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Influent Wastewater Microbiota and Temperature Influence Anaerobic Membrane Bioreactor Microbial Community

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Abstract: Sustainable municipal wastewater recovery scenarios highlight benefits of anaerobic membrane bioreactors (AnMBRs). However, influences of continuous seeding by influent wastewater and temperature on attached-growth AnMBRs are not well understood. In this study, four bench-scale AnMBR operated at 10 and 25 °C were fed synthetic (SPE) and then real (PE) primary effluent municipal wastewater. Illumina sequencing revealed different bacterial communities in each AnMBR in response to temperature and bioreactor configuration, whereas differences were not observed in archaeal communities. Activity assays revealed hydrogenotrophic methanogenesis was the dominant methanogenic pathway at 10 °C. The significant relative abundance of *Methanosaeta* at 10 °C concomitant with low acetoclastic methanogenic activity may indicate possible *Methanosaeta*-*Geobacter* direct interspecies electron transfer. When AnMBR feed was changed to PE, continual seeding with wastewater microbiota caused AnMBR microbial communities to shift, becoming more similar to PE microbiota. Therefore, influent wastewater microbiota, temperature and reactor configuration influenced the AnMBR microbial community.
Keywords: Anaerobic digestion, Illumina, Microbial community

1. Introduction

Sustainable municipal wastewater recovery scenarios have highlighted anaerobic biotechnology with special attention being given to the anaerobic membrane bioreactor (AnMBR) (McCarty et al., 2011). AnMBR configurations have successfully achieved effluent with <40 mg/L chemical oxygen demand (COD) from dilute or municipal wastewaters at temperatures as low as 6 °C (Seib et al., 2016a and Smith et al., 2015). These results indicate that historical anaerobic biotechnology challenges including poor operation at low temperature with low strength wastewater, and high effluent organic concentration can be overcome (Lettinga et al., 2001).

While AnMBR technology shows great promise, remaining challenges require further investigation including high energy requirements for membrane operation (Seib et al., 2016a) and post treatment for nutrient and dissolved methane removal (McCarty et al., 2011), as well as lack of fundamental understanding of microbial communities responsible for system function (Smith et al., 2015). Microbial community composition is of particular interest since anaerobic bioprocesses historically have been operated as "black boxes" without accounting for the relationship between microbiology and process function (McKeown et al., 2012).

In engineered microbial systems, community structure and diversity are considered important factors to achieve process stability (Briones and

Raskin, 2003 and Falk et al., 2009). Highly diverse communities which contain many unique members within different trophic groups (i.e. fermenting bacteria, syntrophic bacteria, methanogens, etc.) are functionally redundant which is important to maintain system function in the event of environmental stress (i.e. pH change, substrate change, toxicity, etc.) (Briones and Raskin, 2003 and Fernandez et al., 2000). Traditional characterizations of community diversity have included richness, evenness, and Shannon-Weaver index, which are broad measures indicating the number of unique members along with general distribution of members within the community (Stirling and Wilsey, 2001). Communities with higher richness and Shannon-Weaver index values are more diverse (Stirling and Wilsey, 2001). A high evenness score indicates unique community members are evenly distributed, which is beneficial for functional redundancy (Fernandez et al., 2000). Additionally, ordination techniques including non-metric multi-dimensional scaling (NMDS) and principal component analysis (PCA) have been useful to compare microbial community differences in separate systems (Bialek et al., 2011 and Bocher et al., 2015).

Increased knowledge of key microbial players is important to understand the potential and limitations of microbially driven processes such as hydrolysis, fermentation, and methanogenesis (McKeown et al., 2012). Links between microbial community composition and function could be used to match inoculum biomass to specific operating conditions including temperature or waste type (McKeown et al., 2012). This information could also be used to warn of impending process upset by identifying adverse shifts in the microbial community before function significantly deteriorates (Collins et al., 2006).

While the importance of microorganisms in biological systems is evident, the body of knowledge describing microbial consortia in anaerobic wastewater reclamation systems is underdeveloped. To date, the majority of studies have focused on microbial communities in anaerobic digesters treating high strength waste. Less attention has been given to microbial community composition in anaerobic systems reclaiming dilute wastes such as municipal wastewater. However, previous studies have shown that microbial communities in otherwise similar conditions will vary due to selective pressures such as temperature and bioreactor configuration (Bialek et al., 2011 and O'Reilly et al., 2009), bacterial communities are typically more even and diverse than archaeal communities in anaerobic systems (Rivière et al., 2009), and hydrogenotrophic methanogenesis becomes the dominant methanogenic pathway at psychrophilic temperatures (McKeown et al., 2009, O'Reilly et al., 2009 and Siggins et al., 2011).

While several examples of low/ambient temperature AnMBRs have been previously described, only two studies have investigated the microbial community composition within the bioreactor (Smith et al., 2013 and Smith et al., 2015). Both studies evaluated completely mixed submerged AnMBRs with gas sparging treating synthetic municipal wastewater, and concluded that biofilm formation on membranes was important to achieve high organic removal. Possible benefits of biofilms such as faster interspecies hydrogen transfer and enhanced syntrophism have already been described (Lettinga et al., 2001). The results of Smith et al. (2015) coupled with existing understanding of the benefits of biofilms highlights the need for further investigation of biofilm microbial consortia in AnMBRs and suggests that reactors relying on biofilm technology such as the fluidized bed reactor (FBR) or downflow floating filter reactor (DFF) may offer advantages over flocculant biomass (Seib et al., 2016b).

The impact of continuous inoculation of anaerobic bioreactors by wastewater microbiota also merits investigation. Municipal wastewater is microbially complex (McLellan et al., 2011) and temporal effects of wastewater microbiota on engineered process microbial community composition have been observed in the aerobic activated sludge process (Lee et al., 2015). Regarding anaerobic systems, no studies have been found which considered the effect of wastewater continuous inoculation on bioreactor anaerobic microbial community.

The objective of this study was to assess AnMBR configurations using different biofilm technologies while treating synthetic and real municipal primary effluent wastewater at low and moderate temperatures. Lab-scale reactors were operated to evaluate treatment performance and bioreactor microbial community composition at common wastewater temperatures (10 and 25 °C). To our knowledge no study currently exists that examines the microbial community structure within AnMBRs utilizing biofilm technology while treating dilute primary effluent municipal wastewater at low temperatures.

2. Methods

2.1. AnMBR configurations

Two different AnMBR configurations utilizing different biofilm technologies and membrane types were used as previously described (Seib et al., 2016b). The first configuration was a downflow floating filter (DFF) bioreactor (2.3 L working volume) combined with a polymeric tubular membrane (1 L working volume). The DFF bioreactor contained buoyant plastic media to support biofilm formation (Aqwise, Herzliya, Israel). The polymeric membrane (polyvinylidene fluoride) had a nominal molecular weight cutoff of 100 kDa (~0.018 μm nominal pore size) (FP100, PCI Membranes, Fareham, UK). The second configuration was a fluidized bed reactor (FBR) (2.3 L working volume) combined with a ceramic membrane (1 L working volume). The FBR contained 0.6 mm × 1.7 mm (12 × 30 mesh) coconut-based granular activated carbon (GAC) (TIGG 5DC 1230, TIGG Corp, Oakdale, PA). The ceramic membrane was composed of aluminum oxide with a 0.05 μm nominal pore size (Type 1/16, Atech Innovations, Gladbeck, Germany).

2.2. Bioreactor inoculation and operational parameters

Each AnMBR configuration was duplicated and individual reactors were operated at different temperatures (10 and 25 °C), yielding a total of four systems (FBR10, FBR25, DFF10, DFF25). All AnMBRs were seeded with 2 g VSS/L of a mix of methanogenic biomass from five different sources as previously described (Seib et al., 2016b). For the first 320 days, all AnMBRs were fed synthetic primary effluent wastewater (SPE) as previously described (Seib et al., 2016b). After day 320, the feed to all AnMBRs was changed to real primary effluent wastewater (PE). PE was collected weekly from a local water reclamation facility (South Shore Water Reclamation Facility, Oak Creek, WI, USA) and stored at 4 °C before use (Table 1). After an initial startup period (day 1–79), total system hydraulic residence time (HRT) in all AnMBRs was 9 h from day 80 to 145. On day 146, HRT was adjusted to the

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minimum time necessary to achieve <10 mg/L BOD_5 in AnMBR permeate in each system. Membranes were operated with flux ranging from 5.9 to 7.4 L/m^2 h and chemically cleaned using NaClO and HNO_3 solutions when transmembrane pressure increased above 0.5 bar (Seib et al., 2016b).

Table 1. Average influent and effluent parameters during SPE and PE operation.

Parameter	**Influent**		**Effluent**							
			Treating SPE influent				**Treating PE influent**			
	SPE	**PE**	**FBR25**	**FBR10**	**DFF25**	**DFF10**	**FBR25**	**FBR10**	**DFF25**	**DFF10**
BOD_5	235 ± 35	160 ± 60	3 ± 1	8 ± 4	6 ± 3	8 ± 7	6 ± 3	6 ± 4	10 ± 7	10 ± 9
TCOD	480 ± 50	310 ± 110	14 ± 7	25 ± 15	25 ± 10	25 ± 13	29 ± 12	29 ± 16	44 ± 16	42 ± 19
NH_3-N	17 ± 1.5	21 ± 7.6	31 ± 2	31 ± 1	29 ± 1	31 ± 2	25 ± 6	25 ± 6	25 ± 5	25 ± 6
TKN	43 ± 2.8	34 ± 6.7	36 ± 2	37 ± 2	35 ± 1	37 ± 2	29 ± 4	29 ± 5	28 ± 4	29 ± 6
PO_4^{-3}-P	2.3 ± 0.3	3.8 ± 1.7	3.9 ± 0.3	3.9 ± 0.3	3.1 ± 0.4	3.7 ± 0.4	4.3 ± 1.6	4.0 ± 1.4	3.6 ± 1.4	3.8 ± 1.7
Total P	5.0 ± 0.4	5.1 ± 1.7	3.9 ± 0.3	4.2 ± 0.4	3.4 ± 0.5	3.8 ± 0.3	4.4 ± 1.6	4.2 ± 1.4	3.8 ± 1.2	3.9 ± 1.6
TSS[a]	120 ± 40	106 ± 40	BD	BD	BD	BD	BD	BD	BD	BD
VSS[a]	115 ± 40	77 ± 25	BD	BD	BD	BD	BD	BD	BD	BD

[a] BD indicates result below method detection limit.

2.3. Analytical procedures

Influent and permeate BOD_5, TCOD, NH_3-N, TKN, PO_4^{-3}, TP, TSS, and VSS concentrations were determined using standard methods (APHA et al., 1999). Bioreactor bulk liquid volatile fatty acid (VFA) concentrations were determined by gas chromatography with a flame ionization detector (FID) (Agilent 7890A, Santa Clara, CA, USA). Methane concentration in biogas was determined using gas chromatography with a thermal conductivity detector (TCD) (Agilent 7890A, Santa Clara, CA, USA).

2.4. DNA extraction

Biomass was collected for DNA analysis from each reactor on days 180, 200, 230, 250, and 355 and from the PE feed on day 355. Biomass

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(~0.5 g) from each reactor was removed from the biocarrier and placed in 2 mL centrifuge tubes. Lysis buffer (120 mM phosphate buffer, pH 8.0, 5% sodium dodecylsulfate) was added to each sample and cells were lysed by performing three freeze-thaw cycles followed by a 90 min incubation at 70 °C. DNA was extracted using a FastDNA Spin Kit (MP Biomedicals, Santa Ana, CA, USA), and then stored at −20 °C until use.

2.5. DNA sequencing

PCR amplification using universal primers for the V4 variable region of 16 s rRNA (515F and 806R) was performed using the HotStarTaq Plus Master Mix Kit (Qiagen, Valencia, CA, USA). PCR consisted of the following steps: 94 °C for 3 min followed by 28 cycles of 94 °C for 30 s, 53 °C for 40 s and 72 °C for 1 min, followed by a final elongation step at 72 °C for 5 min. Ampure XP beads (Beckman Coulter, Indianapolis, IN, USA) were used to purify PCR products. Purified PCR products were used to prepare a DNA library using the Illumina TruSeq DNA library preparation protocol. Sequencing was performed by a commercial laboratory (MR DNA, Shallowater, TX, USA) using an Illumina MiSeq v3 300 base pair sequencing platform (Illumina, San Diego, CA, USA) following manufacturer guidelines. Barcodes and primers were removed from Q25 filtered sequences and processed as previously described (Dowd et al., 2008). Briefly, data were refined by removing sequences <200 bp, sequences with ambiguous base calls, and sequences with homopolymers >6 bp. Denoised sequences were clustered into operational taxonomic units (OTUs) having 97% similarity. Singleton sequences and chimeras were removed. BLASTn was used to taxonomically classify OTUs against a curated database derived from GreenGenes, RDPII, and NCBI (CME, 2015, DeSantis et al., 2006 and NCBI, 2015).

2.6. Microbial community analysis

Inter-AnMBR comparisons of richness (S), Shannon-Weaver diversity (H), and evenness (E) indices were performed using Illumina sequence results. Richness was calculated as the number of unique OTUs identified at the genus level from Illumina sequencing. Shannon-Weaver diversity index was determined as follows: $H = -\sum p_i \log(p_i)$, where p_i is the relative

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abundance of genus i of the n genera detected in a sample (i = 1 to n) (Falk et al., 2009). Evenness was calculated as follows: E = H/ln(S) (Falk et al., 2009).

Ordination techniques including non-metric multi-dimensional scaling (NMDS) and principal component analysis (PCA) were used to compare AnMBR microbial communities. Using Ilumina sequencing data, NMDS using a Bray-Curtis similarity distance matrix was performed in R (version 3.2.0 (20015-04-16)) using the VEGAN and MASS packages. NMDS is considered well suited for environmental data because it does not assume a linear distribution (as in PCA) and is unaffected by null values among samples (Ramette, 2007). PCA was also performed using R. Sequencing results were also used to calculate Pearson's correlation coefficients comparing AnMBR and PE microbial communities.

2.7. Methanogenic activity

Specific methanogenic activity (SMA) assays were performed using acetate and H_2/CO_2 while AnMBRs were fed SPE (day 300) and PE (day 355) at 10 °C. Biocarrier was removed from each AnMBR, placed in a serum bottle with basal nutrient medium and agitated in an anaerobic glove box to remove biomass from the biocarrier. Biocarrier was then removed and biomass was placed in 160 mL serum bottles, sparged with O_2-free gas (7:3 v/v N_2/CO_2), sealed with butyl rubber stoppers, and allowed to endogenously produce biogas for two days at 10 °C. Produced biogas was then removed and substrate (either acetate or H_2/CO_2) was added. SMA using acetate was performed for 40 days as described by Bocher et al. (2015) using biomass concentration of 1.5 to 1.8 g VS/L and 10 g/L calcium acetate at 10 °C. For SMA using H_2/CO_2, biomass concentration was 0.2 g VS/L and serum bottle headspace was charged with 100 mL of a 4:1 mixture of H_2/CO_2 gas that had been previously cooled to 10 °C. Decrease in headspace pressure was monitored for 40 days using a glass syringe with wetted glass barrel to stoichiometrically determine hydrogenotrophic methane production.

3. Results and discussion

3.1. AnMBR organic removal and performance

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All AnMBRs were assumed to achieve quasi-steady state after 320 days of SPE operation, since organic removal varied no more than ±3% in each system. Organic removal in all four AnMBRs was >94% while treating both SPE and PE, with average permeate $BOD_5 \leqslant 10$ mg/L in all systems (Table 1). Each AnMBR required a specific bioreactor HRT to achieve low permeate BOD_5, with FBR25 and DFF25 both operated at 4.2 h and FBR10 and DFF10 operated at 5.6 and 9.8 h, respectively. These values correspond to total system HRTs of 6, 6, 8, and 14 h for the FBR25, DFF25, FBR10, and DFF10 systems, respectively, considering membrane system volumes. Average permeate TCOD was $\leqslant$25 mg/L in all AnMBRs while treating SPE and $\leqslant$45 mg/L while treating PE. The increased average effluent TCOD when treating PE was likely due to a combination of higher amount of recalcitrant COD in the PE along with insufficient time for all AnMBRs to acclimate to the PE substrate. Bioreactor bulk liquid total VFA (as acetic acid) concentrations remained low throughout the study, averaging <40 mg/L in all AnMBRs during SPE operation and <15 mg/L during PE operation.

3.2. Microbial diversity and community structure

Diversity indices derived from sequencing analysis of the V4 region of 16S rRNA of biofilm biomass from each bioreactor (Tables S1 and S2) indicate communities dominated by a few OTUs in each AnMBR (Table 2). Analysis revealed greater richness, evenness, and Shannon-Weaver diversity values in the bacterial community of each AnMBR compared to the archaeal community, which is consistent with findings of previous anaerobic studies (Regueiro et al., 2012 and Rivière et al., 2009). All systems contained a similar number of bacterial and archaeal OTUs, with the exception of FBR10, which had fewer bacterial OTUs. Shannon indices were similar among all AnMBRs with an average index for all systems of 1.62 ± 0.08 for Bacteria and 0.56 ± 0.08 for Archaea. These are lower than values of 1.92 to 3.91 previously reported in mesophilic anaerobic studies treating wastes including swine wastewater and synthetic sulfate-rich wastewater (Briones et al., 2007 and Roy et al., 2009). Evenness scores were higher for bacterial communities compared to archaeal communities. Evenness scores were also similar among AnMBRs, with average scores of 0.27 ± 0.01 for Bacteria and 0.20 ± 0.03 for Archaea. Evenness scores found in previous mesophilic digestion studies ranged from 0.73 to 0.91, indicating more even distribution

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of OTUs detected in those studies (Briones et al., 2007 and Roy et al., 2009). Diversity index scores for 10 °C and 25 °C systems were similar but were lower than scores previously reported for mesophilic systems.

Table 2. Diversity indices for Bacteria and Archaea communities during SPE operation.

		FBR25	**DFF25**	**FBR10**	**DFF10**
Bacteria	Richness	384 ± 30	406 ± 10	330 ± 8	403 ± 18
	Evenness	0.28 ± 0.01	0.27 ± 0.01	0.27 ± 0.01	0.28 ± 0.01
	Shannon Index	1.64 ± 0.08	1.64 ± 0.05	1.52 ± 0.05	1.66 ± 0.09
Archaea	Richness	18 ± 3	18 ± 1	15 ± 1	16 ± 2
	Evenness	0.19 ± 0.02	0.19 ± 0.03	0.17 ± 0.01	0.23 ± 0.02
	Shannon Index	0.56 ± 0.06	0.55 ± 0.08	0.47 ± 0.04	0.64 ± 0.03

A small group of 5 of over 700 bacterial OTUs identified, including *Clostridium*, *Bacteroides*, *Cytophaga*, *Geobacter*, and *Trichococcus*, comprised 31–43% of the total relative abundance in all reactors while treating SPE. This is consistent with analysis previously conducted on mesophilic anaerobic communities that describe the predominant bacterial composition in anaerobic systems being composed of only a few OTUs (Harb et al., 2015 and Rivière et al., 2009). This was also observed among Archaea, with only three genera (*Methanosaeta*, *Methanobacterium*, and *Methanospirillum)* accounting for >80% of archaeal relative abundance in all AnMBRs while treating SPE.

Despite each reactor containing similar dominant OTUs, unique microbial fingerprints were observed in each system based on the most abundant bacterial OTUs. A comparison of the 20 most abundant OTUs, which represented >50% of the relative abundance in each system, showed distinct OTU distributions in all AnMBRs (Fig. S1). During SPE operation, the bacterial community in each AnMBR possessed a unique dominant OTU. For FBR10 and FBR25, an OTU most similar to *Clostridium* was dominant and accounted for >20% of all bacterial relative abundance. For the DFF reactors, an OTU most similar to *Geobacter* was dominant in DFF25, whereas DFF10 showed higher abundances of OTUs most similar to *Cytophaga* and *Trichococcus*. All of these genera are contained within the phyla Proteobacteria, Bacteroidetes, and Firmicuties, which have been described as being dominant in mesophilic anaerobic systems (McKeown et al., 2009 and Regueiro et al., 2012) and have been shown to account for over

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65% of relative abundance in a psychrophilic AnMBR treating synthetic domestic wastewater (Smith et al., 2013).

The microbiome within the PE fed to the AnMBRs significantly differed from community structures in the AnMBRs during SPE operation based on Pearson's correlation coefficient and community microbial fingerprint (Fig. S1). Comparison of Pearson's correlation coefficients revealed poor correlation between AnMBR bacterial communities during SPE operation and the PE bacterial community (r = 0.08 to 0.16). Microbial fingerprint analysis showed that OTUs most similar to *Arcobacter* represented 30% of the PE bacterial relative abundance, but these OTUs were ⩽1% of the relative abundance in all the AnMBRs during SPE operation. Other dominant OTUs in the PE included those most similar to *Bacteroides*, *Parabacteroide*s, and *Aeromonas*. These four genera have previously been found to comprise a large portion of the bacterial community in municipal sewage (Fisher et al., 2014 and McLellan et al., 2011).

Bacterial communities in the AnMBR systems shifted after SPE feeding to AnMBRs ceased and real PE began to be fed (Fig. S1). This was due to introduction of organisms within PE fed to the AnMBRs. Specifically, an OTU most similar to *Arcobacter* appeared within AnMBRs in higher relative abundance during PE operation with higher increases in the 10 °C bioreactors.

No significant differences among the AnMBR archaeal populations were observed. A group of 28 unique archaeal OTUs was identified and over 80% of archaeal relative abundance was accounted for by three OTUs during both SPE and PE operation; these OTUs were most similar to *Methanosaeta*, *Methanobacterium*, and *Methanospirillum* (Fig. S1). Unlike the bacterial community composition, the archaeal community during PE and SPE feeding were not significantly different.

Hydrogenotrophic methanogen OTUs made up a larger portion of methanogen relative abundance in the 10 °C AnMBRs, which is consistent with previous observations of methanogen population shifts to favor hydrogen utilization under psychrophilic conditions (Lettinga et al., 2001 and Siggins et al., 2011). OTUs most similar to hydrogenotrophic methanogens made up 16–40% of archaeal relative abundance in the 25 °C systems, whereas these OTUs accounted for 27–58% of relative abundance at 10 °C. Among methanogens, the OTU most similar to *Methanosaeta* was the most dominant, accounting for at least 40% of archaeal relative abundance in all systems (Fig. S1). The facts that bioreactor bulk liquid VFA concentrations remained very low, *Methanosarcina* was virtually absent from all samples, and all AnMBRs were run at temperatures below the mesophilic optimum of 35 °C indicates that acetoclastic methanogenesis was achieved primarily by *Methanosaeta* spp. (Bialek et al., 2011).

A decrease in relative abundance of methanogens was seen over time at 10 °C in this 365 day study, which suggests that biofilms in all AnMBRs primarily contained psychrotolerant mesophilic methanogens as opposed to developing dominant putatively psychrophilic populations. Methanogens comprised 7–12% of total microbial relative abundance in the 25 °C AnMBRs, whereas only 2–5% methanogens were found at 10 °C. Previous psychrophilic anaerobic studies operating up to 300 days have concluded that reactors seeded with mesophilic biomass primarily contained psychrotolerant mesophilic methanogens rather than a population of psychrophilic methanogens (Collins et al., 2006 and Smith et al., 2013). However, putatively psychrophilic microbial populations have been found in long term studies (>1200 days), indicating that psychrophilic organisms are present but require a very long time to establish in significant abundance (McKeown et al., 2009).

3.3. AnMBR microbial comparisons

Unique microbial communities existed in each AnMBR based on NMDS, Pearson's correlation, and PCA analysis despite similar values for gross evenness and diversity index. Cluster analysis using NMDS plots revealed distinct differences among the bacterial communities of AnMBRs during SPE operation (Fig. 1). The distinct grouping of bacterial profiles from each AnMBR indicate that selective pressures of bioreactor configuration and operational temperature cause differences in the microbial communities of reactors seeded with the same inoculum and fed identical substrate. This observation was also made by Bialek et al. (2011) who found methanogenic community profiles in different bioreactor configurations clustered using

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NMDS and by O'Reilly et al. (2009) who indicated that microbial community profiles are affected by temperature. However, in contrast to bacterial communities, the archaeal communities in this study did not cluster separately using NMDS (Fig. 1). Results from the archaeal fingerprints confirm this observation and indicates similar archaeal community structures for all AnMBRs during SPE operation (Fig. S1). Analysis using Pearson's correlation coefficient did not indicate that either bioreactor configuration or temperature had a more significant impact on community differences.

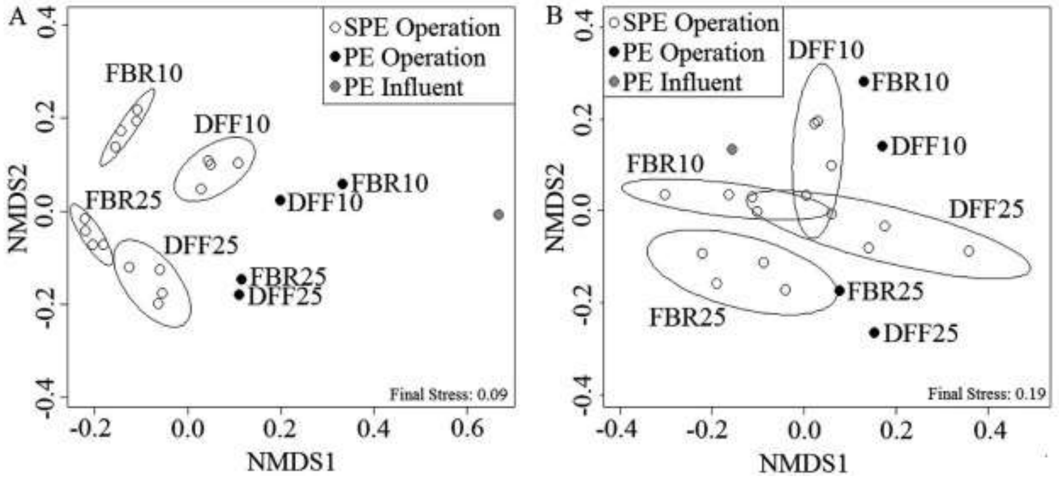

Fig. 1. Non-metric multidimensional scaling (NMDS) analysis of (A) Bacteria and (B) Archaea 16S rRNA sequencing profiles for each AnMBR. Ellipses represent clustering of each AnMBR biomass (95% confidence). During SPE operation samples were taken on day 180, 200, 230, and 250. Samples were taken during PE operation on day 355.

PCA also helps visualize how the most dominant bacterial and archaeal genera are represented among reactors (Fig. 2). For Bacteria, differences observed among OTUs most similar to the genera *Clostridium*, *Arcobacter*, *Geobacter*, *Trichoccous*, *Acinetobacte*r, and *Cytophaga* in each AnMBR explain 73% of the variance observed within bacterial communities during operation with PE and SPE. Additionally, vectors representing specific bacterial OTUs aligned with the AnMBRs possessing the highest relative abundance of each OTU, indicating the microbial community differences across AnMBRs were attributed to a specific dominant OTU in each AnMBR. For Archaea, *Methanosaeta*, *Methanobacterium*, and *Methanospirillum* explain 99% of the variance observed among archaeal communities. Unlike results for Bacteria, the vectors representing archaeal OTUs did not align with different AnMBRs, which reinforces observations made with NMDS and analysis of community fingerprints that unique archaeal community structures did not emerge in each AnMBR.

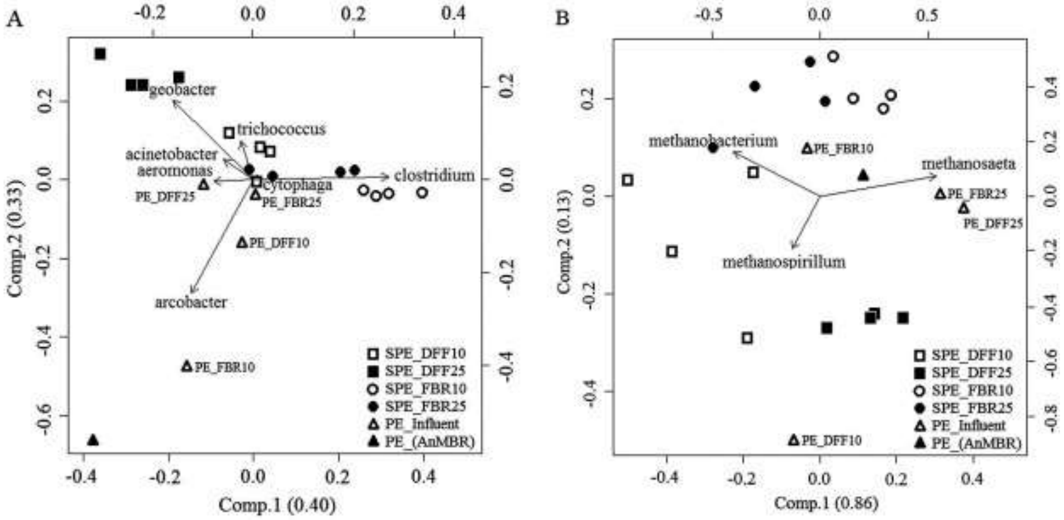

Fig. 2. Principal components analysis (PCA) analysis of (A) Bacteria and (B) Archaea 16S rRNA sequencing profiles for each AnMBR. During SPE operation samples were taken on day 180, 200, 230, and 250. Samples during PE operation were taken on day 355.

3.4. Impact of continuous inoculation

Continuous inoculation by PE caused the community to change in each AnMBR. After wastewater containing a high abundance of *Arcobacter* began to be fed, its relative abundance increased in all bioreactors, ostensibly because reactors were being continuously inoculated (Fig. S1). During PE feeding, the bacterial community in all AnMBRs did not cluster with communities analyzed during SPE operation (Fig. 1). Additionally, *Arcobacter* relative abundance was the primary source of community variance among bioreactor biomass during PE operation, especially for the 10 °C AnMBRs (Fig. 2). Previous work has identified a similar change in microbial community composition within activated sludge systems due to the influent wastewater microbiota (Lee et al., 2015). Influent characteristics are also known to affect microbial community structure (LaPara et al., 2002). The relatively short operation period with PE during this study did not allow time to examine the long term effect of influent continual seeding on AnMBR bioreactor microbial community. Future work should examine the long term

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effect on microbial community structure once a steady state condition has been reached.

3.5. Methanogenic activity and substrate preference

Thermodynamically, hydrogen is a more favorable substrate than acetate at lower temperature (Lettinga et al., 2001). In contrast, acetoclastic methanogenesis has been described by some as the primary methanogenic pathway at low temperatures (Metje and Frenzel, 2007). However, hydrogenotrophic methanogenesis has also been observed in low temperature natural (Metje and Frenzel, 2007) and engineered environments (Bialek et al., 2011 and McKeown et al., 2009).

Methanogenic activity assays in this study revealed hydrogenotrophic methanogenesis became the primary methanogenic pathway at lower temperature (Fig. 3). Comparison of SMA at 10 °C for biomass from all AnMBRs shows hydrogen utilization was similar among all bioreactors during SPE operation but was higher in the FBR10 and DFF10 biomass compared to FBR25 and DFF25 biomass during PE operation. Additionally, while acetate utilization was observed during SPE operation, acetoclastic methanogenesis was not detected during PE operation. These results, combined with the higher relative abundance of hydrogenotrophic methanogens at 10 °C (Fig. S1) indicate that hydrogen utilization was the primary pathway for methanogenesis at 10 °C and prolonged low temperature operation increased biomass hydrogen utilization rate compared to biomass at 25 °C.

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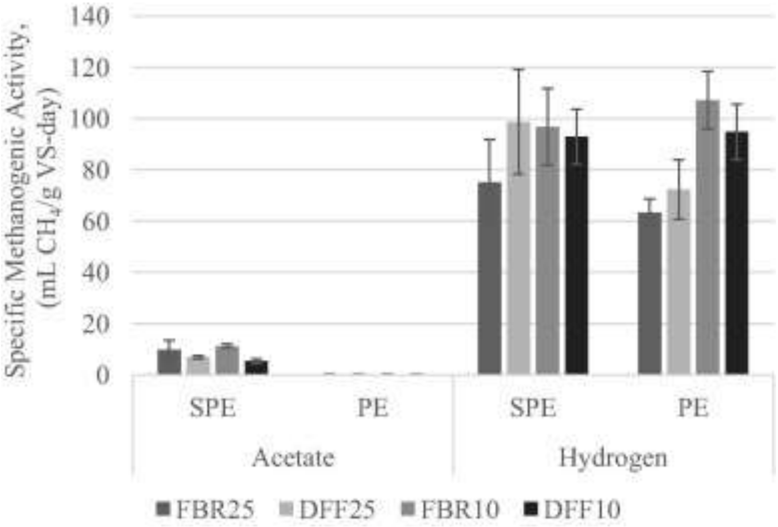

Fig. 3. Specific methanogenic activity (SMA) at 10 °C using acetate and H_2/CO_2 after treating SPE for 300 days (n = 6) and after treating PE for 35 days (n = 6 for H_2/CO_2, n = 3–5 for acetate).

The role of *Methanosaeta* detected in each AnMBR is unclear. *Methanosaeta* is commonly found in methanogenic biomass and is known to be important in forming biofilms in bioreactors such as the upflow anaerobic sludge blanket reactor (Nelson et al., 2012). However, the high relative abundance of *Methanosaeta* in all systems does not correlate to the extremely low or nonexistent methanogenic activity measured with acetate at 10 °C. The primary explanation for high *Methanosaeta* detection may stem from the molecular methods used which relied on sequencing analysis of DNA rather than RNA. DNA-based methods can be biased in that intracellular and extracellular DNA may be included from inactive members within a community (Smith et al., 2015). High detection of *Methanosaeta* coupled with little acetoclastic methanogenic activity suggests that *Methanosaeta* was present but may not have been active. Another possibility is that *Methanosaeta* may have been using a substrate other than acetate. While *Methanosaeta* spp. have been considered to be exclusively acetoclastic since they are not known to use H_2 or formate, a recent study has indicated that *Methanosaeta* may be able to reduce CO_2 to CH_4 via direct interspecies electron transfer in conjunction with *Geobacter* (Rotaru et al., 2014). In this study, *Geobacter* bacterial relative abundance in the 25 °C AnMBRs varied from 5 to 20%, whereas they were only 1.0–2.3% in the 10 °C systems. The presence of *Geobacter* and *Methanosaeta* coupled with low methanogenic acetate utilization suggests that *Methanosaeta* may play a role other than acetate utilizer in low temperature anaerobic systems. The possibility of functionally significant, cold temperature *Methanosaeta*- *Geobacter* direct interspecies electron transfer warrants investigation. Further work utilizing RNA or functional gene-based sequencing methods (such as mcrA) would also be useful to characterize the role of *Methanosaeta* in similar fixed-film anaerobic systems.

4. Conclusions

Unique bacterial communities developed in AnMBRs due to reactor configuration and temperature, but the same was not observed for Archaea. Hydrogenotrophic methanogenesis was dominant at 10 °C, whereas acetoclastic methanogenesis was low or not detected. A shift in AnMBR microbial community occurred when real wastewater was fed due to continuous seeding with influent wastewater microbiota. Future work should determine the influence of long term continuous seeding by wastewater microbiota on bioreactor communities.

Acknowledgements

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Appendix A. Supplementary data

Influent wastewater microbiota and temperature influence anaerobic membrane bioreactor microbial community

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Supporting Materials

Supporting Figures (Figure S1)

Supporting Tables (Table S1 and S2)

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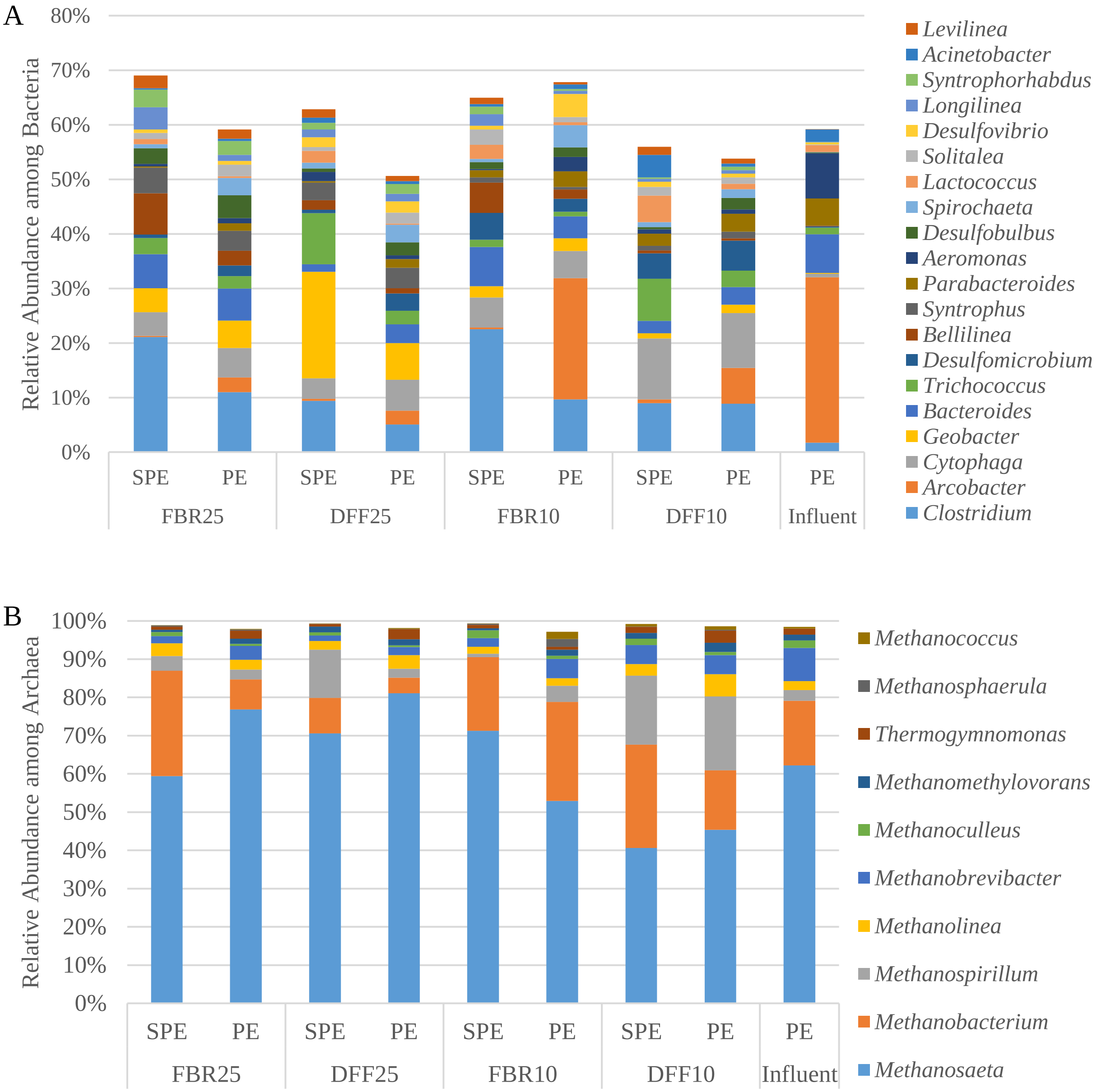

Figure S1: Biofilm community structure at the genus level for A.) Bacteria and B.) Archaea during operation with SPE (day 250) and PE (day 355). Relative abundance is shown for the 20 most abundant genera classified in the domain Bacteria and for the 10 most abundant genera classified in the domain Archaea, respectively.

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Table S1: Relative abundance of the top 98% most abundant genera in FBR10, FBR25, and the real primary effluent (Real PE) detected across all samples analyzed.

	Real PE	FBR10					FBR25				
Genus	Real PE_355	FBR10_180	FBR10_200	FBR10_230	FBR10_250	FBR10_355	FBR25_180	FBR25_200	FBR25_230	FBR25_250	FBR25_355
clostridium	0.01696742	0.287102	0.109447	0.183751	0.084749	0.21106	0.087688	0.099184	0.042286	0.094934	0.085648
geobacter	0.00176911	0.014787	0.018204	0.038343	0.175868	0.019555	0.009234	0.045876	0.056294	0.022795	0.014737
trichococcus	0.01222297	0.013065	0.160805	0.025876	0.084007	0.012195	0.07539	0.020665	0.020449	0.007981	0.029309
cytophaga	0.00630448	0.038682	0.051605	0.037664	0.033589	0.05165	0.109248	0.048864	0.047631	0.048377	0.097542
methanosaeta	0.00254109	0.034147	0.023327	0.075869	0.070874	0.045344	0.00842	0.072957	0.132176	0.009773	0.014256
bacteroides	0.0707485	0.062873	0.025407	0.054537	0.012377	0.067251	0.022443	0.053082	0.028834	0.039906	0.030902
arcobacter	0.30192351	0.002351	0.003465	0.002272	0.003332	0.002641	0.007164	0.024419	0.021143	0.218264	0.063851
bellilinea	0.00078806	0.059417	0.018551	0.066186	0.015751	0.052011	0.005342	0.024369	0.007733	0.016999	0.004532
acinetobacter	0.02286983	0.003083	0.012648	0.00259	0.008205	0.004459	0.040732	0.003553	0.00429	0.008429	0.005672
lactococcus	0.01280196	0.036138	0.049786	0.008602	0.01963	0.024331	0.047729	0.002674	0.002096	0.005041	0.009545
desulfomicrobium	0.00209077	0.038129	0.031953	0.005264	0.005628	0.046123	0.045604	0.017401	0.026821	0.023341	0.053234
aeromonas	0.08301972	0.001696	0.003651	0.003989	0.015345	0.002078	0.007647	0.008211	0.005553	0.02589	0.008076
syntrophus	0.00054682	0.00925	0.017165	0.041044	0.029052	0.009049	0.008213	0.033471	0.031694	0.004593	0.011386
longilinea	0.00054682	0.023112	0.017152	0.035808	0.012601	0.019641	0.005245	0.010132	0.011148	0.005657	0.005974

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levilinea	0.00038599	0.014157	0.024738	0.02003	0.013777	0.010881	0.014134	0.015317	0.008302	0.004033	0.008831
solitalea	0.00062723	0.020041	0.014034	0.009586	0.006132	0.026496	0.015418	0.01941	0.016673	0.009451	0.011441
methanobacterium	0.00069156	0.009455	0.017264	0.03513	0.009311	0.012267	0.005604	0.00742	0.006553	0.004789	0.004889
desulfobulbus	0.00151179	0.013502	0.007957	0.025128	0.005544	0.011603	0.004251	0.038067	0.019935	0.017013	0.020409
fusibacter	0.00083631	0.034057	0.016905	0.004876	0.010053	0.033986	0.026584	0.002272	0.003609	0.003669	0.009614
parabacteroides	0.04937438	0.006077	0.011163	0.001912	0.002436	0.012454	0.021753	0.01248	0.013244	0.028046	0.03174
spirochaeta	0.00062723	0.007027	0.006386	0.006164	0.009563	0.005397	0.008916	0.028751	0.027015	0.040256	0.015465
desulfovibrio	0.00379555	0.005254	0.012214	0.005402	0.016213	0.006408	0.009413	0.00619	0.01745	0.041474	0.00673
syntrophorhabdus	0.00041815	0.012834	0.006782	0.02794	0.011299	0.013075	0.002277	0.023038	0.015243	0.002996	0.005947
dehalococcoides	0.00025733	0.008248	0.005705	0.027123	0.007981	0.006638	0.001946	0.00742	0.006358	0.001666	0.002458
shewanella	0.0111615	0.000732	0.002203	0.003325	0.015023	0.003276	0.03245	0.001444	0.001124	0.005727	0.001964
syntrophomonas	0.00072373	0.015249	0.004925	0.011608	0.015289	0.014807	0.002761	0.006353	0.005248	0.004467	0.002143
anaerophaga	0.00041815	0.002017	0.010185	0.004363	0.003094	0.002323	0.018703	0.016246	0.01069	0.006721	0.010933
compostimonas	0.00020908	0.017061	0.008749	0.008519	0.007267	0.015831	0.005507	0.002272	0.002027	0.004299	0.004134
prolixibacter	0.0006594	0.006449	0.007623	0.001829	0.0028	0.009352	0.015335	0.005323	0.006039	0.008891	0.033031
pelobacter	0.00099714	0.002839	0.010309	0.001815	0.003318	0.003175	0.008088	0.015053	0.012772	0.004187	0.015836
phascolarctobacterium	0.0017852	0.004856	0.005297	0.004059	0.002282	0.007966	0.008268	0.008399	0.004651	0.01172	0.007568

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rikenella	0.00151179	0.007862	0.006287	0.001122	0.001638	0.017881	0.008889	0.002348	0.004831	0.007939	0.004821
acidobacterium	0.00025733	0.00501	0.001992	0.016443	0.001806	0.007447	0.001587	0.011864	0.008552	0.003725	0.004038
caldithrix	0.00040207	0.002749	0.006881	0.004308	0.00182	0.004416	0.017212	0.017376	0.01652	0.002142	0.011619
sulfurovum	0.00083631	0.004573	0.007957	0.002452	0.007813	0.004921	0.005576	0.00231	0.00329	0.003921	0.006249
paludibacter	0.01571295	0.005511	0.00297	0.004267	0.000896	0.011834	0.00588	0.005009	0.002402	0.01333	0.008557
smithella	0.00017691	0.001516	0.005099	0.007162	0.004284	0.001241	0.002222	0.00565	0.015063	0.000266	0.002568
methanospirillum	0.00011258	0.000732	0.005173	0.005001	0.012699	0.000592	0.003727	0.002436	0.003845	0.000784	0.006098
pedobacter	0.00028949	0.00492	0.011719	0.000374	0.000448	0.011141	0.014327	0.001783	0.000986	0.008191	0.005714
eubacterium	0.01318794	0.001747	0.006163	0.001787	0.002758	0.003016	0.005977	0.005286	0.008857	0.006399	0.013927
proteocatella	0.00273409	0.002608	0.006014	0.003477	0.004256	0.003045	0.006349	0.002875	0.002915	0.001134	0.002307
ornithinibacillus	0.00016083	0.009134	0.004715	0.008048	0.00175	0.007591	0.00254	0.004507	0.001069	0.001386	0.00195
leptospira	0.00016083	0.001657	0.002735	0.001468	0.004242	0.002829	0.002415	0.01656	0.010606	0.011902	0.005617
candidatus cloacimonas	0.00119013	0.004766	0.005346	0.00356	0.001974	0.003983	0.002885	0.001871	0.005373	0.003318	0.003351
aminobacterium	0.00017691	0.006359	0.003477	0.004848	0.004494	0.006869	0.002084	0.002448	0.001041	0.00336	0.001291
guggenheimella	0.00024124	0.000193	0.000136	0.011955	0.00021	0.000188	0.000248	0.003377	0.000236	0.000112	0.000206
treponema	0.00022516	0.00158	0.003713	0.002798	0.002898	0.001559	0.005742	0.004758	0.00547	0.003304	0.008694

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acetobacterium	0.00136704	0.000565	0.006707	0.001177	0.005236	0.000346	0.003216	0.00177	0.002402	0.002366	0.002816
pelotomaculum	0.04017498	0.000501	0.000941	0.00054	0.001386	0.000317	0.000635	0.00275	0.002943	0.009773	0.00445
chlorobium	0.00020908	0.000925	0.001188	0.000526	0.003822	0.000693	0.001366	0.008663	0.011106	0.002772	0.005082
thermanaerovibrio	0.00012866	0.000141	0.000334	0.0009	0.01935	0.000159	0.000386	0.002084	0.004387	0.000434	0.000618
ruminococcus	0.00334523	0.004702	0.00344	0.004142	0.002464	0.003868	0.002443	0.003729	0.004179	0.00203	0.003667
anaeromyxobacter	0.00012866	0.001721	0.00297	0.001011	0.00364	0.001818	0.003754	0.00162	0.002846	0.000868	0.00846
fusobacterium	0.00517868	0.000373	0.001559	0.000485	0.004662	0.000274	0.003354	0.001858	0.004137	0.000658	0.002912
paenibacillus	0.00020908	0.000231	0.00297	0.001094	0.0063	0.000779	0.004969	0.00064	0.000458	0.000266	0.000824
synergistes	0.00164045	0.006064	0.005297	0.000887	0.005152	0.00534	0.003658	0.000967	0.001027	0.002198	0.002802
pedosphaera	9.6497E-05	0.000938	0.003119	0.002507	0.002366	0.00101	0.001877	0.006064	0.008815	0.001218	0.004326
anaeromusa	0.0062884	0.000861	0.003849	0.000776	0.00371	0.002078	0.011912	0.000728	0.000652	0.001316	0.001346
blastopirellula	0.00012866	0.000244	0.001584	0.003214	0.008905	0.000245	0.001104	0.002423	0.003262	0.00014	0.001401
syntrophobacter	0.00012866	0.00167	0.005581	0.004405	0.003332	0.001284	0.001684	0.00177	0.0054	9.8E-05	0.001264
anaerovorax	0.00032166	0.00158	0.006324	0.000817	0.004158	0.001169	0.005286	0.000578	0.002582	0.001274	0.0032
zoogloea	0.0017852	0.000475	0.00276	0.000291	0.001316	0.000303	0.004748	0.008751	0.006539	0.004747	0.00912
verrucomicrobium	0.00033774	0.00149	0.002525	0.001233	0.0007	0.002078	0.001477	0.010986	0.004165	0.003655	0.010205
flavobacterium	0.0004664	0.001657	0.00641	0.000554	0.00224	0.002035	0.006266	0.000979	0.00447	0.001498	0.002939

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sulfurospirillum	0.02573257	0.000167	0.000656	0.001787	0.002142	0.000202	0.000773	0.0028	0.002402	0.008499	0.00342
acidaminobacter	0.00049857	0.00307	0.005618	0.00061	0.002198	0.003074	0.011498	0.000527	0.001083	0.00049	0.001662
chondromyces	4.8249E-05	0.001696	0.003081	0.00079	0.0021	0.001544	0.002167	0.000753	0.002068	0.000308	0.001497
deferribacter	8.0414E-05	0.000527	0.000483	0.001773	0.012685	0.000548	0.000538	0.001419	0.004623	0.000308	0.000494
anaerolinea	3.2166E-05	0.000809	0.002574	0.003075	0.003766	0.000563	0.001077	0.002185	0.003679	0.000504	0.00239
ornatilinea	3.2166E-05	0.002351	0.002314	0.003768	0.005544	0.001876	0.001008	0.001142	0.001805	0.000476	0.000522
leptolinea	9.6497E-05	0.001529	0.002104	0.004253	0.00364	0.001371	0.001132	0.003817	0.002249	0.000504	0.001071
holophaga	4.8249E-05	0.004304	0.003576	0.000402	0.000882	0.003998	0.00421	0.000929	0.001652	0.003052	0.006826
pseudomonas	0.01551996	0.000334	0.000854	0.000319	0.00035	0.000592	0.006046	0.001984	0.002138	0.006875	0.005274
sulfuricurvum	0.00106147	0.00122	0.009294	0.000319	0.00063	0.00202	0.011291	0.001004	0.001152	0.002282	0.006112
chitinophaga	8.0414E-05	0.0074	0.000829	0.000166	0.000168	0.011213	0.000207	0.000301	9.72E-05	0.001918	0.000577
methanolinea	9.6497E-05	0.000758	0.0012	0.00417	0.002268	0.001111	0.000635	0.002423	0.005858	0.00035	0.001813
dehalobacterium	4.8249E-05	0.003032	0.002017	0.003532	0.003598	0.003132	0.001822	0.002674	0.002068	0.000966	0.001209
desulfomonile	6.4331E-05	0.000283	0.001077	0.002383	0.004284	0.000188	0.000759	0.007433	0.006192	0.000854	0.000769
rubrobacter	8.0414E-05	0.002646	0.002426	0.003158	0.003164	0.001631	0.001146	0.001858	0.002096	0.000294	0.001126
pedomicrobium	8.0414E-05	0.0014	0.002178	0.004973	0.004536	0.001891	0.001173	0.001783	0.002096	0.000854	0.001758
terribacillus	6.4331E-05	0.000218	0.001436	0.000332	0.004102	0.000981	0.015141	0.000188	0.000139	0.000504	0.006084

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methanobrevibacter	0.00035382	0.001169	0.001361	0.002452	0.001414	0.001472	0.001035	0.003453	0.003429	0.000938	0.001566
thiobacillus	0.00016083	0.000552	0.005841	0.000374	0.00084	0.001443	0.00726	0.000427	0.0005	0.004229	0.008584
thioalkalibacter	0.00012866	0.00379	0.001794	0.000194	0.00035	0.005888	0.002747	0.000226	0.000389	0.00112	0.003186
hespellia	0.00014475	0.002364	0.006782	0.000125	9.8E-05	0.001313	0.003464	0.000188	0.000555	0.000616	0.001525
cetobacterium	0.01560037	0.00045	0.000495	0.000152	0.000322	0.000144	0.001146	0.003013	0.002943	0.005195	0.004587
methylophaga	8.0414E-05	0.002505	0.001955	0.00374	0.000784	0.001934	0.000745	0.001218	0.001111	0.000616	0.000893
rhodocyclus	0.00923156	0.000141	0.00125	0.000125	0.000168	0.000245	0.002057	0.002009	0.002388	0.005027	0.0064
enterobacter	0.01395992	0.000141	0.00047	0.000166	0.00063	0.000188	0.001284	0.000904	0.000736	0.003767	0.001154
dechloromonas	0.00413329	0.000321	0.001126	8.31E-05	0.000252	0.000361	0.001836	0.003114	0.00236	0.006147	0.005411
streptococcus	0.01080768	0.001683	0.001485	0.000388	0.000644	0.00101	0.001491	0.000917	0.001166	0.001932	0.001813
oscillospira	0.00199427	0.000694	0.003007	0.000319	0.000644	0.000693	0.002636	0.00054	0.00118	0.00147	0.007348
solirubrobacter	9.6497E-05	0.000745	0.000619	0.004405	0.002576	0.000823	0.000179	0.001682	0.000666	0.00014	0.000179
cloacibacillus	0.00041815	0.000604	0.004542	0.000249	0.000602	0.000592	0.004113	0.000213	0.000333	0.000504	0.004216
planctomyces	9.6497E-05	0.000244	0.001943	0.000623	0.000938	0.000144	0.003202	0.00349	0.003623	0.00056	0.003461
alkalibacter	0.00012866	0.00212	0.00177	0.002881	0.00196	0.001328	0.000842	0.001055	0.000569	0.000518	0.000714
thermoanaerobacter	0.00017691	0.000462	0.002203	0.000125	0.000252	0.00166	0.007384	0.001507	0.004651	0.00084	0.006098
acetivibrio	0.00012866	0.001799	0.000866	0.001662	0.002184	0.001559	0.001228	0.001544	0.00211	0.000364	0.00239

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ruminiclostridium	0.00093281	0.001079	0.00078	0.002396	0.000434	0.001068	0.000552	0.001105	0.000625	0.00147	0.001209
desulfosporosinus	1.6083E-05	0.002813	0.001497	0.000208	0.000588	0.004084	0.001546	0.000515	0.000555	0.002408	0.002335
tolumonas	0.01770723	1.28E-05	8.66E-05	0.000111	0.000518	2.89E-05	5.52E-05	0.000665	0.000652	0.003753	0.001346
chloroflexus	4.8249E-05	0.000308	0.000705	0.002632	0.00231	0.00026	0.000152	0.003051	0.002152	7E-05	0.000261
faecalibacterium	0.01815755	7.71E-05	4.95E-05	4.16E-05	4.2E-05	2.89E-05	4.14E-05	0.000791	0.001124	0.002282	0.001827
streptomyces	6.4331E-05	0.000591	0.000978	0.001967	0.002814	0.00052	0.000524	0.001833	0.001666	0.00021	0.000481
desulfobacter	0.00027341	0.000103	0.001324	0.000139	0.000168	0.000173	0.001118	0.001431	0.004901	0.002184	0.003653
kushneria	0.00032166	6.42E-05	0.000433	0.000139	0.004816	0.00013	0.000124	8.79E-05	0.000528	5.6E-05	6.87E-05
pelosinus	0.01497314	0.000681	0.000285	0.000388	8.4E-05	0.000462	0.000152	0.000465	0.000347	0.001792	0.000728
aminomonas	3.2166E-05	0.000655	0.00021	0.000887	0.004914	0.000563	9.66E-05	0.000603	0.000361	0.00014	5.49E-05
moorella	4.8249E-05	0.00027	0.000842	0.002022	0.000854	0.000202	0.000152	0.00334	0.002735	8.4E-05	0.000385
desulfofaba	0.00020908	6.42E-05	0.001312	0.000499	0.000994	7.22E-05	0.000966	0.00167	0.004831	0.000126	0.000879
symbiobacterium	8.0414E-05	0.001644	0.000767	0.00061	0.000672	0.002251	0.000635	0.001896	0.002777	0.00035	0.000687
anaerofustis	8.0414E-05	0.003058	0.000495	0.001482	0.001036	0.00254	0.000138	0.000703	0.000764	0.000602	0.000206
thermogymnomonas	6.4331E-05	0.000822	0.000371	0.001053	0.000672	0.000534	0.000331	0.001996	0.004387	0.00014	0.001003
lutispora	4.8249E-05	0.00018	0.001794	0.000776	0.001274	0.000231	0.001339	0.000653	0.001013	0.000168	0.001058
serratia	0.01239988	5.14E-05	8.66E-05	1.39E-05	2.8E-05	7.22E-05	0.000152	0.000402	0.00068	0.004103	0.001621

subdoligranulum	0.012657221	7.71E-05	4.95E-05	6.93E-05	4.2E-05	4.33E-05	5.52E-05	0.000778	0.001027	0.002968	0.001731
mycoplasma	9.6497E-05	0.002736	0.000347	0.000291	0.000322	0.01065	0.000193	0.000527	0.000208	0.001568	0.00044
propionigenium	0.016404052	7.71E-05	6.19E-05	9.7E-05	7E-05	0	6.9E-05	0.000113	0.00018	0.00112	0.00033
ignavibacterium	6.4331E-05	0.000604	0.00078	0.001302	0.000952	0.000707	0.000552	0.002197	0.001708	0.00028	0.000563
methanomethylovorans	6.4331E-05	0.000398	0.00104	0.00072	0.001554	0.000361	0.000317	0.001293	0.002582	0.000294	0.000769
caloramator	3.2166E-05	0.001657	0.00099	0.000956	0.000854	0.002309	0.000773	0.000716	0.000819	0.0007	0.000852
methanoculleus	8.0414E-05	0.001349	0.000928	0.001385	0.000812	0.001284	0.000331	0.00054	0.000666	0.000154	0.000261
desulfuromonas	3.2166E-05	0.00018	0.000545	0.000859	0.00049	0.000173	0.000345	0.005813	0.002929	0.000882	0.001785
arthrobacter	0.00012866	0.001593	0.000619	0.001579	0.000182	0.001097	0.00029	0.001645	0.000486	0.000714	0.000316
acholeplasma	1.6083E-05	0.000938	0.000507	0.000319	0.000112	0.000635	0.000179	0.002461	0.007802	0.000168	0.001785
pirellula	3.2166E-05	0.000411	0.000718	0.000845	0.00154	0.000476	0.000317	0.000515	0.001236	0.000112	0.000398
ralstonia	8.0414E-05	0.000154	0.000631	0.000485	0.002982	0.000173	0.000248	0.000728	0.001416	2.8E-05	0.000302
caldanaerobacter	3.2166E-05	0.000591	0.001386	1.39E-05	0.000224	0.000289	0.001573	0.000176	0.000555	0.000532	0.004656
thermoanaerobaculum	4.8249E-05	7.71E-05	0.000557	0.000748	0.000714	0.000101	0.000552	0.001871	0.001874	0.000168	0.000714
candidatus solibacter	1.6083E-05	0.00045	0.000891	0.000443	0.000644	0.000476	0.000897	0.000942	0.002013	0.000308	0.001552
acidovorax	0.00279842	0.000128	0.001324	6.93E-05	8.4E-05	0.000245	0.002995	0.000741	0.000847	0.00266	0.001882
haliangium	3.2166E-05	0.000437	0.000656	0.000332	0.000588	0.000548	0.000676	0.001243	0.001458	0.001302	0.001387

azospirillum	3.2166E-05	0.001336	0.000804	0.000319	0.000448	0.001587	0.000759	0.001632	0.001985	0.000308	0.000838
leptolyngbya	4.8249E-05	0.001966	0.000408	0.000208	0.000224	0.001919	0.000235	0.002021	0.000764	0.000644	0.001456
desulfocaldus	6.4331E-05	3.85E-05	0.000408	0.000402	0.004396	4.33E-05	0.000138	0.002285	0.002665	8.4E-05	0.000179
desulfonema	4.8249E-05	2.57E-05	0	9.7E-05	0.000672	1.44E-05	0	6.28E-05	0.000208	4.2E-05	6.87E-05
blastomonas	3.2166E-05	0.00027	0.000483	0.000429	0.0007	0.000303	0.000787	0.000691	0.000833	0.000798	0.003049
carboxydocella	1.6083E-05	0.00036	0.002252	0.000235	0.000448	0.000577	0.002471	0.000126	0	0.000182	0.001195
chlamydia	3.2166E-05	0.000257	0.000928	0.00018	0.000392	0.000173	0.000704	0.002034	0.001624	0.001302	0.001442
aminivibrio	0	0.000552	0.000309	0.001842	0.000546	0.000722	0.00011	0.000439	0.000305	9.8E-05	6.87E-05
gaiella	3.2166E-05	0.001079	0.000235	0.000776	0.000336	0.000895	0.000262	0.000465	0.000541	0.000532	0.000288
nitrosovibrio	0.000025733	0.000462	0.000272	0.001164	0.000714	0.000476	0.000373	0.000753	0.000944	0.000392	0.000838
desulfatiferula	3.2166E-05	1.28E-05	0.000149	2.77E-05	4.2E-05	1.44E-05	0.000166	0.002624	0.003582	0.001218	0.002939
cellulosilyticum	8.0414E-05	0.000617	0.000644	0.00115	0.000672	0.000375	0.000607	0.001381	0.000264	0.00021	0.000206
veillonella	0.000202644	0.000707	0.000309	0.000374	0.00028	0.000765	0.000304	0.00054	0.000236	0.00091	0.000371
caldilinea	0	0.000642	0.000446	0.001191	0.00035	0.000534	6.9E-05	0.000427	0.000652	8.4E-05	0.00022
methylotenera	0.000025733	3.85E-05	0.000842	2.77E-05	5.6E-05	0.000216	0.00127	0.000716	0.000583	0.003346	0.002074
cloacibacterium	0.000221943	0.00018	0.000272	0.00018	0.000168	0.000144	0.000193	0.001205	0.001027	0.0007	0.000659
methanomassiliicoccus	1.6083E-05	8.99E-05	0.000186	0.001219	0.000126	0.000216	2.76E-05	0.001105	0.000305	9.8E-05	2.75E-05

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coxiella	3.2166E-05	0.000488	0.00073	0.000402	0.000546	0.000823	0.000428	0.001017	0.000389	0.00014	0.000412
candidatus phytoplasma	4.8249E-05	0.000347	0.001188	0.000166	7E-05	0.000404	0.001366	8.79E-05	6.94E-05	0.000238	0.000714
dethiobacter	3.2166E-05	0.000347	0.000347	0.001413	0.001596	0.000346	0.000166	0.000364	0.000305	7E-05	0.000192
aquabacterium	0.0032809	7.71E-05	0.000223	4.16E-05	7E-05	0.000101	0.000538	0.001193	0.000986	0.00119	0.001112
pelagicoccus	1.6083E-05	0.00176	0.000322	0.000152	0.00014	0.001342	0.000221	7.53E-05	0.000264	0.000504	9.61E-05
intestinimonas	4.8249E-05	0.00018	0.000829	0.000235	0.001148	7.22E-05	0.001463	6.28E-05	0.000208	5.6E-05	0.001085
sedimentibacter	0.0012866	0.000874	0.000644	2.77E-05	5.6E-05	0.000938	0.001532	0.000126	0.00018	0.00042	0.000618
blautia	0.0062884	6.42E-05	3.71E-05	2.77E-05	5.6E-05	0.000101	5.52E-05	0.000239	0.000347	0.000868	0.000797
fibrobacter	9.6497E-05	0.000668	0.000322	0.000277	0.000224	0.000433	0.000386	0.000138	0.000264	0.001162	0.001332
desulforegula	8.0414E-05	6.42E-05	0.000173	5.54E-05	0.000154	5.77E-05	0.000138	0.000527	0.001957	0.002702	0.002239
halothiobacillus	6.4331E-05	0.000257	0.00078	9.7E-05	0.000196	0.000245	0.002346	0.000289	0.000319	0.000154	0.000549
beggiatoa	0	0.000257	0.000433	0.000526	0.001652	0.000231	0.000304	0.000264	0.000194	4.2E-05	0.000275
lysobacter	8.0414E-05	0.000103	0.000161	6.93E-05	0.000588	0.00013	9.66E-05	0.000138	0.000278	0.00028	0.00044
acidithiobacillus	0	0.00018	0.00099	0.000485	0.000196	0.000216	0.000179	0.000113	0.000472	2.8E-05	0.000357
robinsoniella	3.2166E-05	0.000578	0.000322	0.000235	0.00021	0.000563	0.000483	0.000703	0.000305	0.000616	0.000385
gracilibacter	4.8249E-05	0.000257	0.000272	0.000887	0.00063	0.000101	0.000428	0.00113	0.000625	0.000126	0.000179

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lactivibrio	1.6083E-05	0.000334	0.000186	0.001094	0.00091	0.00026	0.00011	0.000515	0.000167	0.00021	0.000151
saccharibacter	0	0.000167	3.71E-05	0.003158	5.6E-05	5.77E-05	2.76E-05	7.53E-05	2.78E-05	7E-05	2.75E-05
peredibacter	9.6497E-05	0.000989	0.000347	0.00036	0.000714	0.000707	0.000317	0.000477	0.000208	0.000196	0.000233
microbulbifer	0	1.28E-05	0	0.000263	0.000812	0	1.38E-05	0.000578	0.002901	1.4E-05	1.37E-05
desulfotignum	9.6497E-05	0	1.24E-05	1.39E-05	2.8E-05	0	5.52E-05	0.000138	0.004276	0.000252	0.003241
maricaulis	0	0.001644	0.00052	2.77E-05	5.6E-05	0.001602	0.000317	0	4.16E-05	0.000322	0.000151
rhizobium	0.00014475	0.000206	0.00078	0.000111	0.000504	0.000115	0.001077	0.000113	0.000194	0.00014	0.000536
acidaminococcus	0.00427804	0	6.19E-05	0	7E-05	2.89E-05	5.52E-05	0.000414	0.000597	0.001246	0.000728
thermaerobacter	1.6083E-05	0	1.24E-05	0.000166	0.000518	1.44E-05	0	0.004256	0.000583	4.2E-05	4.12E-05
simplicispira	0.00209077	6.42E-05	0.000223	8.31E-05	0.000126	4.33E-05	0.000455	0.000465	0.000639	0.001204	0.001126
turicibacter	0.00049857	0.001272	0.000309	0.00018	0.000112	0.001068	0.000152	0.000163	0.000167	0.000658	0.000124
helicobacter	0.00012866	0.000771	0.000149	0.000277	0.00042	0.000678	0.00011	0.000326	0.000347	0.00056	0.00022
rhodopirellula	1.6083E-05	6.42E-05	0.000322	0.000471	0.001008	1.44E-05	0.000152	0.000163	0.000708	0	0.00022
desulfatiglans	1.6083E-05	1.28E-05	7.43E-05	6.93E-05	0.001806	0	0.000221	5.02E-05	0.000944	4.2E-05	4.12E-05
pseudoxanthomonas	0.00011258	3.85E-05	0.000681	5.54E-05	0.000546	7.22E-05	0.001063	0.000126	0.0005	0.000266	0.001291
polyangium	0	0	0	0.000249	0.000294	0	0	0.00172	0.004234	2.8E-05	2.75E-05
barnesiella	0.00088456	0.000244	0.000272	5.54E-05	0.000308	0.000332	0.00138	0.0001	0.000139	0.000378	0.000247

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byssovorax	0	5.14E-05	0.00026	0.000374	0.000308	0.000216	0.000345	2.51E-05	5.55E-05	5.6E-05	0.000247
anaerovibrio	4.8249E-05	0.000848	0.000334	0.000416	0.000196	0.000592	0.000317	0.000213	6.94E-05	0.000238	0.000165
comamonas	0.00123838	0.000116	0.000235	4.16E-05	0.000266	0.000115	0.000566	0.000301	0.000458	0.00042	0.000343
dyella	6.4331E-05	2.57E-05	7.43E-05	0.000222	0.000658	1.44E-05	5.52E-05	0.000239	0.0005	2.8E-05	4.12E-05
solobacterium	1.6083E-05	0.000283	0.000161	0.000346	0.000336	0.000317	0.000124	2.51E-05	0.000153	4.2E-05	4.12E-05
dialister	0.00149571	0.000501	0.000198	0.00018	0.000168	0.000303	0.000207	0.000578	0.000278	0.000616	0.000467
alistipes	0.00392422	2.57E-05	2.48E-05	5.54E-05	7E-05	2.89E-05	1.38E-05	0.000239	0.000333	0.000952	0.000632
devosia	1.6083E-05	3.85E-05	0.000458	0	0.000364	4.33E-05	0.000787	1.26E-05	0.000194	4.2E-05	0.000989
bifidobacterium	0.00345781	1.28E-05	1.24E-05	1.39E-05	0	4.33E-05	0	0.000477	0.000736	0.00084	0.000728
alkaliflexus	0	0.000193	8.66E-05	0.000222	0.000532	0.00026	0.000304	0.000339	0.001222	5.6E-05	0.00044
prochlorococcus	4.8249E-05	0.000565	0.000879	0.000139	7E-05	0.00039	0.000304	0.000163	0.00018	0.000112	0.000371
methylomonas	4.8249E-05	3.85E-05	8.66E-05	1.39E-05	5.6E-05	2.89E-05	0.0004	0.000239	0.000264	0.00287	0.001703
fluviicola	0	0.000514	0.000483	8.31E-05	4.2E-05	0.000332	0.00069	0.000163	0.000167	0.000322	0.000467
haliscomenobacter	1.6083E-05	3.85E-05	3.71E-05	9.7E-05	0.000672	2.89E-05	6.9E-05	5.02E-05	0.000125	4.2E-05	0.000151
sterolibacterium	0	3.85E-05	0.000124	8.31E-05	0.000182	0	0.000262	0.000452	0.000389	4.2E-05	0.000261
desulfitobacterium	3.2166E-05	3.85E-05	3.71E-05	0.000125	0.000154	2.89E-05	4.14E-05	0.001707	0.001971	0.00035	0.000398
calditerricola	3.2166E-05	0.000308	0.000606	0.000305	0.000294	0.00026	0.000635	0.000314	0.000153	0.000126	0.000302

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waddlia	0	2.57E-05	8.66E-05	8.31E-05	0.001442	1.44E-05	4.14E-05	0.000414	0.000819	1.4E-05	4.12E-05
lachnoclostridium	0.00284667	0.000128	0.000111	0.000111	8.4E-05	5.77E-05	0.000193	0.000239	0.000292	0.000602	0.000357
prevotella	0.00500177	1.28E-05	4.95E-05	0	1.4E-05	2.89E-05	2.76E-05	7.53E-05	4.16E-05	0.000406	0.000137
sphingobacterium	0.00739981	0.000244	0.000235	6.93E-05	0.00035	0.000332	8.28E-05	0.000138	0.000861	0.000322	0.000343
phycisphaera	1.6083E-05	1.28E-05	7.43E-05	8.31E-05	0.000672	0	0.000166	0.000703	0.00043	0.000112	0.001882
desulfocapsa	0	5.14E-05	0.000384	0	0.000266	1.44E-05	0.000373	0.000427	0.000888	0.000182	0.000975
thauera	0.00788806	1.28E-05	1.24E-05	0	0	0	1.38E-05	0.00182	0.001499	0.00063	0.000604

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Table S2: Relative abundance of the top 98% most abundant genera in DFF10 and DFF25 detected across all samples analyzed.

	DFF10					DFF25				
Genus	DFF10_180	DFF10_200	DFF10_230	DFF10_250	DFF10_355	DFF25_180	DFF25_200	DFF25_230	DFF25_250	DFF25_355
clostridium	0.102835	0.04844	0.227531	0.100276	0.081943	0.048763	0.243105	0.079674	0.20153	0.06545
geobacter	0.032439	0.17151	0.016197	0.017638	0.046874	0.226915	0.012354	0.008849	0.030268	0.19685
trichococcus	0.035515	0.043177	0.014018	0.18195	0.025115	0.053564	0.017822	0.209246	0.055706	0.04402
cytophaga	0.057347	0.031804	0.044233	0.041861	0.055743	0.026922	0.046186	0.056921	0.021789	0.024977
methanosaeta	0.075057	0.03198	0.041254	0.010746	0.058867	0.037139	0.027956	0.006708	0.047305	0.045504
bacteroides	0.040018	0.008166	0.068521	0.027996	0.066279	0.008441	0.083249	0.024075	0.024983	0.01354
arcobacter	0.004487	0.007401	0.00762	0.004068	0.001863	0.004115	0.002334	0.005857	0.002233	0.010412
bellilinea	0.063689	0.015727	0.077035	0.010626	0.061901	0.013716	0.057431	0.00907	0.069211	0.011693
acinetobacter	0.011273	0.110167	0.006366	0.010345	0.006753	0.074472	0.007768	0.108237	0.003233	0.015132
lactococcus	0.012113	0.014722	0.012136	0.027755	0.004603	0.004361	0.044538	0.055472	0.018854	0.051303
desulfomicrobium	0.005502	0.006906	0.026468	0.02888	0.005086	0.005768	0.042073	0.021839	0.003116	0.007391
aeromonas	0.006722	0.043272	0.003559	0.003827	0.009142	0.092725	0.001665	0.00981	0.005649	0.057601
syntrophus	0.039479	0.01737	0.013673	0.009528	0.031896	0.013962	0.006871	0.006613	0.031359	0.01528
longilinea	0.051338	0.011436	0.027236	0.010438	0.042068	0.007984	0.019454	0.007416	0.040423	0.011248
levilinea	0.023386	0.016317	0.021058	0.037458	0.02054	0.013962	0.013562	0.034704	0.025659	0.010142

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solitalea	0.013318	0.022617	0.035311	0.020288	0.020645	0.010498	0.033358	0.014565	0.005869	0.008753
methanobacterium	0.040335	0.009395	0.015021	0.015136	0.045389	0.007245	0.012664	0.014675	0.049032	0.009009
desulfobulbus	0.023513	0.008087	0.010208	0.010144	0.023482	0.006559	0.008813	0.006818	0.024983	0.003668
fusibacter	0.003123	0.006252	0.011619	0.011482	0.001898	0.00779	0.028544	0.011069	0.00535	0.015064
parabacteroides	0.002061	0.005391	0.003936	0.01191	0.004182	0.004291	0.007262	0.010046	0.001376	0.004073
spirochaeta	0.011764	0.011596	0.010992	0.006089	0.012365	0.009144	0.005386	0.004015	0.006493	0.010007
desulfovibrio	0.005343	0.019634	0.008201	0.018936	0.005745	0.016125	0.005826	0.011998	0.004532	0.011774
syntrophorhabdus	0.018376	0.013318	0.012999	0.002476	0.019573	0.010885	0.00488	0.001354	0.01592	0.01106
dehalococcoides	0.024337	0.006539	0.008702	0.003051	0.022523	0.005733	0.006414	0.002141	0.031905	0.006136
shewanella	0.002537	0.023558	0.001897	0.002021	0.00241	0.015914	0.000849	0.003417	0.003662	0.042186
syntrophomonas	0.009561	0.003286	0.021136	0.002984	0.013738	0.003957	0.011473	0.00222	0.012583	0.005476
anaerophaga	0.006453	0.004913	0.00519	0.024089	0.006053	0.003482	0.004064	0.009841	0.003259	0.004707
compostimonas	0.00899	0.004003	0.01096	0.007989	0.010887	0.004695	0.012452	0.008503	0.00561	0.004006
prolixibacter	0.003425	0.00386	0.007746	0.010479	0.002592	0.003183	0.008976	0.008581	0.001493	0.004019
pelobacter	0.002426	0.004259	0.00392	0.023888	0.002508	0.002972	0.004031	0.013919	0.001441	0.002981
phascolarctobacterium	0.00658	0.003286	0.014378	0.007012	0.005955	0.00211	0.010478	0.006613	0.001883	0.003169
rikenella	0.001522	0.001212	0.017922	0.010211	0.00131	0.000985	0.01694	0.007889	0.000883	0.001834
acidobacterium	0.012668	0.003126	0.006335	0.001673	0.021983	0.002427	0.00426	0.000945	0.004882	0.002482

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caldithrix	0.003599	0.003349	0.001662	0.00985	0.00337	0.002286	0.002105	0.004928	0.001948	0.002859
sulfurovum	0.003266	0.005694	0.003011	0.010184	0.002452	0.005328	0.004488	0.007196	0.009258	0.004909
paludibacter	0.001046	0.002249	0.002822	0.002168	0.002354	0.001671	0.005337	0.004141	0.002298	0.002738
smithella	0.007531	0.011468	0.003042	0.002583	0.008386	0.0067	0.001012	0.001764	0.003	0.007323
methanospirillum	0.002743	0.007496	0.000925	0.00645	0.003482	0.007333	0.000441	0.004708	0.013686	0.009076
pedobacter	0.000428	0.002361	0.00218	0.015577	0.000385	0.000985	0.003476	0.010534	0.000338	0.000755
eubacterium	0.001078	0.000526	0.001474	0.006598	0.001128	0.000791	0.001893	0.00411	0.00361	0.002724
proteocatella	0.008895	0.00689	0.00149	0.003332	0.003713	0.004273	0.002334	0.004488	0.005545	0.007755
ornithinibacillus	0.005549	0.001148	0.009126	0.003854	0.003223	0.001319	0.006675	0.003055	0.008077	0.00151
leptospira	0.002426	0.003812	0.001991	0.002864	0.002179	0.002479	0.001632	0.00148	0.00148	0.002967
candidatus cloacimonas	0.006691	0.002664	0.00508	0.006236	0.010368	0.001565	0.003525	0.002598	0.001818	0.001632
aminobacterium	0.003266	0.002456	0.015805	0.002315	0.002809	0.002831	0.00488	0.001937	0.003947	0.002562
guggenheimella	0.022165	7.97E-05	0.000172	0.000174	0.019594	0.000106	0.000228	0.000299	0.017335	0.000162
treponema	0.004487	0.003541	0.002995	0.003359	0.00648	0.003218	0.001909	0.002015	0.001883	0.004842
acetobacterium	0.00157	0.001531	0.000831	0.012887	0.001429	0.003077	0.000702	0.012817	0.006999	0.003668
pelotomaculum	0.000999	0.000797	0.000423	0.000535	0.001506	0.001196	0.000506	0.000236	0.000857	0.00147
chlorobium	0.002125	0.012712	0.000235	0.000856	0.002046	0.009619	0.00093	0.000661	0.000636	0.005867
thermanaerovibrio	0.000174	0.007927	0.000204	0.000187	0.000196	0.014894	0.000245	0.000331	0.000597	0.015307

ruminococcus	0.002901	0.001739	0.004923	0.003533	0.002046	0.001706	0.004733	0.001984	0.004973	0.00236
anaeromyxobacter	0.002775	0.006555	0.003167	0.003279	0.00482	0.005451	0.002154	0.001417	0.001649	0.007161
fusobacterium	0.001142	0.01134	0.000376	0.001659	0.002263	0.0099	0.000343	0.005606	0.001286	0.007701
paenibacillus	0.004693	0.002217	0.000612	0.008926	0.004231	0.002743	0.000881	0.008581	0.005441	0.009157
synergistes	0.000698	0.002664	0.007809	0.005072	0.000792	0.001706	0.004488	0.004928	0.001221	0.001457
pedosphaera	0.003948	0.004801	0.001568	0.00265	0.006249	0.003271	0.001469	0.001685	0.001818	0.003237
anaeromusa	0.001237	0.002313	0.000659	0.004055	0.000722	0.00211	0.001224	0.006472	0.003129	0.007458
blastopirellula	0.007658	0.005072	0.000267	0.00103	0.005969	0.004713	0.000277	0.00096	0.008349	0.003979
syntrophobacter	0.005407	0.00496	0.00276	0.00285	0.004883	0.003077	0.001028	0.001732	0.003947	0.002643
anaerovorax	0.001506	0.003732	0.002117	0.008699	0.000974	0.003042	0.001518	0.006377	0.001532	0.002616
zoogloea	0.000571	0.002281	0.000564	0.004818	0.000862	0.001653	0.000604	0.00263	0.000961	0.003358
verrucomicrobium	0.003837	0.00142	0.002336	0.002663	0.002445	0.00095	0.002285	0.001433	0.001766	0.000944
flavobacterium	0.000935	0.003429	0.001897	0.008913	0.000602	0.002989	0.001991	0.005149	0.000429	0.002562
sulfurospirillum	0.001411	0.000893	0.000314	0.000562	0.001688	0.001055	0.000212	0.000252	0.001286	0.001443
acidaminobacter	0.00065	0.002967	0.001615	0.003774	0.000399	0.002321	0.004031	0.00444	0.000636	0.004464
chondromyces	0.002426	0.005423	0.004422	0.008806	0.002536	0.00357	0.001616	0.004724	0.001234	0.003453
deferribacter	0.002584	0.005917	0.000423	0.000642	0.00152	0.009021	0.0008	0.000425	0.001467	0.00646
anaerolinea	0.004154	0.003541	0.00149	0.001378	0.00482	0.005715	0.000979	0.001165	0.002727	0.005651

ornatilinea	0.003203	0.00303	0.002744	0.001445	0.003335	0.00313	0.001763	0.001401	0.00596	0.002765
leptolinea	0.003044	0.002169	0.001897	0.001807	0.004217	0.002409	0.001257	0.001984	0.005649	0.002441
holophaga	0.00084	0.001547	0.00472	0.002797	0.000855	0.000686	0.003493	0.002441	0.000247	0.000742
pseudomonas	0.000333	0.001148	0.000172	0.001191	0.000252	0.001741	0.00018	0.001685	0.000234	0.000877
sulfuricurvum	0.00019	0.000622	0.000878	0.003011	0.000252	0.000914	0.001306	0.002929	0.000312	0.000958
chitinophaga	3.17E-05	6.38E-05	0.006444	0.000134	7.71E-05	5.28E-05	0.017283	0.000126	0.000156	5.39E-05
methanolinea	0.005613	0.003397	0.001772	0.000602	0.007503	0.001846	0.000669	0.000441	0.002727	0.002077
dehalobacterium	0.002664	0.001372	0.00174	0.001218	0.001457	0.002198	0.002513	0.001008	0.004869	0.002401
desulfomonile	0.004661	0.001978	0.000157	0.001044	0.002179	0.003658	0.000212	0.000709	0.004116	0.002131
rubrobacter	0.003203	0.002377	0.003497	0.001178	0.002935	0.002673	0.001926	0.000992	0.003805	0.001915
pedomicrobium	0.002933	0.001515	0.002242	0.00095	0.004084	0.0016	0.00111	0.000693	0.004168	0.001659
terribacillus	0.000872	0.000782	0.000251	0.000749	0.003265	0.000492	0.000702	0.000945	0.001597	0.004343
methanobrevibacter	0.002695	0.00319	0.001458	0.001151	0.006942	0.002005	0.001012	0.001008	0.001532	0.001605
thiobacillus	0.000301	0.00051	0.000251	0.002984	0.000588	0.001038	0.00018	0.002598	0.000818	0.000809
thioalkalibacter	0.000143	0.000542	0.001254	0.003801	0.000105	0.000457	0.010445	0.001952	0.000156	0.000283
hespellia	0.000111	7.97E-05	0.003011	0.007066	4.9E-05	1.76E-05	0.002725	0.006566	6.49E-05	0.000108
cetobacterium	0.000127	0.000191	0.000141	0.000147	8.41E-05	0.000123	0.000261	0.000315	0.000338	0.000364
methylophaga	0.004392	0.000957	0.002242	0.000883	0.003734	0.000756	0.001289	0.000598	0.003415	0.001133

rhodocyclus	0.000174	0.000271	0.000267	0.001566	0.000224	0.000281	0.000196	0.00137	2.6E-05	0.000418
enterobacter	0.00046	0.001802	0.000408	0.000348	0.000224	0.00262	0.000114	0.000425	0.000182	0.00379
dechloromonas	0.000143	0.000686	0.000486	0.002422	0.00014	0.000475	0.000294	0.001055	9.09E-05	0.000351
streptococcus	0.000587	0.000383	0.000455	0.00091	0.000392	0.000475	0.001697	0.001023	0.000701	0.001241
oscillospira	0.000444	0.000797	0.000502	0.003747	0.000161	0.000686	0.000832	0.001968	0.000221	0.000674
solirubrobacter	0.004519	0.000702	0.001411	0.000281	0.004252	0.00109	0.000832	0.000488	0.003168	0.001349
cloacibacillus	0.000143	0.000303	0.000847	0.005594	0.000154	0.000264	0.000604	0.005196	0.000441	0.000229
planctomyces	0.001046	0.001563	0.000517	0.003145	0.00131	0.000932	0.000147	0.001307	0.000273	0.000917
alkalibacter	0.001142	0.000925	0.002352	0.001392	0.000967	0.001354	0.00173	0.001228	0.00274	0.001106
thermoanaerobacter	0.000206	0.00067	0.000251	0.000321	0.000154	0.00051	9.79E-05	0.000189	0.000104	0.000647
acetivibrio	0.001585	0.001483	0.000972	0.000669	0.001016	0.001653	0.001795	0.00052	0.000974	0.001956
ruminiclostridium	0.00398	0.000175	0.00058	0.000642	0.00573	0.000475	0.001338	0.000472	0.000935	0.000566
desulfosporosinus	0.000159	0.000415	0.000768	0.001151	0.000105	0.000879	0.004309	0.001165	0.000143	0.000877
tolumonas	0.000127	6.38E-05	6.27E-05	9.37E-05	4.2E-05	0.000141	6.53E-05	6.3E-05	6.49E-05	0.000445
chloroflexus	0.002061	0.00134	0.000439	0.000335	0.003447	0.001372	0.000196	0.000173	0.002636	0.00178
faecalibacterium	4.76E-05	3.19E-05	4.7E-05	0.000134	3.5E-05	8.79E-05	4.9E-05	6.3E-05	3.9E-05	2.7E-05
streptomyces	0.002204	0.001053	0.000847	0.000428	0.002312	0.001002	0.000457	0.000457	0.002701	0.001443
desulfobacter	0.000222	0.000319	0.00022	0.004443	0.000217	0.000193	0.000163	0.002976	6.49E-05	0.000162

kushneria	0.000111	0.00453	6.27E-05	0.000696	0.000357	0.005592	0.000131	0.000189	0.000143	0.0053
pelosinus	0.000365	0.000112	0.00047	0.000254	0.000273	0.000193	0.000555	0.000346	0.000338	0.000108
aminomonas	0.000729	0.002791	0.000894	0.000161	0.000834	0.003324	0.000718	7.87E-05	0.00074	0.003628
moorella	0.002014	0.001228	0.000596	0.000709	0.003783	0.00058	0.000343	0.000252	0.001078	0.000769
desulfofaba	0.000793	0.003094	0.000141	0.000776	0.001079	0.001583	8.16E-05	0.00063	0.00061	0.001416
symbiobacterium	0.000951	0.000925	0.00127	0.000562	0.000694	0.000932	0.001893	0.000614	0.000454	0.00085
anaerofustis	0.001554	0.000223	0.002007	0.000281	0.000841	0.000246	0.002432	0.000346	0.001727	0.000405
thermogymnomonas	0.001031	0.001372	0.001615	0.000348	0.002725	0.000615	0.000245	0.000236	0.00087	0.000674
lutispora	0.000571	0.001531	0.00022	0.002074	0.000385	0.001477	0.000277	0.001653	0.002779	0.001524
serratia	0.000159	9.57E-05	6.27E-05	0.000134	7.01E-05	8.79E-05	4.9E-05	0.00011	7.79E-05	0.000135
subdoligranulum	3.17E-05	1.59E-05	0	6.69E-05	2.8E-05	1.76E-05	8.16E-05	6.3E-05	2.6E-05	2.7E-05
mycoplasma	0.000111	0.000112	0.000235	0.000321	9.81E-05	0.000387	0.000816	0.000173	7.79E-05	0.000121
propionigenium	7.93E-05	0.000335	9.41E-05	5.35E-05	6.3E-05	8.79E-05	6.53E-05	6.3E-05	7.79E-05	0.000324
ignavibacterium	0.001189	0.001212	0.00116	0.001111	0.001429	0.000932	0.000636	0.000504	0.001052	0.000715
methanomethylovorans	0.000698	0.001723	0.000502	0.000816	0.001086	0.001319	0.000539	0.000583	0.000597	0.001767
caloramator	0.000761	0.000797	0.000878	0.000803	0.000595	0.000668	0.001975	0.000472	0.000519	0.000688
methanoculleus	0.001268	0.000478	0.001223	0.000482	0.001541	0.000369	0.001306	0.000378	0.002909	0.000337
desulfuromonas	0.000539	0.000606	0.000486	0.000254	0.000602	0.000475	0.000163	0.000173	0.000221	0.000472

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arthrobacter	0.002125	0.000813	0.001035	0.000589	0.00213	0.000334	0.00062	0.000157	0.000818	0.000391
acholeplasma	0.000365	9.57E-05	0.000533	0.000228	0.000483	7.03E-05	0.00049	0.000205	0.00013	9.44E-05
pirellula	0.002093	0.001611	0.00022	0.000388	0.001779	0.001301	0.000734	0.000252	0.00135	0.000917
ralstonia	0.000935	0.001324	0.000251	0.000736	0.001177	0.001161	0.000228	0.000346	0.001597	0.001268
caldanaerobacter	7.93E-05	0.000351	0.000564	0.003118	4.2E-05	0.000176	0.000294	0.001165	9.09E-05	0.00027
thermoanaerobaculum	0.001792	0.001292	9.41E-05	0.000281	0.002284	0.000686	0.000196	0.000283	0.000753	0.001065
candidatus solibacter	0.000618	0.001324	0.000423	0.000977	0.000645	0.000809	0.000669	0.00074	0.000338	0.000863
acidovorax	9.51E-05	0.000223	7.84E-05	0.000294	7.71E-05	0.000193	0.000114	0.000394	6.49E-05	0.000229
haliangium	0.00092	0.000909	0.000831	0.000977	0.001086	0.000352	0.000196	0.000331	0.000403	0.000796
azospirillum	0.000396	0.000797	0.00058	0.000428	0.000343	0.000563	0.000963	0.000299	0.000208	0.000796
leptolyngbya	0.000238	0.00051	0.00185	0.000428	0.000133	0.000211	0.001387	0.000205	0.000104	0.000189
desulfocaldus	0.000206	0.000797	1.57E-05	0.000201	0.000518	0.00044	6.53E-05	0.000157	0.000714	0.000701
desulfonema	7.93E-05	0.004354	0	0	2.1E-05	0.005434	3.26E-05	3.15E-05	5.19E-05	0.003156
blastomonas	0.000634	0.000766	0.00022	0.001191	0.000441	0.000475	0.000147	0.000457	0.000818	0.000405
carboxydocella	0.000174	0.000303	0.00022	0.002195	9.81E-05	0.000528	0.000163	0.001512	0.000195	0.000486
chlamydia	0.000238	0.000877	0.000157	0.00087	0.000476	0.000369	0.000228	0.000394	0.000416	0.000418
aminivibrio	0.001966	0.000207	0.001223	0.000134	0.002081	0.000264	0.000441	9.45E-05	0.001143	0.000216
gaiella	0.001855	0.000207	0.000768	0.000147	0.001513	0.000229	0.001093	0.000173	0.00087	0.00027

nitrosovibrio	0.000698	0.000383	0.000329	0.000241	0.001149	0.000404	0.000392	0.000173	0.000338	0.000782
desulfatiferula	0.000111	4.78E-05	4.7E-05	0.000201	4.2E-05	5.28E-05	1.63E-05	7.87E-05	1.3E-05	1.35E-05
cellulosilyticum	0.000793	0.000319	0.00047	0.000361	0.000946	0.000317	0.000228	0.000315	0.000714	0.00058
veillonella	0.000349	0.000128	0.000627	0.00091	0.000385	0.000158	0.000783	0.000346	0.000597	9.44E-05
caldilinea	0.001046	0.000558	0.000706	0.000201	0.002123	0.000299	0.000343	6.3E-05	0.000792	0.000364
methylotenera	6.34E-05	0.000159	0.000141	0.000214	0.000126	0.000299	0.000163	0.00022	0	0.000148
cloacibacterium	0.000206	0.000399	0.000157	0.000388	0.000189	0.000651	0.000294	0.00022	9.09E-05	0.000998
methanomassiliicoccus	0.001427	0.000175	0.000517	9.37E-05	0.003075	5.28E-05	0.000228	0	0.001363	0.000135
coxiella	0.00027	0.000239	0.000329	0.001017	0.000399	0.000229	0.000783	0.000803	0.000688	0.000148
candidatus phytoplasma	0.000317	4.78E-05	0.000329	0.002155	0.000645	8.79E-05	0.000441	0.001086	7.79E-05	9.44E-05
dethiobacter	0.000666	0.000159	0.000455	0.000161	0.000469	0.000475	0.000457	0.000142	0.00126	0.000526
aquabacterium	4.76E-05	0.000223	3.14E-05	0.000228	3.5E-05	0.000106	4.9E-05	0.000283	2.6E-05	9.44E-05
pelagicoccus	0.000127	0.000415	0.001584	0.000709	0.000126	0.000211	0.001404	0.00022	1.3E-05	9.44E-05
intestinimonas	0.00046	0.000829	6.27E-05	0.000589	0.000252	0.000774	0.000196	0.000173	0.000312	0.000742
sedimentibacter	1.59E-05	0.000112	0.000502	0.00095	5.6E-05	0.000176	0.001355	0.000583	5.19E-05	8.09E-05
blautia	3.17E-05	1.59E-05	7.84E-05	5.35E-05	1.4E-05	0	3.26E-05	6.3E-05	1.3E-05	1.35E-05
fibrobacter	0.000444	0.000128	0.000564	0.000335	0.000539	0.000193	0.000881	9.45E-05	0.000273	0.000391
desulforegula	6.34E-05	0.000191	3.14E-05	0.000147	9.11E-05	0.000193	1.63E-05	1.57E-05	7.79E-05	0.000108

halothiobacillus	0.000174	0.000431	0.000125	0.001097	0.000343	0.000229	0.000424	0.00063	0.000104	0.000135
beggiatoa	0.000555	0.000574	0.000408	0.000335	0.000441	0.000545	0.000147	0.000283	0.000714	0.000796
lysobacter	0.000317	0.000415	6.27E-05	0.000147	0.001254	0.000774	0.000114	4.72E-05	0.000169	0.000593
acidithiobacillus	0.001078	0.000494	0.000298	0.000428	0.001562	0.000158	0.000163	0.000299	0.000779	0.000297
robinsoniella	0.000365	0.000175	0.001082	0.000549	0.000287	0.000246	0.000881	0.000409	5.19E-05	0.000283
gracilibacter	0.000682	0.000303	0.000282	0.000228	0.000434	0.000633	0.000294	0.000157	0.000649	0.000364
lactivibrio	0.000428	0.000383	0.000706	0.000281	0.000462	0.000598	0.000408	0.000252	0.000792	0.000418
saccharibacter	0.002172	4.78E-05	9.41E-05	0	0.000834	5.28E-05	8.16E-05	3.15E-05	0.001571	2.7E-05
peredibacter	0.000396	0.000223	0.000408	0.000415	0.000553	0.000229	0.000685	0.000283	0.000286	0.000351
microbulbifer	0.000634	0.001212	0	2.68E-05	0.000743	0.000615	0	0	0.000104	0.000526
desulfotignum	3.17E-05	1.59E-05	0	1.34E-05	7.01E-06	1.76E-05	0	3.15E-05	2.6E-05	8.09E-05
maricaulis	9.51E-05	1.59E-05	0.001897	0.000388	5.6E-05	5.28E-05	0.000816	0.000205	3.9E-05	4.05E-05
rhizobium	0.000222	0.000399	9.41E-05	0.000883	0.000154	0.000651	0.000245	0.000882	0.000441	0.000364
acidaminococcus	0.000127	0.000159	4.7E-05	4.01E-05	6.3E-05	5.28E-05	1.63E-05	7.87E-05	6.49E-05	6.74E-05
thermaerobacter	0.000222	0.000303	3.14E-05	0	0.00021	0.000545	0	1.57E-05	0.00048	0.000337
simplicispira	3.17E-05	0.000175	3.14E-05	0.000294	2.1E-05	0.000246	3.26E-05	0.000157	3.9E-05	0.000175
turicibacter	6.34E-05	9.57E-05	0.000988	0.000161	4.2E-05	5.28E-05	0.001355	0.00011	7.79E-05	4.05E-05
helicobacter	0.000143	0.000159	0.000972	0.000201	0.000413	0.000229	0.000816	0.000173	0.000273	0.000175

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rhodopirellula	0.000587	0.000622	7.84E-05	0.000254	0.000616	0.000352	8.16E-05	0.000189	0.001117	0.000472
desulfatiglans	1.59E-05	0.001244	1.57E-05	0.000308	4.2E-05	0.00095	0	9.45E-05	0.000104	0.001443
pseudoxanthomonas	4.76E-05	0.000223	3.14E-05	9.37E-05	0.000308	0.001284	4.9E-05	0.000283	9.09E-05	0.00031
polyangium	6.34E-05	6.38E-05	1.57E-05	0	0.000196	0.000158	1.63E-05	1.57E-05	9.09E-05	0.000243
barnesiella	6.34E-05	0.000638	3.14E-05	9.37E-05	9.81E-05	0.000492	0.000326	0.00022	9.09E-05	0.00089
byssovorax	0.000206	0.000638	0.000204	0.001071	0.000385	0.000457	0.000163	0.000583	0.000467	0.001119
anaerovibrio	0.000349	6.38E-05	0.000596	0.000749	0.000224	0.000123	0.000604	0.000457	0.000506	5.39E-05
comamonas	0.000143	0.000574	0.000172	0.000241	0.00021	0.000615	3.26E-05	0.000268	0.000143	0.000539
dyella	0.000396	0.000766	3.14E-05	0.000107	0.000848	0.001073	0	9.45E-05	0.000701	0.001011
solobacterium	0.00092	0.000654	0.000439	0.000281	0.000806	0.00058	0.000294	0.000268	0.000247	0.000405
dialister	0.000174	1.59E-05	0.000282	0.000281	9.11E-05	3.52E-05	0.000408	0.000189	0.000169	6.74E-05
alistipes	4.76E-05	3.19E-05	1.57E-05	2.68E-05	2.8E-05	0	4.9E-05	6.3E-05	1.3E-05	1.35E-05
devosia	3.17E-05	0.00051	9.41E-05	0.00091	1.4E-05	0.000862	4.9E-05	0.000457	2.6E-05	0.00062
bifidobacterium	1.59E-05	3.19E-05	0	0	3.5E-05	1.76E-05	1.63E-05	1.57E-05	1.3E-05	4.05E-05
alkaliflexus	7.93E-05	0.000558	0.000549	0.000268	0.000203	0.000404	8.16E-05	0.00022	2.6E-05	0.000432
prochlorococcus	0.000143	0.000255	0.000345	0.000589	0.00014	5.28E-05	0.000751	0.000457	0.000143	0.000202
methylomonas	4.76E-05	1.59E-05	4.7E-05	0.000201	1.4E-05	1.76E-05	0	0.000126	2.6E-05	2.7E-05
fluviicola	0.000222	7.97E-05	0.000659	0.000522	0.00021	0.000141	0.000685	0.000236	5.19E-05	0.000189
haliscomenobacter	6.34E-05	0.001116	1.57E-05	4.01E-05	1.4E-05	0.001336	0	3.15E-05	2.6E-05	0.002131

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sterolibacterium	0.000127	0.000782	9.41E-05	0.000816	0.001191	0.000703	6.53E-05	0.000252	1.3E-05	0.000216
desulfitobacterium	4.76E-05	0.000144	0.000188	2.68E-05	4.2E-05	0.000229	0.000114	1.57E-05	5.19E-05	0.000243
calditerricola	0.000285	0.000287	0.000251	0.000321	0.00014	0.000352	0.00018	0.000189	0.00026	0.000324
waddlia	0.000174	0.001021	0	1.34E-05	0.000161	0.000721	9.79E-05	0	0.000156	0.000593
lachnoclostridium	0.000111	9.57E-05	6.27E-05	6.69E-05	8.41E-05	0.000158	0.00018	6.3E-05	3.9E-05	2.7E-05
prevotella	1.59E-05	0	0	1.34E-05	7.01E-06	0	1.63E-05	0	1.3E-05	0
sphingobacterium	4.76E-05	0.000255	0.000314	0.00012	0.000112	0.000229	0.000359	0.000142	1.3E-05	0.000378
phycisphaera	9.51E-05	0.000494	0	4.01E-05	0.000147	0.000299	0	0	7.79E-05	0.000364
desulfocapsa	0.000206	0.000239	0.000125	0.000348	0.000182	0.000369	3.26E-05	0.000236	7.79E-05	0.000283
thauera	3.17E-05	3.19E-05	0	2.68E-05	7.01E-06	0	0	1.57E-05	0	1.35E-05