

Newly Identified Regulatory Roles for Vitamin B₁₂ Suggest Coordination of Community Metabolism

Aaron T. Wright,¹ William C. Nelson,¹ Lindsey N. Anderson,¹ Joshua J. Rosnow,¹ Bryan Killinger,¹ and **Lee Ann McCue**^{1*} (leeann.mccue@pnnl.gov)

¹Pacific Northwest National Laboratory, Richland, WA

<http://www.pnnl.gov/biology/programs/fsfa/>

Project Goals: The goal of the Metabolic and Spatial Interactions in Communities (MOSAIC) Foundational Scientific Focus Area is to understand the fundamental mechanisms by which microbial metabolic interactions and spatial organization impact carbon, nitrogen, and energy dynamics in microbial communities. Our studies focus on the coupling of carbon and nitrogen cycles in microbial communities, the role of environmental variables in governing the rates of these cycles, and the impact of environmental perturbations on microbial community dynamics. We employ tractable model consortia whose member genome sequences have been defined, advanced omics measurements, functional imaging, taxonomic profiling, and modeling to elucidate interaction mechanisms within complex microbial communities. Our research supports the DOE goals to achieve a predictive understanding of Earth's integrated biogeochemical processes.

Individual members of complex microbial communities can interact by exchanging metabolites and signaling compounds. Using genome-informed approaches coupled to proteomics, chemical probing, and other molecular biology studies, we have predicted and tested B vitamin dependencies in a model autotroph-heterotroph community. In particular, our findings suggest requirements for vitamin B₁₂ exchange to support community growth and to regulate microbial metabolism.

Vitamin B₁₂ encompasses a group of closely related corrinoid compounds best known for their role as cofactors of enzymes, and also for playing regulatory roles by binding to riboswitches. Recent studies have also revealed that vitamin B₁₂ is a cofactor of transcriptional regulators. A portion of vitamin B₁₂ is photolabile, thereby when bound to these proteins it provides light-dependent regulation of transcription, controlling processes such as biosynthesis of carotenoids, tetrapyrroles, and photosystems. To identify new roles for B₁₂, we developed a chemical probe that mimics the natural vitamin, in fact to such an extent as to support microbial growth, transcription, and translation. This probe was deployed in individual bacterial isolates, and within a photoautotroph-heterotroph community. Coupling the probe to proteomics analyses, we identified more than 50 B₁₂-binding proteins including enzymes known to use it as a cofactor, a transcriptional regulator, enzymes in the one carbon pool by folate pathway, and enzymes involved in ubiquinone biosynthesis. Importantly, we also identify a B₁₂-dependent role for MetE (methionine synthase) in the photoautotroph when the organism limits vitamin salvage. The unexpected discovery of B₁₂ involvement in these processes suggests a pivotal role in the control

of cell growth, potentially leading to coordination of cell behavior in complex multicellular systems. We predict that these roles for B₁₂ may be generalizable in myriad communities, a possibility that we are now exploring.

This research was supported by the U.S. Department of Energy (DOE), Office of Biological and Environmental Research (BER), as part of BER's Genomic Science Program (GSP). This contribution originates from the GSP Foundational Scientific Focus Area (FSFA) at the Pacific Northwest National Laboratory (PNNL). A portion of this work was performed in the William R. Wiley Environmental Molecular Sciences Laboratory (EMSL), a national scientific user facility sponsored by OBER and located at PNNL. PNNL is a multi-program national laboratory operated by Battelle for the DOE under Contract DE-AC05-76RLO 1830.