Distribution of lipid biomarkers along geochemical gradients in a deep subsurface environment. **M. R. Osburn**[1], F. Schubotz[2], R. E. Summons[3], J. P. Amend[4], [1]Northwestern University, Evanston, 60208, USA; maggie@northwestern.edu [2]Marum Center for Marine Environmental Sciences, Bremen, D-28359, Germany, [3]Massachusetts Institute of Technology, Cambridge, 02139, [4]University of Southern California, Los Angeles, 90089.

Introduction: Lipid biomarkers have been widely applied to environmental interpretation of past and present envrionments on Earth [1] and are in development (and early application) to study extraterrestrial materials [2],[3]. These organic biomarkers display striking spatial variability, mimicking that of biological systems, often ranging broadly across chemical and/or biological gradients [4]. While much is known about the production of individual lipids in model organisms, studies of the natural variability in lipid distributions, particularly intact polar lipids (IPLs), in analog environments are in their infancy and necessary as a comparison to future mission targets. To this end we will discuss the variability in abundace and isotopic composition of biomarkers across geochemical boundaries in a deep subsurface site. While several studies have documented IPL distributions in the marine subsurface [5], [6], [7], [8], [9], this is the first detailed study of IPLs from the terrestrial subsurface.

Study Site: A primary target in the search for extraterrestrial life is the deep subsurface environment. Such sites on Earth are protected from surficial extremes in temperature and radiation, are fed by mineral-rich fluids, and encounter steep chemical gradients ideal to support widespread chemolithotrophy [e.g. 10]. Here we present data from a new deep subsurface research area, the Sanford Underground Resarch Facility (SURF), hosted in the former Homestake Goldmine. This site provides access to 5000 ft below the surface into a geologically complex array of Paleoproterozoic age, iron-rich, metasedimentary rocks [11]. Fluids emanating from drilled boreholes and fracture networks range in age from modern at the surface to > 10,000 yrs at depth [12]. As a team, the NASA Astrobiology Institute *Life Underground* has conducted a broad study of the inhabitants and habitability of SURF that has, thus far, revealed sharp geochemical gradients, evidence for chemolithotrophic metabolisms, and diverse and variable microbial populations [13]. Sampling for this study was conducted with aquisition of other geochemical and molecular data.

Methodology: Samples of biofilms and filtered borehole fluids for lipid extraction and analysis were taken in October 2014 and February 2015. All samples were frozen after collection and lyophilized prior to lipid extraction. A modified Bligh Dyer extraction protocol was applied to all samples prior to their analysis for intact polar lipid composition via lipid chromatography – mass spectrometry. A base hydrolysis procedure was applied to liberate core lipids for quantification via GC-MS and isotope measurements via GC-IRMS.

Intact polar lipid distribution: Our dataset identifies a large diversity of lipid structures including phospholipids, aminolipids, glycolipids, and GDGTs, as well as a number of unidentified compounds. Biofilm samples differ markedly from fluid samples. Bacterial lipids dominate the biofilms with abundant aminolipids (specificically betaine lipids) and limited archaeal contributions in most sites. In contrast the fluid samples contain abundant glycosidic GDGTs, bacterial glycolipids, and phospholipids. The predominance of betaine and glycolipids in these subsurface samples is interesting and divergent from the classical view that the main producers of these lipids are phototrophs. The striking abundance of archaeal glycosidic GDGTs in the fluid samples is reminiscent of observations from the deep marine subsurface [7]. While differential preservation could be at play here (as has been advocated for the marine realm (e.g. [8]), we can at least observe that archaeal lipids are not dominant in the biofilm samples and thus are not always preferentially preserved.

Continuing work: Analysis of core distributions as well as compound-specific carbon and hydrogen isotope measurements are currently underway. We expect that this information will significantly improve our understanding of both carbon cycling and central metabolism within these subsurface ecosystems.

References: [1] Brocks J. J. and Summons R. E. (2004) Treatise on Geochemistry 8, 3. [2] Grotzinger J. P. (2014) Science 343, 386-397. [3] Ming, D. W. et al. (2014) Science 343, 6169. [4] Jahnke et al. (2014) Geobiology 12, 62-82. [5] Biddle et al. (2006) PNAS 103, 3846-3851. [6] Lipp and Hinrichs (2009) Geochimica 73, 6816-6833. [7] Lipp et al. (2008) Nature 454, 991-994. [8] Xie et al. (2013) PNAS 110, 6010-6014. [9] Zink et al. (2003) Org. Geo. 34, 755-769. [10] Chivian et al. (2008) Science 322, 275-278. [11] Caddey S. W. (1991) USGS Bull. 1857-J, 1-67. [12] Murdoch L. C. et al. (2011) Hydrogelogy Journal 20, 27-43. [13] Osburn et al. (2014) Frontiers in Microbiology 5, 1-14.