Geophysical Research Abstracts
Vol. 12, EGU2010-8094, 2010
EGU General Assembly 2010

Development of soft extraction method for structural characterization of boreal forest soil proteins with MALDI-TOF/MS

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Nitrogen (N) is usually the nutrient restricting productivity in boreal forests. Forest soils contain a great amount of nitrogen, but only a small part of it is in mineral form. Most part of soil N is bound in the structures of different organic compounds such as proteins, peptides, amino acids and more stabilized, refractory compounds. Due to the fact that soil organic N has a very important role in soil nutrient cycling and in plant nutrition, there is a need for more detailed knowledge of its chemistry in soil. Conventional methods to extract and analyze soil organic N are usually very destructive for structures of higher molecular weight organic compounds, such as proteins.

The aim of this study was to characterize proteins extracted from boreal forest soil by "soft" extraction methods in order to maintain their molecular structure. The organic layer (F) from birch forest floor containing 78% of organic matter was sieved, freeze dried, pulverized, and extracted with a citrate or phosphate buffer (pH 6 or 8). Sequential extraction with the citrate or phosphate buffer and an SDS buffer (pH 6.8), slightly modified from the method of Chen et al. (2009, Proteomics 9: 4970-4973), was also done. Proteins were purified from the soil extract by extraction with buffered phenol and precipitated with methanol + 0.1M ammonium acetate at -20ºC. Characterization of proteins was performed with matrix assisted laser desorption ionization – time-of-flight mass spectrometry (MALDI-TOF/MS) and the concentration of total proteins was measured using Bradford's method. Bovine serum albumin (BSA) was used as a positive control in the extractions and as a standard protein in Bradford's method.

Our results showed that sequential extraction increased the amount of extracted proteins compared to the extractions without the SDS-buffer; however, it must be noted that the use of SDS-buffer very probably increased denaturization of proteins. Purification of proteins from crude soil extracts by phenol extraction was essential prior to measurement of total proteins; there seemed to be a lot of compounds in crude soil extracts that interfere with the analysis of total proteins, causing overestimation in protein concentration. pH of the buffer solution did not seem to be very crucial for the extractability of soil natural proteins, but at the higher pH, the amount of interfering compounds increased. However, the recovery of BSA added was clearly higher at the higher pH. When the protein precipitates were analyzed with MALDI-TOF/MS, a large curve, most likely formed from wide peaks of several compounds, indicate that most of the compounds in the precipitate were <15 kDa or ~20-50 kDa in molecular weight. It seems that in order to identify individual proteins from mass spectra, a separation of compounds with varying molecular weight is needed before the MALDI-TOF/MS analysis. Due to the fact that a relatively high amount of BSA added was not recovered by the extractions and that the intensity of the signals observed in mass spectra was low, it is questionable whether it is possible to extract soil natural proteins effectively from soils containing a high amount of organic matter without destructing the structures of proteins.