Supplemental Material

Differential Engagement of Fermentative Taxa in Gut Contents of the Earthworm *Lumbricus terrestris*

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Supplemental Figure Legends

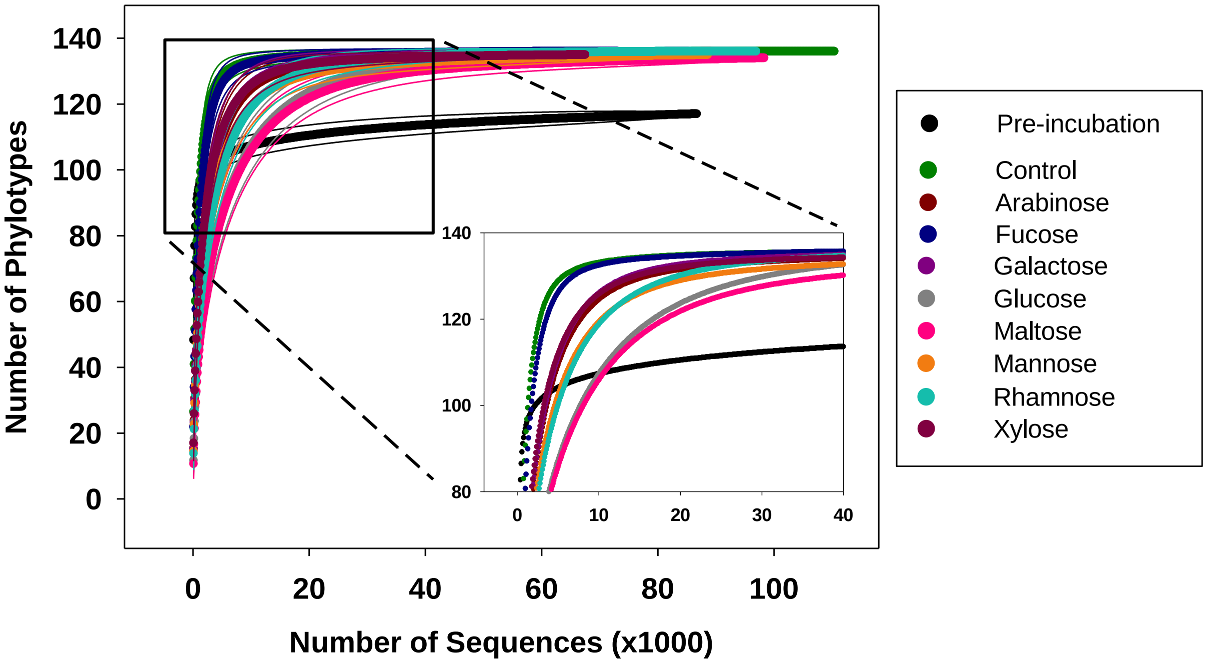
Figure S1. Rarefaction curves of 16S rRNA sequences obtained from different gut content saccharide treatments. Each curve is based on phylotypes with a 99% sequence similarity (1). The narrow solid lines correspond to a confidence interval of 95%. The taxonomic distribution of the sequences is shown in Figure 2.

Figure S2. Rarefaction curves of 16S rRNA sequences obtained from the time-dependent product profiles of the glucose treatment. Each curve is based on phylotypes with a 99% sequence similarity (1). The narrow solid lines correspond to a confidence interval of 95%. The taxonomic distribution of the sequences is shown in Figure 4.

Reference for Supplemental Figure Legends

1. Stackebrandt E, Ebers J. 2006. Taxonomic parameters revisited: Tarnished gold standards. Microb Today 33:152–155.

Figure S1


Number of Phylotypes
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20
40
60
80
100
120
140
Number of Sequences (x1000)
0
20
40
60
80
100
140
120
100
80
0
10
20
30
40
Pre-incubation
Control
Arabinose
Fucose
Galactose
Glucose
Maltose
Mannose
Rhamnose
Xylose

Figure S2

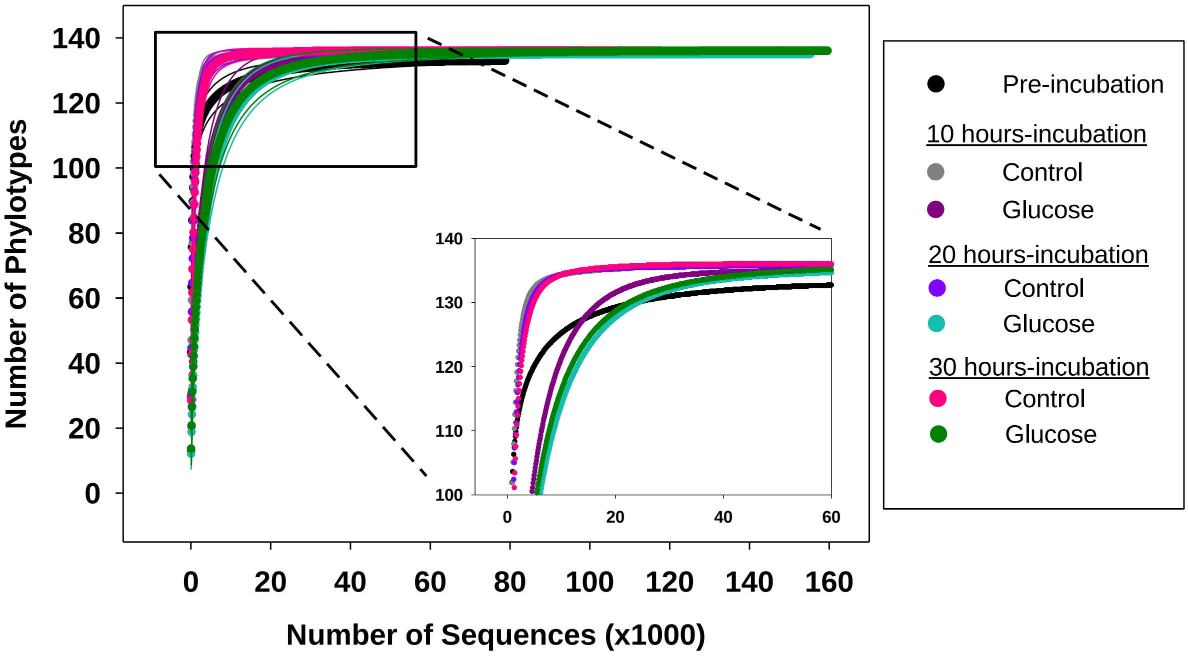
140
120
100
80
60
40
20
0
Number of Phylotypes
0
20
40
60
80
100
120
140
160
Number of Sequences (x1000)
140
130
120
110
100
0
20
40
60
● Pre-incubation
10 hours-incubation
● Control
● Glucose
20 hours-incubation
● Control
● Glucose
30 hours-incubation
● Control
● Glucose

Table S1. Statistical p-values of the products formed in (A) different saccharide treatments and (B) the time-resolved fermentation of glucose.[a,b]

(A) Different saccharide treatments (main data are in Figure 1 and Table 1)

ARABINOSE

	Succinate		Formate		Propionate		Ethanol		Lactate		Acetate		H_2		CO_2	
	C	A	C	A	C	A	C	A	C	A	C	A	C	A	C	A
Mean value	0.00	8.93	1.53	3.1	7.04	9.54	2.41	41.55	-3.11	-3.44	13.3	44.4	1.39	52.96	23.1	87.2
variance	0.00	0.55	0.13	0.05	0.24	2.94	9.45	0.07	0.06	0.08	1.11	5.5	0.19	15.61	8.72	7.89
P value	0.0023		0.0073		0.1360		0.0021		0.1979		0.0002		0.0020		1.08E-05	

FUCOSE

	Succinate		Formate		Propionate		Ethanol		Lactate		Acetate		H_2		CO_2	
	C	F	C	F	C	F	C	F	C	F	C	F	C	F	C	F
Mean value	0.00	1.61	1.53	3.32	7.04	16.2	2.41	0.00	-3.11	-2.3	13.3	21.9	1.39	3.51	23.1	27.6
variance	0.00	1.94	0.13	0.08	0.24	3.16	9.45	0.00	0.06	0.21	1.11	4.42	0.19	0.54	8.72	14.1
P value	0.1836		0.0024		0.0132		0.3073		0.0710		0.0080		0.0233		0.1760	

GALACTOSE

	Succinate		Formate		Propionate		Ethanol		Lactate		Acetate		H_2		CO_2	
	C	Ga	C	Ga	C	Ga	C	Ga	C	Ga	C	Ga	C	Ga	C	Ga
Mean value	0.00	9.71	1.53	2.26	7.04	9.67	2.41	33.47	-3.11	-2.24	13.3	37.3	1.39	57.9	23.1	89.3
variance	0.00	0.60	0.13	0.27	0.24	0.20	9.45	23.76	0.06	0.00	1.11	4.97	0.19	165	8.72	234
P value	0.0021		0.1149		0.0024		0.0026		0.0249		0.0005		0.0168		0.0179	

GLUCOSE

	Succinate		Formate		Propionate		Ethanol		Lactate		Acetate		H_2		CO_2	
	C	Gl	C	Gl	C	Gl	C	Gl	C	Gl	C	Gl	C	Gl	C	Gl
Mean value	0.00	0.00	1.53	0	7.04	10.56	2.41	36.44	-3.11	10.1	13.3	40.2	1.39	65.6	23.1	88.7
variance	0.00	0.00	0.13	0	0.24	2.28	9.45	17.3	0.06	3.75	1.11	15.5	0.19	70.4	8.72	28.2
P value	-		0.0178		0.0617		0.0003		0.0071		0.0075		0.0057		0.0003	

MALTOSE

	Succinate		Formate		Propionate		Ethanol		Lactate		Acetate		H_2		CO_2	
	C	Mt	C	Mt	C	Mt	C	Mt	C	Mt	C	Mt	C	Mt	C	Mt
Mean value	0.00	0.00	1.53	0.00	7.04	15.5	2.41	71.2	-3.11	36	13.3	56.095	1.39	110	23.1	140
variance	0.00	0.00	0.13	0.00	0.24	9.69	9.45	472	0.06	21.6	1.11	126.145	0.19	66.2	8.72	91.3
P value	-		0.0178		0.043		0.0323		0.0047		0.0224		0.0019		0.0024	

MANNOSE

	Succinate		Formate		Propionate		Ethanol		Lactate		Acetate		H_2		CO_2	
	C	Mn	C	Mn	C	Mn	C	Mn	C	Mn	C	Mn	C	Mn	C	Mn
Mean value	0.00	12.4	1.53	0.29	7.04	13.7	2.41	42.5	-3.11	-2.89	13.3	40.3	1.39	70.8	23.1	89.5
variance	0.00	0.55	0.13	0.26	0.24	0.62	9.45	108	0.06	0.06	1.11	3.3	0.19	12.2	8.72	59.1
P value	0.0012		0.0263		0.0011		0.0236		0.3458		0.0002		0.0009		0.0008	

RHAMNOSE

	Succinate		Formate		Propionate		Ethanol		Lactate		Acetate		H_2		CO_2	
	C	R	C	R	C	R	C	R	C	R	C	R	C	R	C	R
Mean value	0	3.59	1.53	0.29	7.04	28.35	2.41	0	-3.11	2.27	13.3	27.9	1.39	12.4	23.1	53.8
variance	0	0.02	0.13	0.25	0.24	7.31	9.45	0	0.06	0.51	1.11	32.5	0.19	1.22	8.72	11.2
P value	0.0006		0.0253		0.0055		0.3073		0.0065		0.0487		0.0005		0.0003	

XYLOSE

	Succinate		Formate		Propionate		Ethanol		Lactate		Acetate		H_2		CO_2	
	C	X	C	X	C	X	C	X	C	X	C	X	C	X	C	X
Mean value	0	2.81	1.53	14.6	7.04	0	2.41	27	-3.11	-2.54	13.3	31.2	1.39	31.5	23.1	59.7
variance	0	4.63	0.13	5.77	0.24	0	9.45	31.5	0.06	0.36	1.11	28.7	0.19	7.74	8.72	24.6
P value	0.1517		0.0113		0.0016		0.007		0.2233		0.0295		0.0029		0.0016	

(B) Time-resolved fermentation of glucose (main data are in Figure 3)

	Succinate		Formate		Ethanol		Lactate		Acetate		H_2		CO_2	
	C	Gl	C	Gl	C	Gl	C	Gl	C	Gl	C	Gl	C	Gl
Mean value	0.1	4.41	-6.94	-3.82	0	54.6	0.31	21.2	6.76	41.1	2.15	158	23.3	162
variance	0.07	0.66	1.16	1.43	0	101	0.07	69.3	0.21	31.8	0.37	447	0.13	447
P value	0.0203		0.0284		0.0112		0.049		0.0089		0.006		0.0076	

[a] Abbreviations: Pre, pre-incubation; C, unsupplemented control; A, arabinose-supplemented; F, fucose-supplemented; Ga, galactose-supplemented; Gl, glucose-supplemented; Mt, maltose-supplemented; Mn, mannose-supplemented; R, rhamnose-supplemented; X, xylose-supplemented.

[b] p-values were calculated by t-Test with different variances. Values are significant by $p \leq 0.05$.

Table S2. pH data of the (A) different saccharide treatments and (B) time-resolved fermentation of glucose.[a]

		pH	
A	**Treatment**	**Pre-incubation**	**Post-incubation**
Gut Content	Unsupplemented Control	7.10 ± 0.01	7.15 ± 0.01
	Arabinose	7.10 ± 0.01	6.75 ± 0.01
	Fucose	7.08 ± 0.01	6.95 ± 0.02
	Galactose	7.08 ± 0.01	6.72 ± 0.07
	Glucose	7.09 ± 0.01	6.70 ± 0.04
	Maltose	7.09 ± 0.01	6.22 ± 0.06
	Mannose	7.08 ± 0.01	6.67 ± 0.04
	Rhamnose	7.06 ± 0.03	6.69 ± 0.00
	Xylose	7.08 ± 0.01	6.78 ± 0.02
Soil	Unsupplemented Control	7.03 ± 0.01	7.04 ± 0.01
	Arabinose	6.98 ± 0.07	7.03 ± 0.01
	Fucose	7.00 ± 0.01	7.03 ± 0.02
	Galactose	6.98 ± 0.06	7.02 ± 0.01
	Glucose	6.99 ± 0.03	6.97 ± 0.02
	Maltose	7.02 ± 0.02	6.97 ± 0.02
	Mannose	7.04 ± 0.05	6.98 ± 0.03
	Rhamnose	7.02 ± 0.01	7.02 ± 0.00
	Xylose	7.04 ± 0.01	7.03 ± 0.01
B			
Gut Content	Unsupplemented Control	7.09 ± 0.01	6.60 ± 0.67
	Glucose	7.10 ± 0.01	6.38 ± 0.12

[a] Values are calculated from three replicate analyses; values and standard deviations are rounded to the nearest second decimal place.

Table S3. Recoveries of carbon (C) and reducing equivalents (e^-) of the different saccharide treatments.

	Recoveries (%)[a]															
	Arabinose		Fucose		Galactose		Glucose		Maltose		Mannose		Rhamnose		Xylose	
Product	C	e^-	C	e^-	C	e^-	C	e^-	C	e^-	C	e^-	C	e^-	C	e^-
Succinate	12	11	5	4	13	12	0	0	0	0	16	14	5	4	5	4
Formate	<1	<1	1	1	<1	<1	0	0	0	0	0	0	0	0	6	3
Propionate	3	3	23	25	3	3	3	4	4	5	6	7	22	24	0	0
Ethanol	26	40	0	0	21	32	21	31	22	33	26	38	0	0	21	32
Acetate	21	21	14	13	17	17	17	17	14	14	17	17	10	9	16	16
Lactate	0	0	0	0	0	0	12	12	19	19	0	0	6	5	0	0
CO_2	22	n.a.	4	n.a.	23	n.a.	20	n.a.	19	n.a.	21	n.a.	11	n.a.	16	n.a.
H_2	n.a.	9	n.a.	1	n.a.	10	n.a.	10	n.a.	9	n.a.	11	n.a.	2	n.a.	7
Total	84	84	47	44	77	74	73	74	78	80	86	87	54	44	64	62

[a] Recoveries were calculated from the fermentation profiles displayed in Figure 1 and Table 1, and values were rounded to the nearest whole number.

Abbreviations: n.a., not applicable.

Table S4. Summary of all detected phylotypes of the different saccharide treatments (the most abundant phylotypes are evaluated in Table 2). [a]

Phylum, Family	**Relative Abundance (%)**[b, c]										
	Pre	**C**	**A**	**F**	**Ga**	**Gl**	**Mt**	**Mn**	**R**	**X**	**No. of Phylo-types**
Acidobacteria,											
Unassigned Families	1.85	0.35	0.11	0.23	0.12	0.04	0.05	0.08	0.06	0.11	3
Actinobacteria,											
Acidimicrobiaceae	1.20	0.22	0.08	0.16	0.10	0.04	0.04	0.07	0.06	0.09	3
Cellulomonadaceae	0.36	0.11	0.05	0.09	0.06	0.02	0.02	0.03	0.02	0.05	1
Gaiellaceae	2.72	0.35	0.10	0.23	0.11	0.05	0.05	0.08	0.08	0.10	3
Intrasporangiaceae	0.32	0.09	0.03	0.05	0.03	0.02	0.01	0.02	0.02	0.02	1
Microbacteriaceae	0.57	0.11	0.04	0.10	0.04	0.02	0.01	0.05	0.02	0.05	2
Micrococcaceae	0.89	0.12	0.05	0.07	0.04	0.01	0.01	0.02	0.02	0.04	1
Mycobacteriaceae	0.50	0.07	0.01	0.05	0.02	0.01	0.01	0.01	0.01	0.02	1
Nakamurellaceae	0.29	0.05	0.01	0.04	0.02	0.00	0.00	0.01	0.01	0.02	1
Nocardioidaceae	2.61	0.32	0.12	0.23	0.12	0.04	0.03	0.09	0.08	0.11	2
Patulibacteraceae	0.94	0.12	0.05	0.12	0.04	0.02	0.01	0.03	0.03	0.04	1
Propionibacteriaceae	1.71	0.19	0.06	0.14	0.08	0.03	0.03	0.05	0.05	0.10	2
Pseudonocardiaceae	1.90	0.30	0.08	0.23	0.08	0.04	0.03	0.08	0.07	0.10	2
Solirubrobacteraceae	6.59	1.18	0.37	0.69	0.32	0.16	0.13	0.26	0.24	0.40	4
Unassigned Families	6.45	0.89	0.32	0.61	0.31	0.13	0.14	0.23	0.21	0.35	7
Bacteroidetes,											
Flavobacteriaceae	0.04	0.61	0.44	0.63	0.96	0.14	0.08	0.24	0.13	0.41	4

Chloroflexi,											
Chloroflexaceae	0.50	0.05	0.01	0.05	0.02	0.01	0.01	0.01	0.01	0.02	1
Unassigned Families	6.20	0.76	0.28	0.61	0.23	0.12	0.12	0.16	0.19	0.28	7
Firmicutes,											
Bacillaceae	2.74	0.56	0.21	0.35	0.13	0.09	0.08	0.13	0.07	0.12	3
Clostridiaceae	0.23	2.05	0.78	1.17	0.39	0.50	0.41	0.65	0.50	0.67	3
Lachnospiraceae	0.00	0.81	0.14	0.27	0.08	0.15	0.00	0.11	0.02	0.12	1
Peptostreptococcaceae	0.14	7.33	1.97	4.21	1.05	1.15	0.53	1.85	1.33	1.76	5
Fusobacteria,											
Unassigned Family	0.00	4.57	0.99	1.49	0.70	0.69	0.04	0.64	1.19	3.27	1
Nitrospirae,											
Unassigned Families	2.57	0.51	0.13	0.40	0.10	0.06	0.06	0.11	0.08	0.13	4
Planctomycetes,											
Planctomycetaceae	7.54	0.85	0.25	0.62	0.32	0.11	0.13	0.18	0.18	0.35	8
Proteobacteria,											
Aeromonadaceae	0.14	62.37	39.32	45.81	52.78	81.65	83.49	82.94	84.43	58.72	15
Bradyrhizobiaceae	5.48	0.84	0.20	0.54	0.26	0.11	0.09	0.15	0.17	0.25	1
Comamonadaceae	0.48	0.11	0.05	0.10	0.03	0.02	0.02	0.02	0.03	0.04	3
Enterobacteriaceae	0.14	6.05	50.93	34.50	37.80	12.88	12.77	8.66	7.79	27.25	6
Methylobacteriaceae	0.75	0.10	0.03	0.06	0.01	0.02	0.01	0.02	0.01	0.02	1
Nitrospinaceae	1.90	0.84	0.24	0.59	0.29	0.12	0.11	0.18	0.15	0.32	2
Phyllobacteriaceae	0.71	0.08	0.03	0.07	0.03	0.01	0.01	0.02	0.02	0.05	1
Pseudomonadaceae	0.02	0.28	0.07	0.10	0.05	0.03	0.03	0.04	0.04	0.06	2
Rhodobacteraceae	0.30	0.04	0.02	0.02	0.01	0.01	0.00	0.01	0.01	0.01	1
Rhodobiaceae	1.25	0.14	0.05	0.08	0.04	0.01	0.03	0.03	0.03	0.05	1
Rhodocyclaceae	0.00	0.05	0.01	0.05	0.02	0.01	0.00	0.01	0.01	0.01	1

Rhodospirillaceae	8.00	1.19	0.30	0.75	0.32	0.13	0.13	0.23	0.22	0.36	7
Shewanellaceae	0.00	1.00	0.61	1.33	1.09	0.19	0.09	0.39	0.74	1.62	1
Xanthobacteraceae	7.50	0.93	0.27	0.65	0.27	0.10	0.11	0.22	0.18	0.29	5
Unassigned Families	4.73	0.99	0.35	0.73	0.44	0.42	0.40	0.53	0.47	0.40	11
Thaumarchaeota,											
Unassigned Families	2.55	0.30	0.10	0.22	0.15	0.05	0.05	0.09	0.09	0.16	3
Tenericutes,											
Mycoplasmataceae	11.96	1.46	0.54	1.15	0.65	0.46	0.50	1.08	0.78	1.37	2
Verrucomicrobia,											
Unassigned Families	5.21	0.63	0.20	0.42	0.26	0.13	0.11	0.19	0.16	0.28	2

[a] See accompanying supplemental text for information on the less abundant families.

[b] Values are rounded to the nearest second decimal place.

[c] Abbreviations: Pre, pre-incubation; C, unsupplemented control; A, arabinose-supplemented; F, fucose-supplemented; Ga, galactose-supplemented; Gl, glucose-supplemented; Mt, maltose-supplemented; Mn, mannose-supplemented; R, rhamnose-supplemented; X, xylose-supplemented;

Table S5. Summary of all detected phylotypes during the time-resolved fermentation of glucose (the most abundant phylotypes are evaluated in Table 3). [a]

Phylum, Family	**Relative Abundance (%)**[b, c]							
		10 h		**20 h**		**30 h**		
	Pre	**C**	**G**	**C**	**G**	**C**	**G**	**No. of Phylo-types**
Acidobacteria,								
Unassigned families	1.92	0.95	0.16	0.88	0.06	0.83	0.09	3
Actinobacteria,								
Acidimicrobiaceae	1.03	0.27	0.06	0.20	0.04	0.15	0.04	3
Cellulomonadaceae	0.68	0.24	0.08	0.20	0.05	0.22	0.06	1
Gaiellaceae	1.70	0.27	0.05	0.24	0.04	0.19	0.04	3
Intrasporangiaceae	0.47	0.24	0.03	0.21	0.01	0.15	0.02	1
Microbacteriaceae	1.71	0.38	0.13	0.21	0.09	0.31	0.07	2
Micrococcaceae	0.30	0.14	0.02	0.11	0.02	0.10	0.02	1
Mycobacteriaceae	0.19	0.12	0.01	0.11	0.01	0.09	0.01	1
Nakamurellaceae	0.37	0.18	0.02	0.14	0.01	0.14	0.02	1
Nocardioidaceae	1.23	0.29	0.05	0.24	0.02	0.25	0.02	2
Patulibacteraceae	0.26	0.10	0.02	0.10	0.01	0.10	0.01	1
Propionibacteriaceae	1.08	0.42	0.07	0.33	0.04	0.30	0.05	2
Pseudonocardiaceae	0.61	0.32	0.07	0.29	0.05	0.27	0.05	2
Solirubrobacteraceae	2.05	1.23	0.19	1.04	0.13	1.07	0.14	4
Unassigned families	3.29	0.93	0.19	0.76	0.11	0.72	0.11	7
Bacteroidetes,								
Flavobacteriaceae	14.28	18.31	4.44	13.82	3.23	12.72	6.52	4
Chloroflexi,								
Chloroflexaceae	0.22	0.09	0.02	0.07	0.01	0.07	0.01	1
Unassigned families	6.89	0.96	0.22	0.73	0.13	0.69	0.16	7
Firmicutes,								
Bacillaceae	0.44	0.51	0.12	0.40	0.13	0.34	0.11	3
Clostridiaceae	0.04	0.57	0.03	2.43	0.10	2.71	0.44	3
Lachnospiraceae	0.00	0.00	0.00	0.00	0.00	0.01	0.00	1
Peptostreptococcaceae	0.07	4.67	0.71	10.61	1.02	14.25	2.28	5
Fusobacteria,								
Unassigned family	0.01	0.21	0.02	0.60	0.02	0.84	0.02	1
Nitrospirae,								
Unassigned family	1.04	0.51	0.06	0.42	0.06	0.35	0.04	4

Planctomycetes,							
Planctomycetaceae	2.08	1.51	0.25	1.25	0.17	0.95	0.18	8
Proteobacteria							
Aeromonadaceae	8.69	45.01	78.04	43.24	69.38	41.03	63.91	15
Bradyrhizobiaceae	3.21	1.20	0.30	0.90	0.17	0.92	0.26	1
Comamonadaceae	2.09	0.67	0.15	0.53	0.12	0.72	0.14	3
Enterobacteriaceae	1.25	5.51	11.51	6.21	22.55	6.00	22.86	6
Methylobacteriaceae	0.34	0.13	0.03	0.09	0.02	0.14	0.03	1
Nitrospinaceae	1.68	1.15	0.17	0.94	0.08	0.63	0.10	2
Phyllobacteriaceae	0.74	0.13	0.03	0.10	0.02	0.10	0.02	1
Pseudomonadaceae	0.86	0.63	0.08	0.53	0.07	0.58	0.09	2
Rhodobacteraceae	0.39	0.11	0.02	0.07	0.01	0.08	0.01	1
Rhodobiaceae	0.68	0.17	0.03	0.13	0.02	0.10	0.02	1
Rhodocyclaceae	0.07	0.39	0.07	0.31	0.05	0.38	0.11	1
Rhodospirillaceae	5.06	1.59	0.24	1.55	0.22	1.30	0.23	7
Shewanellaceae	0.21	2.39	0.35	4.73	0.25	4.86	0.32	1
Xanthobacteraceae	4.20	0.96	0.15	0.76	0.10	0.68	0.10	5
Unassigned families	4.14	1.75	0.49	1.54	0.47	1.52	0.47	11
Thaumarchaeota							
Unassigned families	1.59	0.61	0.11	0.40	0.10	0.27	0.09	3
Tenericutes,							
Mycoplasmataceae	19.39	3.28	0.98	2.01	0.65	2.19	0.48	2
Verrucomicrobia,							
Unassigned families	3.44	0.90	0.25	0.56	0.19	0.70	0.27	2

[a] See accompanying supplemental text for information on the less abundant families.

[b] Values are rounded to the nearest second decimal place.

[c] Abbreviations: Pre, pre-incubation; C, unsupplemented control; G, glucose treatment.

Additional Information on the Less Abundant Families in Tables S4 and S5

Families that were affiliated to the phylum *Proteobacteria* included *Bradyrhizobiaceae, Comamonadaceae, Nitrospinaceae, Phyllobacteriaceae, Rhodobacteraceae, Rhodobiaceae, Xanthobacteraceae, and Pseudomonadaceae* (1–8). These families contain metabolically and ecologically diverse genera that can grow aerobically. The families *Bradyrhizobiaceae*, *Pseudomonadaceae*, and *Xanthobacteraceae* are known for their ability to fix molecular N_2 (1, 6, 7). Chemotrophic members are found in the *Proteobacteria*-affiliated families, but phototrophic members also occur in the families *Bradyrhizobiaceae, Comamonadaceae, Rhodobacteraceae,* and *Rhodospirillaceae* (1, 2, 4, 9).

Some of the families that were affiliated to the phylum *Actinobacteria* (i.e., *Gaiellaceae, Mycobacteriaceae, Patulibacteraceae,* and *Solirubrobacteraceae*) are considered to be strictly aerobic (10–12). Other families belonging to this phylum (e.g. *Cellulomonadaceae*) can be aerobic and also facultatively aerobic (13). *Gaiellaceae, Microbacteriaceae, Patulibacteraceae*, and *Solirubrobacteraceae* are chemoorganotrophic (10, 12, 14).

The phyla *Bacteroidetes*, *Chloroflexi, Firmicutes,* and *Planctomycetes* were also detected. The *Firmicutes*-affiliated families *Clostridiaceae* and *Lachnospiraceae* are mainly anaerobic and capable of diverse fermentations (15, 16). Members of the family *Bacillaceae* are aerobes or facultative aerobes (17), and members of the family *Chloroflexaceae* are phototrophs (18). Most members of the *Flavobacteriaceae* related to the phylum *Bacteroidetes* are cabable of aerobic growth (19).

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