

## **Physical size matters in groundwater bacterial community assembly**

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**Project Goals: ENIGMA -Ecosystems and Networks Integrated with Genes and Molecular Assemblies use a systems biology approach to understand the interaction between microbial communities and the ecosystems that they inhabit. To link genetic, ecological, and environmental factors to the structure and function of microbial communities, ENIGMA integrates and develops laboratory, field, and computational methods.**

A central issue in microbial ecology is to identify key drivers shaping community assembly and functioning, which is also fundamental for ecological modeling. Various physical-chemical and microbial drivers have been extensively investigated, including climate (e.g. temperature), chemistry (e.g. pH, carbon sources), microbial functional genes, etc. However, a basic trait, physical size of cells or the particles they attached, was rarely studied. Considering the porous habitats in subsurface, physical size should be particularly important in groundwater microbial community assembly and functioning. Small-size microbes (e.g. ultramicrobacteria) can be easier to pass through soil pores along groundwater flow; but when flow rate is limited, they may be more difficult to escape from absorption, trapping, or flocculation. In addition, small size generally leads to higher specific surface area and slower growth rate (longer division period) of bacteria. Thus, we hypothesize that physical size plays an essential role in the variation of microbial diversity, assembly, and functioning.

The groundwater in the Oak Ridge Integrated Field Research Challenge site (FRC, Oak Ridge, TN) has large geochemical gradients and diverse subsurface conditions for microbial dispersal. In the spring of 2019, three areas under different contamination levels, three groundwater wells in each area, were selected for 9-week bi-weekly sampling from March to May. Each groundwater sample was filtered in succession through 10- $\mu\text{m}$  filter mainly for large-size and particle-attached bacteria (so-called 'large'), 0.2- $\mu\text{m}$  mainly for normal-size free-living bacteria (so-called 'medium'), and 0.1- $\mu\text{m}$  for small free-living bacteria (so-called 'small'). The results of bacterial communities from 16S rRNA gene sequencing well supported our hypothesis.

The large, medium, and small-size bacteria counted for 7%, 62%, and 31% of the DNA concentrations on average, and the small-size portion was higher than 20% (up to 32% ) in some well in May, indicating these three types were all not negligible. **The size significantly affected bacterial diversity and community structure.** Alpha diversity of small-size bacteria (Shannon  $3.8\pm 1.2$ ) was generally lower than medium ( $5.4\pm 1.2$ ) and large-size bacteria ( $5.1\pm 1.1$ ). The size difference explained a substantial proportion (24.4%,  $P < 0.001$ ; higher than location and time) in the variation of bacterial alpha diversity (Shannon index), and proportion (8.4%,  $P < 0.001$ ; similar to location and time) of beta diversity (Bray-Curtis index). The location, which reflects the influence of contamination and/or spatial distance, showed obvious effect on the alpha (21% and 29%) and beta diversity (26% and 29%) of large and medium-size bacteria, but had much lower impact on small-size bacteria (14% of alpha and 10% of beta). While large and medium-size bacteria from different areas always showed different community structure, small-size bacteria from different areas became convergent after late Apr. The results might be related to better dispersal of small-size bacteria and increased precipitation in late April and May. **The size also affected the dominant phylogeny and some key functional species.** While alpha- and/or beta-Proteobacteria generally dominated in large and medium-size bacteria, the phylum Bacteroidetes significantly increased or even predominate in small-size bacteria after late April, mainly attributed to the genus *Hydrothalea*. Sulfate-reducing bacteria, a relevant functional group in this site, were mainly detected in the orders *Syntrophobacterales* and *Desulfobacterales* and Class *Thermodesulfobivrio* in both large and medium-size bacteria, but were nearly undetectable in small-size bacteria. A newly developed framework based on phylogenetic-bin-level null model analysis (iCAMP) was applied to explore **the different assembly mechanisms of bacteria with different sizes.** Based on the results, dispersal limitation generally played more important roles in large-size ( $46\pm 5\%$ ) than in medium ( $38\pm 6\%$ ) and small-size bacteria ( $23\pm 9\%$ ). In contrast, selection was obviously more influential in small ( $31\pm 12\%$ ) and medium ( $23\pm 5\%$ ) than in large-size bacteria ( $18\pm 2\%$ ). The major assembly processes, selection, dispersal limitation, and drift, all showed dramatically higher (1.5-4.0 fold) temporal variations in small (CV 0.39-0.45) than in medium (CV 0.17-0.29) and large-size bacteria (CV 0.10-0.19).

In conclusion, the size of cells or attached particles is essential in shaping groundwater microbiome. Bacteria with larger sizes or particle-attached bacteria were more affected by dispersal limitation and showed more variation among different locations but higher resistance in temporal dynamics; in contrast, smaller free-living bacteria are more sensitive to temporal change of environmental conditions, and can be easier to migrate under adequate flow rate.

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