Niche differentiation of bacterial communities at a millimeter scale in Shark Bay microbial mats

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Subject category: Microbial ecology and functional diversity of natural habitats

Supplementary Figure S1. Rarefaction curves of smooth and pustular mats layers. The bottom layer of smooth mats (SD10, 19 – 20 mm) has the highest number of different OTUs, followed by the 6th layer of smooth mats (SD6, 11 – 12 mm) and the bottom layer of pustular mats (PD5, 9 – 10 mm). P1 to P5 refer to pustular mat layers 1 - 5, while S1 – S10 refer to smooth mat layers 1 – 10.

Supplementary Figure S2. Abundance of some prominent phyla and taxonomic subgroups by depth in smooth mats. A. Proteobacteria, **B**. Cyanobacteria, **C**. Chloroflexi, **D**. Bacteroidetes, **E**. Planctomycetes, **F**. Spirochaetes, **G**. Firmicutes, **H**. Other low abundance phyla

Supplementary Figure S3. Abundance of some prominent phlya and taxonomic subgroups by depth in pustular mats. **A**. Proteobacteria, **B**. Cyanobacteria, **C.** Chloroflexi, **D.** Bacteroidetes, **E**. Planctomycetes, **F**. Spirochaetes and Firmicutes, **G**. Other low abundance phyla

Supplementary Figure S4. STAMP analysis of taxonomy enriched or depleted between the designated groups and all other groups in smooth mats. A. Surface group compared to the other three groups, showing the surface of smooth mats is characterised by Cyanobacteria, Bacteroidetes, Alpha- and Gammaproteobacteria (Green dots). **B.** Group A compared to all other groups, showing Group A is represented by class Anaerolineae and classes of Spirochaetes (Blue dots) **C.** Group B compared to all other three groups, showing Group B is characterised mainly by Planctomycetes (Red dots). **D.** Bottom layer compared to all other groups, showing the bottom layer is represented by mainly Deltaproteobacteria and candidate phylum OP3 (Brown dots). All tests were performed using Welch's t-tests and subjected to Benjamini-Hochberg FDR correction.

Supplementary Figure S5. STAMP analysis of taxonomy enriched or depleted between the designated groups and all other groups in pustular mats. A. Surface group compared to the other two groups, showing the surface of smooth mats is characterised by Cyanobacteria and Bacteroidetes (Green dots). **B.** Bottom layer of pustular mats compared to all other groups, showing the bottom layer is represented Gammaproteobacteria (Red dots) All tests were performed using Welch's t-tests and subjected to Benjamini-Hochberg FDR correction. Group C cluster in pustular mats showed no bacteria taxa that were significantly enriched or depleted.

Supplementary Figure S6. Network correlation analysis at the bacterial class level of smooth mats. Connections are given for a strong (Pearson's p > 0.6) and significant (*p*-value < 0.001) correlations. Diamond shaped nodes represent elements whilst eclipse shaped nodes represent bacterial classes. Alpha- and Deltaproteobacteria were presented at the order level. Green lines indicate positive correlations whilst red lines indicate negative correlations. There are 48 nodes and 141 edges in the network. Different colours indicate elements and bacterial phyla. Element nodes in yellow represents correlation with bacteria whilst element nodes in white indicates no correlation with bacteria. Green: Cyanobacteria, Red: Bacteroidetes, Blue: Alphaproteobacteria, Orange: Gammaproteobacteria, Pink: Deltaproteobacteria, Purple: Chloroflexi, White: Planctomycetes, Light pink: Caldithrix, Light blue: Lentisphaeria, Light grey: OP8, Brown: Spirochaetes, Dark purple: Firmicutes, Dark green: Others (Bacterial phyla OP3, GN04, TM6, WS3, BRC1, Fibrobacteres and SAR406).

Supplementary Figure S7. Network correlation analysis at the bacterial class level of pustular mats. Connections are given for a strong (Pearson's p > 0.6) and significant (*p*-value < 0.001) correlations. Diamond shaped nodes represent elements whilst eclipse shaped nodes represent bacterial classes. Alpha- and Deltaproteobacteria were presented at the order level. Green lines indicate positive correlations whilst red lines indicate negative correlations. There are 42 nodes and 139 edges in the network. Different colours indicate elements and bacterial phyla. Element nodes in yellow represents correlation with bacteria whilst element nodes in white indicates no correlation with bacteria. Green: Cyanobacteria, Red: Bacteroidetes, Blue: Alphaproteobacteria, Orange: Gammaproteobacteria, Pink: Deltaproteobacteria, Purple: Chloroflexi, White: Planctomycetes, Brown: Spirochaetes, Light green: Verrucomicrobia, Dark purple: Firmicutes, Dark red: Gemmatimonadetes, Dark green: Others (Bacterial phyla Actinobacteria, Acidobacteria, Fibrobacteres and SAR406).

Supplementary Figure S8. Spatial distribution and relative abundance of putative bacterial functional groups in Shark Bay microbial mats. A. Smooth mats **B.** Pustular mats. Functional groups were only assigned to those bacterial taxa for which conserved functions are well described.

Additional Information regarding to Supplementary Figure S6 & S7

Supplementary Figure S6:

48 nodes

141 edges

Rhodobacterales (Alphaproteobacteria) have the most edges (13)

Elements having the most edges with bacteria: Manganese (9), Iron (4), Magnesium (3), Molybdenum (1).

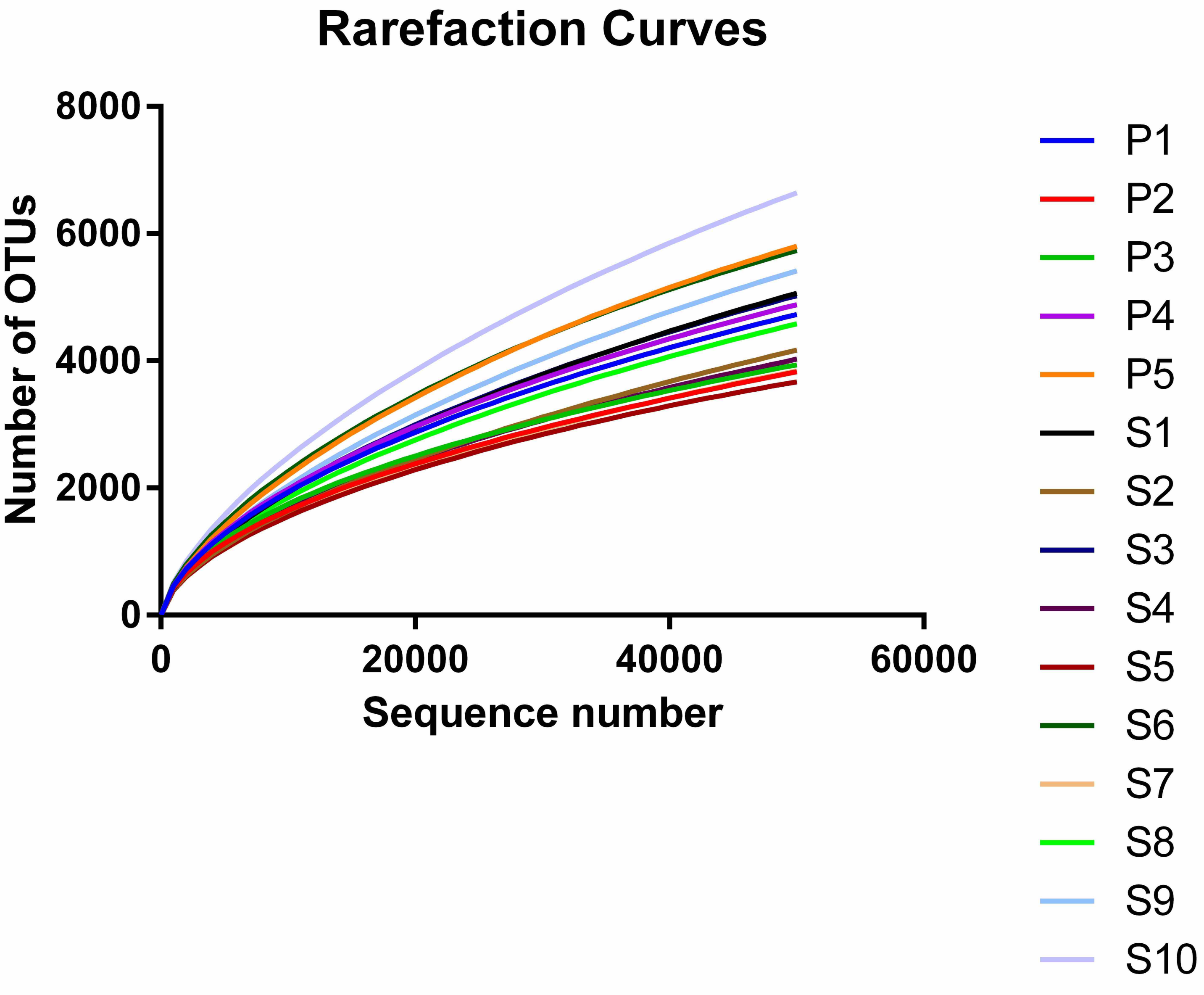
Supplementary Figure S7:

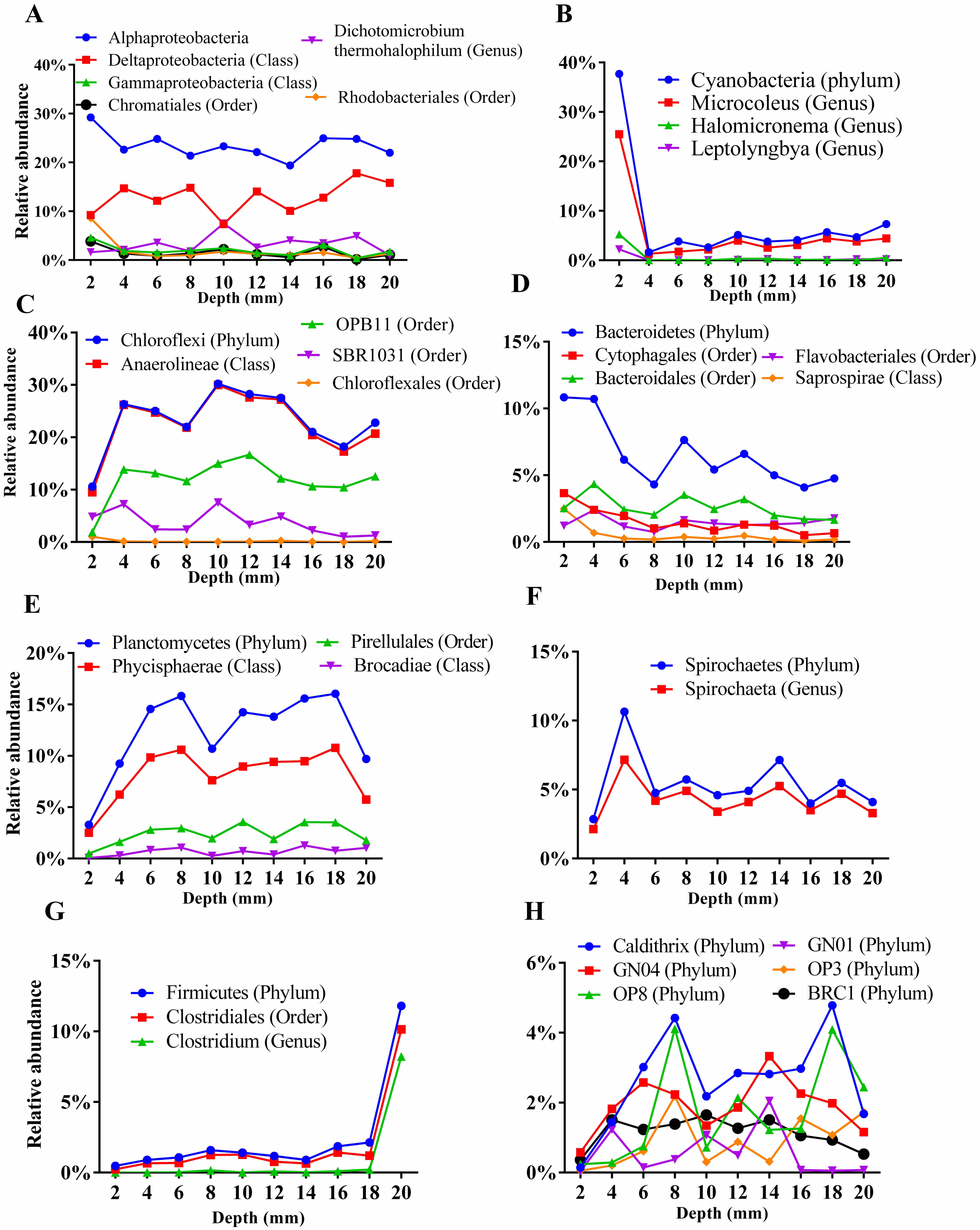
42 nodes

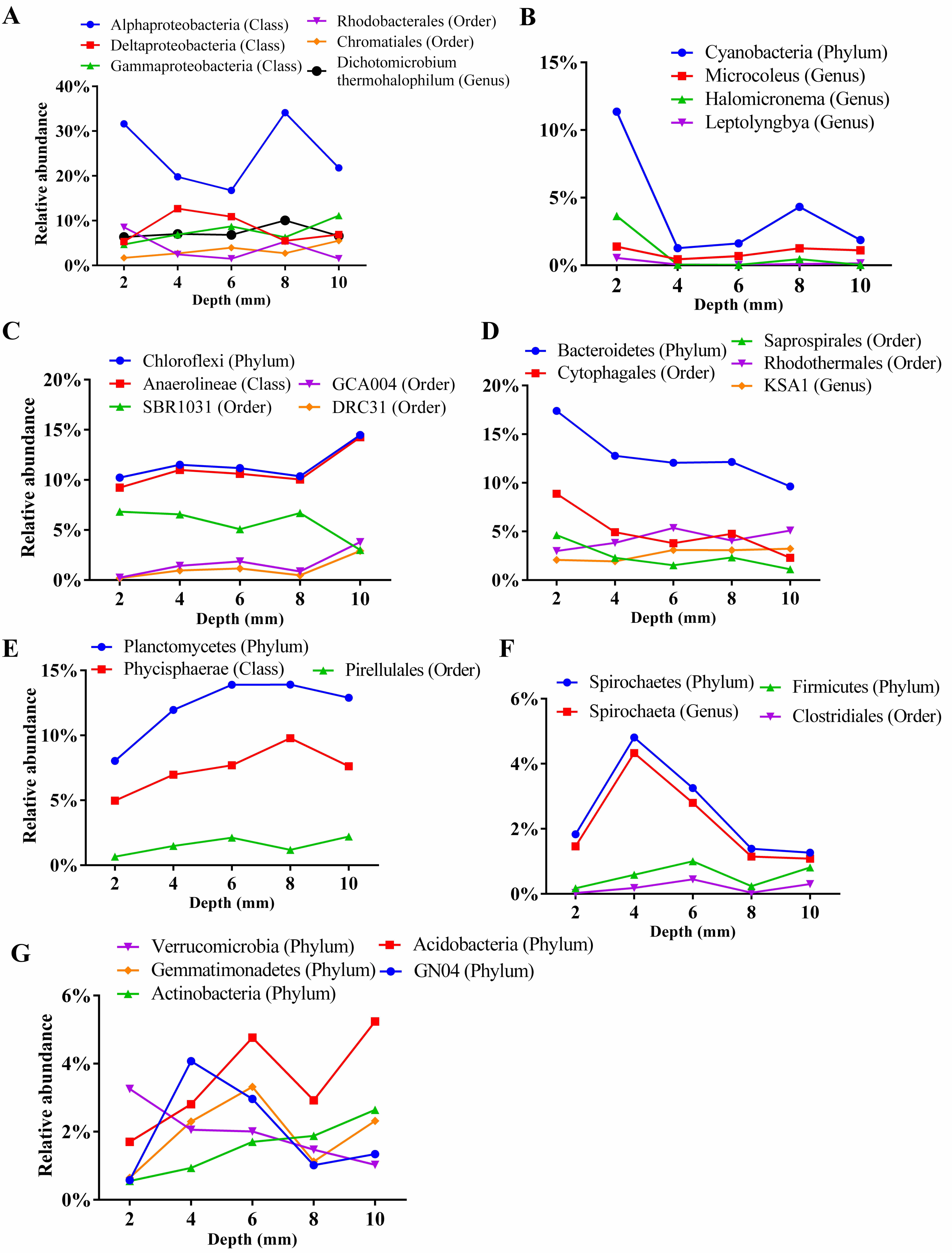
139 edges

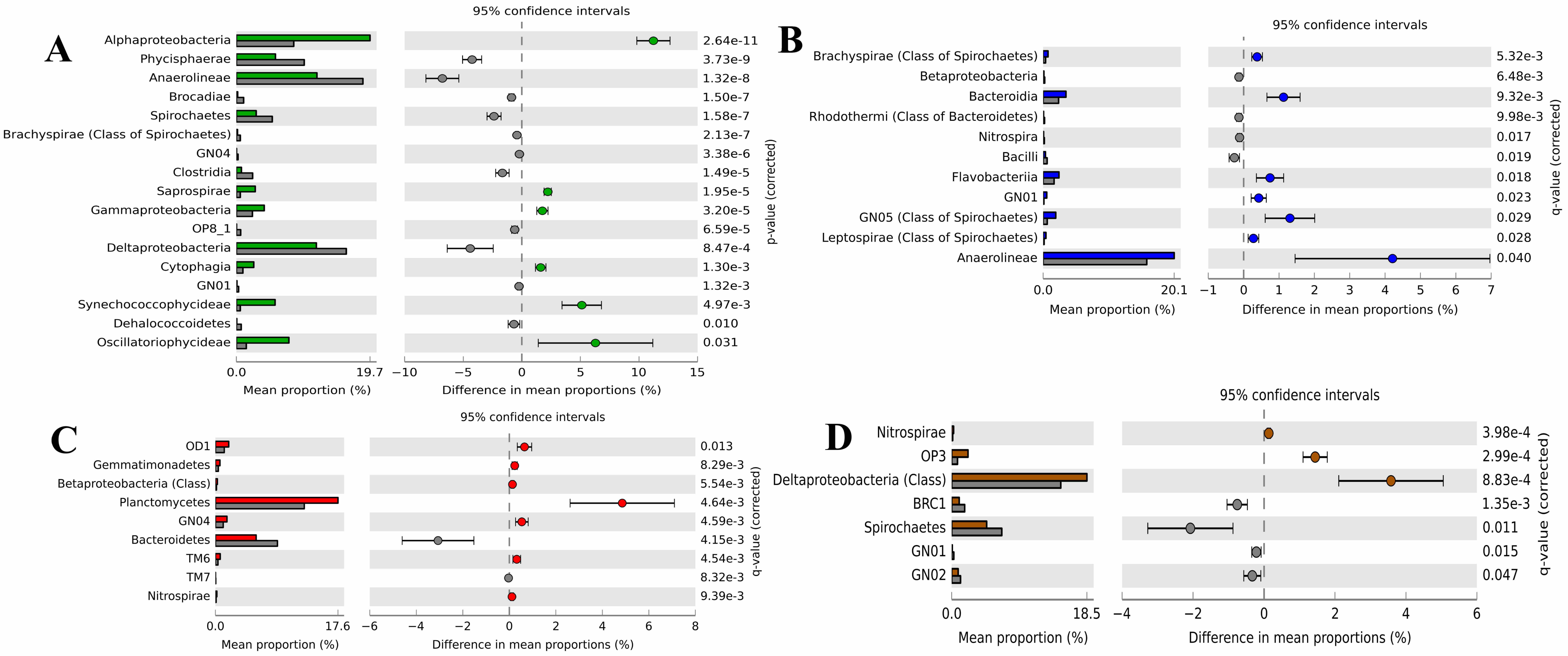
Rhizobiales (Alphaproteobacteria) have the most edges (6)

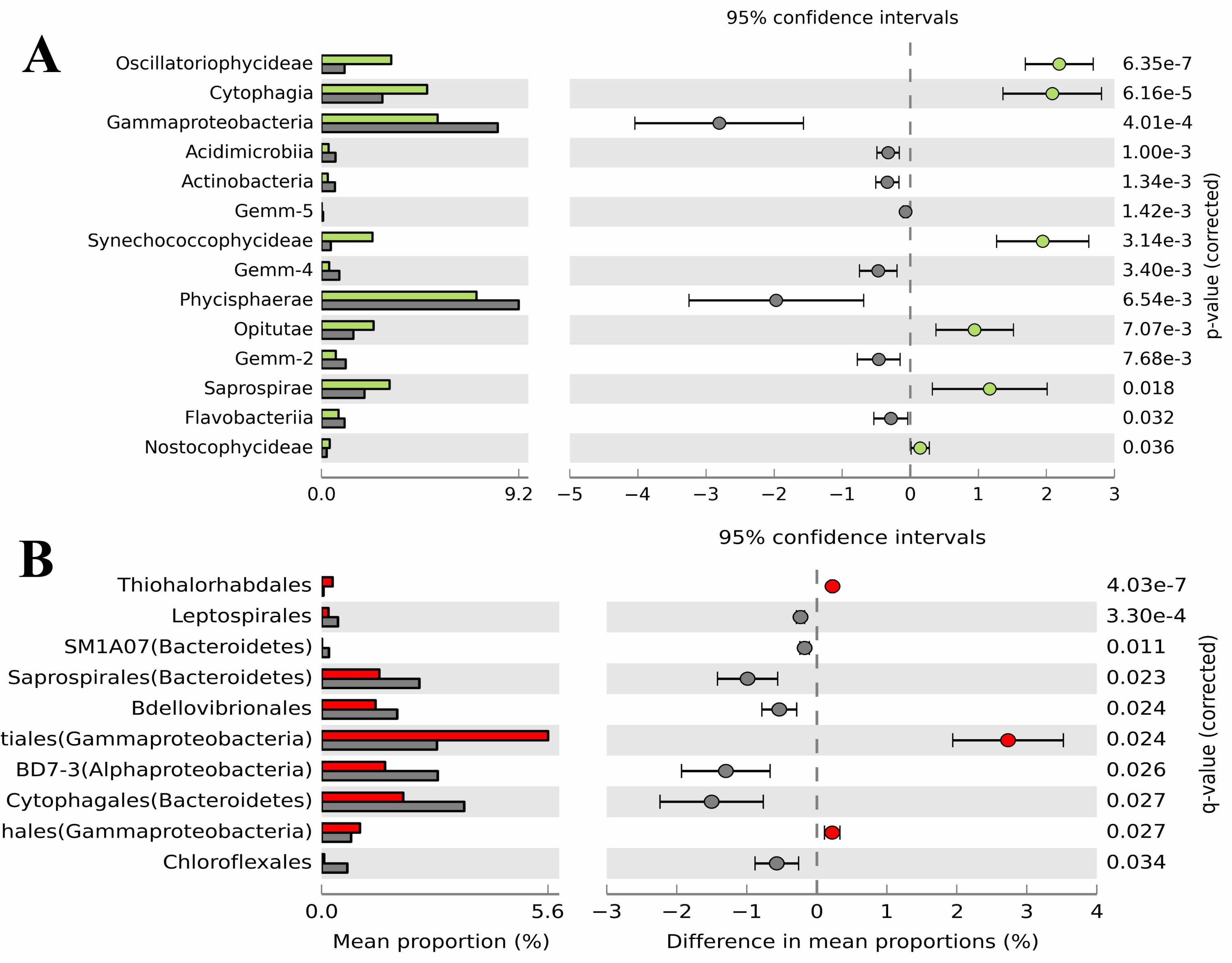
Elements having the most edges with bacteria: Vanadium (3), Phosphorus (1)

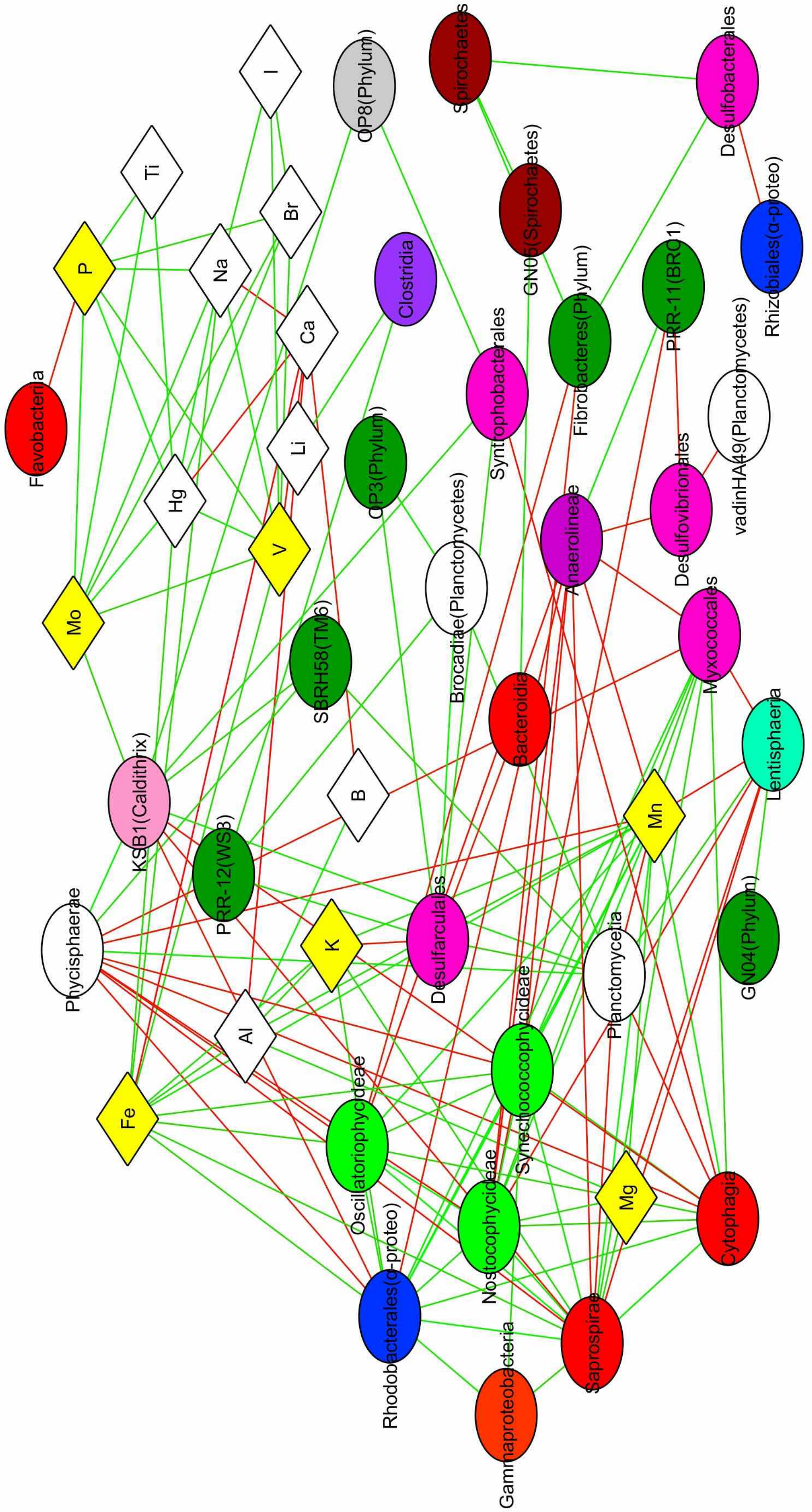
Rarefaction Curves
Number of OTUs
8000
6000
4000
2000
0
0
20000
40000
60000
Sequence number
P1
P2
P3
P4
P5
S1
S2
S3
S4
S5
S6
S7
S8
S9
S10

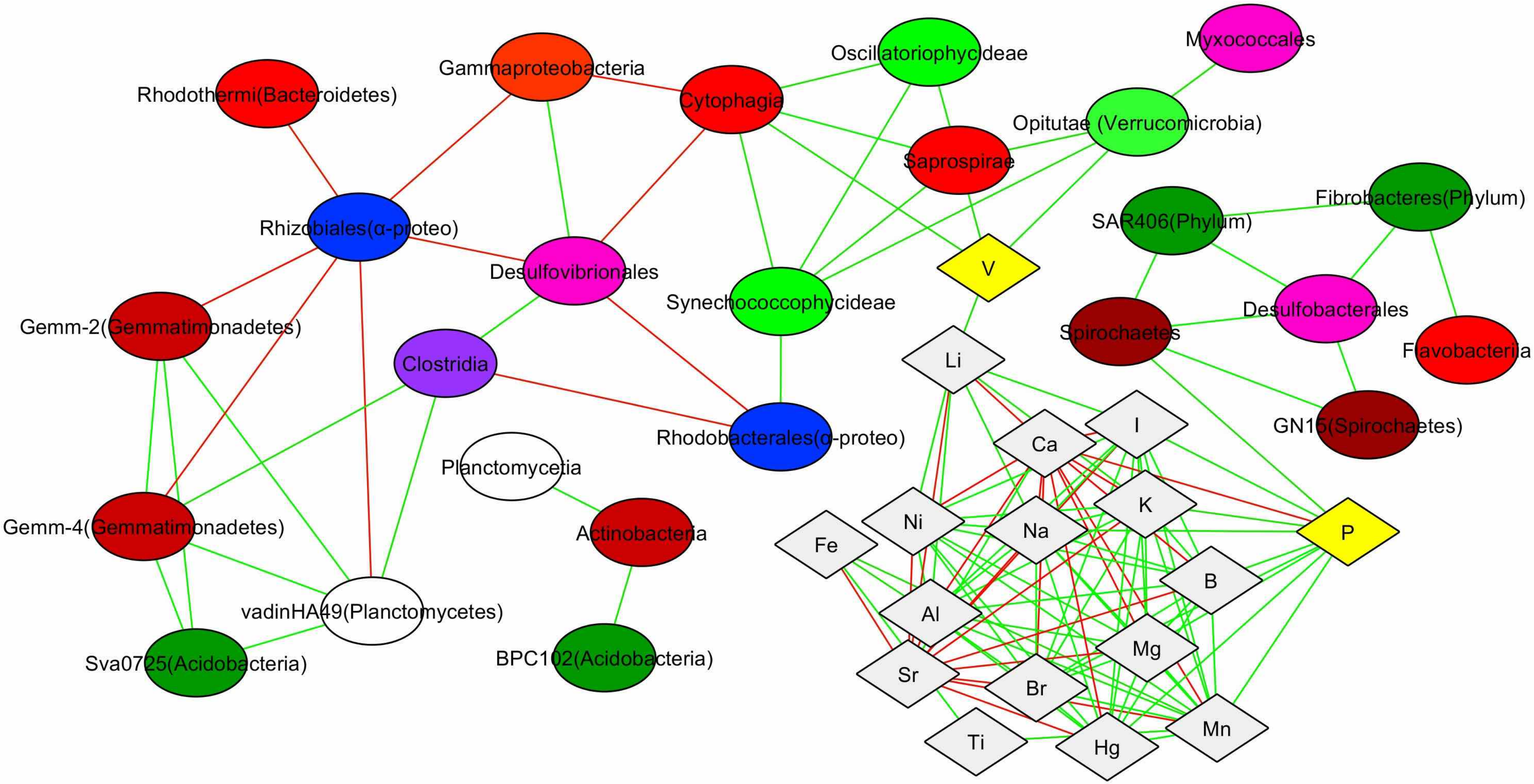

A
Alphaproteobacteria
Deltaproteobacteria (Class)
Gammaproteobacteria (Class)
Chromatiales (Order)
Dichotomicrobium thermohalophilum (Genus)
Rhodobacteriales (Order)
Relative abundance
40%
30%
20%
10%
0%
2 4 6 8 10 12 14 16 18 20
Depth (mm)
B
Cyanobacteria (phylum)
Microcoleus (Genus)
Halomicronema (Genus)
Leptolyngbya (Genus)
40%
30%
20%
10%
0%
2 4 6 8 10 12 14 16 18 20
Depth (mm)
C
Chloroflexi (Phylum)
Anaerolineae (Class)
OPB11 (Order)
SBR1031 (Order)
Chloroflexales (Order)
Relative abundance
40%
30%
20%
10%
0%
2 4 6 8 10 12 14 16 18 20
Depth (mm)
D
Bacteroidetes (Phylum)
Cytophagales (Order)
Bacteroidales (Order)
Flavobacteriales (Order)
Saprospirae (Class)
15%
10%
5%
0%
2 4 6 8 10 12 14 16 18 20
Depth (mm)
E
Planctomycetes (Phylum)
Phycisphaerae (Class)
Pirellulales (Order)
Brocadiae (Class)
Relative abundance
20%
15%
10%
5%
0%
2 4 6 8 10 12 14 16 18 20
Depth (mm)
F
Spirochaetes (Phylum)
Spirochaeta (Genus)
15%
10%
5%
0%
2 4 6 8 10 12 14 16 18 20
Depth (mm)
G
Firmicutes (Phylum)
Clostridiales (Order)
Clostridium (Genus)
Relative abundance
15%
10%
5%
0%
2 4 6 8 10 12 14 16 18 20
Depth (mm)
H
Caldithrix (Phylum)
GN04 (Phylum)
OP8 (Phylum)
GN01 (Phylum)
OP3 (Phylum)
BRC1 (Phylum)
6%
4%
2%
0%
2 4 6 8 10 12 14 16 18 20
Depth (mm)

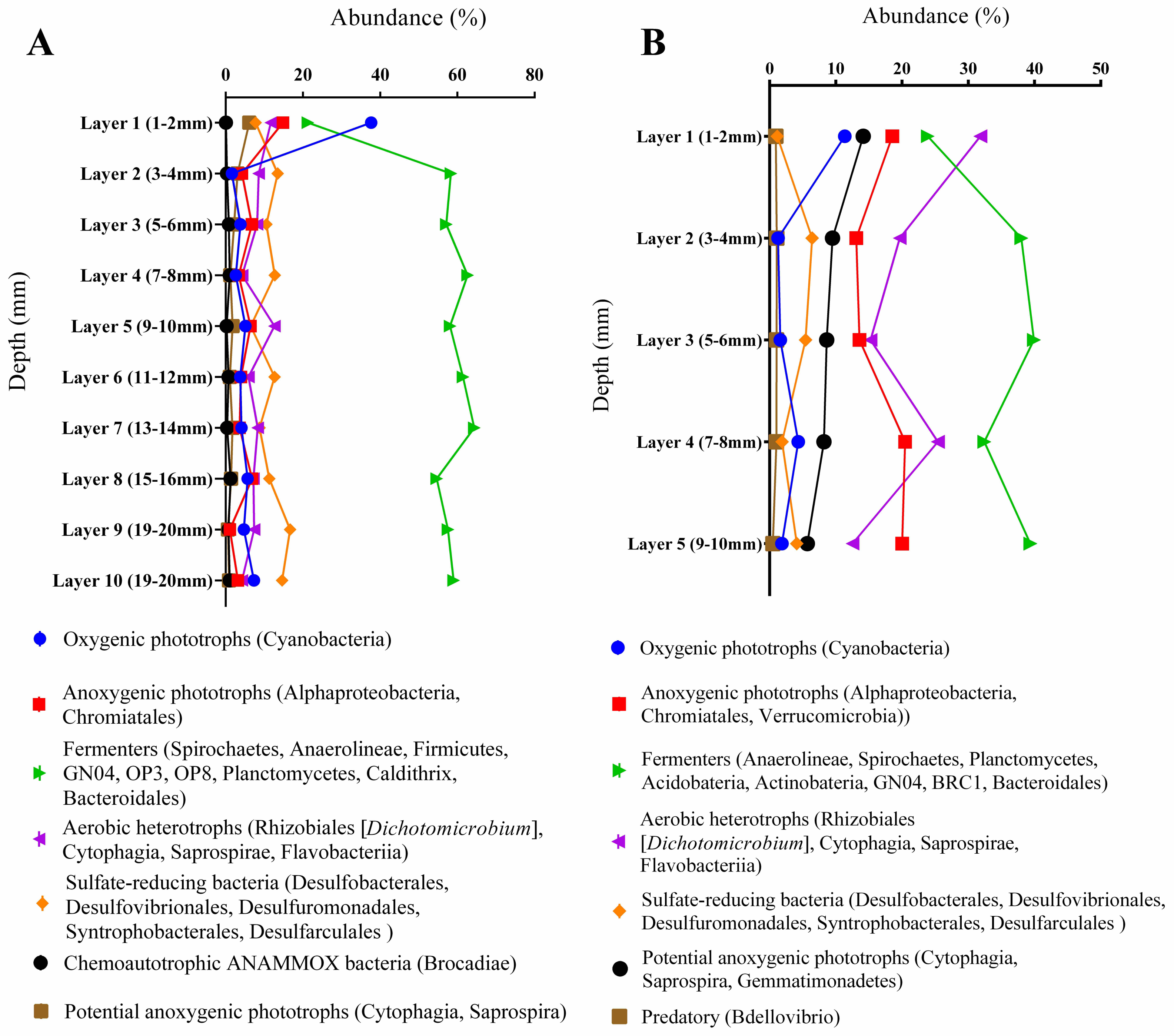

A
Alphaproteobacteria (Class)
Deltaproteobacteria (Class)
Gammaproteobacteria (Class)
Rhodobacterales (Order)
Chromatiales (Order)
Dichotomicrobium thermohalophilum (Genus)
Relative abundance
40%
30%
20%
10%
0%
2
4
6
8
10
Depth (mm)
B
Cyanobacteria (Phylum)
Microcoleus (Genus)
Halomicronema (Genus)
Leptolyngbya (Genus)
15%
10%
5%
0%
2
4
6
8
10
Depth (mm)
C
Chloroflexi (Phylum)
Anaerolineae (Class)
GCA004 (Order)
SBR1031 (Order)
DRC31 (Order)
Relative abundance
20%
15%
10%
5%
0%
2
4
6
8
10
Depth (mm)
D
Bacteroidetes (Phylum)
Cytophagales (Order)
Saprospirales (Order)
Rhodothermales (Order)
KSA1 (Genus)
20%
15%
10%
5%
0%
2
4
6
8
10
Depth (mm)
E
Planctomycetes (Phylum)
Phycisphaerae (Class)
Pirellulales (Order)
Relative abundance
15%
10%
5%
0%
2
4
6
8
10
Depth (mm)
F
Spirochaetes (Phylum)
Spirochaeta (Genus)
Firmicutes (Phylum)
Clostridiales (Order)
6%
4%
2%
0%
2
4
6
8
10
Depth (mm)
G
Verrucomicrobia (Phylum)
Gemmatimonadetes (Phylum)
Actinobacteria (Phylum)
Acidobacteria (Phylum)
GN04 (Phylum)
Relative abundance
6%
4%
2%
0%
2
4
6
8
10
Depth (mm)


A
95% confidence intervals
Alphaproteobacteria
Phycisphaerae
Anaerolineae
Brocadiae
Spirochaetes
Brachyspirae (Class of Spirochaetes)
GN04
Clostridia
Saprospirae
Gammaproteobacteria
OP8_1
Deltaproteobacteria
Cytophagia
GN01
Synechococcophycideae
Dehalococcoidetes
Oscillatoriophycideae
2.64e-11
3.73e-9
1.32e-8
1.50e-7
1.58e-7
2.13e-7
3.38e-6
1.49e-5
1.95e-5
3.20e-5
6.59e-5
8.47e-4
1.30e-3
1.32e-3
4.97e-3
0.010
0.031
p-value (corrected)
0.0
19.7
Mean proportion (%)
−10
−5
0
5
10
15
Difference in mean proportions (%)
B
95% confidence intervals
Brachyspirae (Class of Spirochaetes)
Betaproteobacteria
Bacteroidia
Rhodothermi (Class of Bacteroidetes)
Nitrospira
Bacilli
Flavobacteriia
GN01
GN05 (Class of Spirochaetes)
Leptospirae (Class of Spirochaetes)
Anaerolineae
5.32e-3
6.48e-3
9.32e-3
9.98e-3
0.017
0.019
0.018
0.023
0.029
0.028
0.040
q-value (corrected)
0.0
20.1
Mean proportion (%)
−1
0
1
2
3
4
5
6
7
Difference in mean proportions (%)
C
95% confidence intervals
OD1
Gemmatimonadetes
Betaproteobacteria (Class)
Planctomycetes
GN04
Bacteroidetes
TM6
TM7
Nitrospirae
0.013
8.29e-3
5.54e-3
4.64e-3
4.59e-3
4.15e-3
4.54e-3
8.32e-3
9.39e-3
q-value (corrected)
0.0
17.6
Mean proportion (%)
−6
−4
−2
0
2
4
6
8
Difference in mean proportions (%)
D
95% confidence intervals
Nitrospirae
OP3
Deltaproteobacteria (Class)
BRC1
Spirochaetes
GN01
GN02
3.98e-4
2.99e-4
8.83e-4
1.35e-3
0.011
0.015
0.047
q-value (corrected)
0.0
18.5
Mean proportion (%)
−4
−2
0
2
4
6
Difference in mean proportions (%)


A
95% confidence intervals
Oscillatoriophycideae
Cytophagia
Gammaproteobacteria
Acidimicrobiia
Actinobacteria
Gemm-5
Synechococcophycideae
Gemm-4
Phycisphaerae
Opitutae
Gemm-2
Saprospirae
Flavobacteriia
Nostocophycideae
0.0
9.2
−5
−4
−3
−2
−1
0
1
2
3
6.35e-7
6.16e-5
4.01e-4
1.00e-3
1.34e-3
1.42e-3
3.14e-3
3.40e-3
6.54e-3
7.07e-3
7.68e-3
0.018
0.032
0.036
p-value (corrected)
B
95% confidence intervals
Thiohalorhabdales
Leptospirales
SM1A07(Bacteroidetes)
Saprospirales(Bacteroidetes)
Bdellovibrionales
Chromatiales(Gammaproteobacteria)
BD7-3(Alphaproteobacteria)
Cytophagales(Bacteroidetes)
Thiotrichales(Gammaproteobacteria)
Chloroflexales
0.0
5.6
Mean proportion (%)
−3
−2
−1
0
1
2
3
4
Difference in mean proportions (%)
4.03e-7
3.30e-4
0.011
0.023
0.024
0.024
0.026
0.027
0.027
0.034
q-value (corrected)


Flavobacteria
P
Ti
I
OP8(Phylum)
Spirochaetes
Desulfobacterales
Na
Br
Ca
Clostridia
GN05(Spirochaetes)
Rhizobiales(α-proteo)
PRR-11(BRC1)
Fibrobacteres(Phylum)
Syntrophobacterales
vadinHA49(Planctomycetes)
Hg
Li
OP3(Phylum)
Desulfovibrionales
Anaerolineae
V
Mo
Brocadiae(Planctomycetes)
Myxococcales
SBRH58(TM6)
Bacteroidia
Lentisphaeria
B
KSB1(Caldithrix)
Mn
PRR-12(WS3)
GN04(Phylum)
Phycisphaerae
K
Desulfarculales
Planctomycetia
Al
Synechococcophycideae
Fe
Oscillatoriophycideae
Mg
Nostocophycideae
Cytophagia
Rhodobacterales(α-proteo)
Saprospirae
Gammaproteobacteria


Rhodothermi(Bacteroidetes)
Gammaproteobacteria
Cytophagia
Oscillatoriophycideae
Myxococcales
Opitutae (Verrucomicrobia)
Saprospirae
Fibrobacteres(Phylum)
SAR406(Phylum)
Rhizobiales(α-proteo)
Desulfovibrionales
V
Synechococcophycideae
Desulfobacterales
Gemm-2(Gemmatimonadetes)
Spirochaetes
Flavobacteria
Clostridia
Li
Rhodobacterales(α-proteo)
I
GN15(Spirochaetes)
Ca
Planctomycetia
K
Ni
Na
P
Fe
Gemm-4(Gemmatimonadetes)
Actinobacteria
B
Al
vadinHA49(Planctomycetes)
Mg
Sva0725(Acidobacteria)
BPC102(Acidobacteria)
Sr
Br
Mn
Ti
Hg


A
Abundance (%)
0
20
40
60
80
Depth (mm)
Layer 1 (1-2mm)
Layer 2 (3-4mm)
Layer 3 (5-6mm)
Layer 4 (7-8mm)
Layer 5 (9-10mm)
Layer 6 (11-12mm)
Layer 7 (13-14mm)
Layer 8 (15-16mm)
Layer 9 (19-20mm)
Layer 10 (19-20mm)
Oxygenic phototrophs (Cyanobacteria)
Anoxygenic phototrophs (Alphaproteobacteria, Chromiatales)
Fermenters (Spirochaetes, Anaerolineae, Firmicutes, GN04, OP3, OP8, Planctomycetes, Caldithrix, Bacteroidales)
Aerobic heterotrophs (Rhizobiales [*Dichotomicrobium*], Cytophagia, Saprospirae, Flavobacteriia)
Sulfate-reducing bacteria (Desulfobacterales, Desulfovibrionales, Desulfuromonadales, Syntrophobacterales, Desulfarculales)
Chemoautotrophic ANAMMOX bacteria (Brocadiae)
Potential anoxygenic phototrophs (Cytophagia, Saprospira)
B
Abundance (%)
0
10
20
30
40
50
Depth (mm)
Layer 1 (1-2mm)
Layer 2 (3-4mm)
Layer 3 (5-6mm)
Layer 4 (7-8mm)
Layer 5 (9-10mm)
Oxygenic phototrophs (Cyanobacteria)
Anoxygenic phototrophs (Alphaproteobacteria, Chromiatales, Verrucomicrobia))
Fermenters (Anaerolineae, Spirochaetes, Planctomycetes, Acidobateria, Actinobateria, GN04, BRC1, Bacteroidales)
Aerobic heterotrophs (Rhizobiales [*Dichotomicrobium*], Cytophagia, Saprospirae, Flavobacteriia)
Sulfate-reducing bacteria (Desulfobacterales, Desulfovibrionales, Desulfuromonadales, Syntrophobacterales, Desulfarculales)
Potential anoxygenic phototrophs (Cytophagia, Saprospira, Gemmatimonadetes)
Predatory (Bdellovibrio)

Supplementary Table 1. Bacterial 16S rRNA gene diversity analyses[*] of smooth and pustular mats

Mat layers	Depth	Good's Coverage[a]	No. OTUs	Chao1[b]	Shannon[c]
Pustular layer 1	1 - 2 mm	0.951	4731	8641	6.361
Pustular layer 2	3 - 4 mm	0.961	3827	7434	6.130
Pustular layer 3	5 - 6 mm	0.962	3939	7333	6.271
Pustular layer 4	7 - 8 mm	0.950	4884	8574	6.335
Pustular layer 5	9 - 10 mm	0.941	5802	9063	6.259
Smooth layer 1	1 - 2 mm	0.944	5063	9479	5.602
Smooth layer 2	3 - 4 mm	0.953	4168	8328	6.105
Smooth layer 3	5 - 6 mm	0.947	5027	8518	6.383
Smooth layer 4	7 - 8 mm	0.958	4028	7340	6.130
Smooth layer 5	9 - 10 mm	0.965	3671	5781	5.905
Smooth layer 6	11 - 12 mm	0.944	5738	8705	6.556
Smooth layer 7	13 - 14 mm	0.960	3846	6894	6.005
Smooth layer 8	15 -16 mm	0.952	4583	8198	6.336
Smooth layer 9	17 - 18 mm	0.940	5418	9727	6.227
Smooth layer 10	19 - 20 mm	0.926	6646	12171	6.581

[*] Calculated at 3% genetic divergence

[a] Good’s Coverage measures how well the sample represents the larger environment

[b] Chao1 index measures the species richness (absolute number of species)

[c] Shannon index measures the species diversity (relative species abundance and species eveness)

Supplementary Table 2. Novel candidate bacteria phyla and bacterial phyla that are poorly described, found in Hamelin Pool ecosystems.

Novel bacterial phyla	Pustular	Smooth
Caldithrix	0.01%	2.95%
GN04	2%	1.91%
OP8	N/A	1.73%
GN01	N/A	0.56%
OP3	N/A	0.89%
Fibrobacteres	0.83%	1.05%
TM6	0.34%	0.72%
SAR406	0.42%	0.27%
BRC1	0.66%	1.14%
Gemmatimonadetes	1.94%	0.45%
WS3	0.21%	0.70%
GN02	0.29%	1.22%
OD1	0.34%	1.25%
Verrumicrobia	1.97%	0.54%
Lentisphaerae	0.49%	0.51%
NKB19	0.42%	N/A
KSB3	N/A	0.48%
Hyd24-12	N/A	0.11%
WS1	N/A	0.11%
OP9	N/A	0.10%
Proportion of population	9.92%	16.69%