

Characterizing chemoautotrophy and heterotrophy in marine archaea and bacteria with single-cell multi-isotope nanoSIP

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Figure S3. Top 20 most abundant archaeal OTUs *in situ* and during incubation as assessed by 16S rRNA sequencing of seawater from (A) Pacifica and (B) SPOT. Data is rescaled from Figure 2 to better demonstrate the stability of the archaeal community composition. Each bar represents a single sample or biological replicate (technical replicates were averaged). Red text and bars

indicate incubations amended with bacterial antibiotics. Relative abundance out of total community. Colors are shared between sites. Asterisks indicate samples that were also analyzed via nanoSIMS.

Figure S4. OTU-level changes in community composition over time and between treatments. Relative abundance of the top 50 most abundant OTUs and NMDS ordination of the Bray-Curtis Dissimilarity of the microbial communities *in situ* and during incubation as assessed by 16S rRNA sequencing of seawater from (A,B) Pacifica and (C,D) SPOT are shown. Bray-Curtis Dissimilarity was calculated using all OTUs in a sample. The number adjacent to each symbol in the NMDS plots indicates the day that sample was taken.

Figure S5. Archaeal community composition *in situ* and during incubation as assessed by 16S rRNA sequencing separated by the three major archaeal groups detected: MGI Thaumarchaeota (A+D), MGII Euryarchaeota (B+E) and MGIII Euryarchaeota (C+F), at both Pacifica (A-C) and SPOT (D-F). Shown as relative abundance of archaeal OTUs. The top 20 most abundant OTUs are shown for MGI and MGII, and the top 10 for MGIII. Each color represents a unique OTU.

Table S1. SPOT Contextual Data. BDL indicates below detection limit. Detection limit for ammonium was 10 nM.

Measurement Description	Value	Units	Reference	Method
Depth of chlorophyll fluorescence max	45	m	Parada et al 2017	CTD measurement
Ammonium concentration	BDL	μM	This Study	Standard fluorometric method, Holmes et al. (1999)
Nitrite concentration	BDL	μM	Parada et al 2017	Standard colorimetric measurement, Parsons et al. (1984)
Nitrate concentration	18.485	μM	Parada et al 2017	Standard colorimetric measurement, Parsons et al. (1984)
Oxygen concentration	3.1142	ml/l	Parada et al 2017	CTD measurement
Phosphate concentration	1.48	mM	Parada et al 2017	Standard colorimetric measurement, Parsons et al. (1984)
Salinity	33.7329	PSU	Parada et al 2017	CTD measurement
Temperature	9.4716	°C	Parada et al 2017	CTD measurement
Bacterial productivity	9779.43	cells/ml/day	Parada et al 2017	Leucine incorporation, Fuhrman et al. (2006)
Cell turnover	38.1	Day	Parada et al 2017	Cell abundance divided by leucine incorporation, Fuhrman et al. (2006)

Table S2. Summary of experiments and analyses.

		Incubation Amendments					Analyses				
		Incubation	¹³ C-	¹⁵ N-	¹³ C- ¹⁵ N-Amino						
		Time	bicarbonate	Amino Acids	Acids (nM	Streptomycin	Ampicilin	Cell	16S rRNA	High-Throughput	
		(Days)	(% final)	(nM added)	added)	(mg/ml)	(mg/ml)	enumeration	survey	NanoSIMS Metabolic	FISH-
		Name								Screening	NanoSIMS
SPOT	--	0						Y	Y	Y	
	A6	2	13	50				Y	Y	Y	Y
	A1	2	13	50				Y	Y		
	B2	7	13	50				Y	Y	Y	
	A3	7	13	50				Y	Y		
	A7	7	13	50		0.05	0.05	Y	Y		
	A8	7	13	50		0.05	0.05	Y	Y	Y	
Pacifica	D1	3						Y	Y	Y	
	D4	3	7.5	100				Y	Y		Y
	D6	6	7.5	100				Y	Y	Y	Y
	D9	6	7.5	100		0.05	0.05	Y	Y		
	D8	6			104			Y	Y	Y	Y

Table S3. Average Bray-Curtis Similarities between replicate mock communities and samples. The number of pairwise comparisons are given by n. All replicates include PCR replicates, different filter extractions of the same sample, and different elutions of the same extraction.

	Bray Curtis Similarity Mean \pm SE (n)
Even Mock Communities	90.9 \pm 0.260 (190)
Staggered Mock Communities	90.4 \pm 0.245 (210)
Pacifica and SPOT PCR Replicates	74.1 \pm 4.9 (6)
Pacifica and SPOT All Replicates	73.6 \pm 0.85 (30)

Table S4. Cell density over time in bottle incubations with SPOT water. Results for duplicate incubations are shown.

Chemical Amendments															
Incubation Time (days)	Unamended			¹³ C-bicarbonate + ¹⁵ N-Amino Acids						¹³ C-bicarbonate + ¹⁵ N-Amino Acids + Antibiotics					
	cells/ml			cells/ml			cells/ml	SD	cells/ml			cells/ml			
	x10 ⁵	SD	x10 ⁵	x10 ⁵	SD	x10 ⁵	x10 ⁵	x10 ⁵	x10 ⁵	SD	x10 ⁵	x10 ⁵	SD	x10 ⁵	
0	5.80		1.01												
2				2.98	0.43		2.62	0.30							
7				4.79	0.53		5.29	0.64		4.81	0.72		3.74	0.52	

Table S5. Cell density over time in bottle incubations with Pacifica water.

Incubation Time (days)	Chemical Amendments													
	Unamended			¹³ C-bicarb + ¹⁵ N-AAs			¹³ C-bicarb + ¹⁵ N-AAs + Antibiotics		¹³ C- ¹⁵ N-AAs			¹³ C- ¹⁵ N-AAs + Antibiotics		
	cells/ml			cells/ml			cells/ml		cells/ml			cells/ml		
	SD			SD			SD		SD			SD		
	x10 ⁶	SD	x10 ⁶	x10 ⁶	SD	x10 ⁶	x10 ⁶	SD	x10 ⁶	SD	x10 ⁶	x10 ⁶	SD	x10 ⁶
0	3.84	0.72												
3	5.57	1.41		4.58	1.69		2.44	0.43						
6				2.44	0.64		1.12	0.29		2.37	0.24		1.75	0.48

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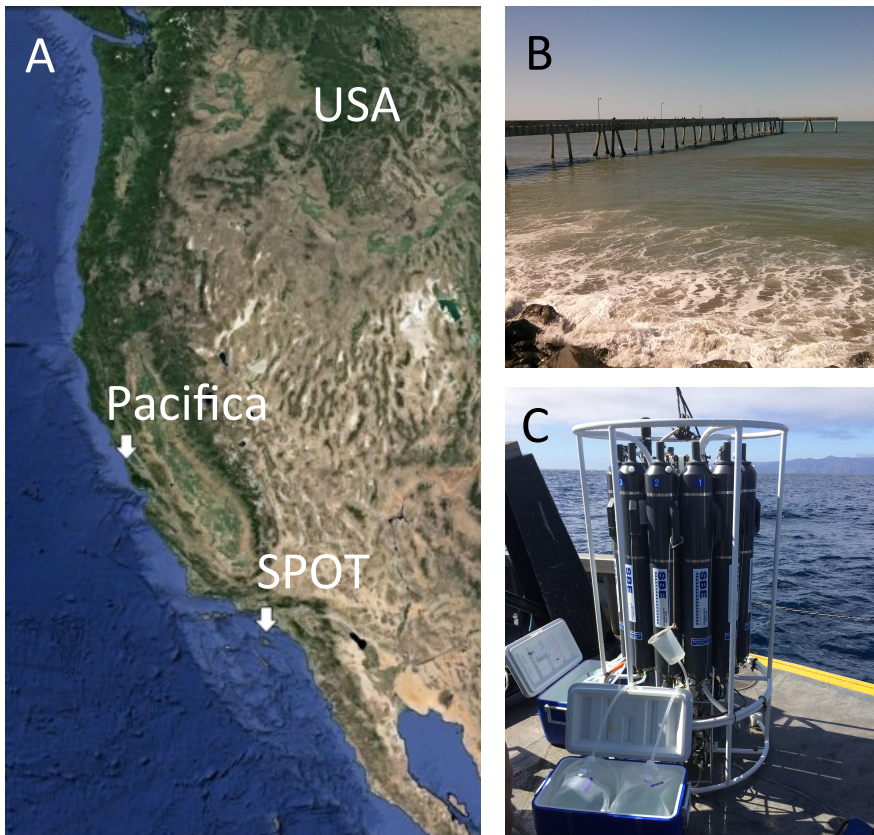


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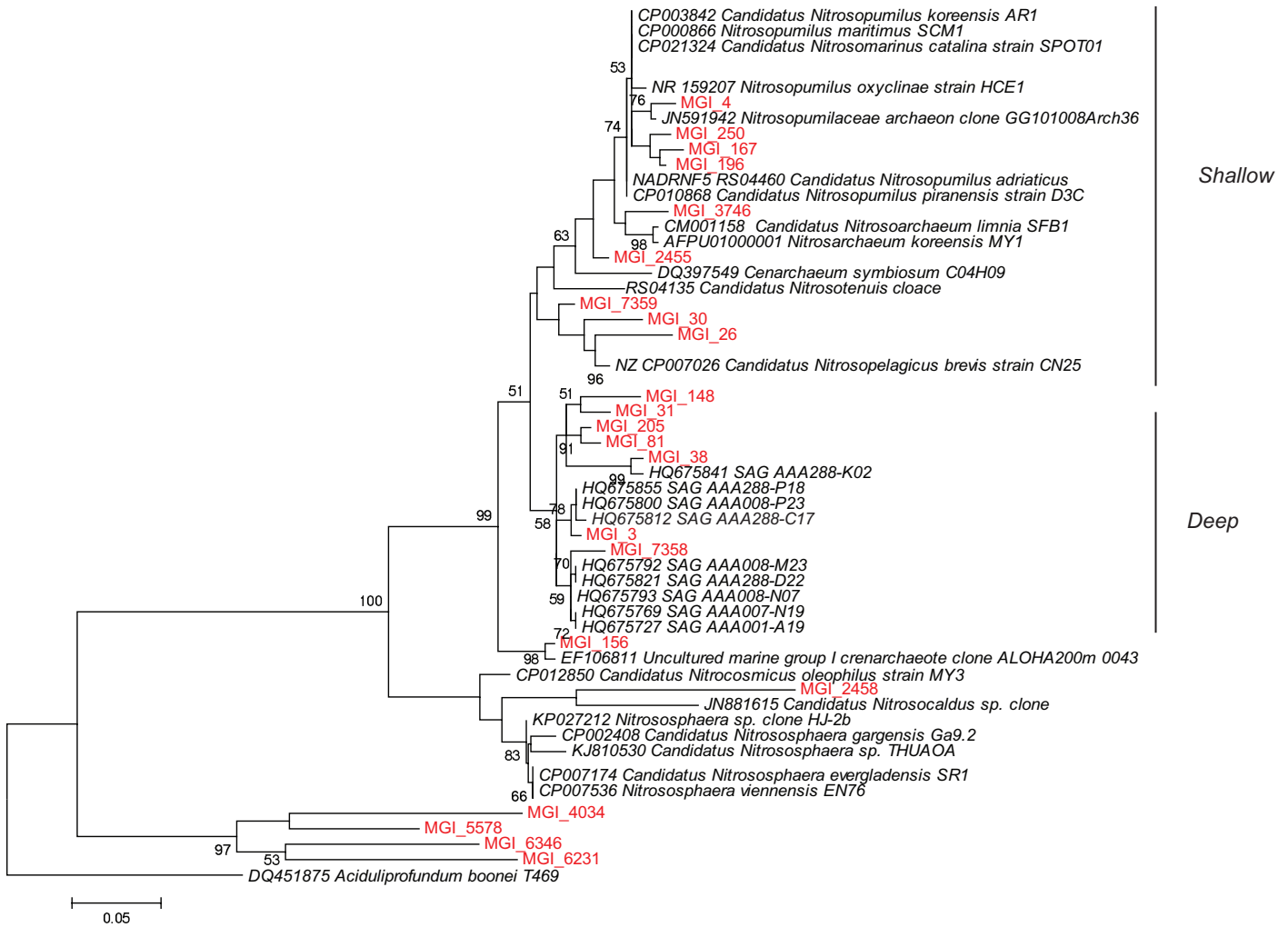


Figure S3. Top 20 most abundant archaeal OTUs *in situ* and during incubation as assessed by 16S rRNA sequencing of seawater from (A) Pacifica and (B) SPOT. Data is rescaled from Figure 2 to better demonstrate the stability of the archaeal community composition. Each bar represents a single sample or biological replicate (technical replicates were averaged). Red text and bars indicate incubations amended with bacterial antibiotics. Relative abundance out of total community. Colors are shared between sites. Asterisks indicate samples that were also analyzed via nanoSIMS.

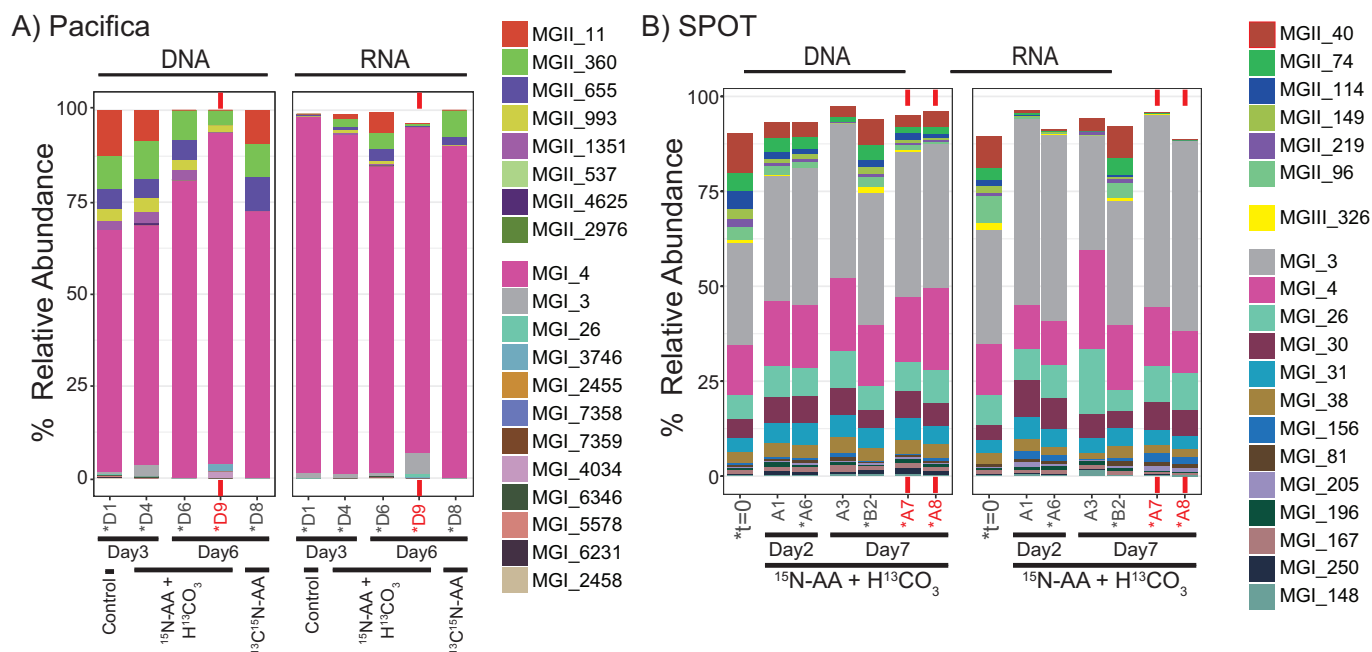
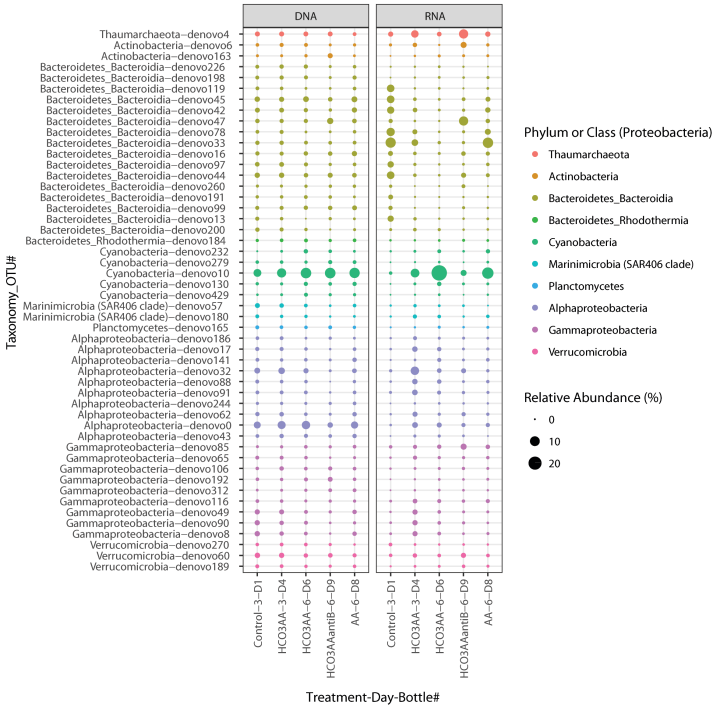
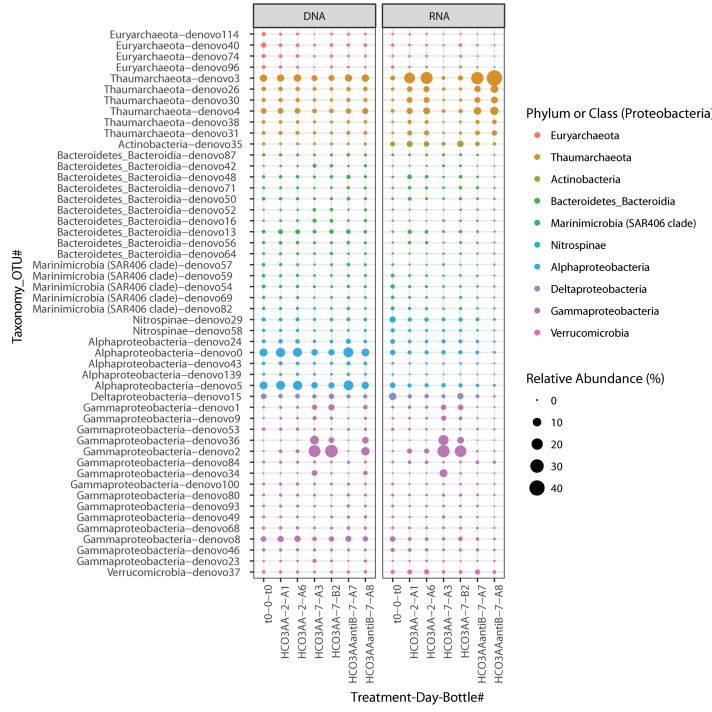


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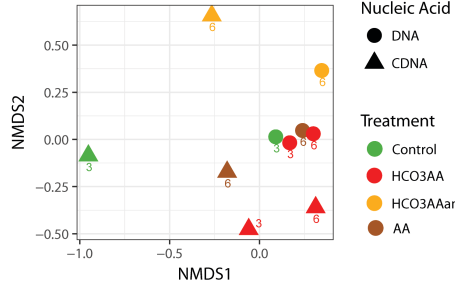
A) Relative Abundance of Top 50 Pacifica OTUs



C) Relative Abundance of Top 50 SPOT OTUs



B) NMDS of Pacifica Communities



D) NMDS of SPOT Communities

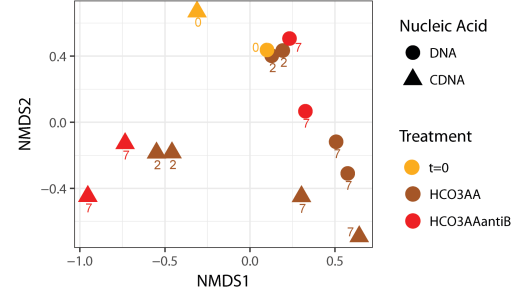


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