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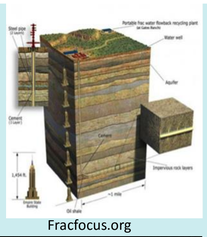
Introduction

- Hydraulic fracturing has become more prevalent over the years in Pennsylvania, which contains gas-rich Marcellus Shale formations.
- This process has been found to reduce species richness and evenness in streams near well pads*.
- **Bacteria communities readily respond** to perturbations in the environment.
- Knowledge of the **biodegradative capabilities** of the microorganisms associated with hydrofracking operations and potential recipients (e.g., aquifers, surface waters, and streambed sediments) enables predictions about the longevity of compounds of environmental concern and the likelihood they will be biologically degraded.



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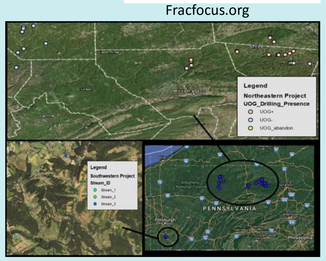


*Trexler, Ryan, Caroline Solomon, Colin J. Brislawn, Justin R. Wright, Abigail Rosenberger, Erin E. McClure, Alyssa M. Grube, et al. 2014. "Assessing Impacts of Unconventional Natural Gas Extraction on Microbial Communities in Headwater Stream Ecosystems in Northwestern Pennsylvania." *Frontiers in Microbiology* 5 (November). doi:10.3389/fmicb.2014.00522.

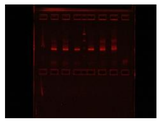
Objectives

- To identify additional microbial taxa in Northeast and Southwest PA that are heavily impacted by fracking.
- Investigate the presence of microbial biomarkers related to biodegradation and biocide resistance.
- Compare impacted sites with unimpacted sites as baseline samples.

Methods

•**Microbial Sample Collection**
- •Impacted and unimpacted water and sediment samples (Northeastern PA n=45 and Southwestern PA n = 12).
- •UOG- (n= 22), UOG+ (n=22), UOG abandoned (n=1), HF+ (n=6), and Control (n=6).

•**Genomic DNA and RNA extraction**
- •Phenol/Chloroform, Qiagen All-prep kit
- Illumina-tag **16S rRNA** library prep and sequencing (Miseq)



•**DNA Amplification**
- •A Polymerase Chain Reaction of the 16S rRNA gene, which is a good thumbprint for this research
- •PCR products are approximately 390 bp

- Analysis of 2,977,011 million 16S sequences from the Northeast region and 593,700 sequences from the Southwest region with **Qiime and R**
  - •Greengenes 97% similarity
  - •Computation of **Beta Diversity and LefSe plots**

Results

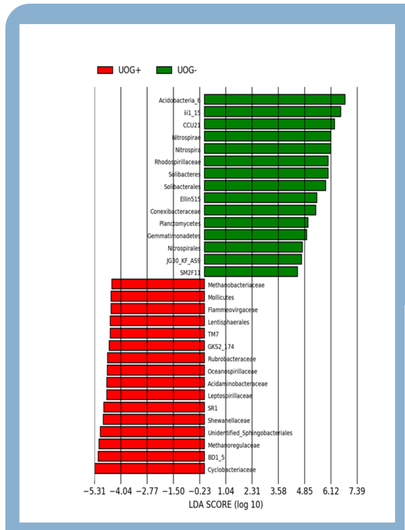


Figure 1: LEfSe plot of biomarkers between UOG+ and UOG- sites in Northeastern PA. Samples are plotted by relative abundances including sample identities.

Several of the enriched taxa have been found in saline environments, showing they can tolerate saline conditions.
- For example, Rubrobacteraceae has been found in relatively high abundance in soil derived from saline sediments[1].

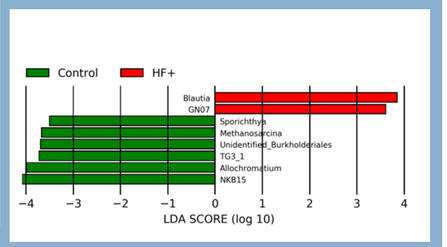


Figure 2: LEfSe plot of biomarkers between HF+ and Control samples in Southwestern PA. Biomarkers such as Blautia and GN07 are abundant in the HF+ sites.
- Not much is known about some OTUs, such as GN07.

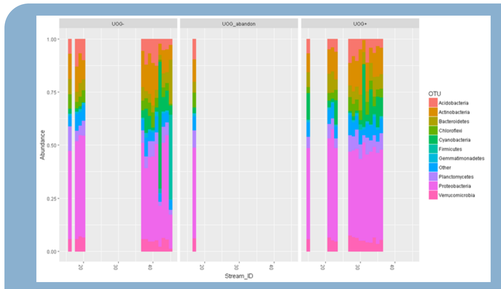


Figure 5: An overview of the phyla in the Northeastern project sample sites. The bars do not show a clear difference. Proteobacteria appears to be the most abundant phylum for almost all of the samples.

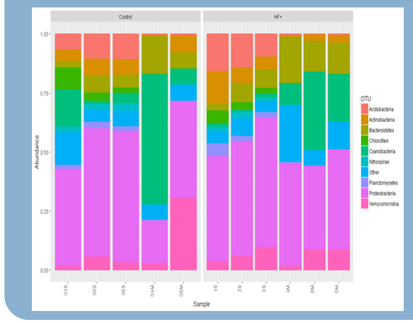


Figure 6: An overview of the phyla in Southwestern PA.
- Acidobacteria seem to be more common in sediment samples than in water samples.

Table 1. General Information for Northeastern and Southwestern Samples.

Classification	Number of Samples	Number of OTUs	Number of Sequences	Average Quality before Filtering (Phred score)	Average Quality after Filtering (Phred Score)
UOG+	14	15,611	1,679,434	34.6783	36.4434
UOG_abandoned	1	4,281	34,851	34.3676	36.3500
UOG-	13	13,924	1,262,726	34.8683	36.4463
Northeastern Project	28	17,308	2,977,011	34.7562	36.4436
HF+	6	8,657	252,716	40.2534	39.4394
Control	5	8,914	340,984	40.2622	40.5882
Southwestern Project	11	10,203	593,700	40.2584	40.5856

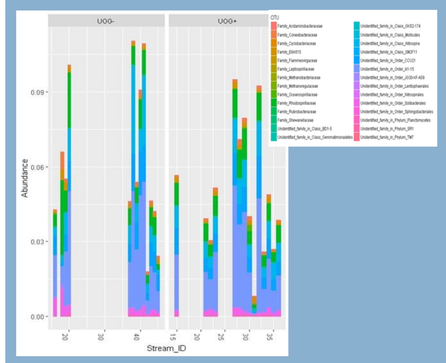


Figure 3: A bar plot showing the enriched taxa for the UOG+ and UOG- sites. The OTUs are classified to the family level.
- Several of the enriched taxa for UOG+ sites, such as Methanoregulaceae[2] and Methanobacteriaceae[3], can tolerate acidic conditions.

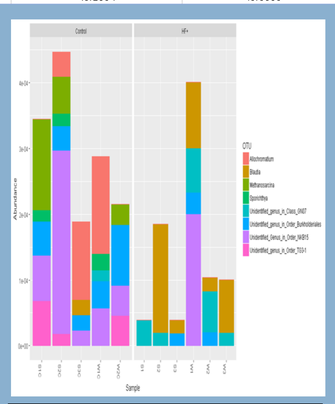


Figure 4: A bar plot showing the enriched taxa for the HF+ and control sites. They are classified to the genus level.
- Half of these enriched OTUs could not be identified to the genus level.
- Blautia is an anaerobic bacteria[4] that can tolerate acidic conditions[5].

Discussion & Future Directions

- This study shows that there may be some new biomarkers for fracking activity in addition to other biomarkers that were identified in our previous study.
- Several biomarkers identified are known to survive saline conditions and biocides.
  - The phylum bar plots provide an overview of the microbial communities in the different samples (Figures 3&4)
  - Acidobacteria appear to be more common in samples with fracking activity.
    - However, LEfSe analysis showed several taxa in that phylum, such as CCU21, were significantly enriched in UOG- sites.
- We will continue our studies by sequencing more DNA samples for the Southwestern project and analyzing the functional profile of several of the Northeastern project samples.
- We will also continue researching information to explain why certain taxa are enriched based on the presence or absence of fracking activity.

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