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Publication date:
2018

Document Version
Version created as part of publication process; publisher's layout; not normally made publicly available

Citation (APA):

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Biofilm thickness controls the contribution of stochastic and deterministic processes in microbial community assembly

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Niche and neutral theories provide diverging viewpoints on the importance of selection and neutral processes in community assembly. In practice, both deterministic and stochastic factors play a role in microbial community assembly, though little is known about manipulating their relative importance. We investigated the effect of biofilm thickness on community assembly using Z-carriers®, biofilm carriers where biofilm thickness is controlled by grid height. Duplicate Z-carriers of five thicknesses (50-500 um), influent and effluent were sampled at intervals from steady-state nitrifying reactors. Extracted DNA was subjected to 16S rRNA amplicon sequencing and qPCR for Bacteria. The biofilm communities were distinct from influent and effluent communities and exhibited greater temporal stability which increased with thickness. Biofilm communities were strongly influenced by selection as few sequence variants (SVs) were shared between the carriers and influent, however, the number of shared (SVs) increased with biofilm thickness. Neutral modelling revealed that a greater percentage of shared SVs were neutrally assembled with increasing thickness, corresponding to a linear relationship between biofilm thickness and migration rate. These observations suggest that biofilm thickness modulates the relative contribution of neutral and deterministic processes on community assembly, with selection dominating in all biofilms, but stochastic factors playing a greater role in thicker biofilms. Furthermore, biofilm communities exhibited high temporal stability, which increased with thickness. We propose that the small, active volume of thin biofilms is subject to greater competition compared to thicker biofilms, where the presence of less active basal layers increases the contribution of neutral processes in community assembly.