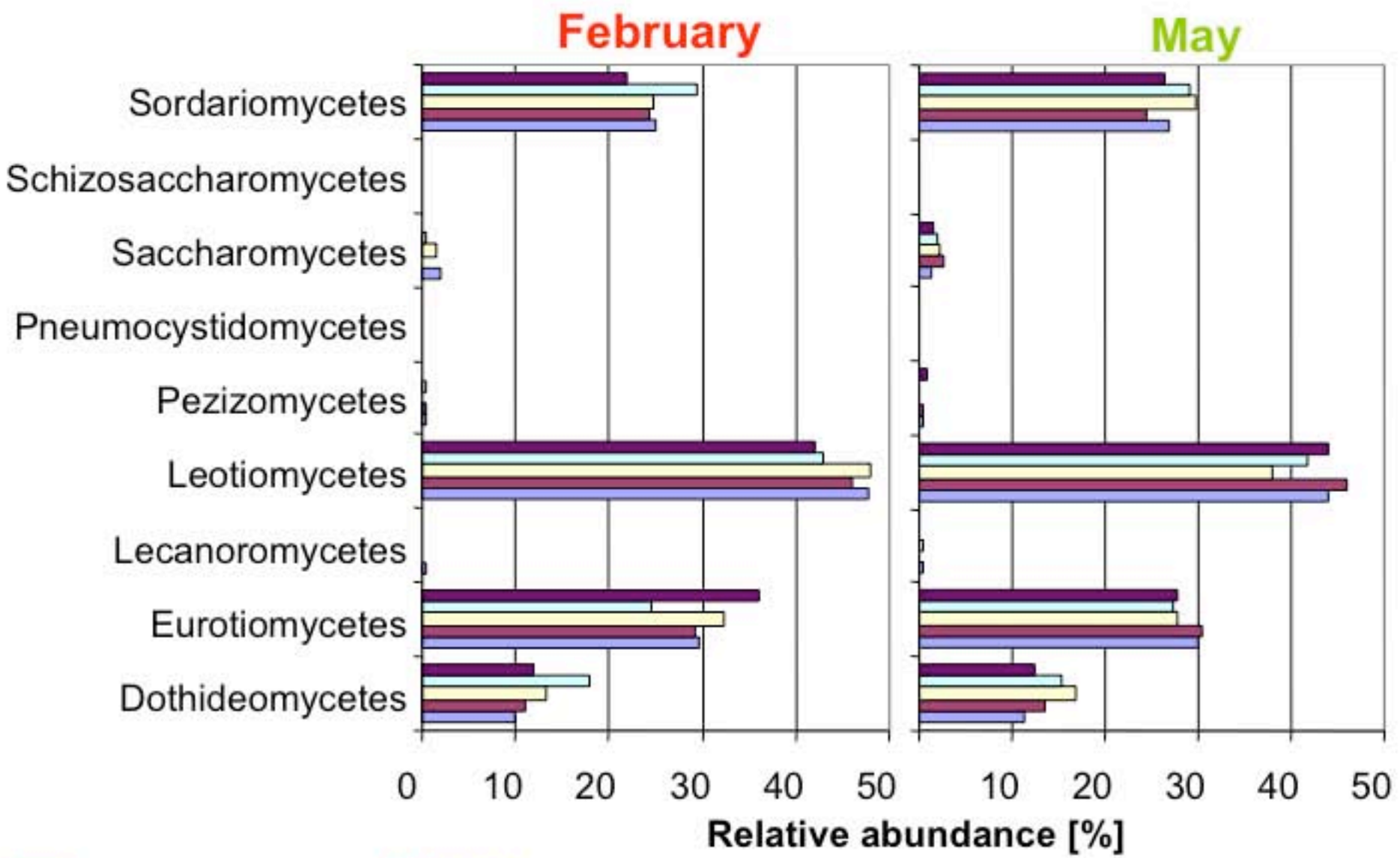
COMMUNITY STRUCTURE - OVERVIEW



- 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

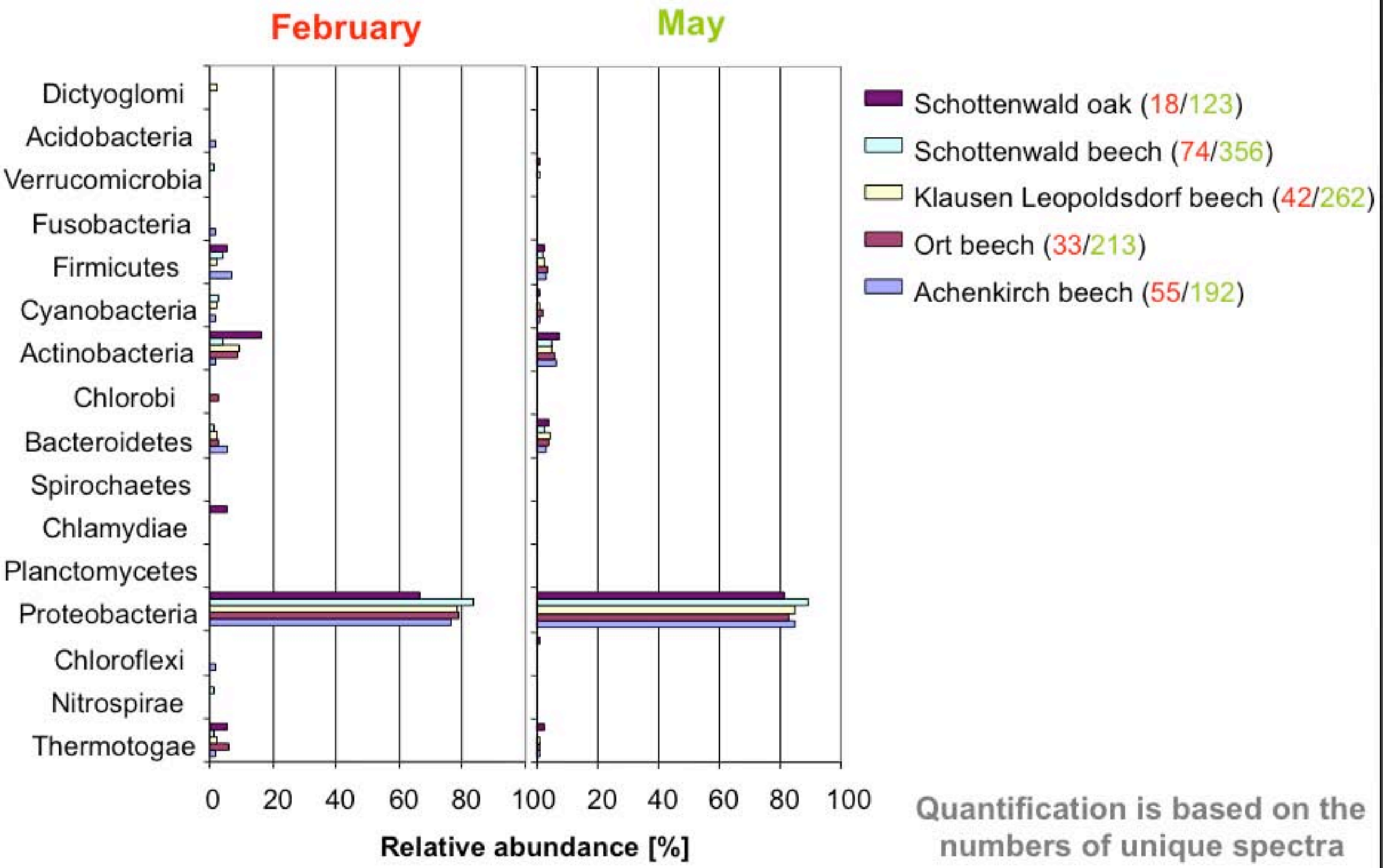
COMMUNITY STRUCTURE - FUNGI



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

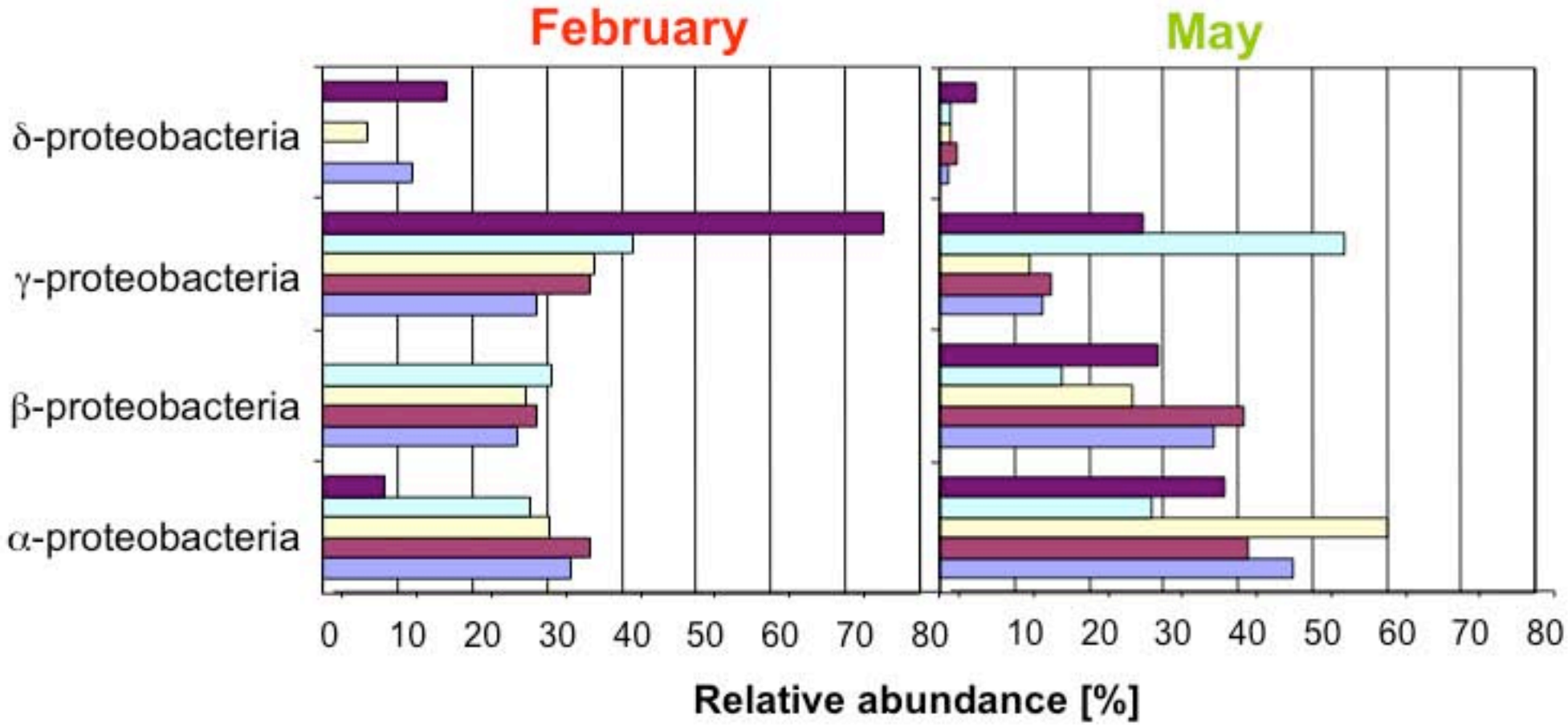
Quantification is based on the numbers of unique spectra

COMMUNITY STRUCTURE - BACTERIA



Quantification is based on the numbers of unique spectra

COMMUNITY STRUCTURE - BACTERIA



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

Quantification is based on the numbers of unique spectra

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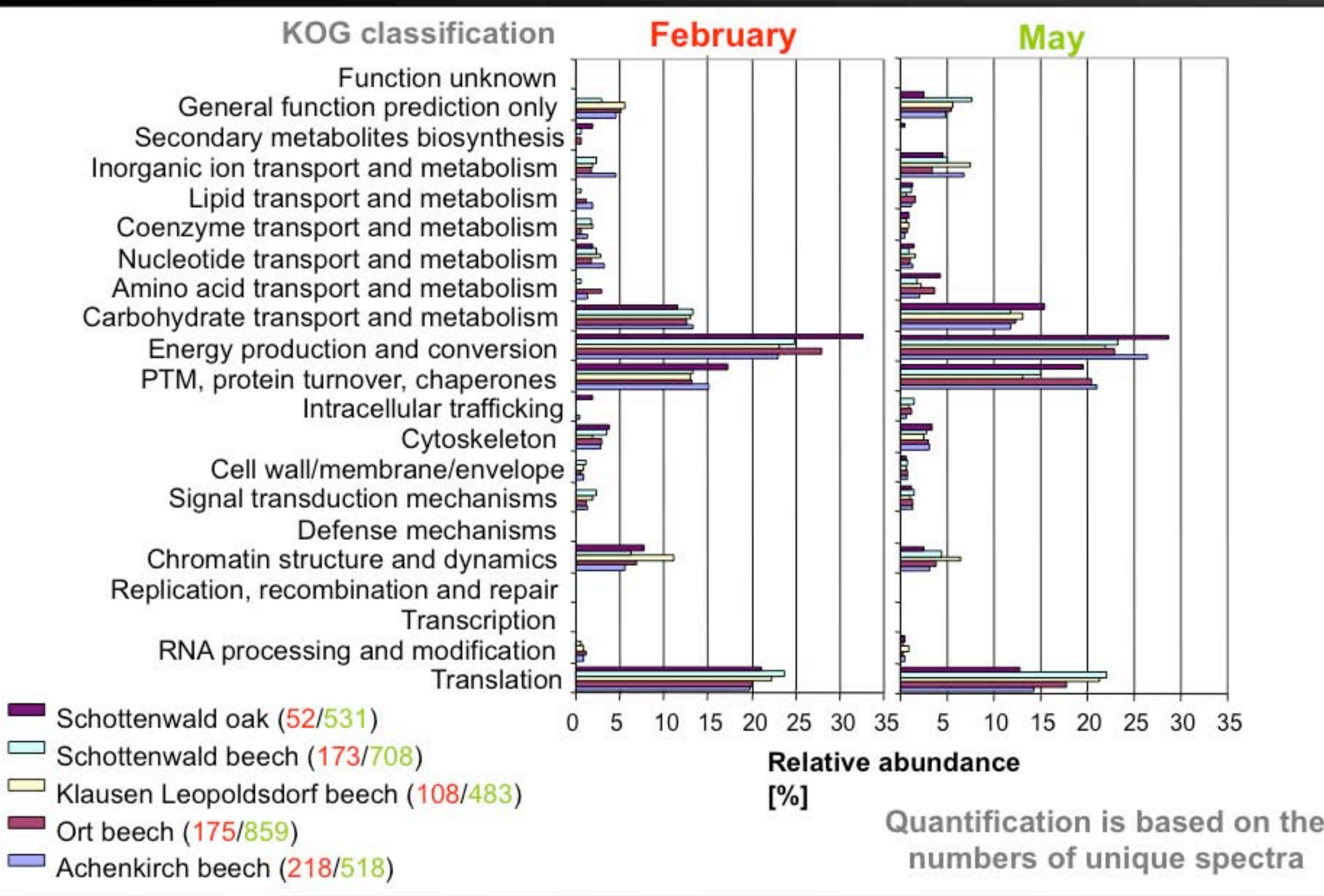
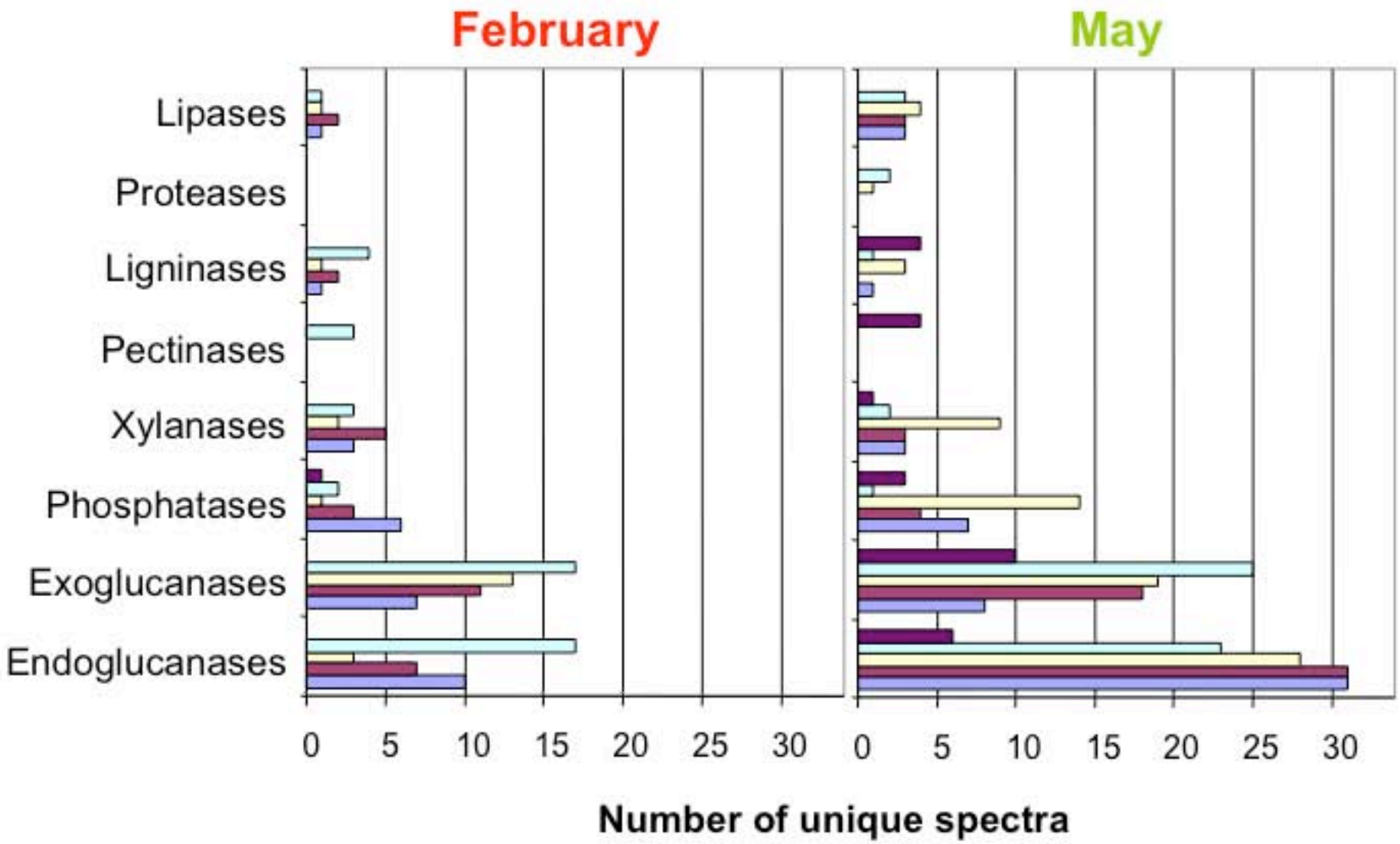
COMMUNITY FUNCTION - FUNGI



Quantification is based on the numbers of unique spectra

COMMUNITY FUNCTION – FUNGAL EXOENZYMES



Hydrolytic enzymes are only of fungal origin

- Schottenwald oak
- Schottenwald beech
- Klausen Leopoldsdorf beech
- Ort beech
- Achenkirch beech

Quantification is based on the numbers of unique spectra

IMPACT OF LITTER BIOGEOCHEMISTRY?

Sampling site Litter type		N	P	Mn	Plant Proteins	Fungal Proteins	Bacteria Proteins	Fungal Exoenzymes
Achenkirch	Feb	+++	+	+	+++	++	+	++
Beech	May	++	+	+	+++	++	++	+++
Klausen-Ld.	Feb	++	+	++	++	++	+	+
Beech	May	+	+	++	+	+++	+++	+++
Ort	Feb	+	++	++	++	+++	+	++
Beech	May	++	++	++	+	+++	++	++
Schottenwald	Feb	+++	+++	+++	+	+++	++	+++
Beech	May	+++	+++	+++	+	+++	+++	+++
Schottenwald	Feb	+++	++	+++	+++	+	+	(+)
Oak	May	++	++	++	++	++	++	++

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

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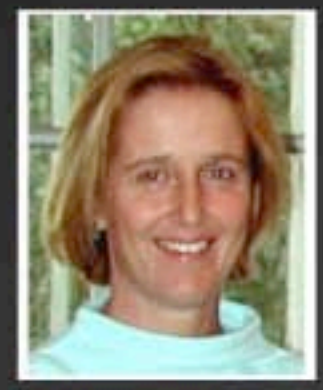
DATA VALIDATION

ENZYMATIC ACTIVITY MEASUREMENTS

Sampling Site Litter Type	Cellulase (Units / ml)		Protease (Units / ml)		Xylanase (Units / ml)	
	Feb	May	Feb	May	Feb	May
Achenk. Beech	0.007	0.01	0.12	0.1	0.025	0.04
Klau.-L. Beech	0.008	0.01	0.12	0.13	0.032	0.039
Ort Beech	0.013	0.018	0.18	0.2	0.04	0.06
Sch.W. Beech	0.009	0.016	0.25	0.3	0.035	0.075
Sch.W. Oak	0.007	0.012	0.04	0.5	0.025	0.065



Sophie Zechmeister-Boltenstern
Katharina Keiblinger
BFW, Vienna

⇨ 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

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