Applying Metabolic Models to Mechanistically Understand and Predict Interactions Between ANME and SRB Strains in Geochemical Cycling Processes

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Project Goals:

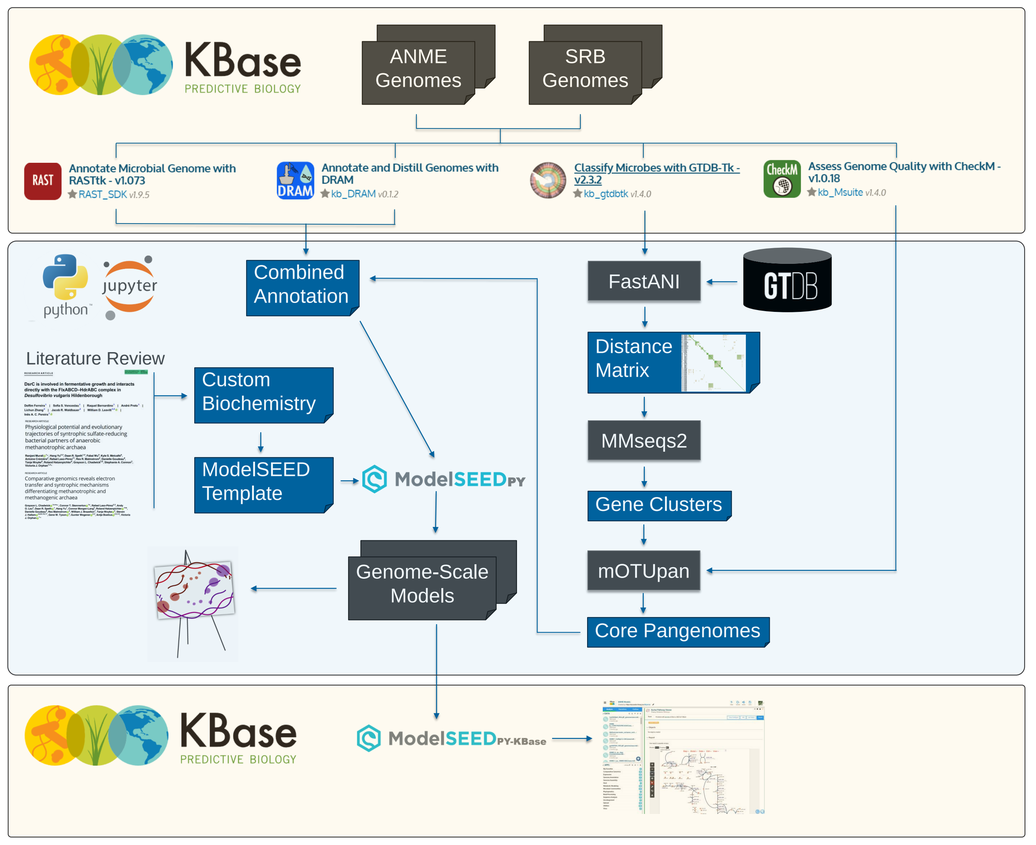
1) Investigate metabolic syntropy between anaerobic methane oxidation (AOM) archaea with sulfate-reducing bacteria.
2) Design a coupled methane (archaea) + sulfate (SRB) ETC model.
3) Evaluate the interaction between diverse strains of AOM archaea and SRB.

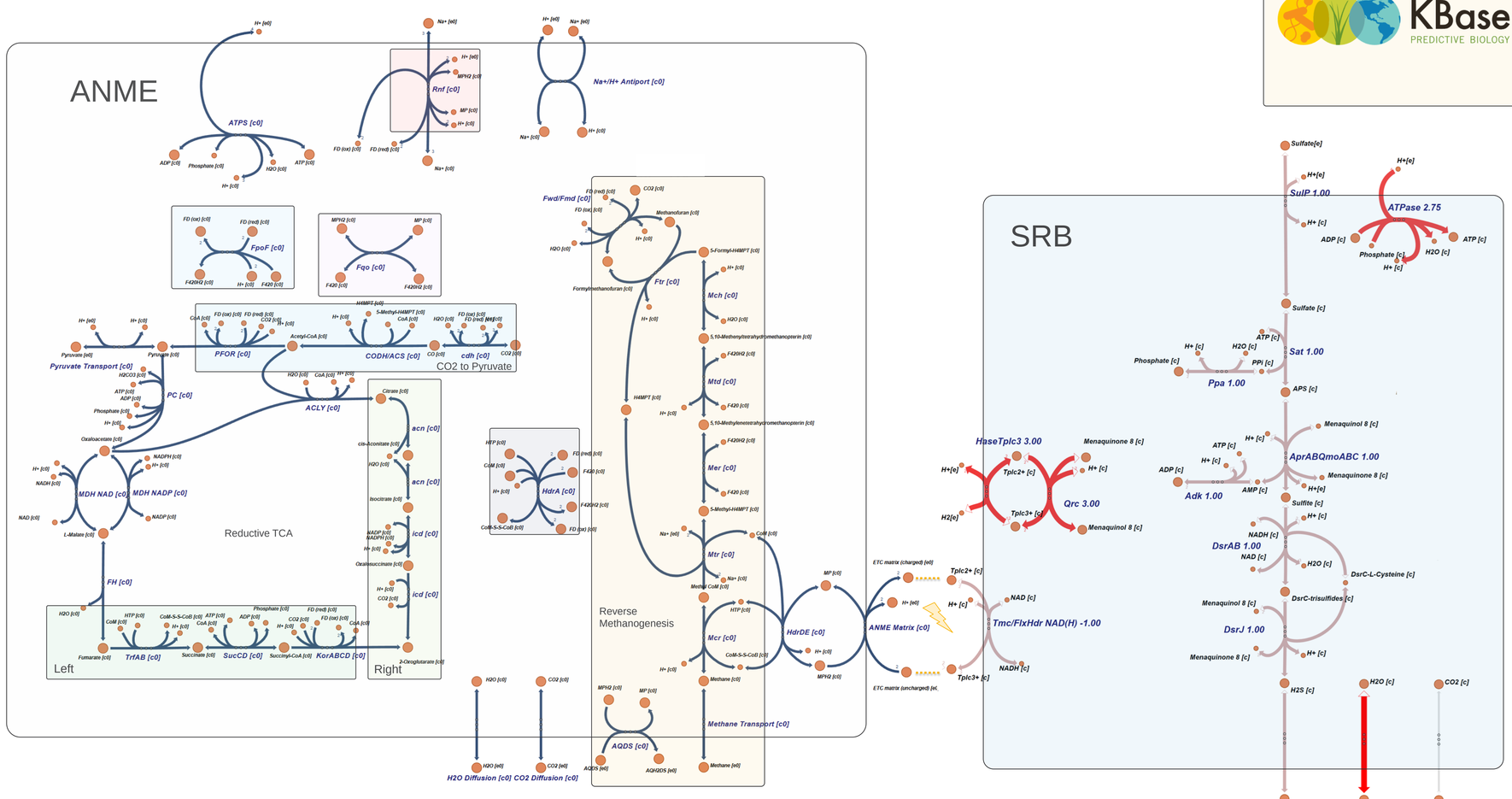
Introduction: Microbial communities of methanotrophic archaea (ANME) and sulfate-reducing bacteria (SRB) annually prevent gigatons of methane from being released into the environment, and therefore are critical agents in climate regulation and geochemical cycling. The anaerobic oxidation of methane (AOM) in ANME is the "reverse methanogenesis" pathway, which requires electron transfer via sulfates to SRB through direct interspecies electron transfer (DIET) mechanisms. Our ANME+SRB communities are an obligate syntrophic species; however, ecological mechanisms of these communities remain opaque, partly because this community is unculturable in laboratory conditions. To model this metabolic system, we improved our ModelSEED reconstruction pipeline for all archaea and bacteria to construct genome-scale metabolic models in the improved ModelSEED2 tool. The improvement process involved 1) annotating >40 genomes, 2) assembling pangenomes to compensate inconsistencies, and 3) developing new biochemical templates, which captures more clade-specific metabolic pathways and reaction intermediates that are unique to archaea and enable understanding this community.

Results: We concurrently developed a suite of community modeling tools to mechanistically simulate the syntrophic interactions under native conditions, which is essential to contextualize the ecological roles of ANME and SRB. These community modeling tools further permit the parameterization of omics data that represent metabolic phenotypes and thermodynamic and uptake constraints that reflect molecular characteristics of the system, which improves simulation accuracy. Further, we developed tools that leverage pangenome information from phylogenetically close strains to improve model reconstruction for incomplete metagenome-assembled-genomes (MAGs), which is essential because the unculturable nature of ANME means that MAGs are their only available sequences and their limited biomass available makes their MAGs incomplete. We therefore applied a pangenome-based approach that enhances our ANME MAG models by including all core genes, which adds hundreds of conserved reactions to ANME MAGs while preserving the distinctive metabolic features that distinguish each ANME clade.

We construct metabolic models from several ANME and SRB MAGs that were assembled and binned from metagenomic data in previous studies [1, 2], and implemented an energy metabolism pathway that couples the anaerobic methane oxidation with the sulfate reduction pathway. We finally perform a detailed accounting for the flow of nutrients and energy within our community model to mechanistically explain low yields and slow growth in these systems.

Conclusions: The improved annotation accuracy of these models empowers community simulations towards evaluating the "reverse methanogenesis" hypothesis and may explain the natural stability and selectivity of these communities, which would clarify vulnerabilities of these communities to anthropogenic influences.





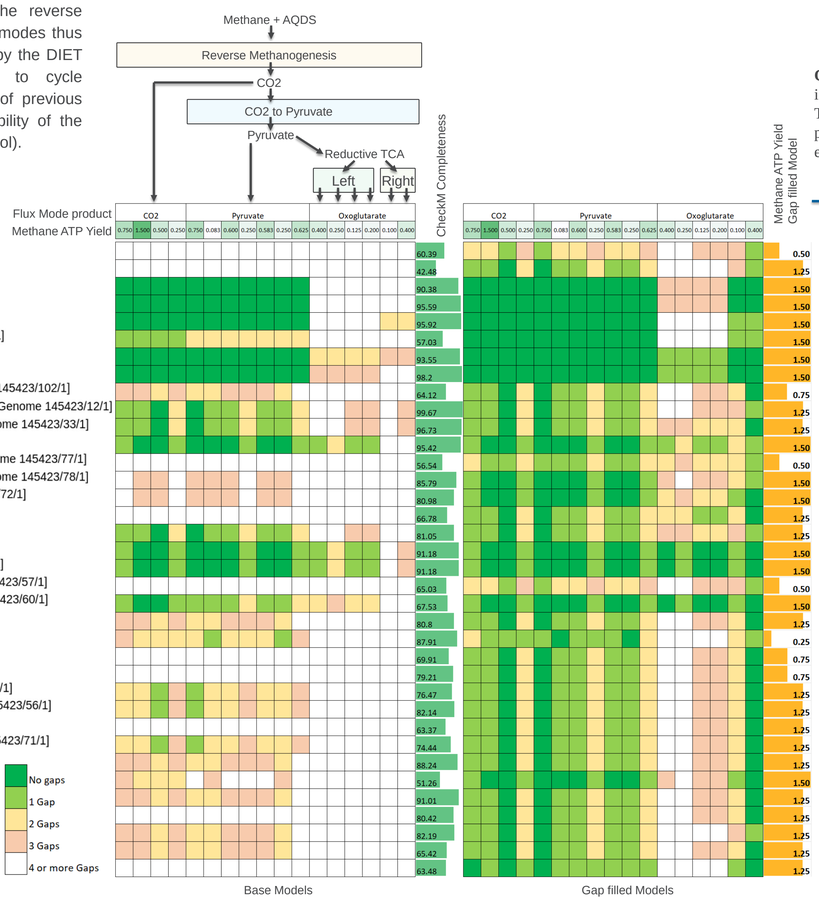
Model Reconstruction Process: The ModelSEED reconstruction framework utilizes RAST to identify gene functions, however many of the DIET genes are poorly characterized and several of the archaeal ETC complexes are often confused with the bacterial homologs (e.g., Nuo and Fpo). For this project we combined RAST and DRAM annotation together to maximize our odds to annotate our genomes since many of the MAGs involved are already in poor quality with a completeness score below 70%.

We compare RAST, DRAM with a curated set of annotation from *Chadwick* et al. [2] to identify genes that might not be possible to be correctly annotated with the current tools from ModelSEED. On the left branch of the reductive TCA pathway, the TfrA is never detected in both RAST or DRAM while TfrB is only assigned by DRAM, in this case we use DRAM to detect the presence of this reaction only if TfrB is present. Most of the DIET genes are never annotated, however, for our models this is not a problem since we assume that the matrix is always present in every of the ANME/SRB strains in this study.

To mitigate the uncertainty of the MAGs we employ a pangenome approach to identify missing genes in our genomes. We use FastANI to search related genomes from the GTDBtk dataset which contains more than 300000 genomes, then we assemble pangenomes to compute the core pangenome of our MAGs. We use the core pangenome to propagate missing functions to our models.

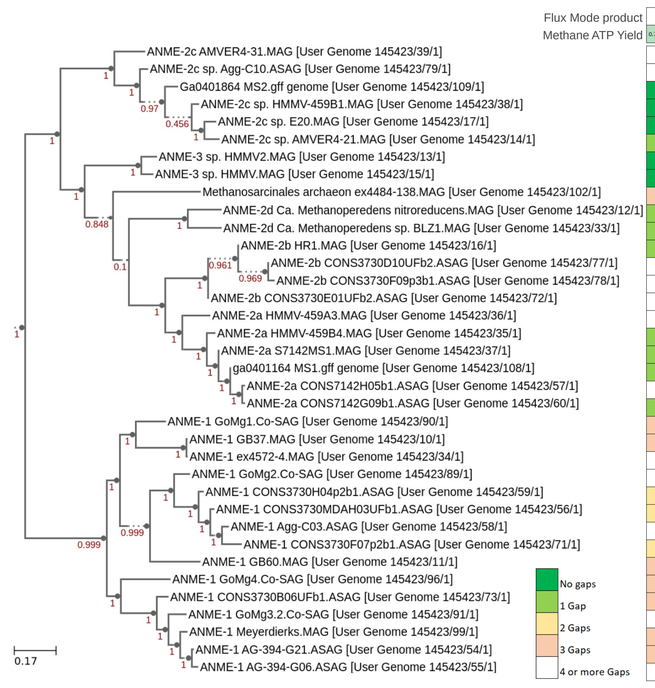
	Features	Core	New
ANME-2b_HR1.MAG	1024	506	0
ANME-2b_CONS3730D10UFb2.ASAG	693	506	137
ANME-2b_CONS3730F09p3b1.ASAG	979	506	32
ANME-2b_CONS3730E01UFb2.ASAG	963	506	29
ANME-2a_HMMV-459B4.MAG	798	520	0
ANME-2a_S7142MS1.MAG	1022	520	0
ANME-2a_CONS7142H05b1.ASAG	787	520	186
ANME-2a_CONS7142G09b1.ASAG	955	520	114
ANME-1_GoMg1.Co-SAG	809	474	52
ANME-1_GB37.MAG	692	403	1
ANME-1_ex4572-4.MAG	545	403	1
ANME-1_GoMg2.Co-SAG	729	474	80
ANME-1_CONS3730H04p2b1.ASAG	818	416	49
ANME-1_CONS3730MDAH03UFb1.ASAG	958	416	15
ANME-1_Agg-C03.ASAG	608	416	95
ANME-1_CONS3730F07p2b1.ASAG	902	416	0
ANME-1_CONS3730B06UFb1.ASAG	961	474	0
ANME-1_GoMg3.2.Co-SAG	814	358	53
ANME-1_Meyerdierks.MAG	880	358	0
ANME-1_AG-394-G21.ASAG	722	358	0
ANME-1_AG-394-G06.ASAG	618	358	59

Pathway Design and Evaluation: We introduced custom biochemistry from the literature to represent the direct interspecies electron transfer between the two microbes. Our latest ANME AOM pathway contains 43 EFMs which 17 are ATP generating modes. The reverse methanogenesis branch is essential for every calculated EFM including non ATP modes thus guaranteeing that Methane is essential for energy. The AQDS which is replaced by the DIET matrix forces our models to form an obligate syntrophic relationship to cycle methanophenazine. The SRB metabolism was adapted from several literature of previous studies of *Desulfovibrio vulgaris* Hildenborough [3]. We knocked out the possibility of the system to receive electrons from carbon uptake (previously Lactate, Formate, Ethanol).

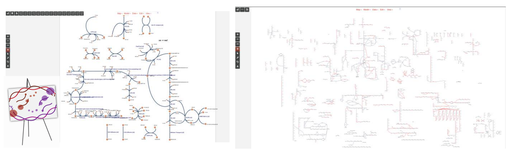


Community Modelling: Experimental fluxomic data from laboratory microcosm studies indicates that only a small fraction of the methane utilized is converted into ANME biomass.

To simulate this behavior, we increase the maintenance ATP of ANME to push more methane into the ANME metabolism. At this stage of the project further research is required in order to validate the interactions between the two-member community, common metabolites are attempted to exchange are Acetate, Formate, Pyruvate. We have not yet conducted an auxotrophy analysis of the strains.



Data Visualization: We provide metabolic network visualization with our Escher tool https://modelseed.org/annotation/projects/anme/. The ETC map allows to display and view all individual EFM of the ANME ETC network. While the Genome-Scale map shows the metabolism that was capture by all ANME, individual clades or strains. The maps are also available in KBase.



https://modelseed.org/annotation/projects/anme/

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