

**Few highly abundant operational taxonomic units dominate within rumen
methanogenic archaeal species in New Zealand sheep and cattle**

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Supplemental Material

Supplemental Figures

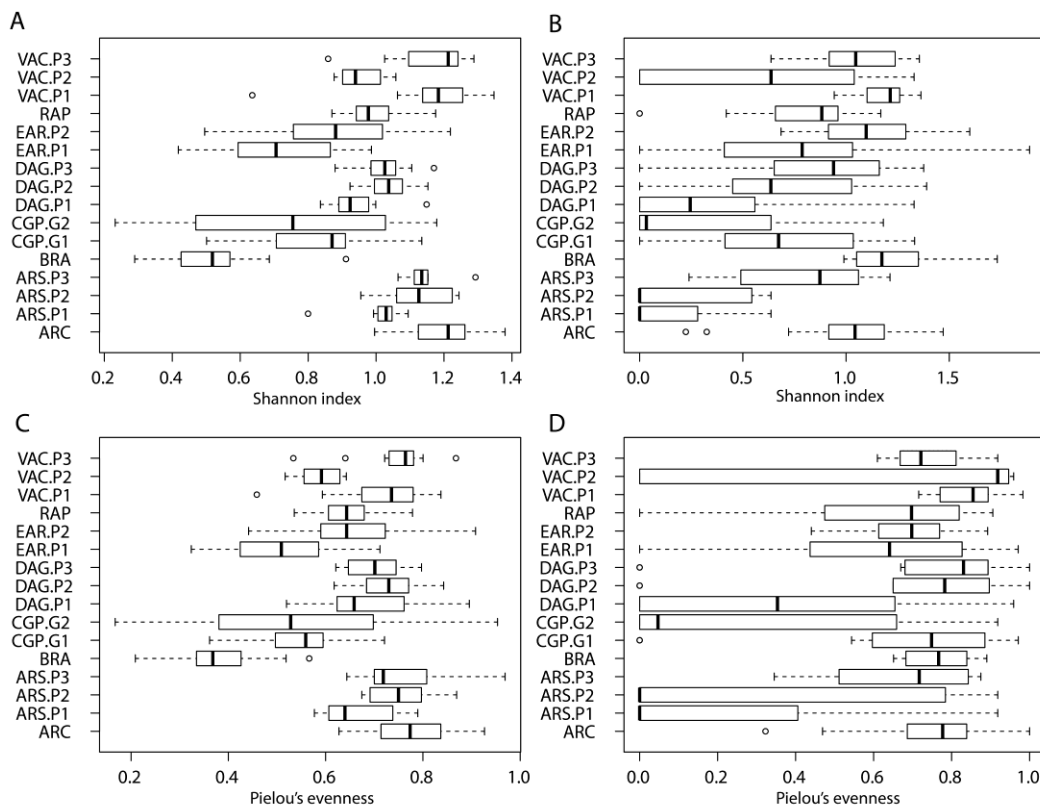


FIG S1 Species diversity and evenness of methanogen communities in New Zealand ruminants. Shown are boxplot representations of species diversity (expressed as Shannon index) of (A) *Methanobacteriales* and (B) *Methanomassiliicoccales* for each of the sample groups. Panels C and D show boxplot representations of species evenness (expressed as Pielou's evenness index) of *Methanobacteriales* and *Methanomassiliicoccales*, respectively, for each sample group. Samples were rarefied to 190 reads. Whiskers represent the maximum and minimum values, outliers are indicated as open circles.

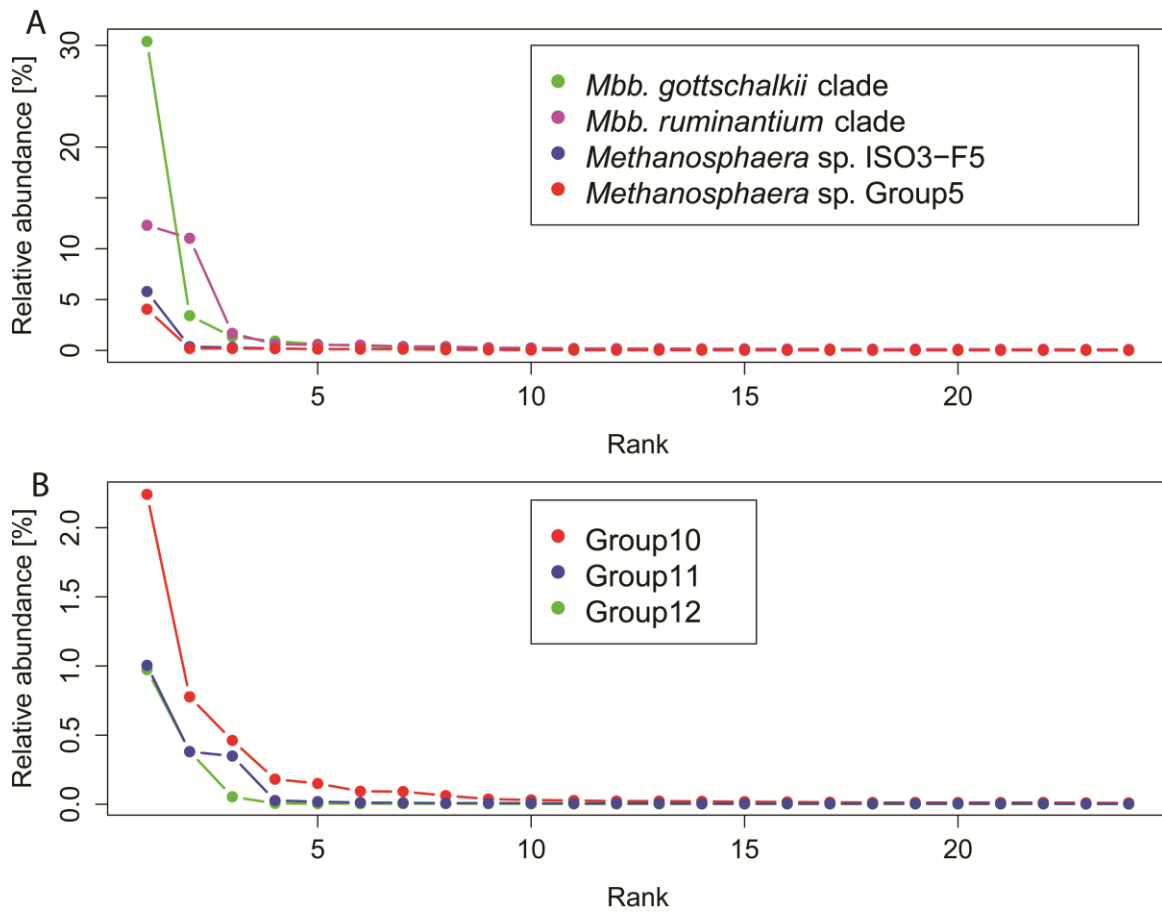


FIG S2 Relative abundance of (A) *Methanobacteriales* OTUs and (B) *Methanomassiliicoccales* OTUs. Shown are the 25 most abundant OTUs of the most abundant species for each order ranked by relative abundance.

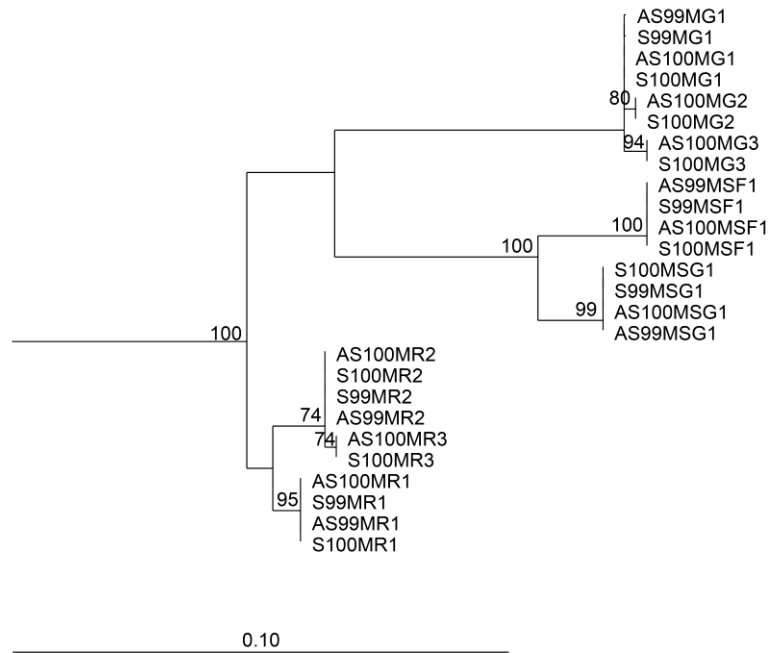
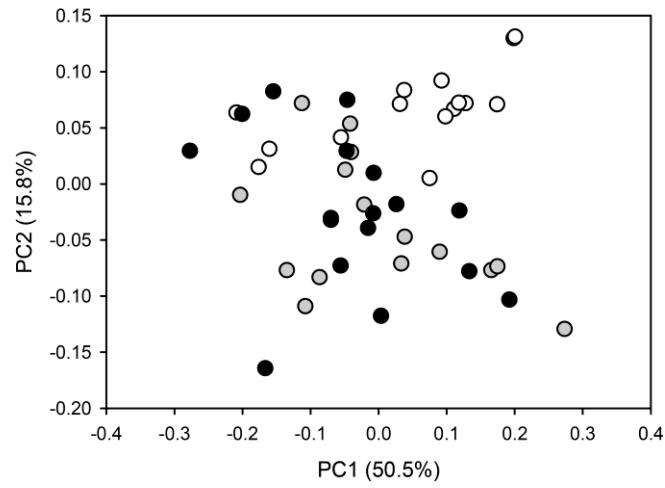


FIG S3 Phylogeny of highly abundant *Methanobacteriales* OTUs constructed from reads prior to and after error correction and at different similarity cut-offs. OTU names indicate the four different ways they were constructed. OTUs starting with “AS” were constructed from error-corrected reads, OTUs starting with “S” from non-error corrected reads. Numbers following “AS” or “S” in the OTU-name indicate the similarity threshold for sequence clustering (“99” = 99% and “100” = 100%, respectively). Species clade designations of OTUs are indicated in the OTU-name by the following abbreviations: “MG” = *Methanobrevibacter gottschalkii* clade; “MR” = *Methanobrevibacter ruminantium* clade; “MSF” = *Methanosphaera* sp. ISO3-F5; “MSG” = *Methanosphaera* sp. Group5. For *Methanobrevibacter* species only most abundant OTUs from analyses that used 99% sequence similarity and the three most abundant OTUs from analyses that used 100% sequence similarity are shown. For *Methanosphaera* species, only the most abundant OTU from each analysis is shown. The dendrogram was rooted with five *Methanopyrus* sequences. The scale bar indicates 0.10 inferred nucleotide substitutions per position. For additional information about sequence similarity between OTUs, see Table S3.

A



B

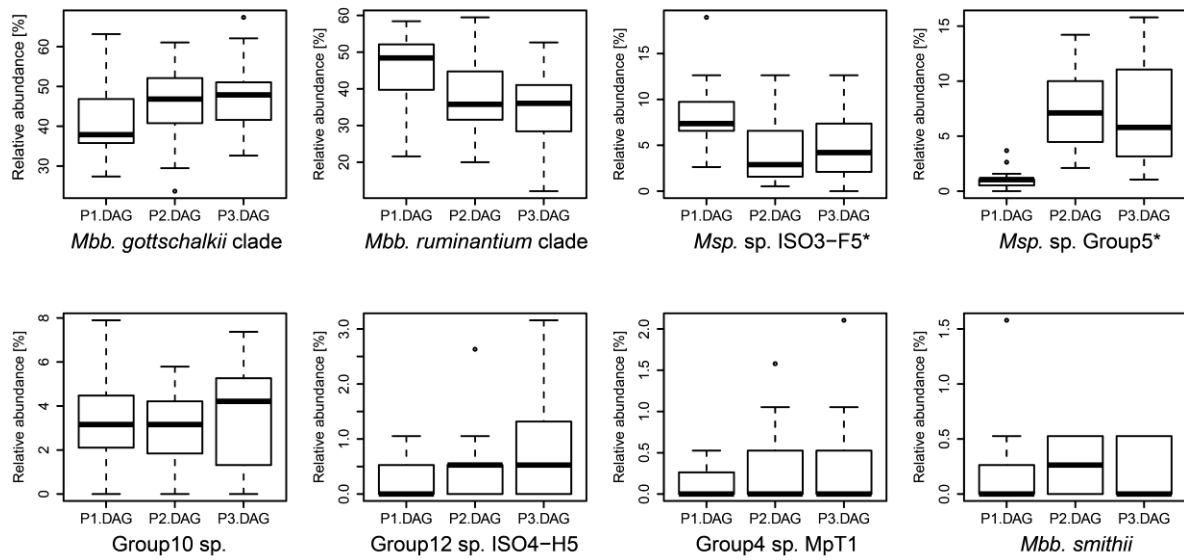


FIG S4 Effects of seasons on methanogen community structure in animals on a pasture diet. (A) Principal coordinate analysis plot of Bray-Curtis dissimilarity distances. The open circles represent samples taken in April (sample group P1.DAG), the grey circles represent samples from the same animals taken in September (P2.DAG), and the black circles represent samples from these animals in October (P3.DAG). Samples were rarefied to 190 reads and calculations of Bray-Curtis-dissimilarities were performed on OTU-tables where OTUs had had been summarized to the species level. (B) Effect of season on the relative abundance of methanogen species. Shown are changes in the relative abundance of methanogens during serial treatments with different feeds (P1.DAG = April, P2.DAG = September, P3.DAG = October). Asterisks after the species name indicate statistically significant different species between P1.DAG and the two other periods, determined by one-way ANOVA followed by a TukeyHSD posthoc test ($p < 0.05$)).

Supplemental Tables

TABLE S1 Accession numbers for sequence data.

Cohort	Database	Accession number
ARC	European Nucleotide Archive	ERP005201
ARS	European Nucleotide Archive	ERP005199
BRA	NCBI Sequence Read Archive	PRJNA239421 (SRX478379 – SRX478388)
CGP	European Nucleotide Archive	ERP005200
DAG	MG-RAST	4491446.3, 4491447.3, 4491448.3, 4491449.3
EAR	European Nucleotide Archive	ERP005194
RAP	NCBI Sequence Read Archive	PRJNA239421 (SRX477717 – SRX477734)
VAC	European Nucleotide Archive	ERP005198

TABLE S2 Similarities of abundant *Methanobacteriales* OTUs to *Methanobrevibacter* and *Methanosphaera* isolates. Listed are the similarities of (A) AS99MG1, (B) AS99MR1, (C) AS99MR2, (D) AS99MSF1, and (E) AS99MSG1.

A) *Methanobrevibacter gottschalkii* (AS99MG1)

Accession	Sequence length	Sequence identity	Isolate
GQ906575	110	100	<i>Methanobrevibacter</i> sp. YE301
GQ906572	110	100	<i>Methanobrevibacter</i> sp. YE300
GQ906573	110	100	<i>Methanobrevibacter</i> sp. YE302
GQ906574	110	100	<i>Methanobrevibacter</i> sp. YE303
GQ906576	110	100	<i>Methanobrevibacter</i> sp. YE304
DQ135988	454	99.78	<i>Methanobrevibacter</i> sp. 1Y
EF112194	446	99.78	<i>Methanobrevibacter</i> sp. 110
EU863827	418	99.76	<i>Methanobrevibacter</i> sp. LGM-AFM03
U55238	457	99.34	<i>Methanobrevibacter gottschalkii</i> strain HO
KF697730	464	99.34	<i>Methanobrevibacter</i> sp. D5
EU919428	454	99.12	<i>Methanobrevibacter</i> sp. WBY1
U55239	456	98.90	<i>Methanobrevibacter gottschalkii</i> strain PG
KF697729	453	98.68	<i>Methanobrevibacter</i> sp. G16
AJ009958	452	98.67	<i>Methanobrevibacter</i> sp. SM9
EU863830	420	98.57	<i>Methanobrevibacter</i> sp. LGM-AFM06
EU863828	420	98.57	<i>Methanobrevibacter</i> sp. LGM-AFM04
EU863829	419	98.33	<i>Methanobrevibacter</i> sp. LGM-AFM05
AY196673	454	98.24	<i>Methanobrevibacter millerae</i> strain ZA-10
U55236	437	97.25	<i>Methanobrevibacter thaueri</i> strain CW

B) *Methanobrevibacter ruminantium* (AS99MR1)

Accession	Sequence length	Sequence identity	Isolate
AY196670	452	100	<i>Methanobrevibacter</i> sp. Z4
AY196671	452	100	<i>Methanobrevibacter</i> sp. Z6
AY196672	452	100	<i>Methanobrevibacter</i> sp. Z8
EU863826	416	100	<i>Methanobrevibacter</i> sp. LGM-AFM02
AY615202	452	99.78	<i>Methanobrevibacter</i> sp. AK-87
DQ150253	422	99.29	<i>Methanobrevibacter</i> sp. NY-1
AY615204	452	99.12	<i>Methanobrevibacter</i> sp. 62
AY615205	452	99.12	<i>Methanobrevibacter</i> sp. 30Y
GQ906570	110	99.09	<i>Methanobrevibacter</i> sp. YE286
GQ906571	108	99.07	<i>Methanobrevibacter</i> sp. YE287
AJ009959	452	98.89	<i>Methanobrevibacter</i> sp. NT7
AY196666	452	98.89	<i>Methanobrevibacter ruminantium</i> strain M1
AY615201	451	98.89	<i>Methanobrevibacter olleyae</i> strain KM1H5-1P

KF697726	448	98.88	<i>Methanobrevibacter</i> sp. Alpaca
KF697732	454	98.45	<i>Methanobrevibacter</i> sp. YLM1
EU863831	416	98.32	<i>Methanobrevibacter</i> sp. LGM-AFM07
EU863832	416	98.32	<i>Methanobrevibacter</i> sp. LGM-AFM08
EU863833	416	98.32	<i>Methanobrevibacter</i> sp. LGM-AFM09

C) *Methanobrevibacter ruminantium* (AS99MR2)

Accession	Sequence length	Sequence identity	Isolate
EU863831	416	100	<i>Methanobrevibacter</i> sp. LGM-AFM07
EU863832	416	100	<i>Methanobrevibacter</i> sp. LGM-AFM08
EU863833	416	100	<i>Methanobrevibacter</i> sp. LGM-AFM09
GQ906570	110	100	<i>Methanobrevibacter</i> sp. YE286
GQ906571	108	100	<i>Methanobrevibacter</i> sp. YE287
KF697726	448	98.66	<i>Methanobrevibacter</i> sp. Alpaca
AY196670	452	98.45	<i>Methanobrevibacter</i> sp. Z4
AY196671	452	98.45	<i>Methanobrevibacter</i> sp. Z6
AY196672	452	98.45	<i>Methanobrevibacter</i> sp. Z8
DQ150253	422	98.34	<i>Methanobrevibacter</i> sp. NY-1
EU863826	416	98.32	<i>Methanobrevibacter</i> sp. LGM-AFM02
AJ009959	452	98.23	<i>Methanobrevibacter</i> sp. NT7
AY615201	451	98.23	<i>Methanobrevibacter olleyae</i> strain KM1H5-1P
AY615202	452	98.23	<i>Methanobrevibacter</i> sp. AK-87
AY615204	452	98.01	<i>Methanobrevibacter</i> sp. 62
AY615205	452	98.01	<i>Methanobrevibacter</i> sp. 30Y
KF697732	454	97.79	<i>Methanobrevibacter</i> sp. YLM1
AY196666	452	97.79	<i>Methanobrevibacter ruminantium</i> strain M1

D) *Methanosphaera* sp. ISO3-F5 (AS99MSF1)

Accession	Sequence length	Sequence identity	Isolate
KF697734	451	100	<i>Methanosphaera</i> sp. ISO3-F5
CP000102	452	96.45	<i>Methanosphaera stadtmanae</i> strain MCB-3
KF697728	447	95.97	<i>Methanosphaera</i> sp. A4
HE582783	453	95.34	<i>Methanosphaera cuniculi</i> strain 1R-7

E) *Methanosphaera* sp. Group5 (AS99MSG1)

	Sequence	Sequence	
Accession	length	identity	Isolate
KF697734	451	97.34	<i>Methanosphaera</i> sp. ISO3-F5
KF697728	447	97.09	<i>Methanosphaera</i> sp. A4
CP000102	452	96.67	<i>Methanosphaera stadtmanae</i> strain MCB-3
HE582783	453	96.67	<i>Methanosphaera cuniculi</i> strain 1R-7

TABLE S3 Effects of read error-correction and sequence clustering threshold on OTU abundance. For an explanation of OTU names see the legend of Fig. 4. Shown are the mean relative abundances of the OTUs, and in parenthesis are the sequence identities to the corresponding “AS99”-OTU (error corrected reads, 99% sequence similarity threshold), which is marked with an asterisk.

Archaeal taxon	Dominant OTUs: abundance (%) with different error corrections and sequence similarities for OTU construction			
	In parentheses are the sequence identities of representative sequences to the most abundant AS99 OTU (marked with an asterisk)			
	Reads error corrected 99% sequence similarity	Reads error corrected 100% sequence similarity	Reads not error corrected 99% sequence similarity	Reads not error corrected 100% sequence similarity
<i>Methanobrevibacter gottschalkii</i> clade	AS99MG1: 30.4%*	AS100MG1: 6.2% (100%)	S99MG1: 28.1% (100%)	S100MG1: 5.2% (100%)
		AS100MG2: 5.0% (99.78%)		S100MG2: 4.0% (99.78%)
		AS100MG3: 2.3% (99.56%)		S100MG3: 1.9% (99.56%)
<i>Methanobrevibacter ruminantium</i> clade OTU1	AS99MR1: 12.3%*	AS100MR1: 8.9% (100%)	S99MR1: 11.6% (100%)	S100MR1: 6.9% (100%)
	AS99MR2: 11.0% (98.45%)	AS100MR2: 3.0% (98.45)	S99MR2: 10.2% (98.45%)	S100MR2: 2.4% (98.45%)
		AS100MR3: 2.9% (98.23%)		S100MR3: 2.2% (98.23%)
<i>Methanobrevibacter ruminantium</i> clade OTU2	AS99MR2: 11.0%*	AS100MR2: 3.0% (100%)	S99MR2: 10.2% (100%)	S100MR2: 2.4% (100%)
	AS99MR1: 12.3% (98.45%)	AS100MR3: 2.9% (99.78%)	S99MR1: 11.6% (98.45%)	S100MR3: 2.2% (99.78%)
		AS100MR1: 8.9% (98.45)		S100MR1: 6.9% (98.45%)
<i>Methanosphaera</i> sp. ISO3-F5	AS99MSF1: 5.8%*	AS100MSF1: 2.6% (100%)	S99MSF1: 5.5% (100%)	S100MSF1: 1.7% (100%)
<i>Methanosphaera</i> sp. Group5	AS99MSG1: 4.1%*	AS100MSG1: 2.3% (100%)	S99MSG1: 3.9% (100%)	S100MSG1: 1.7% (100%)

TABLE S4 Similarity of abundant *Methanomassiliicoccales* OTUs to *Methanomassiliicoccales* sequences. Listed are the similarities of (A) AS99ML1, (B) AS99ML2, and (C) AS99ML3.

A) *Methanomassiliicoccales* OTU 1 (AE99ML1)

Accession	Sequence length	Sequence identity	Isolate
AY995293	450	100	clone CSIRO-Qld25
EF055545	450	100	clone LGMJN56
EF055543	450	100	clone LGMJN54
EF541187	450	100	Uncultured archaeon clone 7
EU413576	450	100	clone SRmetE17
EU413586	439	100	clone SRmetE31
EU413604	450	100	clone SRmetF7
FJ919262	450	100	clone M13
FJ919251	450	100	clone M2
FJ919269	450	100	clone M20
JF302256	450	100	clone AP6-001
JF302296	450	100	clone AP6-049
JF807178	450	100	clone QTPYAK2
EF055544	450	99.78	clone LGMJN55
EF541182	450	99.78	clone 2
EU413575	450	99.78	clone SRmetE16
HM038377	450	99.78	clone MLR 15
HM038382	449	99.78	clone MLR 20
JF302355	450	99.78	clone AP6-143
JF807215	450	99.78	clone QTPYAK39
JF807217	450	99.78	clone QTPYAK41
JF807216	450	99.78	clone QTPYAK40
EF055542	450	99.56	clone LGMJN53
FJ938102	450	99.56	clone 26
JF807177	450	99.56	clone QTPYAK1
JF807210	450	99.56	clone QTPYAK34
JF807211	450	99.33	clone QTPYAK35
GQ339877	450	95.11	Methanogenic archaeon WGK1
DQ445723	421	95.01	Group9 sp. CH1270
GQ339876	450	94.22	Methanogenic archaeon DCM1
JX266068	450	94.00	MPT1
KF697736	401	92.52	ISO4-G1

AB749767	450	92.44	Thermoplasmata archaeon Kjm51a
GQ339875	450	90.89	Methanogenic archaeon CRM1
KF697735	450	90.89	ISO4-H5
CP002916	450	90.89	Thermoplasmatales archaeon BRNA1
CP004049	450	89.56	Candidatus <i>Methanomethylophilus alvus</i> Mx1201
HQ896499	452	89.33	<i>Methanomassiliococcus luminyensis</i> strain B10
CP005934	450	89.33	Candidatus <i>Methanomassiliococcus intestinalis</i> Issoire-Mx1
KF697737	400	89.00	ISO4-G11

B) *Methanomassiliococcales* OTU 2 (AE99ML2)

Accession	Sequence length	Sequence identity	Isolate/clone
JF682961	450	100	clone UVM-H075
JF683103	450	100	clone UVM-J037
KF697735	450	100	ISO4-H5
JF807289	450	99.78	clone QTPYAK113
JF807290	450	99.78	clone QTPYAK114
JF807265	450	99.78	clone QTPYAK89
EU413587	450	99.78	clone SRmetE32
EU413597	450	99.78	clone SRmetE46
EU413645	450	99.78	clone SRmetG19
JF682950	450	99.78	clone UVM-H062
EU413605	441	99.77	clone SRmetF11
EU413660	441	99.77	clone SRmetG39
JF807285	450	99.56	clone QTPYAK109
EU413616	450	99.56	clone SRmetF26
EU413618	450	99.56	clone SRmetF28
JF682920	450	99.33	clone UVM-H032
DQ123869	450	99.11	clone PE-CAN.09
JF682948	450	99.11	clone UVM-H060
CP004049	450	96.44	Candidatus <i>Methanomethylophilus alvus</i> Mx1201
GQ339875	450	95.56	Methanogenic archaeon CRM1
CP002916	450	95.56	Thermoplasmatales archaeon BRNA1
KF697737	400	94.25	ISO4-G11
GQ339876	450	93.56	Methanogenic archaeon DCM1
GQ339877	450	92.67	Methanogenic archaeon WGK1
KF697736	401	92.52	ISO4-G1
JX266068	450	92.22	MPT1

DQ445723	421	91.69	Group9 sp. CH1270
AB749767	450	91.33	Thermoplasmata archaeon Kjm51a
CP005934	450	86.22	Candidatus <i>Methanomassiliococcus intestinalis</i> Issoire-Mx1
HQ896499	452	86.00	<i>Methanomassiliococcus luminyensis</i> strain B10

C) *Methanomassiliococcales* OTU 3 (AE99ML3)

Accession	Sequence length	Sequence identity	Isolate/clone
CP004049	450	98.89	Candidatus <i>Methanomethylophilus alvus</i> Mx1201
KF697735	450	97.11	ISO4-H5
GQ339875	450	96.67	Methanogenic archaeon CRM1
CP002916	450	96.67	Thermoplasmatales archaeon BRNA1
GQ339876	450	94.67	Methanogenic archaeon DCM1
KF697737	400	94.50	ISO4-G11
GQ339877	450	93.78	Methanogenic archaeon WGK1
JX266068	450	92.89	MPT1
AB749767	450	92.44	Thermoplasmata archaeon Kjm51a
DQ445723	421	92.40	Group9 sp. CH1270
KF697736	401	92.02	ISO4-G1
CP005934	450	86.44	Candidatus <i>Methanomassiliococcus intestinalis</i> Issoire-Mx1
HQ896499	452	85.56	<i>Methanomassiliococcus luminyensis</i> strain B10

Table S5 Mismatches of primers Ar915aF and Ar1386R to primer binding sites. Reference sequences in RIM-DB were searched for mismatches to the primers used in this study. Only sequences extending beyond the primer binding site were included in the analysis. Listed are mismatches for (A) primer Ar915aF and (B) primer Ar1386R.

A) Primer Ar915aF					
Order	No. of sequences	0 mismatch	1 mismatch	2 mismatches	≥3 mismatches
<i>Methanopyrales</i>	5	100.0%	0.0%	0.0%	0.0%
<i>Methanococcales</i>	75	97.3%	0.0%	1.3%	1.3%
<i>Methanobacteriales</i>	771	98.0%	1.6%	0.3%	0.1%
<i>Methanomassiliicoccales</i>	194	99.5%	0.5%	0.0%	0.0%
<i>Methanomicrobiales</i>	545	97.6%	2.2%	0.0%	0.2%
<i>Methanocellales</i>	29	93.1%	6.9%	0.0%	0.0%
<i>Methanosarcinales</i>	696	97.0%	2.6%	0.0%	0.4%

B) Primer Ar1386R					
Order	No. of sequences	0 mismatch	1 mismatch	2 mismatches	≥3 mismatches
<i>Methanopyrales</i>	5	100.0%	0.0%	0.0%	0.0%
<i>Methanococcales</i>	58	94.8%	5.2%	0.0%	0.0%
<i>Methanobacteriales</i>	182	98.4%	1.1%	0.6%	0.0%
<i>Methanomassiliicoccales</i>	106	98.1%	1.9%	0.0%	0.0%
<i>Methanomicrobiales</i>	412	98.8%	1.2%	0.0%	0.0%
<i>Methanocellales</i>	27	100.0%	0.0%	0.0%	0.0%
<i>Methanosarcinales</i>	334	96.7%	2.4%	0.6%	0.3%