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Bacterial communities in shallow fractured-rock groundwater evolve over time, exhibiting cyclic patterns in response to multi-annual recharge events.

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Groundwater is an important component of the Earth's critical zone and within this ecosystem, microbial communities play an important role, interacting with, and contributing to, the geology and hydrology of these systems; understanding how microbial communities in this dynamic zone change over time is therefore crucial. However, subsurface aquatic environments are lacking high-resolution temporal data over long time periods. Based on 16S rRNA gene sequences collected monthly over a six-year period (n=230) in groundwater from fractured Triassic limestone-mudstone alternations of the Hainich Critical Zone Exploratory (central Germany), we, therefore, aimed to disentangle the temporal dynamics of bacterial communities. The bacterial communities in the shallow bedrock groundwater showed multi-annual cyclic dissimilarity patterns which corresponded to groundwater level fluctuation and, thus, recharge discharge periods. The impact of groundwater fluctuation and linked cross-stratal exchange on the groundwater microbial communities was associated with the recharge strength and local environmental selection strength. Sampling period was able to explain up to 29.5% of the variability in bacterial community composition (based on a 2-factorial PERMANOVA model). We observed an increase in dissimilarity over time (Mantel $P > 0.001$) indicating that the successive recharge events result in bacterial communities that are increasingly more dissimilar to the communities at the start of the sampling period. Most bacteria in the groundwater originated from the recharge-related sources (mean = 66.5%, SD = 15.1%) and specific bacterial taxa were identified as being either enriched or repressed during recharge events. Overall, we show that seasonal recharge patterns are important for shaping bacterial communities in shallow fractured-rock groundwater and act as drivers of cyclical patterns. Furthermore, the recharge events are successive shocks that perturbed the bacterial communities, leading to decreased similarity to the original state over time. These revelations highlight the importance of high temporal resolution research in the Critical Zone for investigating the complex interplay between surface/subsurface environmental dynamics and the biology of groundwater ecosystems.