

# Ecological stability properties of microbial communities assessed by flow cytometry

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## Supplemental Text S6

### S6: Diversity metrics of cytometric data

In a recent study, Props et al. (2016), showed that microbial community diversity calculation based on single cell data can provide ecological information comparable to 16S rRNA amplicon sequencing. Cytometric  $\alpha$ -diversity measures reveals the phenotypic complexity of a community and is a fast tool to follow community structure changes over time. Here, following Props's study, we used the approach to get an indication on the complexity of the AMC and CMC, respectively. For example, we analysed the diversity of two samples of the AMC and CMC, respectively, before the onset of the temperature disturbance (71 h, 333 h; Text S1 in the supplemental material). The diversities  $D_q$  were determined using order-based Hill numbers (Hill 1973) and named "phenotypic diversity". In contrast to the 128 X 128 binning grid used by Props et al. (2016), we used a gate-template with 34 gates (Text S2 in the supplemental material, Section S2.4). Table S6.1 shows the resulting "phenotypic diversities" for  $D_0$  ( $q = 0$ ),  $D_1$  ( $q = 1$ ) and  $D_2$  ( $q = 2$ ) for both the AMC and the CMC. When  $q = 0$ , only richness is considered. By increasing  $q$  values, additional emphasis is placed on the actual distribution of cells over the gates (relative gate abundances). For both time points,  $D_0$  can reach a high value (34 and 33, respectively). This might be due to random effects because even a gate with only one event will be counted as one "phenotype". To avoid this problem, we introduced the average fraction of cells per gate of 2.94 % for  $D_0$  (see main text section "Cytometric evaluation tools") as a threshold to count a gate as a "phenotype". For  $D_0$  (with threshold),  $D_1$  and  $D_2$  the CMC showed always a higher diversity than the AMC. Still, the

values for the AMC were atypically high which, however, might be caused by the variable phenotypic complexity of its members (discussed in Text S5 in the supplemental material, Section S5.4). These data indicate higher diversity for the CMC which may be one reason why this community showed higher stability properties such as resilience and resistance in our experiment. Many studies discussed similar findings (Hooper et al. 2005).

**Table S6.1:** Phenotypic diversity based on single cell data for AMC and CMC states before any disturbances

States	Time points	D <sub>0</sub>	D <sub>1</sub>	D <sub>2</sub>	D <sub>0</sub> (treshhold 2.94 %)
AMC before temperature disturbance	71h	34	9.55	6.62	8
CMC before temperature disturbance	333h	33	14.23	10.29	11

## References

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- Props, R., Monsieurs, P., Mysara, M., Clement, L. & Boon, N. (2016) Measuring the biodiversity of microbial communities by flow cytometry. *Methods in Ecology and Evolution*, 7, 1376-1385.