Biogeosciences Discussions, 1, S524–S526, 2004
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European Geosciences Union

Biogeosciences Discussions

Interactive comment on "Protein analysis in dissolved organic matter: what free proteins from soil leachate and surface water can tell us – a perspective" *by* W. Schulze

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Received and published: 16 February 2005

Reply to Referee #1: Referee #1 had the following specific comments and questions: - "Extracted proteins from pulverized decomposing plant material do not belong to the DOM per definition ... Therefore, the results are not directly comparable to the results from leachates. The author should clearly state this fact, and in addition should reconsider the title of the publication" Yes, this is correct, indeed the decomposition line presented represents a distinct protein source compared to the other samples. In the revised manuscript, in the Methods section I will added a sentence stating that the decomposition line contains proteins prior to soil adsorption processes. In addition I will modify title and abstract accordingly.

- "The chapter 4.4 and 4.5 should be moved to the beginning of the results. Numbers of proteins should actually be stated." I will follow the suggestion and move theses

sections, and renamed the figures accordingly. In addition, the number of identified proteins will be stated next to the pie charts.

- "Give an example of a typical SDS polyacrylamide gel. Would a 2D-Gel approach lead to better resolution" An SDS-Page image will be included in the revised manuscript. Whether or not a 2D-Gel approach leads to higher resolution may depend on a variety of different factors: it is well known that certain proteins (membrane proteins, etc) do not run well on a 2D-gel. The 2D-Gel approach is possible, as demonstrated by Wilmes and Bond (2004), Environmental Microbiology, 6(9): 911. Although their sample is not extracted from "real" environment, they nicely separate proteins from biofilms of artificial waste water sludge systems on 2D gels. However, it seems that the actual number of proteins identified is roughly the same as by the LC-MS/MS approach. In fact, and I will have to update the Methods section accordingly, as there were rather few proteins present in most of the DOM solutions, the material was digested in-solution (without running a gel). In-solution digestion leads to a 10-times higher digestion efficiency compared to an in-gel-digest.

- "It is generally accepted that the bacterial biomass in soils rich in organic C is dominated by gram positive bacteria like actinomycetes, streptomycetes, and that proteobacteria account for a smaller amount. Does the author have an explanation for the large amount of proteobacterial proteins detected?" I will add a paragraph to the Discussion, where I will state this seemingly contradiction. I do not have a specific answer without actually carrying out a back-to back comparison of methods. It could be, that biomass is not the same as turnover, and different methods measure different things. I found some references, which show that the bacterial community composition is strongly depending on soil type, climate, etc. (Hackel et al, 2004, Applied Environ.Miocrobiol. 70:5057): by TRF analysis, the proportion of gram-positive bacteria varies between 50% and 10% depending on the sampled environment. By PLFA analysis, Proteobacteria can be equal to gram-positive bacteria (on a % mol basis of PLFA components; see Leckie et al, 2004, Microbial Ecology 28: 29), and another study

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revealed that in growth tests, Pseudomonads can be the dominant group (Priha et al, 1999 FEMS Microbiology Letters 30, 187). Clearly, back-to-back method comparisons between DNA-based methods, methods of marker compounds and protein analysis need to be carried out in order to define the bias of different approaches.

- "Please structure your results section similar to the material and methods section." I will restructured the results section following the suggestion mentioned above to first discuss general aspects, then specific ones. In addition I will follow the structuring suggestions of Referee #3.

- "The paper suffers from te missing link between the genomic and proteomic approach." Yes, I absolutely agree with Referee #1 in this respect. Unfortunately the samples and experimental sites analyzed in this study cannot be linked with to genomic and organismic diversity, as these data have not consistently been acquired for the variety of samples analyzed. However, currently we are working on setting up such a detailed comparison between enzyme activities, genomic classification, proteomic analyses and PLFA classification. I hope to be addressing this important question in a future article.

- Technical comments were addressed, some in detail: Page 831, line 18: The statement is unclear Yes, for 30% of the proteins, one tryptic fragment was enough to identify the protein, for 70% there were more than one tryptic fragment per protein. Figures... should be in Black&White and contain the number of proteins as numbers I will modify the figures such that they can be printed in grayscale, and the numbers of proteins are stated next to the pies. Figures 6, and 7, which will be renamed to Figures 2 and 3: In the methods section 2.4. I will state that protein size information was obtained from the NCBI database.

Interactive comment on Biogeosciences Discussions, 1, 825, 2004.