Comparative phylogentic and metagenomic analysis of an ultrabasic continental serpentinizing fluid seep at Yanartaş (Turkey). Kristin Woycheese[1*], Erin Yargıçoğlu[2], Yasemin Güleçal-Pektas[3], Dawn Cardace[4], and D'Arcy Meyer-Dombard[1], [1]Department of Earth and Environmental Sciences, University of Illinois at Chicago, 845 W Taylor Street (M/C 186), Chicago, IL 60607, USA, *kwoych2@uic.edu, [2]Department of Civil and Material Engineering, University of Illinois at Chicago, 842 W Taylor Street (M/C 246), Chicago, IL 60607, USA, [3]Faculty of Sciences, University of Istanbul, PK 34134 Vezneciler, Istanbul, Turkey, [4]Department of Geosciences, University of Rhode Island, 9 E Alumni Ave, Kingston, RI 02881, USA

Introduction: The serpentinization of ultramafic rock in the deep subsurface produces highly reducing, ultra-basic fluid that putatively supports a diverse microbial community fueled by the influx of hydrogen and methane gas. In surface mixing zones, where deep subsurface fluid meets atmospheric conditions, a diverse microbial consortium thrives at Yanartaş (Chimaera), Turkey. The system originates in the Tekirova ophiolite complex, and is one of the largest onshore methane seeps currently described [1]. Isotopic analysis of actively burning gas seeps suggest a thermogenic/abiotic origin for the methane at Yanartaş [1]. Microbial community composition in a biofilm community at Yanartaş was assessed using 16S rRNA gene surveys, metagenomic analysis, and previously reported biogeochemistry [2]. Based on 16S rRNA gene surveys, microbial community composition shifts with increasing distance from the source.

Results: Putative metabolic function based on 16S rRNA gene sequencing surveys and geochemistry of the fluids suggest a predominance of of chemolithotrophy and chemoorganotrophy. Chemolithotrophic microorganisms may partially support components of the microbial population at Yanartaş and are indicative of the highly reducing nature of the serpentinizing fluids. Chemoorganotroph dominance likely arises from the abundance of low molecular weight alkanes in the system, generated abiotically via Fischer-Tropsch-type reactions or by chemolithotrophy.

Methane cycling. Detection of several methanogenic archaeal genera is reported, including *Methanobacterium*, *Methanobrevibacter*, and *Methanolobus*. The identification of these taxa in the 16S rRNA gene sequences of microbial consortia at the source and downstream may suggest an additional, biogenic component to the methane gas at Yanartaş.

Sulfur cycling. The occurrence of sulfate in fluids collected, coupled with 16S rRNA gene analysis indicate the potential for sulfate reduction within the microbial assemblages at Yanartaş. Sulfate-reducing bacteria and archaea may exploit the H_2-rich fluid and reduce available sulfate using H_2 as an electron donor, potentially competing with methanogens for substrate availability. The archaeal order Desulfurococcales, the Deltaproteobacterial orders Desulfuromonadales and Syntrophobacterales, and the bacterial order Nitrospira were all detected in 16S rRNA sequence reads from Yanartaş.

Hydrogen cycling. Major bacterial clades detected in sequence reads include the aerobic hydrogen-oxidizing genus *Hydrogenophaga*, which utilizes H_2 as an electron donor and O_2 as an electron acceptor. This microorganism has been identified in several continental serpentinizing seeps [3, 4] and tends to dominate downstream microbial communities in response to serpentinizing fluid oxidation. Anaerobic fermenting, H_2-producing bacterial classes such as the obligately anaerobic Clostridiales and Bacteroidetes were also reported in 16S rRNA gene sequencing reads and may operate syntrophically with H_2-oxidizing bacteria.

Photolithotrophy. Sequencing analysis supports some modes of photolithotrophy, but not others. Chloroflexi and Cyanobacteria were detected in several of the downstream microbial communities at Yanartaş, and a conspicuous orange pigment was present 6.7 meters from the source. These observations merit further investigation of the phototrophic capacity of the microbial consortia via metagenomic analysis.

PCR screening. PCR screening of function-based nitrogen cycling genes in environmental DNA samples suggested that nifH (nitrogenase) was not detected at the seep source, but was present 3.6 m and 6.7 m downstream. PCR screens for nitrate reductase (narG) and nitrite reductase (nirS) indicated that these genes are present within the microbial community [2].

Metagenomic analysis. Metagenomic analysis is ongoing and will be utilized to determine if putative metabolic inferences based on 16S rRNA gene surveys are robust. In particular, emphasis will be placed on methane, sulfur, hydrogen, and nitrogen cycling to determine the extent of chemolithotrophic activity in the microbial assemblages of Yanartaş.

References: [1] Etiope et al. (2011) *Earth Planet. Sci. Lett., 310, 96-104* [2] Meyer-Dombard et al. (2015) *Front. Microbiol., 5, 723*. [3] Suzuki et al. (2013) *Proc. Natl. Acad. Sci. U.S.A., 110, 15336-15341* [4] Brazelton et al. (2013) *Appl. Environ. Microbiol., 79*, *3906-3916.*