



Fuelling life of groundwater microbiomes in sloping carbonate-/siliciclastic bedrock strata

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The terrestrial deep biosphere hosts up to 20% of the earth's biomass. Whereas life in deep saline waters is suggested to be sustained mainly by H_2 and CO_2 , microbes in shallow groundwaters still receive input from recently fixed organic carbon. Within the research centre AquaDiva, we aim to understand the links between the surface and subsurface biogeosphere, especially how organisms inhabiting the subsurface reflect and influence their environment, and affect water and matter transiting the subsurface. To achieve this, we have constructed a novel infrastructure, the Hainich Critical Zone Exploratory (CZE), which provides an excellent platform to access groundwater and aquifer rock material from oligotrophic limestone aquifer assemblages along with soil and seepage from the respective groundwater recharge areas.

We tracked the formation of the groundwater microbiome and the differentiation of the predominant *Candidatus* Patescibacteria under distinct hydrochemical regimes. Network, correlation analysis and time series allowed us to postulate different biological and hydrochemical drivers for community assembly of planktonic microbiota, with implications for subsurface carbon cycling. Furthermore, our data showed the existence of a unique rock matrix microbiome. Shallow weathered limestones contained bacterial groups most likely originating from soil habitats. Low-permeable mudstones, containing mainly oligotrophic heterotrophs and thiosulfate-oxidizing autotrophs, showed similarities between surface-near samples and deep samples, without relation to rock type and bulk rock permeability. The bacterial communities found on fracture surfaces of limestone subsamples were distinct from their matrix counterparts and ranged from organic matter decomposers in outcrop areas to autotrophs in downdip positions that receive limited surface input. By comparing the stable and radiogenic carbon isotope compositions of microbial phospholipid-derived fatty acids (PLFAs) with those of the potential microbial C sources, we determined that subsurface groundwater microbiomes appeared to assimilate up to 70% ancient organic matter derived from rocks, despite the presence of younger organic matter. These signatures challenge the current paradigm and point to an intensive internal cycling of carbon independent of surface input.