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Nitrogen cycle in Great Basin hot springs

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Introduction

Nitrification and denitrification are two important steps in the nitrogen cycle . Nitrification, a two step process, leads to the production of NO_3^-, (Fig. 1). In the first step, ammonia oxidation, NH_3 is oxidized to NO_2^-, and in the second step, nitrite oxidation, NO_2^- is oxidized to NO_3^-. Until recently, very little was know about nitrification in high temperature environments. However, in 2008 a thermophilic archaeon, named "*Candidatus* Nitrosocaldus yellowstonii", was shown to mediate ammonia oxidation up to 74° C. More recently , NO_2^- oxidizing bacteria were discovered that are active in temperatures up to 48° C(4). While NH_3 oxidation is generally considered to be the rate limiting step, this may not be the case at high temperatures since accumulation of NO_2^- has been reported in some hot springs where NH_3 is the dominant form of inorganic nitrogen (1).

Denitrification is a multistep process in which NO_3^- is reduced to N_2 (Fig. 1). A variety of thermophiles and hyperthermophiles have the ability to respire NO_3^-, but very few studies focus on the denitrification process in nature. To further understand these processes in hot springs, it is necessary to first characterize the chemistry of the hot springs, and then to cultivate and characterize the microbes that are involved.

In order to further elucidate the nitrogen cycle in hot springs, we designed experiments to test the following hypotheses:

- All springs are sourced with ammonium as the primary nitrogen source.
- NO_2^- will first become detectable at the source but NO_3^- will not become detectable until between 50° C and 60° C
- Optimal NO_2^- oxidation activity will occur at 50° C with no activity at 60° C and not activity at 70° C and above
- NO_x respiration will increase with decreasing temperature
- NO_2^- will yield higher denitrification rates than NO_3^- , particularly at temperatures above 60° C
- NO_3^-, NO_2^-, and N_2O reducers are distinct and reduce NO_x as a consortium

The Nitrogen Cycle

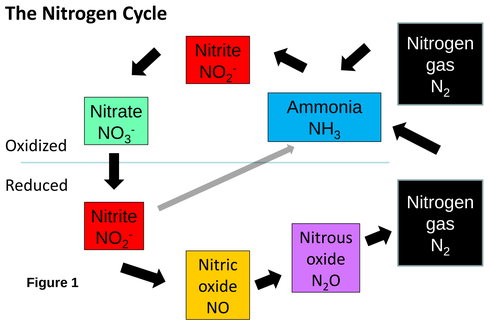

Figure 1

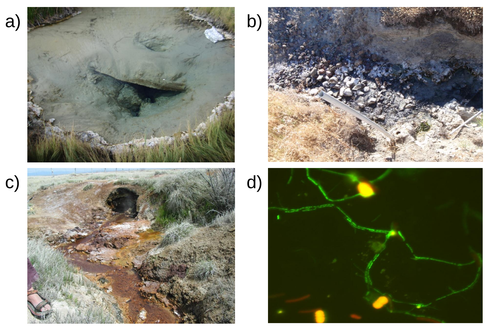

Figure 2. Geothermal springs and microbes that inhabit them: a) Sandy's Spring West , b) Rick's Hot Creek, c) Baker Spring, d) Organisms from Sandy's Spring West stained with Sybr Green.

Biogeochemistry

Samples were collected from sites that had a difference of approximately 10° C in temperature in the outflows of three different hot springs. They were filtered, put on ice, and transported back to the lab to be analyzed using a Lachat QuikChem 8000 FIA nutrient analyzer

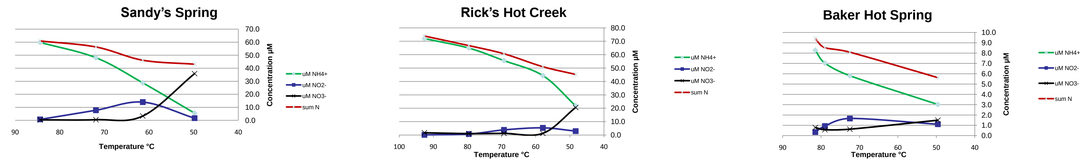

Figure 3. Graphs showing 3 of the approximately18 analytes measured for the three springs. Similar patterns were observed for NH_4^+, NO_2^-, NO_3^- for all three springs.. NO_2^- first became detectable between 70° C. and 80° C. NO_3^- first became detectable around 60° C.

Nitrite Oxidation

$$NO_2^- + O_2 \rightarrow NO_3^-$$

Figure 4. Nitrite oxidation: NO_2^- is the electron donor and O_2 is the electron acceptor.

In situ N pool flux experiments

Enrichments were made using a slurry containing spring water and sediment collected from the outflow of Sandy's Spring at various temperatures. The enrichments were spiked with NO_2^- and incubated in the spring at the temperatures at which they were collected. Samples were taken at 0,5, and 23 hours and the NO_2^- concentration was determined using Lachat nutrient analyzer.

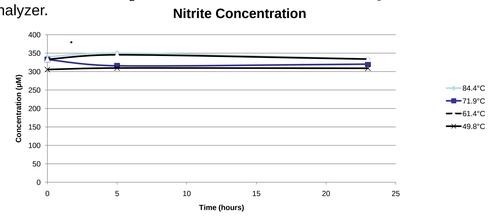

Figure 5. Results from nitrite tests done on enrichments incubated in the spring at 84.4° C, 71.9° C, 61.4° C, and 49.8° C. No NO_2^- oxidation occurred in the samples within 23 hours. These results may be attributed to the unpredictability of these organisms.

Lab experiments

Enrichments consisting of modified Lebedeva medium (3) and spiked with NO_2^- were inoculated with a slurry of sediment from Great Boiling Spring. The enrichments were incubated at 50° C and used to inoculate enrichments incubated at 50° C , 60° C , 70° C and 80° C. Nitrite consumption was monitored using the diazotization method (LaMotte).

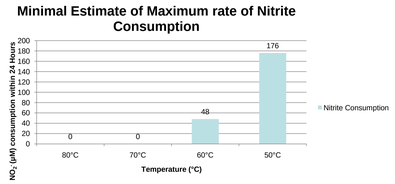

Figure 6. Estimates of rates of NO_2^- consumption for 24 hour period based on experiments done in the lab with enrichments. The results of lab experiments show that NO_2^- oxidizing microorganisms are most active at 50° C and decrease with increasing temperatures. No oxidation is observed at 70° C and above. These results can explain the data obtained from the biogeochemistry experiments since NO_3^- is not measured at 70 ° C and 80° C .

Denitrification

Acetylene Block experiment

Enrichments were made using a slurry containing spring water and sediment collected from the outflow of Sandy's Spring at various temperatures. Acetylene, which blocks the production of N_2 was added. The enrichments were spiked with either NO_2^- or NO_3^- and incubated in the spring at the temperatures at which they were collected.After the incubation period, the headspace was collected for analysis using gas chromatography.

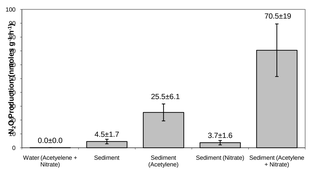

Figure 8. Data from previous Acetylene block experiment comparing N_2O production in water and sediment. The current study compared the difference in N_2O flux when either NO_2^- or NO_3^- was supplied as the electron acceptor. The Acetylene block data from this study will be analyzed in the future

$$NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2$$

Figure 7. Serial reduciton of Nitrate to N2 during denitrification.

Cultivation experiment

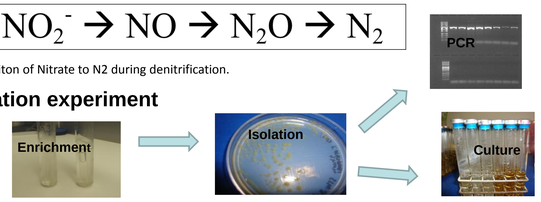

Figure 9. Enrichments were made using a slurry containing spring water and sediment collected from the outflow of Sandy's Spring West at 50° C, 70° C and 80° C. The enrichments were spiked with either NO_2^-, NO_3^- or N_2O and incubated in the spring at the temperatures at which they were collected. Isolates were obtained and used for PCR and sequencing or used to inoculate liquid cultures spiked with NO_2^-, NO_3^-, or N_2O. To test their ability to use different electron acceptors.

					Growth Scored at 36 hours									
Isolate	PCR	Identification	Class	Growth Temp	TPG NO_3^-		TPG NO_2^-		TPG N_2O		D NO_3^-		D NO_2^-		D N_2O	
				Deg. C	Growth	Gas	Growth	Gas	Growth	Gas	Growth	Gas	Growth	Gas	Growth	Gas
D-NO3-50-1	+	Azoarcus aromaticum (93%)	Betaproteobacterium	44	++	(-)	-	-	+	+	+++	+	-	-	++	++
D-NO3-50-2	+	Chelatococcus daeguensis (99%)	Alphaproteobacteria	44	-	-	++	+	++	+	++	(-)	++	+	+	++
D-NO3-50-3	+	Caenispirillum bisanense (100%)	Alphaproteobacteria	44	++	-	++	-	(-)	+	+++	++	++	+	(-)	+
D-NO3-50-4	+	Chelatococcus daeguensis (99%)	Alphaproteobacteria	44	-	-	+	-	++	++	-	-	++	+	+++	++
D-N2O-50-5	+	Azoarcus aromaticum (93%)	Betaproteobacterium	44	++	+	-	-	+	+	+++	+	-	-	++	++
D-NO3-50-6	+	Caenispirillum bisanense (99%)	Alphaproteobacteria	44	+++	-	+++	-	+++	+++	+++	+	++	+	++	++
D-NO3-50-13	-	N/A	N/A	44	+++	-	-	-	+++	+	++	-	-	-	+	+
D-NO3-70-7	+	Thermus oshimai (100%)	Deinococci	70	-	-	-	-	(-)	++	+++	-	-	-	+++	+
D-NO3-70-8	+	Thermus oshimai (100%)	Deinococci	70	-	-	-	-	-	++	+	-	-	-	(-)	++
D-N2O-70-9	-	N/A	N/A	70	+	-	-	-	-	+++	++	-	+	-	+++	+
D-N2O-70-10	-	N/A	N/A	70	-	(-)	-	-	-	+	++	(-)	-	-	+++	+
D-NO3-80-11	-	N/A	N/A	70	-	-	-	-	-	++	++	-	-	-	+++	+
D-NO3-80-12	-	N/A	N/A	70	-	-	-	-	-	++	++	+	+	-	-	++

Table 1. Results from sequencing and liquid cultures including growth and presence of gas with growth on tryptone peptone glucose medium or *Thermus* medium (D.) Scores ranging from none visible (-) to very visible (+++). These results show that the process of denitrification is not done by a single organism but rather distinct organisms as a consortium.

In the Field

Future Work

- Continue trying to optimize of nitrification activity.
- Isolate and Identify nitrite-oxidizing bacteria.
- Use qPCR to quanitify N cycle organisms collected from the outflow.
- Get GC results for acetylene block experiments.
- Further purify microbes obtained in cultivation experiment.
- PCR amplify and sequence 16SrRNA for remaining denitrifiers.
- Look for genes in the Metagenome sequences that are potentially involveved in nitrogen cycling

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