

Supplementary Information

**Comparative Metagenomics of Anaerobic Digester
Communities Reveals Sulfidogenic and Methanogenic
Microbial Subgroups in Conventional and Plug-Flow
Residential Septic Tank Systems**

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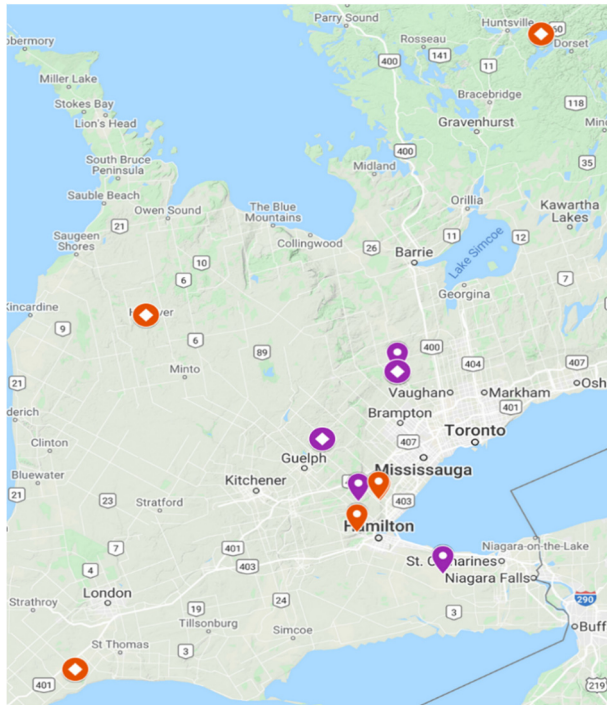
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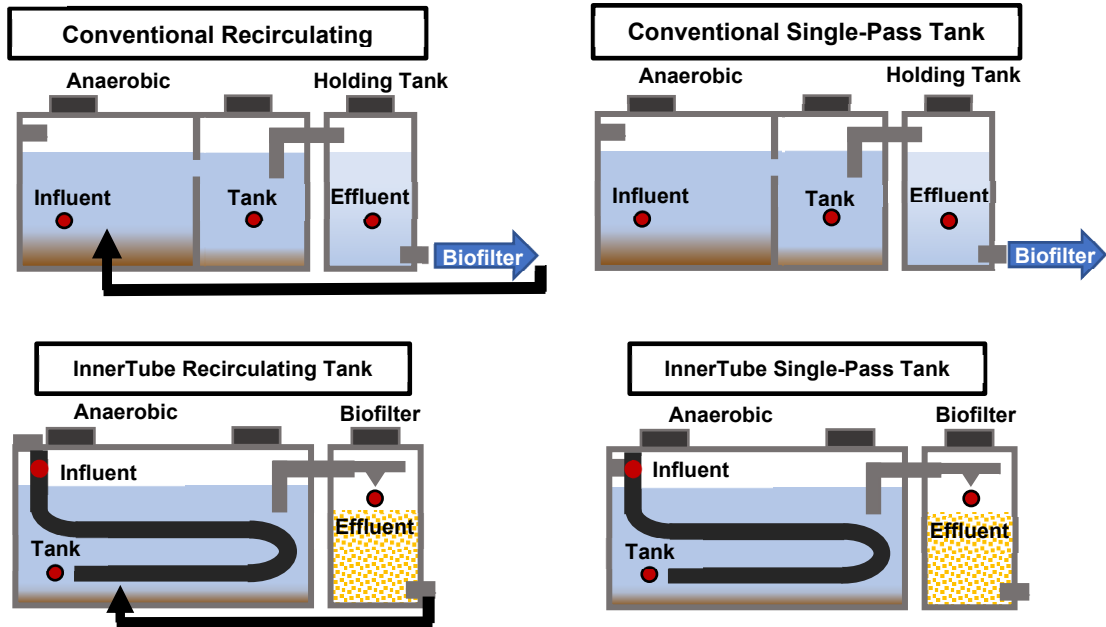
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A.

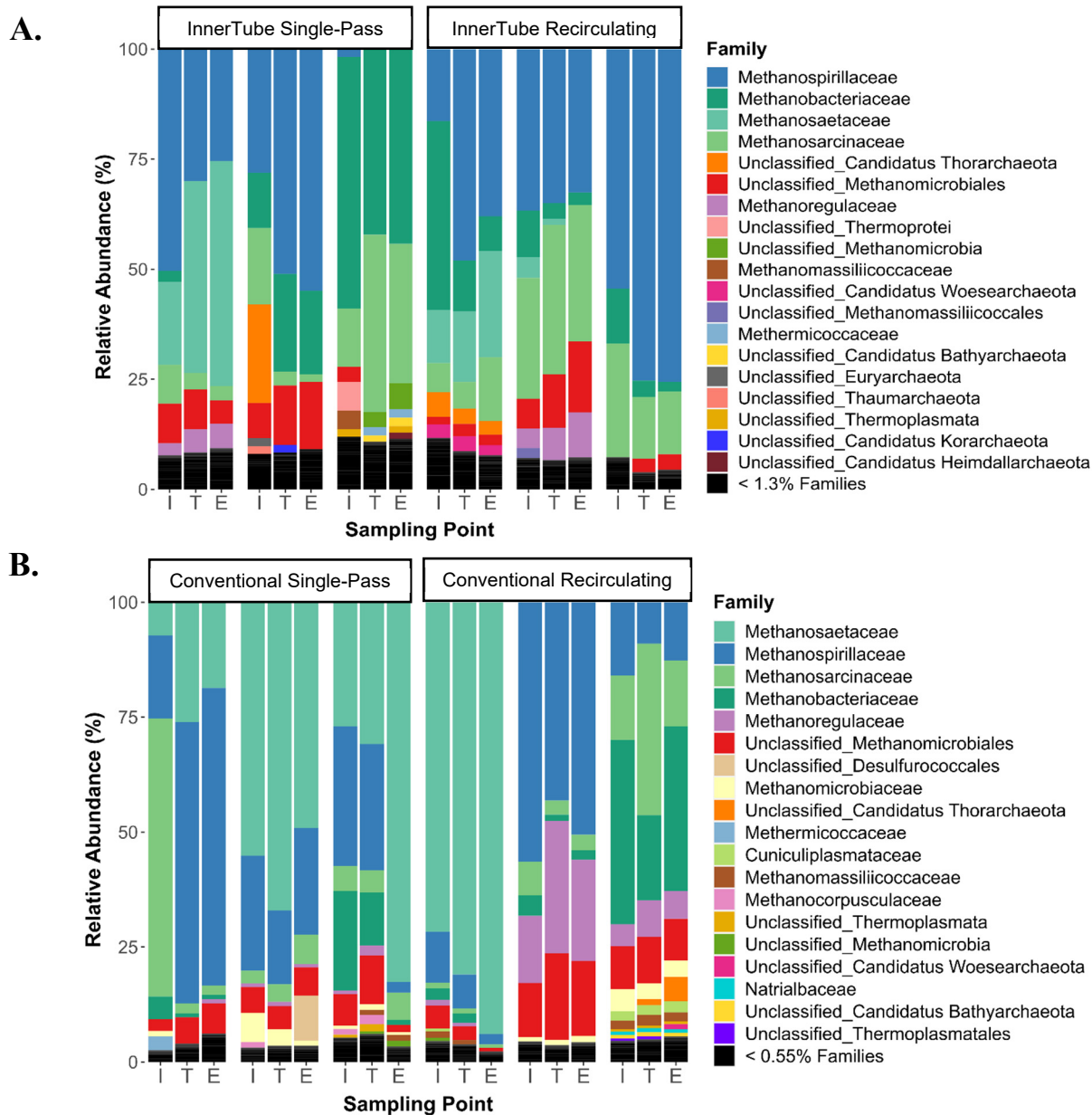


- ◆ Conventional Recirculating
- ◆ Conventional Single-Pass
- ◆ InnerTube Recirculating
- ◆ InnerTube Single-pass

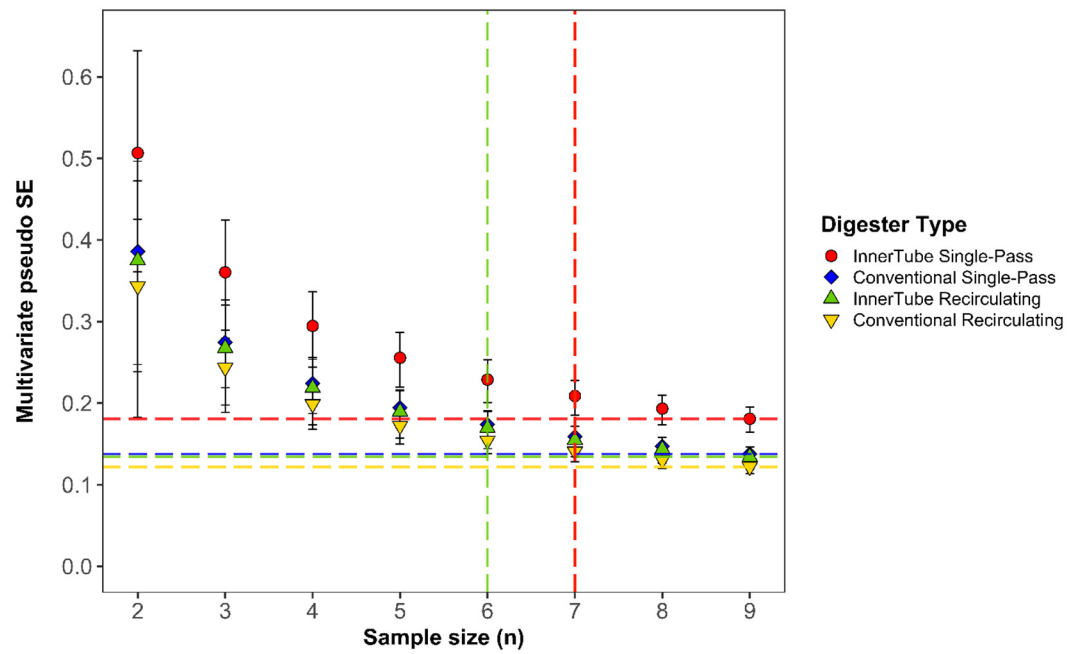
B.



Supplementary Figure S1. a.) Sampling locations of the four digester types. b.) Four digester types examined with sampling points. Three replicate digesters were each sampled at Influent, Tank, and Effluent treatment points.



Supplementary Figure S2. Relative abundance bar graphs showing the top 20 families under Euryarchaeota for triplicate a.) InnerTube Single-pass, InnerTube Recirculating, b.) Conventional Single-Pass, and Convectional Recirculating across sampling points. I=Influent, T=Tank, E=Effluent. The lowest abundant phyla were aggregated into a cutoff percentage labeled in black.



Supplementary Figure S3. Power curve of multivariate dissimilarity-based standard error estimates (multSE) of digester types vs. minimum required sample size. Scatterplot indicates the number of replicates (n=6 for InnerTube Recirculating, n=7 for all other digesters) at which there is no quantitative decrease in the standard error of Bray-Curtis dissimilarities within a given digester type.

Supplementary Table S1. a.) Permutational Analysis of Variance (PERMANOVA) and b.) permutation-based test of multivariate homogeneity of group dispersions (BETADISPER) of Bray-Curtis dissimilarities for species and functional genes of digester types and chemical parameters.

A.

	Taxonomic (Species)					Functional Genes				
Terms ²	df	Sum of Squares	Pseudo <i>f</i>	R2	<i>Adj. p</i> ¹	df	Sum of Squares	Pseudo <i>f</i>	R2	<i>Adj. p</i> ¹
Digester	3	0.810	2.496	0.210	0.001	3	0.082	2.420	0.203	0.001
Sampling Point	2	0.182	0.839	0.047	0.782	2	0.018	0.810	0.045	0.701
Digester:Sampling Point ³	6	0.264	0.407	0.069	1.000	6	0.033	0.488	0.082	1.000
Temperature	1	0.150	1.775	0.039	0.029	1	0.042	4.860	0.103	0.001
pH	1	0.199	2.365	0.052	0.003	1	0.021	2.397	0.051	0.013
DO	1	0.1134	1.346	0.029	0.164	1	0.019	2.257	0.048	0.031
TSS	1	0.126	1.489	0.033	0.098	1	0.011	1.257	0.027	0.237
COD	1	0.109	1.291	0.028	0.189	1	0.008	0.892	0.019	0.520
NH3	1	0.187	2.220	0.049	0.007	1	0.009	1.087	0.023	0.352
TKN	1	0.074	0.874	0.019	0.527	1	0.007	0.760	0.016	0.633

¹:

B.

	Terms	df	Sum of Squares	Pseudo <i>f</i>	Adj. <i>p</i> ¹
Taxonomic (Species)	Digester	3	0.077	16.577	0.001
	Sampling Point	2	0.005	0.523	0.638
Functional Genes	Digester	3	0.009	10.497	0.001
	Sampling Point	2	0.001	0.539	0.606

¹: Significant terms are bolded ($p < 0.05$).

²: Digester and Sampling Point comparisons were performed sequentially (Type I sequential Sum of Squares) while chemical parameter comparisons were performed marginally (Type III marginal Sum of Squares)

³: Colon represents the interaction between Digester and Sampling Point