

# Supplemental Material

## **Methanogenic Food Web in Gut Contents of the Methane-Emitting Earthworm *Eudrilus eugeniae* from Brazil**

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**Table S1.** Number of sequences, number of family-level OTUs, and coverage of bacterial 16S rRNA cDNA sequences of [<sup>13</sup>C]- and [<sup>12</sup>C]-glucose treatments.

Abbreviations: t<sub>0</sub>, sampling point at the start of incubation; t<sub>24</sub>, sampling point after 24 h of incubation; H, 'heavy' fractions; L, 'light' fractions.

Item	t <sub>0</sub> <sup>13</sup> C L	t <sub>0</sub> <sup>13</sup> C H	t <sub>24</sub> <sup>13</sup> C L	t <sub>24</sub> <sup>13</sup> C H	t <sub>24</sub> <sup>12</sup> C L	t <sub>24</sub> <sup>12</sup> C H	Total
Number of sequences	88	92	81	90	91	86	528
Number of OTUs	24	28	29	12	25	23	46
Coverage (%)	85	89	86	94	90	91	98

**Table S2.** Number of sequences, number of species-level OTUs, and coverage of *mcrA* and *mrtA* sequences of [<sup>13</sup>C]- and [<sup>12</sup>C]-glucose treatments. Abbreviations: t<sub>0</sub>, sampling point at start of incubation; t<sub>24</sub>, sampling point after 24 h of incubation; H, 'heavy' fractions; L, 'light' fractions.

Item	t <sub>0</sub> <sup>13</sup> C L	t <sub>0</sub> <sup>13</sup> C H	t <sub>24</sub> <sup>13</sup> C L	t <sub>24</sub> <sup>13</sup> C H	t <sub>24</sub> <sup>12</sup> C L	t <sub>24</sub> <sup>12</sup> C H	Total
Number of sequences	87	93	71	96	68	82	497
Number of OTUs	2	2	5	3	5	4	14
Coverage (%)	100	100	97.2	100	100	100	99.6

**Table S3.** Phylogenetic identity of representative bacterial 16S rRNA cDNA sequences. Abbreviations in sequence identifier code: B0, bacterial 16S rRNA cDNA sequence from sampling point  $t_0$  (i.e., at start of incubation); BE, bacterial 16S rRNA cDNA sequence from sampling point  $t_{24}$  (i.e., at 24 h of incubation); L, 'light' fractions; H, 'heavy' fractions; 13 and 12, [ $^{13}\text{C}$ ]- and [ $^{12}\text{C}$ ]-glucose treatments, respectively.

Phylum and class (*)	Sub-order (*), and family	Representative sequence <sup>1</sup>	Closest related organism or sequence <sup>1</sup>	Similarity (%)
<i>Actinobacteria</i>	<i>Acidimicrobiaceae</i>	B013H_F03 (HG964576)	Uncultured actinobacterium clone SGR131 (JQ793492)	97
	<i>Unclassified Acidimicrobineae*</i>	B013H_E02 (HG964575)	<i>Aciditerrimonas</i> sp. CH22-21 (JN033775)	96
	<i>Corynebacterinae* (Dietziaceae)</i>	B013L_H07 (HG964571)	<i>Dietzia</i> sp. BBDP49 (DQ337508)	99
	<i>Corynebacterinae* (Nocardiaceae)</i>	BE12H_A06 (HG964572)	<i>Rhodococcus pyridinivorans</i> (AB506120)	99
	<i>Corynebacterinae* (Mycobacteriaceae)</i>	B013H_A06 (HG964570)	<i>Mycobacterium moriokaense</i> CIP 105393 (AY859686)	99
	<i>Micrococchineae* (Microbacteriaceae)</i>	BE13L_B12 (HG964569)	<i>Microbacterium takaoensis</i> strain MVC17 (JQ660710)	99
	<i>Micrococchineae* (Micrococcaceae)</i>	B013H_C10 (HG964568)	<i>Citrococcus</i> sp. SeaH-As1s (FJ607345)	99
	<i>Propionibacterinae* (Propionibacteriaceae)</i>	BE13H_D11 (HG964574)	<i>Propioniferax</i> sp. P7 (EU109728)	97
	<i>Propionibacterinae* (Nocardioideaceae)</i>	B013H_B05 (HG964573)	<i>Nocardioides</i> sp. Kmb27 (KF289477)	98
	<i>Coriobacteriaceae</i>	B013H_A05 (HG964578)	Uncultured <i>Coriobacteriaceae</i> bacterium clone Pad-120 (JX505367)	96
	<i>Rubrobacteraceae</i>	B013L_G11 (HG964577)	Uncultured <i>Rubrobacteraceae</i> bacterium clone Elev (EF019514)	94
<i>Bacteroidetes</i>	<i>Cytophagaceae</i>	BE12L_D02 (HG964579)	Uncultured <i>Bacteroidetes</i> bacterium clone X-12 (HQ132391)	91
	<i>Porphyromonadaceae</i>	BE13H_G02 (HG964580)	<i>Dysgonomonas capnocytophagoideis</i> (AB548674)	93
<i>Chloroflexi</i>	<i>Anaerolineaceae</i>	B013H_H11 (HG964581)	Uncultured <i>Chloroflexi</i> bacterium clone RLT4_45 (GU236052)	99
<i>Firmicutes</i>	<i>Bacillaceae</i>	B013H_E05 (HG964592)	<i>Bacillus benzoovorans</i> strain DSM5391 (D78311)	99
	<i>Bacillaceae</i>	BE12H_G09 (HG964593)	<i>Bacillus thuringiensis</i> strain Sn9 (KC683724)	99
	<i>Bacillaceae</i>	BE13L_A05 (HG964594)	<i>Bacillus</i> sp. AP8 (JX101689)	97
	<i>Clostridiaceae 1</i>	B013H_A09 (HG964588)	<i>Clostridium paraputrificum</i> (AY442815)	96
	<i>Clostridiaceae 1</i>	BE12H_A02 (HG964589)	<i>Clostridium butyricum</i> strain IDCC 5101 (EF533982)	99
	<i>Clostridiaceae 1</i>	BE12L_BC9 (HG964590)	<i>Clostridium butyricum</i> strain IDCC 5101 (EF533982)	98

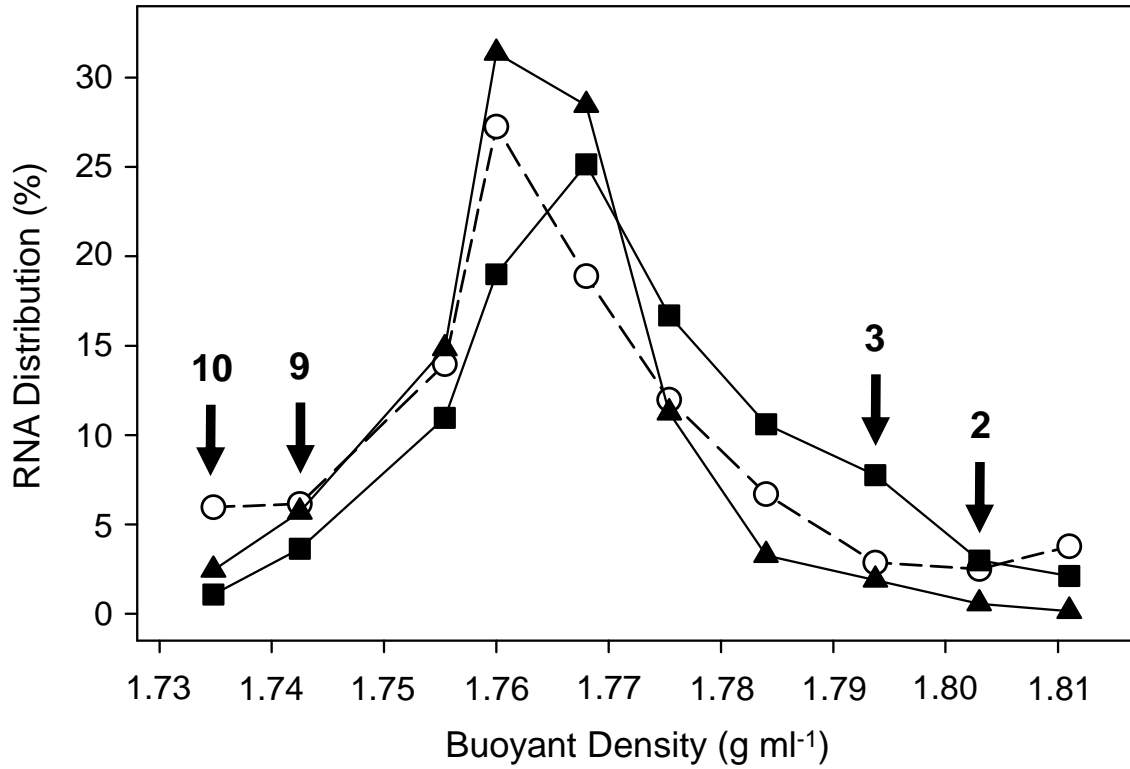
Table S3. (continued)

Phylum and class (*)	Sub-order (*), and family	Representative sequence	Closest related organism or sequence <sup>1</sup>	Similarity (%)
<i>Firmicutes</i>	<i>Lachnospiraceae I</i>	BE13H_H02 (HG964586)	<i>Clostridium propionicum</i> strain: JCM 1430 (AB649276)	99
	<i>Lachnospiraceae I</i>	BE13H_B01 (HG964585)	<i>Clostridium propionicum</i> strain: JCM 1430 (AB649276)	97
	<i>Lachnospiraceae I</i>	BE13L_F11 (HG964587)	<i>Clostridium propionicum</i> strain: JCM 1430 (AB649276)	98
	<i>Lachnospiraceae II</i>	B013H_F04 (HG964591)	<i>Butyrivibrio crossotus</i> strain DSM 2876 (NR_104735)	92
	<i>Lachnospiraceae III</i>	B013H_F06 (HG964597)	<i>Clostridium lentocellum</i> strain DSM 5427 (NR_074536)	97
	<i>Peptostreptococcaceae</i>	B013H_A07 (HG964582)	<i>Clostridium lituseburense</i> strain H17 (AB596881)	98
	<i>Peptostreptococcaceae</i>	BE13H_B07 (HG964583)	<i>Clostridium glycolicum</i> (AY007244)/ <i>Clostridium mayombei</i> (FR733682)	98/ 98
	<i>Peptostreptococcaceae</i>	BE13H_F12 (HG964584)	<i>Clostridium glycolicum</i> (AY007244)/ <i>Clostridium mayombei</i> (FR733682)	99/ 99
	<i>Peptococcaceae (Clostridiales)</i>	B013H_E01 (HG964600)	Uncultured <i>Thermincola</i> sp. clone Pad-28 (JX505275)	94
	<i>Ruminococcaceae</i>	BE13H_E07 (HG964595)	<i>Clostridium viride</i> strain DSM 6836 (NR_026204)	99
	<i>Veillonellaceae</i>	BE13H_G04 (HG964596)	<i>Succinispira mobilis</i> strain 19gly1 (NR_028868)	99
	<i>Erysipelotrichaceae</i>	B013H_D01 (HG964598)	<i>Turicibacter sanguinis</i> strain MOL361 (NR_028816)	99
	<i>Aerococcaceae</i>	B013L_C05 (HG964602)	<i>Aerococcus</i> sp. SBL (HM582941)	94
	<i>Carnobacteriaceae</i>	BE13L_G08 (HG964599)	<i>Alkalibacterium</i> sp. 8B (AY554414)	96
	<i>Planococcaceae</i>	B013L_H02 (HG964601)	<i>Rummeliibacillus pycnus</i> strain Gx4 (JF833091)	99
<i>Fusobacteria</i>	<i>Fusobacteriaceae</i>	B013H_A02 (HG964603)	<i>Fusobacterium varium</i> strain JCM 6320 (AB640694)	92
<i>Planctomycetes</i>	<i>Planctomycetaceae I</i>	B013H_A08 (HG964604)	<i>Gemmata</i> sp. CIL 30 (KC169939)	93
	<i>Planctomycetaceae II</i>	BE13L_B11 (HG964608)	Uncultured planctomycete clone MLS3 (JX240766)	90
	<i>Planctomycetaceae II</i>	B013H_MD9 (HG964606)	Uncultured <i>Planctomycetaceae</i> bacterium clone A09-06C (FJ542900)	99
	<i>Planctomycetaceae II</i>	B013L_A04 (HG964607)	Uncultured planctomycete clone Kir21gry B6.c10 (HM480247)	92
	<i>Planctomycetaceae III</i>	BE12H_BC4 (HG964605)	Uncultured <i>Blastopirellula</i> sp. clone B05-04F (FJ543000)	99
	<i>Planctomycetaceae IV</i>	BE12H_E08 (HG964609)	<i>Isosphaera</i> -like str. CJuq1 (AF239699)	99
	<i>Planctomycetaceae V</i>	B013L_B09 (HG964610)	Uncultured <i>Planctomyces</i> sp. clone P74 (HG917247)	94
	Novel Family	B013L_E07 (HG964611)	<i>Planctomyces</i> sp. (Schlesner 668) (X81956)	85

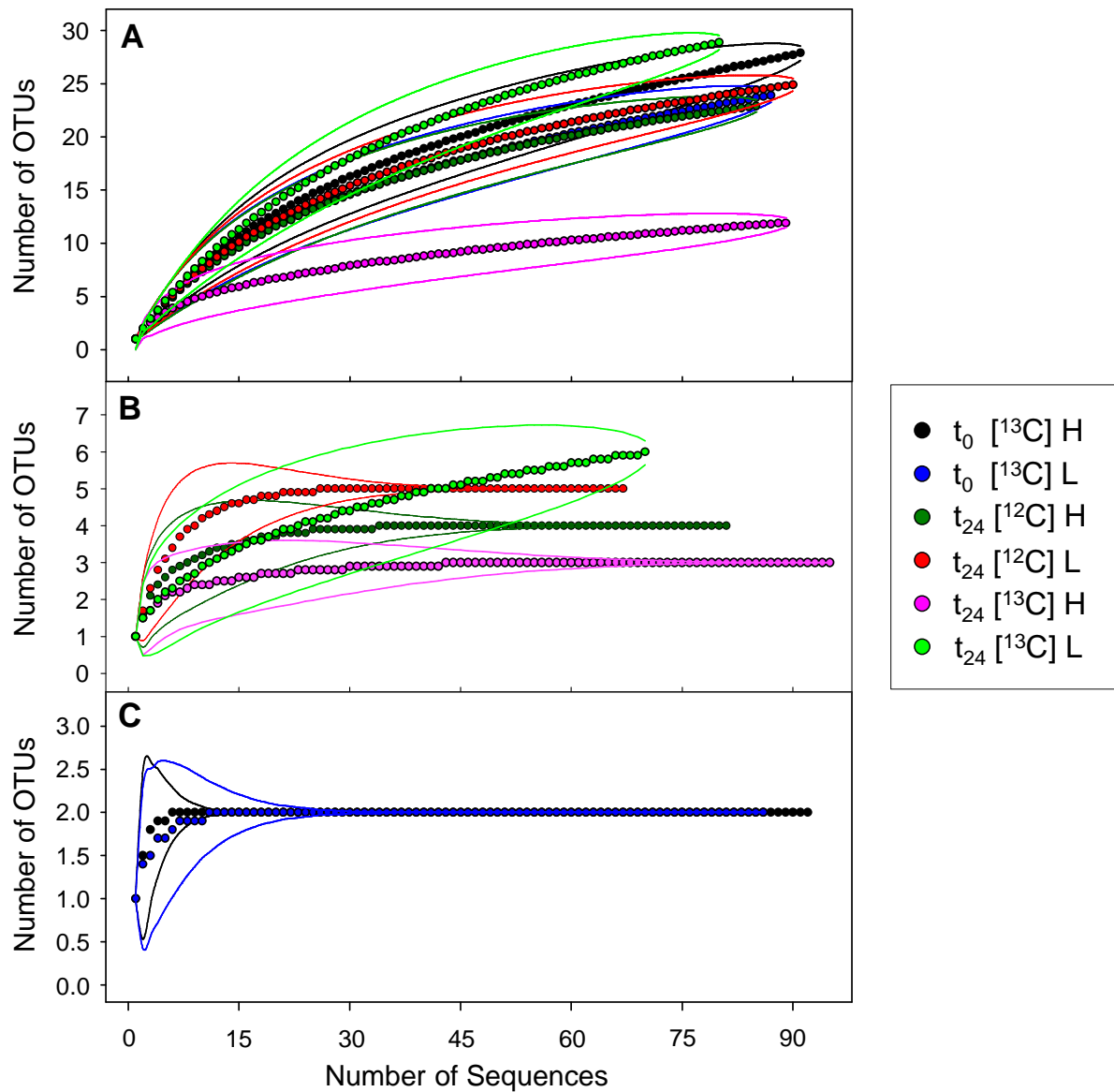
Table S3. (continued)

Phylum and class(*)	Sub-order (*), and family	Representative sequence	Closest related organism or sequence <sup>1</sup>	Similarity (%)
<i>Proteobacteria</i>				
<i>Alpha-proteobacteria</i> *	<i>Hyphomicrobiaceae</i>	B013H_MD10 (HG964617)	<i>Hyphomicrobium</i> sp. NL23 (JX131369)	99
	<i>Phyllobacteriaceae</i>	BE13L_B10 (HG964612)	<i>Mesorhizobium amorphae</i> strain JN37 (KF150349)	99
	<i>Rhodobacteraceae</i>	BE13L_G09 (HG964616)	<i>Paracoccus</i> sp. PA216 (AM900779)	93
	<i>Acetobacteraceae</i>	B013H_F01 (HG964613)	<i>Roseomonas</i> sp. BUT-5 (KF254767)	98
	<i>Acetobacteraceae</i>	BE12H_H07 (HG964614)	<i>Roseomonas</i> sp. WW53 (KF619243)	94
	<i>Rhodospirillaceae</i>	B013H_G10 (HG964615)	<i>Defluviicoccus vanus</i> strain Ben 114 (NR_041771)	88
<i>Beta-proteobacteria</i> *	<i>Oxalbacteraceae</i>	B013H_D05 (HG964618)	<i>Herbaspirillum</i> sp. 14 (AB218285)	99
	<i>Neisseriaceae</i>	BE13L_F12 (HG964619)	<i>Chitinilyticum aquatile</i> strain c14 (NR_043712)	96
<i>Delta-proteobacteria</i> *	Novel family	BE12H_H10 (HG964623)	<i>Anaeromyxobacter dehalogenans</i> strain DCP17 (EU331410)	85
	Novel family	BE13L_F03 (HG964624)	<i>Geobacter bemidjiensis</i> Bem (CP001124)	85
	<i>Polyangiaceae</i>	B013H_C07 (HG964620)	<i>Polyangium fumosum</i> strain PI fu5 (GU207879)	96
	<i>Polyangiaceae</i>	B013L_G04 (HG964621)	<i>Sorangineae</i> bacterium 706 (JF719608)	98
	<i>Polyangiaceae</i>	BE12H_G03 (HG964622)	<i>Sorangium cellulosum</i> strain DSM14627 (NR_044443)/ <i>Polyangium cellulosum</i> strain So ce26 (AF387629)	99/ 99
<i>Gamma-proteobacteria</i> *	<i>Aeromonadaceae</i>	B013H_A04 (HG964625)	<i>Aeromonas trota</i> strain ATCC 49657 (X60415)	99
	<i>Aeromonadaceae</i>	B013L_A08 (HG964626)	<i>Aeromonas veronii</i> strain M-T-TSA 37 (JQ795754)	99
	<i>Aeromonadaceae</i>	BE13H_B04 (HG964627)	<i>Aeromonas hydrophila</i> strain ANSE1 (GU296671)	99
	<i>Enterobacteriaceae</i>	BE12H_D10 (HG964628)	<i>Escherichia coli</i> O145:H28 str. RM13514 (CP006027)	99
	<i>Enterobacteriaceae</i>	BE12L_D10 (HG964629)	<i>Citrobacter freundii</i> strain E087 (JX267103)	98
	<i>Methylococcaceae</i>	BE13L_A11 (HG964630)	<i>Methylobacter</i> sp. LW1 (AF150784)	98
<i>Spirochaetes</i>	<i>Leptospiraceae</i>	BE12H_H09 (HG964631)	Uncultured <i>Leptospira</i> sp. clone Pad-85 (JX505332)	93
<i>Tenericutes</i>	<i>Mycoplasmataceae</i>	B013H_A11 (HG964632)	Candidatus <i>Lumbricincola</i> sp. Lt-A1 (FM165581)	98
<i>Verrucomicrobia</i>	Novel family	B013L_E12 (HG964633)	<i>Verrucomicrobia</i> bacterium YJF2-48 (FJ405888)	87

<sup>1</sup>Accession numbers are in parentheses.



**Figure S1.** RNA distribution in gradient fractions of glucose treatments of *E. eugeniae* gut content. Arrows identify 'heavy' (2 and 3) and 'light' (9 and 10) fractions used for molecular analyses. Symbols: open circles, RNA extracted from [<sup>13</sup>C]-glucose treatments at the start of incubation; filled squares, RNA extracted from [<sup>13</sup>C]-glucose treatments after 24 hours of incubation; filled triangles, RNA extracted from [<sup>12</sup>C]-glucose treatments after 24 hours of incubation.



**Figure S2.** Rarefaction analysis of family level bacterial 16S rRNA cDNA sequences (A) and of species level *mcrA* and *mrtA* sequences (B [ $t_{24}$ ] and C [ $t_0$ ]) of [ $^{13}\text{C}$ ]- and [ $^{12}\text{C}$ ]-glucose treatments. Solid lines represent 95% confidence intervals. Abbreviations:  $t_0$ , sampling point at the start of incubation;  $t_{24}$ , sampling point after 24 h of incubation; H, 'heavy' fractions; L, 'light' fractions; 13 and 12, [ $^{13}\text{C}$ ]- and [ $^{12}\text{C}$ ]-glucose treatments, respectively.