**Age, water source, and sex do not significantly affect the microbiome of the Hadza people of Tanzania**

Ananya Saraph, Maxim Daspe, Mona Golmohammadzadeh, Alina Chalanuchpong

*Department of Microbiology and Immunology, University of British Columbia, Canada*

**SUPPLEMENTARY MATERIAL**

****

**Figure S1: Unweighted UniFrac analysis of Hadza gut microbial community composition does not show a significant difference across seasons.** Individual gut microbiome compositions in the 2013 Dry (light green, N = 133), 2014 Wet (purple, N = 46), and 2014 Dry (light green, N = 63) sub-seasons were analyzed using QIIME2 and plotted on an unweighted UniFrac PCoA plot in R. Samples collected in each sub-season are similar to one another. Percent variance explained on axis 1 = 16.9%, axis 2 = 7.4%.



**Figure S2: There are no differentially abundant ASVs identified in fecal samples from different bush camps at the family level.** Differential abundance testing of fecal samples from different bush camps at the family level was performed in QIIME2 using ANCOM. Two ASVs with high W statistics and clr mean differences were observed, which were identified as k\_\_Bacteria;p\_\_Cyanobacteria;c\_\_4C0d-2;o\_\_YS2;f\_\_ (clr = 15.8, W = 177) and k\_\_Bacteria;p\_\_Proteobacteria;c\_\_Deltaproteobacteria;o\_\_GMD14H09;f\_\_

(clr = 10.3, W = 151). N = 544.



**Figure S3: Gideru, Hukamako, and Sengeli bush camps display similar gut microbiomes when analyzed using weighted UniFrac analysis.** Using QIIME2, human fecal samples were visualized based on weighted UniFrac analysis on a PCoA plot. All samples from bush camps except for Gideru (red spheres), Hukamako (yellow spheres), and Sengeli (blue spheres) were removed from the analysis, as these 3 camps displayed similar gut microbiomes. Percent variance explained on axis 1: 43.73%. Percent variance explained on axis 2: 9.075%. Gideru: N=30. Hukamako: N=104. Sengeli: N=103.

****

**Figure S4: A lack of differentially abundant ASVs are found in the right hand microbiomes of males and females.** Using QIIME2, differential abundance testing between males and females was performed with ANCOM at the genus level. Most genera of bacteria display low clr mean difference and W statistics, with only 2 genera having both a high W statistic and high clr mean differences. These organisms are k\_\_Bacteria;p\_\_Proteobacteria;c\_\_Gammaproteobacteria;o\_\_Pseudomonadales;f\_\_Moraxellaceae;g\_\_Psychrobacter (clr=1.104, W=70) and k\_\_Bacteria;p\_\_Acidobacteria;c\_\_[Chloracidobacteria];o\_\_RB41;f\_\_Ellin6075;g\_\_ (clr=1.007, W=70). N=390.