CLIMMANI – scientific report

Short-term Exchange February 2013, of

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"Linking soil microbial community structure and function with respiration of a soil warming field experiment"

1 Purpose of the visit

The purpose of the visit was to unravel microbial factors that determine respiration dynamics in soils of a CLIMMANI manipulation experiment (Achenkirch - soil warming experiment) using metaproteomics. The metaproteomics technique allows investigating how microbial community composition responds to soil warming in very high phylogenetic resolution, and thus can help to unravel how changing microbial functions alter CO_2 emissions in warmed soil. Three experimental plots of the experimental soil warming CLIMMANI site Achenkirch were sampled in autumn 2012 and the results will be compared to answer the following hypotheses:

H1: Warming significantly enhances microbial metabolic activity in terms of soil respiration per amount of microbial biomass C. Microbial stress biomarkers are elevated in warmed plots.

H2: Warming has a stronger effect on function than on microbial community composition and biomass, and strongly increases microbial metabolic activity

2 Description of the work carried out during the visit

Prior to the visit in Greifswald soil samples were taken at the CLIMMANI manipulation site Achenkirch (soil warming experiment), and proteins were extracted in the laboratory in Vienna, and sent to Greifswald for a state-of-the-art semi-quantitative metaproteomics approach. In Greifswald samples were run on 1D PAGE. Protein lanes from the PAGE were cut into 8 slices and subjected to in-gel tryptic digestion by employing sequencing grade modified trypsin (Promega, reference V5111)

(Shevchenko et al., 1996). The resulting peptide mixtures were analysed on a hybrid LTQ-Orbitrap tandem mass spectrometer (ThermoFisher Scientific) interfaced with a nanoelectrospray ion source. Chromatographic separation of peptides was achieved with C18 reversed-phase HPLC on an Eksigent nano LC system (Eksigent Technologies, Dublin, CA, USA) equipped with an 11 cm fused silica emitter, packed inhouse with a Magic C18 AQ 3 µm resin (Michrom BioResources, Auburn, CA, USA). Peptides were loaded from a cooled (4 ℃) Spark Hol land auto sampler.

The advanced inventory of equipment with their highly sensitive mass spectrometers available at the University of Greifswald allows to detect peptide mass spectra in complex soil samples. These mass spectra can be assigned to extracellular enzymes and other highly-abundant secreted, cell-bound or intracellular proteins. The raw data from the mass spectrometers were then loaded into the software "Proteome Discoverer" (Thermofisher Scientific) and began with searches against a newly created database.

During the 2-week stay of Katharina Keiblinger in Greifswald, a Database was created, which is based on data by Kuffner et al. (2012), who applied a pyro-sequencing approach on soil samples from the CLIMMANI climate-manipulation site Achenkirch.

These data were used to reduce the current NCBI database (which is to date approximately 23GB of data volume) to a smaller database including the phyla obtained with the pyro-sequencing approach as well as Fungi and Archaea to a final volume of approximately 14GB. As the number of sequence entries is increasing rapidly with the current development of molecular approaches, data evaluation of these large amounts of data already represent a big challenge. Even the potent server in Greifswald is already on its limit for such metaproteomic issues, also because soils represent very complex systems. The search for only one sample against the (already reduced!) database lasts for 3-4 days.

After the database search, stringent filters were applied, which only assigned measured peptide mass spectra to a protein if at least two hits on the same protein had been obtained.

Then the assigned proteins were exported into 'prot.xml' and 'csv' format and subjected to a workflow ('PROPHANE', http://prophane.svn.sourceforge.net/viewvc/prophane/trunk/), which is a very useful tool to combine data on microbial taxonomy at different levels with functional categorization of the determined proteins.

Stephan Fuchs introduced Katharina Keiblinger into database generation, database searches, workflow application and final data evaluation with the excel-sheets supplied by the PROPHANE workflow. As it was not possible to finish all the database searches during the two-week stay, Stephan Fuchs will provide the final search results as soon as they are finished. Katharina Keiblinger will then combine the results from the metaproteomics analysis with data about microbial biomass C and nitrogen (N), and microbial metabolic activity as estimated by soil respiration-to-biomass ratios. Additionally, these data will be used for a draft of a scientific paper. The final combination of these data will be done together with Andreas Schindlbacher back in Vienna.

3 Description of the obtained results

3.1 Phylogeny

Starting from the Proteome Discoverer output files, all obtained protein hits were assigned to phylogenetic and functional groups and assignments were validated by the PROPHANE workflow. Because protein abundance is proportional to the number of MS/MS spectra acquired from peptides of the respective protein, protein abundances were calculated based on the normalized spectral abundance factor (NSAF; Florens et al., 2006; Zybailov et al., 2006). This number allows relative comparison of protein abundances over different samples (Bantscheff et al., 2007). To calculate NSAF, the numbers of unique spectra assigned to each protein are divided by the number of the amino acid chain length of the longest candidate in the protein cluster, giving the spectral abundance factor (SAF). The SAF allows the comparison of protein abundances in one sample taking protein molecular weight into account. To allow cross-sample abundance comparison, each SAF is divided by the sum of all SAFs in the respective sample, giving the normalized SAF (NSAF)..

Comparing NSAF from our samples revealed a dominance of *Bacteria* (~60%) followed by *Eukaryota* (~38%) and only some *Archaea* from the phylum Crenarcheota and Euryarcheaota. Among *Bacteria* we found *Proteobacteria, Actinobacteria* and *Nitrospira*, with *Proteobacteria* strongly dominating. Among *Proteobacteria,* the highest NSAF values were found for the classes of *Gamma-, Alpha- and Delta-Proteobacteria*. *Eukaryota* were separated into the phylum of mainly *Basidiomycota* (NSAFs~0.13) and to a minor extent *Ascomycota, Glomeromycota, Microsporidia, Streptophyta, Chlorophyta, Phaeophyceae* and *Bacillariophyta*. The main class within the *Basidiomycota* were the *Agaricomycetes*. The *Ascomycetes* were separated into the classes *Saccharomycetes, Eurotiomycetes, Sordariomycetes, Leotiomycetes, Dothidiomycetes* and *Orbiliomycetes.*

3.2 Functions

For functional assignments, proteins were blasted against the KEGG database, the cluster of orthologous group (COG) database and SEQUEST, and blasted against the HMMER database (http://hmmer.janelia.org/search/phmmer) to cluster main roles and sub roles of functions using TIGRFAMS for bacterial functions and PFAMS for fungal functional categorization. If a protein cluster contained more than two protein hits, clusters were checked for consistency in the phylogenetic and functional assignments (Schneider et al., 2011). Finally, protein abundances were calculated based on the normalized spectral abundance factor (Florens et al., 2006; Zybailov et al., 2006).

Bacterial functions were strongly related to “Post-translational modification, protein turnover, and chaperones”, “Energy production and conversion”, “Amino acid transport and metabolism”, “Coenzyme transport and metabolism”, “Lipid transport and metabolism”, “Translation, ribosomal structure and biogenesis” and “Transcription”. In addition to the COG categorization also a categorization via TIGRFAMS from the HMMER database was done and revealed similar results as can be seen in Figure 1, although the division in the main roles in this database is slightly different from the categorization of the KEGG database.

Functional assignments of fungi were categorized via the KEGG database into KOG, and were strongly related to the following functions: “Cytoskeleton”, “Chromatin structure and dynamics”, “Energy production and conversion”, “Post-translational modification, protein turnover, and chaperones”, “General function prediction only” and “Translation, ribosomal structure and biogenesis”

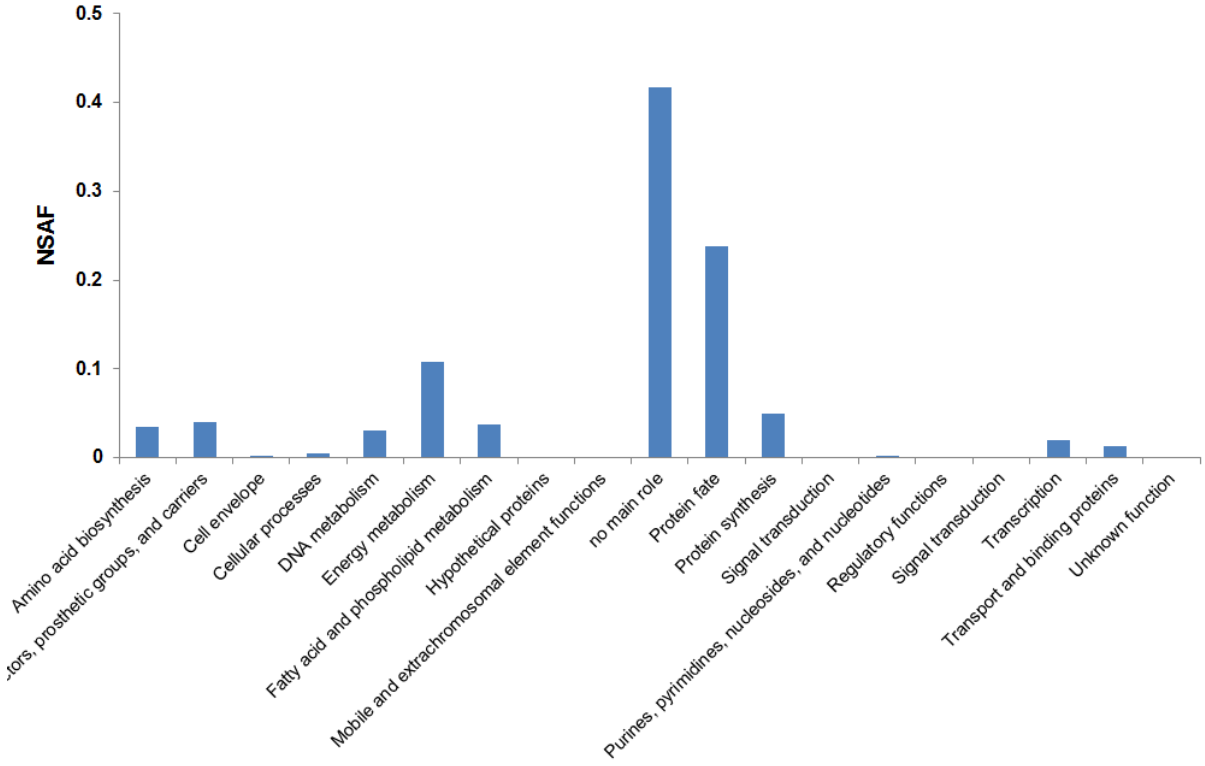

Figure 1 Functional categorization of bacterial proteins.

After completion of database searches, the obtained information about microbial phyla and functions will be combined with results from the CLIMMANI manipulation site Achenkirch. If everything goes to plan (no computer crashes or failure or lost connection to the server for database searches) in at least three weeks we should have all the data for evaluation purposes and to continue to deal with our hypotheses and to work on a draft manuscript.

4 Future collaboration with host institution

During the 2 weeks in Greifswald, a project proposal with the working title “Linking microbial diversity and function” was discussed for a DACH project with the Austrian science fund FWF and the German science fund (DFG) for a further collaboration. This project proposal will aim at combining data of the **climate-manipulation site Rosalia** where a drought and rewetting experiment will start in March 2013. In addition, an exchange of a PhD student from the University of Natural Resources and Life Sciences to the University of Greifswald for a summer school for metaproteomics was arranged.

Final results of the metaproteomics analysis of the climate-manipulation site Achenkirch will be provided to the EXPEER (a major European Infrastructure project in the field of Ecosystem Research facilitating access to experimental and observational platforms as well as analytical and modelling

facilities for the benefit of the international research community) platform after publication. This might help to improve model approaches and extend the basic knowledge about microbes involved in various ecosystem processes. Furthermore, it is of great interest for the scientific community as well as political and economic stakeholders to understand microbial community changes as a consequence of climate change and its further impact on nutrient and respiration dynamics.

5 Projected Publications / articles resulting or to result from the grant

Preliminary results from the exchange to Greifswald will be included into an invited Talk at DOI JGI user meeting Metaproteomics of the Soil workshop March 25 - 28, 2013 in Walnut Creek, California

Keiblinger KM, Liu D, Fuchs S, Schindlbacher A, Riedel K. and Zechmeister-Boltenstern S. “Linking soil microbial community structure and function with respiration of a soil warming field experiment” Abstract for the Annual meeting of the German Soil Science Society (DBG) to be held in Rostock 7th – 13th September 2013 will be submitted.

Draft for a DACH Proposal for future environmental metaproteomics at climate manipulation site Rosalia; Lead Agency is the Austrian Science fund (FWF) with the working title: “Linking microbial diversity and function”

Soil protein analysis from CLIMMANI manipulation site Achenkirch will result in a draft manuscript, but so far results are still not available. Data evaluation will be done during the next months Keiblinger KM, Liu D, Fuchs S, Schindlbacher A, Riedel K. and Zechmeister-Boltenstern S. “A metaproteomic study - Changes in microbial activity and community composition as affected by soil warming a temperate mountain forest soil.”

Methodological aspects of soil protein analysis, maybe resulting in a short communication, results are still not available. Data evaluation will be done during the next months, Keiblinger KM, Liu D, Fuchs S., Riedel K. and Zechmeister-Boltenstern S., Soil protein extraction for metaproteome analysis comparing extraction protocols.

6 Literature

Florens, L., Carozza, M.J., Swanson, S.K., Fournier, M., Coleman, M.K., Workman, J.L., Washburn, M.P., 2006. Analyzing chromatin remodeling complexes using shotgun proteomics and normalized spectral abundance factors. Methods 40, 303-311.

Kuffner, M., Hai, B., Rattei, T., Melodelima, C., Schloter, M., Zechmeister-Boltenstern, S., Jandl, R., Schindlbacher, A., Sessitsch, A., 2012. Effects of season and experimental warming on the bacterial community in a temperate mountain forest soil assessed by 16S rRNA gene pyrosequencing. Fems Microbiology Ecology 82, 551-562.

Schneider, T., Schmid, E., de Castro, J.V., Cardinale, M., Eberl, L., Grube, M., Berg, G., Riedel, K., 2011. Structure and function of the symbiosis partners of the lung lichen (Lobaria pulmonaria L. Hoffm.) analyzed by metaproteomics. Proteomics 11, 2752-2756.

Zybailov, B., Mosley, A.L., Sardiu, M.E., Coleman, M.K., Florens, L., Washburn, M.P., 2006. Statistical analysis of membrane proteome expression changes in Saccharomyces cerevisiae. Journal of Proteome Research 5, 2339-2347.