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Flow-through SIP – A novel stable isotope probing approach limiting cross-feeding

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Stable isotope probing (SIP) is a widely applied tool to link specific microbial populations to metabolic processes in the environment without the prerequisite of cultivation, which has greatly advanced our understanding of the role of microorganisms in biogeochemical cycling. SIP relies on tracing specific isotopically labeled substrates (e.g., 13C, 15N, 18O) into cellular biomarkers, such as DNA, RNA or phospholipid fatty acids, and is considered to be a robust technique to identify microbial populations that assimilate the labeled substrate. However, cross-feeding can occur when labeled metabolites are released from a primary consumer and then used by other microorganisms. This leads to erroneous identification of organisms that are not directly responsible for the process of interest, but are rather connected to primary consumers via a microbial food web.
Here, we introduce a new approach that has the potential to eliminate the effect of cross-feeding in SIP studies and can thus also be used to distinguish primary consumers from other members of microbial food webs. In this approach, a monolayer of microbial cells are placed on a filter membrane, and labeled substrates are supplied by a continuous flow. By means of flow-through, labeled metabolites and degradation products are constantly removed, preventing secondary consumption of the substrate. We present results from a proof-of-concept experiment using nitrifiers from activated sludge as model system, in which we used fluorescence in situ hybridization (FISH) with rRNA-targeted oligonucleotide probes for identification of nitrifiers in combination with nanoscale secondary ion mass spectrometry (NanoSIMS) for visualization of isotope incorporation at the single-cell level. Our results show that flow-through SIP is a promising approach to significantly reduce cross-feeding and secondary substrate consumption in SIP experiments.