

## A microbially-mediated deep terrestrial nitrogen cycle at Henderson Mine, CO

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The existence of life in deep terrestrial subsurface rocks has been established at multiple sites, yet few studies have investigated the origin of nutrients that support such life. At Henderson Mine, CO, subsurface fluids drain from boreholes at 3000' depth, supporting a diverse microbial community based on 16S rRNA gene surveys. The fluids of several boreholes contain nitrogen in multiple oxidation states, including  $\text{NH}_4^+$  at 5-100  $\mu\text{M}$ . Fluid mixing trends show a correlation between  $\text{NH}_4^+$  and degree of water-rock interaction, and so we ask whether subsurface  $\text{NH}_4^+$  is sourced geologically or biologically. As this Mo deposit developed from partial melting of the lower crust, it is plausible that a sedimentary source of nitrogen was mineralized into  $\text{NH}_4^+$ , which then substituted for  $\text{K}^+$  in silicate minerals in the stockworks. We used FTIR microscopy to detect and quantify  $\text{NH}_4^+$  in from biotites and other mineral phases. We also investigate whether biological nitrogen fixation of  $\text{N}_2$  supplies the microbial community with  $\text{NH}_4^+$ . In the borehole fluids with the highest  $\text{NH}_4^+$  (~100  $\mu\text{M}$ ), we amplified the *nifH* gene from DNA extracts of filtered fluids, but not from borehole fluid DNA where  $\text{NH}_4^+$  concentrations were lower. We use a phylogenetic and quantitative PCR-based approach to evaluate whether *nifH* belongs to the novel phylum of bacteria first detected in these samples, the Henderson candidate division.

Our geochemical calculations predict that nitrification ( $\text{NH}_4^+$  and  $\text{NO}_2^-$  oxidation) is favorable in the high  $\text{NH}_4^+$  borehole, and so we next asked whether nitrifiers were present. The DNA from the high- $\text{NH}_4^+$  borehole fluid was the only sample to contain 16S rRNA sequences from archaea and the bacterial genus *Nitrospira*. From this sample, we amplified the gene for archaeal ammonium oxidation, *amoA*, but not the bacterial version of the gene, suggesting that archaeal ammonium oxidizers (AOA) are better adapted to this environment. Finally, we amplified a novel *nxB* gene encoding the beta subunit of nitrite oxidoreductase specific to nitrite-oxidizing *Nitrospira*. Amplification of genes for nitrogen fixation and nitrification support the existence of a subsurface nitrogen cycle. The ubiquity of Mo in enzymes of the nitrogen cycle may dictate the energy sources utilized by microbes in this subsurface Mo mine.

## Is there really a mixing-zone stable carbon and oxygen isotope signal?

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A number of distinctive changes in  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  signals of carbonates in response to different diagenetic phenomena have been proposed. For example, sub-aerial exposure surfaces are typically characterized by extreme depletions in the  $\delta^{13}\text{C}$  and a slight enrichment in  $\delta^{18}\text{O}$ , the vadose zone is characterized by constant and depleted  $\delta^{18}\text{O}$  values, but wildly varying  $\delta^{13}\text{C}$ , the freshwater phreatic zone possesses negative  $\delta^{13}\text{C}$  values which are not so variable as the vadose zone, the mixing-zone is characterized by a co-varying trend from negative to positive  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  values, and finally the marine phreatic zone has values of both  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  which are fairly positive. In this presentation the interpretation of the co-varying trend in the mixing-zone is questioned. Instead it is proposed that this trend really represents a range of varying amounts of recrystallization taking place in the freshwater phreatic zone. The significant change in interpretation is based on observations from several deep cores in the Bahamas which have penetrated shallow-water carbonates which were deposited and sub-aerially exposed during the numerous sea-level changes during the Pleistocene. During the last glacial period sea level fell at least 120 m below its present position. Based on present interpretation of the co-varying stable C and O isotopic record at this level no freshwater water lens would have been present. Clearly a significant freshwater lens was present extending downwards as much as 40 m, coincidentally corresponding to the zone of covariation between the C and O isotopes.