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**Limitations on heterotrophic activity in Subglacial Lake Whillans, West Antarctica**

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Subglacial Lake Whillans (SLW) is one of more than 400 subglacial lakes that have been discovered beneath the Antarctic ice sheet over the past two decades. Taken together, these subglacial environments comprise an estimated  $10^4$  km<sup>3</sup> of liquid water, making them one of the largest unexplored habitats for life on Earth. The lakes and water saturated sediments beneath the East and West Antarctic ice sheets have been isolated from the atmosphere and from sunlight for many thousands of years. As such, organisms living in these environments must rely on inorganic substrates as energy sources, or relict organic matter. SLW lies beneath the West Antarctic Ice Sheet, in a region that has been inundated with seawater during past periods of ice sheet retreat. We used clean hot water drilling to penetrate 800 m of ice overlying SLW in 2013, retrieving the first discrete samples of water and sediment from a subglacial lake. The ~2 m deep SLW water column was characterized by temperature at the pressure freezing point (-0.49°C), low oxygen (71 μM, ~16% of air saturation) and moderate conductivity (720 μS cm<sup>-1</sup>). Inorganic N (3.3 μM), soluble reactive P (3.1 μM) and dissolved organic C (221 μM) concentrations were adequate to support microbial growth. The water contained ~10<sup>5</sup> cells ml<sup>-1</sup>, with diverse cell morphologies present. In spite of relatively abundant nutrients, turnover times, calculated from cell-specific carbon turnover rates determined via <sup>3</sup>H-thymidine and <sup>3</sup>H-leucine incubations, were on the order of hundreds of years. Growth rates (average ~0.0043 d<sup>-1</sup>) obtained from the same incubations, were at least an order of magnitude lower than those measured in Antarctic surface lakes in the McMurdo Dry Valleys and in oligotrophic areas of the ocean; coupled with low bacterial growth efficiency (8%), these data indicate that microbial populations in SLW partition a majority of their carbon demand to activities other than cellular growth. The water column and surficial sediments contained degraded, nitrogen-poor particulate organic matter (PC:PN = 65.4 and 17.9 [molar ratio], respectively; water column PN:PP = 0.78). The δ<sup>15</sup>N of particulate organic matter in SLW (9.9‰) was consistent with that of organic matter that has undergone diagenesis in the presence of oxygen, supporting the contention that particulate organic matter in SLW is a relict of marine productivity during past seawater incursions into the region. The high DOC:DON molar ratio (95.2) suggested that the pool of bulk dissolved organic matter may also be of low nutritional quality. Chemolithoautotrophic productivity was 32.9 ng C L<sup>-1</sup> d<sup>-1</sup>, indicating that contemporaneous new C production via this pathway may support a portion of the heterotrophic activity in SLW. Given the low quality of the standing pools of organic matter in SLW, the rate of organic C production by chemolithoautotrophs may limit heterotrophic growth rates. In addition to regulation by chemolithoautotrophic carbon production, experimental additions of inorganic N and P

and increased temperatures stimulated heterotrophic microbial productivity (N and P = 36% increase versus control; temperature = 5% increase per °C increase). Collectively, our data indicate that microorganisms in SLW persist in a low-energy environment that is limited by the availability of high quality substrates for growth and by temperature. Subglacial lakes, such as SLW, serve as important natural laboratories for physiological and biogeochemical studies of microorganisms in energy limited environments.