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Establishing drinking water biofilms with varying alpha-diversity?

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Introduction

Biofilms are considered the predominant growth mode for bacterial communities in natural environments and may increase community resilience towards environmental stress. The effect of community composition on its function is of fundamental interest in microbial ecology. It has been shown that diversity can protect communities from unstable environmental conditions (Boles *et al.*, 2004), render community functions more stable, (Tilman and Snell-Rood, 2014; Schnitzer *et al.*, 2011) and increase community resistance to invasion by alien types (van Elsas *et al.*, 2012; Fargione and Tilman, 2005) . However, little is known on how to manipulate biofilm community diversity. Here, we postulate that by modifying the substrate loading conditions on biofilms we can affect biofilm community assembly and the resulting biofilm community diversity.

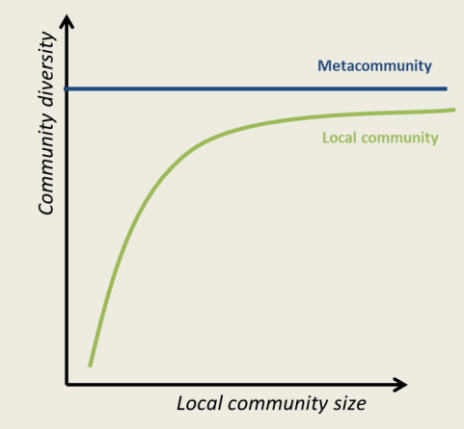

Figure 1.1 Hypothetical relation between community diversity and local community size in neutrally assembled microbial biofilms

We use drinking water biofilms as a model system to experimentally test the effect of surface loading rate on biofilm community dynamics. Our aim is to cultivate nitrite oxidizing bacteria (NOB)–enriched biofilms with varying alpha-diversity. We postulate that at higher nitrite surface loadings the microbial community is larger and has an increased diversity approximating the diversity of the source community (Figure 1.1), compared to biofilms that develop at lower nitrite surface loadings.

Material and Methods

The experimental set-up consisted of 40 parallel flow-through silicone tubes. The biofilm developed on the inner surface (surface area 8.84E-4 m^2) of the tubes by feeding with ambient tap water at a constant flow rate of 0.43 l/day. The first 20 replicates were operated at a nominal 1oading rate of 1 gN/m^2 * day while the second 20 replicates received 10-fold lower nitrogen loading: 0.1 gN/m^2 * day. Tap water was spiked $NaNO_2$ at a concentration of

2 mgN/L or 0.2 mgN/L respectively, to achieve the different loading rates. Biofilm was allowed to develop in the system for 63 days after which the developed biofilm was extracted from the tubes.

Total microbial density was determined by qPCR based on 16S rRNA gene copy numbers as described by Terada *et al.*, (2010) and NOB were quantified by targeting the functional gene *nxrB* for *Nitrobacter* and *Nitrospira* according to Pester *et al.*, (2014) and Vanparys *et al.*, (2007) respectively.

Alpha-diversity of he entire community will be determined from 16S rRNA amplicon libraries obtained via Illumina MiSeq sequencing. The gene amplicons are processed and classified using Mothur software (Schloss *et al.*, 2009) following the MiSeq SOP (Kozich *et al.*, 2013).

Results and Conclusions

We observed significantly higher total bacteria density ($p=1.5*10^{-5}$) in silicone tubes receiving higher loading rates. At higher loading rate *Nitrobacter* (p=0.03) relative abundance was higher (Figure 1.2), consistent with its known advantage over *Nitrospira* under excess nitrite supply (Nowka *et al.*, 2015). However, the abundance of NOB observed in the biofilm was 3-fold lower, than in the source community, while complete NO_2^- oxidation was observed. This may suggest limitation of the experimental system for NOB attachment on selected surfaces.

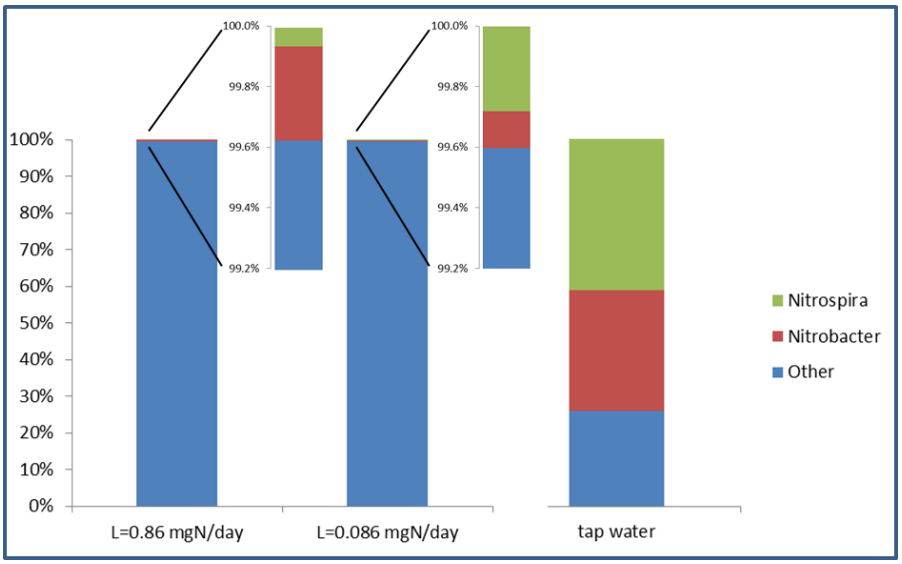

Figure 1.2 Relative abundance of NOB under two different loading conditions compared to the tap water microbial community

Results from the amplicon-library analysis will reveal whether alpha-diversity of a biofilm can simply be manipulated by altering surface loading rate. So far, we have observed significant differences in cell numbers of NOB and total bacteria. If our assumption is valid and the diversity increases with community size, as predicted by neutral assembly community theory, higher alpha-diversities are expected in biofilm communities with higher nitrite loading.

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