Purpose of the visit

The quality of the substrate which is decomposed by soil microorganisms affects decomposition rates, and the chemical composition of resources together with environmental conditions may control the pathways by which elements are lost. Understanding the decomposition process and its controlling factors requires an understanding of the linkage of carbon and nitrogen and the influence of environmental conditions, notably climate change, on nutrient losses and transfers.

We analysed the linkage between respiration rates (an indicator of the decomposition of SOM) and proteome data. The data were collected from sites differing in terms of climate and litter quality.

It is supposed that nitrogen can slow down decomposition due to an inhibitory effect on certain extracellular enzymes or it can accelerate the decomposition process because substrate quality increases. In order to resolve these controversial views we investigated the effect of nutrients on the activity of decomposer community by novel tools offered by environmental proteomics. The experiments acquainted me with the methodological protocol of the novel technique “environmental proteomics”, and the following computational analysis. We analyzed leaf litter samples from CLIMMANI sites (Achenkirch and Klausenleopoldsdorf) with a semi-quantitative proteomics approach.

Description of the work carried out during the visit

During the 6-week stay of Katharina Keiblinger in Zurich, extractions of litter samples from different sites including two climate manipulation (CLIMMANI) sites for metaproteome analysis were performed. Changes of plant litter composition as a consequence of stress conditions (i.e. drought/rewetting, temperature extremes) were analyzed for the protein expression profile of microbial communities in litter to identify altered species composition induced by these changes. In detail, the litter samples (5g) from different sites were ground in liquid nitrogen to a homogenous powder together with 10% Polyvinylpolypyrrolidone (PVPP). The extraction was performed in SDS-buffer 1% (50mM Tris, 1% Sodiumdodecylsulfate pH7.5) and the homogenized extracts were sonicated twice (2x 1min 90% pulsing, max. 40% energy). After sonication the extracts were boiled in a water bath for 20 minutes. The supernatant was separated by a centrifugation step and the proteins were precipitated over night in the 5 fold amount of acetone. This was followed by centrifugation and washing the pellet. The pellet containing the proteins was re-suspended in TAEB-buffer (0.5M Tri-ethyl-ammonium-bicarbonate buffer + 10mM Dithiotreiol (DTT) + 6M Urea + 1M Thiourea). Polyacrylamid gel-electrophoreses were carried out for pre-fractionation purposes on the one hand side with following digestion for each gel piece separately, and to estimate the protein amount extracted from the litter samples for further

protein digestion without a gel on the other side. To improve the methodological practicability, and benefit from a higher resolution of mass spectra, as well as easier handling of single digestion, a gel-free pre-fractionation was attempted to some of the litter samples. The gel-free fractionation was performed by using C18 reverse HPLC to separate peptides in fractions different in their hydrophobic characteristics. Prior to theses analysis was a pre-purification over C18, to ensure the columns were not polluted with poly-phenolic substances or humic acids. Fractions were pooled and another C18 purification step was performed, prior to analyse protein profiles with LC-MS/MS. Database searches with the received mass spectra of the samples were conducted to assign the respective spectra to peptides of microbes and their corresponding function.

Data of litter samples from CLIMMANI manipulation experiments (Klausenleopoldsdorf (N-fertilization experiment) and Achenkirch (soil warming experiment) were evaluated and linked with biogeochemical and respiration data provided by the visitor. The results derived from litter decomposition experiments with proteome data, respiration rates and nutrient fluxes (C, N, P) were combined. We observed shifts in the active microbial community during the decomposition process as well as community shifts after climate change (environmental extremes). The evaluation of how climate change may affect respiration through changes in litter quality and microbial activity and community changes, the preliminary data were integrated, and an abstract submitted to the ISME 2010, in Seattle.

Some effort was invested to adopt this methodology for the extraction of proteins out of soil samples prior to analyze the soil metaproteome. The technical challenge was to extract a sufficient amount of proteins for a gel free LC-MS/MS analysis, significantly over the limit of detection. The extraction of proteins from soils will be a great benefit for further investigations in terms of microbial decomposition and climate change because it reveals new insight in terrestrial ecosystem processes, in terms of microbial community architecture and composition, and ecosystem functioning.. The biogeochemical cycles of C and N in the context of climate change receive increasing attention.

Four extraction protocols were adopted to extract soil protein amounts, for LC-MS/MS over the limit of detection. These methods were applied on two different soils, one forest soil and one nutrient-enriched soil high in organic matter. For a spiking experiment a combination of a pure bacterial culture and a supernatant of a fungal culture were added to sterilized forest soil. The extraction was carried out for the culture combination itself and the culture in combination with the sterilized forest soil.

results

We observed a faster decomposition and higher enzyme activities and higher fungal protein spectral counts and lower plant protein spectral counts in beech leaf litter with narrow C:nutrient ratios than in litter with broader C:nutrient ratios; leaf litter with high nitrogen (N) or phosphorous (P) content relative to their Carbon (C) amount was associated with increased nutrient losses as leachates. Inorganic nitrogen losses for sites with narrow litter C:N ratios and phosphate was released more quickly in sites with narrow C:P ratios. The protein assignment to functional categories revealed that most of the hydrolytic enzymes found in litter samples were of fungal origin.

To assign proteins to phylogenetic and functional groups a comprehensive database has been used including metagenomic and meta-transcriptomics sequence information and a bioinformatic pipeline allowing semi-automatic data evaluation. Metaproteome analysis revealed that the amount of relative Viridiaeplante was significantly different for each site. Lowest amounts were observed in sites with high quality beech litter (high C:N). There is a significant negative relationship between fungal spectral counts and Viridaeplante spectral counts.

Table 1 Geographical location, and tree species of forest field sites, stand age and soil and litter nutrient concentrations

Site	Location	Tree species	Age years	Soil C/N	%C litter	%N litter	%P	Mn
Achenkirch	37°34'N 11°38'E	Spruce (Picea abis) - beech Fagus sylvatica	130	18.0	49.1 ± 1	0.84 ± 0.012	0.04	147 ± 5.6
Klausenleopoldsdorf	48°07'N 16°03'E	Beech Fagus sylvatica	64	16.0	48.0 ± 0.02	0.89 ± 0.011	0.033	1370 ± 48
Schottenwald	48°14'N 15°25'E	Beech Fagus sylvatica	145	13.4	47.0 ± 0.8	0.71 ± 0.015	0.05	2148 ± 46

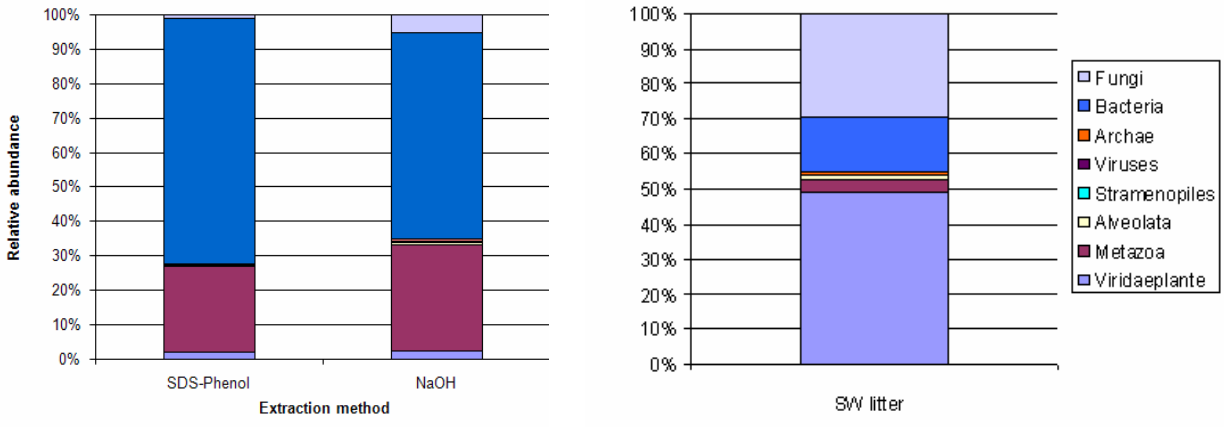

Figure 1 On the left a comparison of two different soil extraction methods is shown (SDS-Phenol and NaOH based protocols) and on the right the microbial community structure of the respective litter sample.

The samples from the sites Orth and Schottenwald which had highest manganese (Mn) and phosphor (P) content, showed a significant increase of fungal proteins in a temporal context. In contrast to the CLIMMANI sites, no temporal changes in the community were observed, at Achenkirch and even a decrease in the fungal/bacterial ratio at Klausen-Leopoldsdorf (low in P and Mn). A significant increase in microbial protein spectral counts was observed over time.

Semi-quantitative proteome analysis, suggest that fungal and bacterial abundance positively correlates with the total amount of P and Mn within the different litter types. Protein counts of extracellular enzymes demonstrated a significant increase of these over time, which was also mirrored by measurements of total enzymatic activities. Comparing the sites of different biogeochemical properties we observed that Achenkirch showed the lowest amount of Cellulases, whereas Klausenleopolsdorf had the highest abundance of Cellulases, Xylanses, Phosphatases and Ligninases. The finding that almost all hydrolytic enzymes identified from litter were of fungal origin suggests a prominent role of fungi during aerobic early litter decomposition. The fungi were predominantly in the branch of Ascomycota, mainly the classes Sordariomycetes, Dothideomycetes, Eurotiomycetes, Leotiomycetes and also Saccharomycetes. To a lesser extent Basidiomycota were found, within the fungal branch, whereas the abundance of bacteria were dominated by Actinobacteria and Proteobacteria. Comparing soil sample results with litter it seems as the main degraders in litter are fungi and a prominent proportion of plant protein was found whereas the soil is dominated by bacteria and secondary importance of metazoan was observed (Figure 1).

Respiration increased upon temperature extremes, especially in the litter with highest C:P ratio. Interestingly there is a negative relationship between litter C:P and respiration rate, this assumes that the higher the carbon content, the more will be respired by the microbial community. In terms of consumer driven nutrient recycling it might be a strategy to make nutrients available. A strong functional response of the microbial community to extreme changes in temperature and moisture were found. The impact of stress on microbes was most pronounced in sites with narrow C:N ratios. A persistent effect of temperature extremes on NH_4 and NO_3 concentrations was observed for three months after temperature changes. However, the effect on PO_4 concentrations was only transient, as were changes in fungal:bacterial ratios according to results of metaproteomics, stronger changes after temperature stress treatment but generally we similar fungal:bacterial three month after treatment. Klausenleopoldsdorf showed a strong increase in alpha – Proteobacteria community after a heat/drought event, but not after the freeze event. After a time period of three month, it was almost

recovered in comparison to the control, but there was a general increase in gamma – Proteobacteria, and also epsilon – Proteobacteria were observed three month after the change in temperature events. The site (Schottenwald) with high quality litter showed a decrease in hydrolytic enzymes after temperature change treatment, but finally the highest amount of secreted enzymes.

Environmental conditions had a strong affect on nutrient losses but only a minor affect on microbial carbon C_{mic} and microbial nitrogen N_{mic}. The impact of environmental stress (heat or freezing) on microbes in terms of C_{mic}, N_{mic} and $C:N_{mic}$ was strongest in sites with narrow litter C:N ratios. Our results indicate a similar stoichiometric demand of microbes, with temporal changes which result in differences in nutrient cycling on substrates with different C:N:P ratios.

Future collaboration with host institution

Further visits to Zurich and / or Braunschweig is planned to evaluate results obtained from the first visit. During the stay in Zurich a proposal for future environmental metaproteomics conducted on samples of the CLIMMANI site Klausenleopoldsdorf was elaborated. The interaction of microbes involved in the decomposition process, their community changes, the effect of climate changes, and the further impact on nutrient and respiration dynamics will be part of a further project submitted to EMSL. "A Metaproteomics Approach to Link Microbial Diversity and Ecosystem Functioning during Leaf Litter Decomposition." https://eus.emsl.pnl.gov/Portal EMSL Proposal ID: 39707

A database with the obtained results to improve model approaches and the basic knowledge of the microbes involved in the decomposition process and to further improve the knowledge into microbial community changes as a consequence of climate change and its further impact on nutrient and respiration dynamics is to be developed in the next months together with the host institution.

Projected publications/articles resulting or to result from your grant

Abstract sent to ISME with data put together. The 13th International Symposium on Microbial Ecology (no. 1585119) "Linking Beech Litter Stoichiometry to microbial decomposer community structure and function."

Proposal "A Metaproteomics Approach to Link Microbial Diversity and Ecosystem Functioning during Leaf Litter Decomposition." for future environmental metaproteomics comparing climate change sites; EMSL https://eus.emsl.pnl.gov/Portal EMSL Proposal ID: 39707

Keiblinger KM, Schneider T., Hämmerle I., Richter A., Riedel K. and Zechmeister-Boltenstern S. The impact of freeze/thaw and drought/rewet events on the decomposition of beech (*Fagus sylvatica)* litter of different provenience.

Soil protein extraction attempts, maybe resulting in a short communication, results are still not available. Data evaluation will be done during a second visit in Zurich, Keiblinger KM, Schneider T., Riedel K. and Zechmeister-Boltenstern S., Soil protein extraction for metaproteome analysis comparing extraction protocols.

Synthesizing data of previous work:

Schneider, T., **Keiblinger, K.,** Roschitzki, B., Gerrits, B., Eberl, L., Richter, A., Battin, T., Zechmeister-Boltenstern, S. and Riedel, K.: *Metaproteome analysis of leaf litter decomposition*. Planned to be published in a high impact factor journal like PLOS ONE.