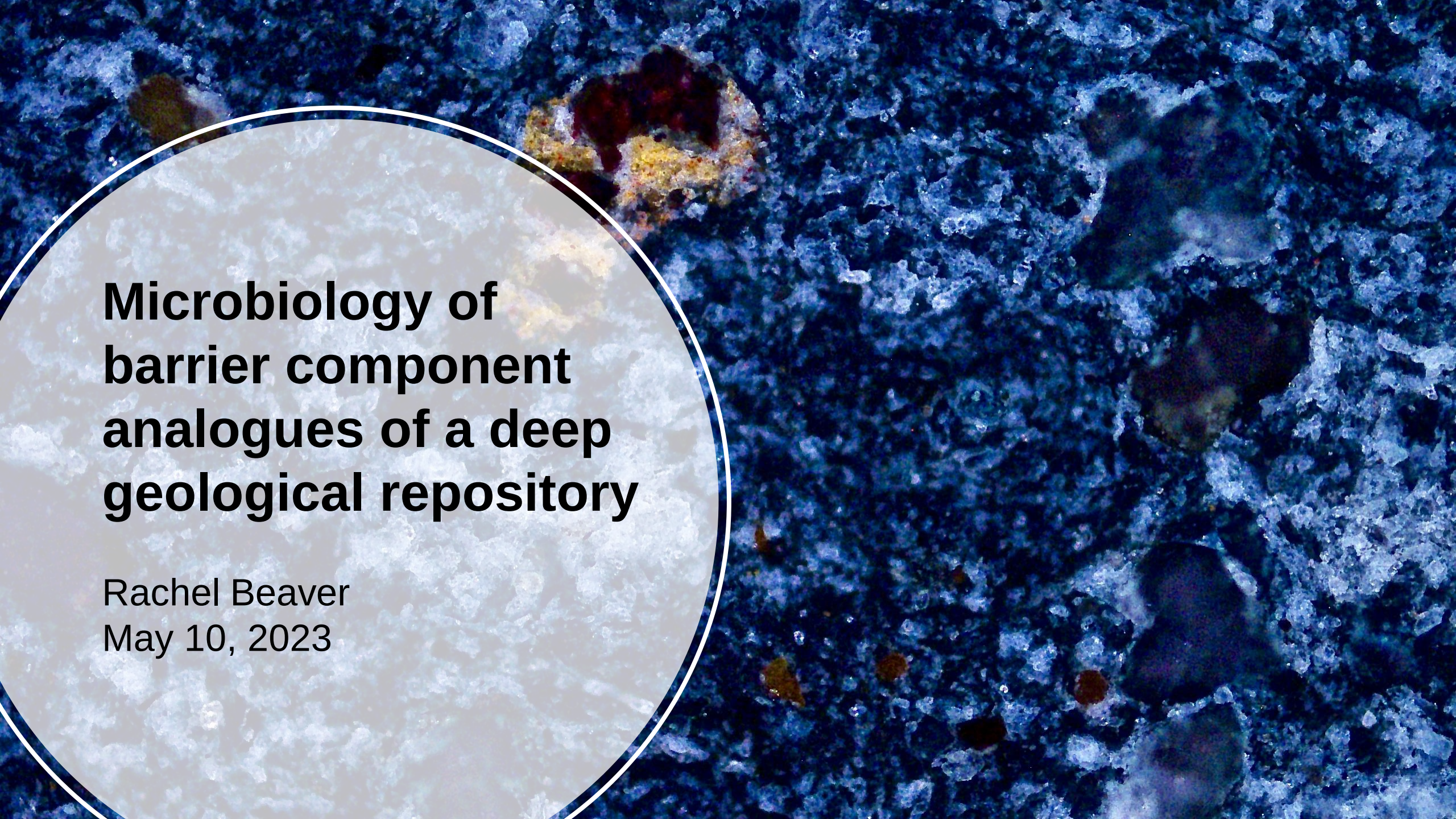
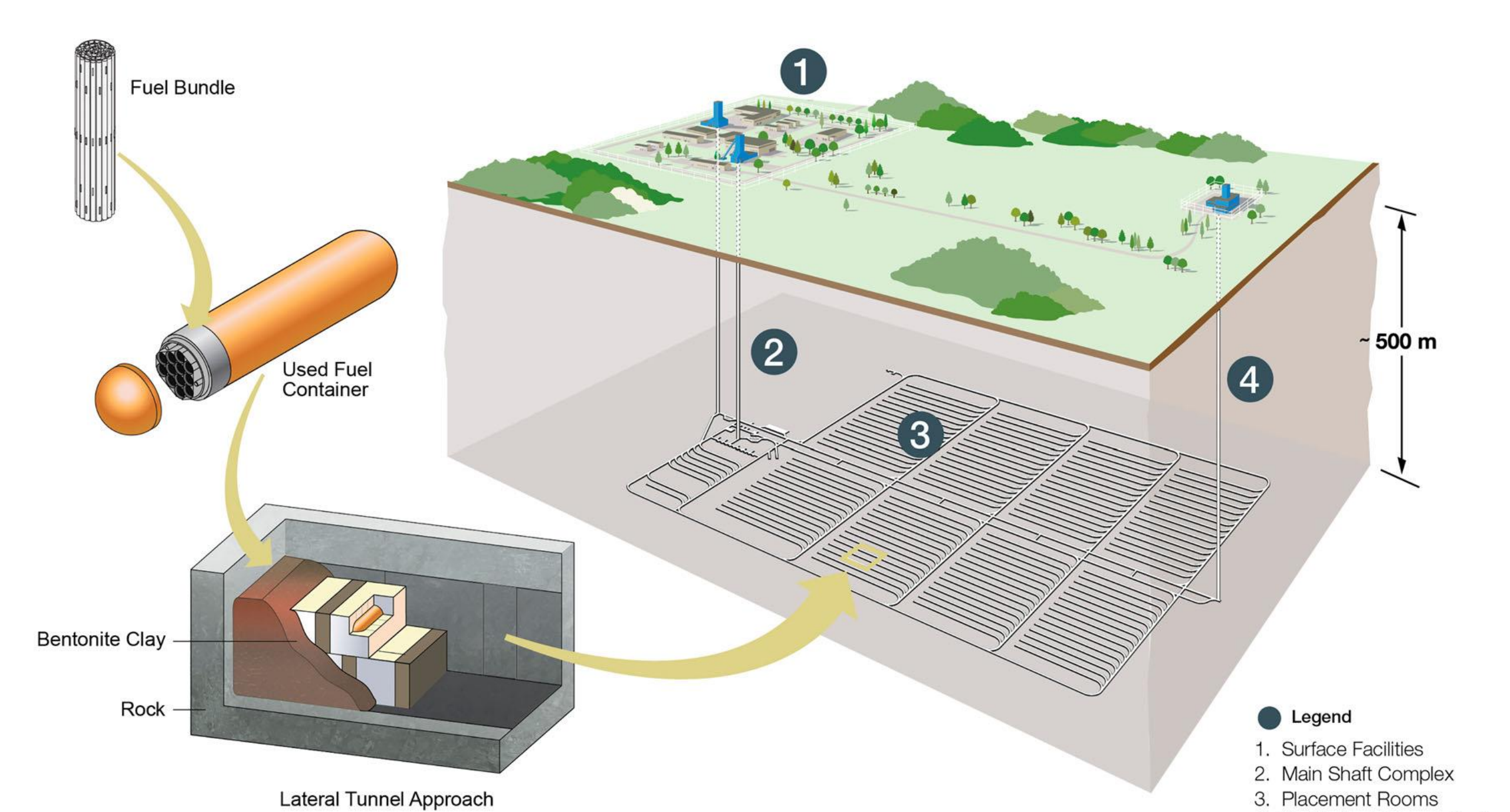


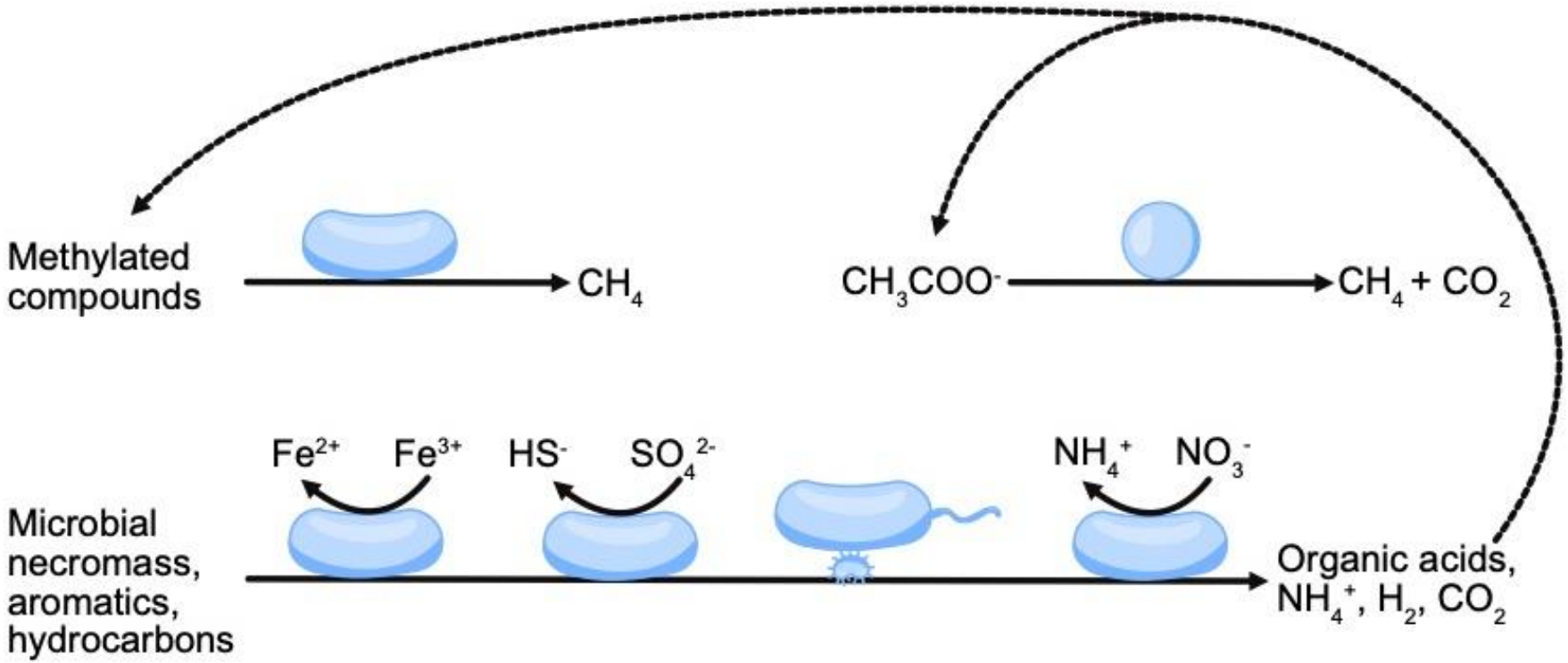
Microbiology of barrier component analogues of a deep geological repository

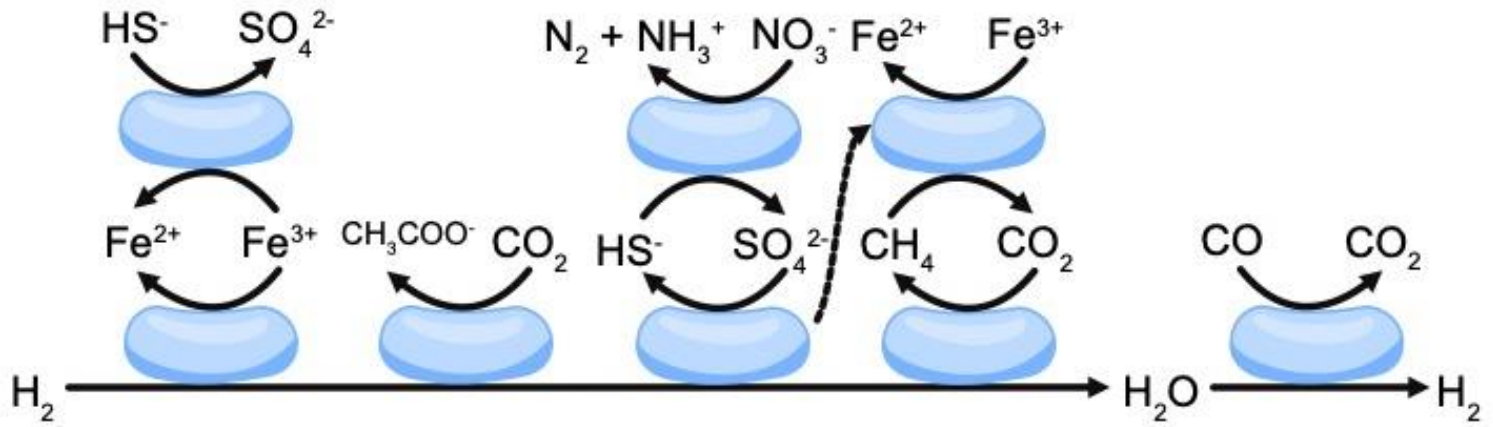
Rachel Beaver
May 10, 2023



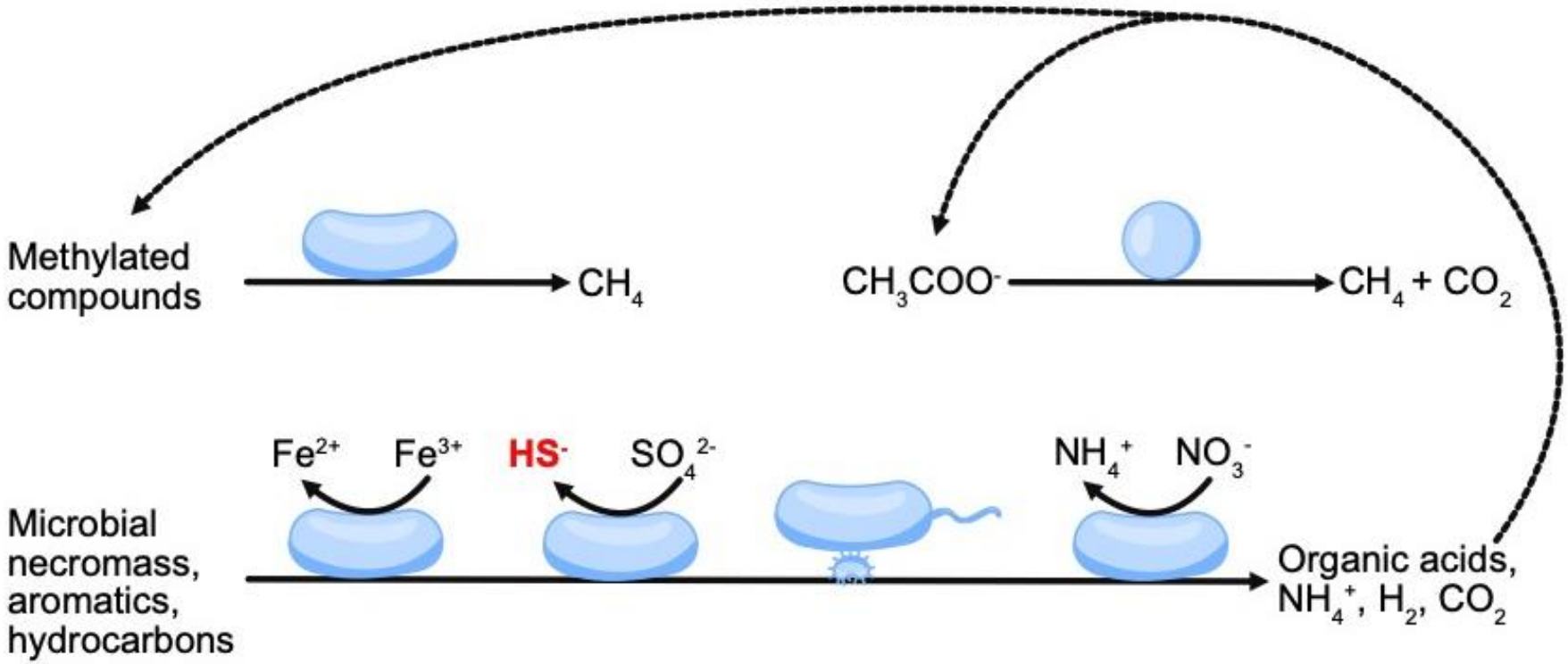
Modified from Hall *et al*., 2017

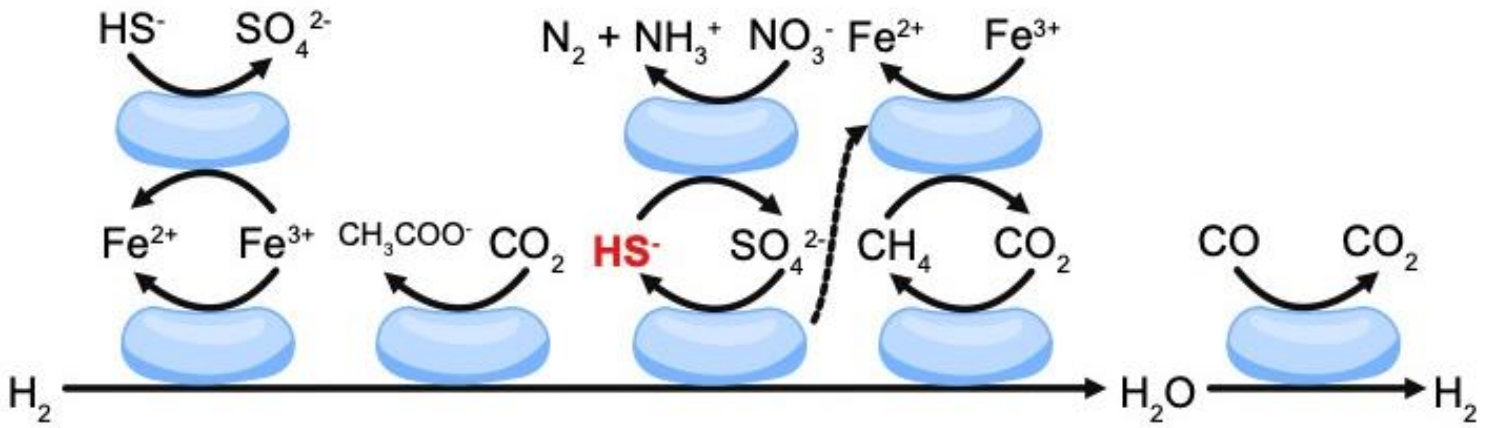
Microbial metabolisms detected in deep subsurface environments





Microbial metabolisms detected in deep subsurface environments



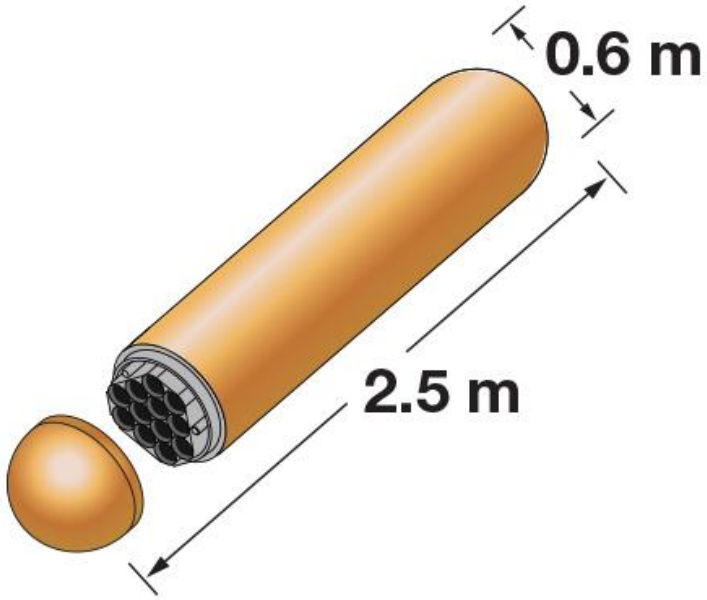


Blake, 2017

Instant Labs, 2018

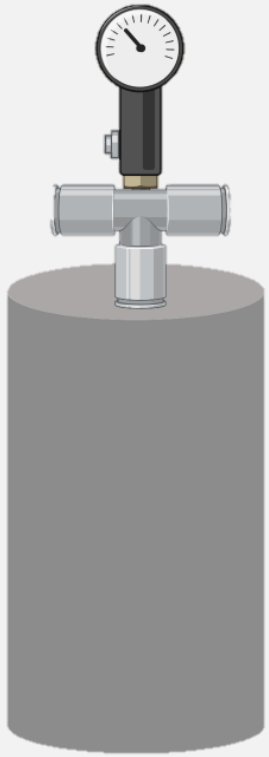
Sherman *et al*., 2015

$$SO_4^{2-} + 9H^+ + 8e^- \rightarrow HS^- + 4H_2O$$



$$Cu + HS^- \rightarrow CuS + H^+$$

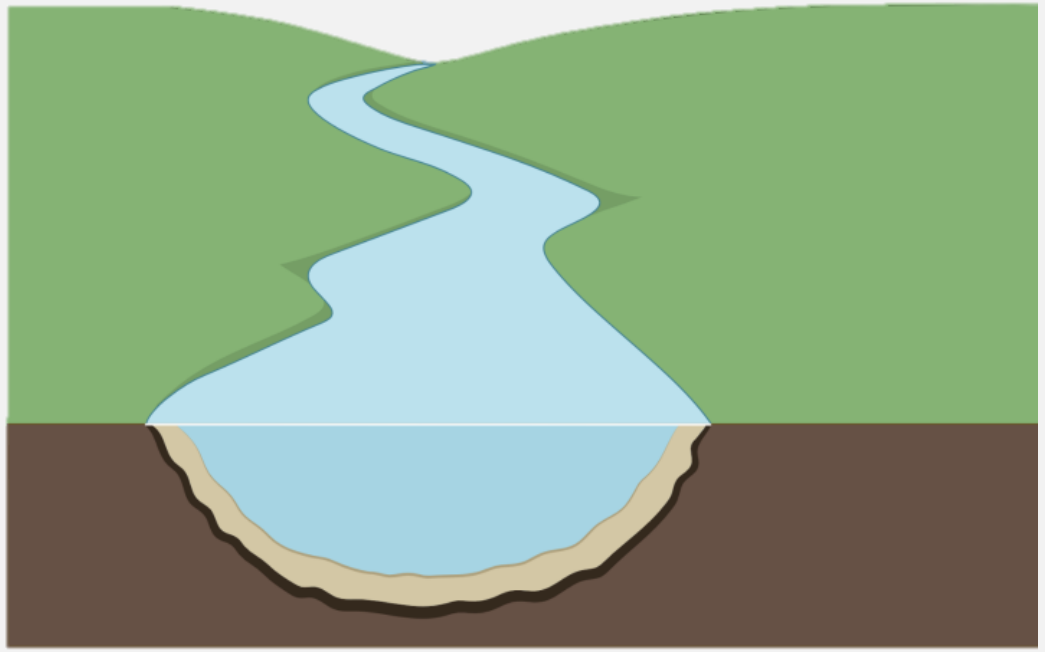
Short-term microcosms



✓ Can test specific conditions

x Challenging to make long-term predictions based on short-term experiments

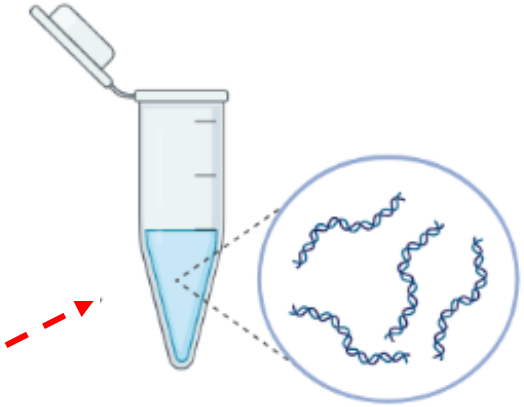
Natural analogue samples



✓ Clay/rock analogues have existed for millennia

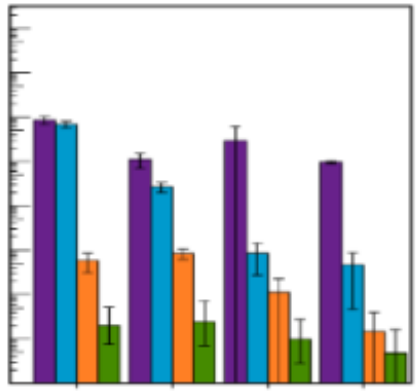
x Challenging to find analogues that mimic all conditions of a DGR

Bentonite



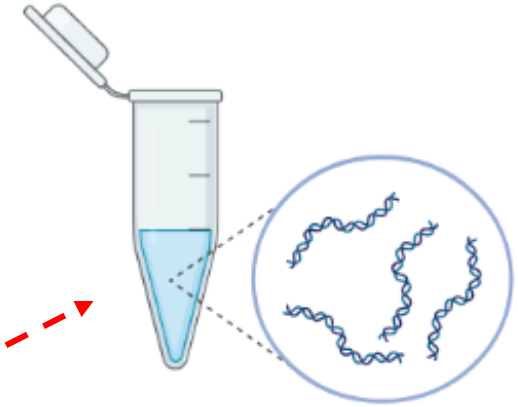
Heterotrophs (aerobic and anaerobic)

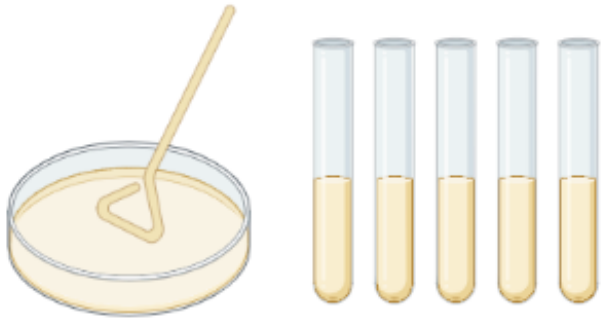
SRB



Abundance data

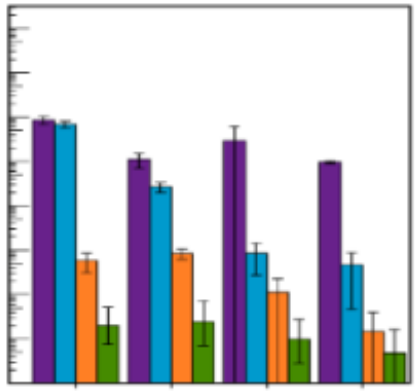
Bentonite





Heterotrophs (aerobic and anaerobic)

SRB



Abundance data

AMERICAN SOCIETY FOR MICROBIOLOGY

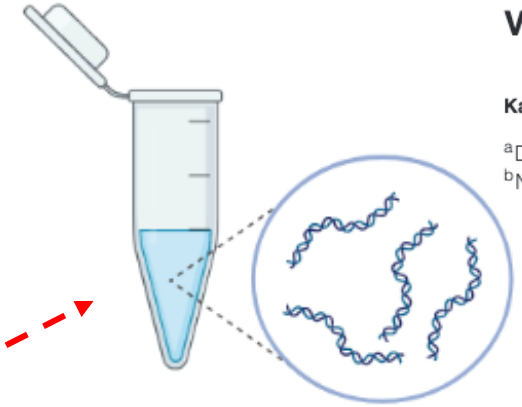
RESEARCH ARTICLE
Applied and Environmental Science
September/October 2019 Volume 4 Issue 5 e00334-19
https://doi.org/10.1128/mSphere.00334-19

Validating DNA Extraction Protocols for Bentonite Clay

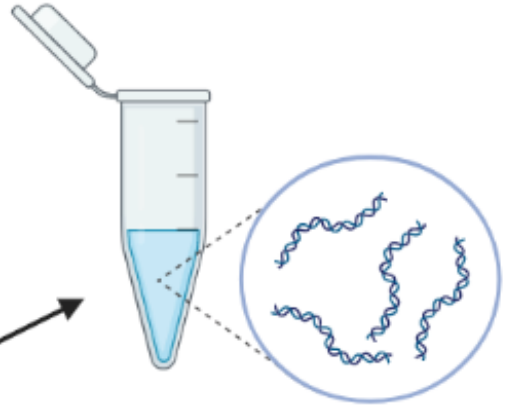
Katja Engel[a], Sara Coyotzi[a], Melody A. Vachon[a], Jennifer R. McKelvie[b], Josh D. Neufeld[a]

[a]Department of Biology, University of Waterloo, Waterloo, Ontario, Canada
[b]Nuclear Waste Management Organization, Toronto, Ontario, Canada

Bentonite

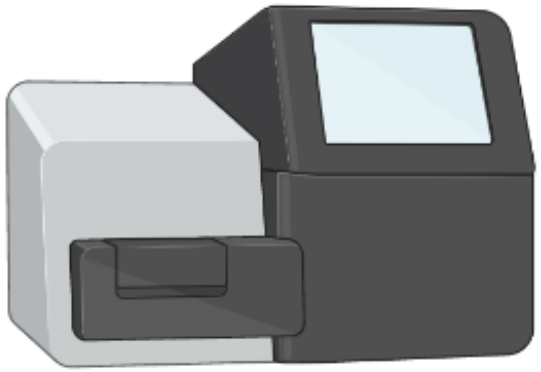


Bentonite

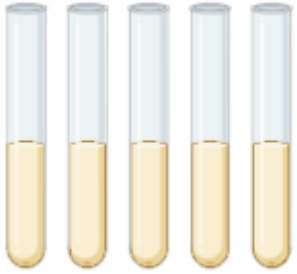


DNA quantification (qPCR)

+

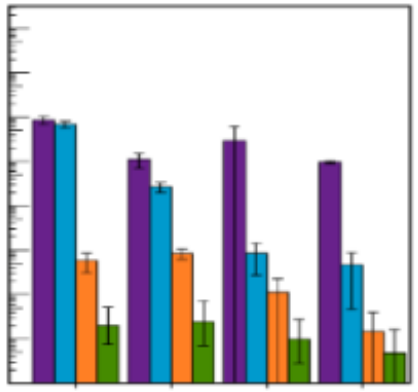


DNA sequencing (MiSeq)



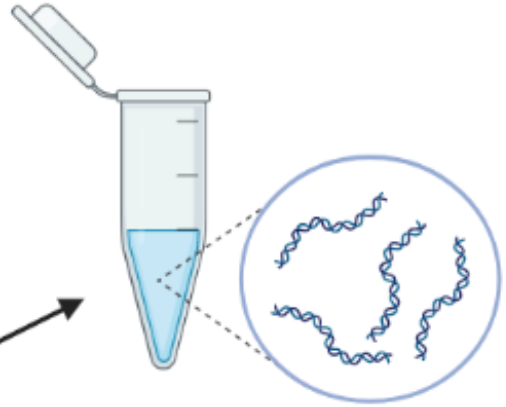
Heterotrophs (aerobic and anaerobic)

SRB



Abundance data

Bentonite



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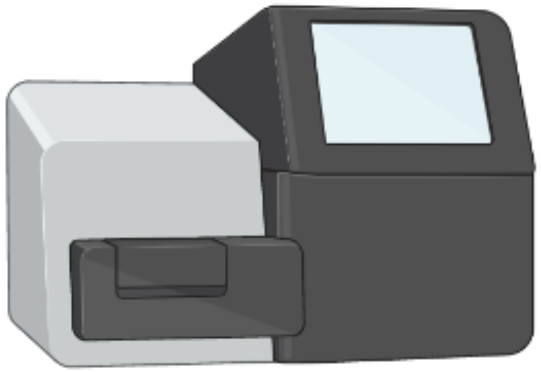

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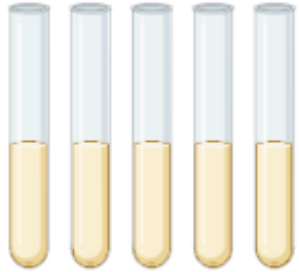
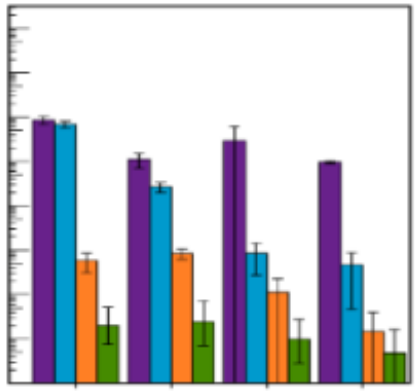


SRB



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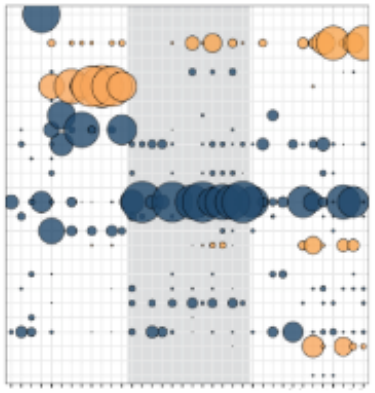


Composition data

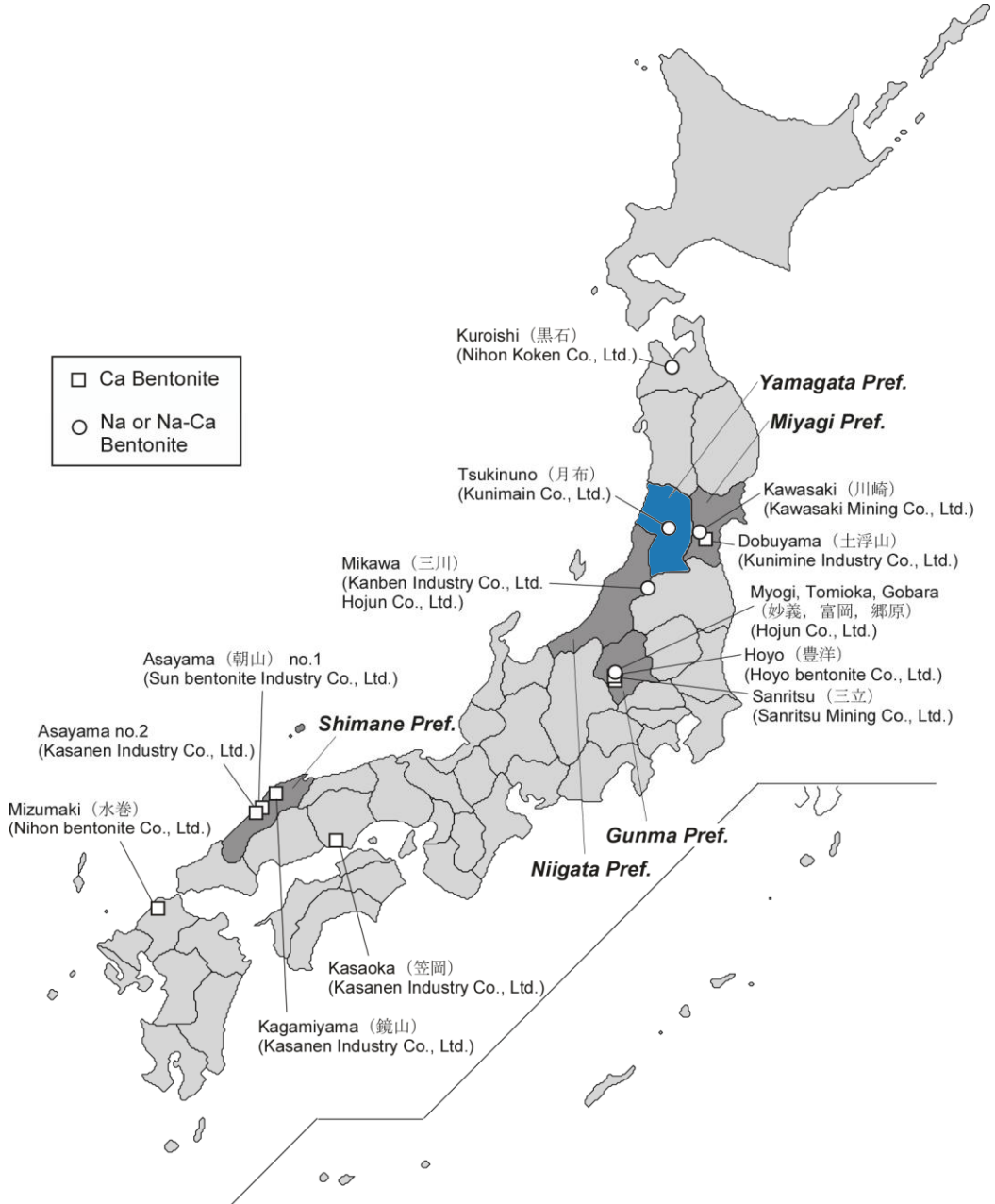
Canadian Science Publishing

ARTICLE

Microbiology of barrier component analogues of a deep geological repository

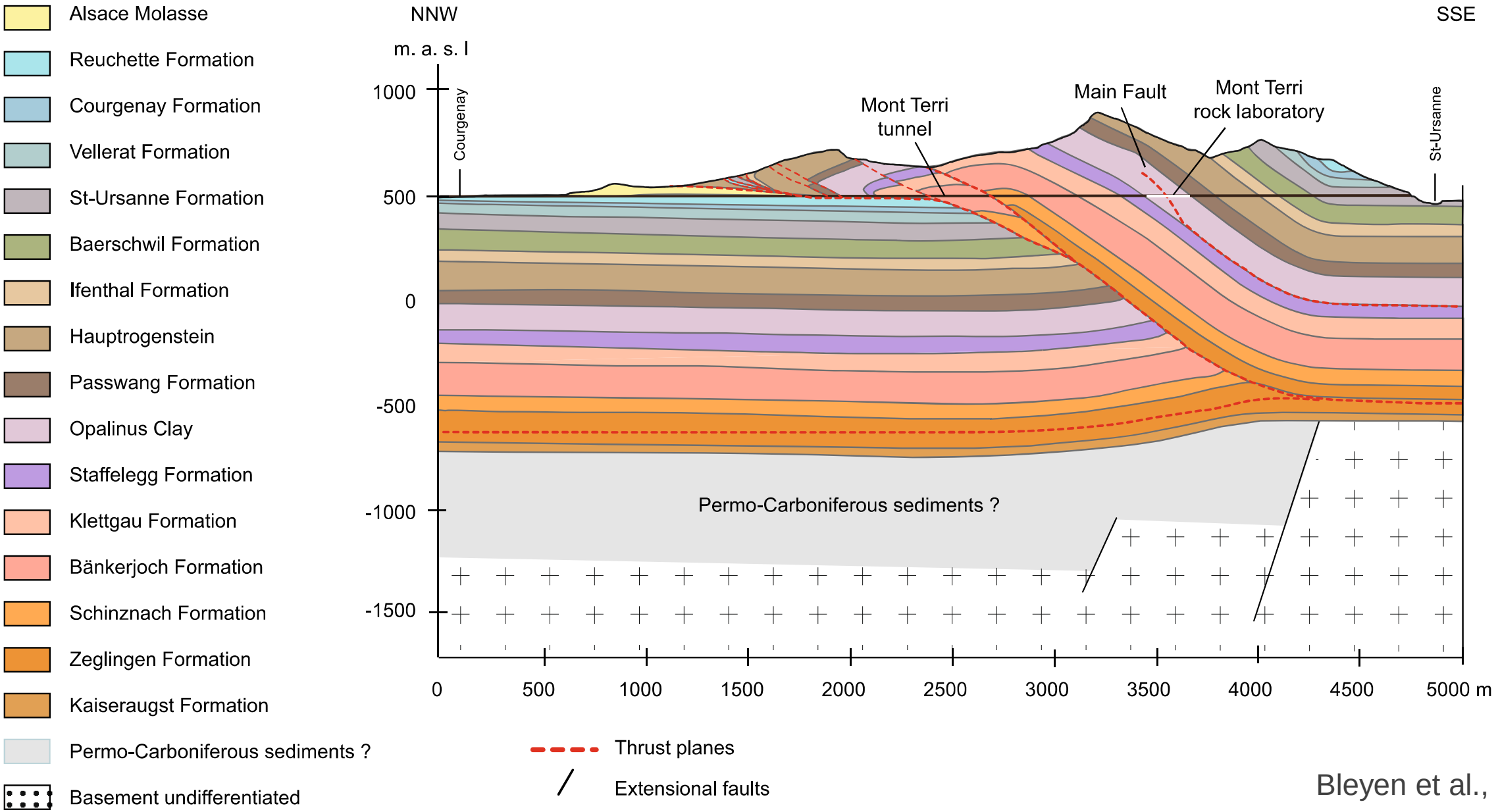
Rachel C. Beaver, Katja Engel, W. Jeffrey Binns, and Josh D. Neufeld

1) The Tsukinuno bentonite deposit, Japan



Takagi, 2005

2) The Opalinus clay formation, Switzerland



Bleyen et al., 2017

3) Rock from Ignace, Ontario (Canada)

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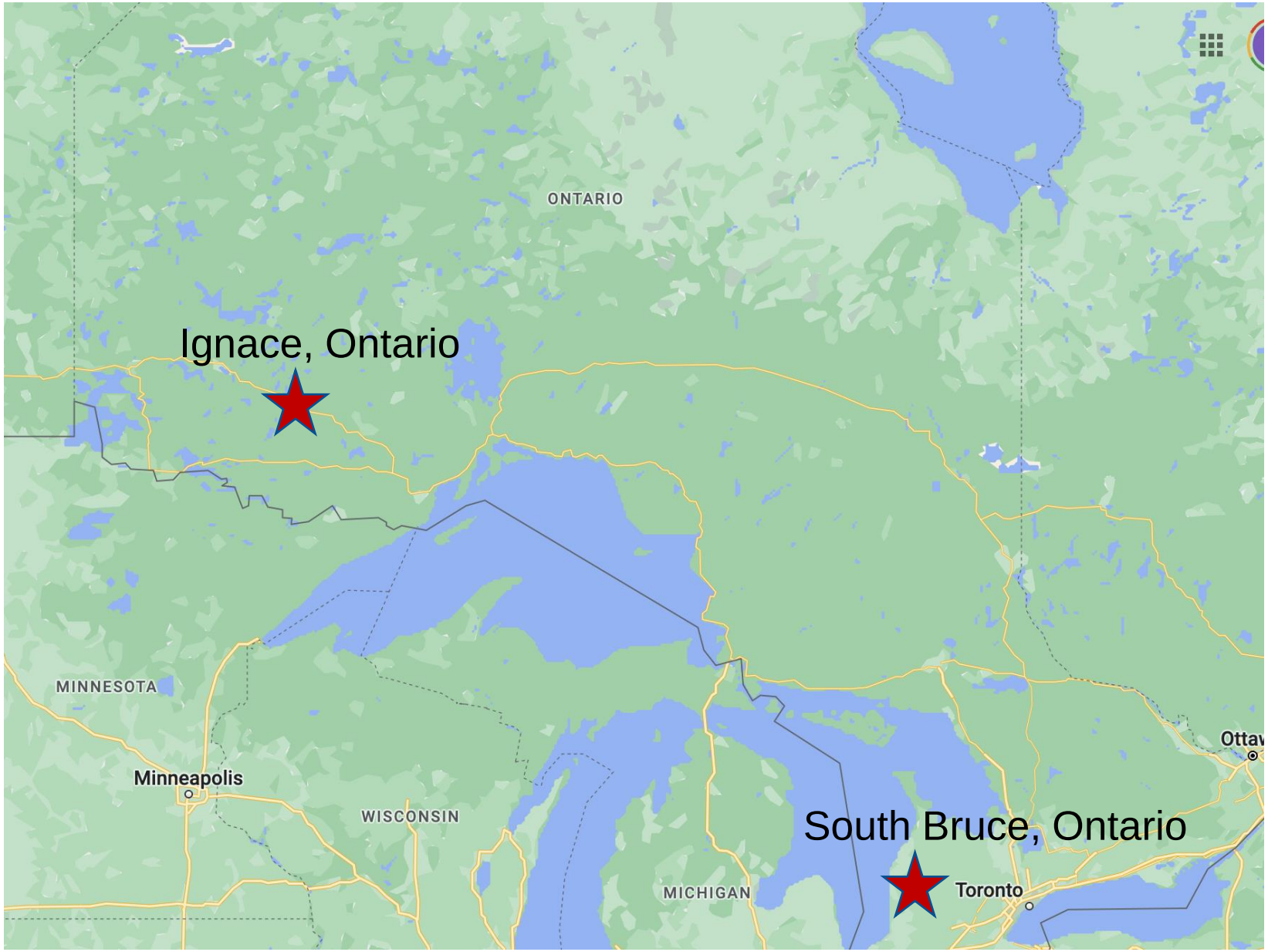
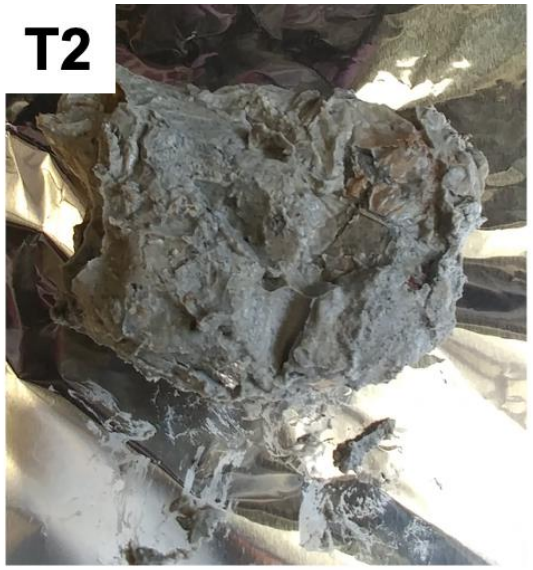


Tsukinuno bentonite samples

Sample	Appearance	Description	Sampling site	Target	Average DNA yield per gram of clay (ng)
1	Rock	Hand specimen taken from the bentonite exposed at the tunnel wall within bentonite bed #19. Sample location 26-27cm up from the lower bentonite-shale contact.	Site 1	Bed 19	BDL
2	Soft	Sample taken from a very wet tunnel wall S side of the tunnel, pH around 6-7.	Site 1	Bed 17	104
3	Stones	Samples taken from tunnel wall, very dry bentonite. No drilling at this site.	Site 2	Bed 2	BDL
4	Soft	Samples taken from cleaned tunnel wall (15 cm from original surface).	Site 1	Bed 20	44
5	Rock and soft	Yellow bentonite, at lower contact of bed #19.	Site 1	Bed 19	BDL
6	Rock and soft	Gray bentonite, 20 cm from lower contact.	Site 1	Bed 19	BDL
7	Rock and soft	Brownish bentonite, 50 cm from lower contact.	Site 1	Bed 19	BDL
8	Soft with some rocks	Orange bentonite, 65 cm from lower contact.	Site 1	Bed 19	16
9	Rock and soft	Yellow bentonite, upper contact of bed #19.	Site 1	Bed 19	8
10	Rock	Massive dry bentonite from upper contact of bed #29.	Site 3	Bed 29	BDL

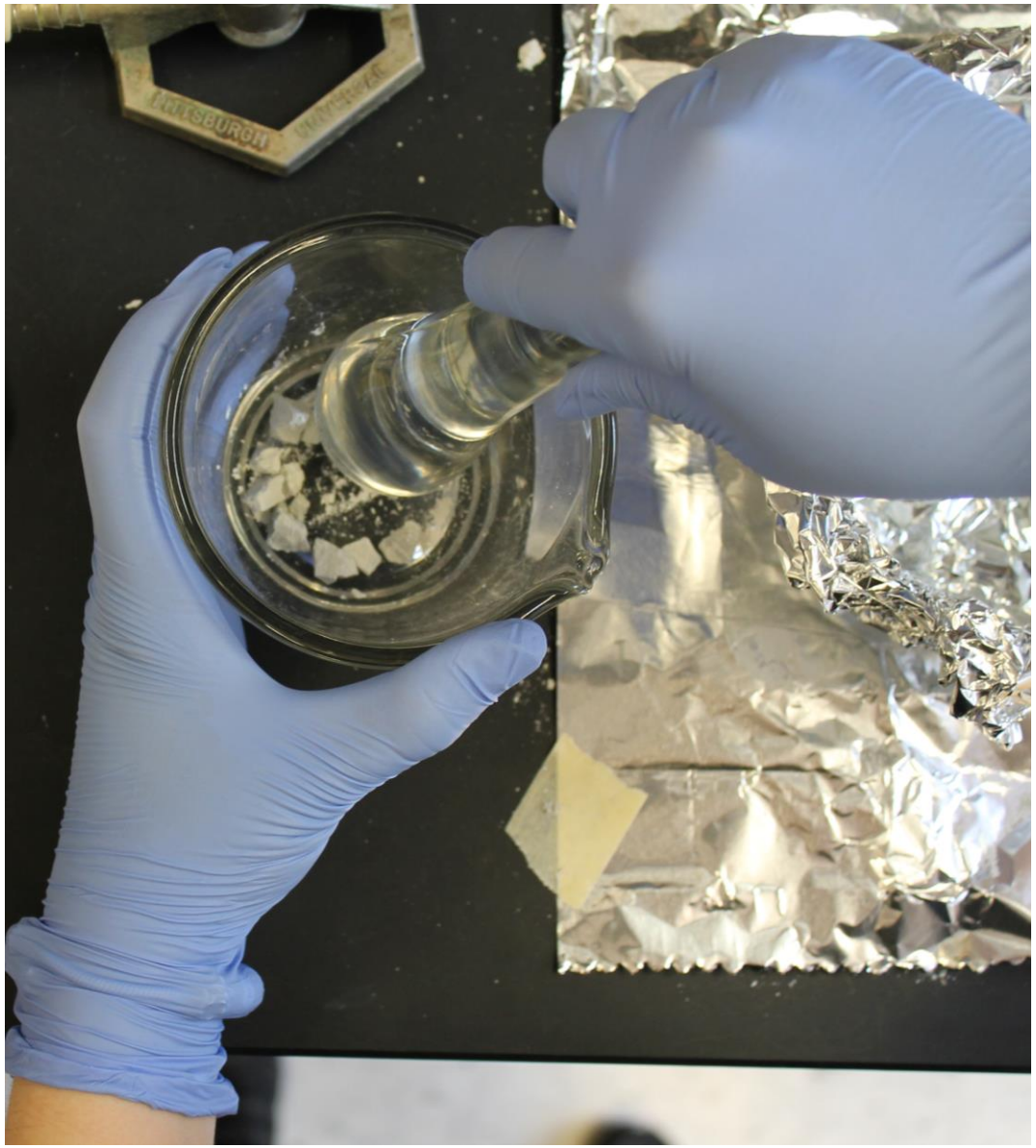
*Values of BDL indicate DNA concentrations below the detection limit of the Qubit fluorometer with 10 μL of sample input.

Tsukinuno bentonite samples

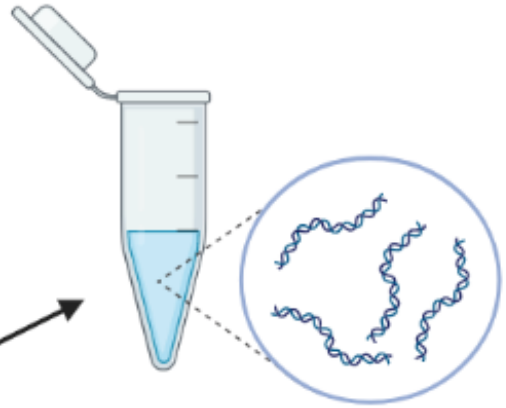


Opalinus clay and Ignace rock samples

Outer layer of the samples was removed, and inner layer was crushed

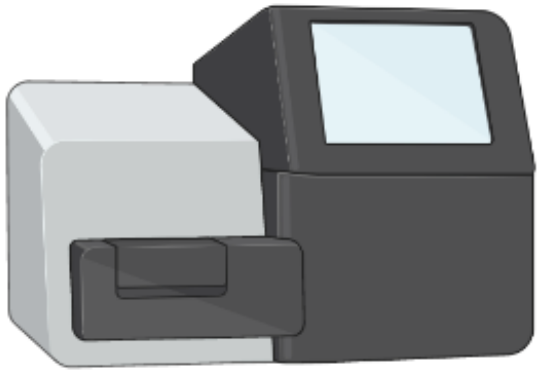


Bentonite



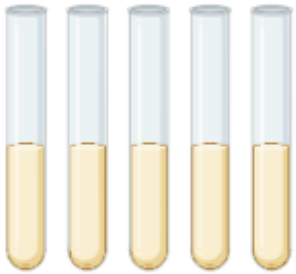
DNA quantification (qPCR)

+

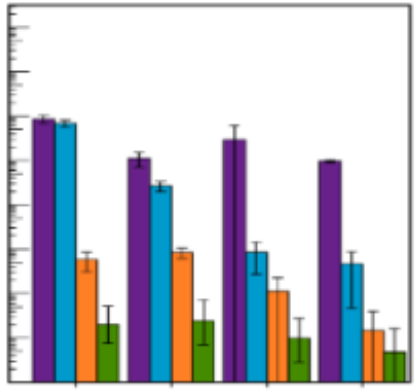


DNA sequencing (MiSeq)

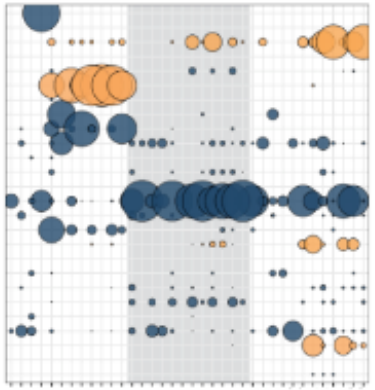
Heterotrophs (aerobic and anaerobic)



SRB

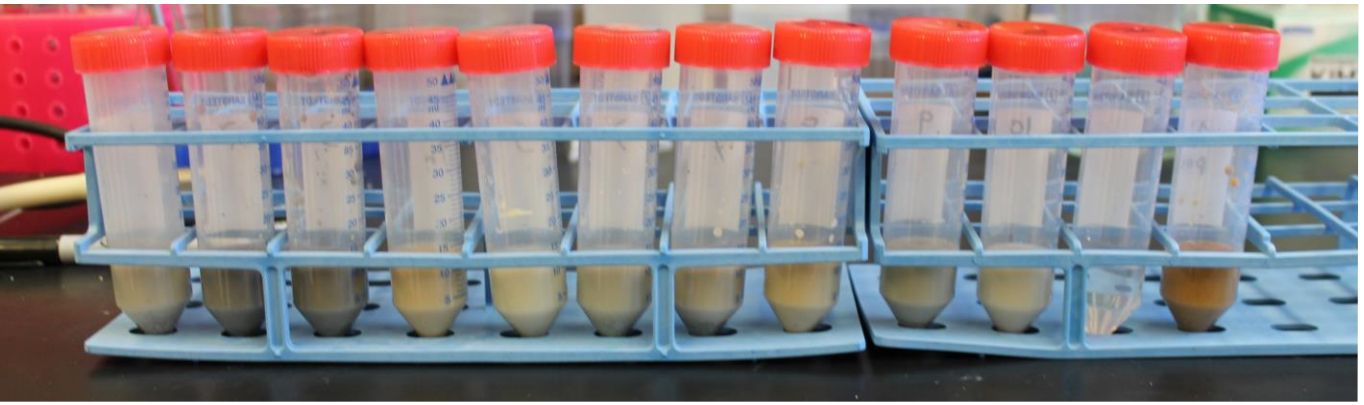


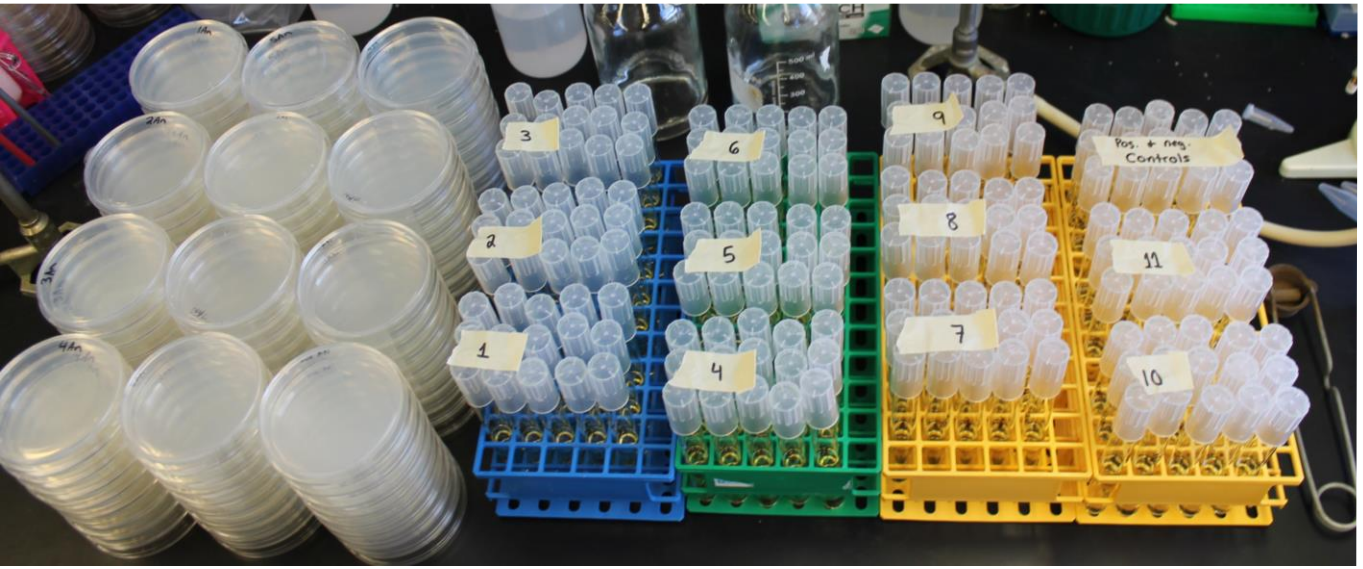
Abundance data



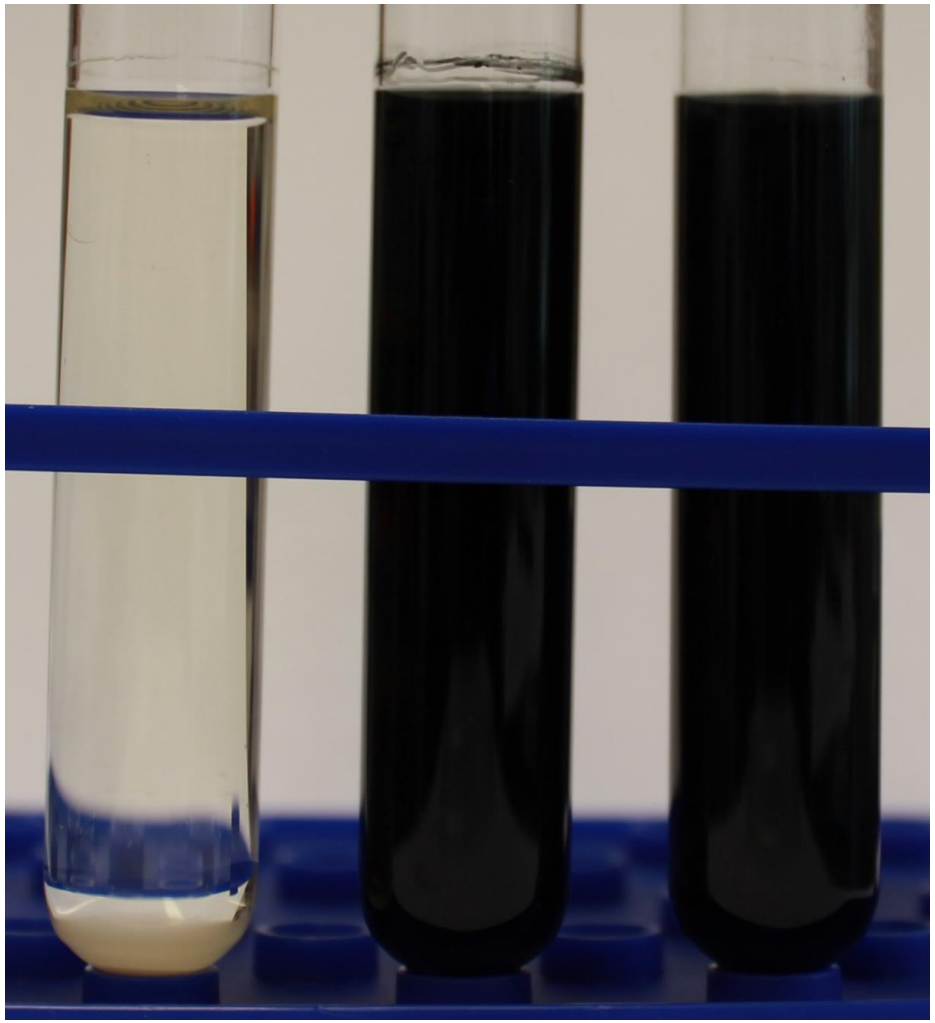
Composition data

Incubations were set up for Tsukinuno bentonite samples

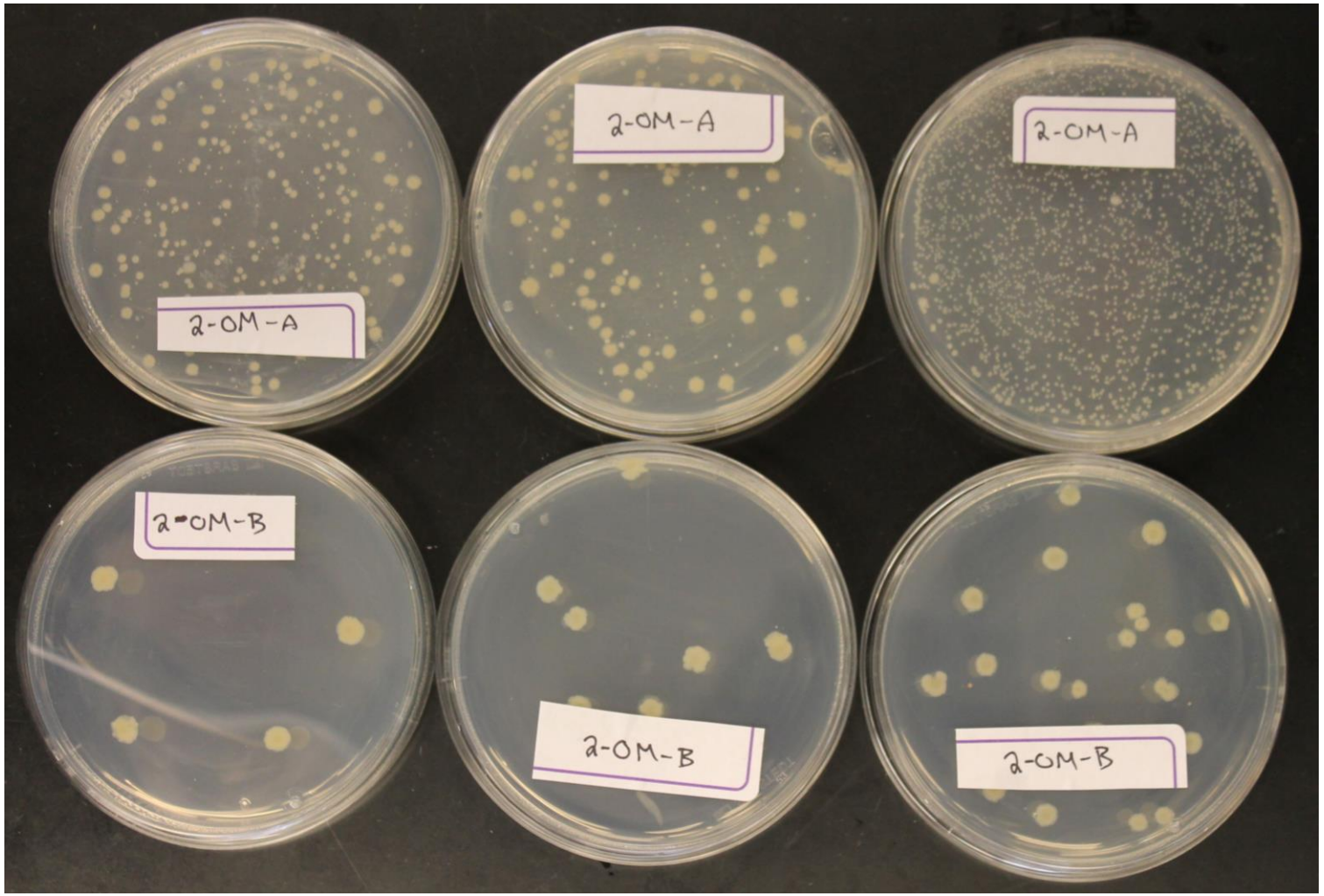


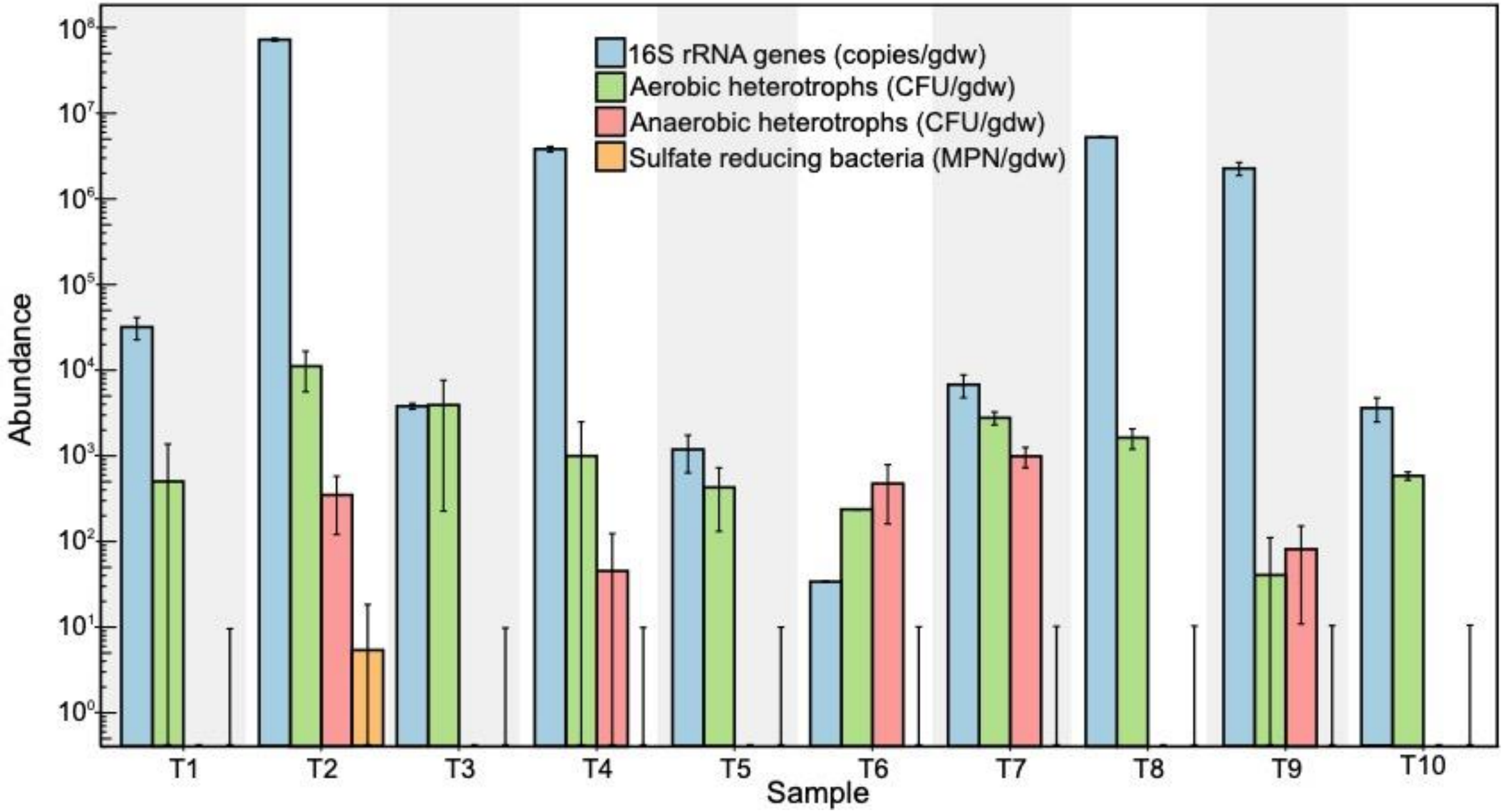


SRB cultures

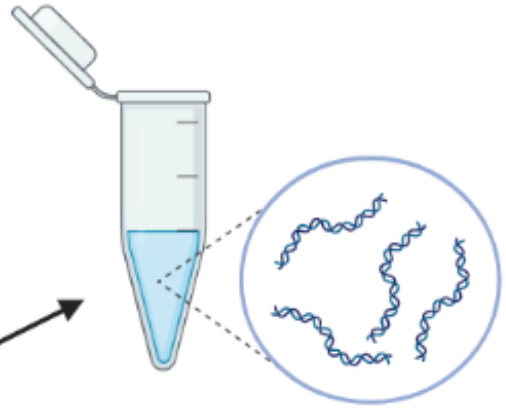


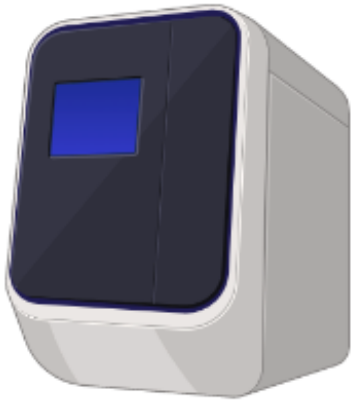
Heterotroph cultures




16S rRNA genes (copies/gdw)
Aerobic heterotrophs (CFU/gdw)
Anaerobic heterotrophs (CFU/gdw)
Sulfate reducing bacteria (MPN/gdw)
Abundance
10^8
10^7
10^6
10^5
10^4
10^3
10^2
10^1
10^0
T1
T2
T3
T4
T5
T6
T7
T8
T9
T10
Sample

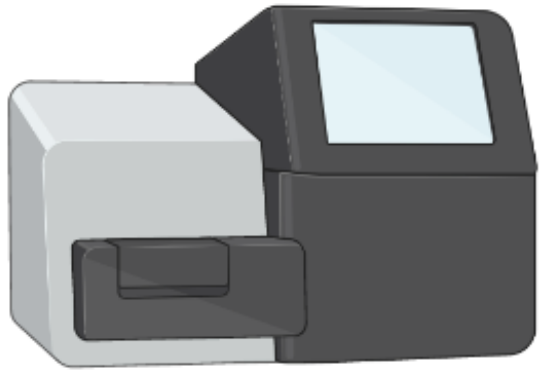
Bentonite



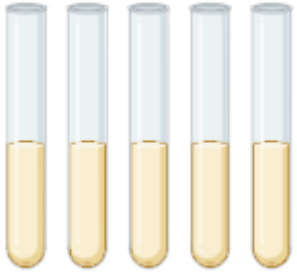


DNA quantification (qPCR)

+

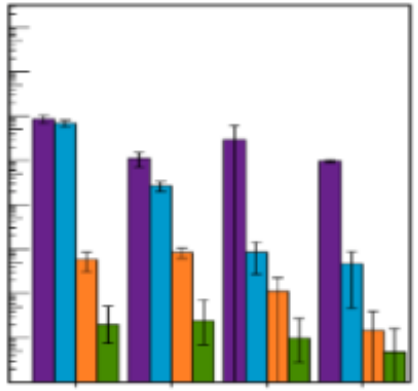


DNA sequencing (MiSeq)

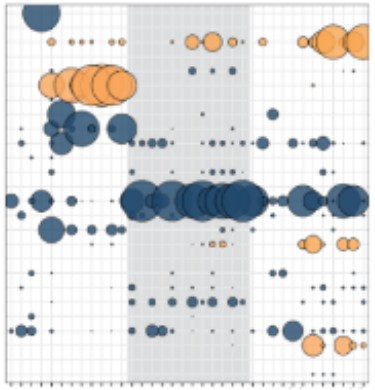


Heterotrophs (aerobic and anaerobic)

SRB

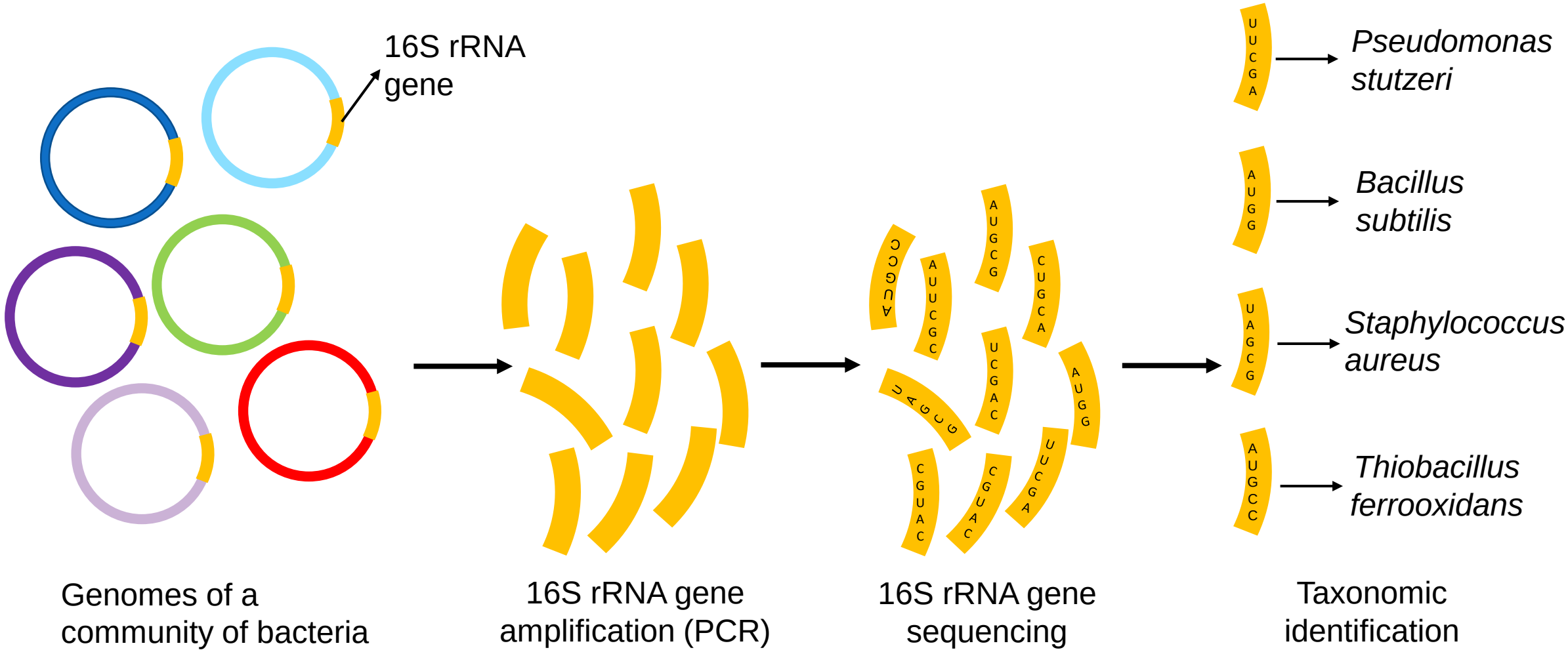


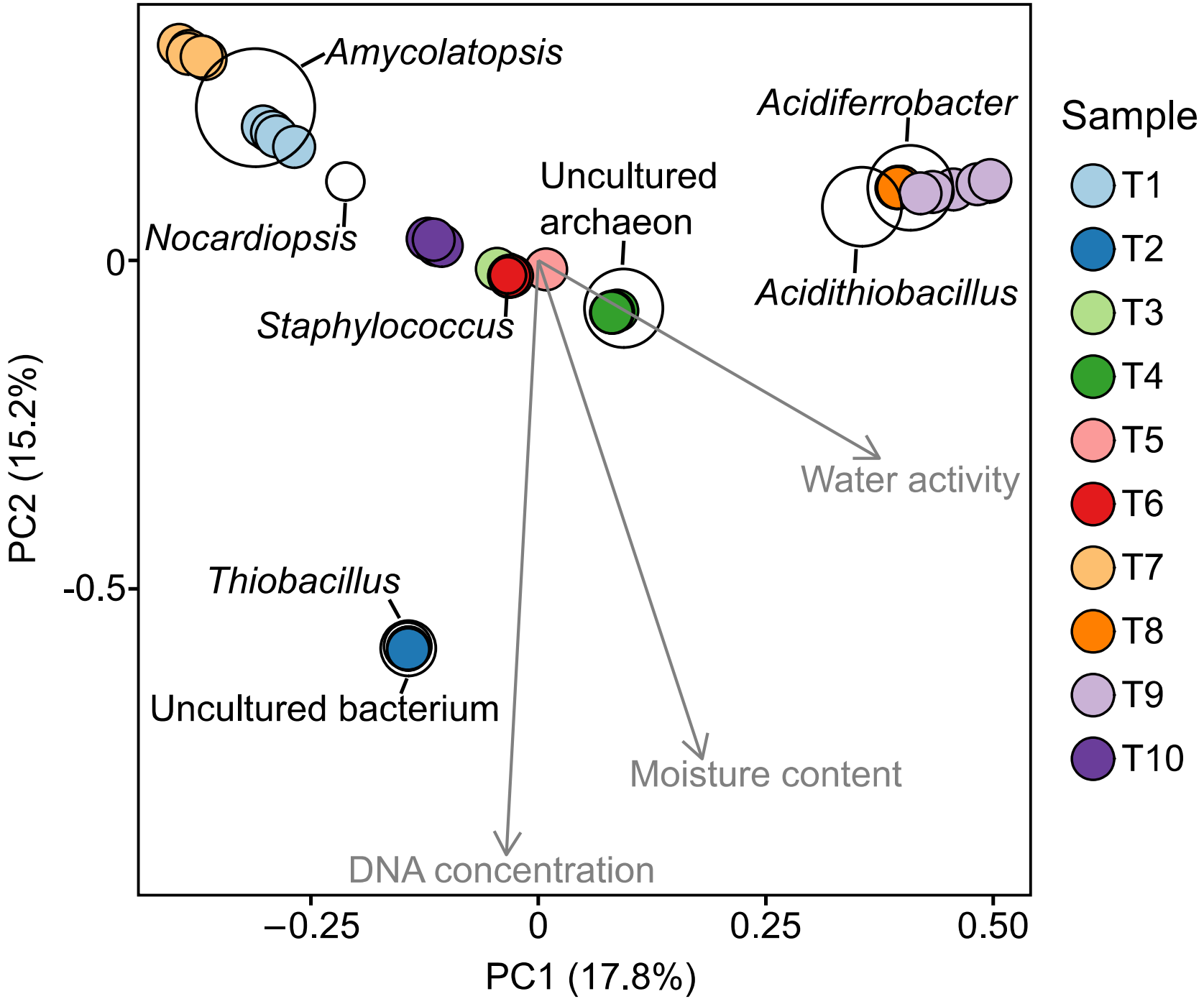
Abundance data

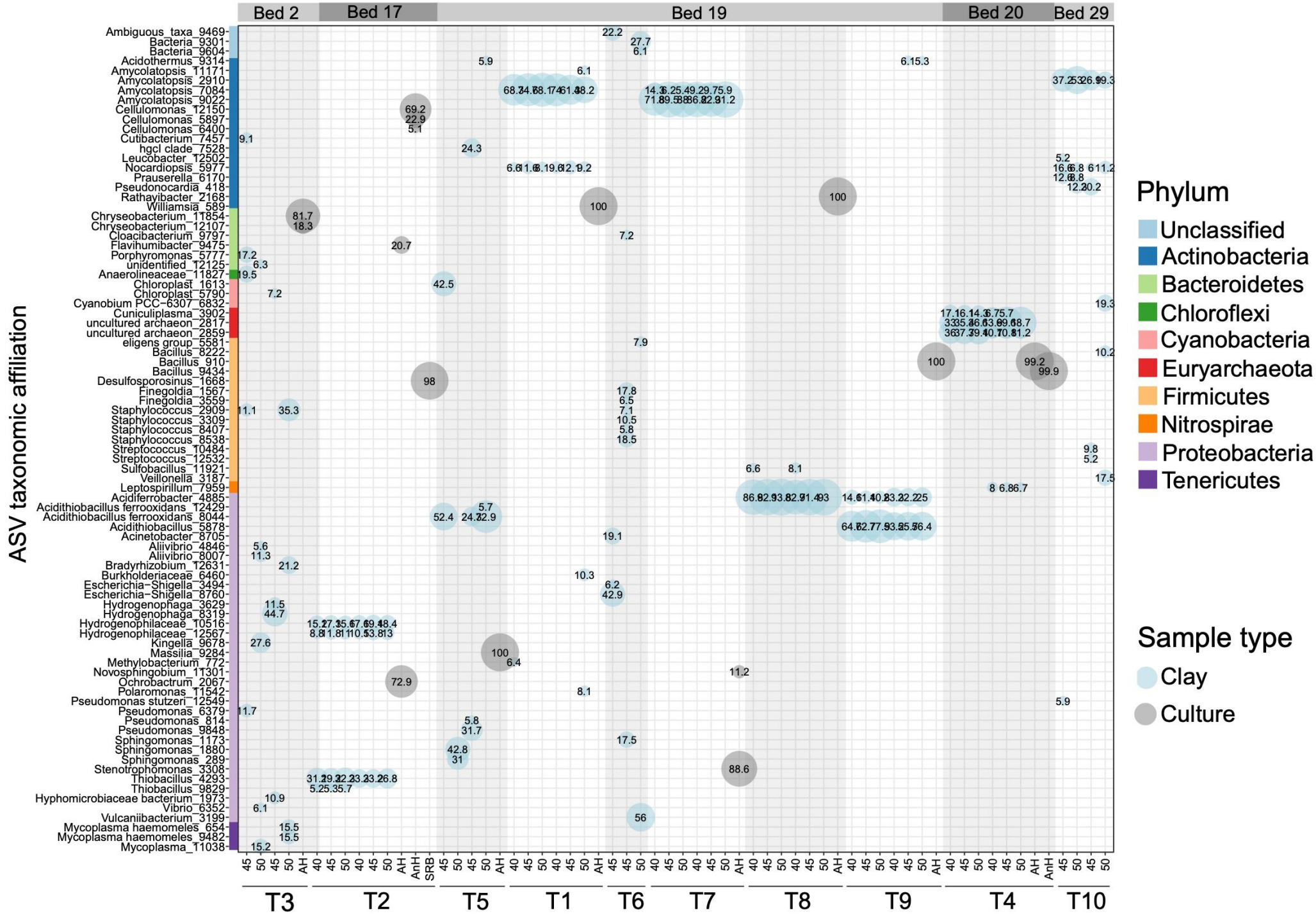


Composition data

16S rRNA gene analysis




Amycolatopsis
Nocardiopsis
Staphylococcus
Uncultured archaeon
Acidiferrobacter
Acidithiobacillus
Thiobacillus
Uncultured bacterium
Water activity
Moisture content
DNA concentration
PC2 (15.2%)
PC1 (17.8%)
0
-0.5
−0.25
0
0.25
0.50
Sample
T1
T2
T3
T4
T5
T6
T7
T8
T9
T10


Bed 2
Bed 17
Bed 19
Bed 20
Bed 29
ASV taxonomic affiliation
Ambiguous_taxa_9469
Bacteria_9301
Bacteria_9604
Acidothermus_9314
Amycolatopsis_11171
Amycolatopsis_2910
Amycolatopsis_7084
Amycolatopsis_9022
Cellulomonas_12150
Cellulomonas_5897
Cellulomonas_6400
Cutibacterium_7457
hgcI clade_7528
Leucobacter_12502
Nocardiopsis_5977
Prauserella_6170
Pseudonocardia_418
Rathayibacter_2168
Williamsia_589
Chryseobacterium_11854
Chryseobacterium_12107
Cloacibacterium_9797
Flavihumibacter_9475
Porphyromonas_5777
unidentified_12125
Anaerolineaceae_11827
Chloroplast_1613
Chloroplast_5790
Cyanobium PCC-6307_6832
Cuniculiplasma_3902
uncultured archaeon_2817
uncultured archaeon_2859
eligens group_5581
Bacillus_8222
Bacillus_910
Bacillus_9434
Desulfosporosinus_1668
Finegoldia_1567
Finegoldia_3559
Staphylococcus_2909
Staphylococcus_3309
Staphylococcus_8407
Staphylococcus_8538
Streptococcus_10484
Streptococcus_12532
Sulfobacillus_11921
Veillonella_3187
Leptospirillum_7959
Acidiferrobacter_4885
Acidithiobacillus ferrooxidans_12429
Acidithiobacillus ferrooxidans_8044
Acidithiobacillus_5878
Acinetobacter_8705
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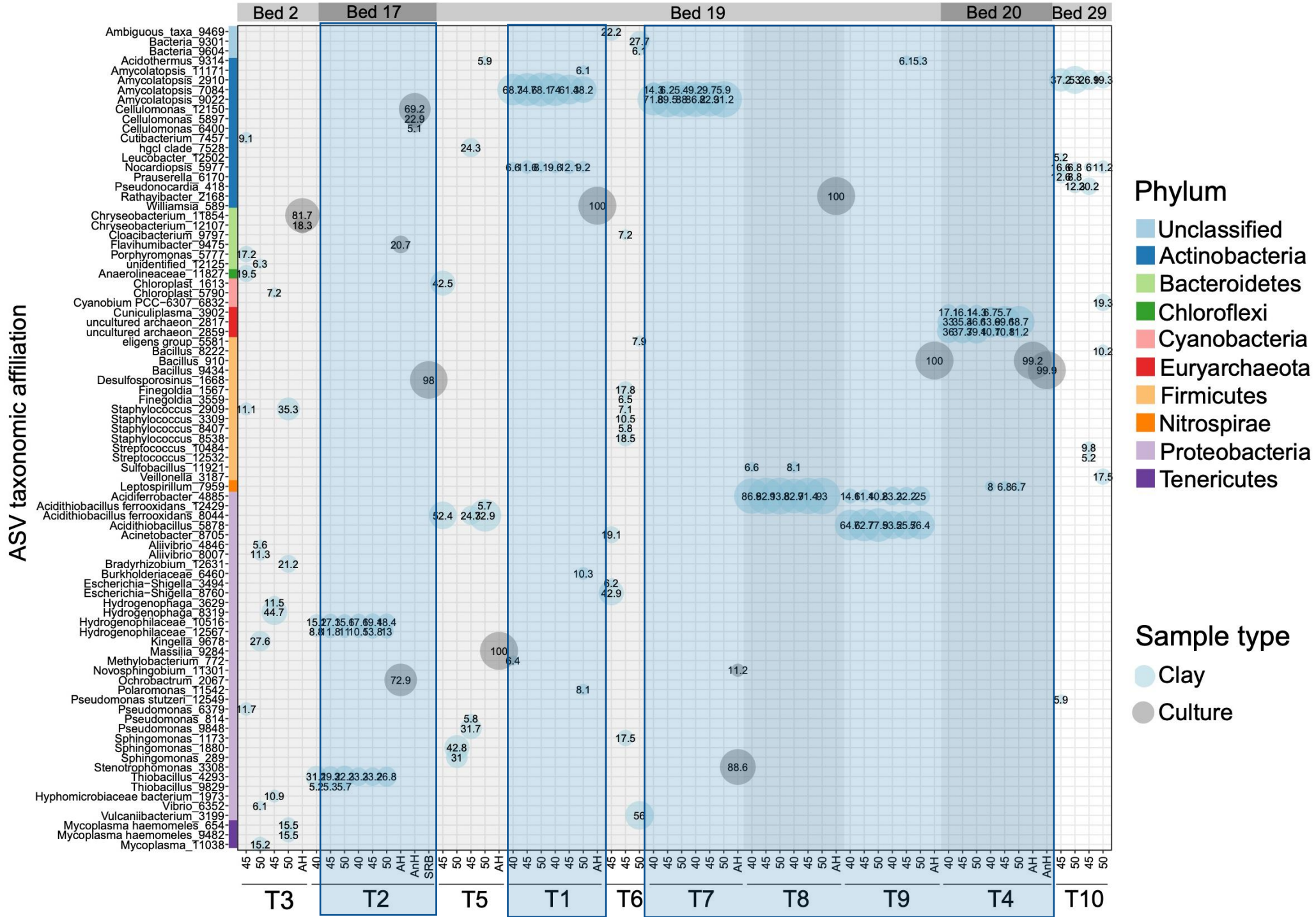
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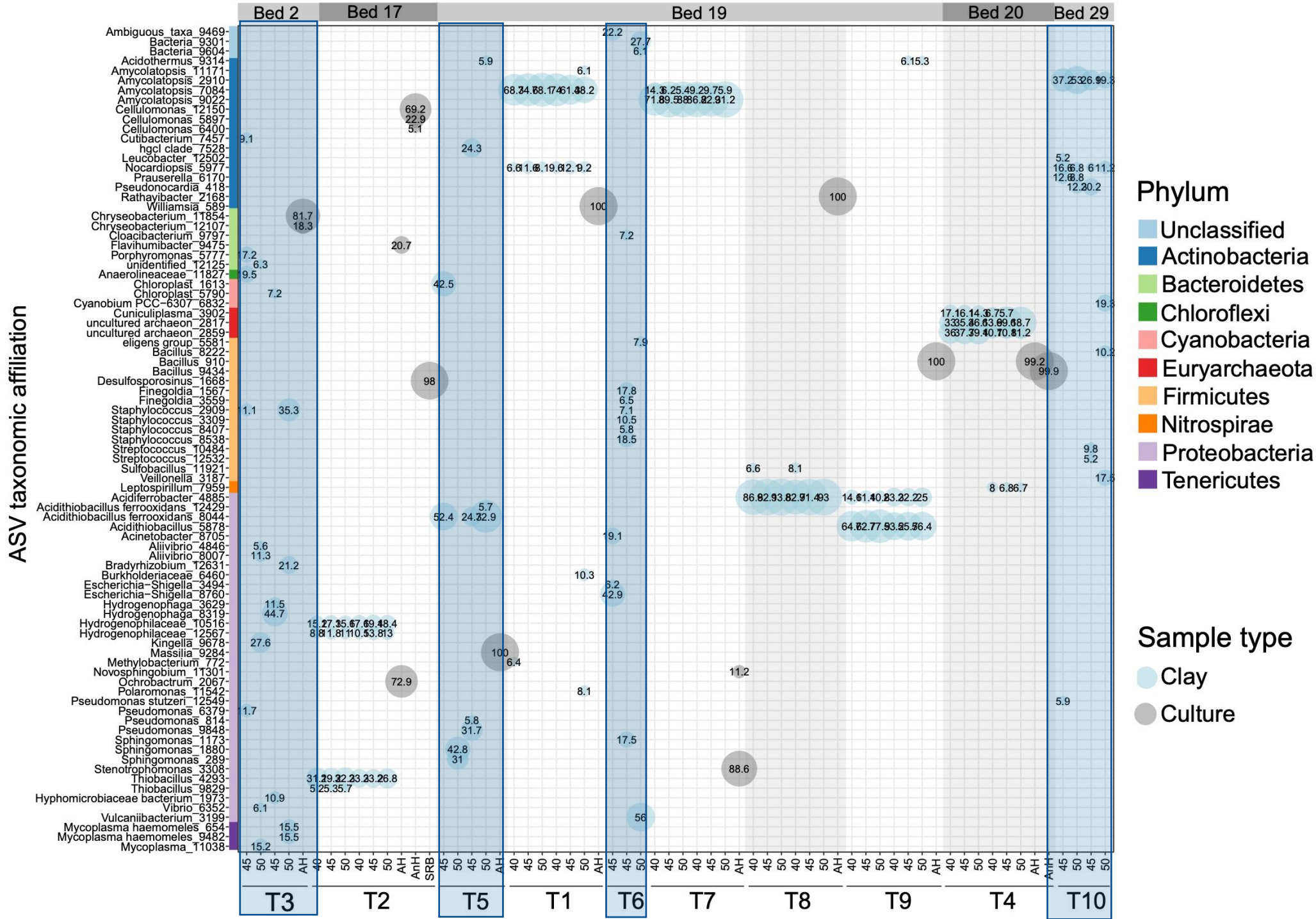
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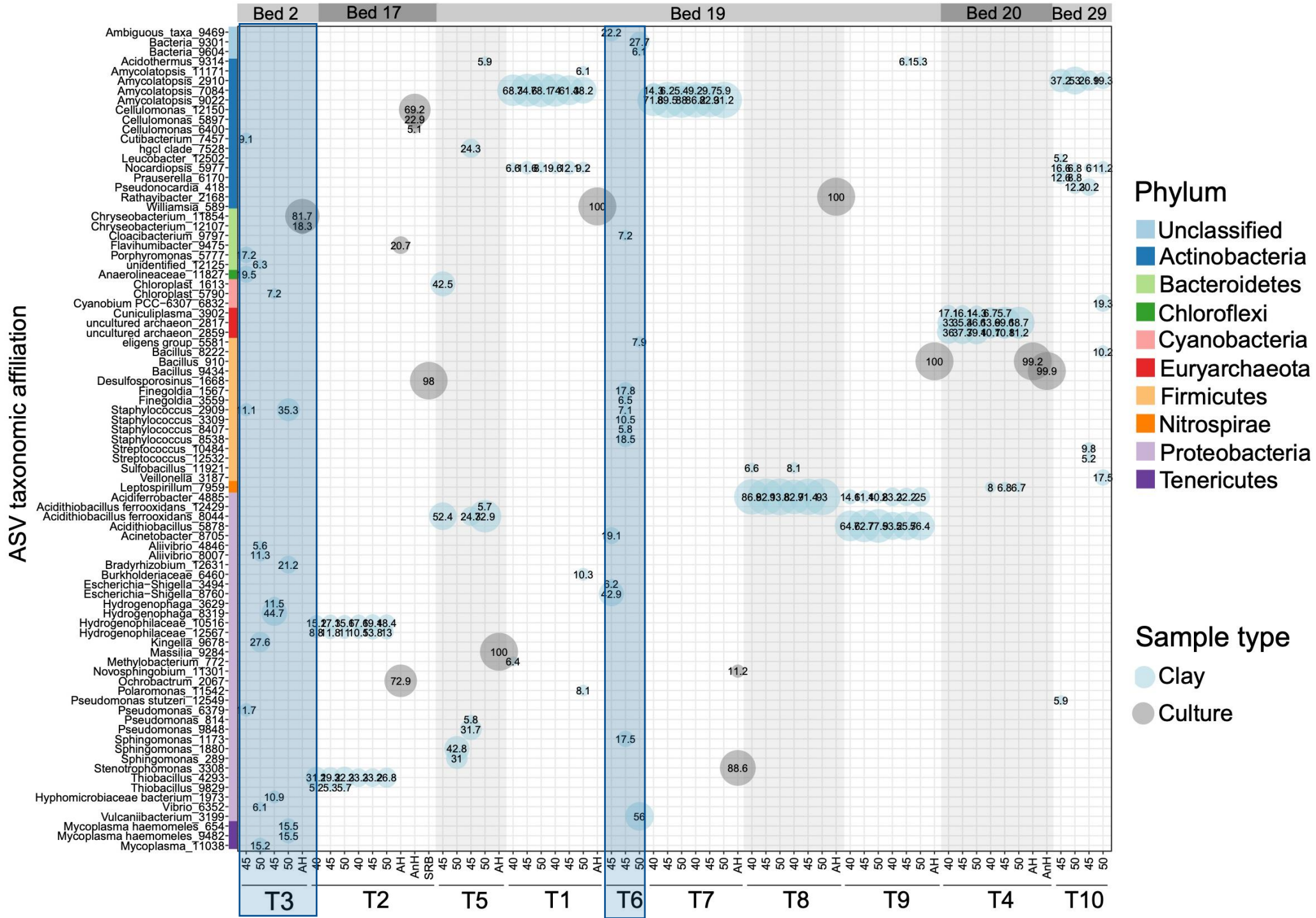
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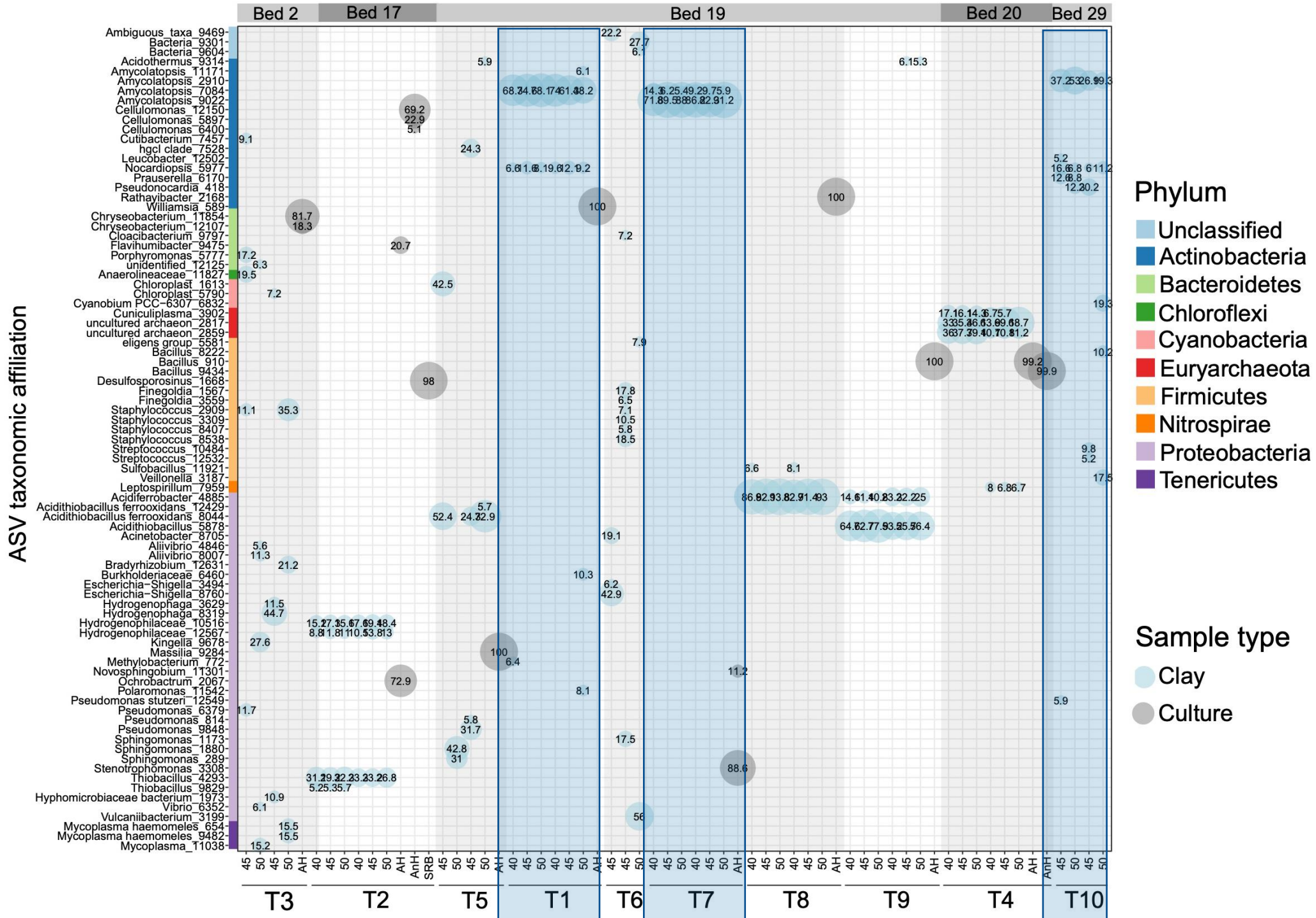
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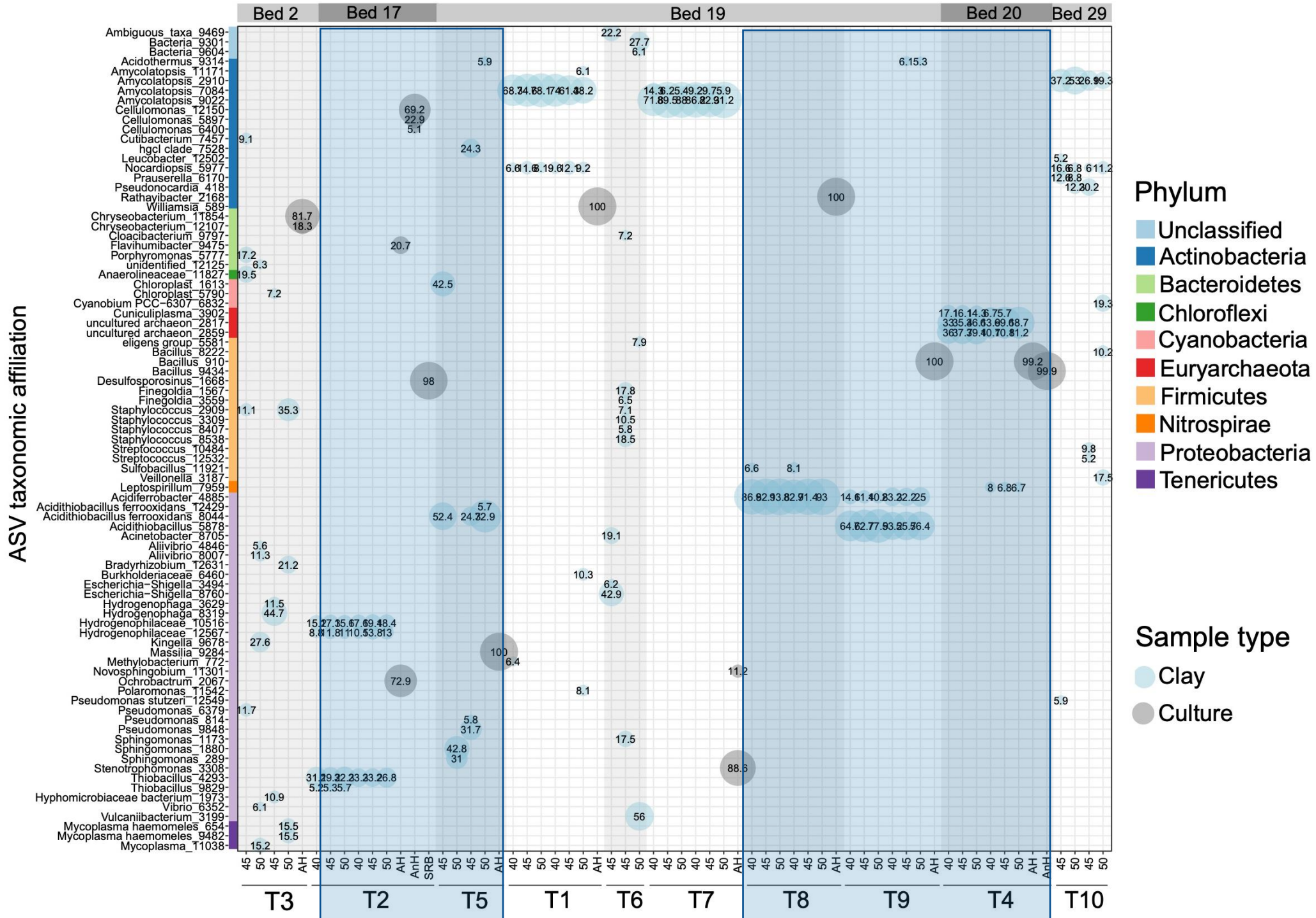
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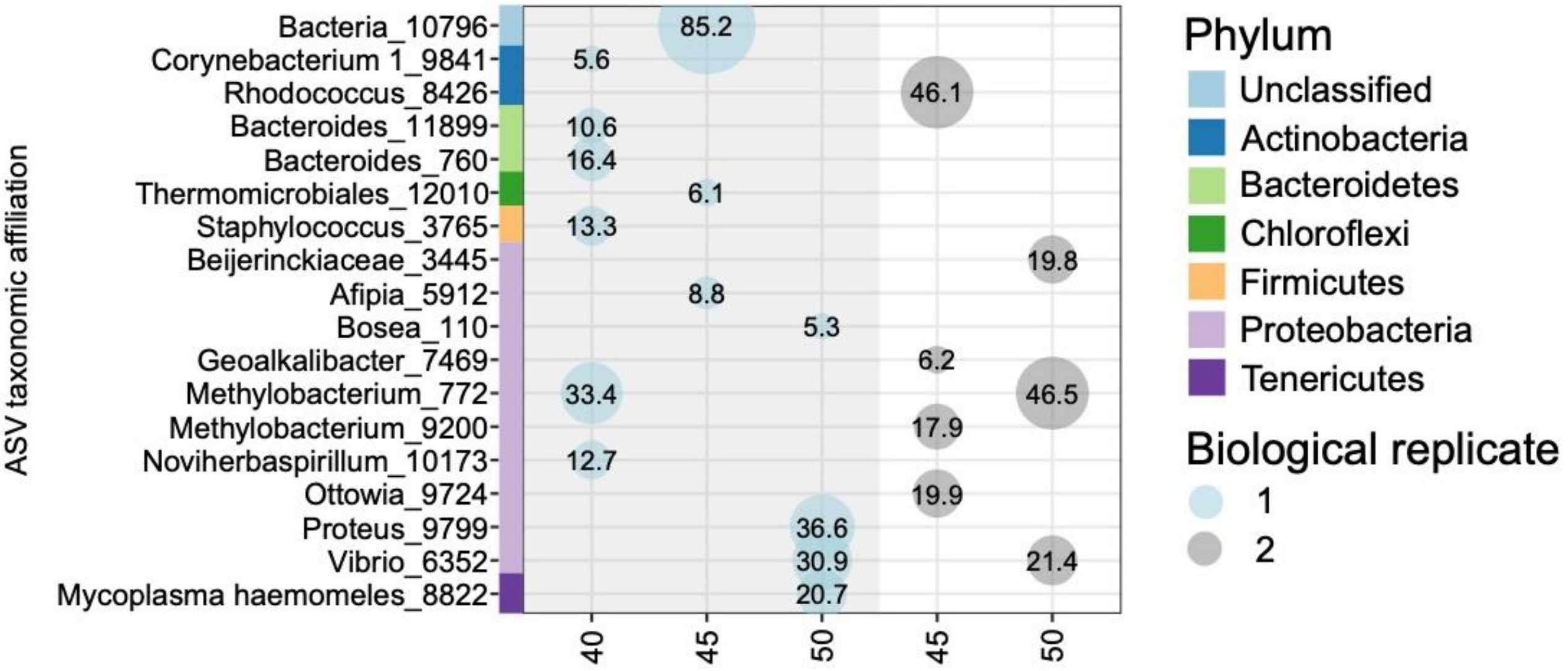
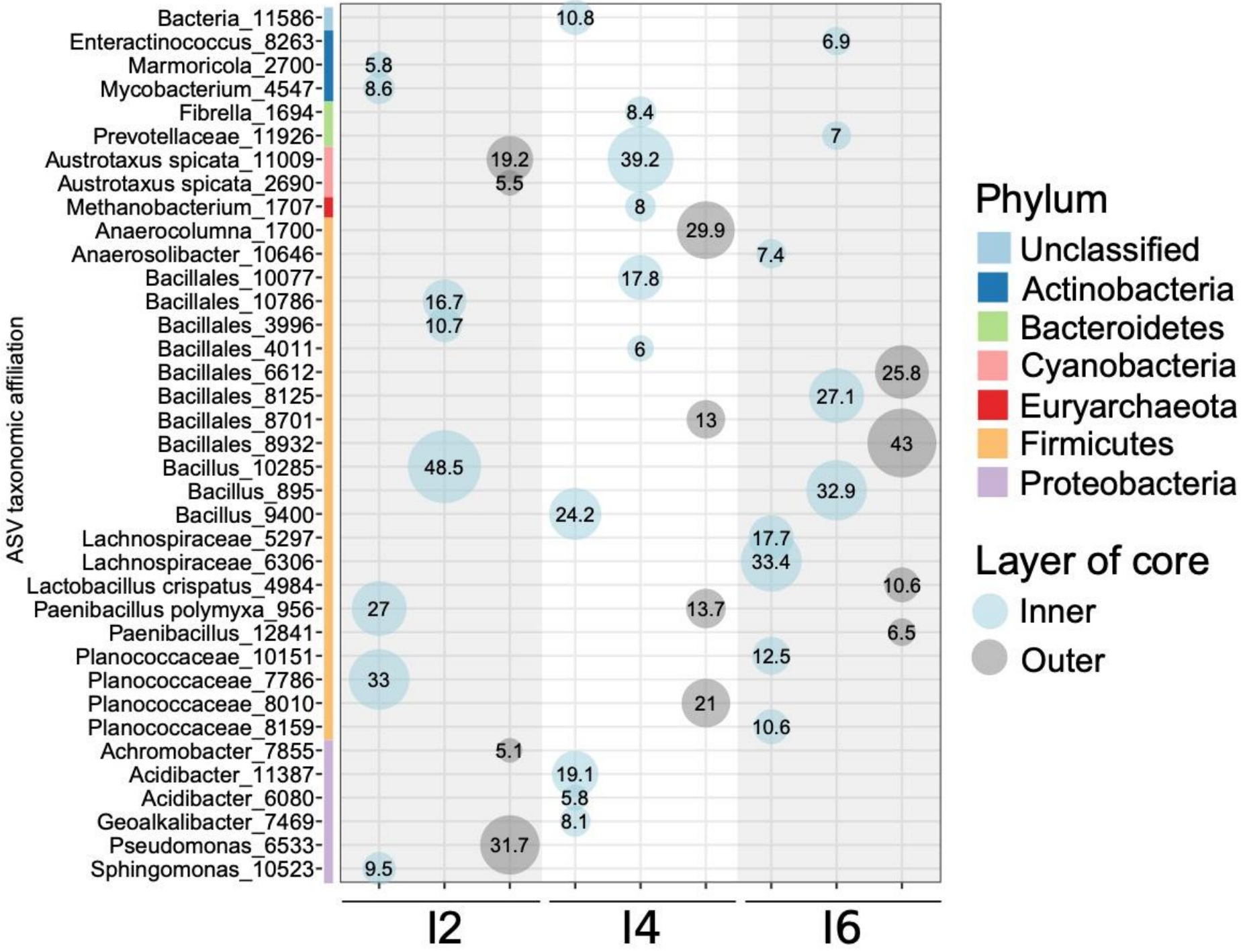
Bubble plot of Opalinus clay samples

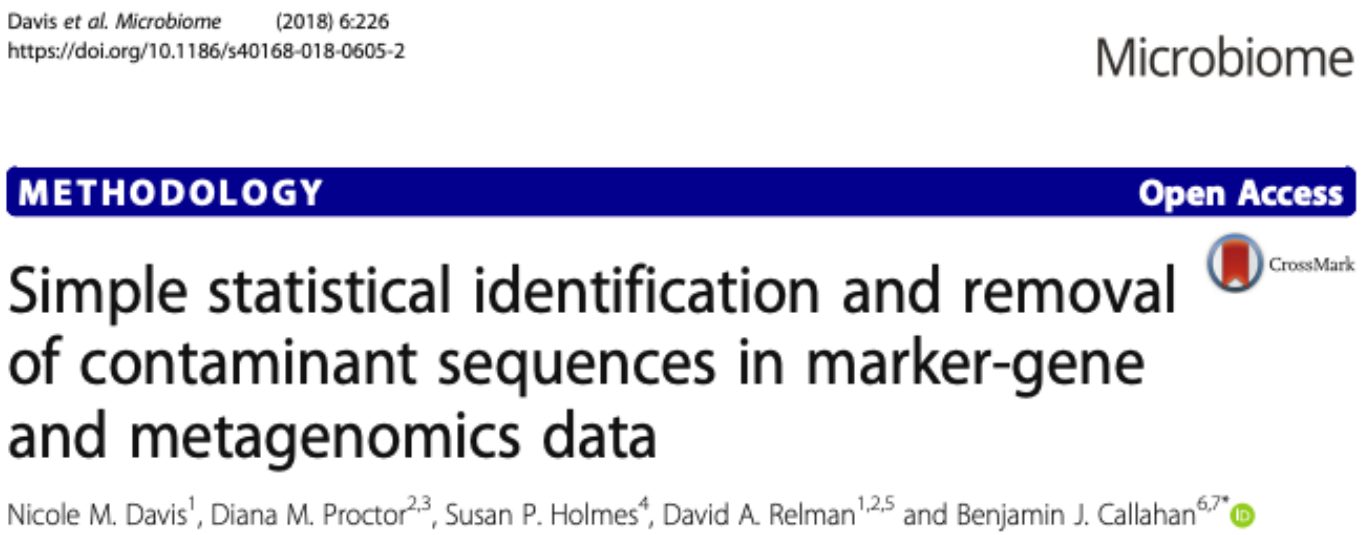


Bubble plot of Ignace rock samples



Working with low biomass samples

- The Decontam R package was used to identify contaminant sequences from all amplicon sequencing data generated from low biomass clay and rock samples.


Davis *et al. Microbiome* (2018) 6:226
https://doi.org/10.1186/s40168-018-0605-2

Microbiome

METHODOLOGY Open Access

Simple statistical identification and removal of contaminant sequences in marker-gene and metagenomics data

Nicole M. Davis[1], Diana M. Proctor[2,3], Susan P. Holmes[4], David A. Relman[1,2,5] and Benjamin J. Callahan[6,7*]

- Replicates are critical to ensure microbial profiles are representative of the clay and not spurious and contaminant-dominated.

Remaining questions

- Would the results differ if samples were taken from anoxic locations?

- Do the microbial profiles represent viable microorganisms or relic DNA?

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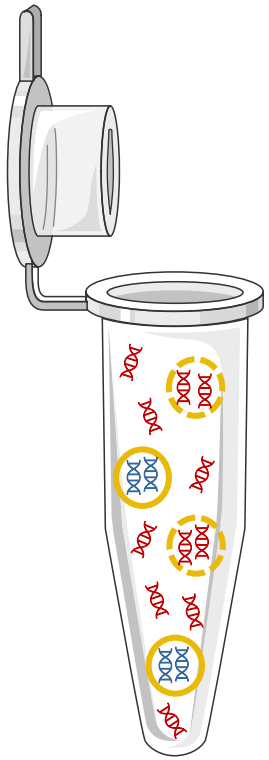
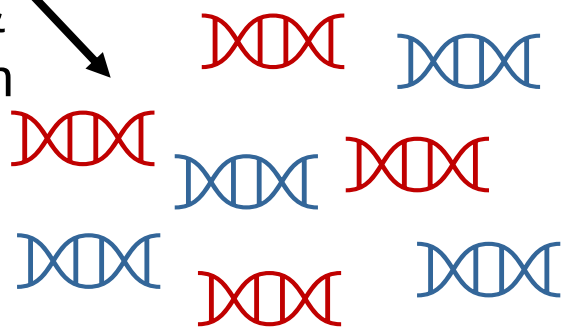
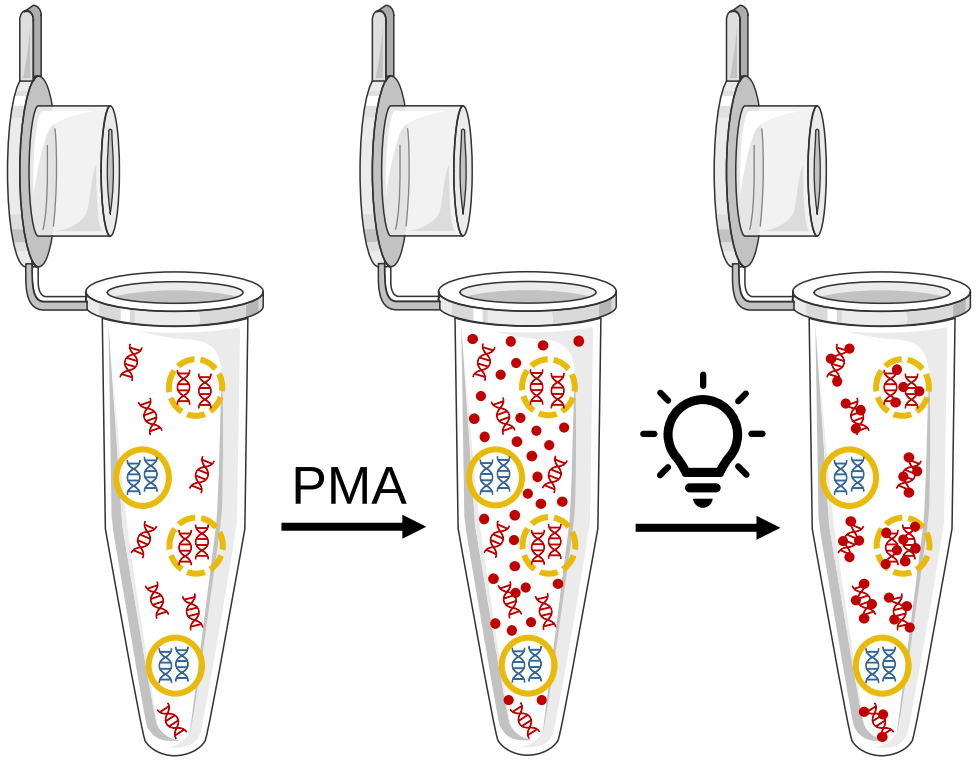


DNA
extraction &
amplification

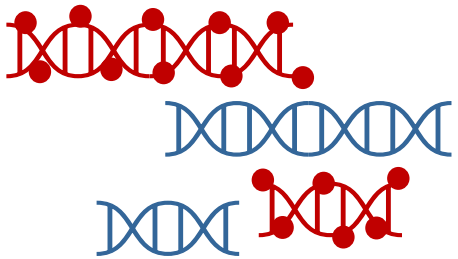


Propidium monoazide prevents the amplification of DNA that is not protected by an intact cell membrane

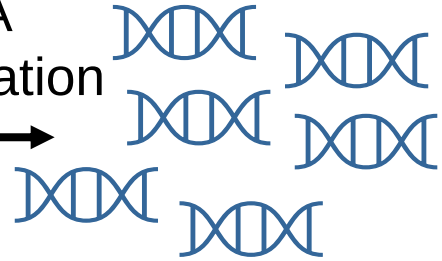


PMA

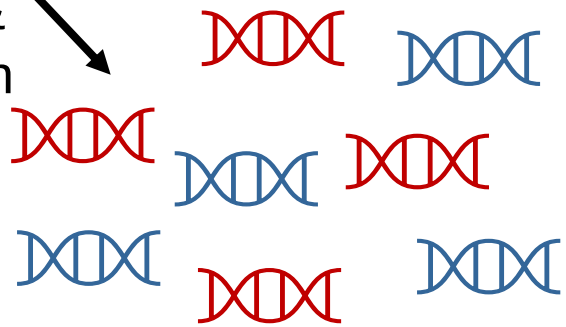
DNA extraction



DNA amplification



DNA extraction & amplification



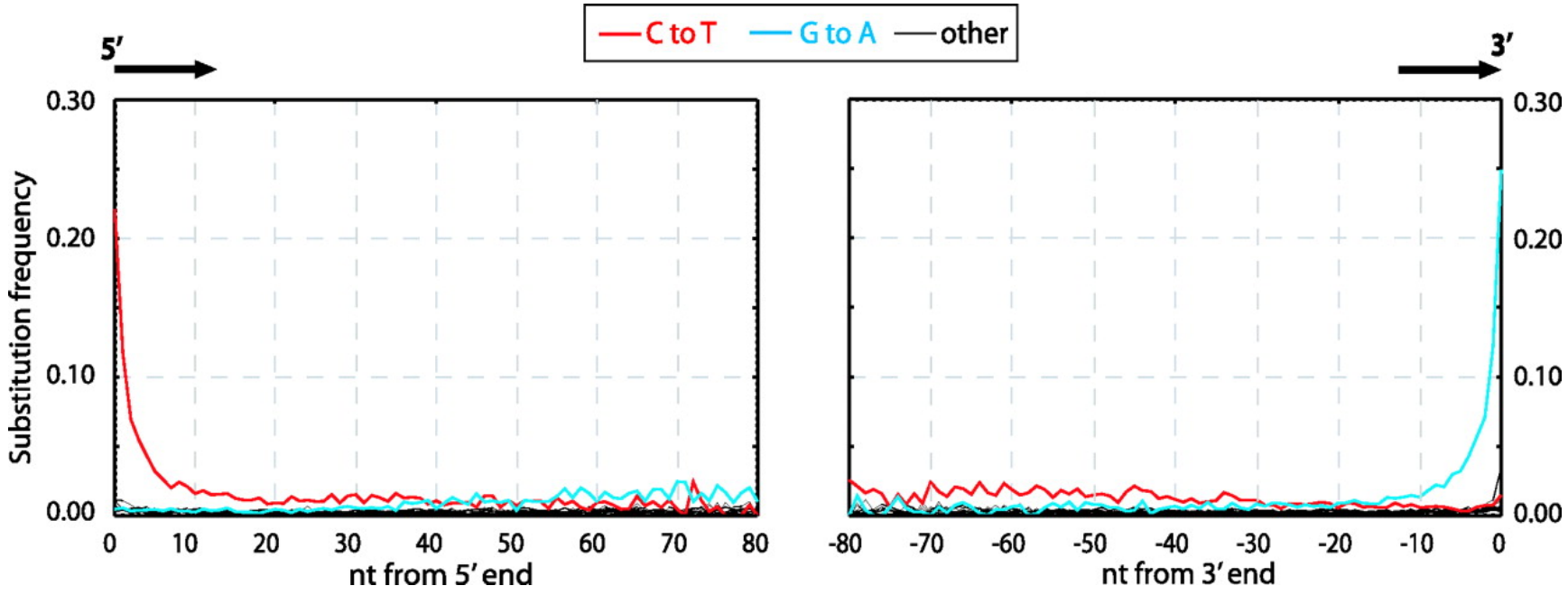
Patterns of damage in genomic DNA sequences from a Neandertal

Adrian W. Briggs*†, Udo Stenzel*, Philip L. F. Johnson‡, Richard E. Green*, Janet Kelso*, Kay Prüfer*, Matthias Meyer*, Johannes Krause*, Michael T. Ronan§, Michael Lachmann*, and Svante Pääbo*†

*Max Planck Institute for Evolutionary Anthropology, Deutscher Platz 6, D-04103 Leipzig, Germany; ‡Biophysics Graduate Group, University of California, Berkeley, CA 94720; and §454 Life Sciences, Branford, CT 06405

Contributed by Svante Pääbo, May 25, 2007 (sent for review April 25, 2007)

- C → T and G → A substitutions occur at an increased frequency in ancient DNA.
- The distribution of C → T and G → A substitutions is not equal across a DNA molecule.
- Other types of substitutions are more rare and are equally distributed across a DNA molecule.



PeerJ. 2021; 9: e11845.
Published online 2021 Jul 27. doi: 10.7717/peerj.11845
PMCID: PMC8323603
PMID: 34395085

PyDamage: automated ancient damage identification and estimation for contigs in ancient DNA *de novo* assembly

Maxime Borry,[1] Alexander Hübner,[1,2] Adam B. Rohrlach,[3,4] and Christina Warinner[1,2,5]

Academic Editor: Rodolfo Aramayo

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Acknowledgements

Josh Neufeld
Katja Engel

Everyone involved
in collecting samples

UNIVERSITY OF WATERLOO

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NUCLEAR WASTE MANAGEMENT ORGANIZATION

Western
UNIVERSITY · CANADA

Ontario
Research Fund

NSERC
CRSNG