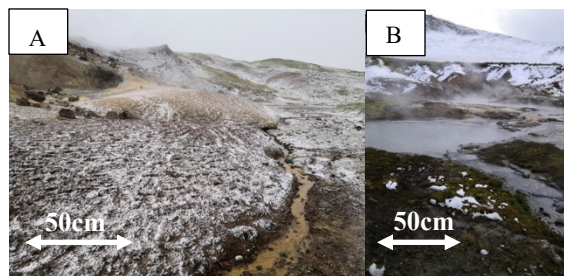


# CARBON AND NITROGEN CYCLING WITHIN A COMPLEX ICELANDIC GEOTHERMAL SYSTEM

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**Introduction:** The study of Mars analogues has provided us with an insight into past and present Martian environments that have the potential to host chemolithotrophic forms of life. Since the discovery of ancient Noachian-age (4.1-3.7Ga) terrestrial hot spring deposits on Mars [1,2,3], many recent studies have focused on their analogues on Earth. Following on from recent studies of hot springs in Iceland [4,5,6] and the discovery of nitrogen compounds on Mars [7], our aim is to examine the biological nitrogen and carbon cycling within geothermal systems in Iceland and how this may link to other metabolic cycles which are relevant to Mars environments.

**Geological setting:** Fieldwork was focused around the Hveragerði region which is within the SE area of the Hengill volcanic system [8]. Hveragerði geothermal systems are heated by a slowly cooling, now-extinct volcano, and tend to have low to moderate temperatures of up to 50°C. Within the area of Ölkelduháls a few km north of Hveragerði, we sampled a spring with iron-oxide-stained microbial streamers and sediments that flowed from the nearby bedrock. This spring was intersected by the outflow from an acidic, iron-rich, bubbling geothermal pool (Figure 1a). We also sampled a stream with black/grey sediment and extremely abundant grey microbial streamers sourced from an uphill pool (Figure 1b).



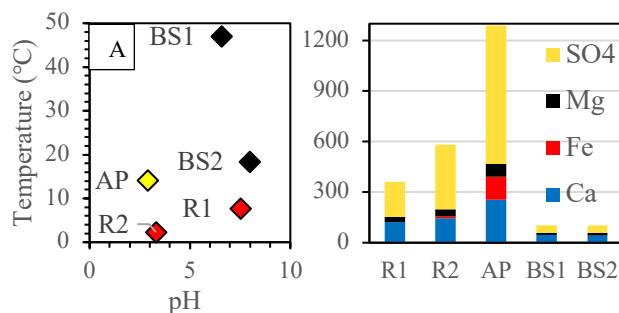
**Figure 1:** Ölkelduháls geothermal system. A) The red spring (R1 and R2) intersected by the acidic pool (AP) outflow. The red spring is ~20cm wide and up to 8cm deep; B) Pool (BS1) and outflow (BS2) of the black stream which is 40-50cm wide and up to 15cm deep.

**Sampling:** All sampling was carried out in late September 2021. We sampled the red spring before (R1) and after (R2) the input of the acidic pool (AP) outflow. We sampled the black stream both upstream near its source pool (BS1) and downstream nearer the red spring system (BS2). In-situ geochemical parameters of pH, temperature, and Eh were measured at the time of sampling. Filtered water samples were also taken for major anions and cations. Additional sediments and microbial material were collected for  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  isotope analyses and for genomic analysis.

**Results and discussion:** The red spring (R1 and R2) showed geochemical evidence of influence from the acidic pool. This pool contained high  $\text{Fe}^{2+}$  concentration of 138ppm (Figure 2B) a low pH of 2.9 (Figure 2A). Meanwhile, the red spring's iron levels increased between R1 and R2, while the pH dropped from 7.5 to 3.3. The high iron concentrations were concurrent with increased magnesium, suggesting that input from the surrounding basaltic bedrock was greater in the acidic pool due to increased dissolution. Modelled iron stability fields under the pool and spring conditions also indicate that  $\text{Fe}^{2+}$  was likely to occur within AP and R2, but other insoluble forms of iron such as iron(III) oxides or pyrite were more likely in the other sites and would precipitate out of the water. Eh values of the red spring increased from 390mV to 680mV between R1 and R2, which is likely linked to the change in oxidation and solubility of iron within this spring.

The black stream showed large variations in temperature from 47°C near its source pool to 18°C further downstream, with a concurrent increase in pH from 6.6 to 8 (Figure 2A). Major geochemistry is similar within both sample sites of the black stream although stability fields of iron, sulphate and nitrate changed distinctly with temperature, suggesting that temperature may have a bigger influence on the water geochemistry and therefore the metabolic pathways that are energetically favourable.

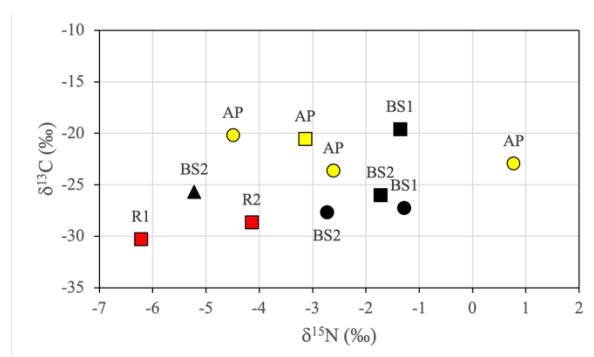
The entire system (acidic pool, and red and black springs) contained very low concentrations of nitrate (<0.1ppm) and undetectable nitrite. Future research will involve measurement of ammonium ion concentrations and nitrogen-based metabolic pathways from metagenomic sequencing.



**Figure 2A:** Temperature and pH of Ölkelduháls springs and pools. Red = red iron-oxide spring, yellow = acidic pool outflow, black = black spring.

**Figure 2B:** Major anions and cations of Ölkelduháls springs and pools.

Measurements of  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  were carried out on sediment and biomass collected from within and around the springs (Figure 3). The results showed negative  $\delta^{15}\text{N}$  values within the red spring and the acidic pool, accompanied by  $\delta^{13}\text{C}$  values as low as -30‰. The red spring and acidic pool biofilm samples in particular showed  $\delta^{15}\text{N}$  values from -3 to -6‰, suggesting that biological  $\text{N}_2$  fixation with alternative nitrogenases may be occurring [9]. If this conclusion can be confirmed with metagenomic data then this site could allow for further exploration of nitrogen-based assimilatory and dissimilatory metabolisms in hot spring settings. Another interesting metabolism which may be relevant in the iron-rich red spring system is nitrate-dependent iron oxidation which has been hypothesized as the earliest iron oxidation pathway to evolve on Earth and may therefore be relevant to iron-rich geothermal systems on Mars [10]. This possibility will be further explored with metagenomic data.



**Figure 3:**  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values for sediment, plant and biofilm samples at the Ölkelduháls site. Red = red iron-oxide spring, yellow = acidic pool outflow, black = black spring. Square = biofilm; triangle = moss/grass, circle = sediment.

The relative wide range of  $\delta^{13}\text{C}$  values from -19 to -30‰ in this system suggest that different carbon fixation mechanisms may be present in the red spring and black spring systems. The specific carbon fixation pathways are uncertain due to the known overlap in the ranges of  $\delta^{13}\text{C}$  values for different  $\text{CO}_2$  fixation pathways, but it has been suggested that the 3-HP/4-HB pathways is often found in low pH, iron(III) oxide and sulphate sediment microbial mats [11]. Future metagenomic analyses will clarify the metabolic pathways used.

**Conclusion:** This system is a useful analogue for Noachian-age hot springs on Mars and will provide more insight into the geochemical biosignatures left behind in the resulting sediments of ancient systems. Nitrogen isotopes show promise as a sensitive geochemical biosignature that will enable examination of Martian hot spring deposits for evidence of past life.

**Future work:** Ammonium concentrations in water samples will be measured and be used along with other ion concentrations to create accurate Eh-pH stability fields. Extracted DNA will be sent for 16S taxonomic sequencing and metagenomic sequencing to establish the taxonomy and metabolic pathways that are present in these springs. These will provide a more comprehensive view of the Ölkelduháls geothermal systems and its relevance to biological nitrogen and carbon cycling on Mars.

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