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Quantifying the contribution of single microbial cells to nitrogen assimilation in aquatic environments

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Nitrogen is a primary productivity-limiting nutrient in the ocean. The nitrogen limitation of productivity may be overcome by organisms capable of converting dissolved N2 into fixed nitrogen available to the ecosystem. In many oceanic regions, growth of phytoplankton is nitrogen limited because fixation of N2 cannot make up for the removal of fixed inorganic nitrogen (NH4+, NO2-, NO3-) by anaerobic microbial processes. The amount of available fixed nitrogen in the ocean can be changed by the biological processes of heterotrophic denitrification, anaerobic ammonium oxidation and nitrogen fixation. For a complete understanding of nitrogen cycling in the ocean a link between the microbial and biogeochemical processes at the single cell level and their role in global biogeochemical cycles is essential. Here we report a recently developed method, Halogen In Situ Hybridization-Secondary Ion Mass Spectroscopy (HISH-SIMS) and its potential application to study the nitrogen-cycle processes in the ocean. The method allows simultaneous phylogenetic identification and quantitation of metabolic activities of single microbial cells in the environment. It uses horseradish-peroxidase-labeled oligonucleotide probes and fluorine-containing tyramides for the identification of microorganisms in combination with stable-isotope-labeling experiments for analyzing the metabolic function of single microbial cells. HISH-SIMS was successfully used to study nitrogen assimilation and nitrogen fixation by anaerobic phototrophs in a meromictic alpine lake. The HISH-SIMS method enables studies of the ecophysiology of individual, phylogenetically identified microorganisms involved in the N-cycle and allows us to track the flow of nitrogen within microbial communities.