Supplementary Tables:

Table S1. 16S rRNA gene clones from the methanogen libraries

			Number of clones/ library						
OTU	No. clones	Family	HRFI HF	LRFI-HF	HRFI-LF	LRFI-LF	Nearest Taxon	Genbank accession[1] no.	% Sequence similarity
0	1	Clostridiaceae	0	0	1	0	Clostridium sp. M62	ACFX02000046	90
1	42	Methanobacteriaceae	3	8	22	9	*Methanosphaera stadtmanae* DSM 3091	NC007681	97
2	230	Methanobacteriaceae	67	57	47	59	*Methanobrevibacter smithii* TS96A	AEMB01000004	98
3	2	Methanobacteriaceae	2	0	0	0	*Methanosphaera stadtmanae* DSM 3091	NC007681	95
4	4	Methanobacteriaceae	1	0	2	1	*Methanosphaera stadtmanae* DSM 3091	NC007681	96
5	5	Methanobacteriaceae	1	3	1	0	Methanobrevibacter smithii TS96A	AEMB01000004	94
6	9	Methanobacteriaceae	5	0	2	2	*Methanobrevibacter ruminantium*	NC013790	94
7	33	Methanobacteriaceae	7	11	6	9	*Methanobrevibacter ruminantium*	NC013790	99
8	1	Methanobacteriaceae	0	0	1	0	Methanobrevibacter smithii TS95D	AEMA01000012	95
9	1	Methanobacteriaceae	0	0	1	0	Methanobrevibacter smithii TS95D	AEMA01000012	96
10	3	Methanobacteriaceae	0	0	2	1	*Methanosphaera stadtmanae* DSM 3091	NC007681	96
11	2	Methanobacteriaceae	0	1	1	0	*Methanosphaera stadtmanae* DSM 3091	NC007681	94
12	12	Thermoplasmatales	7	5	0	0	*Thermoplasma volcanium* GSS1	NC002689	79
13	4	Thermoplasmatales	0	0	1	3	*Thermoplasma volcanium* GSS1	NC002689	80
14	6	Thermoplasmatales	0	3	2	1	*Thermoplasma volcanium* GSS1	NC002689	80
15	1	Thermoplasmatales	0	1	0	0	*Thermoplasma volcanium* GSS1	NC002689	80
16	1	Thermoplasmatales	0	0	1	0	*Thermoplasma volcanium* GSS1	NC002689	80
17	1	Thermoplasmatales	0	0	0	1	*Thermoplasma volcanium* GSS1	NC002689	80

HRFI = High residual feed intake , LRFI = Low residual feed intake, HF = High forage, LF = Low forage

[1]Genbank accession number of closest taxon

Supplementary Figures:

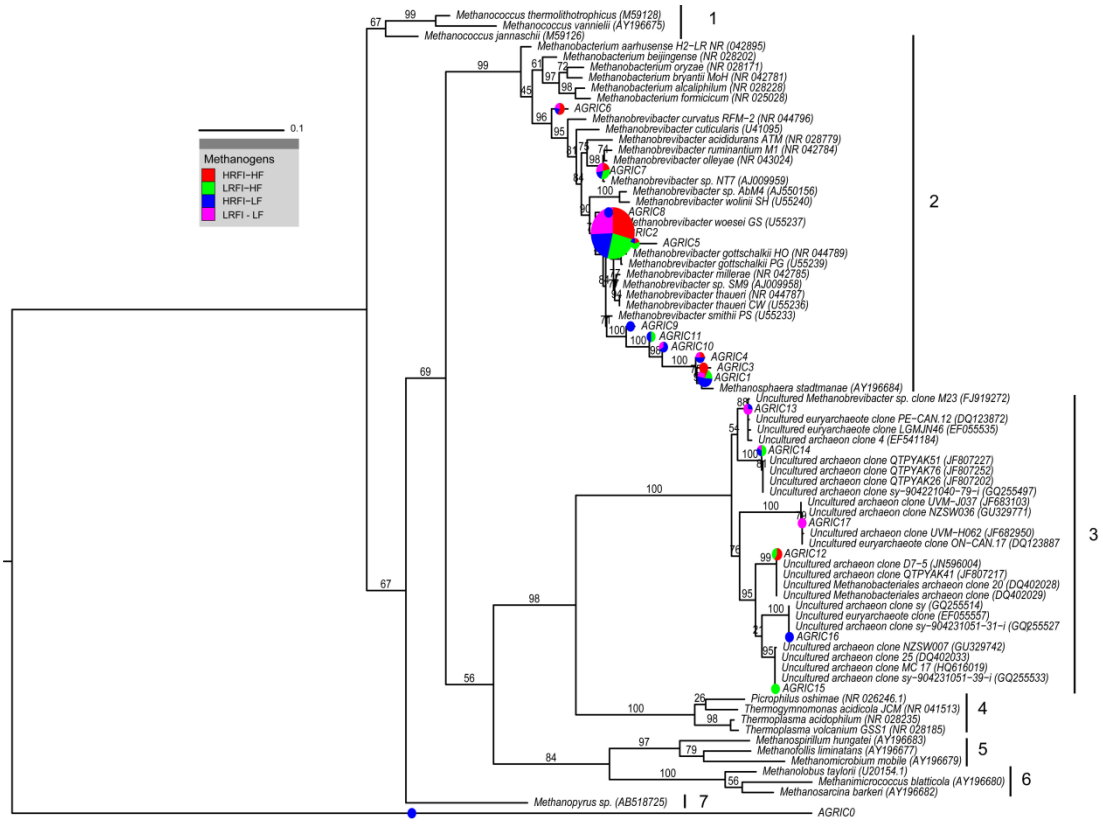


Figure S1. Phylogenetic placement of archaeal clones derived from 16S rRNA gene evolutionary distances produced by using a maximum likelihood approach in PhyML. The tree was created using interactive tree of life (ITOL) http://itol.embl.de/ (33) .The tree was bootstrap-resampled 1000 times and the bootstrap resampling values for 16S rRNA gene sequences (expressed as percentages) are indicated at the nodes. Clostridium sp. (Agric0) was used as the out-group for rooting the tree. The bar represents a sequence divergence of 10%. The Genbank accession numbers for nucleotide sequences are given in parentheses.Higher Taxonomic groupings are indicated as follows: 1, *Methanococcales*; 2, *Methanobacteriales*; 3, uncultured archaea; 4, *Thermoplasmatales*; 5, *Methanomicrobiales*; 6, *Methanosarcinales*; 7, *Methanopyrales*. Piecharts at internal nodes represent the percentage of clones from HRFI-HF, LRFI-HF, HRFI-LF and LRFI-LF libraries from each phylotype.

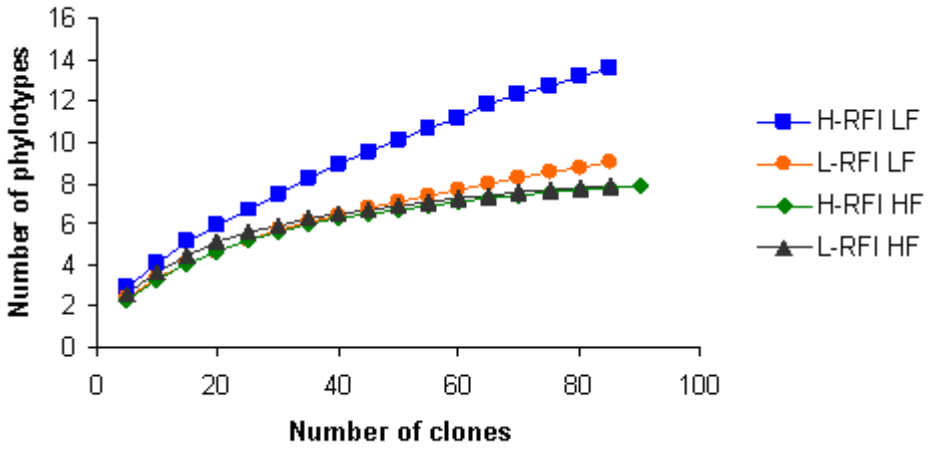


Figure S2. Rarefaction curves for methanogen 16S rRNA gene sequences obtained from HRFI-HF, LRFI-HF, HRFI-LF and LRFI-LF clone libraries at the 97% sequence similarity level.

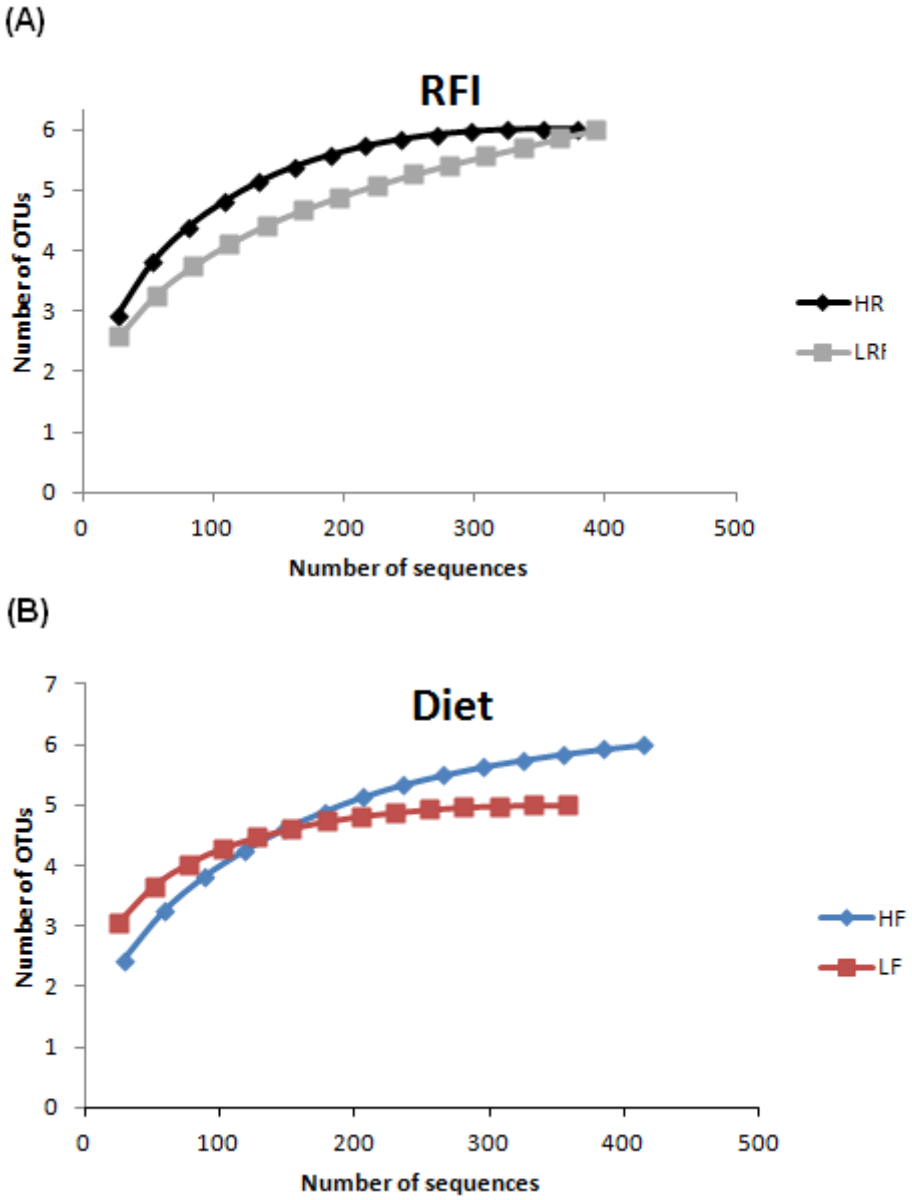


Figure S3. Rarefaction curves for archaeal 16S rRNA gene sequences obtained by tag-encoded FLX-Titanium amplicon sequencing of bovine rumen fluid obtained from **(A)** animals divergently selected for phenotypic RFI and **(B)** while offered a HF and LF diet respectively. The curves indicate the observed number of operational taxonomic units (OTUs) at 98% similarity. The curves generated with the sequences from the high residual feed intake (HRFI) and low residual feed intake (LRFI) phenotypes are marked by black and grey respectively. The curves generated with

sequences from animals while maintained on a high forage (HF) and low forage (LF) diet are marked by blue and red, respectively.

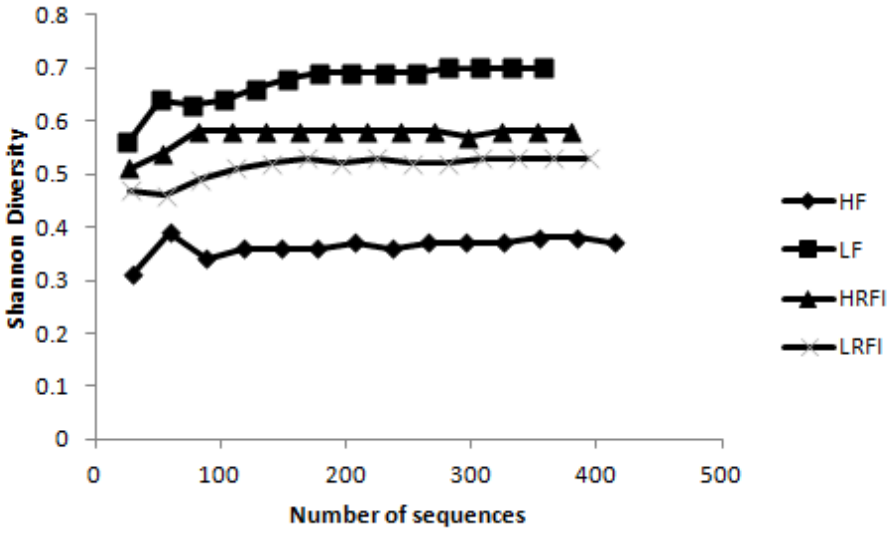
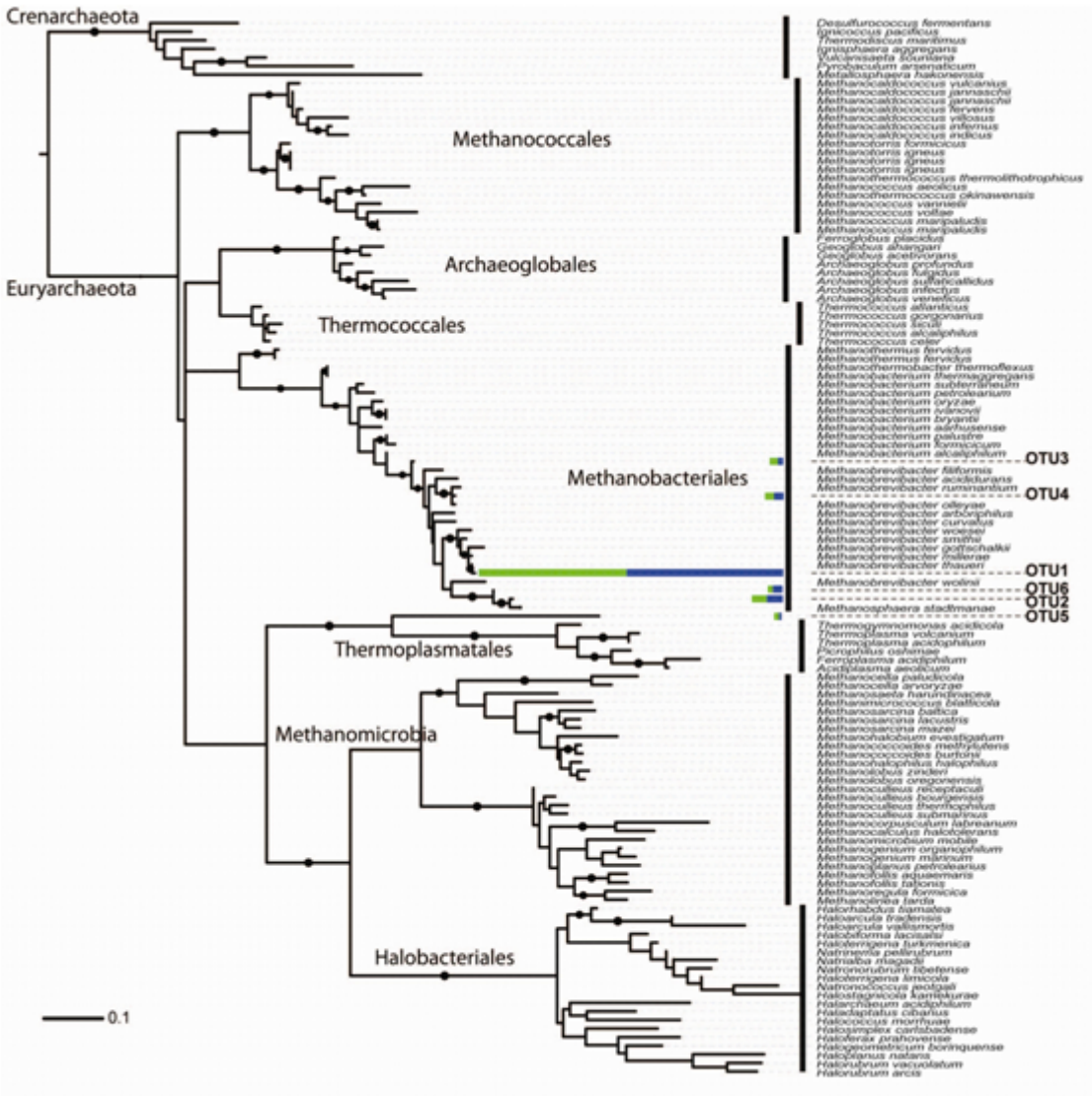


Figure S4. Rarefaction curves using the Shannon diversity index to estimate the diversity of taxa present in both high and low RFI animals and also in both HF and LF dietary phases. The curves generated with the sequences from the high residual feed intake (HRFI) and low residual feed intake (LRFI) phenotypes are marked by a triangle and cross respectively. The curves generated with sequences from animals while maintained on a high forage (HF) and low forage (LF) diet are marked by a diamond and square respectively.



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74 **Figure S5.** Phylogeny of the V3 region of archaeal 16S rRNA type sequences from
75 the Ribosomal Database Project (29). The phylogenetic position of the OTUs
76 discovered from pyrosequencing are indicated. The bars represent the abundance of
77 each of the OTU in HRFI samples (in green) and LRFI samples (in blue). Dots at
78 internal branches indicate bootstrap support grater than 80%. The methanobacteriales
79 clade is shown in Figure 2.

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Supplementary file S1

16S rDNA clone library construction and sequencing

Individual PCR amplicons were pooled by mixing 4 µl of each sample from; HRFI animals (n = 14) on the HF diet (library 1, HRFI-HF); LRFI animals (n = 14) on the HF diet (library 2, LRFI-HF); HRFI animals (n = 14) on the LF diet (library 3, HRFI-LF) and LRFI animals (n=14) on the LF diet (library 4, LRFI-LF) for library construction. Methanogen specific 16S rRNA gene clone libraries were constructed by cloning pooled PCR products into TOP10 vectors (TOPO TA cloning kit; Invitrogen, Carlsbad, CA, USA) by chemical transformation. Colonies with insertion from each of the four libraries (libraries 1, 2 3 and 4) were randomly selected on X-Gal (Sigma-Aldrich Ireland Ltd. Dublin, Ireland) medium, and the plasmid DNA was extracted using a QIAprep® Spin miniprep Kit (Qiagen Ltd, Crawley, UK). All extracted plasmid DNA was diluted to a concentration of 100 ng μl^{-1} and 20 µl of diluted DNA was transferred individually to a well of a 96-well qPCR plate (Applied Biosystems, Warrington, UK). From libraries 1, 2, 3 and 4; 94, 89, 89 and 87 clones were randomly selected, respectively, and subjected to sequence analysis by the dideoxy-chain termination method with an ABI 3730 XL sequencer using the sequencing service provided by Macrogen (Seoul, Korea) with both M13 forward (CGCCAGGGTTTTCCCAGTCACGAC) and M13 reverse (TTCACACAGGAAACAGCTATGAC) primers according to the manufacturer's instructions.

Supplementary file S2

Clone library analysis

All DNA sequence reads of the partial 16S rRNA gene sequences from methanogenic clone libraries were trimmed manually. Sequences were searched using the basic local alignment and search tool from the National centre for Biotechnology Information (BLAST: http://blast.ncbi.nlm.nih.gov/Blast.cgi) for comparison with sequences available in the GenBank database. Sequences were aligned using MUSCLE (22), manually edited and clustered for classification by using the program CD-HIT (23). The number of clusters found at percentage identity cut-offs from 90 % to 100 % were calculated. These values were plotted and used to determine an optimum cut-off for this dataset of 97 %. This value represented an appropriate balance between the number of clusters identified and the number of sequences in each cluster (Figure S1). A phylogenetic tree was constructed from the alignments and a bootstrap analysis of the tree was carried out with 1000 repetitions using maximum likelihood approach in PhyML (24). gamma distribution representing site-rate heterogeneity with an estimated alpha parameter, summarised into four site-rate categories were the settings employed. The model of evaluation used was LG as implemented in PhyML. Rarefaction analysis was performed to determine if the number of methanogenic clones screened in this study was sufficient for accurate estimation of the diversity in each of the four clone libraries. Chao analyses, Shannon diversity index and Simpson's diversity index were calculated to assess the richness and diversity of our clone libraries. Clone library evenness was calculated according to the equation $J' = H'/H'_{max}$, where H' is the Shannon diversity index and H'_{max} is the logarithm of the number of species (61). Community coverage was calculated according to the equation: $C=1-(n/N)$, where *n* is the number of phylotypes

represented by one clone and *N* is the total number of clones examined in each library (34). The Shannon diversity index is a non parametric diversity index that incorporates both richness (S, total number of OTUs) and evenness (E, relative abundance of OTUs). The Chao1 index is a nonparametric estimator of the minimum richness within the studied clone library, thus estimating the number of predicted phylotypes while the Simpson's index of diversity calculates the probability that two individuals selected from the same sample will belong to the same taxonomic category. Rarefaction curves were produced by using an analytical approximation alogrithim utilising the freeware software package: Analytical Rarefaction v1.3 (http://www.uga.edu/strata/software/Software.html). Chao I, Shannon index and Simpson index were calculated using the freeware software package EstimateS v8.2.0 (http://viceroy.eeb.uconn.edu/EstimateS). The nucleotide sequences reported herein have been deposited in the Genbank database under accession numbers JQ952744 to JQ952761 with the prefix "AGRIC".