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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

Anonymous Referee #1

Received and published: 9 February 2005

General comments:

The paper deals with the new application of the proteomics approach in soil leachates and dissolved organic matter (DOM) samples. To my knowledge, this paper (together with a previous publication from the author) is the first one presenting this innovative approach in terrestrial ecosystems which might open the door of the large black box of functional structure and diversity. In recent years, the genomic diversity of terrestrial samples, predominately based on DNA approaches, were in the line of scientific interest and numerous studies show the genomic complexity of environmental samples. Measures of functional genes, RNA, etc. gave first insights into the active fraction of this vast genomic pool. However, the link between structural and functional diversity within terrestrial ecosystems was hardly possible until now. Therefore, I acknowledge

that the author could clearly show that the rather new technique of protein investigation (proteomics) enables to verify at least phylogenetic groups active in the DOM of soils and litter. I strongly agree with the author that the proteomics approach will dramatically increase our knowledge and understanding of the 'functioning' of a given ecosystems.

Specific comments:

Extracted proteins from dried and pulverised decomposing plant material do not belong to the DOM per definition. Based on the extraction method presented in this paper this protein fraction contains proteins from freshly killed cells and adsorbed extracellular proteins which might be not present in soil DOM and water leachate samples. Therefore, the results are not directly comparable to the results from leachates. The author should clearly state this fact. In addition, I suggest reconsidering the title of the publication in this sense.

The chapters 4.4 and 4.5 should be moved to the beginning of the result section, in order to present the results beginning from general to specific matter. In these chapters the author should accurately state the number of proteins identified within the samples (which can be only roughly assumed from fig. 5). In general, it would be rather useful to know the amount of identified proteins in comparison to the total amount of proteins.

Protein extraction and identification from environmental samples is a challenging approach, taking into account the enormous number of proteins of living or dead organisms and their degradation products. There are only few studies until now which have demonstrated that the highly complex protein mixture of terrestrial environmental samples is difficult to deal with. Therefore, I suggest to give an example of a typical sample SDS-polyacrylamid gel to get an idea how things look like. It would be also interesting to know if 2D-gel electrophoresis might be applicable to these protein samples, since the resolution of this technique is much higher than those of a '1D' SDS-polyacrylamid electrophoresis.

I am surprised about the results from the detailed taxonomic view of bacterial proteins

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in the DOM of the forest samples. To my knowledge, it is generally accepted that the bacterial biomass in soils rich in organic C is dominated by gram positive bacteria like actinomycetes or streptomycetes, proteobacteria generally account for a smaller amount. Does the author have any explanation why the protein fraction deriving from proteobacterial is so dominant in these DOM samples?

Please structure your results section similar to the material and method section.

In general, the paper suffers from the missing link between the genomic and proteomic approach. It would have been extremely useful to give details about the organism composition, at least in some cases, and to compare it to the findings presented. I am sure, that this is a challenging task, but it will substantially increase our knowledge about ecosystem functioning.

Technical comments:

Page 830, line 19: please state the amount of extraction solution added Page 830, line 23: please state the approximated molecule size which is excluded by the use of Sepharose 4B Page 831, line 18: The statement is unclear. I suppose the author wants to tell us that one singular peptide fragment from tryptic cleavage is sufficient to identify the protein origin in 30 % of all proteins identified (in 70 % you will need more than one tryptic peptide to identify the protein). The anonymous referee #3 (24 January, 2005) might have misunderstood this statement ('70 % of the proteins not identified'), so please clarify. Page 831, line 22: 'were unique' (in the study presented) or 'are unique' (in general)? Please clarify. Page 837, line 8: omit '(gC 100 gsoil-1)'. This part of the chapter has some obvious typing errors: 'in' the FH layer Ě 'of' a natural beech forest. I'm not sure, if Ellenberg et al. (1986) is the appropriate reference for the amount of bacterial and fungal biomass in soils.

Figures: It's not possible to identify the taxonomic groups or protein type from the figure legends if paper hardcopy is printed in black and white (which is the standard printout in most cases)! Please change it! In addition, the author may state the number

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of identified proteins on the figures (the so called ‘area of pie charts’) in each case. Increase the text in the figure!

Figure 6, 7. The author does not give information on these figures in the material and method section.

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