Biomass distribution and activity of respective subsurface sediments and groundwater within a shallow subsurface ecosystem

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Abstract

Subsurface environments represent diverse microbial communities responsible for mediating biogeochemical cycles linked to the turnover of organic and inorganic carbon important to groundwater used by human society for consumption, irrigation, agriculture and industry. Within the different sediment environments, microorganisms typically reside in two distinct phases (planktonic or biofilm), and significant differences in community composition, structure and activity between free-living and attached communities are commonly accepted. However, largely due to sampling constraints and the challenges of working with solid substrata, the respective contributions of groundwater (planktonic) and sediment-associated (biofilm) cells to subsurface processes is largely unresolved. In order to directly compare the distribution of microbial biomass and activity in a shallow, subsurface environment, total cell numbers, translationally-active cell numbers (Bioorthogonal non-canonical amino acid tagging- BONCAT), and microbial activity (^{3}H-Leucine incorporation) were investigated for a low biomass pristine and contaminated groundwater and corresponding soil cores. The results demonstrated that cell numbers for the 0.2 um fraction were approximately an order of magnitude higher for the pristine groundwater compared to the contaminated groundwater (10^{6} v. 10^{5}). When contaminated groundwater was compared to the pristine, there was a drastic reduction in the BONCAT activity and the contaminated groundwater was between 100-700-fold less. Additionally, the rate of leucine incorporation (^{3}H-leucine) on a per cell basis in pristine groundwater was up to 1,000 times greater than the contaminated groundwater, respectively. Overall, like total cell numbers, activity was lower (both per volume and per cell) in contaminated groundwater compared to pristine groundwater. In pristine soil, activity (^{3}H-leucine) displayed steep gradients of microbial activity in association with

transition zones of water table height (*i.e.*, vadose, capillary fringe, saturated). A similar trend was also observed for the contaminated soil; however, the contaminated soil displayed an overall gradient of decreasing activity with depth. The highest activity for pristine soil was 9,253 ng C/g/d located in the transition depth between the capillary fringe and the saturated zone. Conversely, the highest activity for the contaminated soil was 9,175 ng C/g/d located in the vadose zone, perhaps the zone that is least impacted by contaminant flux. The pristine groundwater had higher activity rates than pristine sediment (per cell), but the contaminated groundwater had slower activity rates than the contaminated sediment (per cell). However, for both pristine and contaminated samples on a per volume basis, sediments had the vast majority of microbial activity compared to groundwater (80-95%). In the absence of strong selection forces compared to the contaminated well, the uncontaminated samples demonstrated more phylogenetic differences between the viable and translationally active populations that could be attributed to growth rate differences. The contaminated groundwater sample was predominated by a single, persistent *Rhodanobacter* strain in the viable fraction, while *Rhodococcus*, *Brevundimonas*, and *Pseudomonas* species dominated the translationally active fraction. Overall, the top active ASVs were prevalent and persistent across the estimated landscape. This is the first quantitative comparison between corresponding groundwater and subsurface sediments as well as predictions of viable and active ASVs (*e.g.*, stable analog probing- SAP) within commonly used sequencing methods. The results suggest that field sampling schemes should consist of both viability and activity-based assessments that can help delineate key microbial populations within diverse microbial communities across and within subsurface systems.

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sediment-associated microorganisms, microbial activity

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