Groundwater Research Report
WR07R007

INFLUENCE OF WETLAND HYDRODYNAMICS ON MICROBIAL REDOX TRANSFORMATION OF NITRATE AND IRON

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INFLUENCE OF WETLAND HYDRODYNAMICS ON MICROBIAL REDOX TRANSFORMATIONS OF NITRATE AND IRON

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Project Summary

Title: Influence of Wetland Hydrodynamics on Microbial Redox Transformations of Nitrate and Iron

Project ID: WRI Project Number WR07R007

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Background/Need:
Nitrogen (N) is a limiting nutrient in many aquatic ecosystems, particularly in estuarine and coastal marine environments. The anthropogenic N delivered in surface waters to coastal environments is a non-point source pollutant that originates primarily from agricultural activities. Thus, understanding controls on the transport and fate of N in inland waters is critical to maintaining the ecological health and economic viability of coastal resources. In addition to effects on ecosystems, nitrate contamination of groundwater is a well-documented problem throughout the world. In Dane County an estimated 30% of private wells contain nitrate concentrations that exceed drinking water standards.

Objectives:
This research sought to explore the interaction of nitrate and iron redox cycles in freshwater aquifers, a poorly understood but potentially significant influence on the fate of nitrate in the environment, through monitoring the geochemical and microbial properties of groundwater over a one year period.

Methods:
Several multilevel sampling devices were installed in the wetland sediments for the collection of groundwater samples. Three different locations in the wetland were chosen for this project and are referred to as ML1, the western transect, and the eastern transect. Groundwater samples were collected monthly using a peristaltic pump and the geochemistry of each sample was determined though a combination of in-field and laboratory analyses. Due to time constraints samples for microbial analysis were collected only once during the sampling period. These samples were analyzed using the most-probable number (Woomer, 1994) and 16S rDNA clone library analyses.

Results and Discussion
At ML1, redox conditions, and therefore iron and nitrate concentrations, varied considerably. Oxic conditions were associated with elevated nitrate concentrations and decreased iron concentrations. When concentrations shifted towards anoxic, a decrease in nitrate concentrations and an increase in iron concentrations were observed. Microbial results from this location

indicate the presence of at least low concentrations of bacteria related to the nitrate and iron cycles. This suggests that coupling of the two redox cycles is likely occurring in the aquifer sediments surrounding ML1.

Nitrate was never observed along the western transect. Conditions were consistently anoxic with significant concentrations of ferrous iron observed throughout the study period. Microbial data indicates the strong presence of nitrate-reducing and iron-reducing bacteria at this location.

Along the eastern transect low levels of nitrate were observed under oxic conditions at the upgradient wells while anoxic conditions were observed downgradient. Although microbial and geochemical sampling failed to identify the coupling of the two redox cycles, some process is removing nitrate from groundwater upgradient of ML8.

Conclusions:
Observations of nitrate and iron concentrations at ML1 are consistent with the conceptual model and are most likely a result of microbial activity. MPN counts and 16S rDNA clone library data indicate the presence of at least low concentrations of bacteria related to the nitrate and iron cycles. This suggests that coupling of the two redox cycles is likely occurring nearby and may occur at this location in the event of a shift to more reducing conditions.

Although nitrate was never observed along the western transect, geochemical and microbial data indicate that iron and nitrate redox cycles are likely coupled in the sediments immediately upgradient of ML4. Geochemical data indicates that nitrate is likely flowing towards the transect after leaching out of the vadose zone of the nearby field. Microbial analyses indicate that microbially-mediated nitrate reduction removes all nitrate from groundwater prior to the groundwater reaching ML4.

Along the eastern transect, nitrate is removed as dissolved oxygen concentrations decrease downgradient. Insufficient microbial data along this transect prevents indentifying the microbial community that may be responsible for nitrate removal. However, abundant concentrations of ferrous iron observed downgradient indicate that the coupling of nitrate and iron cycles may be one of the responsible processes affecting the fate of nitrate along the transect.

Related Publications:
Miller, C.A. 2009, Influence of wetland dynamics on microbial redox reactions of nitrate and iron, MS Thesis, UW Madison Department of Geoscience

Miller, C.A. , J.M. Bahr, and E.E. Roden, Influence of wetland hydrodynamics on subsurface microbial redox transformations of nitrate and iron, AWRA WI Section 33rd Annual Meeting, p. 40.

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Introduction

Nitrate has become one of the most prominent contaminants in groundwater systems world-wide since the 1970's. Consequences of nitrate contamination of groundwater include both health concerns such as methemoglobinemia and environmental concerns such as excessive amounts of nitrate in the Mississippi River Basin flowing into the Gulf of Mexico (Burden, 1982).

Goolsby et al. (1999) showed that nonpoint sources, stemming from the application of fertilizers related to agricultural activity, accounted for approximately 74% of the total nitrogen flux to the Gulf of Mexico. Point sources from other agricultural, domestic, and industrial activities also have the potential to contribute to the flux of nitrate into groundwater systems (Smith and Duff, 1988; Smith et al., 1991). The relative contribution of each of these sources varies greatly depending on seasonal variations in the amount and timing of precipitation. Episodic events, such as the 1993 flooding of Midwestern states, have been shown to double the yearly influx of nitrate to the Gulf as a result of increased runoff and increased leaching from vadose zones, which may act as massive storage reservoirs for nitrate as it accumulates during dry periods (Goolsby et al., 1999).

Wetlands and riparian zones have been shown to be efficient at removing nitrate from groundwater, and several different processes affecting the fate of nitrate may occur in aquifer sediments. One poorly understood mechanism that may act as a pathway for nitrate removal is the coupling of nitrate and iron redox cycles. In this process, ferrous iron is oxidized via the reduction of nitrate under anoxic conditions. Unlike denitrification, this process does not necessarily result in the production of nitrogen gas, and may instead result in several different end products including NH_4^+, NO, NO_2^-, N_2O, or N_2 (Weber et al., 2006; Shelobolina et al., 2003). This process must be microbially-mediated in order to occur under conditions commonly found in aquifers and requires close coupling of the nitrate and iron cycles, such that nitrate and ferrous iron meet in opposing diffusion gradients. Within the zone where the two diffusion gradients meet, nitrate is reduced via the oxidation of ferrous iron, precipitating Fe(III)-oxides that may then be reduced by heterotrophic Fe(III)-reducing bacteria, thus replenishing the available supply of ferrous iron for use in the reduction of nitrate.

The objective of this study was to identify coupled microbial redox metabolism of nitrate and iron in sediments underlying a wetland through geochemical and microbial analyses of groundwater. From the geochemical data, potential locations for active iron and nitrate cycling were identified and the relative abundances of bacteria related to the nitrate and iron cycles were determined. The geochemical parameters combined with the microbial analyses were then used to assess the significance of microbially-mediated nitrate reduction in relation to microbial Fe redox metabolism.

Procedures and Methods

Location of Study

The research for this project was conducted in the Dorn Creek Marsh State Fishery Area in Dane County, Wisconsin. The Dorn Creek sub-watershed has an area of approximately 32.9 km^2, 25.6 km^2 of which are agricultural lands and 5.3 km^2 are wetlands (Rogers, 2006). The high percentage and close proximity of agricultural land use surrounding the Dorn Creek Marsh

results in high nutrient loading in the surface water and groundwater in the watershed, making it an ideal site for studying the coupling of nitrate and iron cycles in wetlands.

Dorn Creek flows from west to east through the wetland and empties into nearby Lake Mendota. Surface water chemistries observed during the summer of 2006 by Craig (2007) indicated that nitrate concentrations decrease by 90% over the reach of the stream, with a significant decrease in nitrate concentrations observed between sampling points 6 and 7 (Figure 1). Based on this information, two transects of multilevel sampling devices and piezometers were installed in the wetland, focusing primarily on the region where the greatest decrease in nitrate concentrations was observed (Figure 2). Additionally, a multilevel sampler (ML1) was installed near County Road Q based on groundwater nitrate concentrations observed in an 11.5 ft deep well installed during the 2006 season (Craig, 2007).

Figure 1: Nitrate concentrations of surface water samples collected during summer of 2006. Nitrate concentrations in mgL^{-1} NO_3-N (Craig, 2007).

Figure 2: Location of multilevel samplers and piezometers installed in the Dorn Creek Marsh State Fishery Area.

Site stratigraphy of the study area is characteristic of post-glacial lake sediments in the region. Core taken at ML1 consists of 0.76 meters of peat, underlain by a silty sand layer, a homogeneous clay layer, and highly variable layers of silt, sand, and gravel (Figure 3). The boring reached a depth of 8.1 meters before hitting bedrock.

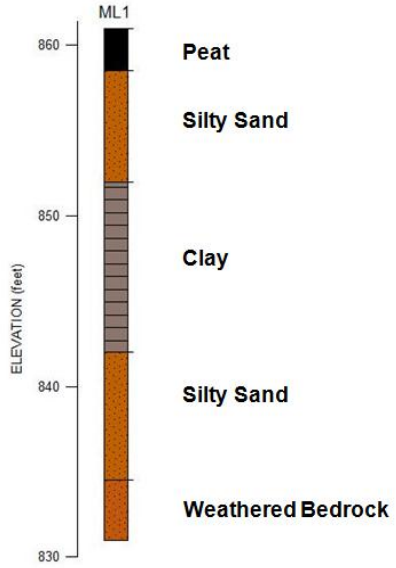

Figure 3: Stratigraphy at ML1 in the Dorn Creek Marsh State Fishery Area.

Core recovered from the eastern transect, which consists of ML5, ML6, ML7, ML8, and ML9, was also very heterogeneous. Groundwater along this transect flowed from south to north (ML9 to ML5), and stratigraphy varied from well-sorted sand and gravel on the southern end of the transect to a complex distribution of peat, clay, silt, and sand on the northern end (Figure 4). The western transect, which consisted of ML2, ML3, and ML4, was relatively uniform by comparison. Along this transect groundwater flows from south to north (ML4 to ML2) and sediments consisted of approximately 1 meter of peat underlain by silty sand, well-sorted sand, and clay (Figure 5).

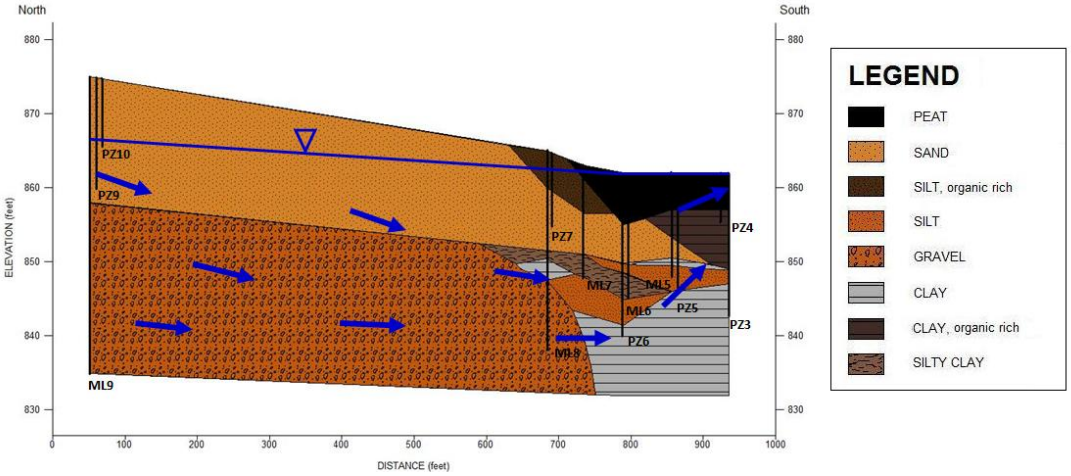

Figure 4: Stratigraphy of the eastern transect in the Dorn Creek Marsh State Fishery Area.

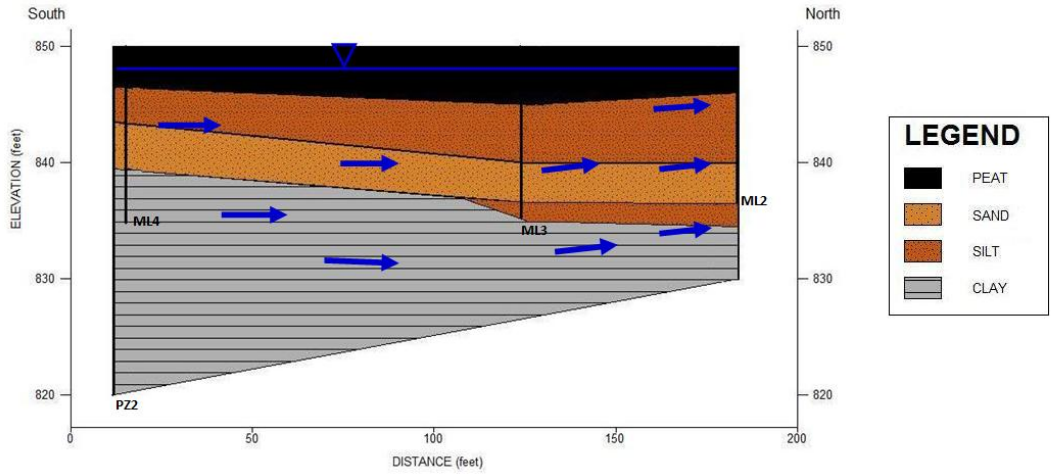

Figure 5: Stratigraphy of the western transect in the Dorn Creek Marsh State Fishery Area.

Field Methods and Laboratory Analysis

Groundwater samples were collected for chemical analyses from every multilevel sampling device except for ML2 and ML6. These two locations failed to produce groundwater in sufficient amounts for sampling due to malfunction of the multilevel sampling device. Groundwater was collected from multilevel samplers using a peristaltic pump and the geochemistry of each sample was determined though a combination of in-field and laboratory analyses. Each sampling point was purged by pumping at least 500 ml of groundwater prior to sampling. Specific conductance and temperature were determined in-situ using a YSI Model 30 handheld conductivity meter. Analyses for dissolved oxygen were also performed in-situ using CHEMets® R-7501 and K-7512 field test kits. Anions chloride, sulfate, and nitrate were analyzed with a Dionex ion chromatograph model ICS 1000. Iron concentrations were determined in the method outline by Stookey (1970) using a Shimadzu UV Mini 1240 Spectrophotometer. Some groundwater samples were also analyzed for various metals, including Ca, Cu, Fe, Mg, and Mn, using a Varian Vista-MPX CCD Simultaneous ICP-OES. Microbial samples were analyzed using the most-probable number (Woomer, 1994) and 16S rDNA clone library analyses.

Results and Discussion

ML1

Geochemical results at ML1 indicate two distinct geochemical environments. The first is located above the clay layer, at depths of less than 3.0 meters. This shallow zone is characterized by highly variable concentrations of nitrate, iron, and oxygen. Increases in nitrate concentrations at this location are associated with decreased ferrous iron and elevated oxygen concentrations, while decreasing nitrate concentrations are associated with elevated ferrous iron and decreased oxygen concentrations (Figure 6a). No microbial data were acquired from sampling points within this zone of groundwater, making it difficult to infer the mechanism responsible for nitrate reduction.

In the deeper zone of groundwater at ML1, geochemical data indicated consistently oxic conditions with high nitrate concentrations and very low ferrous iron concentrations.

Groundwater samples for microbial analysis were obtained from the deepest sampling point at ML1 (Table 1). Results of the microbial analysis and indicated that the dominant process is nitrate reduction, with microbes related to the iron and sulfur cycles also present (Table 2; Table 3).

Western Transect

Groundwater samples from ML3 and ML4 indicated primarily reducing conditions along the western transect, and nitrate was never observed at either location during the sampling period (Figure 6b). Geochemical results indicate two distinct flow paths along the transect, one that is observed in the shallow sampling points and contains high ion concentrations, and one that is deeper in the sediments and contains a low concentration of ions. The distinction between the two flowpaths is well-demonstrated by calculating the molar ratio of calcium to magnesium in groundwater from each sampling point. In the deeper flow path, which is observed in the deepest two sampling points at ML4, the calcium-to-magnesium ratio is approximately 0.4, while in the shallow sampling points the ratio increases to approximately 1.0 (Figure 7). Most likely, the differences observed are a result of fertilizer application on the cornfield immediately south of ML4 which provides a steady influx of nutrients to the shallow groundwater flow path. Aside from the relative concentrations of calcium and magnesium, the fertilizer impact is also observed in concentrations of sulfate and chloride (Figure 8). Typically, elevated nitrate concentrations would also be observed. However, it appears that all nitrate is removed from groundwater via microbially-mediated pathways prior to reaching ML4. Given the significant concentration of ferrous iron along the transect, there is great potential for this to occur lithotrophically, with the nitrate and iron cycles coupled in the vicinity of ML4. Microbial observations at ML4 are consistent with geochemical results. The 16S rDNA clone library developed for ML4 indicated the presence of iron-reducing bacteria (Table 2), while MPN results indicate that nitrate-reducing bacteria dominate the microbial community (Table 3).

East Transect

Groundwater samples from the eastern transect indicate oxidizing conditions at ML9 and a transition to reducing conditions downgradient at ML8, ML7, and ML5. Groundwater samples were not collected at ML6 due to inadequate flow from sampling points. Groundwater from ML9 contained low levels of nitrate during the sampling period (<6.0 mg/L) and very low ferrous iron concentrations. Downgradient, nitrate concentrations were below detection throughout the sampling period and ferrous iron concentrations were significantly elevated (Figure 6c). Overall, sampling points with elevated nitrate concentrations were oxic and contained very low ferrous iron concentrations, while sampling points that did not contain nitrate were anoxic and contained elevated ferrous iron concentrations. This is consistent with the conceptual model that significant concentrations of ferrous iron and nitrate will not be observed together, as the nitrate would be reduced via the microbial oxidation of Fe(II). As would be expected under the oxic conditions observed at ML9, both MPN and 16S rDNA clone library analyses indicated aerobic heterotrophs dominated the microbial community (Table 2; Table 3).

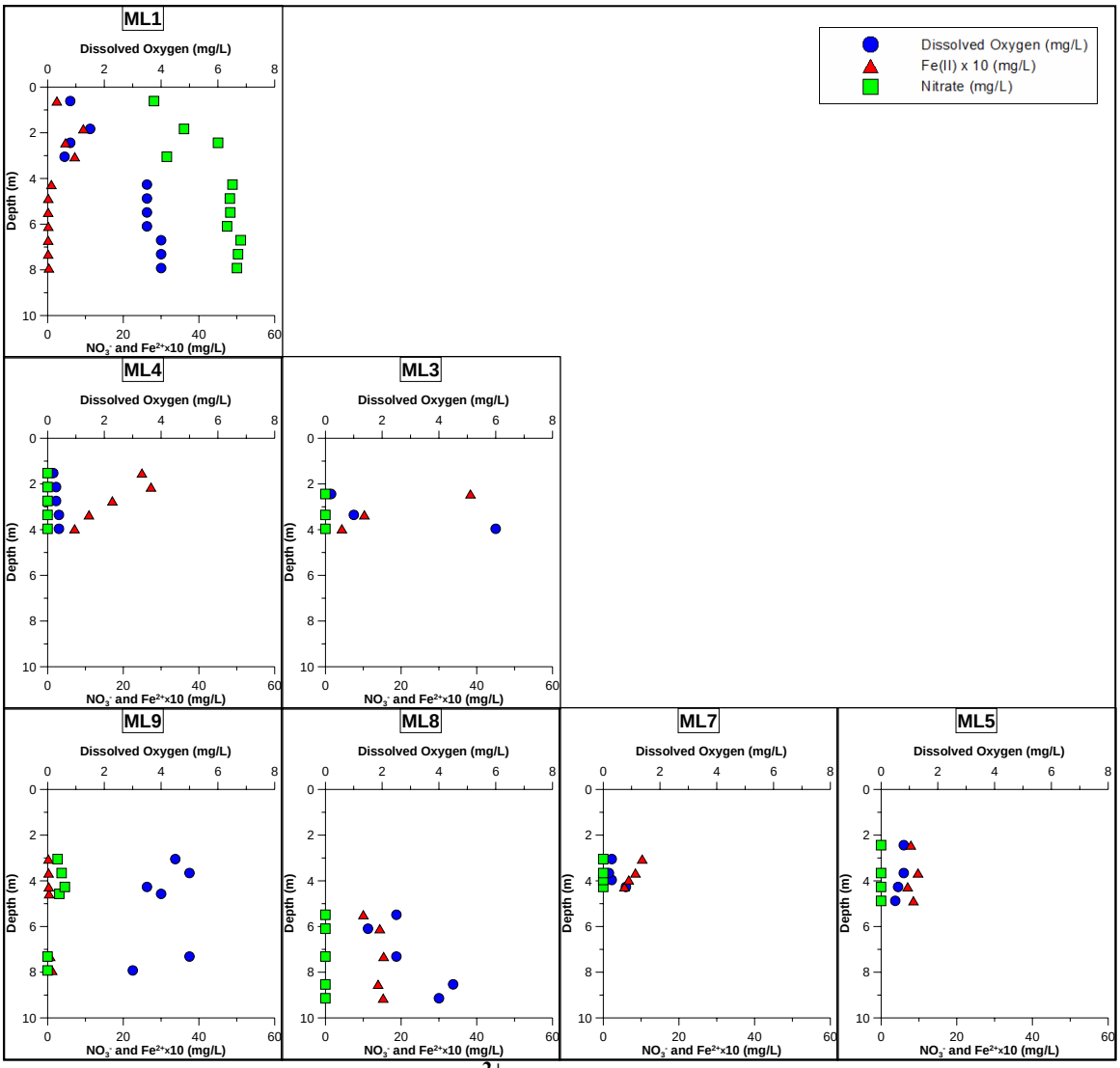

Figure 6: Concentration of NO_3^-, Fe^{2+} and Dissolved Oxygen during the November 2008 sampling round.

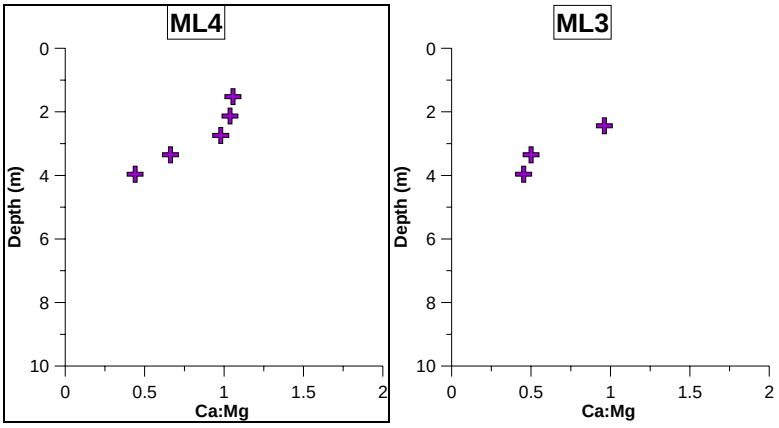

Figure 7: Ratio of calcium to magnesium at the western transect during the November 2008 sampling round.

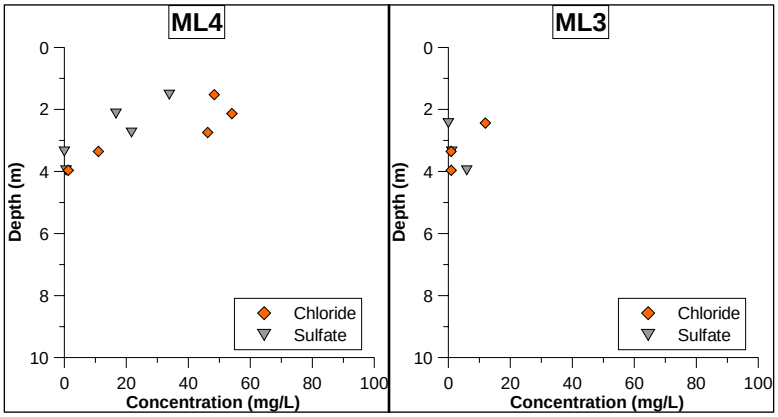

Figure 8: Concentration of sulfate and chloride during the November 2008 sampling round.

Table 1: Geochemistry of groundwater samples collected for microbial analyses.

Well	Depth (m)	T (°C)	Cond. µs/cm	D.O. (mg/L)	Cl^- (mg/L)	SO_4^{2-} (mg/L)	DOC mg/L)	NO_3^- (mg/L)	Fe(II) (mg/L)
ML1	7.9	13.9	682	4.0	44.0	19.2	1.34	65.7	0.06
ML4	2.7	14.0	738	0.3	42.9	25.0	1.69	0.0	1.36
ML7	4.0	10.9	551	0.4	1.42	12.5	1.54	0.0	0.69
ML9	4.6	13.3	897	2.5	3.19	38.4	2.41	3.7	0.09

Table 2: Summary of phylogenetic affiliation and distribution of 16S rDNA gene clones from clone libraries of groundwater from wells ML1, ML4, and ML9

Phylogenetic group	Nearest relative (GenBank accession no.)	Base pairs considered	% similarity	Potential physiology	No. of related clones in groundwater from:		
					ML1	ML4	ML9
α-Proteobacteria	*Sphingomonas mathurensis* strain SM13 (EF424400)	1422	94%	Aerobic heterotroph	0	0	6
	Uncultured α-Proteobacterium (EU244149)	652	98%	?	4	0	2
β-Proteobacteria	*Polaromonas jejuensis* strain JS12-13 (EU030285)	1288	98%	Aerobic and denitrifying	2	0	14
	Uncultured β-Proteobacterium (GQ105041)	592	91%	Aerobic and nitrate-reducing	0	0	3
	Massilia aurea strain AP13T (AM231588)	1441	99%	Aerobic heterotroph	0	0	10
δ-Proteobacteria	*Geobacter psychrophilus* strain P35 (AY653549)	665	96%	Fe(III) reduction	4	6	0
	Desulforegula conservatrix strain Mb1PaT (AF243334)	1436	98%	Sulfate reduction	8	12	0

Table 2 (continued)							
ε-Proteobacteria	Uncultured ε-Proteobacterium (AM086106)	1399	92%	?	4	0	0
	Pseudomonas veronii strain S3 (AY179328)	1463	99%	Aerobic and denitrifying	0	0	136
	Pseudomonas fragi strain B531 (AY581136)	604	97%	Aerobic heterotroph	0	0	1
Verrucomicrobia	Uncultured Verrucomicrobia bacterium (DQ676300)	1467	89%	Aerobic heterotroph	4	4	0
Cyanobacteria	Uncultured Cyanobacterium (EF520515)	1436	97%	Fermentation or aerobic heterotrophy	2	4	0
Acidobacteria	Uncultured Acidobacterium (FJ479521)	506	97%	Fermentation	0	0	2
Bacteroidetes	*Pedobacter agri* strain PB92 (EF660751)	1454	99%	Aerobic heterotroph	0	0	6
Clostridia	Syntrophomonas zehnderi strain OL-4 (DQ898277)	1420	89%	Syntrophic organic matter degrader	0	4	0
	Uncultured Desulfosporosinus (EU981221)	634	96%	Sulfate reduction	0	2	0
	Clostridium cellulolyticum strain H10 (X71847)	1415	92%	Fermentation	4	0	0
Chloroflexi	Uncultured Chloroflexi (DQ811890)	639	84%	Fermentation	2	0	0
Candidate divisions OD1 and OP11	Uncultured bacterium (AB252962)	1414	85%	Sulfur cycle	6	6	6

Table 3: Results of the most-probable number method

Microbial Group	Most probable number (MPN) per ml of GW			
	ML1A	ML4	PZ9	ML7
Nitrate reducers using acetate as the e donor	$(2.39 \pm 1.74)^* 10^2$	$(2.39 \pm 1.74)^* 10^2$	$(4.62 \pm 1.75)^* 10$	$(1.10 \pm 0.39)^* 10$
Fe(III) reducers using acetate as the e donor	$(1.12 \pm 0.64)^* 10^{-1}$	2.40 ± 1.74	< 0.03	$(1.12 \pm 0.64)^* 10^{-1}$
Lithotrophic Fe(II) oxidizers using nitrate as the e acceptor	$(1.12 \pm 0.64)^* 10^{-1}$	$(1.12 \pm 0.64)^* 10^{-1}$	$(1.12 \pm 0.64)^* 10^{-1}$	< 0.03
Fe(II) oxidizers using nitrate as the e acceptor and acetate as C source	$(1.12 \pm 0.64)^* 10^{-1}$	$(1.12 \pm 0.64)^* 10^{-1}$	$(1.12 \pm 0.64)^* 10^{-1}$	< 0.03
Aerobic heterotrophs using acetate as the e donor	4.62 ± 1.75	$(2.39 \pm 1.74)^* 10$	$(2.39 \pm 1.74)^* 10^3$	$(4.62 \pm 1.75)^* 10$

Conclusions and Recommendations

In the upper sediments at ML1 the geochemical data indicate that redox cycles of iron and nitrate are coupled at this location. Redox conditions appear to be highly variable and fluctuate between

oxic and anoxic conditions during the monthly sampling rounds. Oxic conditions are associated with high concentrations of nitrate and low concentrations of dissolved iron. When anoxic conditions are present, however, nitrate concentrations decrease significantly and soluble iron concentrations increase. These observations are consistent with the conceptual model and are most likely a result of microbial activity. Iron reducing bacteria are likely creating abundant supplies of soluble iron under anoxic conditions. The soluble Fe(II) is then available for use by lithotrophic nitrate-reducing bacteria, which use the reduced iron species as an election donor. At depths greater than 10 feet at ML1, conditions were consistently oxic, with high concentrations of nitrate and low concentrations of soluble iron observed throughout the 12 month sampling period. Although the geochemical data do not indicate the coupling of nitrate and iron redox cycles at this depth, MPN counts and 16S rDNA clone library data indicate the presence of at least minor concentrations of bacteria related to the nitrate and iron cycles. This suggests that coupling of the two redox cycles is likely occurring nearby and may occur at this location in the event of a shift to more reducing conditions.

Although nitrate was not detected along the western transect during the sampling period, geochemical and microbial data indicate that iron and nitrate redox cycles are likely coupled in the sediments immediately upgradient of ML4. At ML4, reducing conditions were consistently observed throughout the sampling period and also in the sediment iron extractions. These anoxic conditions were characterized by high concentrations of soluble iron and an absence of nitrate. As stated previously, the impact of agricultural fertilizers along this transect is detected through elevated measurements of sulfate, chloride, calcium, magnesium, and conductivity. Although fertilizers also contain high concentrations of nitrate, it appears that all nitrate is removed prior to the groundwater reaching ML4. Microbial analysis results indicate that heterotrophic nitrate reducers are primarily responsible for nitrate removal as they dominate the MPN counts. However, the abundance of reduced iron at this location and the presence of lithotrophic Fe(II) oxidizing bacteria that use nitrate as an electron donor suggests that the two redox cycles are coupled at this location and are at least partially responsible for the reduction of nitrate upgradient of ML4.

Along the eastern transect, geochemical and microbial data did not indicate where iron and nitrate redox cycles are potentially coupled. However, that does not preclude coupling of the two redox cycles, as water chemistry and sediment iron extractions indicate that redox conditions along the transect transition from oxic conditions with low concentrations of nitrate and iron at ML9 to anoxic conditions with no nitrate and high dissolved iron concentrations at ML8, ML7 and ML5. Nitrate is likely leaching from the vadose zone of the nearby field and is removed from the groundwater prior to reaching ML8. Although attempts to build a 16S rDNA clone library from groundwater samples at ML7 failed due to low microbial concentrations, MPN analysis results at this location do indicate the presence of heterotrophic nitrate reducers as well as iron reducing bacteria. Therefore, the redox cycles of nitrate and iron are likely coupled upgradient of ML8. However, further evaluation of this transect is necessary to fully understand the processes occurring in the subsurface.

Future work at this location that would enhance the knowledge obtained through this project includes a more comprehensive microbial analysis. A comprehensive microbial analysis of all sampling points in the wetland at three different points throughout the year would be a

significant step towards meeting the goal of the project by revealing the spatial and temporal variations in the microbial community throughout the wetland.

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