Quantitative Metaproteomics of Microbial Communities

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Proteins are the molecular agents that organisms use to drive biogeochemical cycles. Understanding of protein-level gene expression patterns – which can be quite distinct from their mRNA-level counterparts – in natural settings is a key quantitative link between community-level measurements of biogeochemical fluxes on the one hand and catalogs of taxonomic and functional diversity generated by high-throughput sequencing on the other. Obtaining this protein-level view of microbial community metabolism has been hampered, however, by the technical difficulty of identifying and quantifying proteins in heterogeneous natural samples. Here we describe a set of novel techniques for precise quantification of complex mixtures of proteins, applicable to both cultured and field-collected samples, and for identifying proteins from microbial communities using a combination of database-dependent and –independent methods. Using *in vitro* isotopic peptide labeling, we demonstrate a strategy for precise protein quantitation with limited susceptibility to analytical interferences. We show the importance of nucleic acid sequence information selection, in particular metagenomes as compared to metatranscriptomes, and processing in construction of an effective database for peptide-spectrum matching in metaproteomics. Our isotope-labeling methodology also enables high-quality *de novo*, database-independent peptide sequencing to broaden the analytical window into community metabolism. We present illustrations of the utility of these quantitative metaproteomic analyses for gaining insight into biogeochemical processes in planktonic communities, microbial mats, and marine and terrestrial sediments.