## PLFA spatiotemporal patterns for characterization of microbial communities in limestone aquifers

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Spatiotemporal distribution patterns and  $\delta^{13}C$  values of phospholipid fatty acids (PLFA) were determined for eight wells sampling two superimposed alkaline Muschelkalk aquifers in the Hainich region (Thuringia, Germany) to characterize changes of microbial communities and their relation to physicochemical properties of the groundwater. The lower aquifer was oxic (O2 up to 5.6 mgL<sup>-1</sup>) and had sulfate concentration of up to 280 mgL<sup>-1</sup>, whereas the upper aquifer was anoxic to suboxic ( $O_2 < 0.1 \text{ mgL}^{-1}$ ) and had ammonium concentration of up to 0.8 mgL<sup>-1</sup>. Principal component analysis of the PLFA revealed two microbial communities within the anoxic aquifer. They differ by the prevalence of the C16:1w7c, 10-MeC16:0 and [3]-, [5]-ladderanes PLFA, typical of Gramnegative, sulfate reducer and anaerobic ammonia oxidation (anammox) bacteria, respectively, and by the prevalence of the C18:1w9c and C18:2w6,9 typical of fungi. The oxic aquifer had higher contribution of C20:4n6, C20:5n3, C22:5 and C22:6 typical of eukaryotes suggesting higher input of surface organic matter. Redundancy analysis showed that Fe total, O2 and  $\mathrm{NH_4^+}$  concentrations explain 39 % of the PLFA distribution. The ladderanes and 10-MeC16:0 were present in both aquifers, but increased in abundance with decreasing O2 and increasing NH4+ concentration. The ladderanes were 13Cdepleted by up to 50 % relative to DIC. In wells with high ladderane concentration, <sup>13</sup>C-depleted iC15:0, C16:0 and 10-MeC16:0 ( $\Delta_{PLFA-DIC}$  up to 50 ‰) suggested a high contribution of anammox bacteria to those PLFA. In other wells,  $\Delta_{\text{PLFA-DIC}}$ values between 10 to 25 ‰ suggested higher PLFA contribution from heterotrophic metabolism. Our results indicated that in the anoxic to suboxic aquifer a large fraction of bacteria performed autotrophic CO<sub>2</sub> fixation (sulfate reducer and/or anammox bacteria), whereas high DOC likely favored heterotrophy.