



Linking Microbial Communities to Degradation Processes Occurring in a VIP and Pour-Flush Latrines

Francis L. de los Reyes III

Ling Wang¹, Aoife Byrne², Konstantina Velkushanova², and Chris Buckley²

¹Department of Civil, Construction, and Environmental Engineering,
North Carolina State University

²Pollution Research Group, University of KwaZulu-Natal, Durban, South Africa



NC STATE UNIVERSITY

Department of
Civil,
Construction, &
Environmental
ENGINEERING





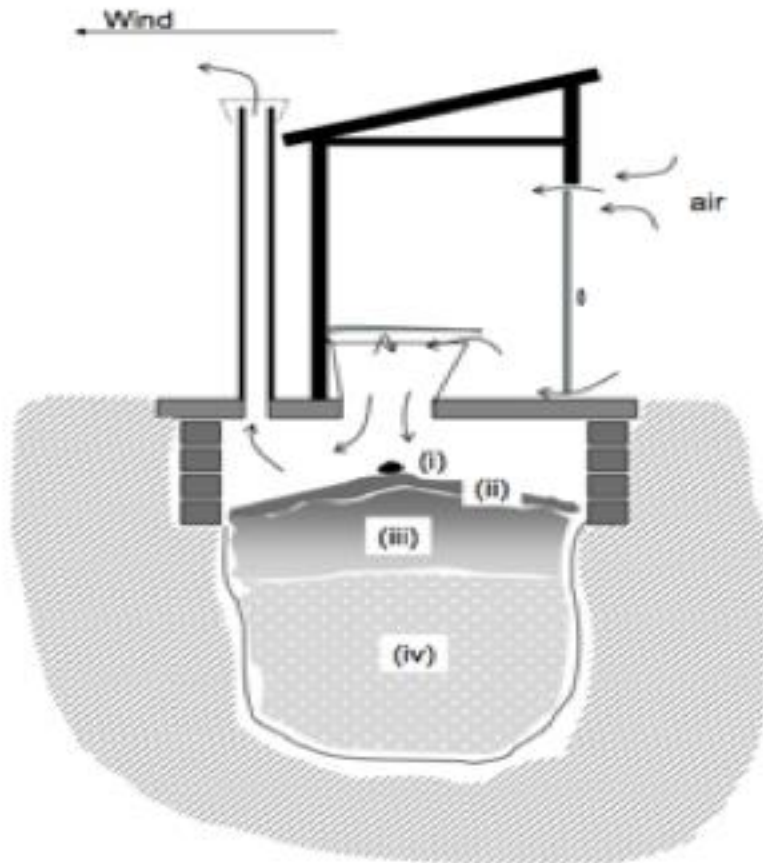
Ventilated Improved Pit Latrine (VIP)

What is going on inside the pit?



Biodegradation in pits

Buckley model, 2008

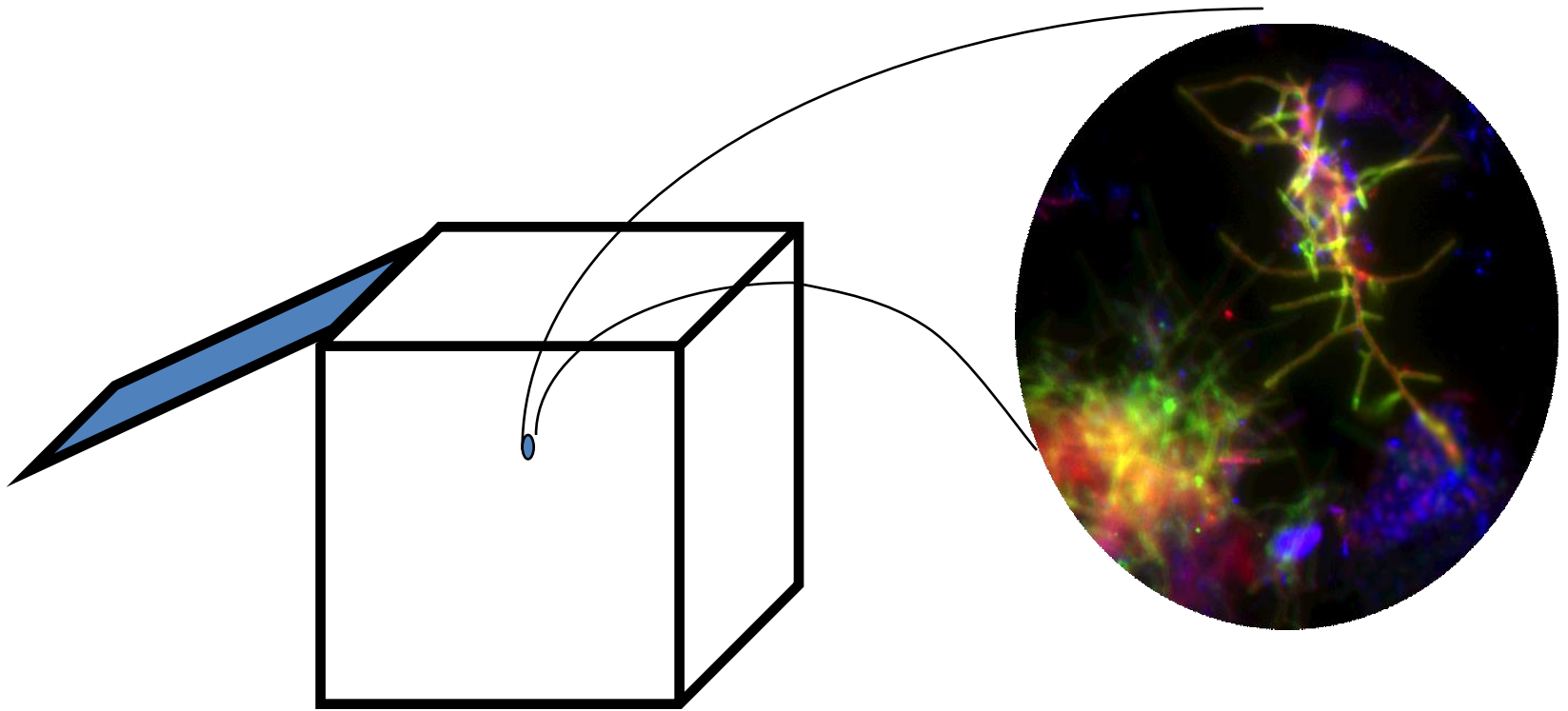


- i) **Rapid aerobic degradation** of the readily biodegradable portion of freshly deposited faeces
- ii) **Slow aerobic degradation** of the biodegradable material remaining on the surface
- iii) **Anaerobic degradation** to methane and CO_2
- iv) **Negligible degradation** of the bottom layer, the material accumulating here is considered biologically stabilised.

Pour flush toilet



Microbial Populations → Inside the environmental “Black Box”



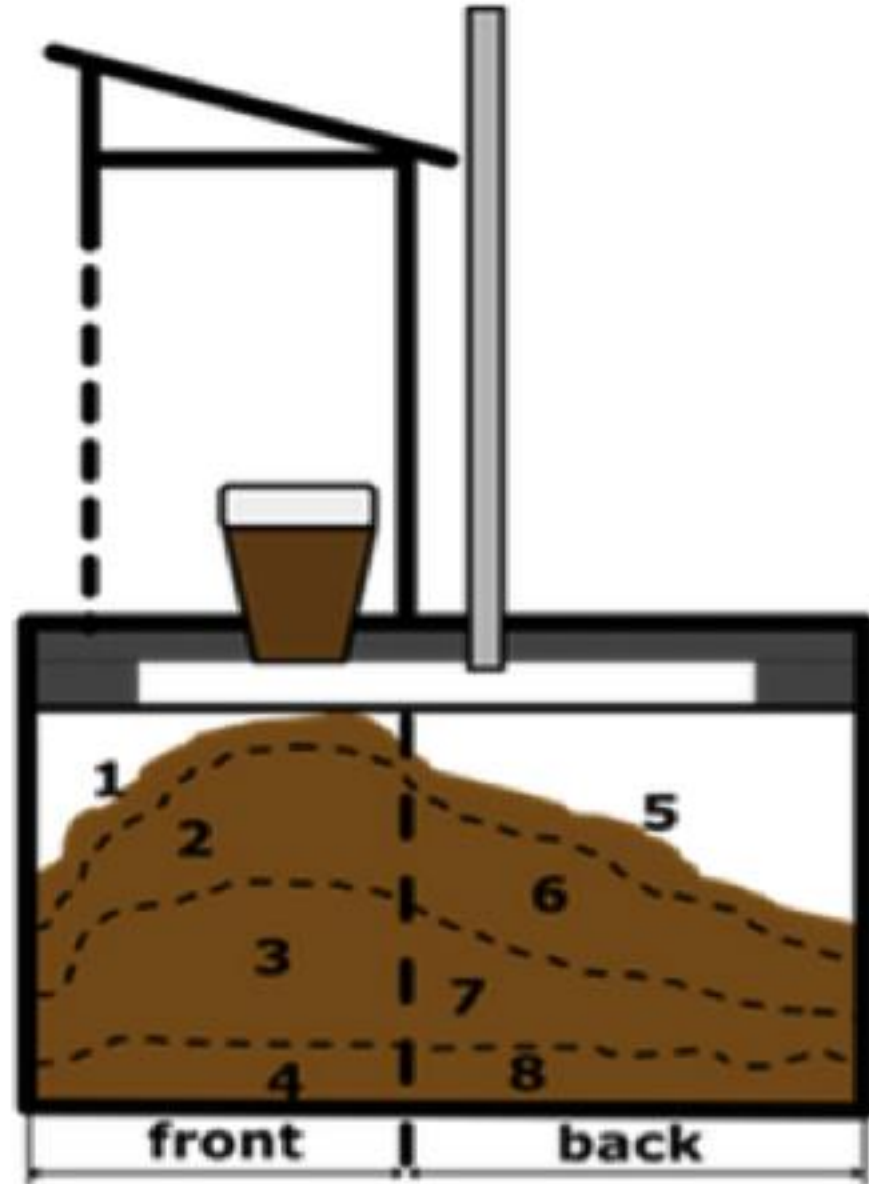
Who's there? What are they doing (causative)?
Can we influence them?

Sampling a VIP

A pit in Bester's Camp, eThekweni municipality

8 representative FS samples from 4 different depth layers from the front and back of the pit

Sample	Layer	Type_ID
VIP1	Top	VIP_front
VIP2	Second	VIP_front
VIP3	Third	VIP_front
VIP4	Forth	VIP_front
VIP5	Top	VIP_back
VIP6	Second	VIP_back
VIP7	Third	VIP_back
VIP8	Forth	VIP_back





Pour-flush toilet (PF) sampling

Four Sites: 1, 2, 3, 4

Sites 1 and 2 have two pits on site

Sites 3 and 4 have a single pit on site

a: active pit

b: standing pit

s: single pit

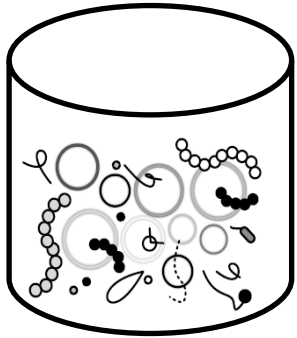
Freshness: front>back

Sample	Site	Type_ID
PF1	1	PF_active_front
PF2	1	PF_active_back
PF3	1	PF_standing_front
PF4	1	PF_standing_back
PF5	2	PF_active_front
PF6	2	PF_active_back
PF7	2	PF_standing_front
PF8	2	PF_standing_back
PF9	3	PF_single_front
PF10	3	PF_single_back
PF11	4	PF_single_front
PF12	4	PF_single_back

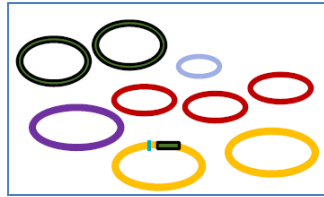


Metagenomic analysis

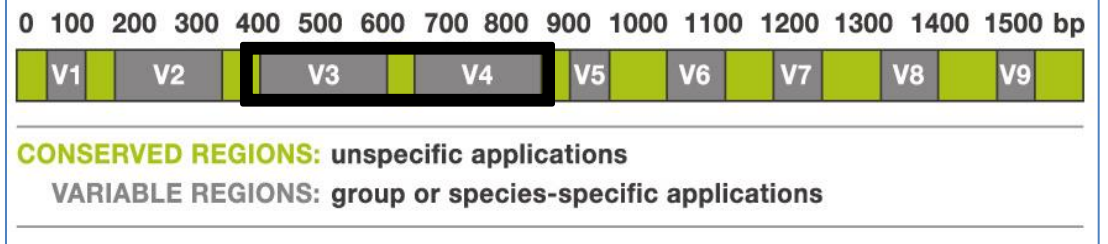
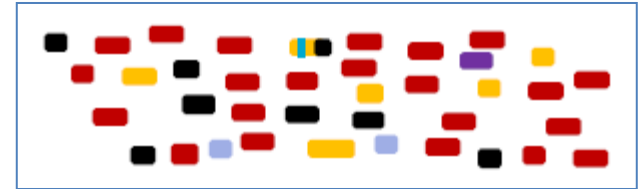
Community profiling of environmental samples through amplicon sequencing



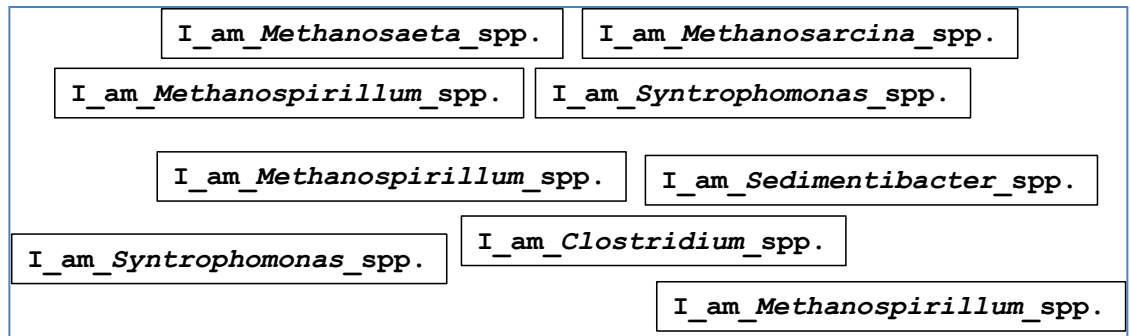
Bulk DNA extraction



16S PCR amplification

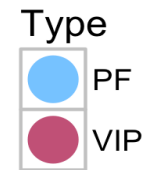
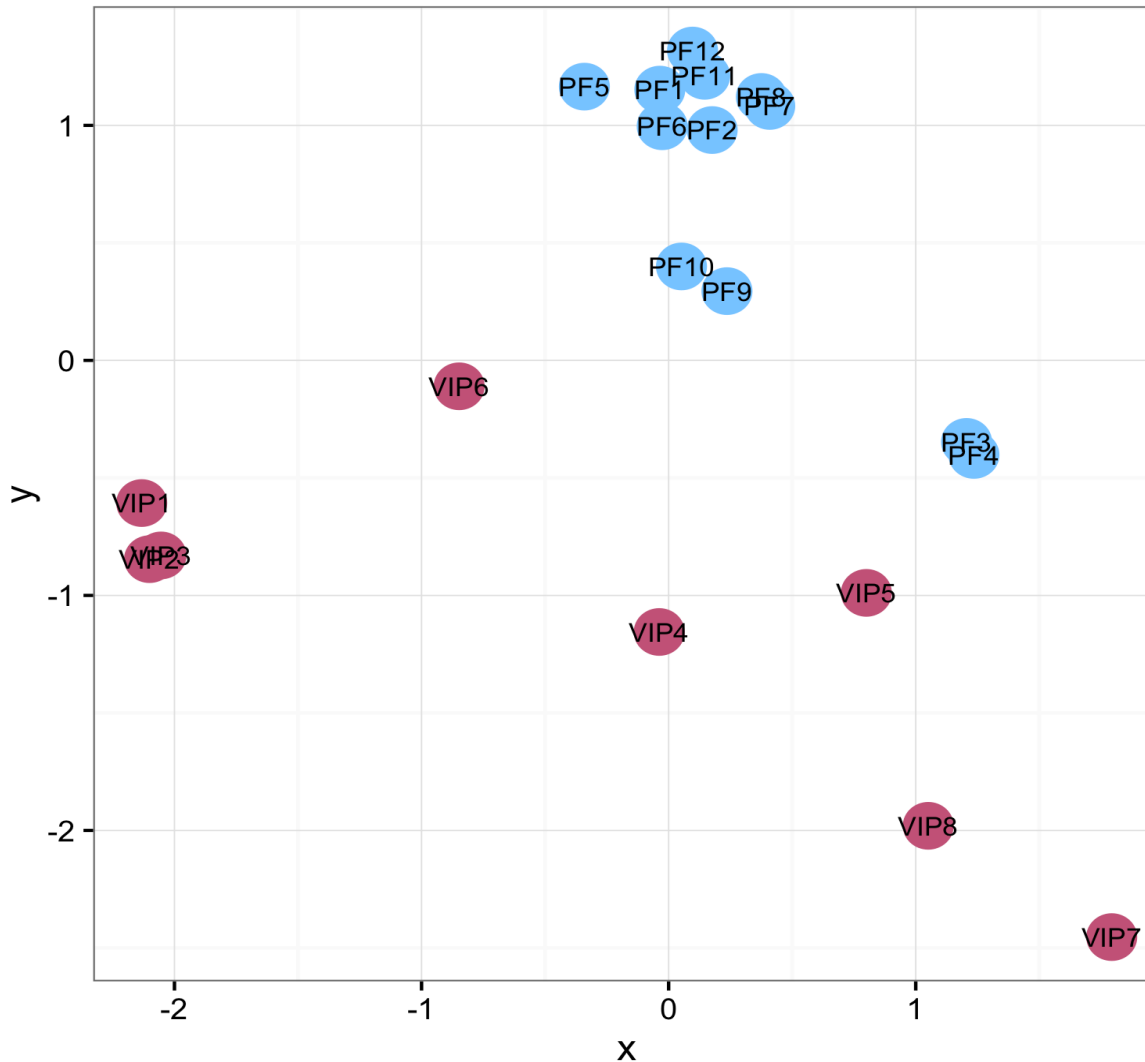


Bioinformatic analysis



Microbial community distribution

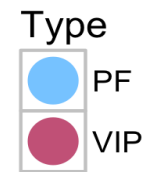
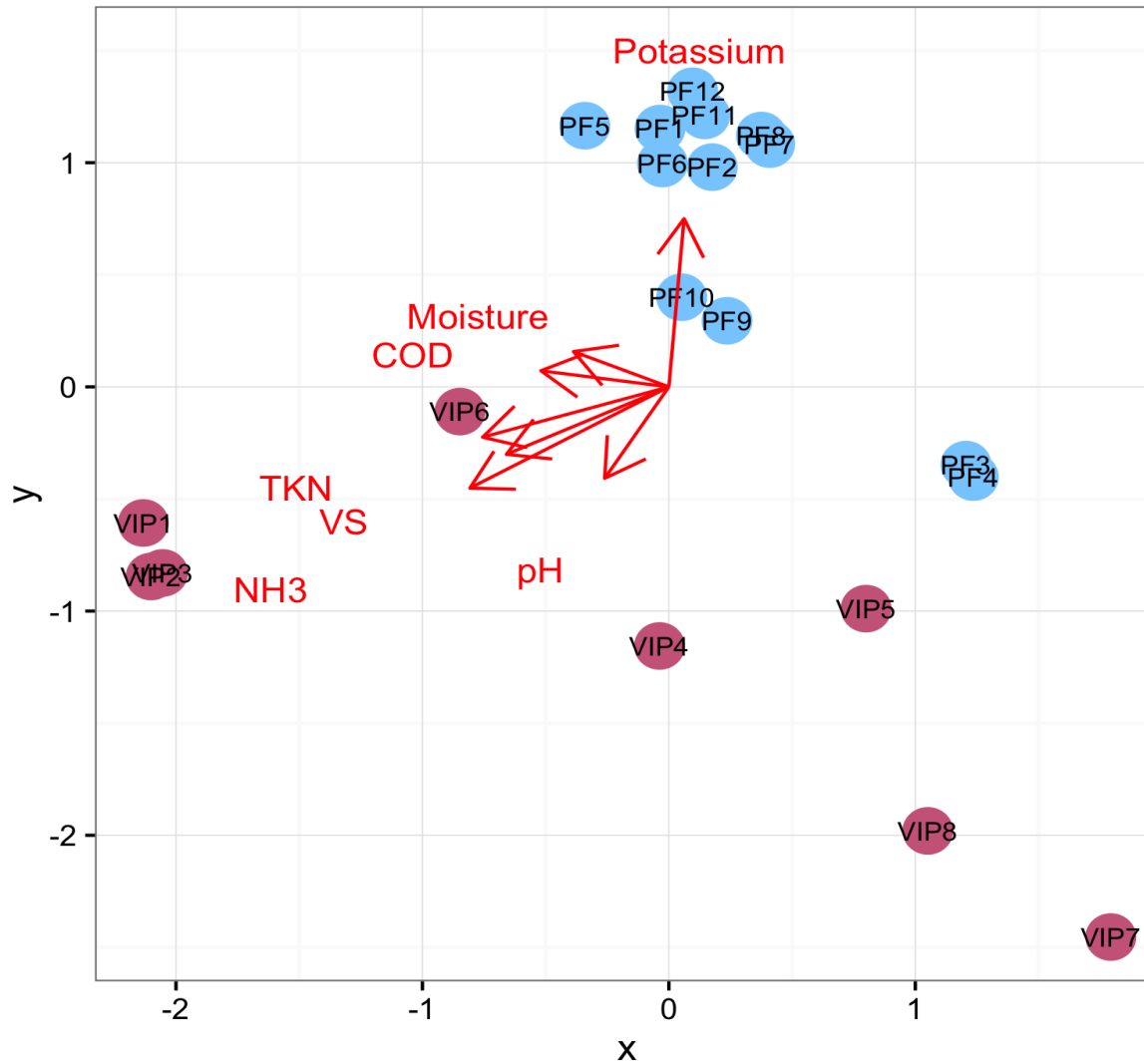
VIP very different from PF communities



Sample	Layer	Type_ID
VIP1	Top	VIP_front
VIP2	Second	VIP_front
VIP3	Third	VIP_front
VIP4	Forth	VIP_front
VIP5	Top	VIP_back
VIP6	Second	VIP_back
VIP7	Third	VIP_back
VIP8	Forth	VIP_back

Sample	Site	Type_ID
PF1	1	PF_active_front
PF2	1	PF_active_back
PF3	1	PF_standing_front
PF4	1	PF_standing_back
PF5	2	PF_active_front
PF6	2	PF_active_back
PF7	2	PF_standing_front
PF8	2	PF_standing_back
PF9	3	PF_single_front
PF10	3	PF_single_back
PF11	4	PF_single_front
PF12	4	PF_single_back

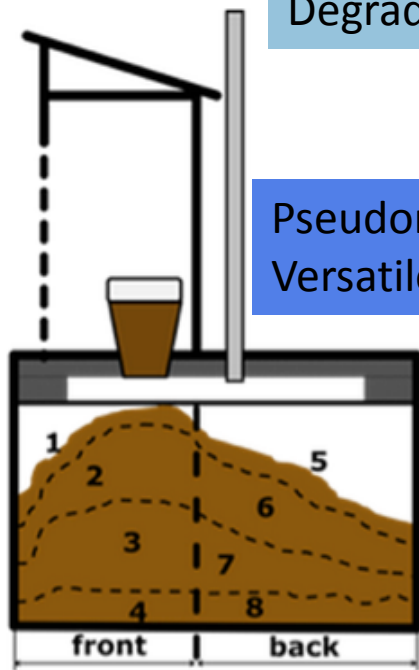
Linking microbial community to conditions



Sample	Layer	Type_ID
VIP1	Top	VIP_front
VIP2	Second	VIP_front
VIP3	Third	VIP_front
VIP4	Forth	VIP_front
VIP5	Top	VIP_back
VIP6	Second	VIP_back
VIP7	Third	VIP_back
VIP8	Forth	VIP_back

Sample	Site	Type_ID
PF1	1	PF_active_front
PF2	1	PF_active_back
PF3	1	PF_standing_front
PF4	1	PF_standing_back
PF5	2	PF_active_front
PF6	2	PF_active_back
PF7	2	PF_standing_front
PF8	2	PF_standing_back
PF9	3	PF_single_front
PF10	3	PF_single_back
PF11	4	PF_single_front
PF12	4	PF_single_back

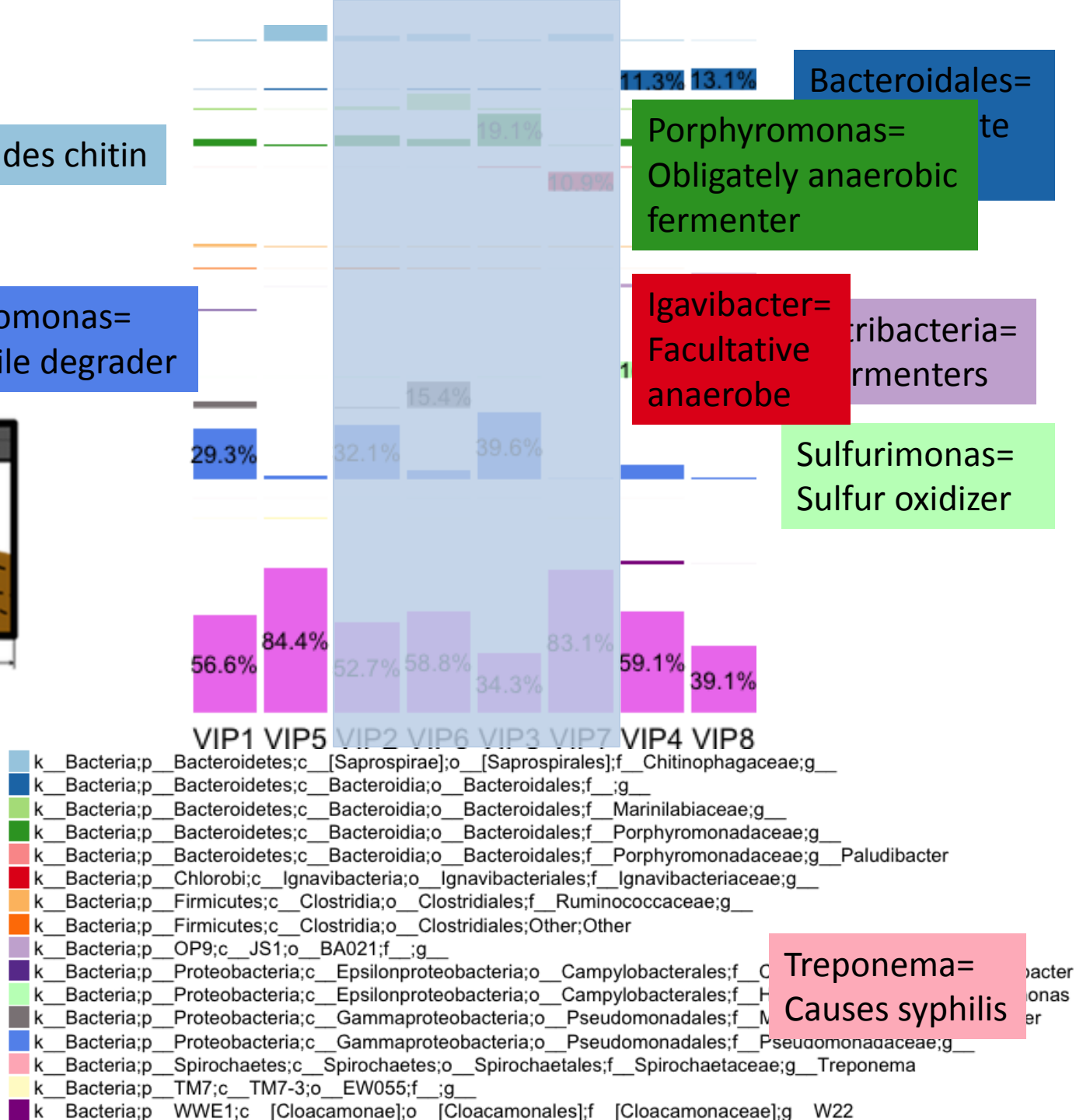
VIP Results



Degrades chitin

Pseudomonas=
Versatile degrader

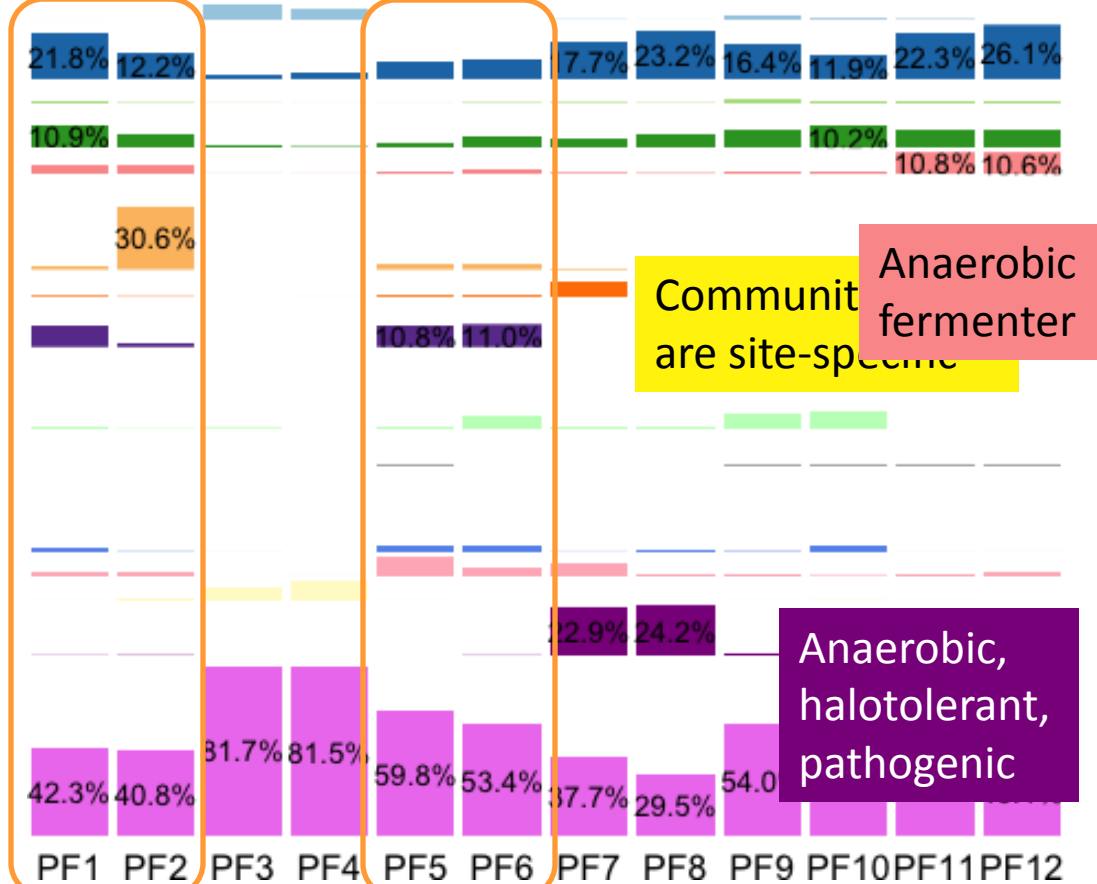
Sample	Layer	Type_ID
VIP1	Top	VIP_front
VIP2	Second	VIP_front
VIP3	Third	VIP_front
VIP4	Forth	VIP_front
VIP5	Top	VIP_back
VIP6	Second	VIP_back
VIP7	Third	VIP_back
VIP8	Forth	VIP_back



Pour-flush toilet (PF)

Sample	Site	Type_ID
PF1	1	PF_active
PF2	1	PF_active
PF3	1	PF_standing_front
PF4	1	PF_standing_back
PF5	2	PF_active_front
PF6	2	PF_active_back
PF7	2	PF_standing_front
PF8	2	PF_standing_back
PF9	3	PF_single_front
PF10	3	PF_single_back
PF11	4	PF_single_front
PF12	4	PF_single_back

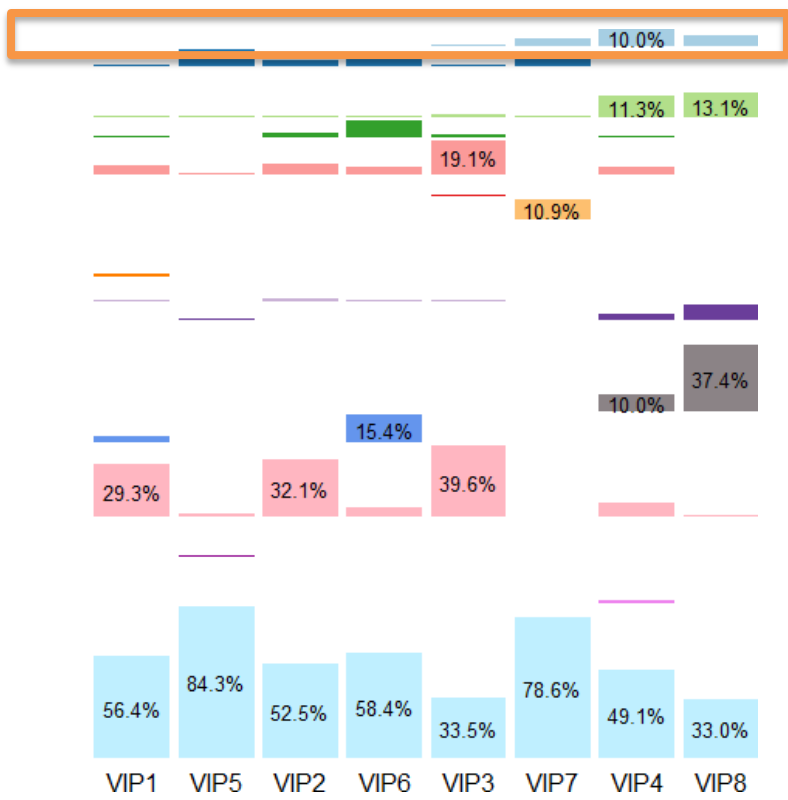
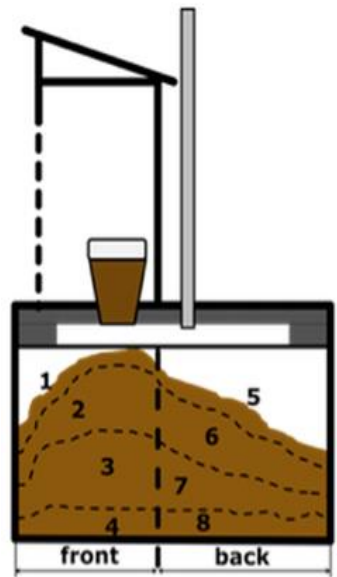
Fresh material have similar communities



- k_Bacteria;p_Bacteroidetes;c_[Saprosirae];o_[Saprosirales];f_Chitinophagaceae;g__
- k_Bacteria;p_Bacteroidetes;c_Bacteroidia;o_Bacteroidales;f__;g__
- k_Bacteria;p_Bacteroidetes;c_Bacteroidia;o_Bacteroidales;f_Marinilabiaceae;g__
- k_Bacteria;p_Bacteroidetes;c_Bacteroidia;o_Bacteroidales;f_Porphyrimonadaceae;g__
- k_Bacteria;p_Bacteroidetes;c_Bacteroidia;o_Bacteroidales;f_Porphyrimonadaceae;g_Paludibacter
- k_Bacteria;p_Chlorobi;c_Ignavibacteria;o_Ignavibacteriales;f_Ignavibacteriaceae;g__
- k_Bacteria;p_Firmicutes;c_Clostridia;o_Clostridiales;f_Ruminococcaceae;g__
- k_Bacteria;p_Firmicutes;c_Clostridia;o_Clostridiales;Other;Other
- k_Bacteria;p_OP9;c_JS1;o_BA021;f__;g__
- k_Bacteria;p_Proteobacteria;c_Epsilonproteobacteria;o_Campylobacteriales;f_Campylobacteraceae;g_Arcobacter
- k_Bacteria;p_Proteobacteria;c_Epsilonproteobacteria;o_Campylobacteriales;f_Helicobacteraceae;g_Sulfurimonas
- k_Bacteria;p_Proteobacteria;c_Gammaproteobacteria;o_Pseudomonadales;f_Moraxellaceae;g_Acinetobacter
- k_Bacteria;p_Proteobacteria;c_Gammaproteobacteria;o_Pseudomonadales;f_Pseudomonadaceae;g__
- k_Bacteria;p_Spirochaetes;c_Spirochaetes;o_Spirochaetales;f_Spirochaetaceae;g_Treponema
- k_Bacteria;p_TM7;c_TM7-3;o_EW055;f__;g__
- k_Bacteria;p_WWE1;c_[Cloacamonae];o_[Cloacamonales];f_[Cloacamonaceae];g_W22

Archaea in VIP

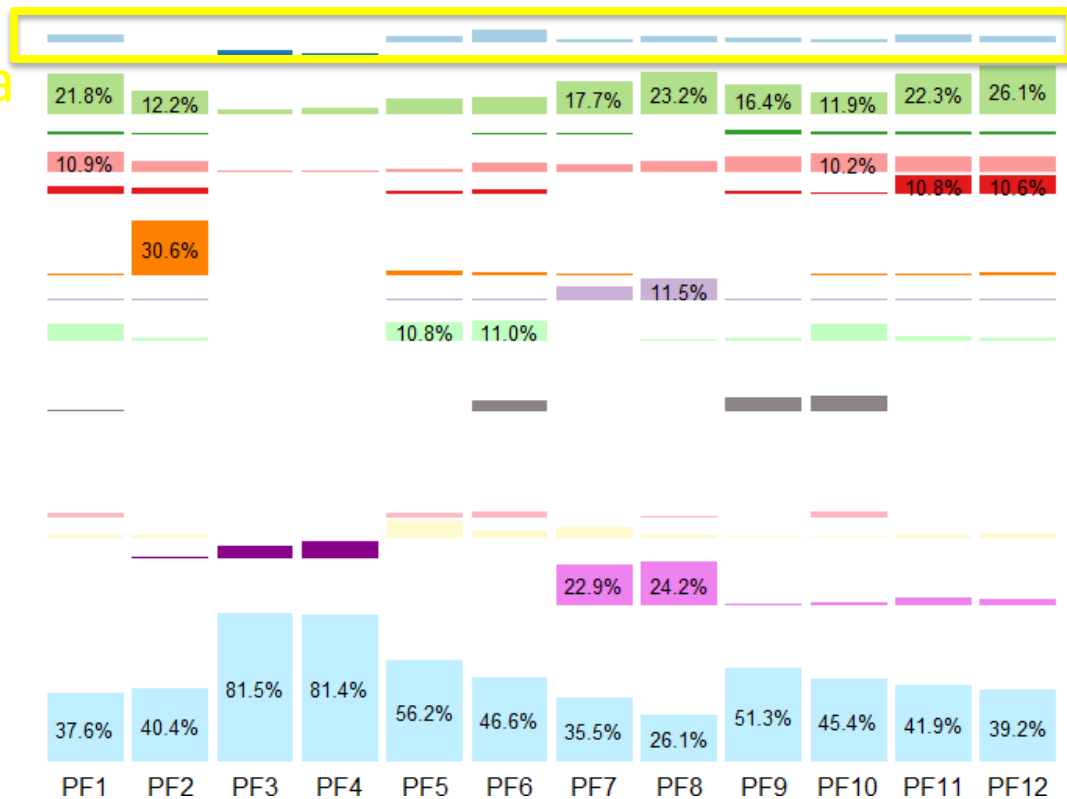
Archaea



- k_Archaea
- k_Bacteria;p_Bacteroidetes;c_[Saprosirae];o_[Saprosirales];f_Chitinophagaceae;g_
- k_Bacteria;p_Bacteroidetes;c_Bacteroidia;o_Bacteroidales;f_;
- k_Bacteria;p_Bacteroidetes;c_Bacteroidia;o_Bacteroidales;f_Marinilabiaceae;g_
- k_Bacteria;p_Bacteroidetes;c_Bacteroidia;o_Bacteroidales;f_Porphyrmonadaceae;g_
- k_Bacteria;p_Bacteroidetes;c_Bacteroidia;o_Bacteroidales;f_Porphyrmonadaceae;g_Paludibacter
- k_Bacteria;p_Chlorobi;c_Ignavibacteria;o_Ignavibacteriales;f_Ignavibacteriaceae;g_
- k_Bacteria;p_Firmicutes;c_Clostridia;o_Clostridiales;f_Ruminococcaceae;g_
- k_Bacteria;p_Firmicutes;c_Clostridia;o_Clostridiales;Other;Other
- k_Bacteria;p_OP9;c_JS1;o_BA021;f_;
- k_Bacteria;p_Proteobacteria;c_Epsilonproteobacteria;o_Campylobacteriales;f_Campylobacteraceae;g_Arcobacter
- k_Bacteria;p_Proteobacteria;c_Epsilonproteobacteria;o_Campylobacteriales;f_Helicobacteraceae;g_Sulfurimonas
- k_Bacteria;p_Proteobacteria;c_Gammaproteobacteria;o_Pseudomonadales;f_Moraxellaceae;g_Acinetobacter
- k_Bacteria;p_Proteobacteria;c_Gammaproteobacteria;o_Pseudomonadales;f_Pseudomonadaceae;g_
- k_Bacteria;p_Spirochaetes;c_Spirochaetes;o_Spirochaetales;f_Spirochaetaceae;g_Treponema
- k_Bacteria;p_TM7;c_TM7-3;o_EW055;f_;
- k_Bacteria;p_WWE1;c_[Cloacamonae];o_[Cloacamonales];f_[Cloacamonaceae];g_W22
- Taxa <8%

Pour-flush toilet (PF)

Archaea



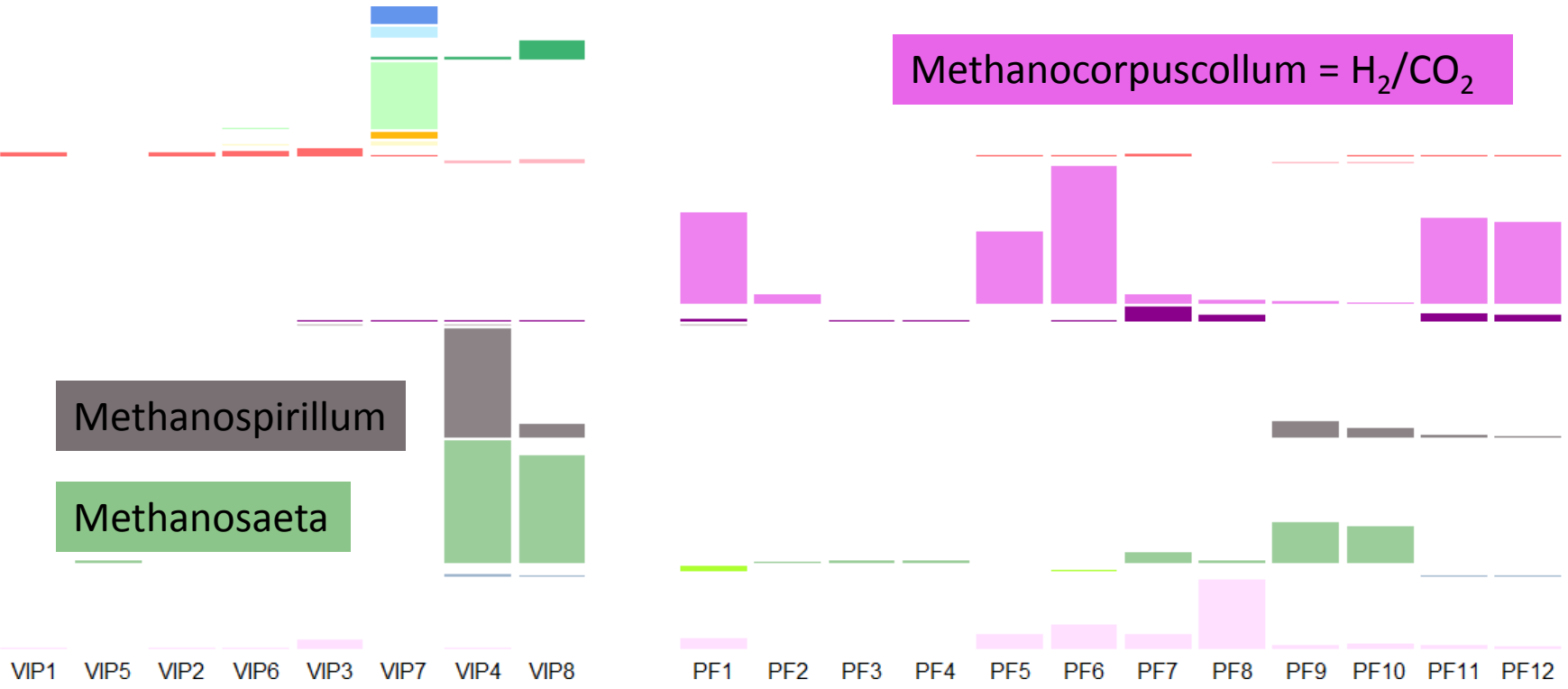
- k_Archaea
- k_Bacteria;p_Bacteroidetes;c_[Saprospirae];o_[Saprospirales];f_Chitinophagaceae;g_
- k_Bacteria;p_Bacteroidetes;c_Bacteroidia;o_Bacteroidales;f_;g_
- k_Bacteria;p_Bacteroidetes;c_Bacteroidia;o_Bacteroidales;f_Marinilabiaceae;g_
- k_Bacteria;p_Bacteroidetes;c_Bacteroidia;o_Bacteroidales;f_Porphyrimonadaceae;g_
- k_Bacteria;p_Bacteroidetes;c_Bacteroidia;o_Bacteroidales;f_Porphyrimonadaceae;g_Paludibacter
- k_Bacteria;p_Chlorobi;c_Ignavibacteria;o_Ignavibacteriales;f_Ignavibacteriaceae;g_
- k_Bacteria;p_Firmicutes;c_Clostridia;o_Clostridiales;f_Ruminococcaceae;g_
- k_Bacteria;p_Firmicutes;c_Clostridia;o_Clostridiales;Other;Other
- k_Bacteria;p_OP9;c_JS1;o_BA021;f_;g_
- k_Bacteria;p_Proteobacteria;c_Epsilonproteobacteria;o_Campylobacterales;f_Campylobacteraceae;g_Arcobacter
- k_Bacteria;p_Proteobacteria;c_Epsilonproteobacteria;o_Campylobacterales;f_Helicobacteraceae;g_Sulfurimonas
- k_Bacteria;p_Proteobacteria;c_Gammaproteobacteria;o_Pseudomonadales;f_Moraxellaceae;g_Acinetobacter
- k_Bacteria;p_Proteobacteria;c_Gammaproteobacteria;o_Pseudomonadales;f_Pseudomonadaceae;g_
- k_Bacteria;p_Spirochaetes;c_Spirochaetes;o_Spirochaetales;f_Spirochaetaceae;g_Treponema
- k_Bacteria;p_TM7;c_TM7-3;o_EW055;f_;g_
- k_Bacteria;p_WWE1;c_[Cloacamonae];o_[Cloacamonales];f_[Cloacamonaceae];g_W22
- Taxa <8%

Archaeal populations

Methanocorpuscollum = H₂/CO₂

Methanospirillum

Methanosaeta



- VIP1
 - VIP5
 - VIP2
 - VIP6
 - VIP3
 - VIP7
 - VIP4
 - VIP8
 - PF1
 - PF2
 - PF3
 - PF4
 - PF5
 - PF6
 - PF7
 - PF8
 - PF9
 - PF10
 - PF11
 - PF12
- k__Archaea;p__[Parvarchaeota];c__[Parvarchaea];o__WCHD3-30;f__;g__
 - k__Archaea;p__[Parvarchaeota];c__[Parvarchaea];o__YLA114;f__;g__
 - k__Archaea;p__Crenarchaeota;c__MCG;o__pGrfC26;f__;g__
 - k__Archaea;p__Crenarchaeota;c__Thaumarchaeota;o__Cenarchaeales;f__Cenarchaeaceae;g__
 - k__Archaea;p__Crenarchaeota;c__Thaumarchaeota;o__Cenarchaeales;f__Cenarchaeaceae;g__Nitrosopumilus
 - k__Archaea;p__Crenarchaeota;c__Thaumarchaeota;o__Cenarchaeales;f__SAGMA-X;g__
 - k__Archaea;p__Euryarchaeota;c__Methanobacteria;o__Methanobacteriales;f__Methanobacteriaceae;g__Methanobrevibacter
 - k__Archaea;p__Euryarchaeota;c__Methanobacteria;o__Methanobacteriales;f__WSA2;g__
 - k__Archaea;p__Euryarchaeota;c__Methanomicrobia;o__Methanomicrobiales;f__Methanocorpusculaceae;g__Methanocorpusculum
 - k__Archaea;p__Euryarchaeota;c__Methanomicrobia;o__Methanomicrobiales;f__Methanomicrobiaceae;g__Methanoculleus
 - k__Archaea;p__Euryarchaeota;c__Methanomicrobia;o__Methanomicrobiales;f__Methanomicrobiaceae;g__Methanogenium
 - k__Archaea;p__Euryarchaeota;c__Methanomicrobia;o__Methanomicrobiales;f__Methanospirillaceae;g__Methanospirillum
 - k__Archaea;p__Euryarchaeota;c__Methanomicrobia;o__Methanosarcinales;f__Methanosaetaceae;g__Methanosaeta
 - k__Archaea;p__Euryarchaeota;c__Methanomicrobia;o__Methanosarcinales;f__Methanosarcinaceae;g__Methanimicrococcus
 - k__Archaea;p__Euryarchaeota;c__Methanomicrobia;o__Methanosarcinales;f__Methanosarcinaceae;g__Methanosarcina
 - k__Archaea;p__Euryarchaeota;c__Thermoplasmata;o__E2;f__[Methanomassiliicoccaceae];g__vadinCA11

Findings

- Unique insights using Illumina sequencing- first study on PF
- Microbial community differences between VIP and PF latrines
- Primary driver in difference are salts (potassium)
- More anaerobic organisms at bottom layers of VIP, consistent with model
- A variety of degradation processes can be identified:
 - carbohydrate, protein, fermentation, methanogenesis, sulfide oxidation
- More acetoclastic methanogenesis in VIPs, so pathway is through acetate
- Methanogenesis in PF is through hydrogen