PHASE 1 MICROBIAL CHARACTERIZATION OF MOAB UMTRA PROJECT GROUNDWATER INTERIM ACTION

Final report

October 12, 2006

Prepared by

Lee Landkamer, Ph.D. and Linda Figueroa, Ph.D., P.E.
Environmental Science and Engineering Division
Colorado School of Mines
Golden, CO

Prepared for

Dave Peterson
S.M. Stoller Corporation
Grand Junction, CO

This document is intended to provide nitrogen biotransformation assessment information to be used by Dave Peterson in the preparation of the ***Groundwater Interim Action Performance Assessment*** *report for the Moab UMTRA Project site.*

Executive Summary

This report summarizes a short-term microcosm study designed to characterize the potential for ammonia transformation via microbial processes in the hyporheic zone sediment at the Moab UMTRA Project Site. The focus of the study was to assess the extent of nitrification and denitrification processes stimulated by the interactions of the sediment microbial community with a mixture of contaminated groundwater and Colorado River water.

Sediment samples collected from the Colorado River bank at the Moab Project Site, water samples from well 0480 and river water were used to establish laboratory microcosms to monitor the ability of the subsurface microbial population to catalyze transformations of ammonia and nitrate to $N_{2(g)}$ at the Moab UMTRA Project site. Six sets of experimental conditions were established in the sediment microcosms. They consisted of three different water mixtures in both anaerobic and aerobic conditions. The groundwater and river water mixtures used were: (1) 100% groundwater, (2) 50% groundwater, and (3) 10% groundwater. River water made up the balance of the water mixture. Over the course of 39 days of incubation, the total nitrogen and ammonia concentrations decreased dramatically in all microcosms. However, the presence of oxygen accelerated the biological transformation of the nitrogen species and the relative amounts of ground water and river water in the microcosms affected nitrogen removal.

The nitrogen/ammonia removal in the microcosms is likely the result of microbial nitrification and denitrification, additionally, anaerobic oxidation of ammonia, a process known as anammox is thought to be occurring in the microcosms. Anammox is a process where bacteria use ammonia as an electron donor and nitrite as an electron acceptor and produce nitrogen gas and a smaller amount of nitrate. The results of the microcosm tests suggest that ammonia biotransformation from both nitrification and anammox and nitrate biotransformation via denitrification are occurring in groundwater at the Moab Site.

Because the microcosm tests demonstrated that nitrogen concentration changes vary depending on the relative mixing of site groundwater and river water, the mixing caused by ground water extraction and injection of river water at ground water interim action (GWIA) wells is also likely to influence microbial mediated nitrogen removal. Also, the application of oxygenated river water on the irrigation test plots may be stimulating biological conversion of the nitrogen compounds by supplying oxygen and dissolved organic matter to the contaminated groundwater. Additionally, introduction of river water to the groundwater may be increasing biological activity by simply diluting the very high salinity of the groundwater, which can be detrimental to microorganisms.

The data from the batch microcosms clearly establishes a high potential for nitrogen removal in the Colorado River hyporheic zone. However, flow-through column studies, which were beyond the scope of this preliminary study, would better estimate the nitrogen biotransformation potential at the Moab UMTRA Project site. Batch experiment artifacts not seen in column studies include: retention of products, high liquid to solids ratio and high gas volume to solid/liquid ratio. These artifacts may have reduced the observed rates of nitrogen biotransformation. A column study would give more insight into the effects of interim action operations on nitrogen chemistry and may suggest operational changes to the GWIA that would increase total nitrogen removal in the subsurface environments at the Moab UMTRA Project site.

Background

Performance of the ground water interim action (GWIA) extraction and injection systems at the Moab Project Site is currently being assessed using both chemical and hydraulic measurements. The two main contaminants of interest from the ground water plume are ammonia and uranium. Chemical data collected in 2004 and 2005 suggests microbially mediated changes in ammonia concentrations are stimulated in the hyporheic zone of the Colorado River by the interim action. Very little microbial data has been collection to date in parallel with chemical data to elucidate the microbial processes stimulated in the hyporheic zone by the interim action. This report summarizes a short-term microcosm study designed to characterize the microbial process potential in the hyporheic zone sediment. The focus of the study was to assess the extent of nitrification and denitrification processes stimulated by the interactions of the sediment microbial community with a mixture of the groundwater plume and Colorado river-water.

Experimental Procedure

Colorado School of Mines (CSM) and S.M. Stoller Corporation (Stoller) personnel determined the best sampling scheme based on detailed analysis of the groundwater monitoring data via conference calls and meetings at the Stoller office in Grand Junction, Colorado. CSM and Stoller personnel collected field samples for microcosm experiments on August 23, 2006. Liquid samples were collected from well 0480 and nested piezometers 0562 and 0563, and from the river adjacent to the piezometer locations. A single composite sediment sample was collected adjacent to piezometer number 0562 from a depth of approximately 2 feet below ground level and sieved, retaining the fraction less than 2 mm. All sampling was done aseptically as possible; samples were stored in sterilized containers and packed in ice until they could be refrigerated.

The sediment sample collected from the riverbank and the water samples from well 0480 (GW) and the river water (RW) were used to establish microcosms to monitor the ability of the subsurface microbial population to catalyze transformations of ammonia and nitrate to $N_{2(g)}$ (nitrification/denitrification). Six sets of experimental conditions were established in the sediment microcosms. They consisted of three different water mixtures in both anaerobic and aerobic conditions. The groundwater and river water mixtures used were: (1) 100% groundwater, (2) 50% groundwater, and (3) 10% groundwater. River water made up the balance of the water mixture. Each microcosm contained 200 g of sediment (damp weight: damp with pore-water but no free water) and 450 ml of water. Duplicate microcosms were established for each set of conditions for a total of 12 microcosms that were designated as shown in Table 1. The microcosms were incubated at room temperature in the dark. Ammonia, nitrate, nitrite, inorganic carbon, organic carbon and pH were measured weekly using filtered (0.45 um) liquid phase samples collected periodically from the microcosms over a 39 day period. Additionally, the microcosms were sampled at the end of week 2 and 4 for anion and cation analysis. The headspace gases of the anaerobic microcosms were examined by gas chromatography at the end of week 2. Water from both anaerobic and aerobic 50% groundwater microcosms was examined microscopically for bacteria at the end of week 3.

When the anaerobic microcosms were established, they were sealed and argon was circulated in the headspace for two hours to remove oxygen. The aerobic microcosms were loosely capped so that oxygen transfer could occur continuously within the headspace and 120 cc of filtered air was injected into the aerobic microcosms with a syringe twice weekly. The microcosms were shaken vigorously twice a week to facilitate mass transfer. This procedure

removed chemical gradients that may have developed (e.g., oxygen or ammonia gradient throughout the sediment layer) but does not ensure that such gradients did not develop between shakings. When the microcosms settled, a layer of fine silt covered the coarser material. It is likely that the sediments in the aerobic microcosms may have gone anaerobic at times due to the high oxygen demand of nitrification and mass transfer limitations. The microcosms were sampled with a syringe and needle to minimize disturbance of the microcosms. Argon was injected into the anaerobic microcosms while the liquid sample was being withdrawn (in equal amounts) to maintain the headspace pressure.

Table 1. Microcosm conditions and designations.

Microcosm Designation	Headspace	Groundwater	River-water
MC 1 A&B	Argon (anaerobic)	100 %	0 %
MC 2 A&B	Argon (anaerobic)	50 %	50 %
MC 3 A&B	Argon (anaerobic)	10 %	90 %
MC 4 A&B	Air (aerobic)	100 %	0 %
MC 5 A&B	Air (aerobic)	50 %	50 %
MC 6 A&B	Air (aerobic)	10 %	90 %

The groundwater, piezometer and river-water samples were also analyzed for ammonia, nitrite, nitrate, chloride, sulfate, fluoride, bromide, phosphate and a suite of metals (Ag, Al, As, B, Ba, Be, Ca, Cd, Co, Cr, Cu, Fe, K, Li, Mg, Mn, Mo, Na, Ni, P, Pb, S, Sb, Se, Si, Sn, Sr, Ti, V, and Zn) after being filtered (0.45um).

Results

The total nitrogen removal in the microcosms is displayed in Figures 1, 2 and 3. The relative amounts of the three nitrogen species are presented along with the measured pH. Note that the sampling periods 1 through 5 correspond to microcosm initialization and weeks 1 through 4, respectively, but period 6 corresponds to a sampling period 12 days after period 4 (total elapsed time is 39 days). The actual sampling time could not be used in the plots due to software limitations. All points are an average of microcosms A and B except period 6 when only microcosm A was sampled due to time constraints.

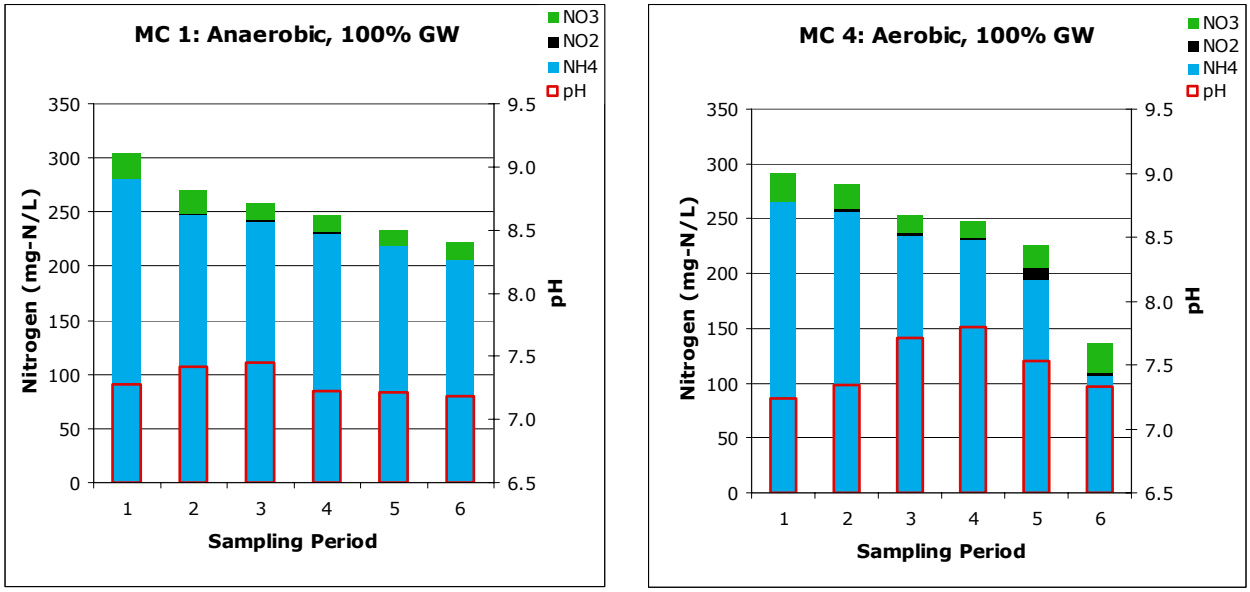


Figure 1. Total nitrogen (NH_4^+, NO_2^- and NO_3^-) concentration and pH (red line, right-hand ordinate) for microcosms 1 and 4 (values are averaged between duplicates A and B).

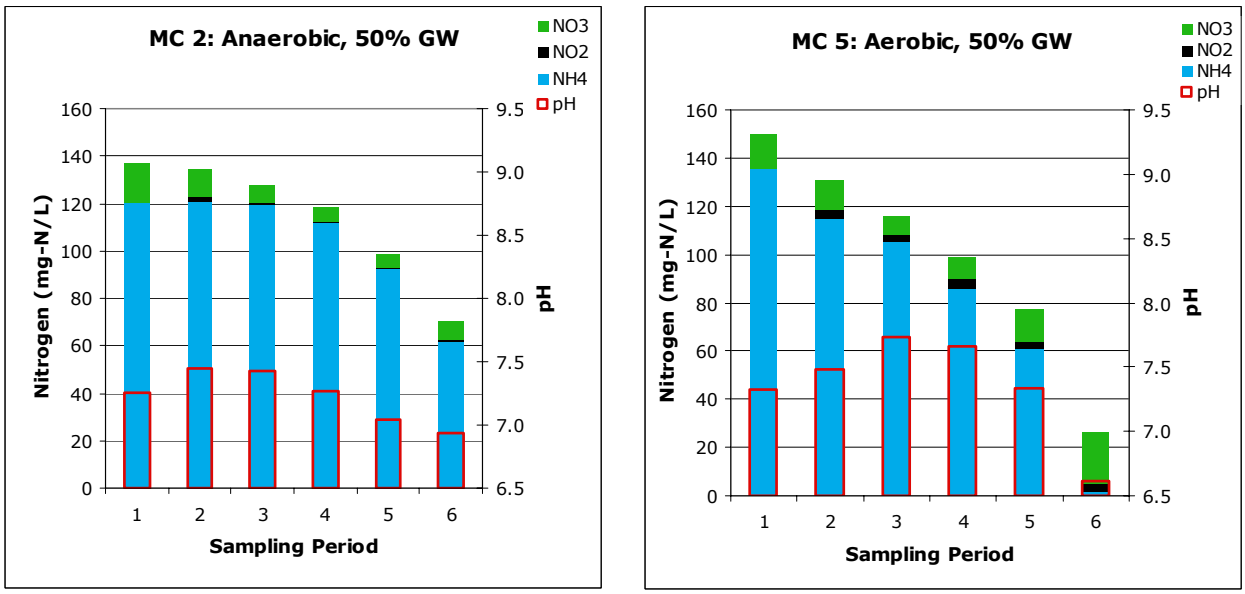


Figure 2. Total nitrogen (NH_4^+, NO_2^- and NO_3^-) concentration and pH (red line, right-hand ordinate) for microcosms 2 and 5 (values are averaged between duplicates A and B).

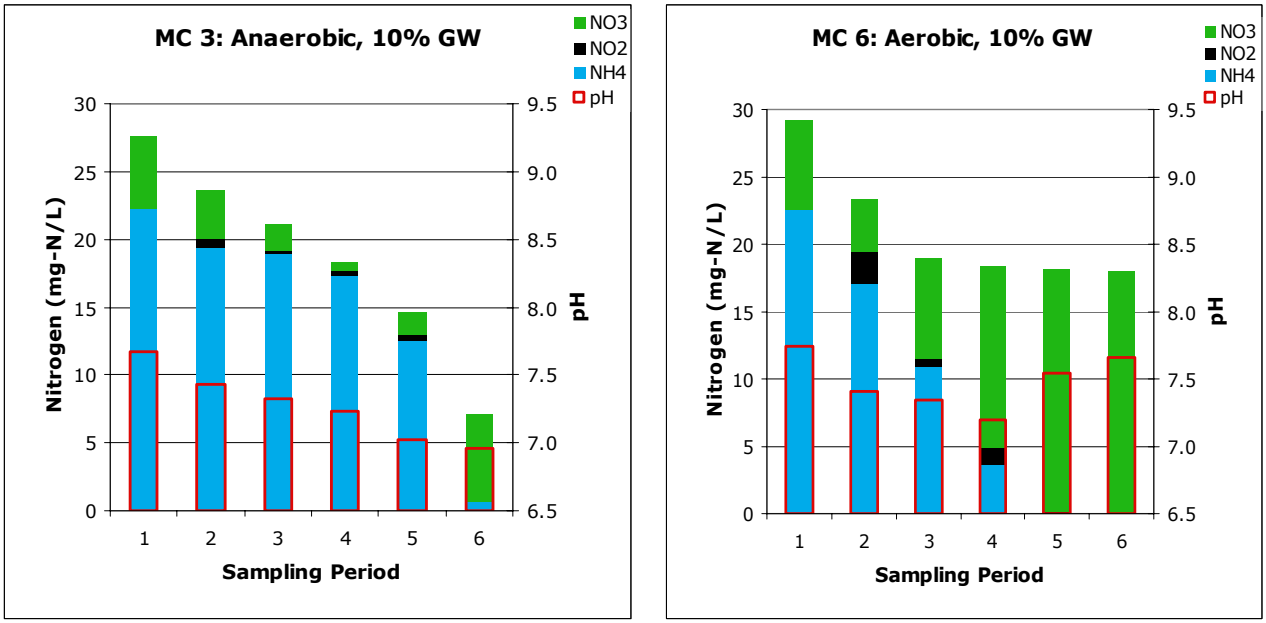
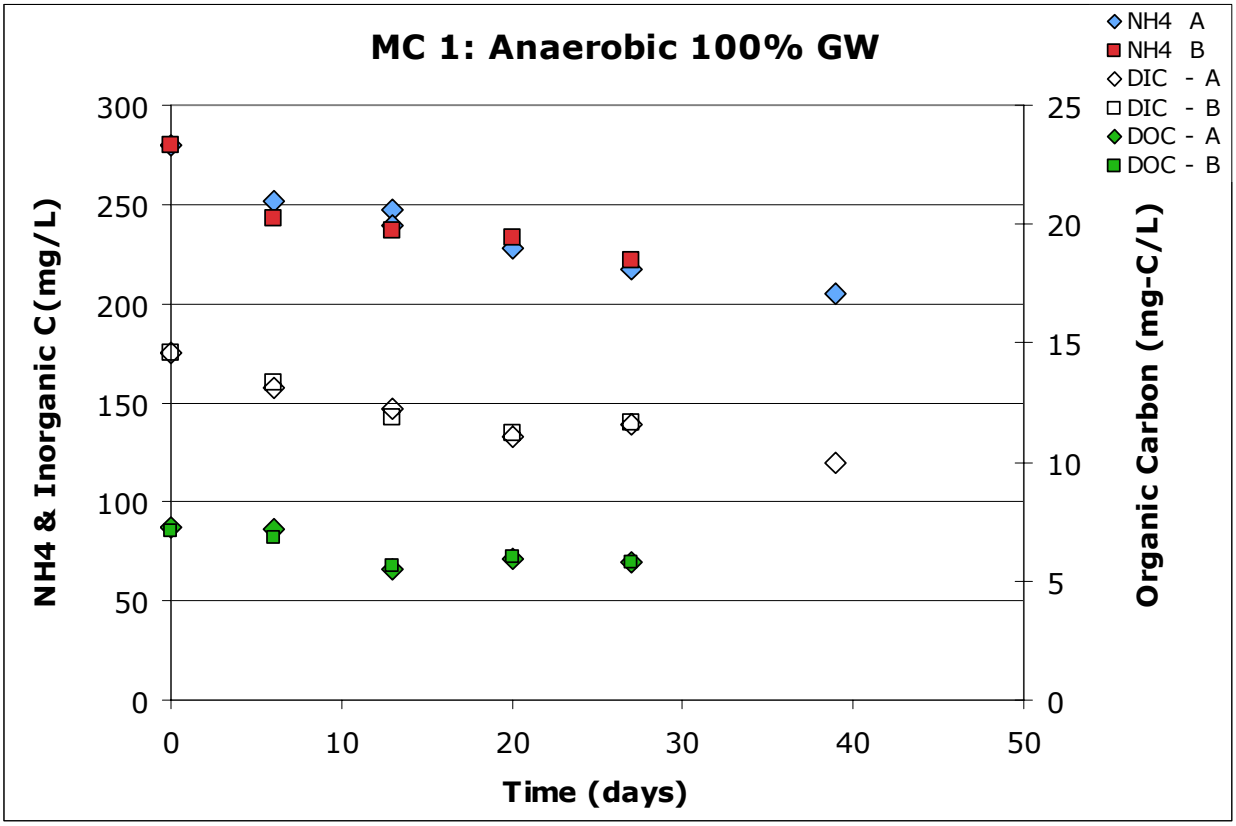


Figure 3. Total nitrogen (NH_4^+, NO_2^- and NO_3^-) concentration and pH (red line, right-hand ordinate) for microcosms 3 and 6 (values are averaged between duplicates A and B).

Figures 4, 5 and 6 show ammonia concentrations along with the dissolved organic and inorganic carbon (DOC & DIC) concentrations plotted as a function of time. Dissolved is operationally defined as passing through a 0.45 um filter. Note that the symbol shapes correspond to the duplicate number (diamond = A, square = B) for all constituents in all three figures.



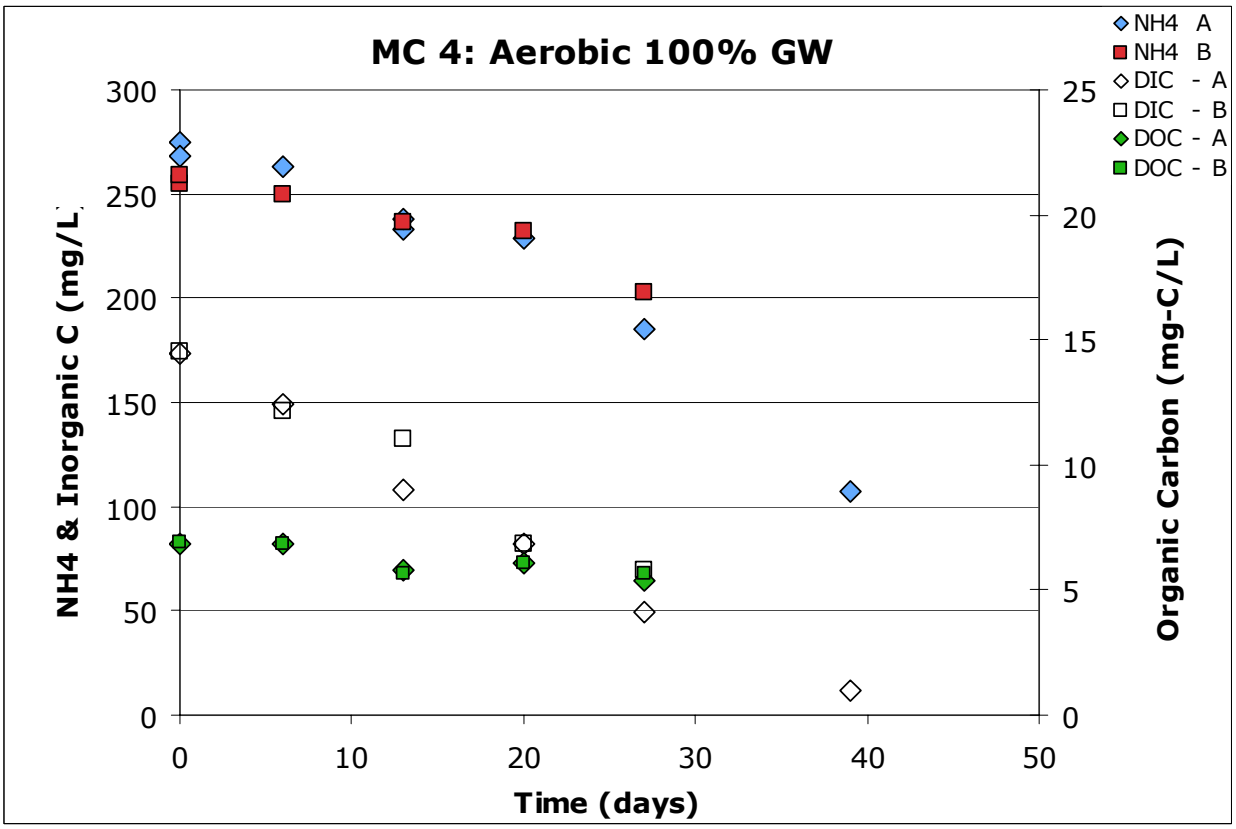
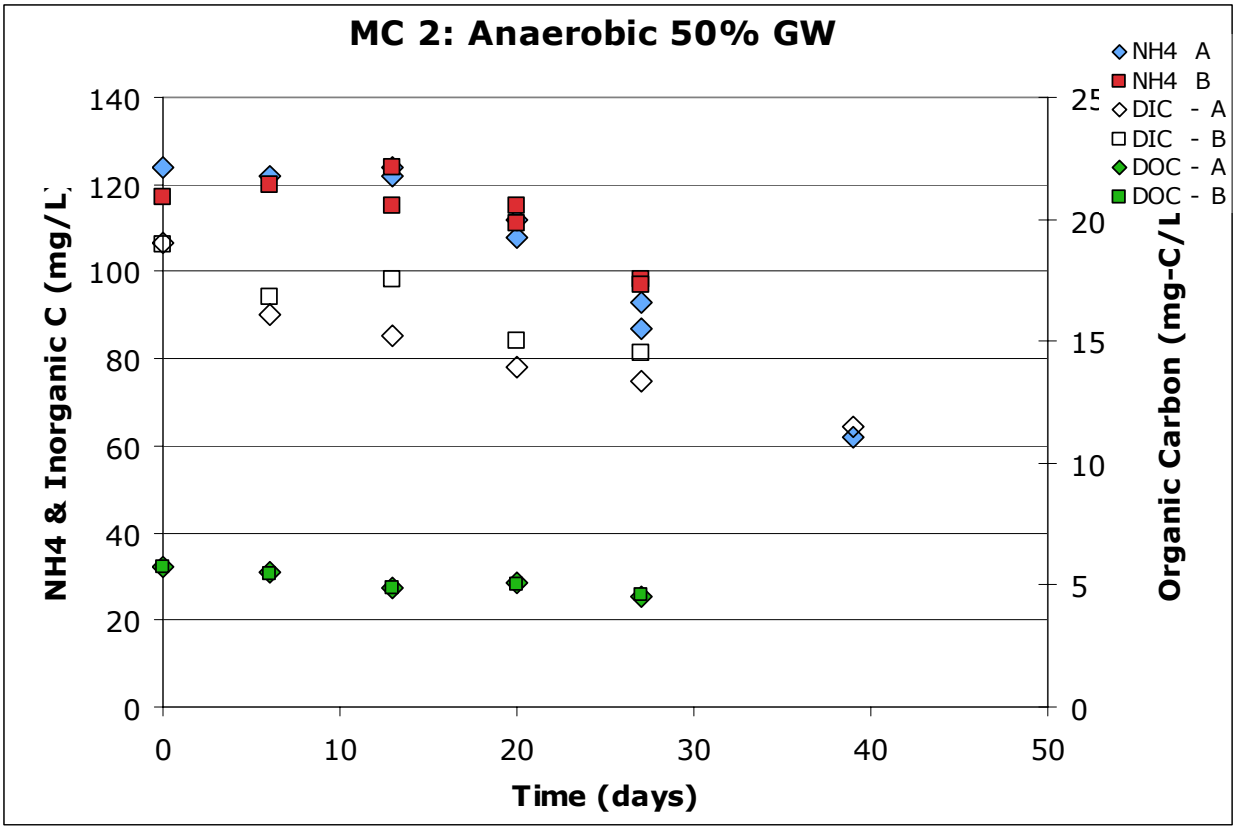


Figure 4. Concentrations of total ammonia (NH_4^+ and NH^3, mg-N/L), dissolved inorganic carbon (DIC, mg-C/L) and dissolved organic carbon (DOC, right-hand ordinate, mg-C/L) for microcosms 1 and 4.



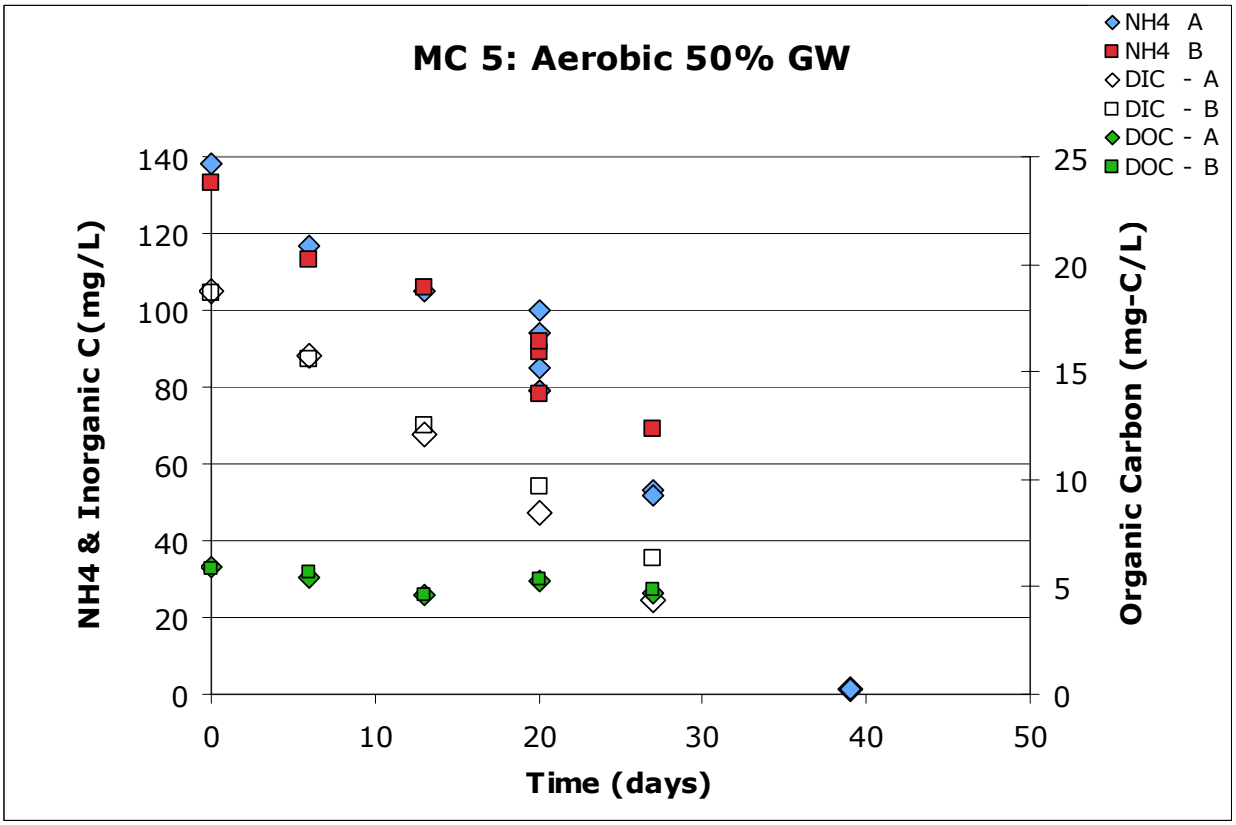
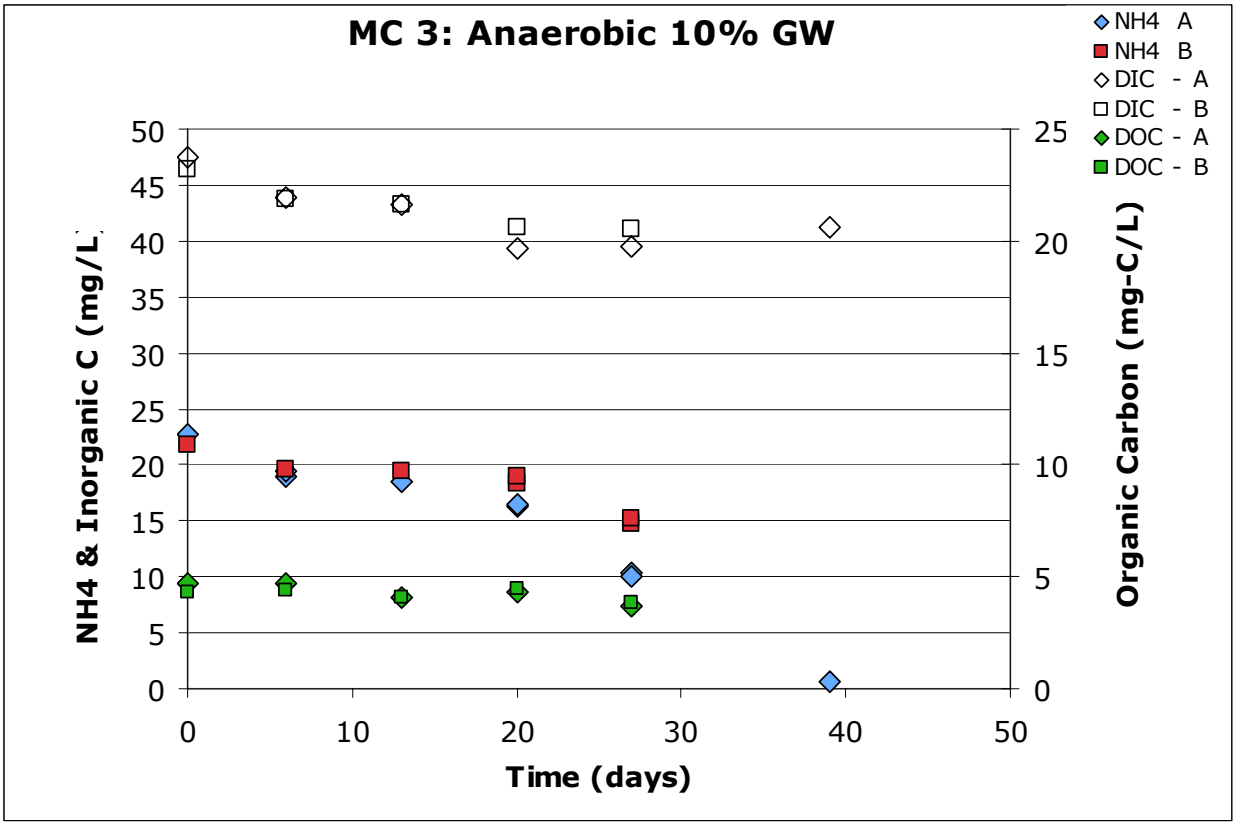


Figure 5. Concentrations of total ammonia (NH_4^+ and NH^3, mg-N/L), dissolved inorganic carbon (DIC, mg-C/L) and dissolved organic carbon (DOC, right-hand ordinate, mg-C/L) for microcosms 2 and 5.



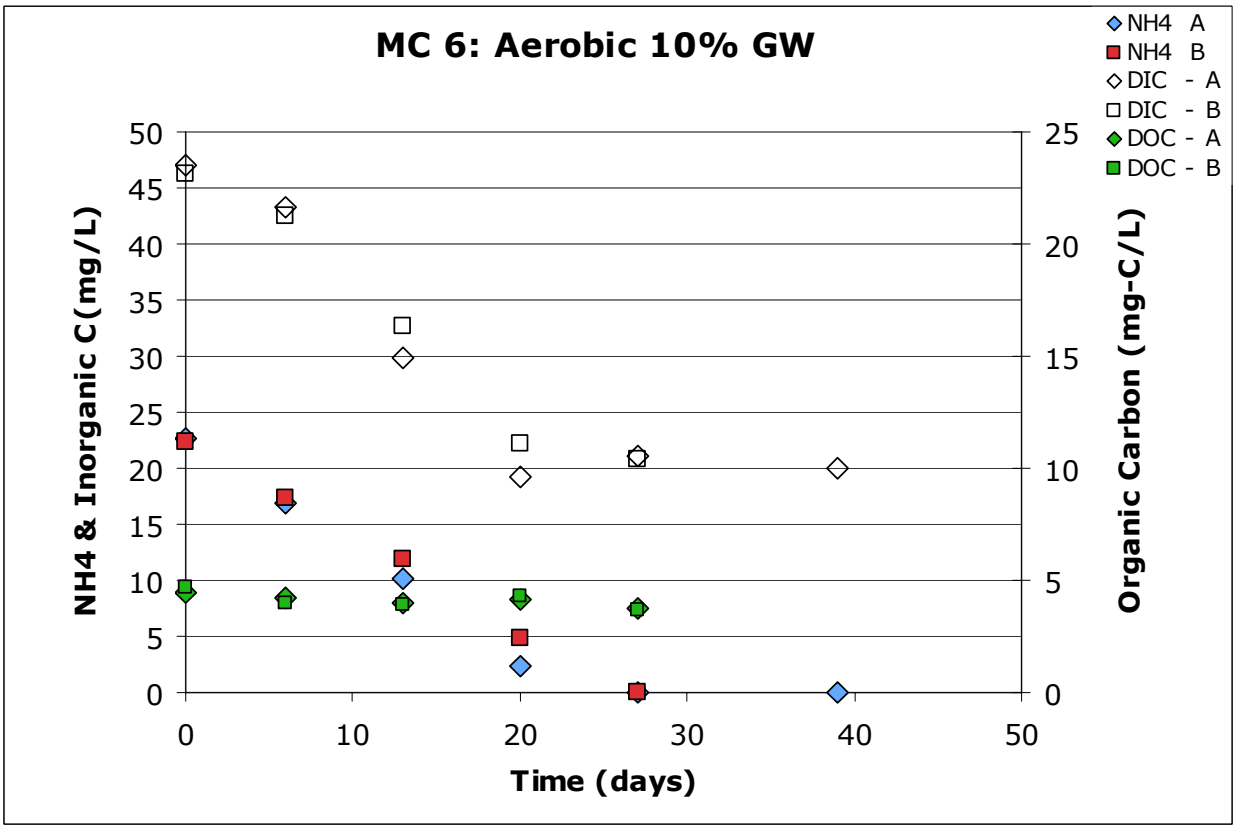


Figure 6. Concentrations of total ammonia (NH_4^+ and NH^3, mg-N/L), dissolved inorganic carbon (DIC, mg-C/L) and dissolved organic carbon (DOC, right-hand ordinate, mg-C/L) for microcosms 3 and 6.

A short term experiment was performed to assess the potential for ammonia and inorganic carbon volatilization from the aerobic microcosm because these microcosms were nominally open to the atmosphere (loosely capped). Briefly, 100 ml of filter sterilized (0.22 um) water (100, 50 and 10% groundwater (well 0480) with the balance being DI water) was added to three microcosm bottles that were then sampled, loosely capped for 7 days and then sampled again. The results are displayed in Table 2. No appreciable loss of ammonia was observed but a substantial amount of inorganic carbon was lost due to the volatilization of CO_2. A caveat to these results is that the headspace in the volatilization experiments was greater (approximately 500 ml) than the microcosm experiments which started with approximately 100 ml of head space. The larger head space in the volatilization controls resulted due to the limited amount of groundwater that was available by the time these experiments were performed. For this reason it is possible that the controls are over estimating the CO_2 loss.

Table 2. Ammonia and dissolved inorganic carbon (DIC) volatilization control experiment results.

Control Description	Elapsed Time	DIC mg/L	pH	NH_4 (mg/L)
100% GW	0	160.0	~7.3	281
“	7 days	74.0	8.35	280
50% GW	0	79.6	~7.3	139
“	7 days	50.3	8.54	139
10% GW	0	16.3	~7.3	28.8
“	7 days	14.5	8.15	28.3

In the aerobic microcosms, the DIC decreases more or less constantly with time with the exception of the last 2 points in the 10% GW microcosm corresponding to ammonia depletion. In the anaerobic microcosms the DIC decreases at a slower rate and plateaus towards the end of the experiments.

Slight decreases in DOC are noted in all microcosms. Note that the DOC measurements do not reveal anything about the character of the DOC, i.e., if it is biologically available or refractory. Sediment samples collected from the microcosms at the end of the experiment and a sample of the sediment used to start the microcosms that had been kept at 4 C (labeled “source sediment”) were analyzed for particulate organic matter by combusting the samples at 900 C and measuring the CO_2 produced. The results of these measurements are presented in Table 3. Due to the heterogeneity of the sediment (sediment grain size and discrete pieces of organic matter such as small roots) and the small sample size used for the analysis (70-100 mg), there was some variability in replicate measurements but the results seem to indicate that some particulate organic matter in the microcosm sediments was consumed.

Table 3. Soil organic matter values of the microcosm sediments at the end of the experiment (percent organic matter by weight). Five replicate measurements of the source sediment and three replicates of each microcosm were performed (a new sample for each measurement).

	Source Sediment	1A	2A	3A	4A	5A	6A
% Organic Matter	0.74	0.59	0.60	0.58	0.58	0.60	0.62
Std. Deviation	0.21	0.07	0.13	0.07	0.19	0.11	0.13

Gas production was noted in all microcosms as evidenced by pockmarks in the silt layer the day after shaking the microcosms (Figure 7). If the microcosms were tapped (vibrated), bubbles could be seen rising from the sediment surface. Gas production was higher in the 100% GW and 50% GW microcosms than in the 10% GW microcosms and at the end of the experiment no bubbles were being generated in the aerobic 10% GW microcosms.

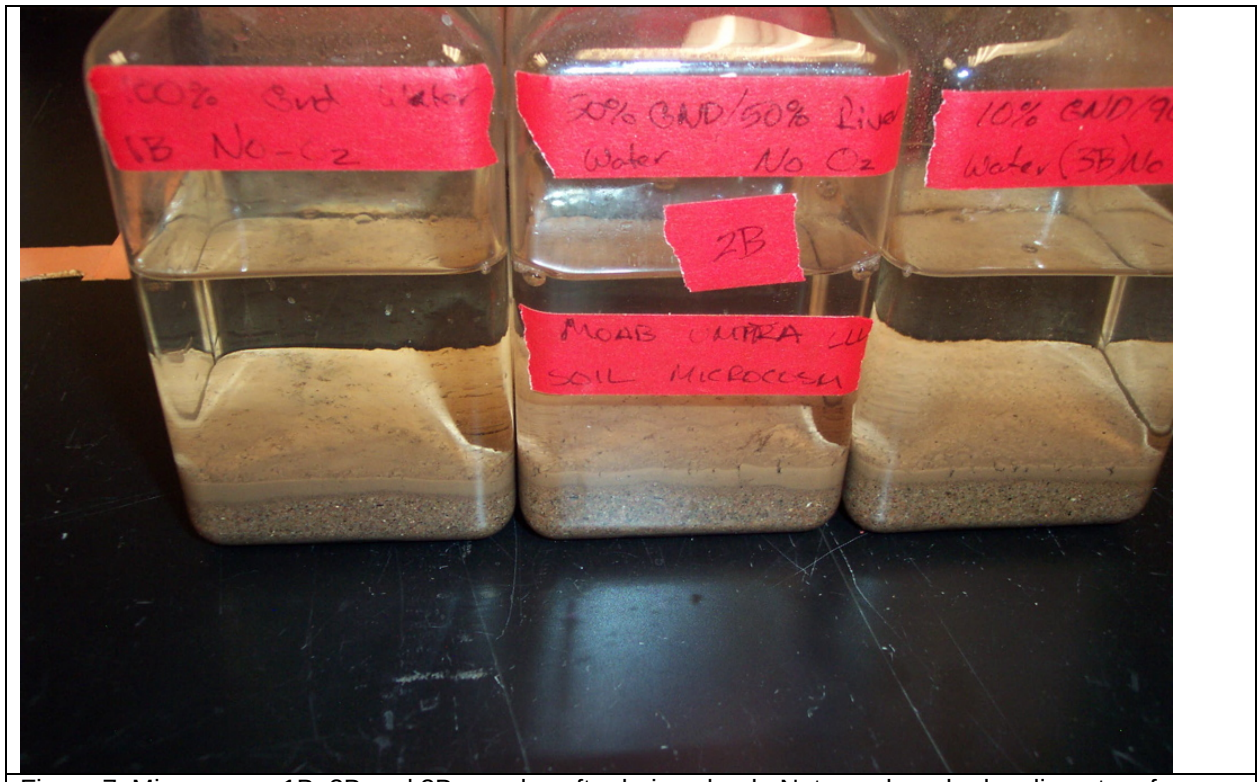


Figure 7. Microcosms 1B, 2B and 3B one day after being shook. Note pockmarked sediment surface and the bubbles that have collected the fluid-air interface. The sediment surface is initially very smooth once the sediment has settled after shaking.

Gas chromatograph analysis of the headspace gases in the anaerobic microcosms revealed only CO_2 and argon. No H_2S, methane or N_2 was detected. The aerobic headspace gases were not analyzed because the microcosms were open to the atmosphere (air is mostly nitrogen so any N_2 generation would not have shown up). It is possible that the argon in the anaerobic microcosms interfered with N_2 detection.

The anion and cation analyses of the groundwater, river-water and the piezometer samples are contained in the attached spreadsheet (microcosm data.xls) under the tab labeled "Auxiliary Data". The analysis of the microcosm water from week 2 and 4 is in the tab "MC ICP Results". There was no iron detected in the microcosm water samples even though there was a small amount (0.12 mg/L) in the river water. Manganese, which is also a potential electron donor/acceptor, was present in the microcosms at small and variable amounts but not at levels that could substantially affect the biochemistry of the nitrogen biotransformations.

Microscopic examination of water samples stained with DAPI (4',6'-diamidino-2-phenylindole, a DNA specific stain) and filtered onto a 0.22 um filter revealed the presence of bacteria in water samples from well 0480, both piezometers and the microcosms. Figure 8 displays two examples of the images taken. In general, the bacteria are the regular shaped rods and spheres (cocci in microbial terms) while the more amorphous shapes are likely soil particles

or organic matter. The higher bacterial numbers in the image from well 0480 are due in part to the fact that a 5-fold higher volume of well 0480 water was filtered relative to the volume used for the piezometer 0563 image.

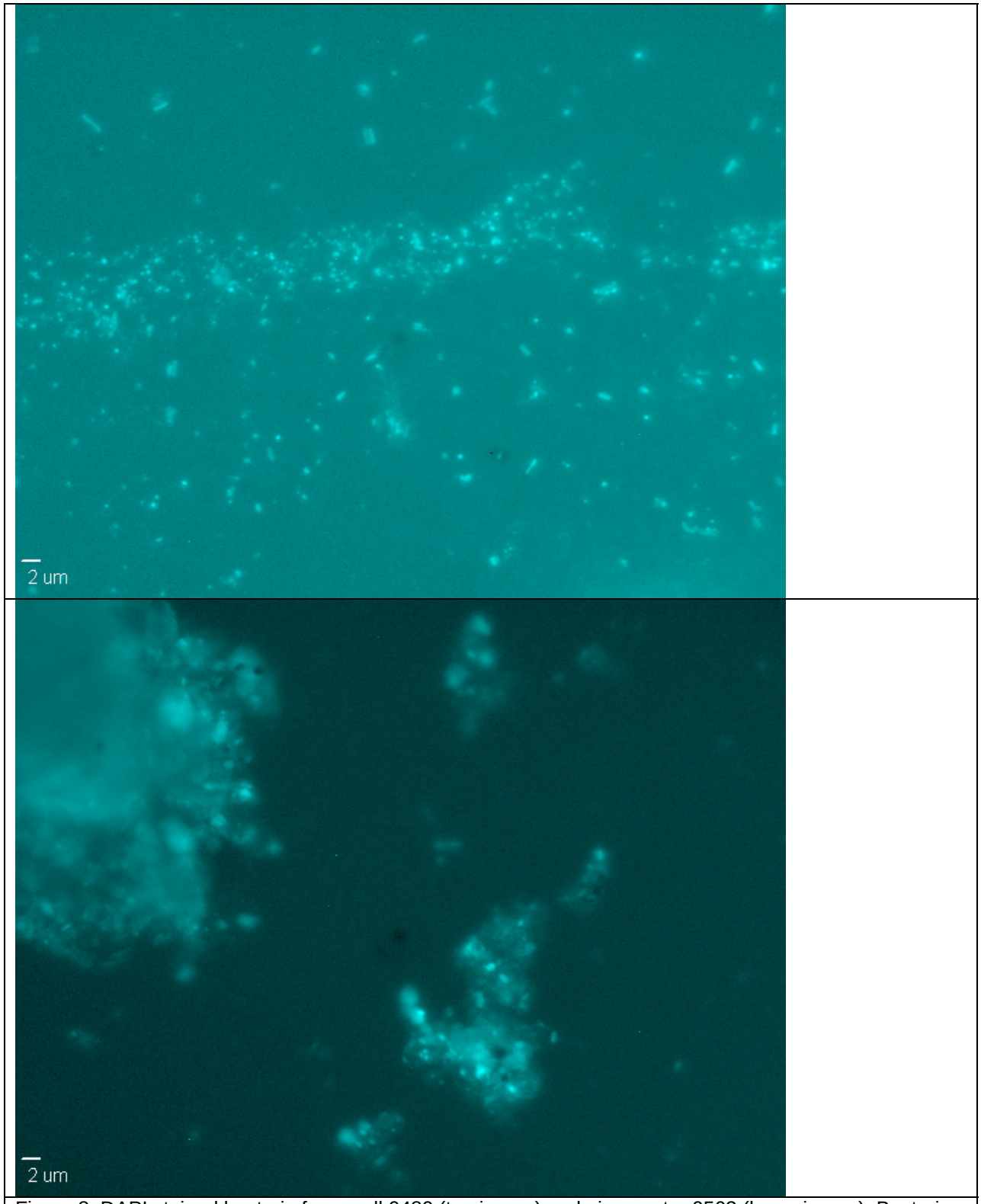


Figure 8. DAPI stained bacteria from well 0480 (top image) and piezometer 0563 (lower image). Bacteria can be seen in the lower image associated with amorphous masses of unknown composition.

Discussion

The total nitrogen and ammonia removal after 39 days of incubation are displayed on a percentage basis (Figure 9) and on a mass basis (Figure 10) as a function of the amount of groundwater (well 0480) in the microcosms for both the anaerobic and the aerobic cases. Table 4 presents this data in a tabular format.

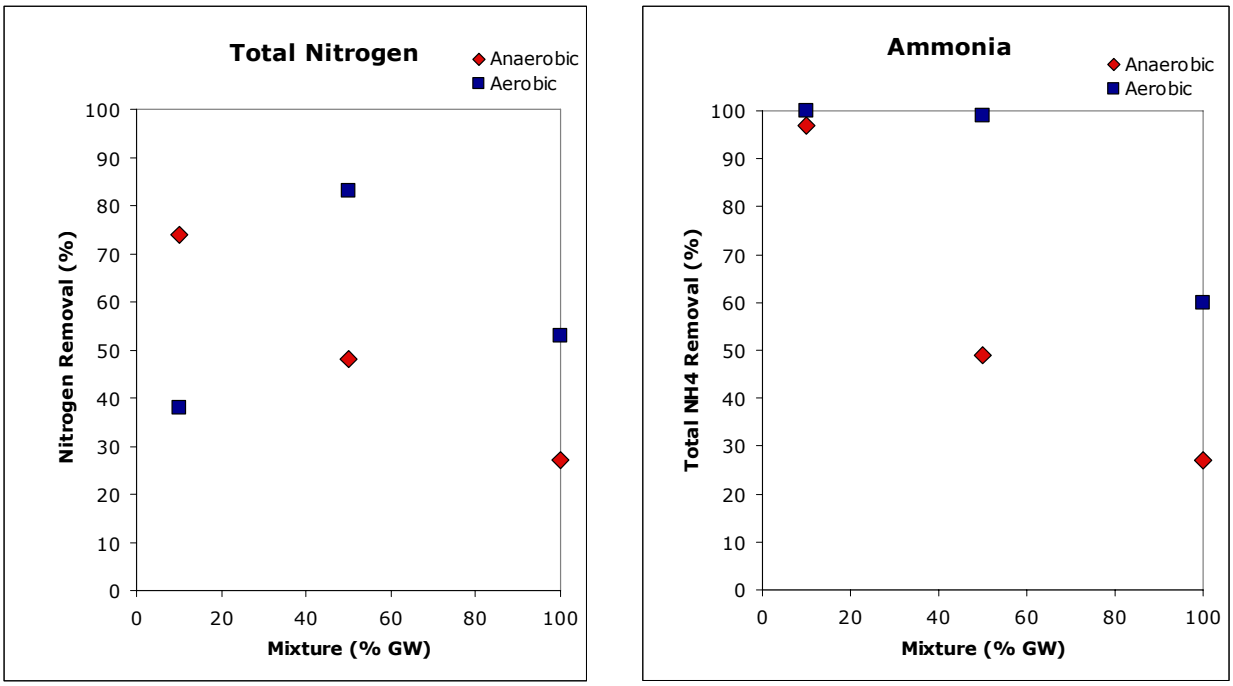


Figure 9. Total nitrogen and ammonia removal on a percentage basis as a function of headspace composition and percent groundwater in mixture.

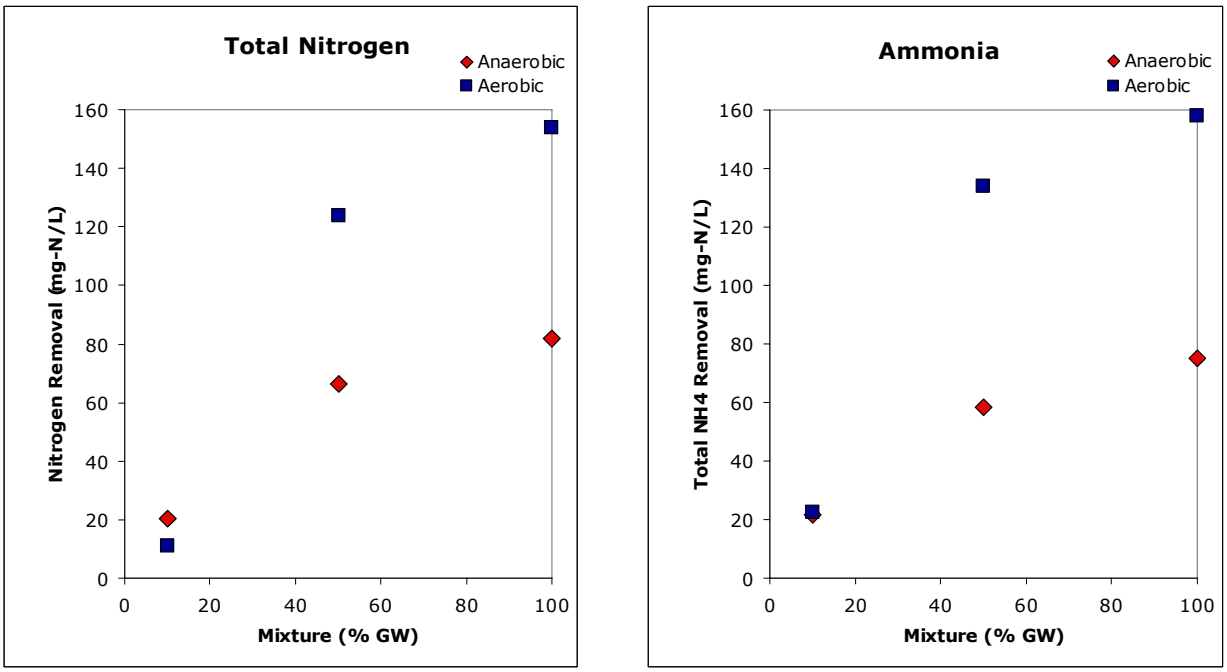


Figure 10. Total nitrogen and ammonia removal on a mass basis (mg-N/L) as a function of headspace composition and percent groundwater in mixture.

Table 4. Ammonia and total nitrogen removal.

Microcosm	Total N %	Total N mg-N/L	NH_4 %	NH_4 mg-N/L
Anaerobic 100% GW	27	82	27	75
Anaerobic 50% GW	48	66	49	59
Anaerobic 10% GW	74	20	97	22
Aerobic 100 % GW	53	154	60	158
Aerobic 50 % GW	83	124	99	134
Aerobic 10 % GW	38	11	100	23

The total nitrogen and ammonia removal was substantially greater in the aerobic microcosms with the exception of the 10% GW microcosms. While all or most of the ammonia was removed in the aerobic and anaerobic 10% GW microcosms, nitrate began to accumulate in the 10% GW microcosms after the ammonia concentrations became low. It is possible that the nitrate may have continued to decrease in the anaerobic 10% GW microcosm if the experiments had continued for a longer time period but it appears that denitrification had stalled in the aerobic 10% GW microcosm. In general, the fractional nitrogen removal increased as the GW was diluted with RW but the total removal on a mass basis was higher when the fraction of GW was higher. There are multiple biochemical processes that may be affecting the nitrogen species in the microcosms; the most prominent are outlined below (Ahn 2006).

Autotrophic Nitrification: In aerobic environments certain bacteria are capable of oxidizing ammonia to nitrate, utilizing inorganic carbon (e.g., HCO_3^-) as a carbon source. This occurs in two steps; one consortium of bacteria oxidizes the ammonia to nitrite and a separate consortium oxidizes the nitrite to nitrate. The overall stoichiometry is approximated by the following equation.

$$NH_4^+ + 1.83\ O_2 + 0.10\ HCO_3^- \Rightarrow 0.021\ C_5H_7NO_2 + 0.98\ NO_3^- + 1.04\ H_2O + 1.88\ H^+ \qquad (1)$$

In general, autotrophic nitrifiers are slow growing and nitrification consumes 4.3 mg O_2/ mg NH_4- N based on Equation 1. The process produces acidity that tends to decrease the solution pH and consumes alkalinity.

Denitrification: In anaerobic environments, other bacteria species utilize nitrate and nitrite as electron acceptors in order to oxidize organic matter and produce dinitrogen gas in a two step process ($NO_3^- \Rightarrow NO_2^- \Rightarrow H_{2(g)}$) , a representative overall stoichiometry is shown in Equation 2.

$$NO_3^- + 1.08\ CH_2O + 0.24H^+ \Rightarrow 0.056\ C_5H_7O_2N + 0.47\ N_{2(g)} + 1.68\ H_2O + 0.76\ HCO_3^- \qquad (2)$$

The overall denitrification process produces inorganic carbon (alkalinity) and tends to drive the pH of a solution up but the first step ($NO_3^- \Rightarrow NO_2^-$) may decrease the pH. Note that denitrification also produces nitrite as an intermediate.

Anammox: A more recently discovered process in the nitrogen cycle is anaerobic ammonia oxidation (anammox). Anammox is a process in which certain bacteria use ammonia as an electron donor and nitrite as an electron acceptor producing nitrogen gas and a small amount of nitrate. The exact stoichiometry of this process can vary substantially but the following has been proposed.

$$1\ NH_4^+ + 1.32\ NO_2^- + 0.066\ HCO_3^- + 0.13\ H^+ \Rightarrow 1.02\ N_2 + 0.26\ NO_3^- + 0.066\ CH_2O_{0.5}N_{0.15} + 2.03\ H_2O \qquad (3)$$

As can be seen from Equation 3, the bacteria performing anammox are autotrophic (use inorganic carbon as a carbon source) and tend to drive the pH up. The process of anammox requires nitrite production by either nitrification or denitrification.

We think it is possible that all three processes may be occurring in the aerobic microcosms and that both denitrification and anammox are occurring in the anaerobic microcosms. A brief discussion of the processes occurring in the microcosms will be broken into two parts: (1) anaerobic, and (2) aerobic microcosms.

Anaerobic Microcosms: If only denitrification were occurring the nitrate present in the microcosms would be converted to nitrogen gas but no transformation of ammonia would occur. As can be seen in Figures 1 through 3, ammonia is disappearing which leads us to believe that anammox is occurring concurrently with denitrification. The fact that inorganic carbon is being consumed in the anaerobic microcosms provides further evidence that anammox is occurring because denitrification produces inorganic carbon.

Aerobic Microcosms: The most obvious mechanism expected to be operating in the aerobic microcosms is nitrification because denitrification and anammox are nominally anaerobic processes. However, we believe that other processes are occurring concurrent to nitrification because nitrate is not accumulating in amounts equivalent to the rate at which ammonia is disappearing. Even though the headspace of the aerobic microcosms is oxic, due to mass transfer limitations and the high oxygen demand of nitrification, the sediments in the microcosms are likely to become anoxic very quickly. This would create microenvironments where denitrification and anammox could occur. The fact that the total nitrogen (rather than ammonia alone) in the aerobic microcosms is decreasing is an indicator that either anammox or denitrification is taking place, or both processes may be occurring simultaneously.

Possible Inferences from the Microcosm Testing

The results of the microcosm tests suggest that ammonia biotransformation from both nitrification and anammox and nitrate biotransformation via denitrification are occurring in groundwater at the Moab Site. Nitrification in groundwater near shallow well 0480 appears possible, particularly since the aquifer is recharged from the application of oxygenated river water to irrigation test plots located a short distance hydraulically upgradient of this well. Nitrification may stimulate anammox by producing nitrite and the organic matter introduced to the groundwater from the irrigation plots may promote denitrification. In addition, all three forms of biologically mediated nitrogen removal have the potential to occur in the hyporheic zone located beneath the bed of the Colorado River near its west bank given that (a) characterization of this zone has indicated that it is recharged by oxygenated water infiltrating from the river, (b) dissolved organic carbon is present in river water, and (c) anaerobic, reducing microenvironments have been identified in sub-river sediments collocated with pockets of relatively high dissolved oxygen concentration.

Because the microcosm tests demonstrated that nitrogen concentration changes vary depending on the relative mixing of site groundwater and river water, the mixing caused by ground water extraction and injection of river water at GWIA wells is also likely to influence microbially mediated nitrogen removal. For example, ground water pumping at extraction wells has been shown to induce inflow of river and hyporheic zone water toward the wells, which may in turn augment the nitrogen biodegradation processes that take place in sub-river sediments where river and ground water mix naturally. Similarly, injection of oxygenated river water in other wells not only causes dilution of ammonia-contaminated ground water prior to its discharge

to the river but also has the potential to stimulate nitrification and possibly anammox or denitrification before ground water reaches the river.

In addition to supplying oxygen and organic matter, the injection of river water acts to dilute the very high salinity (ionic strength) of the undiluted groundwater that may be inhibiting microbial growth and biochemistry. Note that the water from well 0480 that was used to establish the 100% GW microcosms appears to have been impacted by the irrigation plot because the DOC was at very high levels for groundwater (5.6 mg-C/L) and the Cl^- levels were much lower than historical concentrations (2100 mg/L vs. 5500 to 9400 mg/L) for that well. It is postulated that lower microbiological activity would have been observed in the microcosms with undiluted groundwater due to the extremely high ionic strength.

The data from the batch microcosms clearly establishes a high potential for nitrogen removal in the Colorado River hyporheic zone. However, flow-through column studies, which were beyond the scope of this preliminary study, would better estimate the nitrogen biotransformation potential at the Moab UMTRA Project site. Batch experiment artifacts not seen in column studies include: retention of products, high liquid to solids ratio and high gas volume to solid/liquid ratio. These artifacts may have reduced the observed rates of nitrogen biotransformation. A column study would give more insight into the effects of interim action operations on nitrogen chemistry and may suggest operational changes to the GWIA that would increase total nitrogen removal in the subsurface environments at the Moab UMTRA Project site.

Ahn, Y.-H. (2006). "Sustainable nitrogen elimination biotechnologies: A review." *Process Biochemistry* **41**: 1709-1721.