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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.





Methane + AQDS

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 Cor	e New	
1024	506	0

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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

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).

> —ANME-2c AMVER4-31.MAG [User Genome 145423/39/1] -ANME-2c sp. Agg-C10.ASAG [User Genome 145423/79/1]

> ANME-3 sp. HMMV2.MAG [User Genome 145423/13/1]

¹ ANME-3 sp. HMMV.MAG [User Genome 145423/15/1]

0.848

-Ga0401864 MS2.gff genome [User Genome 145423/109/1]

ANME-2c sp. E20.MAG [User Genome 145423/17/1]

ANME-2b HR1.MAG [User Genome 145423/16/1]

Pathway Design and Evaluation: We introduced custom biochemistry from the literature to

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.

Community Modelling

	HOTSEE	R SRB13	SRB18	RB		Hotseer	SRB13	SRB18	SRBJ		Hotseen	58818	SRB18	SRB2		Hotseep	SRB13	5RB18	SRBI		Hotseet	ې چ	18 ¹² 5	1818 c	RBL
	0.08546	0.117592	0.13747 0.	.205311		60.88825	96.07288	92.02827	63.57201		14.31%	19.69%	23.01%	34.37%		-2.86%	-2.60%	-2.90%	-3.44%			5	7	7	10
	0.154451	0.194372	0.240373 0.	.242238		45.9131	58.34655	43.56617	46.35706		25.86%	32.54%	40.24%	40.55%		-2.04%	-2.40%	-2.94%	-2.38%		1	2	13	13	17
	0.145891	0.210569	0.249573 0.	.228934		63.68243	59.0041	50.46541	44.09355		24.42%	35.25%	41.78%	38.33%		-1.63%	-2.35%	-2.79%	-2.56%		1!	5	15	15	15
	0.14528	0.20733	0.249088 0.	.228029		63.62267	59.39853	50.35469	44.07737		24.32%	34.71%	41.70%	38.17%		-1.62%	-2.31%	-2.78%	-2.54%		1!	5	15	15	15
	0.14582	0.207632	0.249767 0.	.228705		63.56016	59.32437	50.14688	43.87332		24.41%	34.76%	41.81%	38.29%		-1.63%	-2.32%	-2.79%	-2.55%		1!	5	15	15	15
	0.184573	0.231274	0.253603 0.	.235105		55.76249	56.74562	47.9232	51.07279		30.90%	38.72%	42.46%	39.36%		-2.06%	-2.58%	-2.83%	-2.62%		1!	5	15	15	15
()	0.130219	0.205608	0.215507 0.	.213382		66.50962	60.88173	57.31269	55.17023		21.80%	34.42%	36.08%	35.72%		-1.45%	-2.29%	-2.41%	-2.38%		1!	5	15	15	15
ane	0.130455	0.205623	0.217027	0.21489		66.44125	60.79143	56.89535	54.75936		21.91%	34.54%	36.45%	36.09%		-1.46%	-2.30%	-2.43%	-2.41%		1!	5	15	15	15
th	0.181781	0.181781	0.181781 0.	.181781		178.1382	75.00231	78.82303	86.61494		30.43%	30.43%	30.43%	30.43%		-4.06%	-4.06%	-4.06%	-4.06%			7	7	7	7
Ĕ	0.105581	0.188598	0.21543 0.	.214254		70.89162	45.19131	43.02171	43.27815		17.68%	31.57%	36.07%	35.87%		-1.36%	-2.37%	-2.69%	-2.46%	SS	1	2	13	13	14
Σ	0.105867	0.180305	0.217304 0.	.217002		70.77294	53.22264	43.10457	43.52511		17.72%	30.19%	36.38%	36.33%	4	-1.37%	-2.26%	-2.71%	-2.49%	na:	1	2	13	13	14
<u>L</u>	0.222994	0.238778	0.263848 0.	.243962		63.94241	60.54394	48.43626	43.87992		37.33%	39.97%	44.17%	40.84%	A	-1.49%	-1.45%	-1.50%	-1.49%	io	19	9	19	19	19
10	0.105024	0.129614	0.168265	0.15328		47.04139	69.57717	60.06214	57.42595	SS	17.58%	21.70%	28.17%	25.66%	JCe	-1.76%	-1.74%	-1.96%	-2.05%	В	1	0	12	14	12
RB	0.162217	0.210152	0.252123	0.23		60.73736	58.24872	49.68608	43.61015	Ша	27.16%	35.18%	42.21%	38.50%	nar	-1.81%	-2.35%	-2.81%	-2.57%	e to	1!	5	15	15	15
% S	0.223501	0.239307	0.264452 0.	.244514	9	63.90588	60.50087	48.36397	43.79822	3io	37.42%	40.06%	44.27%	40.93%	Itel	-1.50%	-1.46%	-1.51%	-1.49%	ane	19	9	19	19	19
ñ	0 123644	0 186141	0 231364 0	227681	č	47 09589	55 93264	43 91392	44 54774		20 70%	31 16%	38 73%	38 12%	2.	-1 61%	-2 46%	-3.09%	-3 04%	Ž	1	2	12	12	12





1304	0.227001	—	47.09509	55.55204	43.91392	44.34774	ш	20.7076	51.10%	30.7370	30.1270		-1.0170	-2.4070	-3.0970	-3.0470	Ŧ	 12	12	
6544	0.197406	cha	71.42282	50.89376	42.52138	42.31545	Σ	17.13%	28.38%	32.90%	33.05%	Ĕ	-1.33%	-2.13%	-2.46%	-2.27%	Å	12	13	
0991	0.225295	ĔŇ	66.07867	60.33589	52.39097	45.39473	AN	22.25%	34.67%	40.48%	37.84%	, ,	-1.48%	-2.31%	-2.70%	-2.52%	2%	15	15	
0991	0.225295	n	66.07867	60.33589	52.39097	45.39473	ę	22.25%	34.67%	40.48%	37.84%	bel	-1.48%	-2.31%	-2.70%	-2.52%	5	15	15	
7342	0.152514	ŭ	46.96226	69.47774	59.98111	57.40856	e	17.51%	21.60%	28.01%	25.53%	stl	-1.75%	-1.73%	-1.95%	-2.04%	1%	10	12	
5096	0.222716	lec	66.25489	60.58944	53.89239	46.42695	hai	22.04%	34.53%	39.36%	37.29%	P	-1.47%	-2.30%	-2.62%	-2.49%	til	15	15	
7483	0.289432	ш	149.8804	214.2785	119.7309	61.61481	let	35.22%	48.30%	48.13%	48.45%	ne	-2.82%	-2.74%	-2.73%	-2.75%	n	12	17	
0844	0.060844		28.98474	27.20698	27.20698	26.40033	Σ	10.19%	10.19%	10.19%	10.19%	hal	-4.07%	-4.07%	-4.07%	-4.07%	Σ	2	2	
5811	0.185811		110.2268	46.23266	42.74001	40.01188	%	31.11%	31.11%	31.11%	31.11%	let	-4.15%	-4.15%	-4.15%	-4.15%	ЧL	7	7	
5641	0.185641		126.1681	75.59853	43.96139	43.96139		29.64%	31.18%	31.18%	31.18%	2	-3.95%	-4.16%	-4.16%	-4.16%	A X	7	7	
7707	0.289432		143.7884	236.5574	180.9371	102.8704		33.71%	45.86%	48.17%	48.45%	%	-2.70%	-2.63%	-2.74%	-2.75%	Ja)	12	17	
6071	0.236071		159.1279	126.664	58.27429	51.37196		34.81%	39.52%	39.52%	39.52%		-2.78%	-3.16%	-3.16%	-3.16%	2	12	12	
2312	0.243741		45.94737	58.61928	43.66024	46.64473		25.98%	32.72%	40.57%	40.81%		-2.05%	-2.41%	-2.97%	-2.39%		12	13	
9932	0.227198		48.84057	55.57511	43.61956	44.17098		24.22%	31.04%	38.49%	38.04%		-1.91%	-2.45%	-3.08%	-3.04%		12	12	
7483	0.289432		149.9185	115.3723	66.25679	60.1877		48.45%	48.30%	48.13%	48.45%		-3.80E-17	-1.75E-17	1.75E-17	-5.55E-17		19	19	
7483	0.289432		71.42727	65.19049	49.35282	44.87306		48.45%	48.30%	48.13%	48.45%		3.80E-17	1.05E-12	-2.05E-17	2.05E-17		19	19	
4302	0.244457		45.92425	58.75728	43.78774	46.78176		26.04%	32.80%	40.68%	40.92%		-2.05%	-2.41%	-2.97%	-2.40%		12	13	
3049	0.244657		45.9324	58.72149	43.74155	46.70552		26.08%	32.80%	40.69%	40.96%		-2.05%	-2.41%	-2.97%	-2.40%		12	13	
3082	0.219984		83.42987	53.94751	42.62869	42.09828		19.60%	30.11%	35.67%	36.83%		-1.53%	-2.22%	-2.61%	-2.16%		12	13	
8072	0.289432		150.6609	215.361	120.5174	61.61481		35.28%	48.40%	48.23%	48.45%		-2.82%	-2.74%	-2.73%	-2.75%		12	17	

33.57% 39.84%

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.



References:

49.49339 60.09443 45.17543 44.93

H2S[e]

H2S Transport 1.00

H2O Diffusion -4.00 CO2 Diffusion (nd)

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-1.95% -2.66% -3.19%

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