

Release Notes 2021-11-08 : This is a slightly reorganized version of a draft from 2018. It took on more significance after a discussion on LinkedIn regarding plant-based dog food and my posts getting deleted after mentioning Sarcina which is thought to be an issue with vegetarian diets. It is also now the victim of a sudden time constraint. This paper provides some background information for later works (while referencing prior unpublished works) discussing the health of some dogs as it relates mostly to diet but also to soil exposure. One dog, Moe, is discussed in more depth due to Sarcina detection and his death from bloat. Sarcina remains an open research topic and detection (of two species in dogs) itself may be noteworthy in conjunction with the dogs' play-area soil parameters. The rest of this looks at all the unknowns in the soil, which did not appear to be mito/chloroplast, and a sequence which appears common to many unknowns here and on the NCBI databases. As a technical report, it contains a lot of data and method details, failures and criticisms, not found in scientific papers. The "Results" wander into the Discussion as they are derivative results that make a point about the Discussion as it wandered. Note that the original draft was more or less complete but a lot of errors not fixed and they may have been forgotten in the last few years and missed in this update. **For information only, not for any particular purpose. Intended for audiences familiar with the topic and controversial issues. Caveat Emptor**

16S rRNA Analysis of 3 Canine Fecal Samples and the Dogs' Play-Area Soil

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This technical report explores the bacterial contents of three canine stool samples and one sample of the dogs' play-area soil. It documents some highlights of the fecal results in the context of the illnesses the dogs experienced. All of them had some GI disturbances at the time that were also likely present at their deaths. As each case is rather involved, this report focuses on presenting the 16S rRNA results related to existing literature and delegates the detailed case studies to follow on works. The single soil sample is considered in more depth here as the major issues are self contained or are a diversion from the main focus of the overall effort. Diet and soil exposure were considered as factors potentially effecting the fecal samples. All dogs were fed commercial products along with cooked and raw meats and various vitamin supplements which can be compared to prior works. With the benefit of additional experience in the intervening years, these diets can be related to more successful outcomes from modified diets developed later. One of the dogs, Moe, who later was rendered moribund by bloat, had interesting reads for taxa possibly relating to the soil including Romboutsia, Sarcina ventriculi, Turicibacter, and Helicobacter with more Peptoclostridium than the other dogs. The rest of this work discusses the soil results given the existence of scattered waste and recent applications of algeacide and copper, and the winder sampling time. The soil reads were notable for a large number of unidentified sequences but results could still be compared to published works with various earth environments such as landfills and fields. Many of the unidentified reads had surprisingly good similarity to Limisphaera rather than mitochondria or chloroplasts , of unknown significance, using an exact string matching technique. Two subsequences of Limisphaera were identified which blasted largely to uncultured bacteria. A partial subsequence appears in known common soil bacteria including Pseudomonas.

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1. INTRODUCTION

Microbiome has become a popular research topic especially as it relates to human diet and health. Canine microbiome has been investigated [74] [96] most often versus commercial and raw diets. Outdoor dogs can eat a variety of substances including soil or items contaminated with soil possibly exposing them to important groups of organisms independent of intended diet. The microbiomes of many environmental niches have also been explored and published as some excellent online resources.

One popular tool for analysis of microbial populations is 16S rRNA, the method used in this work. While very good, it has some fundamental and practical limitations [87]. Practical issues include the sample handling, preparation, and processing along with quality control issues common to many lab procedures. Fundamental limitations are largely due to the use of one small DNA sequence to infer the abundance of a particular organism or worse assume a particular phenotype or "other genes" in chromosomes and plasmids that normally correlate with a given 16S sequence. A recent investigation into a pathogenic role for Sarcina in chimpanzee GI diseases illustrates many of the issues [70]. Ideally, it is a survey tool and is unbiased toward any particular bacteria that provides context for focused efforts to find specific less common pathogens.

One problem with relating soil to health is the variable nature of the soil and difficulties finding all viable low abundance organisms. Many microbiome analyses discuss the predominant organisms or taxa groups but ignore low abundance ones even though these may be significant pathogens if present at all. This work begins to motivate an interest in more comprehensive approaches to relating soil to dog health but leaves that actual work for the future.

The present work documents results of fecal 16S rRNA from 3 dogs with varying GI abnormalities and a single soil sample from their play area in which they would routinely dig and eat. Their diet was a mixture of common components plus vitamins but perhaps noteworthy for inclusion of D-serine and large amounts of vitamin K. As a technical report, this is largely a data reference to hopefully be used in later works. However, one of the dogs, Moe, illustrates the possible importance of rare soil dwelling organisms thought to be pathogenic and the possible issues with soil sampling.

This work also investigates the relatively large number of unidentified reads from the soil sample using exact string matching to isolate some sequences that appear to segregate with unidentified sequences in the NCBI database at the time (2018). While the technique produced variable results, eventually two subsequences from Limisphaera seemed to be common to many of the unknowns while also failing to BLAST against anything other than uncultured bacteria. This is surprising as Limisphaera, while a member of phylum Verrucomicrobia, is a thermophile [5]. A partial sequence appears to be common in soil bacteria including Pseudomonas which may be interesting in light of its adaptability to soil treatments including copper [64] [33] and quats [43] [90] which had been applied some time prior to sampling. Pseudomonas may make an interesting OTU which may interact strongly with soil treatments and diet/vitamins. For example, L-serine was found to inhibit an important virulence factor in *P. aeruginosa* [68], but depending on genetics, it could grow solely on d-serine [44] and research interest continues on d-serine's mechanism against other organisms [71].

Thinking outloud

2. METHODS

2.1. 16S RNA Sample Procedures

Fecal samples were collected immediately after excretion and placed in DNA/RNA shield vials provided by Zymo Research. Care was taken during fecal sample collection to avoid including any material which had touched the soil and only Greta's sample was collected from the play area. They were shipped within a few days. The 16S RNA analysis was performed by Zymo Research using their normal commercial procedures including a bead-beating step for extraction and Illumina sequencing. Two runs were performed as the first pass did not give satisfactory results for Moe. The differences in analysis pipeline are noted in the discussion.

Thinking outloud

this is just copied from Emily's email, need to integrate and explore abundance biases and extraction in light of culturability and persister states etc

As per the company [17],

Materials and Methods The samples were processed and analyzed with the ZymoBIOMICST Service - Targeted Metagenomic Sequencing (Zymo Research, Irvine, CA).

DNA extraction: One of three different DNA extraction kits was used depending on the sample type and sample volume. In most cases, the ZymoBIOMICST DNA Miniprep Kit (Zymo Research, Irvine, CA) was used. For low biomass samples, such as skin swabs, the ZymoBIOMICST DNA Microprep Kit (Zymo Research, Irvine, CA) was used as it permits for a lower elution volume, resulting in more concentrated DNA samples. For a large sample volume, the ZymoBIOMICST-96 MagBead DNA Kit (Zymo Research, Irvine, CA) was used to extract DNA using an automated platform.

16S library preparation: Bacterial 16S ribosomal RNA gene targeted sequencing was performed using the Quick-16ST NGS Library Preparation Kit (Zymo Research, Irvine, CA). The bacterial 16S primers used amplified the V3-V4 region of the 16S rRNA gene. These primers have been custom-designed by Zymo Research to provide the best coverage of the 16S gene while maintaining high sensitivity. They amplify the V3-V4 region of the 16S rRNA gene. The sequencing library was prepared using an innovative library preparation process in which PCR reactions were performed in real-time PCR machines to control cycles and therefore prevent PCR chimera formation. The final PCR products are quantified with qPCR fluorescence readings and pooled together based on equal molarity. The final pooled library was cleaned up with Select-a-Size DNA Clean & ConcentratorT (Zymo Research, Irvine, CA), then quantified with TapeStation and Qubit.

Sequencing: The final library was sequenced on Illumina MiSeqT with a v3 reagent kit (600 cycles). The sequencing was performed with \sim 10% PhiX spike-in.

Bioinformatics analysis: Amplicon sequences were inferred from raw reads using the Dada2 pipeline (Callahan et al, 2016) [12]. Chimeric sequences were also removed with the Dada2 pipeline. Taxonomy assignment, composition barcharts, alpha-diversity and beta-diversity analyses were performed with Qiime v.1.9.1 (Caporaso et al., 2010)[13]. Taxa that have an abundance significantly different among groups were identified by LEfSe (Segata et al., 2011)[85] with default settings if applicable. Other analyses were performed with in-house scripts.

2.2. Diet

Histories were recorded for diet, vitamins, and some observations and outcomes in a text format. This work pre-dates MUQED [56] although the format and software are earlier versions that led to MUQED. Commercial pet foods, foods and vitamins for human consumption were mixed with specific rules that have only been partially published [49] [57] [55] [54]. All products with an ingredient list were verified to be safe for dogs at least twice even on repeat purchases as details may vary between purchases. Details are given as needed for each dog in the following sections.

2.3. Analysis

Much of the analysis of the fecal samples compares and contrasts the more abundant OTU's in the sample set (including the soil sample) with rare sequence analysis consisting of reads that segregated with a subset of samples (detectable or non-detectable).

The global gross population properties were summarized with a simple entropy or diversity calculation having various names within different fields, (logs taken to base 2 creating "units of bits") ,

$$\eta = \sum p_i * \log_2 (p_i) \quad (1)$$

Further analysis relied on general distribution of the dominant OTU's at varied taxonomic levels as needed for comparison with other work. No principal component or similar analysis has been performed.

Thinking outloud

I'm not sure the linear methods like PCA or LDA really help here although it is nice when you can get clusters that separate well on a 2D plot. Maybe with linear combinations of $\log[n]$ although need to see if linear is normally used

Several prior works on dog fecal microbiome related to disease and diet were compared to the second run OTU's provide by the Zymo pipeline. Plots of the log abundance of various select OTU's from the different sources were made. Two sources were generally used for the soil comparisons, the Earth Microbiome Project (EMP) [92] and one work describing geographically distributed landfills [86]. This was complicated by taxonomical issues and lack of low abundance organisms in prior discussions.

The unassignable reads were compared to various groups of organisms using a developmental exact string matching (ESM) technique as described in unpublished manuscripts [52] [53] although the details are not of much significance at this point. Briefly, it only identifies exact matches of 8 or more consecutive bases without regard to alignment. Tests with known samples against knowns generally appeared as accurate as the commercial pipeline assignments, at least to the genus level, with a few significant errors that do not appear to be relevant here. The details of the method are not needed here but rather the significance is in the specific patterns they found that can be explored with better means. The groups of "known" sequences used for resolution of these unknowns included random generated sequences, the NCBI 16S database, the entire NCBI nucleotide data base, sets of chloroplast and mitochondrial hits, and the other unknown sequences although only a few results are presented here.

3. CASES

16S rRNA analysis was performed on fecal samples from three rescue dogs living at a residence in rural Northern Georgia, USA, as well as on one soil sample from their play area. Applicable details of the sample collections are in Appendix D. The soil is situated in the backyard of the residence which was largely shaded at the time and occupied by 8 or more dogs . It is largely clay with some inclusions of rubbish remnants suggestive of prior use as a landfill. It had been recently cultivated slightly after being left unattended for a few months and had been sprayed about 1 year ago with a copper sulfate and quaternary algeacide mixture. The surface consisted of common weeds with various grasses as well as many bare areas. It was rather heterogeneous being exposed clay in elevated areas and containing more organic matter at bottom of slopes with new debris being exposed largely near tops of slopes. The sample was collected in a low area decorated by many mole tunnels .

The three dogs are Greta, Peapod, and Moe. Greta and Peapod are described somewhat more in earlier unfinished manuscripts [50][51] but will hopefully be the basis for follow on works that reference this one. All dogs received similar diets varied in response to possible needs. Most of the time the "raw snacks" were given that consisted of beef, boiled chicken thighs, Eggland hard-boiled eggs, grated carrot and cooked spinach with olive oil, lecithin, KCl and citric acid commonly added [49]. Common supplements included most B vitamins with emphasis on SMVT substrates, vitamin K2, taurine, d-serine, arginine, lecithin, copper, and garlic. Additional vitamins chosen daily were given individually or to all as recorded with excerpts shown in Appendix D and monthly summarizies in Appendix E.



FIG. 1: Moe, undated photograph courtesy of Barbara Cade

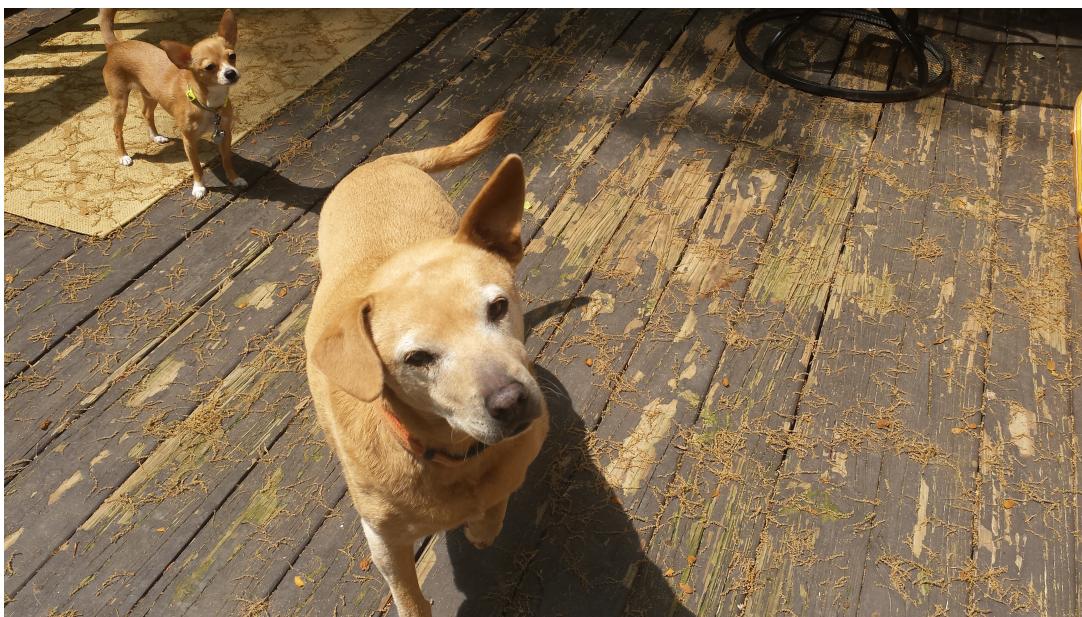


FIG. 2: Greta , 2017-04-14, courtesy of Barbara Cade



FIG. 3: Peapod , 2017-04-29, courtesy of Barbara Cade

	Greta	Moe	Peapod	Soil
Born	2005-07	2005-11	2000	
Sample Date	2018-01-15	2018-01-15	2018-01-15	2018-01-05
Put to Sleep	2020-10-09	2018-08-13	2018-04-16	
Afflictions	plasmacytoma uveitis	chronic diarrhea fluid sacs	blood streaked poop possible brain infection	household waste moles

TABLE I: Brief summary of the dogs and soil from which samples were obtained.

Briefly Greta had intermittent uveitis largely in right eye as well as a plasmacytoma removed after observation of rectal bleeding. She had a mixed raw-kibble diet with no vitamins between surgery and collection. She also had perioperative cephalexin. Prior to surgery she had been getting the vitamin enriched diet similar to Moe.

Peapod had a variety of conditions including possible oral and brain infection. She had blood streaks in her stool from time to time. Her diet was largely raw, vitamin enriched, with intermittent kibble as noted in the appendix.

Moe's diet was a mix of kibble and raw with vitamins similar to Peapod. He was generally in good health except for some skin bubbles that were surgically removed. Locations were front paw and rear legs with one pair possibly growing back. The growth on his front paw, and possible others, predated his exposure to the soil and not all of them appeared to inflate as much as one on his rear leg. He also had chronic diarrhea at the time of sampling. Later he had apparently insignificant abdominal distention until he suddenly developed from X-ray diagnosed moribund bloating.

Their exposure to the soil varied significantly between them. A year or so prior to this work Peapod would commonly eat feces and yard dirt but her activity decreased as she declined. Greta consumed feces and dug significantly during most of the period prior to the sampling. Moe was the most energetic digger and would eat grass but not so much feces.

4. RESULTS

As noted above, pipelines differs slightly between the two runs giving different results for all samples except Moe who only ran the second time. Generally only the latter run is reported except where noted to examine pipeline effects. This is also discussed in procedures used to check the known and unknowns with a novel assignment method based on exact string matching.

The first table, Table II, is a brief summary of the total number of different OTU's and reads found for each sample as well as entropies calculated from read and OTU probabilities (log base is 2 and entropy is in bits). The soil contains a large number of reads but many were assigned to a single "unknown" OTU. Moe and Peapod has similar statistics and Greta, with prior antibiotic exposure, slightly lower entropy.

Name	soil	peapod	moe	greta
Richness, number of OTU's	296	39	46	33
OTU entropy	6.58	4.28	4.27	3.72
Richness, number of reads	1063	91	86	46
read entropy	9.68	5.32	5.07	4.29

TABLE II: Simple count and entropies. This is of questionable utility except for a quick sanity check. The entropy is normal definition, $H = -\sum p_i \log_2 (p_i)$. The soil is quite diverse even with many reads lumped into a few unknowns. Moe and Peapod remarkably similar and Greta much lower as may be expected from recent antibiotic exposure.

Name	soil	peapod	moe	greta
p..Actinobacteria	0.218	.023	.0132	.0323
p..Acidobacteria	0.188			
p..Acidobacteria;o..Subgroup 6;f..NA	0.0968			
None;Other;Other;Other;Other;Other;Other	0.0935			
p..Verrucomicrobia	0.0599			
p..Chloroflexi;o..NA;f..NA	0.0342			
o..Gaiellales;f..NA	0.0341			
o..Chthoniobacterales;f..DA101 soil group	0.0272			
g..Variibacter;s..NA	0.0237			
o..Acidimicrobiales;f..NA	0.0212			
o..Rhizobiales;f..Xanthobacteraceae	0.0184			
c..Betaproteobacteria;o..SC-I-84;f..NA	0.018			
g..Gaiella;s..NA	0.0179			

TABLE III: Most common OTU's identified in soil. Note that many were not identifiable to any precision. Subgroups are subsets of Acidobacteria.

Name	soil	peapod	moe	greta
g..Alloprevotella;s..NA		0.16	0.111	
g..Fusobacterium;s..perfoetens		0.108	0.105	0.0665
g..Megamonas;s..funiformis		0.0971	0.0584	0.163
g..Prevotella;s..NA		0.0827		
g..Bacteroides;s..plebeius		0.0569	0.0322	0.0933
g..Bacteroides;s..NA		0.054	0.12	0.1
g..Fusobacterium;s..mortiferum		0.0525	0.0704	0.181
g..Blautia;s..NA		0.0364	0.0172	0.0842
g..Phascolarctobacterium;s..NA		0.0328	0.0218	
g..Lachnoclostridium;s..NA		0.0327	0.0286	0.0263

TABLE IV: Most common OTU's identified in Peapod. All of the dogs had a pretty well defined set of OTU's with no unknowns.

Name	soil	peapod	moe	greta
g..Bacteroides;s..NA		0.054	0.12	0.1
g..Clostridium;s..perfringens	0.000901	0.0165	0.114	0.00194
g..Alloprevotella;s..NA		0.16	0.111	
g..Fusobacterium;s..perfoetens		0.108	0.105	0.0665
g..Peptoclostridium;s..NA		0.0318	0.0783	0.0397
g..Fusobacterium;s..mortiferum		0.0525	0.0704	0.181
g..Megamonas;s..funiformis		0.0971	0.0584	0.163
g..Sutterella;s..stercoricanis		0.0141	0.0405	0.0117
g..Bacteroides;s..plebeius		0.0569	0.0322	0.0933
g..Lachnoclostridium;s..NA		0.0327	0.0286	0.0263

TABLE V: Most common OTU's identified in Moe. This misses the most interesting suspected pathogens.

Name	soil	peapod	moe	greta
g..Fusobacterium;s..mortiferum		0.0525	0.0704	0.181
g..Megamonas;s..funiformis		0.0971	0.0584	0.163
g..Bacteroides;s..NA		0.054	0.12	0.1
g..Bacteroides;s..plebeius		0.0569	0.0322	0.0933
g..Blautia;s..NA		0.0364	0.0172	0.0842
g..Fusobacterium;s..NA		0.0239	0.0279	0.071
g..Fusobacterium;s..perfoetens		0.108	0.105	0.0665
g..Peptoclostridium;s..NA		0.0318	0.0783	0.0397
g..Blautia;s..hansenii-producta		0.0202	0.0119	0.0377
g..Collinsella;s..intestinalis-stercoris		0.0207	0.011	0.0316

TABLE VI: Most common OTU's identified in Greta. Greta had recently had antibiotics after her plasmacytoma surgery and this is what grew back.

Relative rankings of the more common OTU's are shown in Table VII. At some point all the OTU's identified in the dogs are shown but many lower abundance soil contents are not referenced.

Name	soil	peapod	moe	greta
g..Megamonas;s..funiformis		3	7	2
g..Fusobacterium;s..mortiferum		7	6	1
g..Fusobacterium;s..perfoetens		2	4	7
g..Bacteroides;s..NA		6	1	3
g..Alloprevotella;s..NA		1	3	
g..Bacteroides;s..plebeius		5	9	4
g..Peptoclostridium;s..NA		11	5	8
g..Blautia;s..NA		8	14	5
g..Clostridium;s..perfringens	160	20	2	21
g..Fusobacterium;s..NA		13	11	6
o..Subgroup 6;f..NA	1			
None;Other;Other;Other;Other;Other	2			
g..Lachnoclostridium;s..NA		10	10	12
g..Prevotella;s..NA		4		
g..Blautia;s..hansenii-producta		16	16	9
g..Sutterella;s..stercoricanis		21	8	14
g..Collinsella;s..intestinalis-stercoris		15	18	10
g..Bacteroides;s..coprocola		18	22	11
g..Phascolarctobacterium;s..NA		9	13	
g..Blautia;s..glucerasea		14	25	17
o..NA;f..NA	3			
o..Gaiellales;f..NA	4			
g..Lachnoclostridium-Pseudobutyribrio;s..NA		23	29	13
g..Allobaculum;s..stercoricanis		19	24	20
g..Succinivibrio;s..NA		12	39	
o..Chthoniobacterales;f..DA101 soil group	5			
g..Helicobacter;s..bilis-canis			12	
g..Variibacter;s..NA	6			
g..Bacteroides;s..vulgatus		17	34	
o..Acidimicrobiales;f..NA	7			

TABLE VII: Some more common OTU's and ranks in each sample. Note that the ranks exclude manually entered higher level sums such as total of a given phylum when it was needed for the later discussion.

OTU's are shown in the following tables grouped by their presence of absence in a given set of sample. A total of 16 such groups would be possible but not all of them are populated. The tables Table VIII through Table XXII show the most important ones.

Name	soil	peapod	moe	greta
o..Subgroup 6;f..NA	0.0968	0	0	0
None;Other;Other;Other;Other;Other;Other	0.0935	0	0	0
o..NA;f..NA	0.0342	0	0	0
o..Gaiellales;f..NA	0.0341	0	0	0
o..Chthoniobacterales;f..DA101 soil group	0.0272	0	0	0
g..Variibacter;s..NA	0.0237	0	0	0
o..Acidimicrobiales;f..NA	0.0212	0	0	0
o..Rhizobiales;f..Xanthobacteraceae	0.0184	0	0	0
o..SC-I-84;f..NA	0.018	0	0	0
g..Gaiella;s..NA	0.0179	0	0	0
o..Nitrosomonadales;f..Nitrosomonadaceae	0.0167	0	0	0
g..Gemmatimonas;s..NA	0.0166	0	0	0
g..Haliangium;s..NA	0.0158	0	0	0
g..Candidatus;s..NA	0.0155	0	0	0
o..Gemmatimonadales;f..Gemmatimonadaceae	0.0152	0	0	0
g..Candidatus;s..Koribacter	0.0151	0	0	0
o..NA;f..NA	0.0147	0	0	0
g..Afipia-Bradyrhizobium;s..NA	0.0118	0	0	0
o..Rhodospirillales;f..DA111	0.0114	0	0	0
g..Sphingomonas;s..jaspsi	0.0106	0	0	0
o..Solirubrobacterales;f..480-2	0.00933	0	0	0
g..Chthoniobacter;s..NA	0.00913	0	0	0
o..Subgroup 7;f..NA	0.00904	0	0	0
o..Subgroup 17;f..NA	0.0085	0	0	0
g..Bryobacter;s..NA	0.00814	0	0	0
g..Flavobacterium;s..NA	0.0079	0	0	0
o..NA;f..NA	0.00777	0	0	0
g..Blastocatella;s..NA	0.00751	0	0	0
g..Pseudonocardia;s..NA	0.00682	0	0	0

TABLE VIII: Most common OTU's occurring only in soil sample. 291 OTU's were listed these are the top few. Again Subgroups are subsets of Acidobacteria.

Name	soil	peapod	moe	greta
g..Prevotella;s..NA	0	0.0827	0	0
g..Prevotella;s..copri	0	0.00603	0	0
g..Oscillospira;s..NA	0	0.000565	0	0
g..Ruminiclostridium;s..NA	0	0.00047	0	0
g..Bacteroides;s..uniformis	0	0.000391	0	0
g..Bacteroides;s..clarus	0	0.0002	0	0

TABLE IX: All OTU's occurring only in Peapod sample

Name	soil	peapod	moe	greta

TABLE X: No OTU's were shared only with Peapod and soil exclusively.

Name	soil	peapod	moe	greta
g..Helicobacter;s..bilis-canis	0	0	0.0242	0
g..Bacteroides;s..stercoris	0	0	0.00119	0
g..Sarcina;s..ventriculi	0	0	0.000929	0
g..Terrisporobacter;s..glycolicus-mayombei	0	0	0.00069	0
g..Ruminococcaceae;s..NA	0	0	0.000305	0

TABLE XI: All OTU's occurring only in Moe. Note that these are also very interesting organisms.

Name	soil	peapod	moe	greta
o..Clostridiales;f..Peptostreptococcaceae	0.000361	0	0.0119	0
g..Romboutsia;s..NA	0.00108	0	0.00081	0
g..Turicibacter;s..sanguinis	0.00113	0	0.000451	0

TABLE XII: OTU's were shared only with Moe and soil. Note that Romboutsia is an interesting candidate for the gaseous odorless bubbles on Moe.

Name	soil	peapod	moe	greta
g..Alloprevotella;s..NA	0	0.16	0.111	0
g..Phascolarctobacterium;s..NA	0	0.0328	0.0218	0
g..Succinivibrio;s..NA	0	0.0265	0.000849	0
g..Bacteroides;s..vulgatus	0	0.0197	0.00155	0
g..Roseburia;s..NA	0	0.0106	0.00223	0
g..[Ruminococcus];s..NA	0	0.00234	0.00119	0

TABLE XIII: OTU's shared only with Moe and Peapod.

Name	soil	peapod	moe	greta
g..Clostridium;s..NA	0.000676	0.00164	0.00792	0

TABLE XIV: Single OTU shared only with Moe, Peapod and soil.

Name	soil	peapod	moe	greta
g..Faecalitalea;s..NA	0	0	0	0.00167

TABLE XV: Single OTU unique to Greta

Name	soil	peapod	moe	greta

TABLE XVI: No OTU's shared only by Greta and soil

Name	soil	peapod	moe	greta
g..Sutterella;s..wadsworthensis	0	0.00752	0	0.00139

TABLE XVII: Single OTU shared only by Greta and Peapod

Name	soil	peapod	moe	greta

TABLE XVIII: No OTU's shared only by Greta and Peapod and soil only

Name	soil	peapod	moe	greta
g..Faecalibacterium;s..prausnitzii	0	0	0.00941	0.0106
g..Clostridium;s..baratii	0	0	0.0104	0.00263
g..Sarcina;s..maxima	0	0	0.00973	0.000617
g..Erysipelatoclostridium;s..NA	0	0	0.0046	0.00108
o..Clostridiales;f..Lachnospiraceae	0	0	0.000703	0.00162
g..Lachnospiraceae;s..NA	0	0	0.000584	0.000499

TABLE XIX: OTU's shared by only Moe and Greta. Interestingly Greta also had a Sarcina.

Name	soil	peapod	moe	greta

TABLE XX: No OTU shared by only Moe, Greta and soil

Name	soil	peapod	moe	greta
g..Megamonas;s..funiformis	0	0.0971	0.0584	0.163
g..Fusobacterium;s..mortiferum	0	0.0525	0.0704	0.181
g..Fusobacterium;s..perfoetens	0	0.108	0.105	0.0665
g..Bacteroides;s..NA	0	0.054	0.12	0.1
g..Bacteroides;s..plebeius	0	0.0569	0.0322	0.0933
g..Peptoclostridium;s..NA	0	0.0318	0.0783	0.0397
g..Blautia;s..NA	0	0.0364	0.0172	0.0842
g..Fusobacterium;s..NA	0	0.0239	0.0279	0.071
g..Lachnoclostridium;s..NA	0	0.0327	0.0286	0.0263
g..Blautia;s..hansenii-producta	0	0.0202	0.0119	0.0377
g..Sutterella;s..stercoricanis	0	0.0141	0.0405	0.0117
g..Collinsella;s..intestinalis-stercoris	0	0.0207	0.011	0.0316
g..Bacteroides;s..coprocola	0	0.0186	0.00893	0.0308
g..Blautia;s..glucerasea	0	0.0215	0.00743	0.00538
g..Lachnoclostridium-Pseudobutyryvibrio;s..NA	0	0.00772	0.00385	0.0165
g..Allobaculum;s..stercoricanis	0	0.0175	0.00752	0.00261
g..Clostridium;s..colicanis	0	0.00129	0.0158	0.0036
g..Sutterella;s..NA	0	0.00738	0.00183	0.00835
o..Clostridiales;f..Ruminococcaceae	0	0.000939	0.00721	0.00063
g..Erysipelatoclostridium;s..spiroforme	0	0.000583	0.00419	0.00067
g..Slackia;s..piriformis	0	0.00223	0.00226	0.000722
g..Butyricicoccus;s..pullicaeorum	0	0.00171	0.00191	0.000657
g..Erysipelatoclostridium;s..ramosum	0	0.0025	0.000385	0.000696
g..Lachnoclostridium-Lachnospiraceae;s..NA	0	0.000957	0.00121	0.0013

TABLE XXI: OTU's shared by all dogs and not in soil

Name	soil	peapod	moe	greta
g..Clostridium;s..perfringens	0.000901	0.0165	0.114	0.00194

TABLE XXII: Single OTU common to all samples

Also of interest is the set of OTU's in the soil that occur in at least one dog .These are tabulated in Table **XXIII**.

Name	soil	peapod	moe	greta
g..Clostridium;s..perfringens	0.000901	0.0165	0.114	0.00194
g..Clostridium;s..NA	0.000676	0.00164	0.00792	0
o..Clostridiales;f..Peptostreptococcaceae	0.000361	0	0.0119	0
g..Romboutsia;s..NA	0.00108	0	0.00081	0
g..Turicibacter;s..sanguinis	0.00113	0	0.000451	0

TABLE XXIII: Soil OTU's in at least one dog. Note that these are low abundance and some prior works tend to dismiss these but see text on possible importance.

Name	soil	peapod	moe	greta	Comment
g..Clostridium;s..perfringens	0.000901	0.0165	0.114	0.00194	
g..Clostridium;s..NA	0.000676	0.00164	0.00792	0	unknown suspect
g..Peptoclostridium;s..NA		0.0318	0.0783	0.0397	pathogen
g..Romboutsia;s..NA	0.00108	0	0.00081	0	gas former
g..Sarcina;s..ventriculi	0	0	0.000929	0	bloating, gas former
g..Helicobacter;s..bilis-canis	0	0	0.0242	0	pathogen
g..Turicibacter;s..sanguinis	0.00113	0	0.000451	0	unknown

TABLE XXIV: Suspicious organisms in Moe and abundances in other samples.

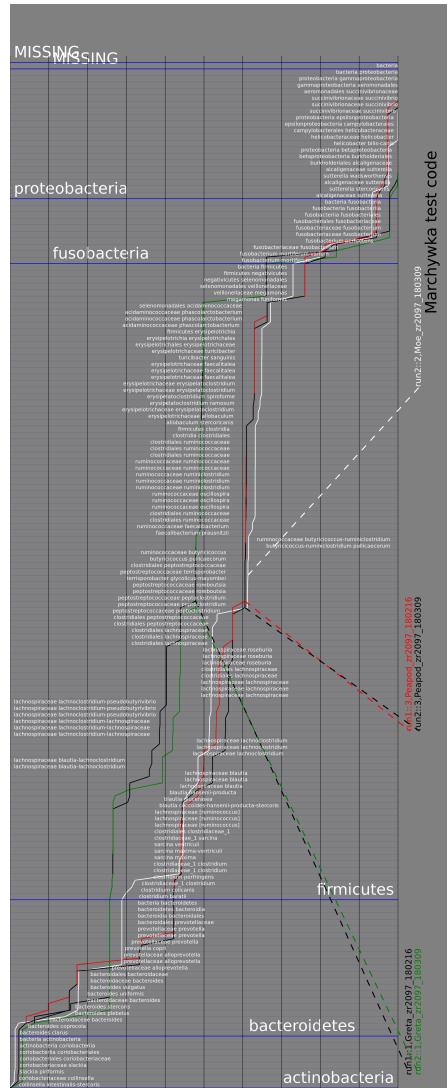


FIG. 4: Species level cumulative organism percentages from run2 for each dog . In the original svg, there is detail at many levels allowing easy zoom to see trends or specific organisms identified.

Thinking out loud

Some of the SVG's appear to have the text shifted one pixel on my eog viewer. This is a problem when the organism columns are only one "pixel" wide lol.

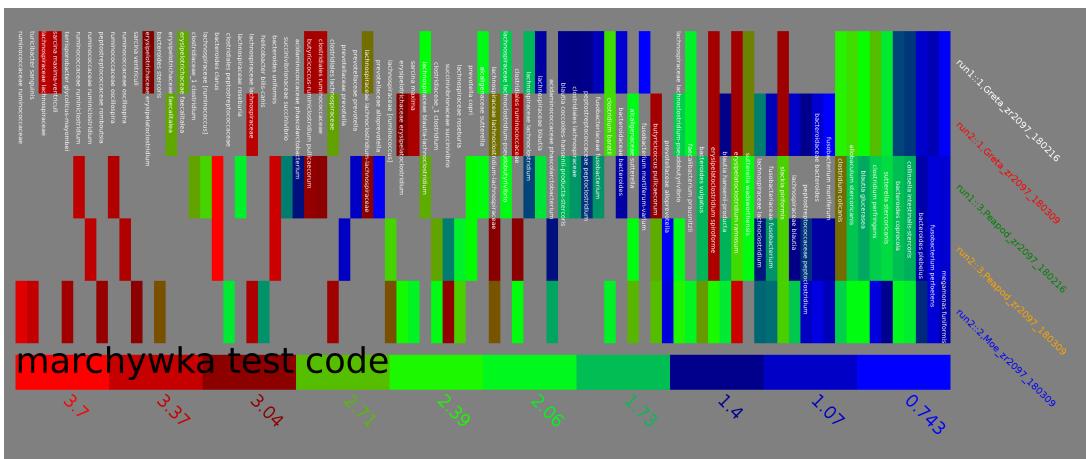


FIG. 5: Organism distribution in dogs showing comparative distributions and run to run variations as pipeline changed. Blue is most abundant and numerical scale is negative log abundance base 10 .

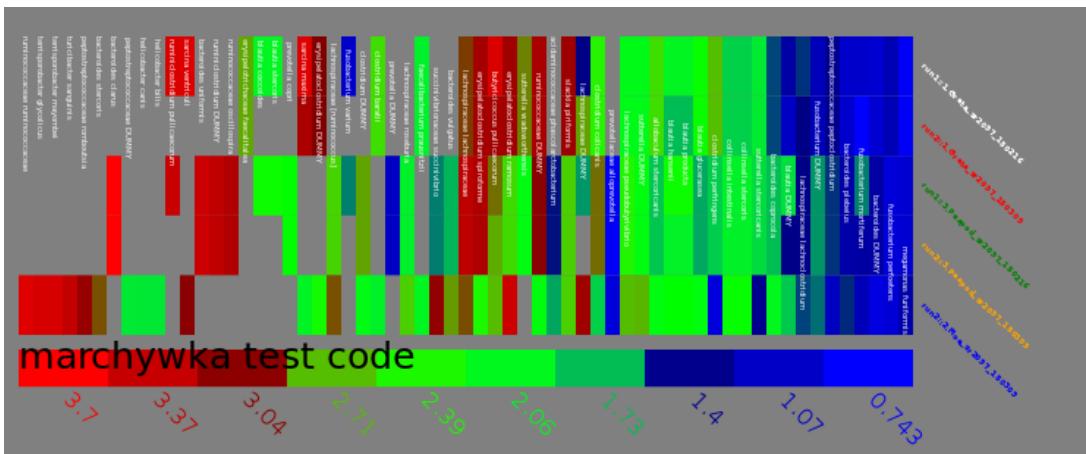


FIG. 6: As above but with names made more useful using options to remove meaningless placeholders like NA or unidentified. Genus and species are not always available so the most specific identifiable taxonomic level is used. Blue is most abundance and numerical scale is negative log abundance base 10 although svg alignment may be off .

The distributions are shown graphically in a few different ways. Some detail is lost to the png format but the original svg files are available see Supplemental Information.

Cumulative abundances, similar to a survival curve, are shown in Fig. 4. The original svg is more useful as the annotations are visible although under some conditions there may be an "off by one issue" that confuses the labels.

The two runs are also compared in the frequency plots shown in Fig. 5. The two sets of data for each sample except Moe reflect different pipelines applied to the same set of reads.

Some of the difference reflects small changes in species level detail, some is just an artifact from changing "NA" to "unidentified" that this software did not remove when finding meaningful names.

As explained more fully in the discussion, post hoc exploration of the unknown soil reads with developmental exact string matching code demonstrated a robust similarity to a thermophilic Limisphaera. The following results demonstrate alignments in the form of vertical-sized dot-plots. As the sequences are nominally already aligned as 16S rRNA, the points of agreement should remain vertical and level although some shifts are noted and of course areas of difference exist. Only areas of 8 or more consecutive base matches are considered here but there is no bound on difference in location generating short vertical lines at various offsets from the main. Total hits for each base are indicated by the blue histogram at the top.

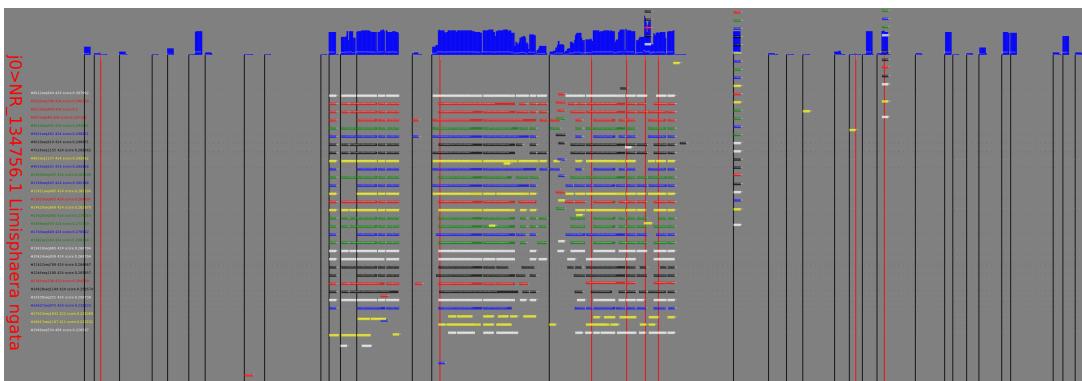


FIG. 7: Selected example of unknown read that matches well to *Limisphaera*. This a dot plot modified to make alignment horizontal rather than diagonal with shifts becoming vertical offsets. Only strings of length 8 or greater are considered hits rather than individual base matches. Note that the alignments are more or less of constant offset with little indel apparent.

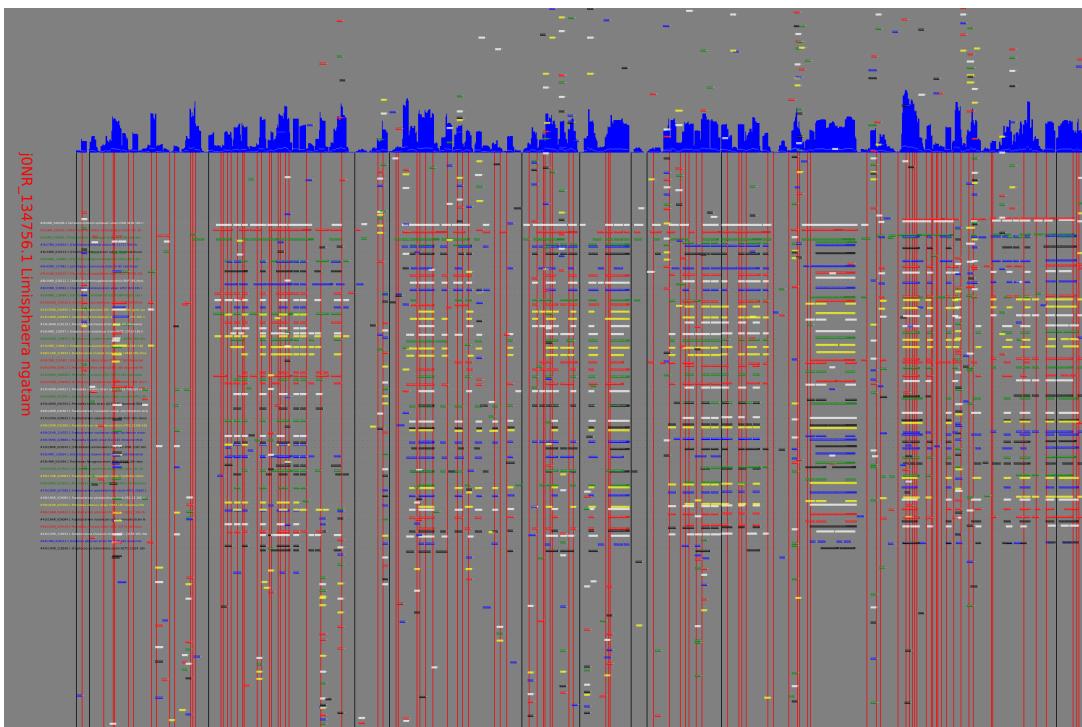


FIG. 8: *Limisphaera* horizontal dot plots to best matches. Generally they seem to shift in same direction which seems a bit surprising as you would expect similar insert and delete amounts. The shifts are often separated by areas lacking matches and some of the areas contain short complementary C/G strings which may be associated with polymerase slippage.

Frequency	Organism
34	Garcilla:nitratireducens
27	Uncultured:Limisphaera
23	Uncultured:Pedosphaera
13	Uncultured:Verrucomicrobia
7	Aetherobacter:rufus
7	Desulfofaba:gelida
7	Jahnella:thaxteri
7	Racemicystis:persica
6	Occallatibacter:riparius
6	Uncultured:Conexibacteraceae

Entry	Organism Description
QNRS01000042.1	Garcilla nitratireducens strain GHB3 Ga0255681_142, whole genome shotgun sequence
MF002263.1	Uncultured Limisphaera sp. clone 716_51 16S ribosomal RNA gene, partial sequence
MF002299.1	Uncultured Pedosphaera sp. clone 716_32 16S ribosomal RNA gene, partial sequence
KT122308.1	Uncultured Bellilinea sp. clone TGRWLFZ-16s-SI328 16S ribosomal RNA gene, partial sequence

TABLE XXV: Most common organisms scoring well against the unknown sequences from our soil sample using the subset of NCBI fasta entries that match ribosomal NOT protein and length constrained . Most if not all appeared be bacterial 16S hits or wgs likely covering 16S.

Zymo Seq No	score	entry	Cite
jseq297	0.997	jKT122308.1:Uncultured:Bellilinea	soil thermophile [99]
jseq912	0.976	jKT122315.1:Uncultured:Gemmamimonadetes	ag flood soil[72] [104]
jseq521	0.974	jMF002263.1:Uncultured:Limisphaera	
jseq462	0.962	jMF002263.1:Uncultured:Limisphaera	
jseq1002	0.947	jKT122308.1:Uncultured:Bellilinea	
jseq490	0.935	jKX035369.1:Uncultured:Chloroflexaceae	
jseq1076	0.915	jKT122342.1:Uncultured:Syntrophobacterales	
jseq132	0.912	jKX035369.1:Uncultured:Chloroflexaceae	
jseq1065	0.907	jNR_151988.1:Brevitalea:deliciosa	savanna [98]

TABLE XXVI: Unknown sequences scoring .9 or above against the DB composed of a truncated download of ribosomal non-protein entries.

The resulting interesting sequences are labelled SO1-SO4 in Table **XXVII**.

hits	hits>=	length/pos	sequence	Comment
12/1191	3	GTAGCGGAAGAGGAAGGGACGGCTAACTCTGTGCCAGCAGCCGCGGTAAATACAGAGGTCCC		SO1
12	21/127	39/505	AGCGTTGTTCGGATTCACTGGCGTAAAGGGTGCCTAGG	
12	40/1191	39/505	AGCGTTGTTCGGATTCACTGGCGTAAAGGGTGCCTAGG	
-	19		Verrucomicrobia, class OPB35 soil group	
13	26/127	54/450	AAGAGGAAGGGACGGCTAACTCTGTGCCAGCAGCCGCGGTAAATACAGAGGTCCC	
13	56/1191	54/450	AAGAGGAAGGGACGGCTAACTCTGTGCCAGCAGCCGCGGTAAATACAGAGGTCCC	
-	30		Verrucomicrobia, class OPB35 soil group and Chthoniobacter	
-	39/1191		1.2, both of above with one in between	
-	19		Verrucomicrobia, class OPB35 soil group	
	33/127	38	CGGCTAACTCTGTGCCAGCAGCCGCGGTAAATACAGAGG	
187/1191	38		CGGCTAACTCTGTGCCAGCAGCCGCGGTAAATACAGAGG	
187/1191			overall hits	common base
63/200			Acidobacteria	common base
53/58			Verrucomicrobia	common base
28/280			Proteobacteria	common base
9/34			Gemmamimonadetes	common base

TABLE XXVII: Limisphaera sequences in common with best match "no-hit" reads. The exact string matching code found 30 no-hit reads with the best similarity to Limisphaera. 12 and 13 of them, respectively has this string in common . And more interestingly 21 and 26 respectively had these in common as roots of longer matches with same number in larger set of 127 no-hit reads. The common base occurred exactly or as a substring in 33 of the unknown reads. Sequences were name SO("soil organism") with SO4 being a common base sequence.

sequence	position	length	sequence	Comment
li39	975	8	AAAGGGTG	
li39	505	39	AGCGTTGTTCGGATTCACTGGGCGTAAAGGGTGCCTAGG	
li39	152	9	TAAAGGGTG	
li54	450	54	AAGAGGAAGGGACGGCTAACTCTGTGCCAGCAGCCGCGTAATACAGAGGTCCC	
li54	1143	8	GAGGAAGG	

TABLE XXVIII: Running the sequences and their reverse complements against Limispheara produced no hits for the rc strings and only 3 off target matches.

5. DISCUSSION

Some of the most interesting prior microbiome results from the literature are summarized in Table [XXIX](#). Differences between the present results and prior ones are complicated by many factors but the comparisons among these dogs, summarized in Fig. [9](#), are informative in isolation.

5.1. The present dogs

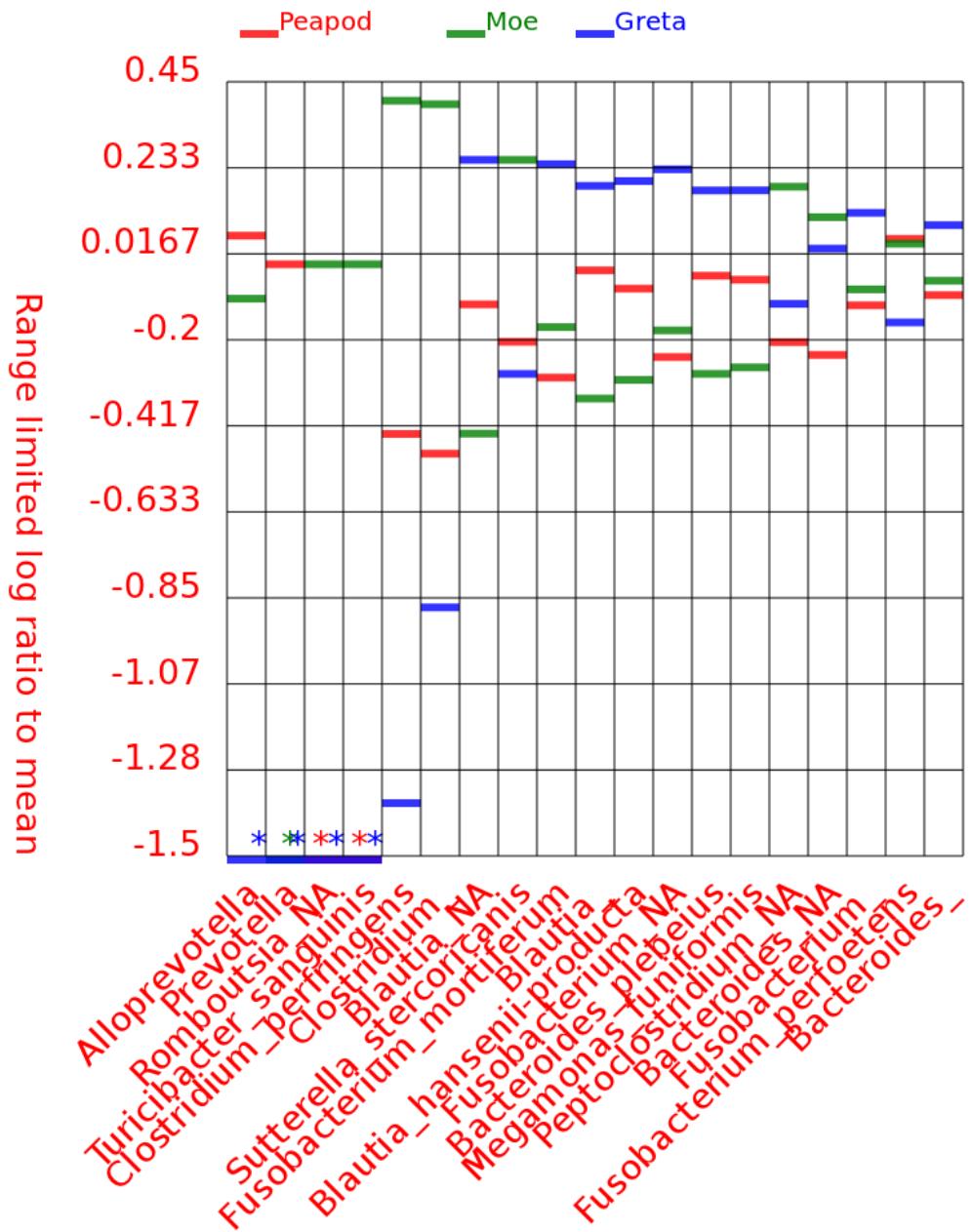


FIG. 9: Log ratio of sample abundance to mean of non-zero amounts. Note that some samples had zero values as indicated with a ”*”. Note Greta depleted of Clostridium and even more so of C. perfringens. Peapod: Red, Moe: Green, Greta: Blue

The dogs are all different breeds with different medical histories but with similar diets and known variations in soil exposure history. Symptoms just prior to the sample period varied with Peapod having some blood in stool with likely infections elsewhere, Moe having chronic runny stool and recent skin bubbles, and Greta more or less recovering well from surgery with intermittent uveitis (bilateral but largely confined to right eye). Just prior to sampling, Peapod was eating a home made raw diet with only intermittent kibble, Moe had both everyday, and Greta ate largely kibble with raw ground beef but with none of the vitamins Moe and Peapod were getting. Prior to her surgery Greta was eating similarly to Moe with all the vitamins. Moe and Greta both dug a lot but Peapod had become somewhat disabled over the prior year and was not ingesting as much soil. The results can be discussed partially in

terms of terminal GI ecology but with the caveat of changing genera names and questions about generalizability of metabolism of one organism *in vitro* to the entire genus *vivo*. Another component of the 16S results could be from highly prevalent organisms elsewhere that pass small amounts of DNA to the stool. One concern was a reverse process in which overwhelming exposure to soil organisms created a transient increase in GI tract and blood but leaving some to found colonies in various niches or create biofilms.

Thinking outloud

needs some kind of organization, still a collection of facts as encountered in the literature search

One immediate observation from Fig. 9 is Greta's comparatively low Clostridium totals, perfringens amount, and their ratio. Greta was the only dog to have had recent antibiotics prior to the sampling due to a plasmacytoma removal surgery and no supplemental vitamins during the prior month. The antibiotic was cephalaxin (and possibly others IV) which has been reported to have variable efficacy on strains of Bacteroides and Fusobacterium [65] but it also associated with concerns about (formerly) Clostridium difficile related diseases [97] while some activity against Clostridium perfringens has been observed [93]. Greta's fraction of perfringens to total Clostridium is 2/8 versus 11/15 and 1.65/1.95 for the others. She has higher Bacteroides along with the lower Clostridium compared to Moe and Peapod. One work showing the metabolic relationship between these genera [22] suggests that Clostridium requires an external source of biotin, pantothenate and pyridoxine (citing the case of C. difficile which is now in a different genus although C. perfringens does require biotin, pantothenate, and pyridoxamine [11]) while Bacteroides require CO₂ and sulfur. . Peapod and Moe would have had a much richer set of these vitamins during the prior month(indeed Peapod was thought to benefit greatly from all SMVT substrates given at differing times) including sulfur sources such as garlic and taurine as well as vitamin K2. Biotin limitation, similar to other nutrients, can provoke toxin expression in C. difficile [100] making clinical symptoms worse without higher abundances. Interestingly, biotin deprivation of a mouse host by vancomycin induced Lactobacillus overgrowth resulted in alopecia rescued by dietary biotin [30] . Greta's Blautia fraction was higher than the others. Blautia can apparently convert hydrogen into acetate making it fit better with hydrogen producers [22] as may occur in Stickland metabolism without an amino acid to prevent hydrogen evolution [34]. In human transplant patients higher Blautia has been associated with better outcomes and an absence of anaerobe targeting antibiotics (not considered anaerobe targeting, "intravenous vancomycin, ceftriaxone, ceftazidime, cefepime, aztreonam, and trimethoprim-sulfamethoxazole") [34]. Greta's overall Fusobacterium abundance is approximately 1.5 times that of Moe and Peapod. Some of that may be due to differential antibiotic sensitivity or recovery but in other works it appeared to increase with raw diets, detailed later, contrary to what is observed here. Fusobacterium is quite interesting as it is thought to differ in roles between human and dog IBD [94] [69]. One study explored the fecal microbiome of dogs with adenoma or carcinoma or polyps near the rectum [31] but apparently did not include plasmacytoma or raw diets with vitamins. The relationship between cancer and bacteria remains confusing (for example, [80]) and Greta's recent antibiotic exposure precludes much interpretation along these lines.

Peapod generally had middle or lower abundances of these organisms and likely was suffering from oral and brain infections. Prevotella, unique to Peapod, has been considered a defining feature of the gut microbiome of humans eating a plant-based non-Western diet and contrasted with dominance of Bacteroides and Clostridiales [28] [35] . It is dominant in the rumen [39]. Prevotella has also been associated with infections in humans of "infections of the head and neck, respiratory tract, central nervous system, and abdominal and urogenital tracts, as well as bacteremia" [3] . Prevotella intermedia and nigrescens can apparently turn glucose into formate and succinate as well as a polysaccharide similar to glycogen thought to be beneficial for gingival area survival [89] . Apparently P. ruminicola can obtain nitrogen from ammonium and peptides but not amino acids [39]. Prevotella is thought to be a part of various oral infections (with a high potential for recombination) [105] . Peapod and Moe had similar abundances of Alloprevotella which has been observed to increase abundance in oral cancer [106]. Oscillospira, unique to Peapod, is routinely observed in farm animals GI tract and thought to fluctuate seasonally [47] . It remains difficult to culture although it appears related to Clostridial cluster IV [26] while being associated with leanness and health [40]. It dominates along with Bacteroides, Parabacteroides, and Petromonas in iron reducing lake sediment communities [103]. A competitive advantage for iron suggests it could flourish in the case of either an iron rich diet or GI bleeding. Peapod was the only one with obvious blood in the sample.

Moe had chronic runny stool at the time and a few isolated inflated skin bubbles. Compared to the other dogs, he was notable for higher Clostridium, C. perfringens, Peptoclostridium, lower Blautia, and non-zero Sarcina ventriculi, Helicobacter, Romboutsia, and Turicibacter. Of these, Romboutsia, Turicibacter, and C. perfringens were also in the soil and Sarcina is supposed widely distributed in soils.

In some particulars, Sarcina is an interesting OTU,

Sarcina ventriculi was first observed in 1842 by Goodsir (13) in the contents of a human stomach. The organism has been cultivated from garden soil (2, 3) and stomach contents (4), and it has also been enriched and isolated from sand (26), river mud (8), and peat bog sediments (14). The prevalence of this organism in sedimentary environments and acid or alkaline soils that have been stored for months to years

(26) suggests the presence of resistant structures or spores. Examination of soil and sand by numerous workers has failed to reveal the presence of spores of *S. ventriculi*, even though the organism was readily cultivated from these samples. To date there has been only one, nondetailed report on the sporulation of *S. i'entriculi* (20). In addition, the ability of *S. ventriculi* to grow under conditions from pH 2.0 to 9.0 (15) has raised some interesting questions about physiological adaptations of this organism to extremes in pH. Goodwin and Zeikus (15) demonstrated that the internal pH of *S. ventriculi* shifted in relation to the external pH, with an internal pH of 7.1 during growth at an external pH of 7.0, versus an internal pH of 4.3 during growth at pH 3.0. In the present communication we report the ultrastructural and physiological changes which occur when this organism is grown at low versus high pHs. These findings represent the first detailed descriptions of the sporulation cycle of *S. 'entriculi*. [45]

A number of widely accepted errors existed early in the 1900's [7] but it appears to be well adapted to low pH [25] as may occur in the functioning stomach.

Sarcina has been implicated in a variety of human and animal stomach conditions but its relation to pathogenesis remains unclear [76] [1] [70] although it or similar organisms are associated with bloat in cattle [20] and has been suggested that it "can normally be found in the soil" [2]. Oddly, it seems associated with vegetarian diets [42] [60] (probably all cite the same primary source from 1971 and its unclear if larger studies have been done) although Moe's grass and dirt eating may have created a sufficient inoculum to allow infection with a predominantly meat diet even if it derives energy solely from carbohydrates [60]. Interestingly, it elicits a characteristic frothy vomit named for the organism [32].

Turicibacter was notable for its absence in Peapod and Greta despite being considered important in other works [4]. It is generally considered beneficial with abundance being modulated by diet and exercise [18] while decreased in several diseases [59] including multicentric lymphoma [23]. However, the first isolate of a *Turicibacter* was from a human blood culture described as a non-spore forming strict anaerobe with culture pH range between 6.5 and 8 using only the carbohydrates maltose and 5-ketogluconate [9]. The impact of dietary cholesterol has been examined along with cecal butyrate. These results suggested a correlation with cholesterol as it was not observable in mice fed a no cholesterol diet [19]. The diet for these dogs included Eggland's best hard-boiled eggs and ground beef normally with 20 percent fat and there was no real attempt to limit cholesterol intake.

Moe and Peapod also shared the single unidentified *Clostridium* with the soil, see Table XIV, and it is interesting to speculate that it may have infected Greta prior to her antibiotics.

5.2. Relationship to prior results with Healthy and Diseased Dogs

OTU	Peapod	Moe	Greta	Prior Work	Condition	Citation
Bacteroides	15	16.3	22.5	0-4 commercial diet lab dogs	Healthy	[29]
Bacteroides				.05-.85 commercial diet lean vs obese	Healthy	[29]
Bacteroides				14-15 raw meat based diet	Healthy	[8]
Bacteroides				as low as 6 with inulin	Healthy	[8]
Bacteroides NA	5.4	12	10			
B. plebeius	5.69	3.22	9.33	1 raw meat based diet	Healthy	[8]
B. plebeius						
Blautia	7.78	3.7	12.7	7.2-13.6 BARF vs commercial	Healthy	[84]
Blautia				9-11 commercial diet lean vs obese	Healthy	[29]
Blautia				9.9(Healthy) 0.2(NHD), 0.2(ABD), 5.9(IBD) 3.6(cIBD) pyro	IBD	[88]
Blautia				9.7(Healthy) 6.3(NHD), 8.2(ABD), 9.2(IBD) 9.5(cIBD) qPCR	IBD	[88]
Blautia NA	3.64	1.72	8.42			
B. hansenii-producta	2.02	1.19	3.77			
Clostridium	1.95	14.8	0.8	1.2-5.3 BARF vs commercial	Healthy	[84]
Clostridium				33.7(Healthy) 81.3(NHD), 44.0(ABD), 13.7(IBD) 14.2(cIBD)	IBD	[88]
Clostridium				16-17 raw meat	Healthy	[8]
Clostridium				39-49(+/-27) commercial diet lean vs obese	Healthy	[29]
Clostridium				45-79 commercial diet lab dogs	Healthy	[29]
C. perfringens	1.65	11.4	0.194	.09-.21 BARF vs commercial	Healthy	[84]
C. perfringens				2.0(Healthy) 4.0(NHD), 6.2(ABD), 3.0(IBD) 2.4(cIBD) qPCR	IBD	[88]
C. perfringens				< 1 raw meat based diet	Healthy	[8]
C. perfringens				8.90 raw, 0 natural	Healthy	[38]
Fusobacteria						
Fusobacterium	18.6	20.4	31.8	7.3(Healthy) 6.9(NHD), 8.2(ABD), 6.4(IBD) 7.1(cIBD) qPCR	IBD	[88]
Fusobacterium				7.3-12.1 BARF vs commercial	Healthy	[84]
Fusobacterium				.3-.6 commercial diet lean vs obese	Healthy	[29]
Fusobacterium				0-04 commercial diet lab dogs	Healthy	[29]
Fusobacterium				no changes	IBD	[94] [69]
Fusobacterium				31-36 raw meat based diet	Healthy	[8]
Fusobacterium				18 raw meat+inulin (LCFA)	Healthy	[8]
Fusobacterium				ca. 3 raw, 0 natural computed from table	Healthy	[38]
Fusobacterium NA	2.39	2.79	7.1			
F. mortiferum	5.25	7.04	18.1			
F. perfoetens	10.8	10.5	6.65			
Megamonas				1.7-3.1 commercial diet lean vs obese	Healthy	[29]
Megamonas				.6-1.7 raw meat based diet	Healthy	[8]
Megamonas				5-13 raw meat+inulin (LCFA)	Healthy	[8]
M. funiformis	9.71	5.84	16.3			
Peptoclostridium NA	3.18	7.83	3.97			
Romboutsia NA	0	0.081	0			
Sutterella				.09-.16 BARF vs commercial	Healthy	[84]
S. stercoricanis	1.41	4.05	1.17			
Turicibacter				.5 - 2.5 BARF vs commercial	Healthy	[84]
Turicibacter				.3-2.9 commercial diet lean vs obese	Healthy	[29]
Turicibacter				5.2-22.2 commercial diet lab dogs	Healthy	[29]
Turicibacter				1.28-3.76 raw meat based diet	Healthy	[8]
Turicibacter	0	0.0451	0	2.9(Healthy) 0(NHD), 0(ABD), 1.5(IBD) 3.8(cIBD) qPCR	IBD	[88]
T. sanguinis						

TABLE XXIX: Some results from prior work analyzing 16S content of dog feces. Generally group means are reported but check references for distributions and p values. Note that "healthy" likely just means absence of visible disease without regard to latent or developing problems like cancer. NOTE: These are percentages not fractional abundance to make comparison to literature easier. Abbreviations: NHD = acute non-hemorrhagic diarrhea; AHD=acute hemorrhagic diarrhea; BARF= bones and raw food; IBD=inflammatory bowel disease, cIBD=controlled IBD, Healthy = absence of overt disease.

Generally the works in Table **XXIX** examine a range of diets similar to those used here, excluding the high doses of vitamins given Peapod and Moe, and a limited set of GI related diseases. The comparisons are rather tenuous and only serve as a reference for the current results. Group means have been quoted in the table although in many cases variation within groups is much larger than inter-group differences and original sources should be consulted for full details on observed distributions.

Differences between this and prior work may also include methodological artifacts with DNA extraction and assignment pipeline. The method used here employs a calibrated procedure including bead beating for good extraction. While the analysis pipeline varied, that should not matter for general trends above the genus level.

One work examined obese and lean pet dogs along with research dogs all fed commercial diets with little difference between them [29]. While the ranges in the table reflect means in the lean and obese groups, the variation within groups tended to be much larger (quoted errors greater than means). Also the presence of cyanobacteria etc in the client owned pets but not in the research dog samples is suggestive of soil contamination during sample collection(which was carefully avoided here due to the nature of the investigation) or maybe real ingestion.

Prior work with healthy dogs fed various beef and chicken raw diets analysed with pyrosequencing demonstrated 31-36 percent Fusobacterium in the control diets although lower with the addition of inulin [8]. In the present case only Greta had this much Fusobacterium and her diet contained the largest fraction of kibble but again there are many other factors to consider.

The impact of excess vitamins on fecal microbiome must rely on less directly relevant prior work. Work with broilers distinguished their cecal microbiome based on vitamin supplementation finding more clostridium and undetectable Escherichia/Shigella that contrasted with the non-supplemented group [46]. No Escherichlia was observed in these dogs.

All of these dogs had a diet with large amounts of menaquinine supplementation although Greta's supplements were stopped after surgery. This could remove a competitive disadvantage for non-synthsizers. Prior work exploring K2 synthesis in isolates from neutropenic cancer patients demonstrated that, *Bacteroides fragilis* produced menaquinone but " [n]o menaquinones were detected in organisms of the genera *Fusobacterium*, *Clostridium*, *Bifidobacterium*, *Lactobacillus*, *Actinomyces*, *Peptococcus*, or *Peptostreptococcus*" [75]. *Fusobacterium* and *Clostridium* were generally higher than in some other dogs although the other genera were not examined. Similar to siderophores in marine organism pairs, menaquinone synthesizing organisms were found to boost populations of anaerobic non-synthesizers in culture with the result largely obtainable from quinone added to media [21] (this is a very interesting article illustrating confusion about iron and vitamin K and also need for solubility factors to make vitamin K accessible). Vitamin K has been observed to restore defective mutants in low iron conditions and may have less obvious impact on microbial communities [73].

This previously unreported identification of the strains E[nterobacter] agglomerans, S[erratia] marcescens and E[nterococcus] faecium as menaquinone producers will in the first instance contribute to aiding in their microbiological classification (11). In addition, the sourcing of these additional vitamin K-producing strains from the neonatal gut flora shows their potential as bacteria that could contribute in the neonate to the overall requirements of vitamin K, an essential vitamin in the human blood clotting process (6). In the study presented here, the newly identified vitamin K producing strains together with the several other previously reported vitamin K-producing strains identified in this study namely, *Bacteroides ovatus*, *Citrobacter freundii*, *Enterococcus faecalis*, *Escherichia coli*, *Prevotella buccae*, *Staphylococcus capitis*, *Staphylococcus epidermidis*, *Staphylococcus haemolyticus* and *Staphylococcus warneri*, [27]

The gut microbiome is becoming more recognized as an intermediary between diet and host in shaping actual nutritional value of consumed food [63]. The work [63] singles out BCAA in a cocktail of 18 amino acids that promotes insulin resistance in humans but the reason for this appear to relate to increased plasma levels of BCAA and aromatic amino acids as being predictive of T2DM. They then point to the worst microbial offenders as, " *Bacteroides*, *Clostridium*, *Propionibacterium*, *Fusobacterium*, *Streptococcus*, and *Lactobacillus*." Culture medium for *Fusobacterium varium* was found to require large amounts of aspartate, asparagine, cysteine, glutamine, glutamate, histidine, lysine, serine and threonine suggested to be used as energy sources [78]. Presumably in extreme cases this could deprive the host of important amino acids.

Clostridium perfringens is shared by all samples and generally recognized as a pathogen. However none of the dogs had typical diseases such a gangrene. Apparently *Clostridium difficile* regulates toxin production depending on availability of Stickland amino acids [10] similar to toxin production with iron limitation in other settings. All dogs did have some abnormalities in stool although only Peapod and Greta had obvious blood with Greta's thought to be due to tumor. Peapod's bleeding did not appear to stop with antibiotics (and IIRC worming) suggesting possibility of similar tumor although resistant organisms can not be excluded- *H pylori* for example is difficult to eradicate.

Thinking outloud

want to try to integrate this idea somewhere, note the factor of 4 flopping in d-serine dose should be fixed

Also note the possible contributions of d-serine (which unfortunately is subject to some uncertainty in dosing amounts). One recent results suggests that hydrogen peroxide production by DAO in the GI tract can modulate intestinal

microbiota [83] and indeed it appeared to help Peapod but that was assumed to be due to other mechanisms.

5.3. Contributions of the soil

Unfortunately the timing of the soil sample was not optimal to capture the conjectured pathological microbial blooms in warm wet conditions and the higher organic content component with mole tunnels was sampled rather than the clay surface decorated with waste remnants. Comparing the abundances of select OTU's between the play area sample and typical EMP environments as well as several landfills, illustrated in Fig. 12 and Fig. 13, this sample generally aligns with the environmental samples although methodological differences can not be excluded. These major taxa were chosen largely due to their prevalence but are also the dominant ones discussed in other works on transitioning of soil ecology such as [77].

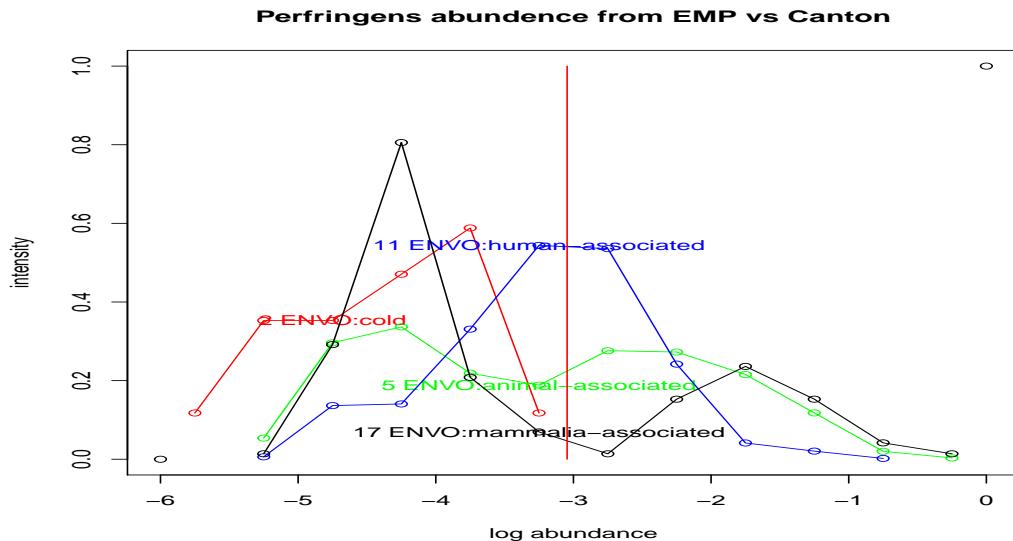
The landfills in [86] did not seem to match the gross features of this soil. as shown in Table XXXI. While it may be interesting to project the present soil abundances against some existing LDA models. the log rather than linear abundances with suitable appreciation of zero appear instructive at this point. Notably, there is zero fusobacteria and epsilonproteobacteria with higher alphaproteobacteria, aciobacteria, and actinobacteria than the landfills but within bounds for the EMP means. The landfill samples were dominated by Proteobacteria but with little Alpha and varying Beta,Delta, Epsilon, and Gamma. The present sample was largely Alpha(which interestingly have been investigated for gene sharing mechanisms [61]). The landfill work finds 4 clusters dominated by Clostridiales (within Fimicutes), Campylobacterales(Proteobacteria), or more diverse including Chlorobi and OP9 [66] , or finally largely candidate division OP3. It is noteworthy for the discussions that follow however that the OP phyla were found in various extreme environments[81]. The largest contributor to this soil, from Table III is Acidobacteria Subgroup 6. While Acidobacteria appear to increase in abundance with decreasing pH, Subgroup 6 has the opposite relationship [36]. Further it appears to increase as percentage of all Acidobacteria with soil fertility [62]. Our sample and the landfills both had a significant number of unidentified reads but their work considered primarily higher abundance organisms with less interest in some of the rare but potentially important ones. The sample was taken at the bottom of a slope which would be expected to accumulate nutrients eroded from the higher lying exposed clay and this may be one factor influencing microbial abundances. Possibly any landfill-like communities in this sample reorganized as the debris was digested. Prior spraying with copper sulfate and algeacide may have had some impact even as lower lying soil had been exposed in the following months due to erosion. The quaternary ammonium chloride algeacide may be of some concern for allowing Pseudomonad proliferation [91] [14] [102] [58] although in this sample it was at $3.3e - 4$ (data not shown) . Such a proliferation may inhibit fungal growth [37]. Effects of copper on soil have been studied with confusing results [67] for the bacteria of interest here.

There are also some interesting observations about Moe and soil bacteria. Its tempting to try to point to a specific "bug" and attribute his diarrhea to that "infection" but that would be difficult for several reasons. First, there are multiple possible pathogens possibly with variable virulence phenotype. Related work looking at *H. pylori* and *E. Coli* found that a better suspect was low stomach chloride [16] consistent with recent diet design goals that motivate the addition of citric acid and potassium chloride to the diet. Organisms shared by the soil sample and any dog are listed in Table XXIII. The most interesting organisms are the well known *C. perfringens* , Romboutsia and the common soil organism Sarcina which was not observed in our soil sample. . The observed *C. perfringens* amount is typical of several of the EMP environments as illustrated in Fig. 10. All dogs had it and it is difficult to determine any relationship to soil or disease. Moe's blisters that were ultimately removed surgically had torn prior to surgery and appeared to contain gas but with no odor. One of the organisms shared only by him and the soil, Romboutsia, has metabolism consistent with odorless gas evolution. Aparently Romboutsia ilealis CRIBT is common in rat small intestine and appears to require several exogenous vitamin sources while being notable for odorless gas evolution (as well as high copy number of the 16S rRNA gene) [24] . The 4 known species of Roboutsia have been isolated from rat intestine, mud, lake sediment, and human GI tract [79] . It was found along with Fonticella and Gracilibacteri in hydrogen producing digested sludge enriched soil and cattle feces [41]. It should be considered as an organism of interest but little definitive data is available at this time.

Moe's Sarcina is also a known producer of hydrogen and carbon dioxide associated with bloating conditions in other animals but was not oberved in his play area soil while generally thought to be common. Considered against statistics from the EMP [92] and landfills [86] however its detection by 16S methods does not seem to occur in most cases. The average amount of Sarcina in the EMP sample types with the highest abundance is shown in Table XXX. The Sarcina abundance distribution in the EMP environment with largest mean , animal associated, is show in Fig. 11. Only 15 percent of these samples had non-zero amounts. After including the low abundance organisms in the data, 8 of the 58 landfill samples (14 percent) appeared to contain Sarcina of undetermined species at the $1e-4$ level [86]. Detection of Sarcina then in soils by 16S with typical extraction and amplification methcanisms does not appear to be uncommon but it is not found in most cases. Literature however suggests it is easily cultivated from many soils pointing to either

a bias in sample types, misleading use of adjectives, or an undetectable persister state.

Contributions from non-bacterial environmental organisms should be considered too. The soil may harbor fungi and virus as well as important metabolites effecting both fecal microbiome and disease. For example, prior work suggested infection with canine parvovirus enriched bacterial genera Shigella, Peptoclostridium, Peptostreptococcus, Streptococcus, and Fusobacterium [107]. Prior treatment with copper-quat may have reduced viable non-prokaryotic organisms however.



Thinking outloud

The log ratio to median plots look more informative. More histograms of abundances in different environments would be useful to explore before PCA etc. Have not verified proper usage of the emp data however caveat emptor.

FIG. 10: Using results from various environments described by EMP at <http://www.earthmicrobiome.org/data-and-code/> the perfringens relative abundance observed in the present soil sample (vertical red line) is plausible for several environments and at the peak for animal associated. Note that mammal and animal associated appear bimodal suggesting more exploratory work before leaping to various linear combination models.

Thinking outloud

Prior work listed a canine associated environment at EMP, none of the files I downloaded seemed to contain one.

Serial	Catagory	sarcina, any	maxima	ventriculi	total
57	run2::2 Moe	0	9.7e-03	9.3e-04	0.01063
56	run2::1 Greta	0	6.2e-04	0	0.00062
38	emp::ENVO:animal-associated	2.6e-04	0	0	0.00026
40	emp::ENVO:mammalia-associated	1.1e-04	0	0	0.00011
42	emp::ENVO:plant-associated	8.7e-05	0	0	8.7e-05
27	emp::ENVO:human-associated	6.5e-05	0	0	6.5e-05
48	emp::ENVO:river	2.1e-05	0	0	2.1e-05
13	emp::ENVO:pond	1.7e-05	0	0	1.7e-05
36	emp::ENVO:coastline	9.7e-06	0	0	9.7e-06
24	emp::ENVO:fluvisol	8.8e-06	0	0	8.8e-06
16	emp::ENVO:nest	7.6e-06	0	0	7.6e-06
28	emp::ENVO:estuary	5.2e-06	0	0	5.2e-06
32	emp::ENVO:cove	4.6e-06	0	0	4.6e-06
23	emp::ENVO:steppe	3.8e-06	0	0	3.8e-06
30	emp::ENVO:lagoon	2.5e-06	0	0	2.5e-06
2	emp::ENVO:forest	1.2e-06	0	0	1.2e-06

Thinking outloud

should remove the emp::ENVO:: prefix

TABLE XXX: EMP sample types with the highest average Sarcina although likely many samples contain zero. Note that the present samples from Moe and Greta are fecal rather than soil shown for comparison. [92]

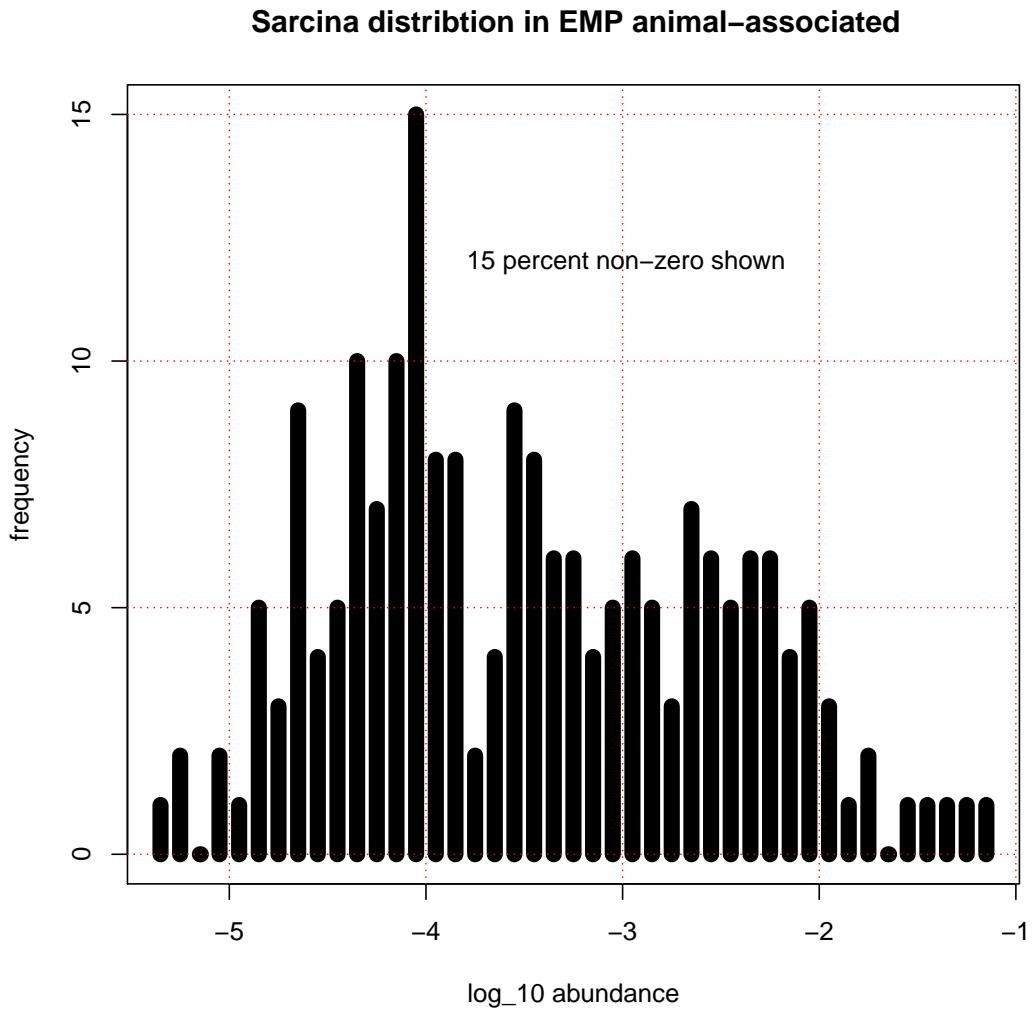


FIG. 11: Distribution of log abundance in the EMP animal associated samples with non-zero Sarcina. Note that only 14 percent had any and most are in the very low end.

Group	Landfill max	Landfill min	Landfill mean	Present sample	
Other acidobacteria actinobacteria proteobacteria	0.78	0.21	0.568772	0.32	
	0.012	0	0.00164509	0.19	
	0.023	0.00073	0.00530754	0.22	
	0.79	0.21	0.421228	0.27	
alphaproteobacteria	0.086	0.016	0.0300702		0.14
betaproteobacteria	0.3	0.021	0.0678772		0.065
deltaproteobacteria	0.23	0.044	0.0977719		0.045
epsilonproteobacteria	0.6	0.04	0.125877		0
gammaproteobacteria	0.31	0.033	0.0997719		0.024
fusobacteria	0.055	0.00069	0.0043114		0

TABLE XXXI: Some OTU abundances from landfills taken from [86] compared to the present sample. The play area soil is much higher in Acidobacteria and Actinobacteria and the proteobacteria are dominated by alphaproteobacteria rather than epsilon and gamma. No fusobacteria were observed in the current sample.

Sample	Other	acidobacteria	actinobacteria	fusobacteria	Proteobacteria				
					alpha	beta	delta	epsilon	gamma
cultivated(n=708)	0.53	0.19	0.033	4.5e-07	0.075	0.067	0.067	0	0.037
Forest(n=21)	0.41	0.32	0.07	0	0.11	0.044	0.02	1.9e-06	0.025
stromatolite(n=21)	0.4	0.16	0.061	0	0.24	0.035	0.034	0.0055	0.067
tropical(n=28)	0.28	0.59	0.011	0	0.037	0.032	0.021	1.6e-06	0.022
luvisol(n=32)	0.41	0.23	0.093	0	0.17	0.044	0.0092	0	0.048
mountain(n=22)	0.42	0.35	0.04	0	0.11	0.033	0.018	0	0.026
podzol(n=4)	0.3	0.42	0.048	0	0.14	0.038	0.0059	0	0.039
sandy(n=2)	0.46	0.11	0.27	0	0.11	0.017	0.022	0	0.0039
run2::1 Greta	0.63	0	0.032	0.32	0	0.021	0	0	0
run2::2 Moe	0.72	0	0.013	0.2	0	0.042	0	0.024	0.00085
run2::3 Peapod	0.74	0	0.023	0.18	0	0.029	0	0	0.026
run2::4 soil	0.32	0.19	0.22	0	0.14	0.065	0.045	0	0.024

TABLE XXXII: Some EMP results with zero across the samples. Notably the zeroes were confined to fusobacteria and epsilon proteobacteria. Many ways you may want to decompose or resolve the present soil by EMP type but benefit not clear yet. More complete results in the appendix.

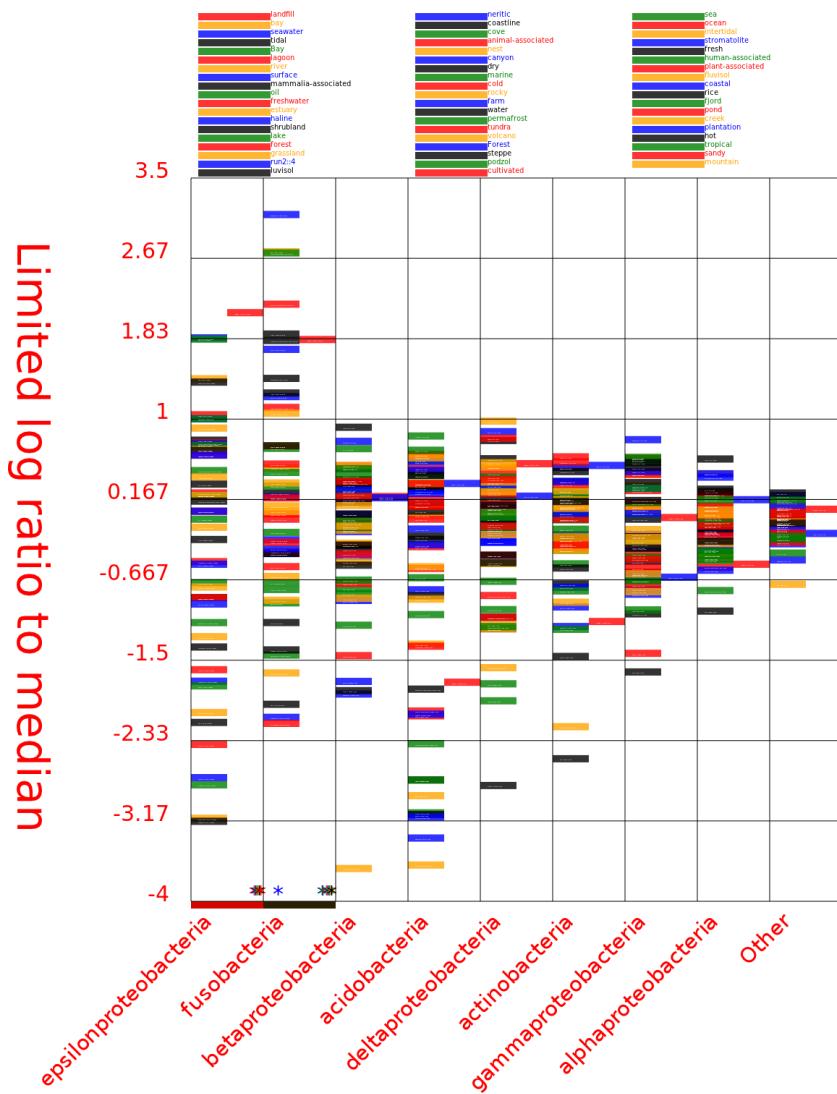


FIG. 12: Relative abundances of select OTU's or organism groups from EMP environments and the the backyard sample. The y-axis is the log ratio between median and sample abundance value (note in other plot the mean was used). In the right halves, red is the landfill data and blue is our backyard sample. Note differentiation by epsilon proteobacteria and fusobacteria. The svg is more clear with better labels .

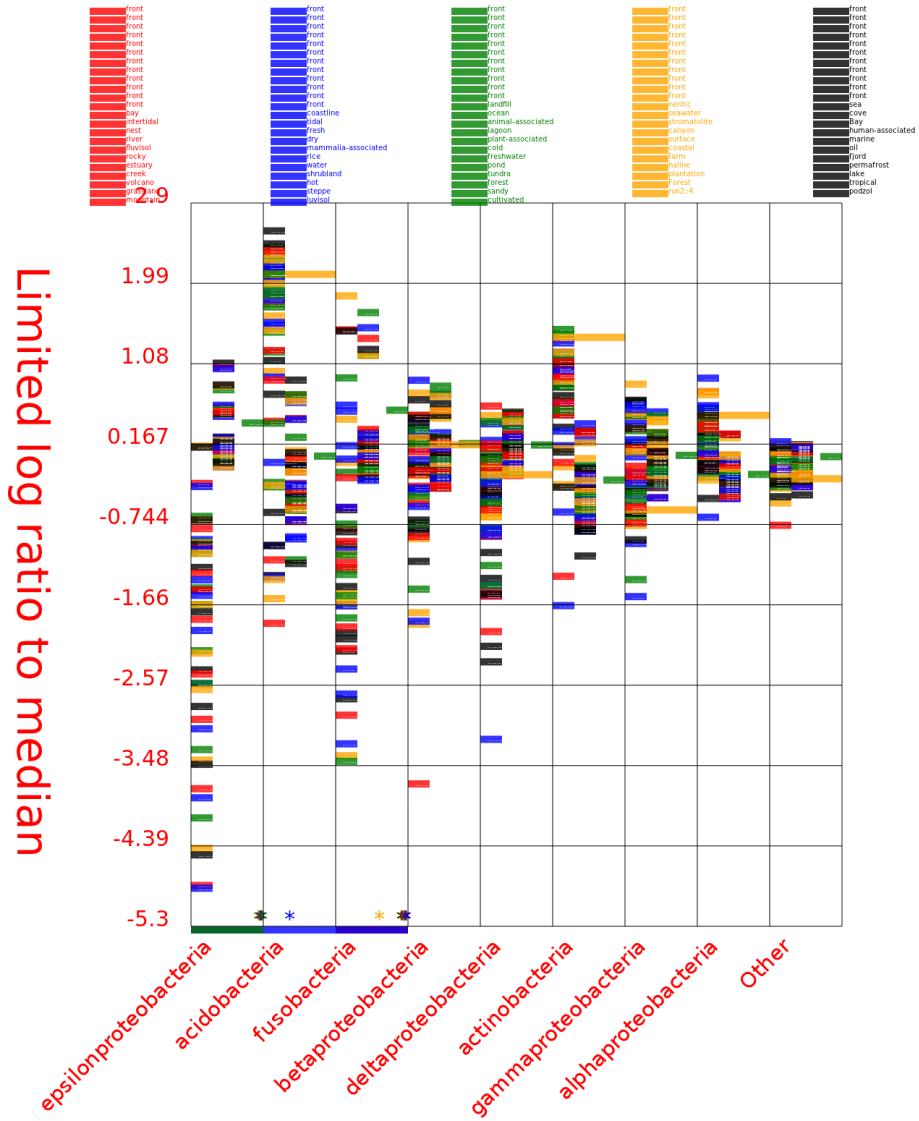


FIG. 13: A variety of EMP environment means, individual landfills, a landfill mean, and the present soil sample. Each column contains distribution of an OTU abundance as labelled at the bottom. Within each, the left third are means from various EMP environments, the middle third a specific landfill, the right third in green is landfill group means, and the present sample extending across the entire column in orange. "Front" refers to the landfills from the Frontiers paper [86]. The specific EMP environments labels are not legible here, although clear in the source svg file, as the immediate point of this graph is to show trends in the groups. Only the betaproteobacteria column shows overlap between our soil sample and the landfill mean. In most cases it occurs outside the range of lanfill observations although that may be due to DNA processing or taxonomic issues. Epsilonproteobacteria and fusobacteria were zero for our sample although the landfills tended to cluster higher than the EMP environments in these organisms. The svg is more clear with better labels .

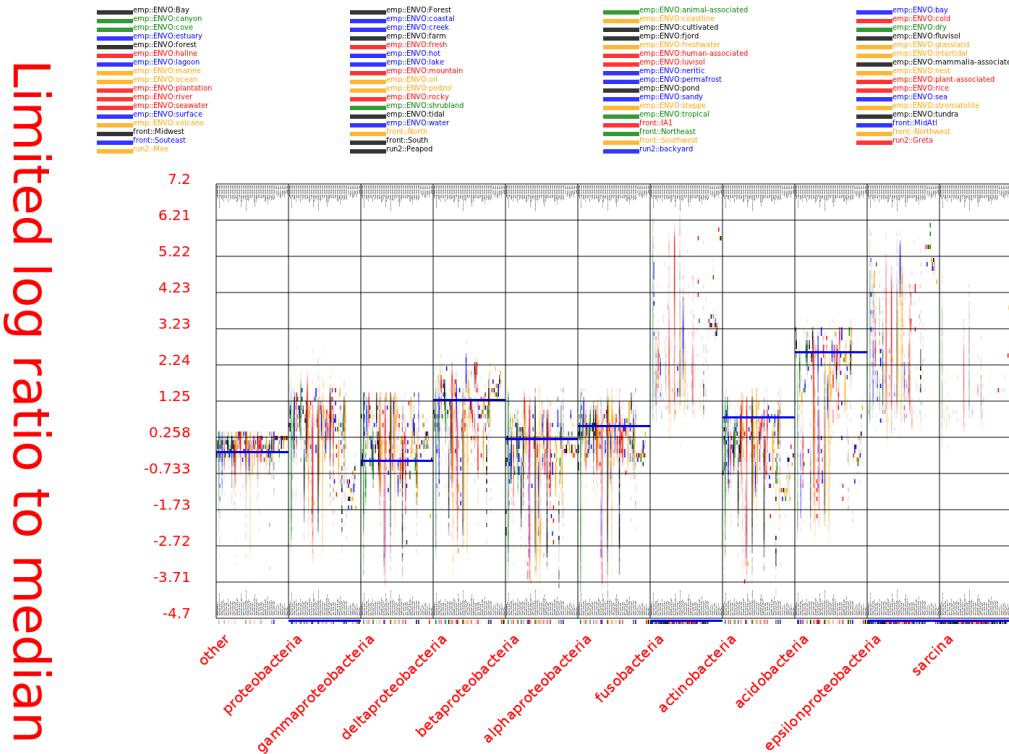


FIG. 14: All available EMP ground along with the landfills arbitrarily classified by gross geography just for illustration. The current sample generally falls into the EMP groups but differs from all the landfill groups. The svg is more clear with better labels .

5.4. The unknown soil reads

The unidentifiable sequences may reflect uncultured fastidious organisms native to the soil of no clinical relevance but may also be cryptic indications of something relevant. BLAST hits were generally not informative but ESM turned up some patterns. Originally the motivation for ESM was to look for segment transfer but the same approach may be useful to find regular modifications that may make DNA more robust during times of stress which may give it similarities to known extremophiles. Prior work has explored specific sequences for classification into meaningful groups such as pathogens or select agents [15] and maybe there is a "persister sequence" or state that has a signature in the 16S reads.

The landfills in [86] did not seem to match the gross features of this soil except that both had a significant number of unidentified samples. That work points to the unknowns as being "most closely related to mitochondrial 16S rRNA gene sequences from the eukaryotic fungal-like Oomycetes". While beyond the scope of this report, quick attempts to use mitochondrial and chloroplast databases and 18S for control did not appear to help with these unknowns. Isolated good scores did come up between known 16S sequences and non-16S, perhaps of interest for other reasons but requiring more investigation. A demonstration sample provided by Zymo Research did contain some unknown reads which did BLAST and ESM score well against 18S sequences but that was not the case for the present soil sample. Some exploration on larger ribosomal non-coding DNA collections further demonstrated good scores against Limisphaera and recently added organisms, apparently all prokaryotic hits, with the most frequent listed in Table **XXV**. Presumably the *Garcinia* WGS picked up a 16S gene but no attempt was made to verify. The best matches, above .9 with 1 being complete coverage irrespective of misalignment, are listed in Table **XXVI**. The matches are plausible as either relatives of known soil bacteria or thermophiles of arguable significance as either new species or modified DNA. It is interesting that Limisphaera came up twice although that too could be coincidence.

One work determined that soil dessication reduced abundance of the most abundant organisms and those remaining were resistant to dessication and gamma radiation [82]. One suprising result from the ESM method was the number of extremeophilic organisms represented in the best hits. While exact numbers fluctuated as the algorithims were developed and code debugged, the predominance of Limisphaera [6] persisted. Issues surrounding the stability and fidelity of 16S rRNA have been investiggated for decades . For example, slippage of DNA polymerase at hairpins [95] and some sequences near shifts appear to be complementary short poly-G/C regions, and it may be possible to

interpret these sequences in this context. Presumably the sequences would reflect some kind of stability improvement which may be apparent in secondary structure either measured or predicted even if the latter does not match real life the trends may reflect real properties of the reads. Attempts to look at some organisms from <http://www.rna.cccb.utexas.edu/SIM/> and predictions from gtofld did not show any obvious patterns by looking at 2D plots of the connectivity in the CT files (results not shown).

Consistent with the known existence of conserved and hypervariable regions, the ESM shows regions with good agreement and essentially no agreement. In most cases alignment is preserved though the variable regions but not always and the shifts tend to be in the same direction versus Limisphaera. Most of the shifts appear to occur outside the read region which only attempted to capture V3 and V4.

Thinking outloud

I still need to do a good alignment and check base numbers but the 450-550 matches appear near V3 so far :) Need to distinguish DNA mods from post transcriptional mods that presumably would not show up although I guess a primer and polymerase could find them if they fit complement

During sporulation, it is thought that some DNA modification occurs largely in methylation pattern but much remains unknown. Possibly modifications to the packaging as well as covalent modifications could confuse the sequencing. Recently, backbone sulfur modification has been described [101].

Two sequences were common among Limisphaera and the no-hit or unassignable reads as shown in Table XXVII. Interestingly the third entry, "common base" is a subset of the 54 long sequence and appears to be an exact string match to Pseudomonas and Acinetobacter two possibly pathogenic groups of soil bacteria often suggested to be favored by or resistant to quaternary disinfectants [48]. Requesting more blast nits from the NCBI nucleotide DB also turned up hits to Psychrobacter and Corallococcus and many of low frequency. However, on a quick grep of the 16S DB fasta file it turns out to be very common in other soil bacteria including Korneobacter.

6. CONCLUSIONS

Moe, the most exuberant digger, harbored several soil associated bacteria that make good suspects for contributing to his diseases even if they were of low abundance. Greta, with recent antibiotic exposure, and Peapod who had not been digging much did not have as clear a link to the soil organisms. The Prevotella in Peapod may reflect abundance in non-GI locations causing clinical symptoms. Future work may be able to help point to both the source of exposure and a route to elimination of a range of pathogens allowing for both cure and prevention. Follow up to the 16S could include serial 16S of more dogs and soil locations as well as targeted approaches which amplify pathogen DNA. Two OTU's of interest were identified , Sarcina and Pseudomonas, for follow up. Dietary components of interest include vitamin K and both L and D amino acids such as D-serine which subjectively may have shown some benefit for Peapod but has subsequently been difficult to find. Future work may also consider dogs on trendy diets such as vegan or plant based and hopefully fecal microbiomes and health outcomes can be found in the existing literature. Prevotella, which were abundant in Peapod, may be interesting too in the context of a plant based diet.

It is likely important to consider the impact of low abundance organisms during interpretation of soil or animal samples. Gross measures of community structure may capture some but not all of the effects on the host or, in the case of environmental samples, visitors. These may be missed in models or discussions that consider only linear combinations of dominant organisms.

While a bit of a tangential curiosity and more speculatively, the unknown soil reads remain as possible uncultured bacteria but their resemblance to a thermophile suggests either a relationship to these known organisms, segment transfer, or transition into a latent state that involves DNA modifications or confuses the sequencing but is still recognized by the primer(s).

7. SUPPLEMENTAL INFORMATION

Some data files and svg plots are available in the archived version along with the latex and bibtex source code.

7.1. Computer Code

Thinking outloud

this does not work right, check the zip file as the underscores are wrecking the next char Other files should be ok
....

1. *zymo-txt-files-2018.zip* : Most of the 16S results excluding raw data and QC
2. *muqed-data-rRNApoop.zip* : Later data in almost MUQED format. Older data in cases.tex not included.
3. *./keep/violin.svg* : Original scalable artwork
4. *./keep/violinlf.svg* : Original scalable artwork
5. *./keep/violinxxx.svg* : Original scalable artwork
6. *./keep/kmboth.svg* : Original scalable artwork
7. *./keep/pmg_ratios.svg* : Original scalable artwork
8. *./keep/r1vr2na.svg* : Original scalable artwork
9. *./keep/r1vr2.svg* : Original scalable artwork
10. *./keep/pmg_ratio.svg* : Original scalable artwork

Command to extract sample specific taxa,

```
Link to questionable EMP sample map,
ftp://ftp.microbio.me/pub/EarthMicrobiomeProject/emp_14_1/sample-map.txt.gz
```

category tables:

```
./mjz_linc_graph.out -cmd "read-ragged foo $zymorun2/taxa_plots/sorted_otu_L7.txt 6" -cmd "set-param maxcnt 30" -cmd "string-ragged doh soil,peapod,moe,greta" -cmd "zymo-rags foo 1 4 doh" -quit
```

rank tables:

```
./mjz_linc_graph.out -cmd "read-ragged foo $zymorun2/taxa_plots/sorted_otu_L7.txt 6" -cmd "set-param maxcnt 30" -cmd "string-ragged doh soil,peapod,moe,greta" -cmd "zymo-rags foo 2 4 doh" -quit | more
```

Heat map,
\mjmgr\{r1vr2.png}

2176 cat z11v_microbes.txt |ftocmd -cmd

```
./mjz_zymo.out -cmd "read-catagory-map phyla phyla.txt" -cmd "read-catagory-map genera genera_plus.txt" -cmd "dump-catagory-map phyla" -cmd "set-param catagory_map phyla" -cmd "set-param use_sample_filter 0" -cmd "parse-biom-json run2 /home/marchywka/d/zymo/run1/zr2097.180309.zymo/WithSoil.Bac16Sv34/otus/otu_table.biom" -cmd "copy-biom run2 run2" -cmd "clear-biom run2" -cmd "parse-biom-json run1 /home/marchywka/d/zymo/run1/zr2097.180216.zymo/WithSoil.Bac16Sv34/otus/otu_table.biom" -cmd "copy-biom run1 run1" -cmd "clear-biom run1" -cmd "adhoc-tree list2.txt 0x0100 run2.svg" -quit
```

Heatmap with options turned on to ignore things like NA and unidenfitieid,
\mjmgr\{r1vr2na.png}

```
./mjz_zymo.out -cmd "read-catagory-map phyla phyla.txt" -cmd "read-catagory-map genera genera_plus.txt" -cmd "dump-catagory-map phyla" -cmd "set-param catagory_map phyla" -cmd "set-param use_sample_filter 0" -cmd "parse-biom-json run2 /home/marchywka/d/zymo/run1/zr2097.180309.zymo/WithSoil.Bac16Sv34/otus/otu_table.biom" -cmd "copy-biom run2 run2" -cmd "clear-biom run2" -cmd "parse-biom-json run1 /home/marchywka/d/zymo/run1/zr2097.180216.zymo/WithSoil.Bac16Sv34/otus/otu_table.biom" -cmd "copy-biom run1 run1" -cmd "clear-biom run1" -cmd "adhoc-tree list2.txt 0x0103 run2.svg" -quit
```

The EMP relaed plots,

```
./mjz_zymo.out -cmd "read-catagory-map phyla phyla.txt" -cmd "read-catagory-map genera genera_plus.txt" -cmd "dump-catagory-map phyla" -cmd "set-param catagory_map phyla" -cmd "set-param use_sample_filter 0" -cmd "load-tax tax-info" -cmd "parse-biom-json emp /dohs/rom/junk/emp.json" -cmd "copy-biom-conform emp emp" -cmd "clear-biom emp" -cmd "add-ragged grclass emp emp" -cmd "read-ragged xlate /home/
```

```

marchywka/junk/s2" -cmd "parse-biom-json run2 /home/marchywka/d/zymo/run1/zr2097.180309.zymo/WithSoil.
Bac16Sv34/otus/otu_table.biom" -cmd "copy-biom run2 run2" -cmd "clear-biom run2" -cmd "adhoc-tree
xxlist2.txt 0x10004 run2.svg" -cmd "clear-ragged xlate" -cmd "read-ragged xlate /home/marchywka/junk/s4
" -cmd "adhoc-tree xxlist2.txt 0x10020 run4.txt" -cmd "clear-ragged xlate" -cmd "read-ragged xlate /
home/marchywka/junk/s5" -cmd "adhoc-tree xxlist2.txt 0x10020 run5.txt" -cmd "clear-ragged xlate" -cmd "
read-ragged xlate /home/marchywka/junk/sample_ha" -cmd "adhoc-tree xxlist2.txt 0x10010 runha.txt" -quit
2> xxx

2297 more runha.txt | grep -i "[^a-z]sarcina" | grep [0-9] | sed -e 's/[ \t][ \t]*//g' >> runha1382.txt
2298 cat runha1382.txt | more
2299 vi runha1382.txt
2300 ./mjm_linc_graph.out -source zt.txt -quit
2301 cat runhat.txt

2309 cat runhat.txt | sed -e 's/|/ /g' | sort -g -k 3| mjm zed 2 > xxx
=====
2218 ./mjm_zymo.out -cmd "read-catagory-map phyla phyla.txt" -cmd "read-catagory-map genera genera_plus.txt
" -cmd "dump-catagory-map phyla" -cmd "set-param catagory_map phyla" -cmd "set-param use_sample_filter
0" -cmd "load-tax tax-info" -cmd "parse-biom-json emp /dohs/rom/junk/emp.json" -cmd "copy-biom-
conform emp emp" -cmd "clear-biom emp" -cmd "add-ragged grclass emp emp" -cmd "read-ragged xlate /
home/marchywka/junk/s2" -cmd "parse-biom-json run2 /home/marchywka/d/zymo/run1/zr2097.180309.zymo/
WithSoil.Bac16Sv34/otus/otu_table.biom" -cmd "copy-biom run2 run2" -cmd "clear-biom run2" -cmd "adhoc-
-tree xxlist2.txt 0x10004 run2.svg" -cmd "clear-ragged xlate" -cmd "read-ragged xlate /home/marchywka/
junk/s4" -cmd "adhoc-tree xxlist2.txt 0x10020 run4.txt" -cmd "clear-ragged xlate" -cmd "read-ragged
xlate /home/marchywka/junk/s5" -cmd "adhoc-tree xxlist2.txt 0x10020 run5.txt" -cmd "clear-ragged xlate"
-cmd "read-ragged xlate /home/marchywka/junk/sample_envo" -cmd "adhoc-tree xxlist2.txt 0x10010 run3.
txt" -quit 2> xxx
2227 head -n 2 run3.txt > sa1.txt
2228 grep -A 3 -i [^a-z]sarcina run3.txt >> sa1.txt
2233 vi sa1.txt
2249 cat sa1.txt | sed -e 's/[ \t][ \t]*//g' > sa1t.txt
2250 run_linc_graph -opt -compile
2251 vi zt.txt
2252 ./mjm_linc_graph.out -source zt.txt -quit
2268 more sa1t.txt | sed -e 's/|/ /g' | awk '{ x=$3+$4+$5; print $0" "x}' | sort -g -k 6
=====
```

Limishpaera dot plots,

```

./mjm_string_seq.out -cmd "set-param mflags 2" -cmd "read-fasta knowns ncbitax_16S.fasta" -cmd "index-fasta
knowns 8" -cmd "set-param fasta_dir /home/marchywka/d/jdft/jdftx/svn/cpp/mjm_libmesh/junk3/" -cmd "mt
-explore-streaming-hits knowns /home/marchywka/d/zymo/run1/zr2097.180309.zymo/WithSoil.Bac16Sv34/
SVs_poor_annotated/SV_no_hits.fasta" -quit
2521 ii=0; while [ "$ii" != 137 ] ; do echo $ii ; ./mjm_string_seq.out -cmd "set-param mflags 10" -cmd "
read-fasta knowns junk3/comp.fasta$ii.fastaa" -cmd "index-fasta knowns 8" -cmd "set-param maxscores
15" -cmd "set-param save_aligns 1" -cmd "mt-explore-streaming-hits knowns temp.fasta_dummy.fasta" -cmd
"write-align junk3/comp$ii.svg default 0x019" -quit; ii=$((ii+1)) ; done
```

Log ratio of selected microbes in Moe, Peapod, and Greta,

```
. ./mjm_linc_graph.out -cmd "read-ragged r relative_abund.txt 0" -cmd "write-svg-ragged pmg_ratio.svg r 0" -
quit
```

Entropy and read entropy calculations,

```
. ./mjm_linc_graph.out -cmd "read-ragged foo $zymorun2/taxa_plots/sorted_otu_L7.txt 6" -cmd "set-param maxcnt
10000" -cmd "string-ragged doh soil,peapod,moe,greta" -cmd "zymo-rags foo 8 4 doh" -quit
. ./mjm_linc_graph.out -cmd "read-ragged fooy $zymorun2/Dada2/abundance.table.csv 0x010" -cmd "transpose-
ragged foo fooy 0" -cmd "set-param maxcnt 10000" -cmd "string-ragged doh soil,peapod,moe,greta" -cmd "
```

```
zymo-rags foo 8 4 doh " -quit
```

Interesting unknown sequences,

```
./mjm_string_seq.out -cmd "set-param mflags 10" -cmd "read-fasta knowns best_limis.fasta" -cmd "index-fasta knowns 8" -cmd "set-param maxscores 30" -cmd "set-param save_aligns 1" -cmd "mt-explore-streaming-hits knowns temp_fasta_dummy.fasta" -cmd "write-drift string_list_limis.txt default 0x04" -quit

more string_list_limis.txt | mjm eq | mjm zed 11 | sort -g -k 2 | awk '{print $2" "$4}' | uniq -c | sort -k 3

echo AGCGTTGTTCCGATTCACTGGCGTAAAGGGTGCCTAGG | blastn -ungapped -perc_identity 100 -word_size 39 -remote -db nt -num_alignments 3 -num_descriptions 500 | tee limibl

echo AGCGTTGTTCCGATTCACTGGCGTAAAGGGTGCCTAGG | blastn -ungapped -perc_identity 100 -word_size 39 -db /opt/blast/ncbi-blast-2.5.0+/bin/16SMicrobial -num_alignments 3 -num_descriptions 500 | tee limibl

marchywka@marchywka-Latitude-E6510:~/d/jdft/jdftx svn/cpp/mjm_libmesh$ more string_list_limis.txt | grep AGCGTTGTTCCGATTCACTGGCGTAAAGGGTGCCTAGG | wc
21      315     3801
marchywka@marchywka-Latitude-E6510:~/d/jdft/jdftx svn/cpp/mjm_libmesh$ more string_list_limis.txt | grep AAGAGGAAGGGACGGCTAACTCTGTGCCAGCAGCCGCGTAATACAGAGGTCCC | wc
26      390     4931
```

adhoc-rags to output individual taxa counts with group names,

```
./mjm_zymo.out -cmd "parse-otu front front3.otu" -cmd "parse-front-reads front frontiers.csv" -cmd "read-ragged xlate front_geo.txt" -cmd "add-ragged grclass front front" -cmd "parse-biom-json run2 /home/marchywka/d/zymo/run1/zr2097.180309.zymo/WithSoil.Bac16Sv34/otus/otu_table.biom" -cmd "copy-biom run2 run2" -cmd "clear-biom run2" -cmd "add-ragged grclass run2 run2" -cmd "add-ragged otu-hash betaproteobacteria betaproteobacteria" -cmd "read-ragged otu-hash bact.txt" -cmd "set-param otu_depth_limit 3" -cmd "add-ragged xlate 4 backyard" -cmd "adhoc-rags xxlist2.txt 0xa0000 yyy.yyy" -quit
```

Additions for group density plots, tbd for inclusion, may be same as above

```
2306 ./mjm_zymo.out -cmd "parse-otu front front3.otu" -cmd "parse-front-reads front frontiers.csv" -cmd "read-ragged xlate front_geo.txt" -cmd "add-ragged grclass front front" -cmd "parse-biom-json run2 /home/marchywka/d/zymo/run1/zr2097.180309.zymo/WithSoil.Bac16Sv34/otus/otu_table.biom" -cmd "copy-biom run2 run2" -cmd "clear-biom run2" -cmd "add-ragged grclass run2 run2" -cmd "add-ragged otu-hash betaproteobacteria betaproteobacteria" -cmd "read-ragged otu-hash bact.txt" -cmd "set-param otu_depth_limit 0" -cmd "add-ragged xlate 4 backyard" -cmd "adhoc-rags xxlist2.txt 0xa0000 yyy.yyy" -quit 2>xxx
```

For the emp file,

```
grep www.www 'which run_zymo'
./mjm_zymo.out -cmd "parse-biom-json emp /dohs/rom/junk/emp.json" -cmd "copy-biom-conform emp emp" -cmd "clear-biom emp" -cmd "add-ragged grclass emp emp" -cmd "read-ragged xlate /home/marchywka/junk/sample_envo" -cmd "parse-biom-json run2 /home/marchywka/d/zymo/run1/zr2097.180309.zymo/WithSoil.Bac16Sv34/otus/otu_table.biom" -cmd "copy-biom run2 run2" -cmd "clear-biom run2" -cmd "add-ragged grclass run2 run2" -cmd "add-ragged xlate 4 backyard" -cmd "add-ragged xlate 3 Peapod" -cmd "add-ragged xlate 2 Moe" -cmd "add-ragged xlate 1 Greta" -cmd "read-ragged otu-hash bact.txt" -cmd "set-param otu_depth_limit 0" -cmd "adhoc-rags xxlist2.txt 0xa0000 www.www" -quit 2>xxx
```

With some editing, grep for envo and edit the local results to make fff.fff

```

2604 cat yyy.yyy > fff.fff
2605 vi fff.fff
2606 cat www.wwwee >> fff.fff
./mjm_violin_box_etc_plots.out -cmd "add-ragged param group_idx 0" -cmd "read-tragged in fff.fff" -cmd "add
-ragged pr bm 200" -cmd "add-ragged pr tm 250" -cmd "add-ragged pr lm 350" -cmd "add-ragged pr catpitch
100" -cmd "add-ragged pr ynames 10" -cmd "add-ragged pr xsn-run2::backyard 1" -cmd "add-ragged pr xin-
run2::backyard 0" -cmd "add-ragged pr xhn-run2::backyard .5" -cmd "add-ragged pr szleg 20" -cmd "add-
ragged pr disperse 1" -cmd "group-stats in 3 pr param violinxxx.svg" -quit > xxx

```

Investigation against larger data sets,

```

./mjm_string_seq.out -cmd "set-param mflags 2" -cmd "read-fasta knowns /home/marchywka/junk/uniq.fasta" -
cmd "set-param write_align_marks 0" -cmd "index-fasta knowns 8" -cmd "set-param maxscores 3" -cmd "mt-
explore-streaming-hits knowns /home/marchywka/d/zymo/run1/zr2097.180309.zymo/WithSoil.Bac16Sv34/
SVs_poor_annotated/SV_no_hits.fasta" -quit | tee unk_ribo.xxx

cat unk_ribo.xxx | sed -e 's/[0-9]:/\n/g' | sed -e 's/ .*//g' | grep ":" | sort | uniq -c | sort -g

```

uniq.fasta truncated during download not unbaised,

```

eutilsnew -out xxx -v -report fasta -db nucleotide "ribosomal AND 400:4000[slen] AND mito* NOT protein "
i
./mjm_linc_graph.out -cmd "stream-edit-fasta /home/marchywka/junk/ribo_mito_notp.fasta /home/marchywka/junk
/xxx" -quit
mjm_linc_graph.h638 entries=418018 wrote=27888

```

Frontiers stuff,

```

2342 vi bact.txt
2343 ./mjm_zymo.out -cmd "read-catagory-map phyla phyla.txt" -cmd "read-catagory-map genera genera_plus.
txt" -cmd "dump-catagory-map phyla" -cmd "set-param catagory_map phyla" -cmd "set-param
use_sample_filter 0" -cmd "load-tax tax-info" -cmd "parse-otu front front3.otu" -cmd "parse-front-
reads front frontiers.csv" -cmd "read-ragged xlate front_geo.txt" -cmd "add-ragged grclass front
front" -cmd "parse-biom-json run2 /home/marchywka/d/zymo/run1/zr2097.180309.zymo/WithSoil.Bac16Sv34/
otus/otu_table.biom" -cmd "copy-biom run2 run2" -cmd "clear-biom run2" -cmd "add-ragged grclass run2
run2" -cmd "add-ragged otu-hash proteobacteria proteobacteria" -cmd "set-param otu_depth_limit 3" -
cmd "add-ragged xlate 4 backyard" -cmd "adhoc-tree xxlist2.txt 0xa0010 xxx.xxx" -quit
2344 cat xxx.xxx | awk '{ i=2; n=0; s=0; max=0; min=1; while (i<=(NF-4)) { if ( ($i*1.0)>max) max=($i*1.0)
; if (($i*1.0)<min) min=($i*1.0); s=s+$i; n=n+1; i=i+1} if (n>0) { print $1"& "max"& "min"& "(s/n)"&
"($NF*1.0)"\\\"}}'

```

EMP tables,

```

2394 ./mjm_zymo.out -cmd "read-catagory-map phyla phyla.txt" -cmd "read-catagory-map genera genera_plus.txt
" -cmd "dump-catagory-map phyla" -cmd "set-param catagory_map phyla" -cmd "set-param use_sample_filter
0" -cmd "load-tax tax-info" -cmd "parse-biom-json emp /dohs/rom/junk/emp.json" -cmd "copy-biom-
conform emp emp" -cmd "clear-biom emp" -cmd "add-ragged grclass emp emp" -cmd "read-ragged xlate /
home/marchywka/junk/sample_envo" -cmd "parse-biom-json run2 /home/marchywka/d/zymo/run1/zr2097.180309.
zymo/WithSoil.Bac16Sv34/otus/otu_table.biom" -cmd "copy-biom run2 run2" -cmd "clear-biom run2" -cmd "
read-ragged otu-hash bact.txt" -cmd "set-param otu_depth_limit 3" -cmd "add-ragged xlate 4 backyard" -
cmd "adhoc-tree xxlist2.txt 0x090010 xxxx.xxx" -quit

```

```

2404 more xxx.xxx > emp_proteo.txt
2408 cp emp_proteo.txt x
2409 ./transpose yyyy x

```

```

2446 cat x | sed -e 's/[\t][\t]*/* /g'
cat transpose
./mjm_linc_graph.out -cmd "read-ragged in $2 0x08" -cmd "transpose-ragged out in 0" -cmd "write-ragged $1
out" -quit

```

EMP median ratio plots,

```
./mjm_violin_box_etc_plots.out -cmd "read-ragged in proteo_datar" -cmd "add-ragged pr catpitch 100" -cmd "add-ragged pr tm 300" -cmd "add-ragged pr rm 300" -cmd "add-ragged pr ysz 1000" -cmd "add-ragged pr szleg 30" -cmd "add-ragged pr lm 300" -cmd "transpose-ragged out in -1" -cmd "write-svg-ragged violin.svg out 0 pr" -quit

./mjm_violin_box_etc_plots.out -cmd "read-ragged in proteo_datar" -cmd "add-ragged pr catpitch 100" -cmd "add-ragged pr tm 300" -cmd "add-ragged pr rm 300" -cmd "add-ragged pr ysz 1000" -cmd "add-ragged pr szleg 30" -cmd "add-ragged pr lm 300" -cmd "add-ragged pr xfrac .5" -cmd "add-ragged pr xin-run2::4 .5" -cmd "add-ragged pr xsn-rin2::4 .5" -cmd "transpose-ragged out in -1" -cmd "write-svg-ragged violin.svg out 0 pr" -quit

./mjm_violin_box_etc_plots.out -cmd "read-ragged in proteo_datarf.txt" -cmd "add-ragged pr catpitch 100" -cmd "add-ragged pr tm 300" -cmd "add-ragged pr rm 300" -cmd "add-ragged pr ysz 1000" -cmd "add-ragged pr szleg 30" -cmd "add-ragged pr lm 300" -cmd "add-ragged pr xfrac .5" -cmd "add-ragged pr xin-landfill .5" -cmd "add-ragged pr xsn-landfill .5" -cmd "add-ragged pr xin-run2::4 .5" -cmd "add-ragged pr xsn-rin2::4 .5" -cmd "transpose-ragged out in -1" -cmd "write-svg-ragged violin.svg out 0 pr" -quit

./mjm_violin_box_etc_plots.out -cmd "read-ragged in lfxp3.txt" -cmd "add-ragged pr catpitch 100" -cmd "add-ragged pr tm 300" -cmd "add-ragged pr rm 300" -cmd "add-ragged pr ysz 1000" -cmd "add-ragged pr szleg 30" -cmd "add-ragged pr lm 300" -cmd "add-ragged pr xfrac .3" -cmd "add-ragged pr xin-landfill .7" -cmd "add-ragged pr xsn-landfill .3" -cmd "add-ragged pr xin-run2::4 0" -cmd "add-ragged pr xsn-run2::4 1" -cmd "add-ragged pr xsn-front .3" -cmd "add-ragged pr xin-front .3" -cmd "transpose-ragged out in -1" -cmd "write-svg-ragged violinlf.svg out 0 pr" -quit
```

Additional commands not used,

```
./mjm_zymo.out -cmd "read-catagory-map phyla phyla.txt" -cmd "read-catagory-map genera genera_plus.txt" -cmd "dump-catagory-map phyla" -cmd "set-param catagory_map phyla" -cmd "set-param use_sample_filter 0" -cmd "load-tax tax-info" -cmd "parse-otu front front.otu" -cmd "parse-front-reads front frontiers.csv" -cmd "read-ragged xlate front_geo.txt" -cmd "add-ragged grclass front front" -cmd "parse-biom-json run2 /home/marchywka/d/zymo/run1/zr2097.180309.zymo/WithSoil.Bac16Sv34/otus/otu_table.biom" -cmd "copy-biom run2 run2" -cmd "clear-biom run2" -cmd "add-ragged grclass run2 run2" -cmd "add-ragged xlate 4 backyard" -cmd "adhoc-tree xxlist2.txt 0x10004 run2.svg" -quit 2> xxx

./mjm_zymo.out -cmd "read-catagory-map phyla phyla.txt" -cmd "read-catagory-map genera genera_plus.txt" -cmd "dump-catagory-map phyla" -cmd "set-param catagory_map phyla" -cmd "set-param use_sample_filter 0" -cmd "load-tax tax-info" -cmd "parse-otu front front.otu" -cmd "parse-front-reads front frontiers.csv" -cmd "read-ragged xlate front_geo.txt" -cmd "add-ragged grclass front front" -cmd "parse-biom-json run2 /home/marchywka/d/zymo/run1/zr2097.180309.zymo/WithSoil.Bac16Sv34/otus/otu_table.biom" -cmd "copy-biom run2 run2" -cmd "clear-biom run2" -cmd "add-ragged grclass run2 run2" -cmd "add-ragged xlate 4 backyard" -cmd "adhoc-tree xxlist2.txt 0x10010 run2.svg" -quit 2> xxx
```

Monthly diet tables,

```
2632 vi txt/peapod.txt
2633 ./run_linc_graph -dt-mo txt/peapod.txt
2634 texfrag -include xxxtable
2635 cp xxxtable /home/documents/latex/proj/rRNAPoop/keep/peapod_mo.tex
2636 cp txt/peapod.txt txt/moe.txt
2637 vi txt/moe.txt
2638 ./run_linc_graph -dt-mo txt/peapod.txt
2639 ./run_linc_graph -dt-mo txt/moe.txt
2640 texfrag -include xxxtable
2641 cp xxxtable /home/documents/latex/proj/rRNAPoop/keep/moe_mo.tex
```

```

2642 cp txt/peapod.txt txt/greta_mo.txt
2643 vi txt/greta_mo.txt
2644 ./run_linc_graph -dt-mo txt/greta_mo.txt
2645 texfrag -include xxxtable
2646 cp xxxtable /home/documents/latex/proj/rRNAPoop/keep/greta_mo.tex
2647 cp xxxtable /home/documents/latex/proj/rRNAPoop/keep/greta_mo.tex
2648 history
marchywka@happy:/home/documents/cpp/proj/linc_graph$ cat txt/peapod.txt | grep -v "^\#"
maxsz 50
datemin 2017-08-01
datemax 2021-11-01
dogs Peapod
scope
variable datemin mjmdatemin
variable datemax mjmdatemax
variable footnotes mjmsuperscripts
caption A summary of most dietary components and events for selected months between \\mjmdatemin and \\
mjmdatemax. Format is "average daily amount ;maximum; days given/ days in interval ". Units are
arbitrary except where noted. Any superscripts are defined as follows: \\mjmsuperscripts
foodalias vmap xxx 0.0
foodalias multiB B-multi 1.0
datealias 2017-11 "2017-11 Nov"
datealias 2017-12 "2017-12 Dec"
datealias 2018-01 "2018-01 Jan"
grouping vitamin 2 "{\\bf VITAMIN}"
grouping medicine 3 "{\\bf MEDICINE}"
grouping outcome 4 "{\\bf RESULT}"
grouping food 1 "{\\bf FOOD}"
grouping 0 "{\\bf UNCLASSIFIED}"
superscript biotin(mg) a
superscript pantothenate(mg) a
superscript Iodine(mg) a
superscript lipoicacid(mg) a
footnote a SMVT substrate. Biotin, Pantothenate, Lipoic Acid, and Iodine known to compete.
superscript lysine b
superscript lysinehcl b
superscript arginine b
superscript b7ngnc c
superscript b10ngnc c
superscript b15ngnc c
superscript b20ngnc c
footnote c hamburger with varying fat percentages- 7,10,15,20, etc. .

```

```

The script run_linc_graph invokes this code,
# for using the glob parser
CODE=0x0b
#FOODS="Iodine K1 K2 furosemide diroban tyrosine"
# TODO the new files have units and need bit 8 set although crashing with
# logic error could be improved...
CMD="$EXE -cmd \"read-ragged in $DATAFILE\" -cmd \"read-ragged p $PIN\" -cmd \"snacks-time $OFILE $CODE in
p 4\" -quit 2>fuck"

```

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3. Pubmed eutils facilities and the basic research it provides.
4. Free software including Linux, R, LaTex etc.
5. Thanks everyone who contributed incidental support.

Appendix A: Statement of Conflicts

No specific funding was used in this effort and there are no relationships with others that could create a conflict of interest. I would like to develop these ideas further and have obvious bias towards making them appear successful. Barbara Cade, the dog owner, has worked in the pet food industry but this does not likely create a conflict. We have no interest in the makers of any of the products named in this work.

Zymo Research contributed free 16S sequencing to this work.

Appendix B: About the Authors and Facility

This work was performed at a dog rescue run by Barbara Cade and housed in rural Georgia. The author of this report, Mike Marchywka, has a background in electrical engineering and has done extensive research using free online literature sources. I hope to find additional people interested in critically examining the results and verify that they can be reproduced effectively to treat other dogs.

Appendix C: Symbols, Abbreviations and Colloquialisms

TERM definition and meaning

Appendix D: Sample notes and surrounding diet

Vial	Date	Time	Source	Type	Conditions	Notes
1	2018-01-15	1145	Greta	Fecal	Fresh, likely. Well formed maybe greasy	Top of hill near shed sample to middle. normal. Possibly but unlikely Beauty
2	2018-01-15	0945	Moe	Fecal	Fresh, runny inhomogeneous inclusion mucus etc	Church field in dormant grass near tall grass
3	2018-01-15	0900	Peapod	Fecal	Good, red blob	dining room carpet , normal with red blob, sample adjacent to blob and central
4	2018-01-15	1150	MoleArea	Soil	top frozen friable 4 in down	2nd post from propane tank. Use machete to dig. Moe and Greta sniffed, sample black clay soil mix.

FIG. 15: Some sample collection details for the 16S RNA analysis.

Diets were recorded in terse structured line oriented files and parsed to create daily quantities of coded nutrients or foods. Most are self explanatory although the units should be regarded as "arbitrary." In many cases estimated milligram consumption of a nutrient is indicated or can be computed but not documented here. See prior tech report for details [50]. This predates MUQED used in later works.

While Greta did have similar diet to Peapod and Moe, between surgery and collection she generally just had commercial kibble, raw beef, carrots and shrimp.

Greta's diet history from surgery to collection,

```

17497 2017-11-27 Greta 01KC 2 11KC 0.3 B-1 300 B-12 0.3 B-2 20 B-6 20 Cu 0.6 GED
  1 HMDB 1 Iodine 0.25 K2 4.5 Mg 13.3 SnAgOx 1 b20ngnc 2.75 biotin 10 carrot 0.3
  ctbroth 0.5 d-serine 17.5 egg03 0.15 garlic 0.3 kibble 0.2 lecithin 360 oliveoil
  0.3 pantothenate 550 spinach 0.3 taurine 300 vmap[cw] 202
17498 2017-11-28 Greta 11KC 0.3 B-100 0.5 Cu 0.6 GED 1 HMDB 1 K2 4.5 Mg 13.3 b20
ngnc 0.5 carrot 0.3 ctbroth 0.5 egg03 0.15 garlic 0.3 kibble 0.2 lecithin 360 ol
iveoil 0.3 pantothenate 50 spinach 0.3 taurine 300 vmap[cw] 203
17499 2017-11-29 Greta 01KC 1 b20ngnc 1 cephalexin 0 ctbroth 1 deramaxx 0 eyeser
um 1 shrimp 26.9461 vmap[cw] 204
17500 2017-11-30 Greta GED 1 cephalexin 2 ctbroth 2 ctskinbs 1 deramaxx 1 eyeser
um 1 garbage 1 shrimp 8.98202 vmap[cw] 205
17501 2017-12-01 Greta cephalexin 1000 ctbroth 1.2 swings 2 vmap[cw] 206

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17502 2017-12-02 Greta b20ngnc 0.1 cephalixin 1 ctbroth 1 etc 1 vmap[cw] 207
 17503 2017-12-03 Greta 11KC 0 B-6 0 Cu 0 K2 0 Mg 0 b20ngnc 2 carrot 0 cephalixin
 2 ctbroth 2 ctskinbs 1 d-serine 0 egg03 0 garlic 0 lecithin 0 oliveoil 0 pantot
 henate 0 spinach 0 taurine 0 vmap[cw] 208
 17504 2017-12-04 Greta 11KC 0 B-6 0 Cu 0 K2 0 KibbleAmJrLaPo 1 Mg 0 b20ngnc 1 ca
 rrrot 0 cephalixin 1 ctbroth 2 ctskinbs 1 d-serine 0 egg03 0 garlic 0 lecithin 0
 oliveoil 0 pantothenate 0 spinach 0 taurine 0 vmap[cw] 209
 17505 2017-12-05 Greta KibbleAmJrLaPo 1 b20ngnc 2 cephalixin 2 ctbroth 2 deramax
 x 1 shrimp 9.96905 vmap[cw] 210
 17506 2017-12-06 Greta KibbleAmJrLaPo 1 b20ngnc 1 cephalixin 2 ctbroth 3 shrimp
 9.96905 vmap[cw] 211
 17507 2017-12-07 Greta KibbleAmJrLaPo 1 b20ngnc 1 cephalixin 1 ctbroth 2 ctskinb
 s 1 shrimp 9.96905 vmap[cw] 212
 17508 2017-12-08 Greta KibbleAmJrLaPo 1 cephalixin 2 ctbroth 2 vmap[cw] 213
 17509 2017-12-09 Greta KibbleAmJrLaPo 1 b20ngnc 1 cephalixin 3 ctbroth 3 ctskinb
 s 1 vmap[cw] 214
 17510 2017-12-10 Greta KibbleAmJrLaPo 1 cephalixin 1 ctbroth 2 goatmilk 1 shrimp
 9.96905 vmap[cw] 215
 ... mostly the same ...
 17540 2018-01-09 Greta KibbleAmJrLaPo 1 b20ngnc 2 ctbroth 2 egg03 0.4 shrimp 29.
 9072 vmap[cw] 245
 17541 2018-01-10 Greta b20ngnc 2 carrot 1 ctbroth 2 shrimp 19.9381 vmap[cw] 246
 17542 2018-01-11 Greta KibbleAmJrLaPo 1 b20ngnc 2 carrot 1 ctbroth 2 ctskinbs 1
 egg03 0.25 vmap[cw] 247
 17543 2018-01-12 Greta b20ngnc 2 carrot 1 ctbroth 2 egg03 0.25 vmap[cw] 248
 17544 2018-01-13 Greta KibbleAmJrLaPo 1 b20ngnc 2 ctbroth 2 shrimp 9.96905 vmap[
 cw] 249
 17545 2018-01-14 Greta KibbleAmJrLaPo 1 b20ngnc 2 ctbroth 2 vmap[cw] 250
 17546 2018-01-15 Greta KibbleAmJrLaPo 1 b20ngnc 2 carrot 1 ctbroth 2 ctskinbs 1
 shrimp 9.96905 vmap[cw] 251

Peapod's recent diet prior to collection. Note the vitamins and only intermittent kibble.

17538 2018-01-07 Peapod 11KC 2.75 B-1 150 B-6 25 Cu 3 Iodine 0.25 K2 3.75 KibbleAmJrLaPo 0.4 Mg 33.25
 SnAgOx 1 arginine 250 b20ngnc 3.75 biotin 10 carrot 0.5 ctbroth 1.5 d-serine 43.75 egg03 0.25 garlic
 1.5 lecithin 3300 lipoicacid 1 oliveoil 0.75 pantothenate 1125 spinach 0.5 taurine 500 vmap[cw] 242
 17539 2018-01-08 Peapod 11KC 3.7 B-1 150 B-6 25 Cu 2.5 Iodine 0.25 K2 3.75 Mg 33.25 arginine 225 b20ngnc
 3.7 biotin 10 carrot 1.45 ctbroth 1.45 d-serine 35 egg03 0.225 garlic 1.45 lecithin 4440 oliveoil 3.45
 pantothenate 1125 shrimp 29.9072 spinach 1.45 taurine 1450 yeggo3 0.5 vmap[cw] 243
 17540 2018-01-09 Peapod 11KC 2.45 B-1 150 B-12 0.375 B-6 20 K2 3 KibbleAmJrLaPo 0.3 Mg 26.6 arginine 100
 b20ngnc 2.45 biotin 10 carrot 0.45 coffee 1 ctbroth 0.45 d-serine 43.75 egg03 0.225 garlic 1.2 lecithin
 2940 oliveoil 1.7 pantothenate 1100 spinach 0.45 taurine 450 vmap[cw] 244
 17541 2018-01-10 Peapod 11KC 3.45 B-1 150 B-12 0.375 B-2 25 B-6 20 Cu 0.9 K2 3 Mg 26.6 b20ngnc 3.7 biotin
 10 carrot 1.45 chili 0.25 ctbroth 1.45 d-serine 43.75 egg03 0.475 garlic 1.45 lecithin 4440 oliveoil
 3.7 pantothenate 1100 shrimp 19.9381 spinach 1.45 taurine 1450 teeth 1 vmap[cw] 245
 17542 2018-01-11 Peapod 11KC 2.5 B-1 150 B-12 0.375 B-3 50 B-6 25 Cu 3 K2 3.75 KibbleAmJrLaPo 0.4 Mg 33.25
 b20ngnc 4.5 biotin 10 carrot 0.5 chili 1 ctbroth 0.5 d-serine 43.75 egg03 0.25 garlic 1.5 lecithin 4200
 lipoicacid 1 oliveoil 3.5 pantothenate 1125 spinach 0.5 taurine 500 teeth 2 vmap[cw] 246
 17543 2018-01-12 Peapod 11KC 3.5 B-1 150 B-2 25 B-6 25 Cu 3 K2 3.75 Mg 33.25 b20ngnc 3.7 biotin 10 carrot
 1.5 chili 0.2 coffee 1 ctbroth 1.5 d-serine 43.75 egg03 0.5 garlic 1.5 lecithin 4440 oliveoil 3.7
 pantothenate 1125 shrimp 19.9381 spinach 1.5 taurine 1500 teeth 1 vmap[cw] 247
 17544 2018-01-13 Peapod 11KC 3.5 B-100 0.5 Cu 3 K2 3.75 Mg 33.25 b20ngnc 4.5 biotin 10 carrot 1.5 chili 1
 ctbroth 1.5 d-serine 43.75 egg03 0.25 garlic 1.5 lecithin 4200 oliveoil 3.5 pantothenate 625 shrimp
 29.9072 spinach 1.5 taurine 1500 teeth 1 worm 0.75 vmap[cw] 248
 17545 2018-01-14 Peapod 11KC 2.5 B-1 150 B-6 25 Cu 0.5 K2 3.75 KibbleAmJrLaPo 0.4 Mg 33.25 arginine 125
 b20ngnc 3.5 biotin 10 carrot 0.5 ctbroth 1.5 d-serine 43.75 egg03 0.25 garlic 1.25 lecithin 3000
 oliveoil 2.5 pantothenate 1125 spinach 0.5 taurine 500 teeth 1 vmap[cw] 249
 17546 2018-01-15 Peapod 11KC 3.75 B-1 150 B-12 0.375 B-6 25 K2 7.5 Mg 33.25 b20ngnc 3.75 biotin 10 carrot
 1.5 chili 0.25 ctbroth 1.5 d-serine 43.75 egg03 0.25 lecithin 4500 oliveoil 3.75 pantothenate 1125
 shrimp 19.9381 spinach 1.5 taurine 250 teeth 1 yeggo3 0.5 vmap[cw] 250

Moe's diet history prior to collection. Kibble everyday plus the raw "snack" including various vitamins. Greta had a similar diet prior to surgery.

17538 2018-01-07 Moe 11KC 2.75 B-6 25 Cu 1 Iodine 0.25 K2 3.75 Mg 33.25 SnAgOx 1 arginine 250 b20ngnc 2.95 biotin 10 carrot 0.5 ctbroth 0.7 d-serine 43.75 egg03 0.25 garlic 0.5 kibble 0.2 lecithin 3300 lipoicacid 1 oliveoil 0.75 pantothenate 625 spinach 0.5 taurine 500 vmap[cw] 243

17539 2018-01-08 Moe 11KC 1.75 B-1 150 B-6 25 Cu 0.5 Iodine 0.25 K2 3.75 Mg 33.25 SnAgOx 1 arginine 250 b20ngnc 1.95 biotin 10 carrot 0.5 ctbroth 0.7 d-serine 43.75 egg03 0.25 garlic 0.5 kibble 0.2 lecithin 2100 oliveoil 1.5 pantothenate 625 spinach 0.5 taurine 500 vmap[cw] 244

17540 2018-01-09 Moe 11KC 0.5 B-1 150 B-12 0.375 B-6 25 K2 3.75 Mg 33.25 arginine 125 b20ngnc 0.7 carrot 0.5 ctbroth 0.7 d-serine 43.75 egg03 0.25 garlic 0.25 kibble 0.2 lecithin 600 oliveoil 0.75 pantothenate 625 shrimp 9.96905 spinach 0.5 taurine 500 vmap[cw] 245

17541 2018-01-10 Moe 11KC 1.5 B-12 0.375 B-2 25 B-6 25 Cu 1 K2 3.75 Mg 33.25 b20ngnc 1.95 biotin 10 carrot 0.5 chili 0.25 ctbroth 0.7 d-serine 43.75 egg03 0.25 garlic 0.5 kibble 0.2 lecithin 2100 oliveoil 1.75 pantothenate 125 spinach 0.5 taurine 500 vmap[cw] 246

17542 2018-01-11 Moe 11KC 1.5 B-12 0.375 B-6 25 Cu 1 K2 3.75 Mg 33.25 b20ngnc 2.7 biotin 10 carrot 0.5 chili 1 ctbroth 0.7 ctskinbs 0.2 d-serine 43.75 egg03 0.25 garlic 0.5 kibble 0.2 lecithin 3000 lipoicacid 1 oliveoil 2.5 pantothenate 625 spinach 0.5 taurine 500 vmap[cw] 247

17543 2018-01-12 Moe 11KC 1.5 B-1 150 B-2 25 B-6 25 Cu 1 K2 3.75 Mg 33.25 b20ngnc 1.9 biotin 10 carrot 0.5 chili 0.2 ctbroth 0.7 d-serine 43.75 egg03 0.25 garlic 0.5 kibble 0.2 lecithin 2040 oliveoil 1.7 pantothenate 125 spinach 0.5 taurine 500 vmap[cw] 248

17544 2018-01-13 Moe 11KC 0.5 B-100 0.5 Cu 1 K2 3.75 Mg 33.25 b20ngnc 0.7 carrot 0.5 ctbroth 0.7 d-serine 43.75 egg03 0.25 garlic 0.5 kibble 0.2 lecithin 600 oliveoil 0.5 pantothenate 125 shrimp 9.96905 spinach 0.5 taurine 500 vmap[cw] 249

17545 2018-01-14 Moe 11KC 2.5 B-6 25 Cu 0.5 K2 3.75 Mg 33.25 arginine 125 b20ngnc 2.7 biotin 10 carrot 0.5 ctbroth 0.7 d-serine 43.75 egg03 0.25 garlic 0.25 kibble 0.2 lecithin 3000 oliveoil 2.5 pantothenate 625 spinach 0.5 taurine 500 vmap[cw] 250

17546 2018-01-15 Moe 11KC 2.75 B-1 150 B-12 0.375 B-6 25 K2 7.5 Mg 33.25 b20ngnc 2.95 biotin 10 carrot 0.5 chili 0.25 ctbroth 0.7 d-serine 43.75 egg03 0.25 kibble 0.2 lecithin 3300 oliveoil 2.75 pantothenate 1125 spinach 0.5 taurine 250 vmap[cw] 251

Appendix E: Monthly Diet Tables

Name	2017-11 Nov	2017-12 Dec	2018-01 Jan
FOOD			
b20ngnc ^(c)	2.4 ;1;29/30	1.6 ;1;28/31	2 ;1;31/31
canned		0.23 ;1;5/31	
carrot	0.54 ;1;28/30	0.032 ;1;1/31	0.42 ;1;20/31
chili			0.048 ;1;3/31
coffee	0.067 ;1;2/30		
cooked		0.035 ;1;2/31	
ctbrothbs	1.6 ;1;30/30	1.9 ;1;30/31	1.7 ;1;31/31
ctbrothb	0.11 ;1;2/30	0.13 ;1;3/31	
ctskinbs	0.13 ;1;4/30	0.77 ;1;19/31	0.35 ;1;11/31
ctskin	0.033 ;1;1/30		
eggo3	0.21 ;0.5;28/30		0.088 ;0.4;15/31
garlic	0.54 ;1;27/30		0.093 ;0.12;12/31
oliveoil	0.43 ;1;27/30		0.22 ;1;13/31
serrano			0.032 ;1;1/31
shrimp(grams)	3.5 ;50;4/30	6.4 ;30;15/31	5.8 ;30;12/31
spinach	0.34 ;0.2;27/30		0.093 ;0.12;12/31
VITAMIN			
B-100(count)	0.1 ;1;5/30		
B-12(mg)	0.14 ;0.3;14/30		0.024 ;0.19;4/31
B-1	0.59 ;1;20/30		
B-2(mg)	7.7 ;20;12/30		1.2 ;12;3/31
B-6(mg)	28 ;40;22/30		3.2 ;12;8/31
Cu(mg)	0.55 ;0.4;27/30		0.089 ;0.25;7/31
Iodine(mg) ^(a)	0.097 ;0.25;15/30		8.06e-03 ;0.25;1/31
K2(mg)	6.6 ;15;26/30		1.9 ;15;12/31
Mg(mg)	2.9 ;27;5/30		3.8 ;17;7/31
Mg	0.087 ;0.2;12/30		
arginine ^(b)	6.67e-03 ;0.2;1/30		0.044 ;0.12;11/31
biotin(mg) ^(a)	8.6 ;10;26/30		1.6 ;10;5/31
d-serine	0.13 ;0.2;21/30		0.044 ;0.12;9/31
lecithin(lecu)	410 ;240;27/30		392 ;1200;14/31
lipoicacid(mg) ^(a)	133 ;200;20/30		
pantothenate(mg) ^(a)	464 ;500;27/30		46 ;500;8/31
taurine(mg)	227 ;200;27/30		60 ;81;12/31
MEDICINE			
cephalexin	0.067 ;1;1/30	0.52 ;2;10/31	
deramaxx	0.033 ;1;1/30	0.032 ;1;1/31	
gentamicin	0.3 ;1;9/30		
swings		0.065 ;1;1/31	
RESULT			
weight(kg)	0.8 ;24;1/30		
01KC	0.78 ;1;18/30		
11KC	0.34 ;0.2;27/30		0.32 ;1;13/31
B-3(mg)	3.3 ;100;1/30		
B-3	0.033 ;1;1/30		
HMDB	2.6 ;1;28/30		
KibbleAmJrLaPo		0.9 ;1;28/31	0.92 ;1;29/31
KibbleEliteSeriesBeef	0.78 ;1;24/30		

TABLE XXXIII: Part 1 of 2. Events Summary for Greta from 2017-11-01 to 2018-01-31A summary of most dietary components and events for selected months between 2017-11-01and 2018-01-31. Format is average daily amount ;maximum; days given/ days in interval . Units are arbitrary except where noted. Any superscripts are defined as follows: **a)** SMVT substrate. Biotin, Pantothenate, Lipoic Acid, and Iodine known to compete..**c)** hamburger with varying fat percentages- 7,10,15,20, etc. ..

Name	2017-11 Nov	2017-12 Dec	2018-01 Jan
SnAgOx	0.63 ;1;19/30		
etc		0.032 ;1;1/31	
goatmilk		0.39 ;1;12/31	
kelp	0.017 ;0.25;2/30		
kibble	0.017 ;0.5;1/30		
worm	0.083 ;2.5;1/30		

TABLE XXXIV: Part 2 of 2. Events Summary for Greta from 2017-11-01 to 2018-01-31A summary of most dietary components and events for selected months between 2017-11-01and 2018-01-31. Format is average daily amount ;maximum; days given/ days in interval . Units are arbitrary except where noted. Any superscripts are defined as follows: **a)** SMVT substrate. Biotin, Pantothenate, Lipoic Acid, and Iodine known to compete..**c)** hamburger with varying fat percentages- 7,10,15,20, etc. ..

Name	2017-11 Nov	2017-12 Dec	2018-01 Jan
FOOD			
b20ngnc ^(c)	2.9 ;1;30/30	3.2 ;1;31/31	2.7 ;1;31/31
carrot	0.68 ;1;30/30	0.5 ;0.25;31/31	0.53 ;1;31/31
chili			0.19 ;1;15/31
coffee	0.067 ;1;2/30		
ctbrothbs	1.6 ;1;30/30	1.4 ;1;31/31	1.5 ;1;31/31
ctbrothb	0.11 ;1;2/30	0.081 ;1;3/31	
ctskinbs	0.033 ;1;1/30	0.16 ;1;5/31	0.23 ;1;7/31
eggo3	0.26 ;1;30/30	0.3 ;0.5;31/31	0.3 ;1;31/31
garlic	0.74 ;1;30/30	0.58 ;1;31/31	0.46 ;0.25;30/31
habanero			8.06e-03 ;0.25;1/31
jalapeno			8.06e-03 ;0.25;1/31
oliveoil	0.47 ;1;30/30	0.3 ;0.25;31/31	1.2 ;1;31/31
serrano			0.048 ;0.25;6/31
shrimp(grams)	2 ;50;2/30	0.96 ;10;3/31	5.1 ;60;7/31
spinach	0.44 ;1;30/30	0.5 ;0.25;31/31	0.5 ;0.25;31/31
VITAMIN			
B-100(count)	0.067 ;0.5;4/30	0.065 ;0.5;4/31	0.048 ;0.5;3/31
B-12(mg)	0.2 ;1.5;16/30	0.2 ;0.38;17/31	0.15 ;0.38;12/31
B-1	0.3 ;1;14/30	0.27 ;1;15/31	0.21 ;1;12/31
B-2(mg)	11 ;100;12/30	7.1 ;25;9/31	6.5 ;25;8/31
B-6(mg)	33 ;50;25/30	21 ;25;26/31	20 ;25;25/31
Cu(mg)	1.2 ;2;30/30	1.1 ;2;31/31	0.65 ;0.5;25/31
Iodine(mg) ^(a)	0.13 ;0.25;19/30	0.22 ;0.26;27/31	0.097 ;0.25;12/31
K2(mg)	5.6 ;15;29/30	4 ;3.8;31/31	4.7 ;3.8;31/31
Mg(mg)	6.2 ;33;6/30	33 ;33;31/31	29 ;33;27/31
Mg	0.11 ;1;12/30		
Mn(mg)			2.52e-04 ;0.0078;1/31
arginine(mg)		48 ;250;8/31	
arginine ^(b)	0.023 ;0.25;3/30	0.14 ;0.5;12/31	0.26 ;0.25;24/31
biotin(mg) ^(a)	5.9 ;10;29/30	5.3 ;10;29/31	5.5 ;10;17/31
d-serine	0.18 ;0.3;24/30	0.17 ;0.25;22/31	0.25 ;0.5;27/31
lecithin(lecu)	574 ;1200;30/30	1717 ;1200;31/31	1771 ;1200;31/31
lipoicacid(mg) ^(a)	10 ;100;3/30	17 ;33;21/31	13 ;32;16/31
lipoicacid	2.4 ;4;25/30	0.68 ;3;10/31	
pantothenate(mg) ^(a)	351 ;500;29/30	406 ;500;31/31	419 ;500;28/31
taurine(mg)	294 ;650;30/30	325 ;250;31/31	320 ;162;31/31
MEDICINE			
clindamycin(mg)			1.6 ;50;1/31
RESULT			
weight(kg)	0.67 ;20;1/30		
01KC	0.88 ;1;23/30	0.29 ;1;9/31	
11KC	0.48 ;1;30/30	1.5 ;1;31/31	1.4 ;1;31/31
HMDB	0.2 ;1;5/30		
KibbleEliteSeriesBeef	1 ;1;30/30	1 ;1;31/31	1 ;1;31/31
SnAgOx	0.33 ;1;9/30	1.2 ;1;21/31	0.55 ;1;14/31
goatmilk		0.48 ;1;15/31	
kelp	0.025 ;0.25;3/30		
worm	0.067 ;2;1/30		

TABLE XXXV: Part 1 of 2. Events Summary for Moe from 2017-11-01 to 2018-01-31A summary of most dietary components and events for selected months between 2017-11-01and 2018-01-31. Format is average daily amount ;maximum; days given/ days in interval . Units are arbitrary except where noted. Any superscripts are defined as follows: **a)** SMVT substrate. Biotin, Pantothenate, Lipoic Acid, and Iodine known to compete..**c)** hamburger with varying fat percentages- 7,10,15,20, etc. ..

Name	2017-11 Nov	2017-12 Dec	2018-01 Jan

TABLE XXXVI: Part 2 of 2. Events Summary for Moe from 2017-11-01 to 2018-01-31A summary of most dietary components and events for selected months between 2017-11-01and 2018-01-31. Format is average daily amount ;maximum; days given/ days in interval . Units are arbitrary except where noted. Any superscripts are defined as follows: **a)** SMVT substrate. Biotin, Pantothenate, Lipoic Acid, and Iodine known to compete..**c)** hamburger with varying fat percentages- 7,10,15,20, etc. ..

Name	2017-11 Nov	2017-12 Dec	2018-01 Jan
FOOD			
b20ngnc ^(c)	3.1 ;1;30/30	3.5 ;1;31/31	3.9 ;1;31/31
carrot	1.6 ;1;30/30	1.3 ;1;31/31	1.2 ;1;31/31
chili			0.28 ;1;17/31
coffee	0.1 ;1;3/30	0.065 ;1;2/31	0.097 ;1;3/31
ctbrothbs	1.7 ;1;30/30	1.5 ;1;31/31	1.5 ;1;31/31
ctbrothb	0.11 ;1;2/30	0.048 ;1;3/31	
eggo3	0.34 ;1;30/30	0.38 ;0.5;31/31	0.3 ;0.5;31/31
garlic	1.5 ;1;30/30	1.6 ;1;31/31	1.5 ;1;30/31
habanero			0.032 ;1;1/31
jalapeno			8.06e-03 ;0.25;1/31
oliveoil	1.5 ;1;30/30	0.65 ;1;31/31	2.5 ;2;31/31
serrano			0.073 ;1;6/31
shrimp(grams)	26 ;30;29/30	16 ;30;21/31	18 ;30;20/31
shrimp	0.033 ;1;1/30	0.032 ;1;1/31	
spinach	1.5 ;1;30/30	1.3 ;1;31/31	1.2 ;1;31/31
VITAMIN			
B-100(count)	0.067 ;0.5;4/30	0.065 ;0.5;4/31	0.048 ;0.5;3/31
B-12(mg)	0.24 ;1.5;16/30	0.19 ;0.38;16/31	0.14 ;0.38;12/31
B-1	0.47 ;0.5;26/30	0.44 ;0.5;27/31	0.39 ;0.5;24/31
B-2(mg)	11 ;100;12/30	7.3 ;25;9/31	6.5 ;25;8/31
B-6(mg)	38 ;200;25/30	28 ;100;26/31	27 ;100;25/31
Cu(mg)	2.8 ;2;30/30	3.1 ;2;31/31	2.4 ;2;29/31
Iodine(mg) ^(a)	0.12 ;0.25;19/30	0.21 ;0.25;26/31	0.1 ;0.25;13/31
K2(mg)	7 ;15;28/30	5.9 ;15;31/31	5.8 ;15;31/31
Mg(mg)	5.5 ;33;6/30	46 ;133;31/31	39 ;133;27/31
Mg	0.11 ;1;12/30		
Mn(mg)			2.52e-04 ;0.0078;1/31
arginine(mg)		48 ;250;8/31	
arginine ^(b)	0.023 ;0.25;3/30	0.15 ;0.5;12/31	0.3 ;1;24/31
biotin(mg) ^(a)	11 ;10;30/30	9 ;10;29/31	9.7 ;10;30/31
d-serine(mg)	2.9 ;88;1/30		
d-serine	0.23 ;1;24/30	0.28 ;1;23/31	0.55 ;1;30/31
lecithin(lecu)	1819 ;1200;30/30	2836 ;1200;31/31	3787 ;1200;31/31
lipoicacid(mg) ^(a)	5 ;50;3/30	16 ;29;20/31	10 ;24;16/31
lipoicacid	1.8 ;2.5;25/30	0.79 ;2.5;10/31	
pantothenate(mg) ^(a)	758 ;500;30/30	1020 ;500;31/31	952 ;1000;30/31
taurine(mg)	989 ;650;30/30	847 ;650;31/31	657 ;650;31/31
MEDICINE			
clindamycin(mg)			17 ;50;6/31
swings		0.032 ;1;1/31	0.065 ;1;1/31
RESULT			
weight(kg)	0.2 ;5.9;1/30		
01KC	0.94 ;1;23/30	0.29 ;1;9/31	
11KC	1.5 ;1;30/30	2.4 ;1;31/31	3.1 ;1;31/31
B-3	0.05 ;0.5;3/30	0.097 ;0.5;6/31	0.081 ;0.5;5/31
HMDB	0.13 ;1;4/30		
KibbleAmJrLaPo		0.18 ;1;17/31	0.14 ;0.4;11/31
SnAgOx	0.3 ;1;7/30	1.2 ;1;23/31	0.9 ;1;18/31

TABLE XXXVII: Part 1 of 2. Events Summary for Peapod from 2017-11-01 to 2018-01-31A summary of most dietary components and events for selected months between 2017-11-01and 2018-01-31. Format is average daily amount ;maximum; days given/ days in interval . Units are arbitrary except where noted. Any superscripts are defined as follows: a) SMVT substrate. Biotin, Pantothenate, Lipoic Acid, and Iodine known to compete..c) hamburger with varying fat percentages- 7,10,15,20, etc.

..

Name	2017-11 Nov	2017-12 Dec	2018-01 Jan
brush			0.097 ;1;3/31
goatmilk		0.097 ;1;3/31	
kelp	0.023 ;0.25;3/30		
kibble	0.23 ;1;16/30	3.23e-03 ;0.1;1/31	
noeggo3	0.37 ;1;11/30	8.06e-03 ;0.25;1/31	
teeth			0.55 ;1;16/31
worm	0.025 ;0.75;1/30		0.024 ;0.75;1/31
yeggo3			0.081 ;0.5;5/31

TABLE XXXVIII: Part 2 of 2. Events Summary for Peapod from 2017-11-01 to 2018-01-31A summary of most dietary components and events for selected months between 2017-11-01and 2018-01-31. Format is average daily amount ;maximum; days given/ days in interval . Units are arbitrary except where noted. Any superscripts are defined as follows: **a)** SMVT substrate. Biotin, Pantothenate, Lipoic Acid, and Iodine known to compete..**c)** hamburger with varying fat percentages- 7,10,15,20, etc. ..

Appendix F: Full Comparison to select EMP samples and bacteria

Sample	Other	acidobacteria	actinobacteria	fusobacteria	Proteobacteria				
					alpha	beta	delta	epsilon	gamma
animal-associated	0.72	0.00083		0.037	0.01	0.028	0.062	0.0042	0.0056
Bay	0.4	0.25		0.12	8e-06	0.041	0.0066	0.054	0.0053
bay	0.095	0.00011		0.099	0.035	0.26	0.075	0.0021	0.026
canyon	0.49	0.064		0.21	0.0001	0.12	0.06	0.033	0.0042
coastal	0.36	0.00077		0.042	3.6e-05	0.26	0.029	0.015	0.0011
coastline	0.58	0.013		0.013	0.00011	0.037	0.13	0.11	0.024
cold	0.34	0.00074		0.037	0.00022	0.1	0.021	0.09	0.0011
cove	0.4	0.23		0.021	0.00013	0.067	0.077	0.071	0.01
creek	0.61	0.026		0.034	7.1e-05	0.035	0.14	0.09	5.5e-05
cultivated	0.53	0.19		0.033	4.5e-07	0.075	0.067	0.067	0
dry	0.45	0.18		0.07	5e-06	0.18	0.058	0.026	0.0021
estuary	0.47	0.088		0.082	0.00074	0.12	0.022	0.1	0.00018
farm	0.38	0.26		0.091	0.0034	0.11	0.078	0.017	0.00031
fjord	0.46	7.3e-05		0.0044	1.1e-05	0.11	0.0031	0.00051	0.0002
fluvisol	0.5	0.11		0.029	1.5e-06	0.11	0.094	0.031	0.0016
forest	0.32	0.38		0.061	9.8e-05	0.16	0.032	0.011	4.2e-06
Forest	0.41	0.32		0.07	0	0.11	0.044	0.02	1.9e-06
fresh	0.28	0.05		0.15	0.0012	0.065	0.35	0.042	0.0047
freshwater	0.4	0.0039		0.24	2.7e-05	0.088	0.14	0.0025	0.00033
grassland	0.46	0.19		0.079	1.5e-05	0.098	0.054	0.063	7.2e-07
haline	0.79	6.6e-05		0.012	2.9e-05	0.026	0.00081	0.058	0.00012
hot	0.84	0.098		0.0002	7.1e-07	0.0098	0.013	0.036	7.1e-06
human-associated	0.67	0.00038		0.092	0.034	0.016	0.071	0.002	0.0029
intertidal	0.69	2.1e-05		0.00043	0.0002	0.091	9.3e-06	0.00075	0.008
lagoon	0.26	0.27		0.033	5.9e-05	0.11	0.0084	0.033	0.0042
lake	0.72	0.00016		0.1	4.3e-05	0.033	0.13	0.00034	1.7e-05
luvisol	0.41	0.23		0.093	0	0.17	0.044	0.0092	0
mammalia-associated	0.58	0.0014		0.084	0.0042	0.082	0.092	0.0025	0.0014
marine	0.57	0.0083		0.067	0.00018	0.15	0.0091	0.003	0.0016
mountain	0.42	0.35		0.04	0	0.11	0.033	0.018	0
neritic	0.25	0.015		0.0075	0.0011	0.042	0.0056	0.21	0.069
nest	0.35	0.0041		0.21	0.00034	0.11	0.03	0.0019	0.0048
ocean	0.46	0.042		0.031	6.7e-05	0.16	0.0015	0.078	0.011
oil	0.2	0.02		0.11	1.3e-05	0.043	0.21	0.026	0.00091
permafrost	0.5	0.19		0.06	2.3e-06	0.081	0.11	0.03	7.7e-05
plant-associated	0.4	0.025		0.08	0.00085	0.16	0.1	0.037	0.0017
plantation	0.61	0.15		0.15	5.2e-07	0.048	0.016	0.015	1.9e-05
podzol	0.3	0.42		0.048	0	0.14	0.038	0.0059	0
pond	0.57	0.081		0.037	1.9e-05	0.059	0.039	0.18	0.00014
rice	0.44	0.17		0.034	0.00034	0.067	0.088	0.17	0.00056
river	0.57	0.012		0.0084	0.00014	0.065	0.0059	0.27	0.0025
rocky	0.38	0.38		0.12	8.5e-06	0.052	0.03	0.009	0.00075
sandy	0.46	0.11		0.27	0	0.11	0.017	0.022	0
sea	0.37	0.33		0.029	0.00032	0.074	0.0074	0.062	0.067
seawater	0.17	4e-05		0.0047	0.085	0.063	0.0006	0.02	0.01
shrubland	0.47	0.12		0.19	0.0017	0.16	0.021	0.023	4.3e-05
steppe	0.45	0.054		0.04	2.6e-06	0.052	0.019	0.011	6.7e-07
stromatolite	0.4	0.16		0.061	0	0.24	0.035	0.034	0.0055
surface	0.31	0.044		0.11	2.6e-05	0.064	0.25	0.019	0.0016
tidal	0.38	0.00016		0.0023	0.0049	0.37	0.00065	0.0019	0.006
tropical	0.28	0.59		0.011	0	0.037	0.032	0.021	1.6e-06
tundra	0.45	0.13		0.14	3.4e-05	0.12	0.096	0.048	2.5e-05
volcano	0.41	0.36		0.097	8.1e-05	0.049	0.014	0.05	9e-06
water	0.51	7e-05		0.019	2.6e-05	0.034	0.1	4.5e-05	0.00014
run2::1	0.63	0		0.032	0.32	0	0.021	0	0
run2::2	0.72	0		0.013	0.2	0	0.042	0	0.024
run2::3	0.74	0		0.023	0.18	0	0.029	0	0.026
run2::4	0.32	0.19		0.22	0	0.14	0.065	0.045	0

TABLE XXXIX: Some mean values for various EMP environments. Notably absent is zero except for a few cases discussed in the text believed to be meaningful.

Appendix G: Some of the sequences used here

```

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>seq1076 426
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GAGCAA
>seq686 403
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>seq918 407
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GCAA

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CGA
```

>seq462 424

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>seq1002 401

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Appendix H: General caveats and disclaimer

This document was created in the hope it will be interesting to someone including me by providing information about some topic that may include personal experience or a literature review or description of a speculative theory or idea. There is no assurance that the content of this work will be useful for any particular purpose.

All statements in this document were true to the best of my knowledge at the time they were made and every attempt is made to assure they are not misleading or confusing. However, information provided by others and observations that can be manipulated by unknown causes ("gaslighting") may be misleading. Any use of this information should be preceded by validation including replication where feasible. Errors may enter into the final work at every step from conception and research to final editing.

Documents labelled "NOTES" or "not public" contain substantial informal or speculative content that may be terse and poorly edited or even sarcastic or profane. Documents labelled as "public" have generally been edited to be more coherent but probably have not been reviewed or proof read.

Generally non-public documents are labelled as such to avoid confusion and embarrassment and should be read with that understanding.

Appendix I: Citing this as a tech report or white paper

Note: This is mostly manually entered and not assured to be error free.
This is tech report MJM-2021-010.

Version	Date	Comments
0.01	2021-10-30	Create from empty.tex template
0.01	2021-10-30	Copy in text from microbepub.tex
0.01	2018-10-03	Most of the data and text in microbepub.tex is coherent.
0.10	2021-11-08	Release draft due to Sarcina importance and time pressure.
-	November 7, 2021	version 0.10 MJM-2021-010
1.0	20xx-xx-xx	First revision for distribution

Released versions,
build script needs to include empty releases.tex

Version	Date	URL
0.10	2021-11-08	https://www.researchgate.net/publication/355982163_16S_rRNA_Analysis_of_3_Canine_Fecal_Samples_and_their_Relationship_to_Disease_Status
0.10	2021-11-08	https://www.academia.edu/s/16646631ed
0.10	2021-11-08	https://www.linkedin.com/posts/marchywka_notes-from-2018-rrna-analysis-of-sick-dogs-activity-6863258

```

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type ="techreport" ,
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contact ="marchywka@hotmail.com" ,
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pages =" 60"
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```

Supporting files. Note that some dates,sizes, and md5's will change as this is rebuilt.

This really needs to include the data analysis code but right now it is auto generated picking up things from prior build in many cases

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42082 Nov 7 18:19 rRNApoop.aux 2ecc9a5c2db08a60546f59d73503124c
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4096 Nov 7 18:20 rRNApoop.bundle_checksums 7a60e9cfcdac4e2beba3da89d5f00ab
33703 Nov 7 18:19 rRNApoop.flts 38741d577d54a1764c65e7031dfca60f
3 Nov 7 18:19 rRNApoop.last_page ecf27a776cdfc771defab1c5d19de9ab
78090 Nov 7 18:19 rRNApoop.log 63f4e9c9c84128d7ca4f4b2f1b5760b3
1743 Nov 7 18:19 rRNApoop.out 8d607b9eeb7f14688a9b60c444dc96f2
19376667 Nov 7 18:19 rRNApoop.pdf a6452d709b094b402cdcffff648fd7bd
28827 Nov 7 18:18 rRNApoop.tex 0b952040b116bee55e4f497497ed8ae1
2307 Nov 7 18:19 rRNApoop.toc 3a88b4fc0a51db121d4e8294bd512220
7448 Oct 31 09:52 seq_usedf.tex 8ae2fbf41ab33435511914b394a94e62
8624 Nov 7 09:31 soil_art.tex b8f6b2bede669a68d9d73f388222ec63
31050 Jul 21 2011 /usr/share/texlive/texmf-dist/bibtex/bst/urlbst/plainurl bst
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15 Jul 23 2019 /var/lib/texmf/fonts/map/pdfmap/updmap/pdftex.map -> pdftex_dl14.map 341

e900b5c25cd7029252688d79936e4
1431330 Jul 23 2019 /var/lib/texmf/web2c/pdftex/pdflatex.fmt c2a1d33979bfd7080ba601d397e5e158
2983299 Nov 3 19:08 zymo-txt-files-2018.zip 1281123ee6bafe64f6da6aa1d6fa5119
19376667 Nov 7 18:19 rRNApoop.pdf a6452d709b094b402cdcffff648fd7bd