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Understanding microbial ecology can help improve biogas production in AD

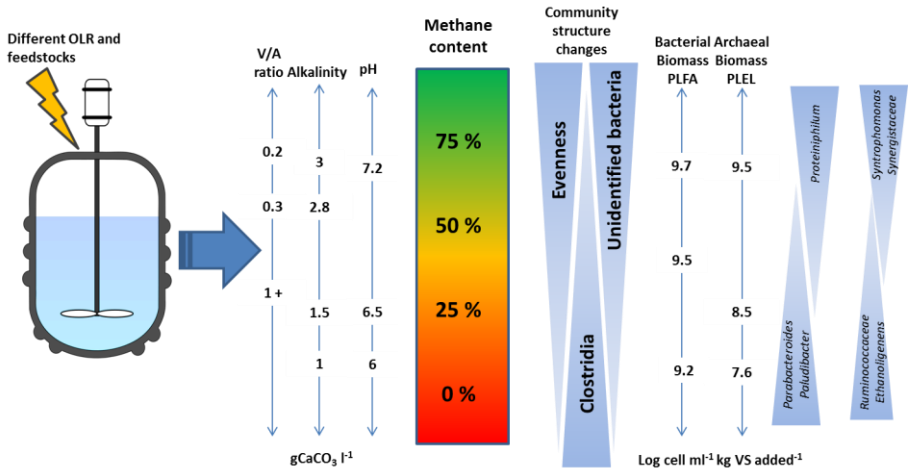
Robert M. W. Ferguson[a], Frédéric Coulon[b], Raffaella Villa[b*]

[a]School of Biological Sciences, University of Essex, Wivenhoe Park, Colchester CO4 3SQ, UK
[d]School of Water, Energy and Environment, Cranfield University, Cranfield, MK43 0AL, UK.
*Corrisponding author: Raffaella Villa, r.villa@cranfield.ac.uk

Highlights

- For all feedstocks, bacterial community composition was governed by methane content
- Reactors with > 60% methane had a more even distribution of bacterial diversity
- Methane < 30 % correlated to a 50% increase in Firmicutes (Ruminococcaceae)
- Methane > 60% correlated to unidentified OTUs and Synergistaceae

Graphical abstract



Abstract: 454-pyrosequencing and lipid fingerprinting were used to link anaerobic digestion (AD) process parameters (pH, alkalinity, volatile fatty acids (VFAs), biogas production and methane content) with the reactor microbial community structure and composition. AD microbial communities underwent stress conditions after changes in organic loading rate and digestion substrates. 454-pyrosequencing analysis showed that, irrespectively of the substrate digested, methane content and pH were always significantly, and positively, correlated with community evenness. In AD, microbial communities with more even distributions of diversity are able to use parallel metabolic pathways and have greater functional stability; hence, they are capable of adapting and responding to disturbances. In all reactors, a decrease in methane content to less than 30 % was always correlated with a 50 % increase of Firmicutes sequences (particularly in operational taxonomic units (OTUs) related to Ruminococcaceae and Veillonellaceae). Whereas digesters producing higher methane content (above 60 %), contained a high number of sequences related to Synergistetes and unidentified bacterial OTUs. Finally, lipid fingerprinting demonstrated that, under stress, the decrease in archaeal biomass was higher than the bacterial one, and that archaeal Phospholipid etherlipids (PLEL) levels were correlated to reactor performances. These results demonstrate that, across a number of parameters such as lipids, alpha and beta diversity, and OTUs, knowledge of the microbial community structure can be used to predict, monitor, or optimise AD performance.

Keywords: FOGs, Glycerol, Synergistaceae, Ruminococcaceae, Operational Taxonomic Units (OTUs), Next-generation sequencing.

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1. Introduction

Anaerobic digestion (AD) is a widely implemented technology for the treatment of wastewater and organic mixed solid wastes. Notwithstanding this, poor anaerobic digester performance and system failure are still common issues. Most of these problems originate from inadequate operational and process control and a lack of understanding of the dynamics of the microbial processes taking place in the digesters (Leitao, van Haandel, Zeeman, & Lettinga, 2006). Plant management is mainly achieved through the monitoring of physicochemical parameters rather than biological ones. However, there is a general consensus among the scientific community that in-depth understanding of the AD microbial communities and their ecology is vital to optimise and adequately manage the process (Ferguson et al. 2014; Rittmann et al. 2006). Developments in culture independent molecular methods have led to a number of studies analysing the microbial communities in AD reactors, both at laboratory (Ferguson et al. 2016; Goux et al. 2015; Vanwonterghem et al. 2015) and at full-scale (Werner et al. 2012; Valentin-Vargas et al. 2012). Most of these studies demonstrated that the microbial ecology of AD is highly diverse and dynamic. Unstable communities have been observed in digesters with stable performance and functional redundancy renders it difficult to formulate any generic trends/relationships between microbial community response and digester performance (Fernández et al., 1999; Goux et al., 2015; X. Wang et al., 2011). Recently, studies of the microbial ecology of wastewater anaerobic digesters showed that it was possible to link digester performance with fundamental ecological parameters such as community evenness, as well as ecological theories such as the biogeography model, the species-area relationships and the taxa-time relationships (Wells et al. 2011; Valentin-Vargas et al. 2012; Werner et al. 2012). Therefore, as discussed by a number of authors, the possibility of integrating the engineering of anaerobic digesters with microbial ecological theory is now a genuine prospect (Rittmann et al. 2006; Vanwonterghem et al. 2014).

In this context, there are still a number of key problems that need to be addressed; in particular and the relationship between AD performance and microbial community structure (alpha and beta diversity) and the consistency of these correlations. In short, to what extent do deterministic or stochastic processes determine the structure of microbial communities in AD. If stochastic processes dominate then unique functionally redundant microbial communities will exist in different digesters, making prediction of AD based on microbial community impossible. However, if deterministic processes dominate it will be possible predict species turnover and hence use this to monitor and predict AD performance (Måren, Kapfer, Aarrestad, Grytnes, & Vandvik, 2018). The syntrophic relationships involved in methanogenic degradation of most organic substrates in AD mean that species co-occurrence will be relatively even, and that species with similar ecological requirements will respond in similar ways (Schink, 2002). This means that it is probable that monitoring based on the presence of certain phylogenetic or functional groups should be possible after a deeper understanding of the AD microbial community.

Molecular based lipid fingerprinting and PCR-based 454-pyrosequencing analyses were carried out to investigate the microbial community structure, biomass and dynamics in digesters running under different conditions (with varying co-digestion substrates and changing organic loading rate (OLR)). Molecular based lipid fingerprinting analysis provided insights into the microbial biomass changes and microbial community structure in the digesters. Whereas, 454-pyrosequencing was used to gain detailed phylogenetic

information on both the dominant and minor important members of the microbial community.

2. Methods

2.1 Digester operational parameters

Laboratory-scale semi-continuous digesters consisted of 1-L borosilicate glass bottles with a 700 ml working volume and 5-L bottles with a 4.5-L working volume maintained at 38 °C using a water bath. All reactors were seeded with digested sludge from a commercial Sewage Treatment digester (in a ratio of 30:70 %) and fed with autoclaved primary sludge three times a week to achieve a retention time of 7 days and an organic loading rate (OLR) of 1.4 kg VS m^{-3} d^{-1}. A different organic waste (glycerol or fat rich – FOG waste collected from a restaurant grease trap) was used to induce periods of unstable performance in the digesters (see table 1 for details of feedstocks). Glycerol or FOG was added to the autoclaved primary sludge to increase the OLR from 1.4 kg VS m^{-3} d^{-1} to 2.9 for one hydraulic retention time (HRT = 7 days) and then returned to 1.4 kg VS m^{-3} d^{-1}. These OLRs were selected as they were known to cause digester failure based on our preliminary work. All the reactors were run for more than 130 days (18-20 HRT) depending on the substrate. The effects of one or two sequential changes in OLR were investigated using the same feedstock (glycerol - glycerol) or with a different feedstock (glycerol - FOG waste). Feedstock and feeding regimes are those reported previously (Ferguson et al. 2016).

Table 1. Summary of seed, primary sludge and co-digestion feedstock composition. Triplicate average error bars show standard deviation.

Characteristic	Unit	Seed	Primary Sludge	Co-digestion		
Co-digestion substrate				Glycerol waste + PS		FOGs waste + PS
Co-digestant concentration	g l^{-1}			30	50	1.5
pH		7.73 ± 0.005	7.09 ± 0	7.40 ± 0.04	7.43 ± 0.03	7.53 ± 0.1
TS*	%	4.59 ± 0.46	1.46 ± 0.56	2.34 ± 0.43	2.53 ± 1.65	2.33 ± 1.36
VS**	% of TS	63.17 ± 0.04	65.93 ± 0.13	88.38 ± 2.31	91.66 ± 3.42	97/82 ± 1
sCOD***	g l^{-1}	237 ± 0.65	43.0 ± 1.45	84.46 ± 0.97	115.65 ± 0.62	141.43 ± 3
Alkalinity	g l^{-1} $CaCO_3$	5.5 ± 0.5	2.5 ± 0.7	2.3 ± 0.1	2.4 ± 0.6	2.1 ± 1.2

*TS = total solids, **VS = volatile solids, ***sCOD soluble chemical oxygen demand

2.2. Biogas production, methane concentration and physicochemical characterisation

Gas production was measured daily by water displacement in a glass column (150 x 5 cm) and volumes corrected to standard atmospheric conditions. Methane content was measured using a SERVOPRO1400 CH_4 gas analyser (Servomex, UK) according to manufacturer recommendations. pH and alkalinity were measured according to standard APHA methods (APHA 1989).

2.3. Volatile fatty acids analysis

A 40 ml aliquot of the digestate was centrifuged at 5000 g for 5 min and the supernatant was filtered to < 0.45 µm with a syringe filter (Eduok, Ferguson, Jefferson, Villa, & Coulon, 2017). 5 µl of 97 % sulphuric acid was added (to avoid acid degradation during storage) and the sample was stored at – 20 °C until analysis. 100 µl of the sample was injected into a

HPLC (535 Kontron, Bio-TEK, UK) equipped with a Bio-Rad fermentation column (Cat 125-0115) 300 x 7.8 mm maintained at 65ºC, and a UV detector at 210 nm. The mobile phase was 0.001 M sulphuric acid in HPLC grade water with a flow rate of 0.8 ml/min. Acetic, propionic, n-butyric, iso-butyric and lactic acids were quantified using an external multilevel calibration ranging from 0.1 g l-1 to 5 g l-1. The % error in the repeatability of measurements for each acid was less than 4 %.

2.4. Phospholipids (PLFA) and ether-linked isoprenoids (PLEL) analysis

For PLFA, total lipids were extracted from 40 g aliquot of freeze-dried digestate using a modified version of the Bligh-Dyer technique as described by Frostegård, et al. (1991). The dried fatty acid methyl esters (FAMEs) were resuspended in 0.2 ml of hexane and analysed by gas chromatography equipped with flame ionisation detector (GC-FID Agilent Technologies 6890N) as described by Pankhurst et al. (2012). FAMEs were identified by comparison of retention times with the 26 bacterial acid methyl ester (BAME) mix standard (SUPELCO, Sigma, UK). Nonadecanoic acid methyl ester (Sigma, UK) was added (24.44 µg ml-1) as an internal standard to each sample after solid phase extraction (SPE).

For PLEL another aliquot of the phospholipids fraction, equivalent to 40 g of the digestate was used for PLEL analysis according to the method described by Gattinger, et al. (2003). The dried ether-linked isoprenoids were reconstituted in 0.2 ml of hexane and analysed by gas chromatography coupled to mass spectrometry (GCMS Agilent Technologies 6890N) according to the operating conditions described by Gattinger, et al. (2003). Nonadecanoic acid methyl ester (Sigma, UK) was added as an internal standard to each sample after SPE. The taxonomic affiliations are summarised in TS1. Gram-positive bacteria were represented by the series of iso and anteiso branched saturated PLFA. Gram-negative bacteria were represented by cyclopropane, hydroxyl and monounsaturated PLFA. The 16:0 straight chain PLFA has been previously demonstrated as an ubiquitous bacterial marker (Piotrowska-Seget and Mrozik 2003). The PLFA 18:2w9cis and 18:1w7trans used as markers for clostridia. The PLEL i20:0 was used as a marker for the *Euryarchaeota*, i20:1 as a marker of the aceticlastic methanogens belonging to *Methanosarcina* and i40:0 as a marker for hydrogenotrophic methanogens belonging to *Methanobacterium*, *Methanococcus*, *Methanopyrus*, and *Methanothermus* (Gattinger et al. 2002).

2.5. 454-pyrosequencing analysis and Bioinformatics

The microbial diversity and dynamics of the digesters was investigated by extracting total genomic DNA from 200 mg wet weight digestate samples using a MoBio Power Soil kit (MO BIO Laboratories, Inc, UK). Samples were then processed for NGS by 454-Pyrosequencing on the GS FLX System (Roche) as described in Eduok et al. (2015) using the following primers: for amplification of the bacterial 16S rRNA gene PCR primers were adapted for 454 amplicon sequencing by attaching the M13 adapter (italics) to the target forward primer M13-16S-IA-FL (5'-*CACGACGTTGTAAAACGA*CCATGCTGCCTCCCGTAGGAGT-3'), whereas the 25-mer Lib-L specific sequence adapter B (italics) was followed by the reverse template specific primer sequence 16S-IA-RL (5'-*CCTATCCCCTGTGTGCCTTGGCAGTC*TCAGAGAGTTTGATCCTGGCTCAG -3'). For amplification of the archaeal 16S rRNA gene PCR primers ARC-344F (5'-*CACGACGTTGTAAAACGA*ACGGGGYGCAGCAGGCGCGA) and ARC-915R (5'-*CCTATCCCCTGTGTGCCTTGGCAGT*CTCAGGTGCTCCCCCGCCAATTCCT- 71 3') where used; and

were adapted for 454 sequencing as described above. To multiplex the samples unique 10-mer barcode sequences were included in the M13 adapter.
The sequences obtained were processed as described in Dumbrell *et al.* (2017). Denoising of 454-Pyrosequencing amplicons was carried out by the sequencing provider using AmpliconNoise (Quince, Lanzen, Davenport, & Turnbaugh, 2011). The obtained sequence data were then processed in QIIME using Biolunix version 8; Sequences with > 6 ambiguous bases, a homopolymer run of > 6, a read length of < 300 or > 800, and a quality score < 25 were excluded (Caporaso et al., 2010; Ferguson, Gontikaki, Anderson, & Witte, 2017; Field et al., 2006). After quality control there were 19,633 reads. *De novo* operational taxonomic units (OTUs) were picked using Usearch (with *de novo* chimera removal) at 97 % percent similarity using the script pick_otus.py, 2137 OTUs were identified (Edgar, 2010; Edgar, Haas, Clemente, Quince, & Knight, 2011). A representative sequence for each OTU was then identified with pick_rep_set.py and used to assign taxonomy with using assign_taxonomy.py and the default parameters and the Green Genes reference taxonomy (13_8_99) (McDonald et al. 2012; Werner et al. 2012; Wang et al. 2007). Cumulative sum scaling was used to normalise the OTU table and account for differing sampling depth using the QIIME script normalize_table.py (Paulson, Stine, Bravo, & Pop, 2013)

2.6. Statistical analysis

Statistical analysis was carried out in R (v 3.2.0) and cited packages (R Development Core Team, 2015). Analysis of variance (ANOVA) was used to test for significant differences between digester group means (e.g. lipid biomass and alpha diversity metrics) significance was accepted at $P < 0.05$. To investigate patterns of beta diversity in the digesters a distance matrix using the Bray-Curtis method was calculated in Vegan 2.3.0 (Bray & Curtis, 1957; Oksanen et al., 2015). Permutational multivariate analysis of variance using distance matrices (PERMANOVA) was used to determine if the microbial communities were significantly different for the 6 digester groups (Anderson, 2001). Generalized additive models (GAMs) were used to correlate physicochemical parameters to the microbial community (e.g. pH, biogas methane content, biogas production, acetic acid concentration, and propionic acid concentration) with significance accepted at $P < 0.05$ (Oksanen, 2013). To test for significant changes in OTU abundance between digester groups the QIIME script group_significane.py was used to carry out a Kruskal-Wallis test. Ecological indexes were calculated as: Shannon-weaver index (*H'*), Simpsons index (*D*) and Pielou's evenness (*J*).

3. Results

3.1. Reactors performances: VFAs, alkalinity, biogas and methane production

Reactor performances over time and detailed results of the analysis have been reported previously (Ferguson et al. 2016). For the purpose of this work methane percentages were averaged across each HRT and reported in Figure 1 (a-d). The figures clearly show the periods of low methane production following an increase in OLR. At times of 'balanced' anaerobic performance, when the OLR was maintained at 1.4 kg VS m^{-3} $d^{-}1$, biogas production was around 0.28 m^3 kg^{-1} VS day $^{-}1$ and methane content around 75 %. When organic loading rate was increased to 2.9 kg VS m^{-3} d^{-1} biogas production dropped to around 1 m^3 kg^{-1} VS day $^{-}1$ and methane content was lower than 30 % for a period of one or more HRTs, depending on the digester history. For example, reactors that received two OLR

change with the same feedstock (Gly-Gly; Figure 1b) returned to 'balanced' conditions much faster after the second change (3HRTs vs 4HRTs; Figure 1a and 1b).

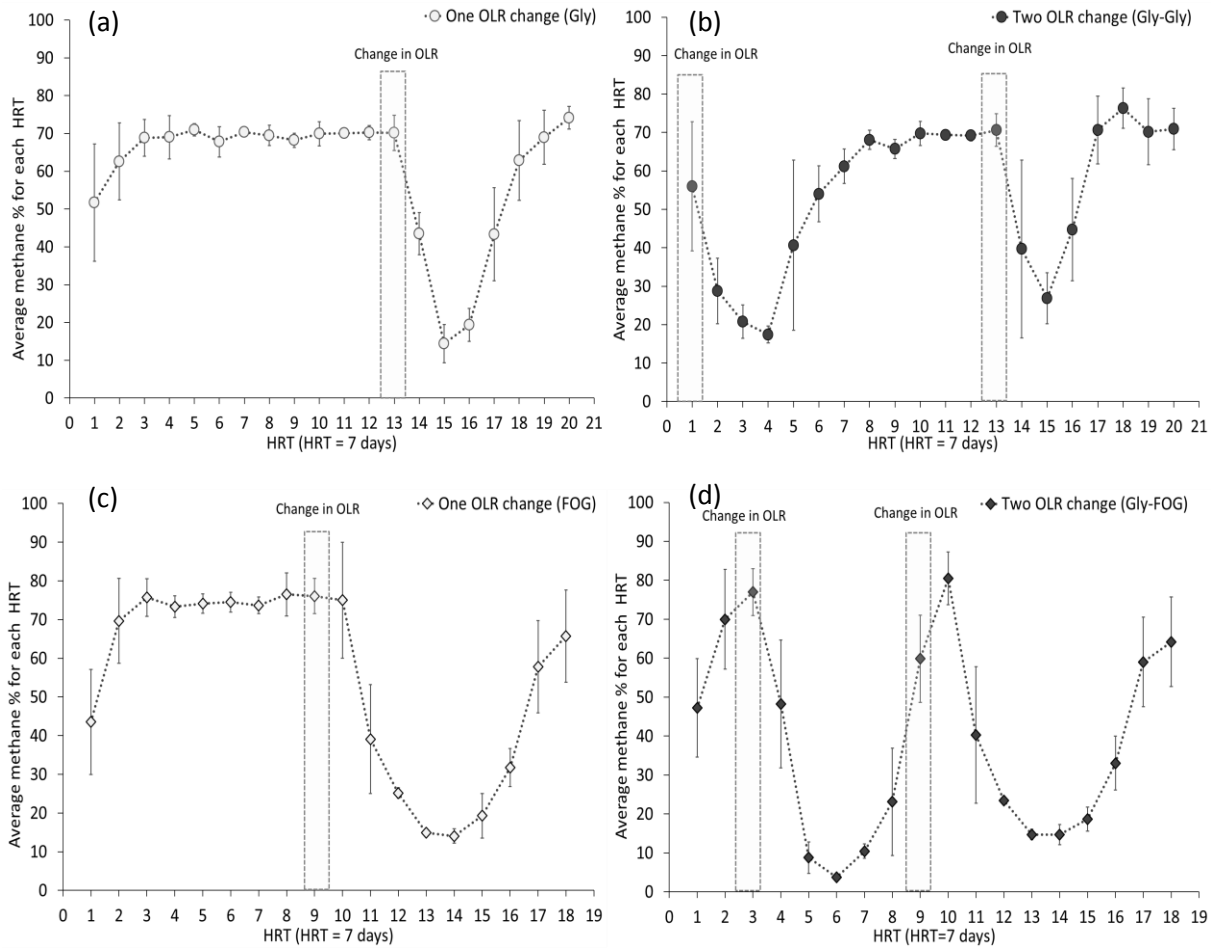


Figure 1 Methane evolution over the four experimental settings. Reactors were fed at OLR 1.4 kg VS m^{-3} d^{-1} during stable periods with primary sludge (PS). OLR was increased to 2.9 kg VS m^{-3} $d^{-}1$ for a whole HRT with different co-digestant at different times: (a) one OLR increase with glycerol; (b) two OLR increase with glycerol; (c) one OLR increase with FOG; (d) two OLR increase first with glycerol and then with FOG.

VFA content, alkalinity, pH and VFA/alkalinity ratio changed as expected with changes in methane content (table 2).

In order to identify whether there were any general trends, correlation between microbial community structure and dynamics and digesters performance, the digesters were grouped according to the methane content of their biogas. Overall 6 groups were identified which further related to VFA production, alkalinity and VFA/Alkalinity ratio (table 2).

3.2. Microbial lipid fingerprinting (PLFA and PLEL)

Many of the bacterial lipid markers (PLFA) were associated to *Actinobacteria*, low GC Gram positive bacteria, CFB, *δ-Proteobacteria*, *Bacillus*, and *Clostridia* (table 3). There were relatively little changes in the contribution of many of the individual PLFAs to the total fingerprint at different biogas methane content. The only PLFAs that varied between the digester groups were the PLFA18:1w9cis and 18:1w9trans, used as marker for clostridia, (Table 3). The trans oleic acid (18:1w9trans) doubled in concentration when methane

content was ≥ 60 % in comparison to digesters with a methane content ≤ 20 %. In contrast, the cis oleic acid (18:w9cis) doubled in digesters with a methane content ≤ 30 %.

Table 2. Summary of the key physico-chemical parameters of the digesters.

Digester group	No of samples	CH_4	pH	Alkalinity	Volatile fatty acids						V/A ratio*
					Total	Acetic	Propionic	Butyric		Lactic	
								Iso-	n-		
		%		$gCaCO_3\ l^{-1}$	$g\ l^{-1}$	$g\ l^{-1}$	$g\ l^{-1}$	$g\ l^{-1}$	$g\ l^{-1}$	$g\ l^{-1}$	
1	7	0 – 20	5.8	1	8 ± 2	2 ± 1	4± 1	1.1 ± 2	0.8 ± 2	1.3 ± 2	8 ± 3
2	7	21- 30	5.9	1.1	6.3 ± 1	2.6 ± 1	1.9 ± 1	0.9 ± 1	0.7 ± 0.6	0.7 ± 1	5.6 ± 1
3	3	31- 45	6.5	1.3	6.4 ± 3	2.7 ± 1	1.8 ± 1	1.0 ± 1	0.7 ± 0.4	1.6 ± 1	5.4 ± 0
4	5	46 – 60	6.9	2.2	2.6 ± 2	0.9 ± 1	0.7 ± 1	0.3 ± 1	0.2 ± 0.3	0.1 ± 1	0.9 ± 0
5	9	61- 70	7.2	2.4	1.8 ± 1	0.6 ± 1	0.7 ± 1	0.2 ± 1	0.3 ± 0.3	0.5 ± 0	1.1 ± 0
6	5	> 70	7.3	3.0	2.2 ± 2	0.3 ± 0	0.7 ± 0.9	0.0 ± 0	0.04 ± 0	0.0 ± 0	0.4 ± 0

*Ratio of total VFA/total alkalinity

This finding suggests changes in the community structure and/or metabolic function of the associated bacterial groups (Anaerobes/CFB group). No change in the ratio of cyclopropyl to mono-unsaturated fatty acids (cy17:0/16:1ω7c and cy19:0/18:1ω7c) was observed suggesting that the microbial community of the digesters were not experiencing significant stress conditions despite varying the OLR of the digesters (Frostegård et al. 2011).

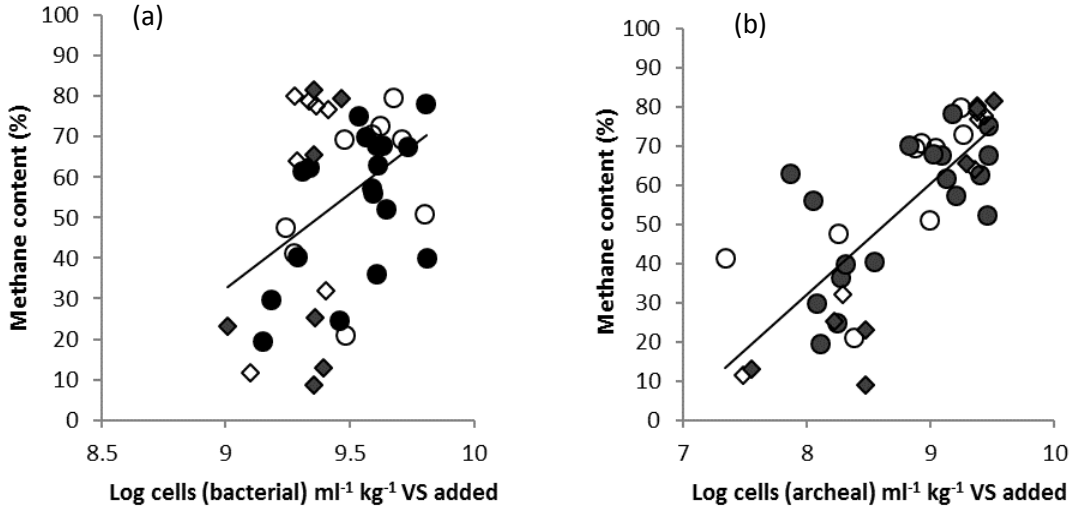


Figure 2 Scatter plot of bacterial biomass (left panel) and archaeal biomass (right) against methane content in all conditions tested. White circles: one OLR change (Gly); black circles: two OLR change (Gly-Gly); white diamonds: one OLR change (FOG); black diamonds: two OLR change (Gly-FOG). The solid line represents the linear regressions both are significant at $P < 0.01$ and with R^2 of 0.6 for archaea and 0.2 for bacteria.

The PLFA 18:2w6,9 which is associated with fungi made up approximately 3.5 for mol % in all digesters conditions. Fungi are not often considered in studies of the microbial

communities in AD and their potential role in AD remains to date unresolved. It is possible that they could play a role in cellulose digestion as this has been observed in cattle rumen, therefore fungi could be a key hydrolytic group in AD (Bauchop & Mountfort, 1981).
The archaeal biomass, calculated from PLEL as number of cells ml^{-1} kg^{-1} VS added, was always correlated with methane content for all the digestion substrates (sludge, sludge and glycerol and sludge and FOG) at $P < 0.001$ (Figure 2b). The digesters with biogas production higher than 0.2 m^3 kg^{-1} VS day^{-1} had an average archaeal biomass of 9.1 ± 0.4 log cells ml^{-1} kg^{-1} VS added, in contrast digesters with biogas production < 0.2 had average archaeal biomass of 8.3 ± 0.4 log cells ml^{-1} kg^{-1} VS added. In contrast, no clear relationship between the bacterial biomass, calculated from PLFA as number of cells $ml^{-1}kg^{-1}$ VS added, and biogas methane content was observed (Figure 2a).

Table 3. Summary of PLFA and PLEL (mol %) in digesters with varying biogas methane content. Taxonomic affiliations given in supplementary table 1. superscript numbers are standard deviation.

Lipid	**Digester group (% methane)**					
	0 – 20	21 - 30	31 - 45	46 - 60	61 - 70	> 70
11:00	1.7 ± 0.3	1.5 ± 1.4	1.5 ± 1.4	0.8 ± 0.2	0.6 ± 0.4	0.4 ± 0.3
12:00	0 ± 0	0 ± 0.2	0 ± 0.2	0 ± 0	0 ± 0.1	0.1 ± 0
13:00	0.1 ± 0	0.2 ± 0	0.2 ± 0	1 ± 0	0.1 ± 0	0.1 ± 0.1
14:00	3.6 ± 0	3.6 ± 0.2	3.6 ± 0.2	4.2 ± 0	2.8 ± 0.1	2.7 ± 0.1
15:00	1.6 ± 0	1.6 ± 0	1.6 ± 0	1.6 ± 0	1.4 ± 0	1.5 ± 0
16:00	16.8 ± 0	16.3 ± 0	16.3 ± 0	16.7 ± 0	15.8 ± 0	16.8 ± 0.4
17:00	0.6 ± 0.4	0.5 ± 2.3	0.5 ± 2.3	0.6 ± 0.8	0.5 ± 0.7	0.4 ± 0.9
18:00	7.6 ± 0.2	7.7 ± 4	7.7 ± 4	8.8 ± 1.2	10.3 ± 2.5	10.7 ± 3.2
20:00	0.4 ± 0.3	0.4 ± 2.9	0.4 ± 2.9	0.3 ± 1.7	0.4 ± 2.6	0.3 ± 2.9
iso-15:0	9.3 ± 0.1	8.7 ± 0.9	8.7 ± 0.9	9.5 ± 0.2	8.5 ± 0.4	8.4 ± 0.5
a-15:0	8.5 ± 0	7.9 ± 0.7	7.9 ± 0.7	9.8 ± 0.4	8.7 ± 0.6	8.5 ± 0.6
iso-16:0	1.8 ± 0	2.2 ± 2.4	2.2 ± 2.4	1.2 ± 0.4	1 ± 0.5	1.4 ± 1.1
iso-17:1	1 ± 0.4	0.9 ± 0.7	0.9 ± 0.7	1 ± 2.9	1.2 ± 4.5	0.9 ± 4
cyc-17:0	0.2 ± 0	0.2 ± 4.3	0.2 ± 4.3	0 ± 3.1	0.4 ± 5.1	0.4 ± 3.3
cyc-19:0	0.4 ± 0	0.4 ± 0.5	0.4 ± 0.5	0.4 ± 0.2	0.3 ± 1	0.3 ± 0.3
16:1 w7cis	14.2 ± 0	12 ± 0	12 ± 0	16.3 ± 0	14.5 ± 0.3	14.1 ± 0.3
18:2 w6cis	15.3 ± 0	14.7 ± 0.3	14.7 ± 0.3	12.5 ± 0.1	12.9 ± 0.2	13.1 ± 0.2
18:1 w9cis	5.4 ± 0	5.5 ± 0.4	5.5 ± 0.4	4.1 ± 0	2.4 ± 0.5	2.2 ± 0.6
18:1w9trans	6.9 ± 4.3	9.8 ± 0.6	9.8 ± 0.6	10.8 ± 0.3	12.8 ± 1.7	12.9 ± 1.3
18:2w6,9	4 ± 0.2	3.8 ± 3.2	3.8 ± 3.2	0 ± 1.9	4.1 ± 2.9	3.5 ± 4.2
2OH-10:0	0 ± 1.7	0.1 ± 3.2	0.1 ± 3.2	0 ± 3.2	0 ± 3.4	0 ± 3.4
2OH-12:0	0 ± 0.6	0 ± 1.8	0 ± 1.8	0 ± 1.9	0.1 ± 2	0.1 ± 2.9
3OH-12:0	0 ± 0	0 ± 0.2	0 ± 0.2	0 ± 0.1	0 ± 0.1	0.2 ± 0.1
i20:1	33.5 ± 10.1	21.8 ± 17.3	21.8 ± 17.3	21.2 ± 11.9	34.5 ± 13.2	26.8 ± 12.1
i20:0	40.3 ± 20.6	60.2 ± 25.4	60.2 ± 25.4	34.3 ± 27.2	48.7 ± 15.6	47.6 ± 22.5
i40:0	26.1 ± 15.6	18.1 ± 16.5	18.1 ± 16.5	44.5 ± 29.9	16.8 ± 10	25.5 ± 20.3

Thus, archaeal biomass is a key parameter in biogas quality, this is not surprising as the methanogens do not have metabolic pathways available other than methane production

(Garcia et al. 2000). In contrast the bacterial biomass growth is not restricted to methane production; bacteria are more resilient and are able to switch to other pathways when methane production is inhibited. Indeed, the high concentration of VFA and changes in VFA profiles to longer chain VFA would suggest that bacteria have switched to other fermentation pathways (Table 2). These results are consistent with previous research that showed that increasing biogas production is correlated with distinct changes in lipid fingerprinting, which could be used to monitor AD performance (Schwarzenauer & Illmer, 2012).

3.3. DNA based molecular analysis (454-pyrosequencing)

Over 75 % of the archaeal diversity was dominated by Euryarchaeota, especially the genus *Methanosarcina* and to a lesser extent (only 1%) to the genus *Methanobrevibacter*. Further to this, 20 % of the OTUs identified were related to unidentified Archaea and a small number (< 2 %) of members belonging to Thermoprotei, a class of the Crenarchaeota. Only three OTUs accounted for 54 % of all sequences, and no other OTUs had greater than 3 % of sequences. The closest matches to these OTU are all from the genus *Methanosarcina* which is the most metabolically diverse methanogenic genus and has also been shown to be the most stress resilient (Karakashev et al. 2005; Vavilin et al. 2008). It is therefore unsurprising that this group should dominate in all conditions analysed.

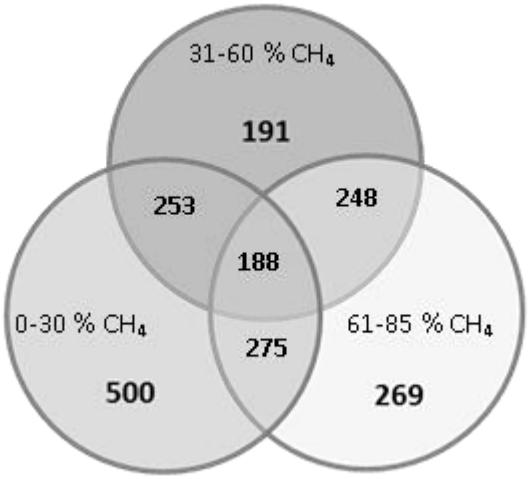


Figure 3. Venn Diagram showing number of unique and shared OTUs in digesters with 0-30 %, 31-60 % and 61-85 % methane content.

A total of 19,363 bacterial sequences were clustered into 2137 OTUs. The rank abundance curve revealed that 17 % of the total OTUs were comprised of > 5 sequences (Figure S1). This indicates that there was a core group of approximately 15 % of OTUs present across the whole data set (figure 3). The OTUs were assigned to Bacteroidetes (21 %) Firmicutes (16 %) Synergistetes (6.8 %) Proteobacteria (5.6 %) and unknown (45 %) (figure 4). Predominance of these bacterial phyla and a high number of unidentified OTUs is consistent with results reported in other studies using NGS techniques, the high number of unknown OTUs indicates that a large amount of the diversity in AD is poorly described in reference databases (Schlüter et al. 2008; Kröber et al. 2009; Lee et al. 2012; Werner et al. 2012; Rivière et al. 2009).

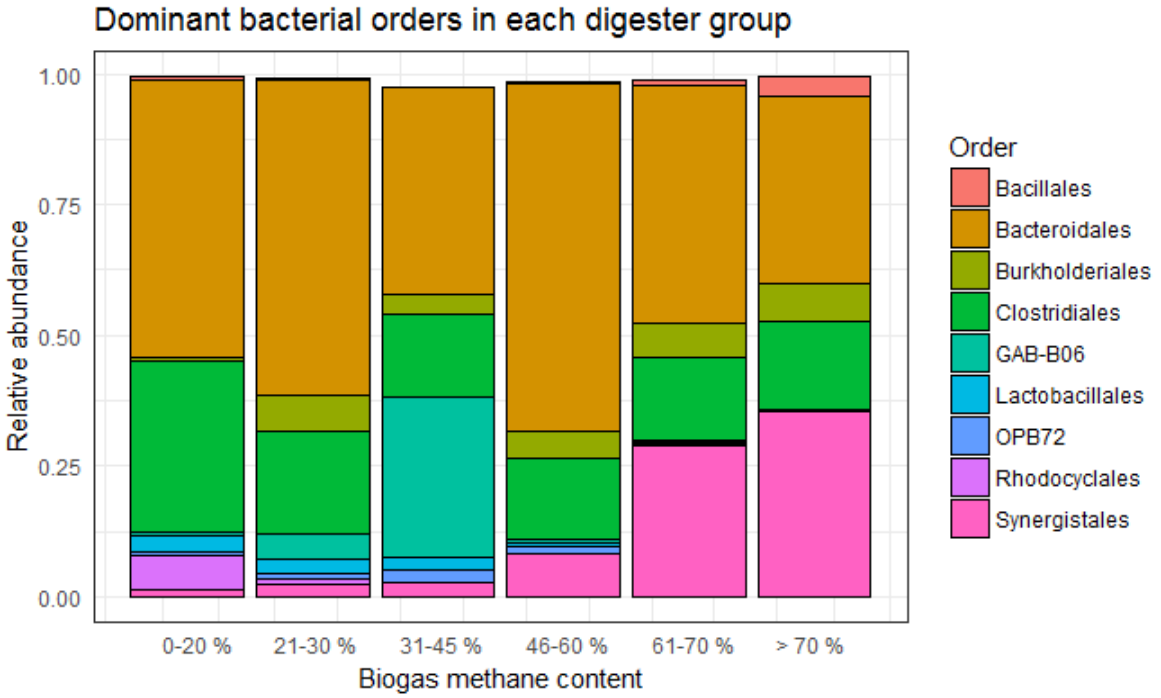


Figure 4. Relative proportions of the dominant orders based on the number of sequences assigned to that taxonomic group.

3.3.1 Alpha diversity. Bacterial OTU richness was 134 ± 83 across all samples, however there was no significant difference in OTU richness between the digester performance groups (ANOVA, $F_{5,31}$ = 0.95, P = 0.5). Shannon-weaver index (*H'*) did slightly increase from 2.6 ± 0.3 to 2.8 ± 0.1 between group 1 (0-20 % methane) and 6 (> 70 % methane) however, this was not significant (ANOVA, $F_{5,31}$ = 0.99, P = 0.43). In contrast there were significant increases for "*D*" Simpsons index (ANOVA, $F_{5,31}$ = 2.3, P = 0.006) and "*J*" Pielou's evenness (ANOVA, $F_{5,31}$ = 1.7, P = 0.02) with increased biogas methane content across the digester groups. This is clearly shown in Figure 5 and it indicates that the better performing digesters (in terms of biogas production and methane content) had more even communities. A number of studies have reported that improved digester performance was related with higher community evenness (Wittebolle et al. 2009; Merlino et al. 2012; Werner et al. 2012). Community evenness is particularly important in a system such as AD, as it signposts equitable distribution between the various AD functional groups; this enables the community to fully exploit all metabolic pathways, as well as the co-metabolic pathways, which are known to play an important in AD performance (Hashsham et al., 2000). Further to this, communities with uneven distributions of diversity tend to be dominated by groups of microorganisms specialised to the current conditions, when exposed to external changes (e.g. pH) they are unable to adapt rapidly and require long recovery times.

3.3.2 Beta Diversity. Links between the bacterial community structure and physiochemical parameters were further demonstrated by PERMANOVA. The results showed a significant shift in the bacterial community structure between the pre-defined digester groups (table 2) (PERMANOVA, $F_{5,31}$ = 2.7, P = 0.001 and, R = 0.31), specifically there was a significant correlation between the community structure and methane content and biogas production (GAMs P = 0.017 r^2 = 0.21, and P = 0.01, r^2 =0.25 for methane content and biogas production

respectively). In contrast there was no significant effect of individual reactor (PERMANOVA, $F_{5,31}$ = 2.7, P = 0.3, and r^2 = 0.31). Further understanding of the key taxonomic groups and OTUs involved is required to develop this into a predictive framework for optimising AD.

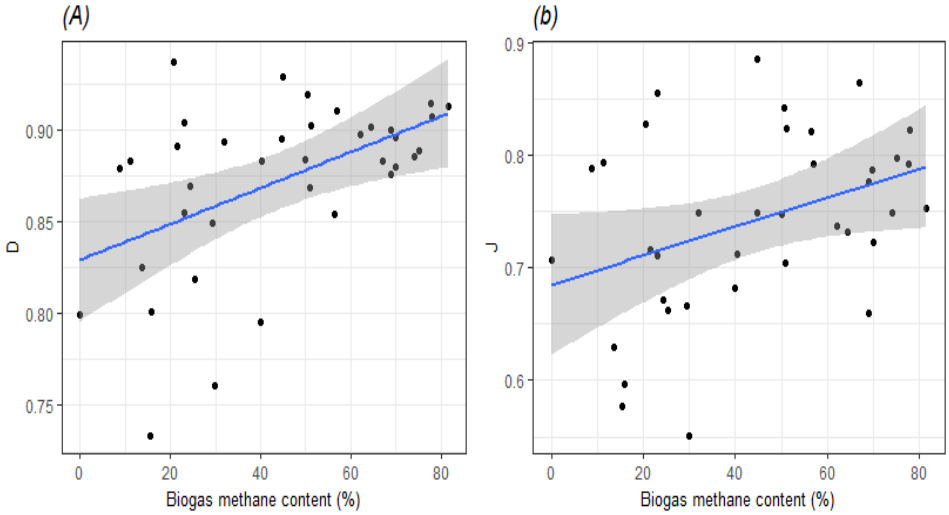


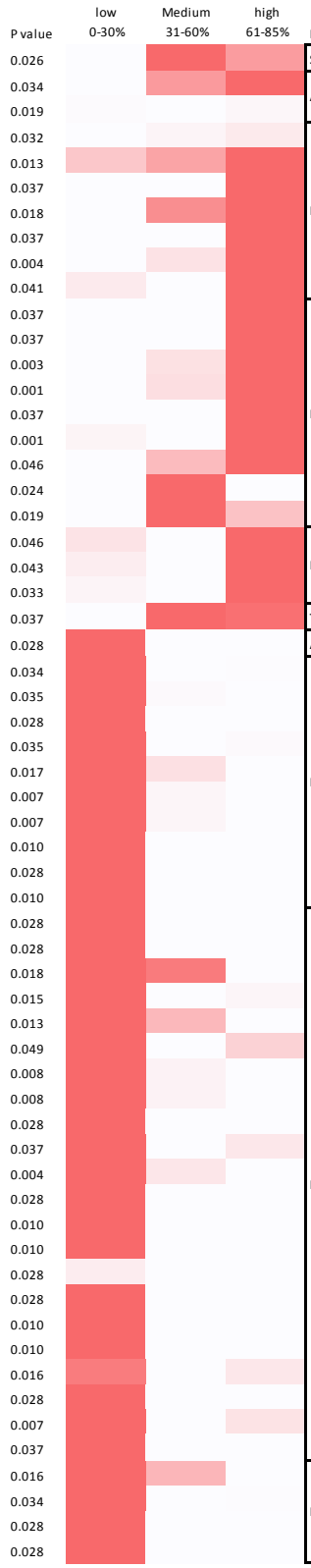
Figure 5. Scatter plot of Simpsons diversity index (a) and Pielou's evenness index (b) against % methane. (Blue lines represent linear regression between data points and shaded area are 95 % confidence intervals of the predicted model).

3.4. Relationships between methane content and bacterial community

Overall Bacteroidales dominated in all digesters and all conditions making up approximately 20 % of the community (Figure 4). The phyla Bacteroidetes and Proteobacteria, which comprised at the order level mainly of Bacteroidales and the Betaproteobacteria order Burkholderiales remained at fairly constant levels in all conditions, ranging from 17-25 % and 5-7.6 % respectively. For optimising AD performance, it is important to understand if there are core groups of bacteria that are important for good performance (high biogas production, methane content, and stable production) and also to identify those that are associated with the poor performance. To do this the six groupings defined earlier in section 3.1, table 2 were further consolidated into three groups defined as low, medium, and high biogas methane content (0-30, 31-60, and 61-85 %). A Kruskal–Wallis one-way analysis of variance was then carried out to identify OTUs correlated with these performance groups (Figure 6).

3.4.1. Dominant OTUs at low methane content. A number of Firmicutes OTUs (12), including OTUs related to the families Ruminococcaceae, Lachnospiraceae, Clostridiaceae, Lactobacillaceae, Acidaminococcaceae, and Veillonellaceae were significantly associated with digesters with biogas methane content < 30 %. Firmicutes, and in particular Clostridia are an important fermentative group in AD; and indeed as a key AD group they are common to AD systems (Nelson et al. 2011). Most of the increase in Fermicutes was down to two families, Ruminococcaceae and Veillonellaceae, which made up approximately 7 % the community each in the bioreactors with the lowest biogas methane content and production (group 1). Digesters with low methane concentration also had high concentration of VFA (Table 2). Other studies have also shown Ruminococcaceae to be associated with poor AD

performance (Tian, Cabrol, Ruiz-Filippi, & Pullammanappallil, 2014; Vanwonterghem et al., 2015).



P value	low 0-30%	Medium 31-60%	high 61-85%	Phylum	Class	Order	Family	Genus
0.026				Synergistetes	Synergistia	Synergistales	Synergistaceae	*Aminobacterium*
0.034				Armatimonadetes	Armatimonadetes gp2	Formerly phylum OP10		
0.019								
0.032				Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidales incertae sedis	*Phocaeicola*
0.013							Porphyromonadaceae	*Petrimonas*
0.037								
0.018								*Proteiniphilum*
0.037								
0.004								
0.041					Cytophagia	Cytophagales	Flammeovirgaceae	*Aureibacter*
0.037				Firmicutes	Clostridia	Clostridiales	Clostridiaceae	*Anaerovirgula*
0.037							Clostridiales Incertae SedisXIII	*Anaerovorax*
0.003								*Fervidicola*
0.001							Clostridiales IncertaesedisXI	*Sedimentibacter*
0.037								*Tepidimicrobium*
0.001							Gracilibacteraceae	*Lutispora*
0.046							Ruminococcaceae	*Acetanaerobacterium*
0.024								*Saccharofermentans*
0.019							Syntrophomonadaceae	*Pelospora*
0.046				Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	*Malikia*
0.043					Deltaproteobacteria	Myxococcales	Sorangiineae	*Polyangiaceae*
0.033					Gammaproteobacteria	Chromatiales	Halothiobacillaceae	*Thiofaba*
0.037				Thermotogae	Thermotogae	Thermotogales	Thermotogales incertae sedis	*Oceanotoga*
0.028				Actinobacteria	Coriobacteridae	Coriobacteriales	Coriobacterineae	*Olsenella*
0.034				Bacteroidetes	Bacteroidia	Bacteroidales	Porphyromonadaceae	*Butyricimonas*
0.035								*Paludibacter*
0.028								*Parabacteroides*
0.035								
0.017							Prevotellaceae	*Hallella*
0.007								*Prevotella*
0.007								
0.010								*Xylanibacter*
0.028								
0.010								
0.028				Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	*Lactobacillus*
0.028					Clostridia	Clostridiales	Clostridiaceae	*Geosporobacter*
0.018								
0.015								*Oxobacter*
0.013								*Proteiniclasticum*
0.049								
0.008							Lachnospiraceae	*Sporobacterium*
0.008								
0.028							Ruminococcaceae	*Anaerotruncus*
0.037								
0.004								*Butyricicoccus*
0.028								
0.010								
0.010								
0.028								
0.028								*Faecalibacterium*
0.010								
0.010								*Oscillibacter*
0.016								
0.028								
0.007					Negativicutes	Selenomonadales	Acidaminococcaceae	*Phascolarctobacterium*
0.037							Veillonellaceae	*Selenomonas*
0.016				Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	*Acidovorax*
0.034						Rhodocyclales	Rhodocyclaceae	*Azospira*
0.028					Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	*Pseudomonas*
0.028								

Figure 6. Relative number of sequences assigned to dominant OTUs that varied significantly according to methane content of the digester groups. (Darker grey indicates relative increase in numbers within that OTU).

Tentative exploration of the metabolic capabilities of the Firmicutes OTUs (by looking at the closest matches to the OTU sequence in BLASTn) revealed that the ones identified in this study are probably acidogens, with the capability to produce longer chain fatty acids such as butyric, propionic, lactic, and valeric acid. For example, *Butyricicoccus* can convert acetic acid into butyric acid, directly competing with methanogens. The other major phyla in the low group were Bacteroidetes, mainly represented by the Porphyromonadaceae and Prevotellaceae families (Figure 6). The exact role of Prevotellaceae in AD is unknown but the closest matches to the representative sequences for the OTUs were both isolated from rumen and related to acidogenic bacteria (Ramšak et al., 2000; Whitford, Forster, Beard, Gong, & Teather, 1998). Matching these OTUs using BLASTn (Altschul, Gish, Miller, Myers, & Lipman, 1990) also suggested that they could be acidogens, again capable of producing a wide range of VFA. In summary, there was a significant association between fermentative long chain fatty acid producing bacteria and digesters with low biogas methane content; as described previously the production of long chain fatty acids in AD reduces pH and undermines the syntrophic relationships between bacteria and archaea to support methane production (Ferguson et al., 2016).

3.4.2. Dominant OTUs at high methane content. The OTUs correlated with the high-methane content group diverged at the family level from those in the low-methane one. The phylum Synergistetes showed a marked increase from 1 % to 18 % as biogas methane content and production increased (Figure 4). The phylum Synergistetes can produce a range of organic acids that can be processed by other bacteria, or produce substrates such as acetic acid and hydrogen that are directly used by methanogens; indeed it has been shown that the range of substrates they use and produce is enhanced by co-culture with methanogens (Baena et al., 2000). Syntrophic relationships between bacteria and methanogens are required for stable AD (McMahon et al. 2004; Hattori. 2008; Stams and Plugge 2009). For example, the closest match to the *Aminobacterium* OTU found in this study was *Aminobacterium colombiense,* which has been detected in biogas reactors in other studies and can produce acetic acid from amino acids and hydrogen; significantly these functions are enhanced in via syntrophic associations with methanogens (Chertkov et al., 2010). Also, *Proteiniphilum* OTUs were identified that were matched to a strain isolated from a USAB reactor which can enhance rates of propionic acid conversation into methane (via acetic acid) when added to a syntrophic propionate-degrading co-culture (*Syntrophobacter sulfatireducens* and *Methanobacterium formicicum*) (Chen & Dong, 2005).

4. Discussion

4.1. How can microbial community structure and dynamics information be used to monitor and optimise AD?

The microbial communities in AD are often treated as a black box and there is a general perception amongst AD operators that optimisation will not be achieved through an improved understanding of the microbial ecology. In this study, consistent shifts in the structure of the microbial communities were observed with increase in biogas methane content, regardless of the feedstock used. Such information can help to develop new strategies for monitoring and optimising AD process, and further assist AD operators to predict unstable digester performance.

4.1.1. Predicting performance. Results presented here show that there are general and consistent relationships between performance and microbial community structure. However, further research is needed as other authors have shown that microbial communities in different digesters diverge over time; even when those digesters are under stress, which you might expect to cause communities to converge due to selection pressure (Goux et al. 2015; Werner et al. 2012). As counterpoint to this other studies (including ours) have found that deterministic processes dominate over stochastic in AD microbial communities, supporting microbial monitoring as a viable tool for AD (Vanwonterghem et al. 2014; Vanwonterghem et al. 2015). It is probable that the answer falls somewhere in between. For example, despite finding that communities in AD were unique to individual digesters, Werner et al. (2012) was still able to show links between community structure and function that were common to all digesters; and Goux *et al,* (2015) was able to find predictive shifts in the archaeal component of the microbial community. It may therefore be possible to monitor AD performance based on these OTUs without needing a full shotgun sequencing analysis. This could conceivably be done with portable qPCR machines or even loop mediated DNA amplification (LAMP) which can identify specific bacteria, without the need for DNA extraction, in under an hour (Notomi et al., 2000). Indeed LAMP has been used to identify Ebola in remote locations in Guinea, we therefore think that its use for an AD plant is not beyond the realms of possibility (Kurosaki et al., 2016). We were also able to show consistent links between digester function and microbial community structure, but the stochastic element to the assembly of AD microbial communities needs to be taken into account. There will be inconsistencies between digesters, and decisions will need to be made considering a wide range of microbial and physiochemical parameters, including past knowledge of the specific digester. We therefore suggest a combined molecular approach using lipid fingerprinting and DNA based technologies could be employed to provide process monitoring in AD by application of existing technology. However take-up of these technologies for monitoring AD has been slow, a great deal of development and collaboration between industry and research is required for this becomes a realistic prospect.

4.1.2. Bioaugmentation and AD optimisation. The core groups of bacteria specific to particular levels of performance revealed a number of unique OTUs in digesters with high biogas methane content (Figure 4 and 6). This information can further contribute to AD optimisation via bioaugmentation. Although it has been demonstrated in principle (Enright et al. 2009; Guo et al. nd; Schauer-Gimenez et al. 2010; Tale et al. 2011; Westerholm et al. 2012), bioaugmentation is logistically challenging; the bacteria need to be isolated and cultivated in sufficient quantity and finally there is no guarantee that the community will take hold in the digester. As an alternative it has previously been shown that changes in digester performance can be used to optimise the community to improve recovery from process imbalance (Goux et al. 2015; McMahon et al. 2004; McMahon et al. 2007; Stroot et al. 2001; Ferguson et al. 2016). Ultimately a vast quantity of knowledge needs to be collected on the relationship between community structure, function, and process control in AD so that operators can take full advantage of the possibility of process manipulation as a means of control for AD.

5. Conclusions

The results clearly demonstrate a relationship between the community structure and the performance of AD. There were consistent increases in Clostridia, specifically Ruminococcaceae and Veillonellaceae, in digesters with low biogas methane content; and an increase in the numbers of Synergistetes in those with high methane content. A statistically significant correlation between community evenness and AD performance was also demonstrated, highlighting that a more equitable distribution of diversity in AD is related to higher methane production, possibly due to improved balance between the functional groups present.

It was also demonstrated that lipid fingerprinting, due to its ability to detect changes in biomass, is a valuable companion to sequence based analysis, or even on its own as a monitoring tool. Pyrosequencing analyses of multiple digester conditions in this study also revealed that a large proportion of sequences could not be assigned to taxonomic affiliations even at the phylum/class levels. This highlights that further work is required to fully understand the identity and function of the microbial diversity present in AD.

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References

Altschul, S. F., Gish, W., Miller, W., Myers, E. W., & Lipman, D. J. (1990). Basic local alignment search tool. *Journal of Molecular Biology*, *215*(3), 403–10. http://doi.org/10.1016/S0022-2836(05)80360-2

Anderson, M. J. (2001). A new method for non-parametric multivariate analysis of variance. *Aust. Ecol.*, *26*(1), 32–46. http://doi.org/10.1111/j.1442-9993.2001.01070.pp.x

Baena, S., Fardeau, M. L., Labat, M., Ollivier, B., Garcia, J. L., & Patel, B. K. (2000). Aminobacterium mobile sp. nov., a new anaerobic amino-acid-degrading bacterium. *International Journal of Systematic and Evolutionary Microbiology*, *50*(1), 259–264. http://doi.org/10.1099/00207713-50-1-259

Bauchop, T., & Mountfort, D. O. (1981). Cellulose Fermentation by a Rumen Anaerobic Fungus in Both the Absence and the Presence of Rumen Methanogens. *Applied and Environmental Microbiology*, *42*(6), 1103–1110.

Bray, J. R., & Curtis, J. T. (1957). An Ordination of the Upland Forest Communities of Southern Wisconsin on JSTOR. *Ecological Monographs*, *27*(4), 325–349.

Caporaso, J. G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F. D., Costello, E. K., … Knight, R. (2010). QIIME allows analysis of high-throughput community sequencing data. *Nature Methods*, *7*(5), 335–336. http://doi.org/10.1038/nmeth.f.303

Chen, S., & Dong, X. (2005). Proteiniphilum acetatigenes gen. nov., sp. nov., from a UASB reactor treating brewery wastewater. *International Journal of Systematic and Evolutionary Microbiology*, *55*(Pt 6), 2257–61. http://doi.org/10.1099/ijs.0.63807-0

Chertkov, O., Sikorski, J., Brambilla, E., Lapidus, A., Copeland, A., Glavina Del Rio, T., … Klenk, H.-P. (2010). Complete genome sequence of Aminobacterium colombiense type strain (ALA-1). *Standards in Genomic Sciences*, *2*(3), 280–9. http://doi.org/10.4056/sigs.902116

Dumbrell, A. J., Ferguson, R. M. W., & Clark, D. R. (2017). Microbial Community Analysis by Single-Amplicon High-Throughput Next Generation Sequencing: Data Analysis -- From Raw Output to

Ecology. In T. J. McGenity, K. N. Timmis, & N. Balbina (Eds.), *Hydrocarbon and Lipid Microbiology Protocols: Microbial Quantitation, Community Profiling and Array Approaches* (pp. 155–206). Berlin, Heidelberg: Springer Berlin Heidelberg. http://doi.org/10.1007/8623_2016_228

Edgar, R. C. (2010). Search and clustering orders of magnitude faster than BLAST. *Bioinformatics (Oxford, England)*, *26*(19), 2460–1. http://doi.org/10.1093/bioinformatics/btq461

Edgar, R. C., Haas, B. J., Clemente, J. C., Quince, C., & Knight, R. (2011). UCHIME improves sensitivity and speed of chimera detection. *Bioinformatics*, *27*(16), 2194–2200. http://doi.org/10.1093/bioinformatics/btr381

Eduok, S., Ferguson, R. M. W., Jefferson, B., Villa, R., & Coulon, F. (2017). Aged-engineered nanoparticles effect on sludge anaerobic digestion performance and associated microbial communities. *Science of The Total Environment*, *609*, 232–241. http://doi.org/10.1016/j.scitotenv.2017.07.178

Eduok, S., Hendry, C., Ferguson, R. M. W., Martin, B., Villa, R., Jefferson, B., & Coulon, F. (2015). Insights into the effect of mixed engineered nanoparticles on activated sludge performance. *FEMS Microbiology Ecology*, *91*(7), fiv082. http://doi.org/10.1093/femsec/fiv082

Enright, A. M., O'Flaherty, V., Aelst, A. C. van, & Lens, P. N. L. (2009). Bioaugmentation of UASB reactors with immobilized Sulfurospirillum barnesii for simultaneous selenate and nitrate removal. *Applied Microbiology and Biotechnology*, *83*(2), 377–388.

Ferguson, R. M. W., Coulon, F., & Villa, R. (2016). Organic loading rate: A promising microbial management tool in anaerobic digestion. *Water Research*, *100*, 348–356. http://doi.org/10.1016/j.watres.2016.05.009

Ferguson, R. M. W., Gontikaki, E., Anderson, J. A., & Witte, U. (2017). The Variable Influence of Dispersant on Degradation of Oil Hydrocarbons in Subarctic Deep-Sea Sediments at Low Temperatures (0-5 °C). *Scientific Reports*, *7*(1), 2253. http://doi.org/10.1038/s41598-017-02475-9

Ferguson, R. M. W., Villa, R., & Coulon, F. (2014). Bioengineering options and strategies for the optimization of anaerobic digestion processes. *Environmental Technology*, *3*(1), 1–14. http://doi.org/10.1080/09593330.2014.907362

Fernández, A., Huang, S., Seston, S., Xing, J., Hickey, R., Criddle, C., & Tiedje, J. (1999). How stable is stable? Function versus community composition. *Applied and Environmental Microbiology*, *65*(8), 3697.

Field, D., Tiwari, B., Booth, T., Houten, S., Swan, D., Bertrand, N., & Thurston, M. (2006). Open software for biologists: from famine to feast. *Nature Biotechnology*, *24*(7), 801–3. http://doi.org/10.1038/nbt0706-801

Frostegård, A., Tunlid, A., & Baath, E. (2011). Use and misuse of PLFA measurements in soils. *Soil Biology and Biochemistry*, *43*(8), 1621–1625.

Frostegård, Å., Tunlid, A., & Bååth, E. (1991). Microbial biomass measured as total lipid phosphate in soils of different organic content. *Journal of Microbiological Methods*, *14*(3), 151–163. http://doi.org/10.1016/0167-7012(91)90018-L

Garcia, J.-L., Patel, B. K. C., & Ollivier, B. (2000). Taxonomic, Phylogenetic, and Ecological Diversity of Methanogenic Archaea. *Anaerobe*, *6*(4), 205–226.

Gattinger, A., Günthner, A., Schloter, M., & Munch, J. C. (2003). Characterisation of Archaea in Soils by Polar Lipid Analysis. *Acta Biotechnologica*, *23*(1), 21–28.

Gattinger, A., Schloter, M., & Munch, J. C. (2002). Phospholipid etherlipid and phospholipid fatty acid fingerprints in selected euryarchaeotal monocultures for taxonomic profiling. *FEMS Microbiology Letters*, *213*(1), 133–139.

Goux, X., Calusinska, M., Lemaigre, S., Marynowska, M., Klocke, M., Udelhoven, T., … Delfosse, P. (2015). Microbial community dynamics in replicate anaerobic digesters exposed sequentially to increasing organic loading rate, acidosis, and process recovery. *Biotechnology for Biofuels*, *8*(1), 122. http://doi.org/10.1186/s13068-015-0309-9

Guo, J., Wang, J., Cui, D., Wang, L., Ma, F., Chang, C.-C., & Yang, J. (n.d.). Application of bioaugmentation in the rapid start-up and stable operation of biological processes for municipal wastewater treatment at low temperatures. *Bioresource Technology*, *101*(17), 6622–6629.

Hashsham, S. A., Fernández, A. S., Dollhopf, S. L., Dazzo, F. B., Hickey, R. F., Tiedje, J. M., & Criddle, C. S. (2000). Parallel Processing of Substrate Correlates with Greater Functional Stability in Methanogenic Bioreactor Communities Perturbed by Glucose. *Applied and Environmental Microbiology*, *66*(9), 4050–4057.

Hattori., S. (2008). Syntrophic Acetate-Oxidizing Microbes in Methanogenic Environments. *Microbes and Environments*, *23*, 118–127.

Karakashev, D., Batstone, D. J., & Angelidaki, I. (2005). Influence of Environmental Conditions on Methanogenic Compositions in Anaerobic Biogas Reactors. *Appl. Environ. Microbiol.*, *71*(1), 331–338. http://doi.org/10.1128/aem.71.1.331-338.2005

Kröber, M., Bekel, T., Diaz, N. N., Goesmann, A., Jaenicke, S., Krause, L., … Schlüter, A. (2009). Phylogenetic characterization of a biogas plant microbial community integrating clone library 16S-rDNA sequences and metagenome sequence data obtained by 454-pyrosequencing. *Journal of Biotechnology*, *142*(1), 38–49.

Kurosaki, Y., Magassouba, N., Bah, H. A., Soropogui, B., Doré, A., Kourouma, F., … Yasuda, J. (2016). Deployment of a Reverse Transcription Loop-Mediated Isothermal Amplification Test for Ebola Virus Surveillance in Remote Areas in Guinea. *Journal of Infectious Diseases*, *214*(suppl 3), S229–S233. http://doi.org/10.1093/infdis/jiw255

Lee, S. H., Kang, H. J., Lee, Y. H., Lee, T. J., Han, K., Choi, Y., & Park, H. D. (2012). Monitoring bacterial community structure and variability in time scale in full-scale anaerobic digesters. *J Environ Monit*, *14*(7), 1893–1905.

Leitao, R. C., van Haandel, A. C., Zeeman, G., & Lettinga, G. (2006). The effects of operational and environmental variations on anaerobic wastewater treatment systems: A review. *Bioresource Technology*, *97*(9), 1105–1118. http://doi.org/http://dx.doi.org/10.1016/j.biortech.2004.12.007

Måren, I. E., Kapfer, J., Aarrestad, P. A., Grytnes, J.-A., & Vandvik, V. (2018). Changing contributions of stochastic and deterministic processes in community assembly over a successional gradient. *Ecology*, *99*(1), 148–157. http://doi.org/10.1002/ecy.2052

McDonald, D., Price, M. N., Goodrich, J., Nawrocki, E. P., DeSantis, T. Z., Probst, A., … Hugenholtz, P. (2012). An improved Greengenes taxonomy with explicit ranks for ecological and evolutionary analyses of bacteria and archaea. *The ISME Journal*, *6*(3), 610–618. http://doi.org/10.1038/ismej.2011.139

McMahon, K. D., Martin, H. G., & Hugenholtz, P. (2007). Integrating ecology into biotechnology. *Energy Biotechnology / Environmental Biotechnology*, *18*(3), 287–292.

McMahon, K. D., Zheng, D., Stams, A. J. M., Mackie, R. I., & Raskin, L. (2004). Microbial population dynamics during startup and overload conditions of anaerobic digesters treating municipal solid waste and sewage sludge. *Biotechnology and Bioengineering*, *7*, 823–834.

Merlino, G., Rizzi, A., Villa, F., Sorlini, C., Brambilla, M., Navarotto, P., … Daffonchio, D. (2012). Shifts of microbial community structure during anaerobic digestion of agro-industrial energetic crops and food industry byproducts. *Journal of Chemical Technology & Biotechnology*, *87*(9), 1302–1311. http://doi.org/10.1002/jctb.3784

Nelson, M. C., Morrison, M., & Yu, Z. (2011). A meta-analysis of the microbial diversity observed in anaerobic digesters. *Bioresource Technology*, *102*(4), 3730–9. http://doi.org/10.1016/j.biortech.2010.11.119

Notomi, T., Okayama, H., Masubuchi, H., Yonekawa, T., Watanabe, K., Amino, N., & Hase, T. (2000). Loop-mediated isothermal amplification of DNA. *Nucleic Acids Research*, *28*(12), E63. http://doi.org/10.1093/nar/28.12.e63

Oksanen, J. (2013). Multivariate Analysis of Ecological Communities in R: vegan tutorial. Retrieved

April 20, 2015, from http://cc.oulu.fi/~jarioksa/opetus/metodi/vegantutor.pdf
Oksanen, J., Blanchet, F. G., Kindt, R., Legendre, P., Minchin, P. R., Hara, R. B. O., … Wagner, H. (2015). Vegan: Community Ecology Package. R package version 2.3-2. https://CRAN.R-project.org/package=vegan. http://doi.org/10.4135/9781412971874.n145
Pankhurst, L. J., Whitby, C., Pawlett, M., Larcombe, L. D., McKew, B., Deacon, L. J., … Coulon, F. (2012). Temporal and spatial changes in the microbial bioaerosol communities in green-waste composting. *FEMS Microbiology Ecology*, *79*(1), 229–239. http://doi.org/10.1111/j.1574-6941.2011.01210.x
Paulson, J. N., Stine, O. C., Bravo, H. C., & Pop, M. (2013). Differential abundance analysis for microbial marker-gene surveys. *Nature Methods*, *10*(12), 1200–2. http://doi.org/10.1038/nmeth.2658
Quince, C., Lanzen, A., Davenport, R. J., & Turnbaugh, P. J. (2011). Removing noise from pyrosequenced amplicons. *BMC Bioinformatics*, *12*(1), 38. http://doi.org/10.1186/1471-2105-12-38
R Development Core Team. (2015). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org.
Ramšak, A., Peterka, M., Tajima, K., Martin, J. C., Wood, J., Johnston, M. E. A., … Avguštin, G. (2000). Unravelling the genetic diversity of ruminal bacteria belonging to the CFB phylum. *FEMS Microbiology Ecology*, *33*(1), 69–79. http://doi.org/10.1111/j.1574-6941.2000.tb00728.x
Rittmann, B. E., Hausner, M., Löffler, F., Muyzer, G., Okabe, S., DB, O., … Wagner. (2006). A vista for microbial ecology and environmental biotechnology. *Environ Sci Technol*, *40*(4), 1096–1103.
Rivière, D., Desvignes, V., Pelletier, E., Chaussonnerie, S., Guermazi, S., Weissenbach, J., … Sghir, A. (2009). Towards the definition of a core of microorganisms involved in anaerobic digestion of sludge. *The ISME Journal*, *3*(6), 700–14. http://doi.org/10.1038/ismej.2009.2
Schauer-Gimenez, A. E., Zitomer, D. H., Maki, J. S., & Struble, C. A. (2010). Bioaugmentation for improved recovery of anaerobic digesters after toxicant exposure. *Water Research*, *44*(12), 3555–3564.
Schink, B. (2002). Synergistic interactions in the microbial world. *Antonie van Leeuwenhoek*, *81*(1/4), 257–261. http://doi.org/10.1023/A:1020579004534
Schlüter, A., Bekel, T., Diaz, N. N., Dondrup, M., Eichenlaub, R., Gartemann, K.-H., … Goesmann, A. (2008). The metagenome of a biogas-producing microbial community of a production-scale biogas plant fermenter analysed by the 454-pyrosequencing technology. *Journal of Biotechnology*, *136*(1–2), 77–90.
Schwarzenauer, T., & Illmer, P. (2012). PLFA profiles for microbial community monitoring in anaerobic digestion. *Folia Microbiologica*, *57*(4), 331–333. http://doi.org/10.1007/s12223-012-0136-3
Stams, A. J. M., & Plugge, C. M. (2009). Electron transfer in syntrophic communities of anaerobic bacteria and archaea. *Nat Rev Micro*, *7*(8), 568–577.
Stroot, P. G., McMahon, K. D., Mackie, R. I., & Raskin, L. (2001). Anaerobic codigestion of municipal solid waste and biosolids under various mixing conditions 1. Digester performance. *Water Research*, *35*(7), 1804–1816.
Tale, V. P., Maki, J. S., Struble, C. A., & Zitomer, D. H. (2011). Methanogen community structure-activity relationship and bioaugmentation of overloaded anaerobic digesters. *Water Research*, *45*(16), 5249–5256. http://doi.org/http://dx.doi.org/10.1016/j.watres.2011.07.035
Tian, Z., Cabrol, L., Ruiz-Filippi, G., & Pullammanappallil, P. (2014). Microbial ecology in anaerobic digestion at agitated and non-agitated conditions. *PloS One*, *9*(10), e109769. http://doi.org/10.1371/journal.pone.0109769
Valentin-Vargas, A., Toro-Labrador, G., & Massol-Deya, A. A. (2012). Bacterial Community Dynamics in Full-Scale Activated Sludge Bioreactors: Operational and Ecological Factors Driving Community Assembly and Performance. *PLoS ONE*, *7*(8), e42524.
Vanwonterghem, I., Jensen, P. D., Dennis, P. G., Hugenholtz, P., Rabaey, K., & Tyson, G. W. (2014).

Deterministic processes guide long-term synchronised population dynamics in replicate anaerobic digesters. *The ISME Journal*, *8*(10), 2015–28. http://doi.org/10.1038/ismej.2014.50

Vanwonterghem, I., Jensen, P. D., Ho, D. P., Batstone, D. J., & Tyson, G. W. (2014). Linking microbial community structure, interactions and function in anaerobic digesters using new molecular techniques. *Current Opinion in Biotechnology*, *27*, 55–64. http://doi.org/10.1016/j.copbio.2013.11.004

Vanwonterghem, I., Jensen, P. D., Rabaey, K., & Tyson, G. W. (2015). Temperature and solids retention time control microbial population dynamics and volatile fatty acid production in replicated anaerobic digesters. *Scientific Reports*, *5*, 8496. http://doi.org/10.1038/srep08496

Vavilin, V., Qu, X., Mazéas, L., Lemunier, M., Duquennoi, C., He, P., & Bouchez, T. (2008). Methanosarcina as the dominant aceticlastic methanogens during mesophilic anaerobic digestion of putrescible waste. *Antonie van Leeuwenhoek*, *94*(4), 593–605. http://doi.org/10.1007/s10482-008-9279-2

Wang, Q., Garrity, G. M., Tiedje, J. M., & Cole, J. R. (2007). Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. *Applied and Environmental Microbiology*, *73*(16), 5261–7. http://doi.org/10.1128/AEM.00062-07

Wang, X., Wen, X., Yan, H., Ding, K., Zhao, F., & Hu, M. (2011). Bacterial community dynamics in a functionally stable pilot-scale wastewater treatment plant. *Bioresource Technology*, *102*(3), 2352–2357.

Wells, G. F., Park, H. D., Eggleston, B., Francis, C. A., & Criddle, C. S. (2011). Fine-scale bacterial community dynamics and the taxa-time relationship within a full-scale activated sludge bioreactor. *Water Res*, *45*(17), 5476–5488.

Werner, J. J., Knights, D., Garcia, M. L., Scalfone, N. B., Smith, S., Yarasheski, K., … Angenent, L. T. (2012). Bacterial community structures are unique and resilient in full-scale bioenergy systems. *Proceedings of the National Academy of Sciences of the United States of America*, *108*(10), 4158–4163.

Werner, J. J., Koren, O., Hugenholtz, P., DeSantis, T. Z., Walters, W. A., Caporaso, J. G., … Ley, R. E. (2012). Impact of training sets on classification of high-throughput bacterial 16s rRNA gene surveys. *The ISME Journal*, *6*(1), 94–103. http://doi.org/10.1038/ismej.2011.82

Westerholm, M., Leven, L., & Schnuer, A. (2012). Bioaugmentation of Syntrophic Acetate-Oxidizing Culture in Biogas Reactors Exposed to Increasing Levels of Ammonia. *Applied and Environmental Microbiology*, *78*(21), 7619–7625. http://doi.org/10.1128/aem.01637-12

Whitford, M. F., Forster, R. J., Beard, C. E., Gong, J., & Teather, R. M. (1998). Phylogenetic Analysis of Rumen Bacteria by Comparative Sequence Analysis of Cloned 16S rRNA Genes. *Anaerobe*, *4*(3), 153–163. http://doi.org/http://dx.doi.org/10.1006/anae.1998.0155

Wittebolle, L., Marzorati, M., Clement, L., Balloi, A., Daffonchio, D., Heylen, K., … Boon, N. (2009). Initial community evenness favours functionality under selective stress. *Nature*, *458*(7238), 623–626.



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Ferguson, Robert M. W.; Coulon, Frederic; Villa, Raffaella

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