



## **Molecular differentiation of subsoil biopores of different origin by PLFA analysis**

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Biologically generated macropores (biopores) are a key factor for propagation of root growth, nutrient mobilisation and acquisition from the subsoil. However, biopores of different origin, i.e. root-derived, earthworm-derived or of mixed origin, are difficult to distinguish visually in the field. Therefore, the objective of this study was to test molecular differentiation by means of phospholipids fatty acids (PLFA).

24 samples of biopore content of the three aforementioned origins and 8 bulk soil samples were taken from two soil depths (45 – 75 cm; 75 – 105 cm) and extracted twice by a solution of methanol, chloroform and citrate/KOH buffer (pH 4, v:v:v = 1:2:0.8). Following separation of phospholipids, derivatisation was by hydrolysis using NaOH in MeOH and methylation by adding BF<sub>3</sub> and heating at 80°C. After further purification and preparation, samples were measured by gas chromatography – mass spectrometry (GC-MS).

Generally, the abundance of PLFA differed only slightly between the upper and lower soil depth. Gram negative bacteria (16:1w7c, 18:1w7c and Cy17:0) were the most abundant microbial group in both depths and show clear enrichment in biopores, especially in the mixed-pore type. A similar pattern was observed for fungi (18:2w6,9), but it was less pronounced in the deeper section. Actinomycetes (10Me16:0 and 10Me18:0) in contrast, show the highest enrichment in root-derived pores. Interestingly, highest abundance of AM fungi (16:1w5c) was found not in root-derived pores, but in the mixed-pore type. Protozoa (20:4w6) occurred significantly higher in the earthworm-derived biopores. The majority of the gram positive bacteria (a15:0, i15:0, i17:0 and a17:0) showed no significant preference of habitat, i.e. in this case pore type or bulk soil. This is indicative for general decomposers of old soil organic matter.

Thus we showed, that PLFA analysis not only a valuable molecular proxy for the differentiation of biopore types, but also provides deep insight into the role of individual microbial functional groups in nutrient mobilisation and cycling in subsoils.