

## **Indigenous production of core and intact bacteria and archaeal tetraether lipids in the pristine aquifers**

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Groundwater ecosystems host diverse microbial communities in the vast and complex habitats. Current understanding of biogeochemical processes and diversity of the microbes in this ecosystem still largely depends on cultivation and molecular biological methods. Here we investigate the microbial community in aquifers from Hainich Critical Zone Exploratory (CZE) by analysing bilayer membrane lipids – branched and isoprenoidal glycerol dialkyl glycerol tetraethers (brGDGTs and isoGDGTs) – which were used as biomarkers for determining climate and environment change in the past. Core lipid GDGTs (CL-GDGTs), represented the fossil pools of membrane lipids after cell death, are found to be 1–2 orders of magnitude higher than intact polar lipid GDGTs (IPL-GDGTs) that derived from the living microbial cells in both aquifers and the ambient surface soils. In aquifers, brGDGTs shows higher abundance in both core and intact parts. The similar trend is shown in CL-GDGTs of surface soils (with an average of 76% of the total amount), however, isoGDGTs dominants in IPL-GDGTs (average 56%), suggesting branched GDGTs-producing bacteria may have faster degradation and growth rates than archaea in soils. Principal components analysis (PCA) of brGDGTs highlights two out of seven ground water samples (H32 and H53) from the Hainich transect upper (HTU) aquifer assemblage have allochthonous bacteria inputs from the soils of the recharge areas, possibly due to the shallow depth of the two wells to the ground surface. Notably, PCA of isoGDGTs indicates no significant difference between soils and aquifers. Nevertheless, the 16S rRNA gene sequence for archaea provides evidence that pristine aquifers show different Thaumarchaeota communities from those of overlying surface soils, suggesting an in-situ archaea production. This is the first report of the provenance of GDGTs in karstic aquifers, and it implies that both bacteria and archaeal GDGTs are mainly produced in-situ in most ground waters, probably from the heterotrophs that can adapt to the oligotrophic environment.