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Assessing Biomineral Formation by Iron-oxidizing Bacteria in a Circumneutral Creek

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Abstract: Iron oxidizing bacteria and environmental conditions influence the formation of iron biominerals in aquatic environments. This 10-week Research Experience for Undergraduates (REU) study focused on elucidating how water chemistry and iron oxidizing bacteria affect the formation of iron oxides in creeks with circumneutral pH. Two locations, each with multiple microenvironments containing abundant iron oxide deposits, were studied. Water chemistry was assessed via both in situ and laboratory analysis over a 5-week period, revealing correlations between aqueous components that indicate differing groundwater sources may feed nearby discharge points. Microscopy of iron oxide deposits reveals morphologies consistent with the presence of iron oxidizing bacteria. Although efforts to isolate iron oxidizing bacteria did not produce pure cultures, 16S ribosomal DNA analysis also suggests the presence of iron oxidizing bacteria in these sites. Taken together, these results show the diversity of iron oxide forming microenvironments in spatially collocated sites, which may result in unique formations of iron oxide structures, microbial communities, and aqueous chemical cycling.

Keywords: *iron (hydr)oxides, water chemistry, neutrophilic, gradient tubes, iron oxidation, groundwater*

Iron oxidation is an ubiquitous phenomenon in aqueous environments that may control pH (Blowes et al. 2003) and the mobility of nutrients and contaminants (Jambor and Dutrizac 1998). The formation of iron (oxyhydr)oxides (from here on out referred to as "iron oxides") may be driven by the abiotic and biotic oxidation of ferrous (Fe^{2+}) to ferric iron (Fe^{3+}) in the presence of oxygen. These processes commonly occur simultaneously upon mixing of iron-rich, anoxic groundwater with oxygen-rich surface water (Druschel et al. 2008). Iron biomineral formation from microbially mediated processes has been identified in diverse aqueous environments, including acid mine drainage sites (Colmer et al. 1950; Baker et al. 2009; Johnson and Hallberg 2015), hydrothermal vents (Emerson et al. 1999; Peng et al. 2011; Toner et al. 2012), and circumneutral pH surface waters (Emerson and Moyer 1997; Duckworth et al. 2009; Emerson et al. 2010; Fleming et al. 2014). In many circumneutral pH waters, abiotic oxidation is in direct competition with neutrophilic iron oxidizing bacteria (FeOB), organisms that exist at oxic-anoxic interfaces and have metabolic capabilities that promote rapid iron oxidation (Druschel et al. 2008). Multiple neutrophilic, microaerophilic FeOB, such as *Leptothrix*, *Gallionella,* and *Sideroxydans,* have been identified and studied to understand biotic-abiotic interactions in diverse environments (Duckworth et al. 2009; Fleming et al. 2014).

Generally, less is known about the formation and chemistry of these minerals, as well as microbes that oxidize iron at circumneutral pH, than most other aspects of iron biogeochemistry. The importance of FeOB and iron oxides originates from their sorptive capacity and influence on delivered water quality in distribution systems through pipe oxidation (Cullimore and McCann 1978; Søgaard et al. 2001; Fleming et al. 2014; Sowers et al. 2017), importance in microbial

ecology and evolution (Posth et al. 2014), and potential for use in soil remediation (Emerson et al. 2010). These applications, combined with their natural abundance, motivated our interest in understanding the physical, chemical, and biological characteristics of environments capable of supporting biogenic formation of iron oxides. In this study, we analyzed water chemistry and microbial composition of streams in North Carolina over the summer of 2015.

Materials and Methods

Field Site

The first sampling area was located on two small tributaries of Walnut Creek (WC: 35° 45' 45.4" N, 78° 40' 19.7" W) in Raleigh, North Carolina. The sampling sites consisted of a groundwater spring and a midstream site in the first tributary (WC1S and WC1M), and the midstream of the second tributary (WC2). The water was relatively shallow (<10 cm), with the exception of small ponds formed along the streams, and had variable flow (dependent on rainfall) for both tributaries. There was a high abundance of canopy vegetation that limited light penetration. The second location was Rocky Branch Creek (RBC: 35° 46' 48.6" N, 78° 40' 1.9" W) located on North Carolina State University Campus in Raleigh, North Carolina (Andrews and Duckworth 2016; Sowers et al. 2017). There were two site locations along the south side of the creek bank (RBC1 and RBC2), as well as a seep located downstream on the north side of the creek (RBC3) and a midstream location (RBCM). Water depth in RBC1-2 was greater than that observed in WC sites (approximately 50 cm) and had a more continuous flow. RBC3 depth was approximately 5 cm. Iron oxide formation occurred near the bank where the presence of rocks reduced water flow rates. The WC and RBC sites are approximately 1.9 miles apart, and are assumed to be fed by distinct water sources.

*S*ample Collection

Temperature and pH measurements, as well as stream water and iron oxide samples, were obtained weekly on site from June 6, 2015 to July 8, 2015 in Raleigh, North Carolina. Temperature was measured with a portable, analogue thermometer (Fisher) submerged at half depth until temperature equilibrated. The pH was measured on site using pH strips and in the laboratory using a calibrated Accumet excel XL20 pH meter equipped with a combination electrode (Fisher). Temperature and pH measurements were taken in every sampling location. Water samples were collected at half stream depth with a 25 mL polypropylene syringe, and immediately filtered with 0.22 µm filters into 50 mL polypropylene conical centrifuge tubes. Biomass was collected using 25 mL polypropylene syringes. Excess water was decanted to maximize the amount of biomass collected. Samples were stored at -4˚C until further analysis.

Organic and Inorganic Analysis

Samples were thawed and allowed to equilibrate to room temperature before analysis. Samples for dissolved organic carbon (DOC) and metals analysis were acidified with hydrochloric acid and nitric acid, respectively, to a pH ≤ 2. A Shimadzu total organic carbon analyzer was used to measure DOC, and a Thermo Scientific iCE 3000 series flame atomic absorption spectrometer was used to quantify iron (Fe), manganese (Mn), magnesium (Mg), calcium (Ca), and potassium (K). For metal ions, a six-point calibration (0.0–8.0 mg/L in 1% nitric acid (HNO_3)) was performed for each element with a linear fit ($R^2 > 0.995$). Blanks (1% HNO_3) were tested after every 10 samples for quality control. Samples with values over the range of the calibration curve were diluted and re-analyzed.

Statistical Testing

Correlations between measured constituents were tested through regression analysis to determine the relationships between each of the measured constituents, followed by two-tailed t-tests to determine correlation significance (Table 1). Regression analysis was performed in Microsoft Excel.

Growth Media

Gradient tubes, suboxic microcosms that may be utilized to isolate iron oxidizing bacteria, were employed to grow cultures from stream samples (Emerson and Moyer 1997; Emerson and Floyd 2005). The bottom layer of iron sulfide mineral

Table 1. Matrix of correlation (R) between measured constituents.

	Fe	Mn	Ca	Mg	K	Fe	Mn	Ca	Mg	K
			RBCM					**WC1S**		
Fe	1.00	0.18	0.79	0.69	0.56	1.00	0.59	0.61	0.68	0.31
Mn		1.00	0.08	0.05	0.15		1.00	***0.98***	0.42	0.48
Ca			1.00	***0.94***	0.44			1.00	0.44	0.35
Mg				1.00	0.15				1.00	0.34
K					1.00					1.00
			RBC1					**WC1M**		
Fe	1.00	***0.94***	0.66	0.59	0.30	1.00	0.45	0.51	0.44	0.43
Mn		1.00	0.57	0.77	0.24		1.00	***0.97***	0.56	0.42
Ca			1.00	0.06	0.23			1.00	0.68	0.43
Mg				1.00	0.29				1.00	0.57
K					1.00					1.00
			RBC2					**WC2**		
Fe	1.00	0.66	0.68	0.64	0.57	1.00	***0.91***	***0.81***	0.24	0.53
Mn		1.00	0.55	***0.91***	0.39		1.00	***0.96***	0.41	0.68
Ca			1.00	0.25	***0.97***			1.00	0.56	***0.80***
Mg				1.00	0.04				1.00	0.27
K					1.00					1.00
			RBC3							
Fe	1.00	***0.86***	0.75	***0.85***	0.58					
Mn		1.00	0.77	***0.95***	***0.80***					
Ca			1.00	***0.92***	0.27					
Mg				1.00	0.63					
K					1.00					

Correlation significance (p) of correlation coefficient (R) was analyzed using a two-tailed statistical test with Df = N-2, N represents the 5 data points used to calculate R. Values of R>0.8 have significance of p>0.1 (shown in bold italics).

was made using 11.6g of $FeSO_4$, and 9.9g of Na_2S. The cleaned minerals were then mixed with 3.75g of agar mixed in 250 mL of deionized water. The media was composed of 0.75g NH_4Cl, 0.15g $MgSO_4 \cdot 7H_2O$, 0.075g $CaCl_2 \cdot 2H_2O$, 750 mL of deionized water, 0.0375g K_2HPO_4, and 0.315g $NaHCO_3$ (Wolfe's modified mineral media (Wolin et al. 1963)) with 2.25g of 0.3% low melt agarose. All chemicals were obtained from Fisher Scientific and were of analytical grade or higher purity. Media and mineral mixtures were autoclaved then cooled to 60˚C before 1 µL of ATCC vitamin and trace mineral supplement were added to the media.

Bacterial Isolation

Biomass for inoculation was collected on June 12, 2015 throughout each individual site. All tubes and pipette tips were autoclaved, and all aseptic transfers were conducted in a dead air box (Fisher Optimizer PCR workstation). Gradient tubes were made by pipetting 4 mL of the iron sulfide mineral agar solution into 40 mL glass tubes. Following solidification, 12 mL of medium was added to

form a stratified tube. While the top layer was semi-solid, it was purged with N_2 for one minute. Tubes were tightly covered with caps and a rubber septum and stored at 4˚C.

Before inoculation, the gradient tubes were purged with CO_2 at 10 psi for five seconds to obtain neutral pH and provide a carbon source and small amounts of O_2. Using sterile 1 ml syringes equipped with 22-gauge hypodermic needles, 500 µL of biomass (sites with no biomass accumulation were inoculated with collected water samples) sample from each site were inoculated into each tube (first generation). Two negative controls, one without purging and inoculation and one without inoculation, were kept for quality control. Each sample was made in triplicate and incubated at ambient benchtop conditions. Further purification (second generation gradient tubes) was achieved by transferring biomass into new tubes after significant iron oxides (as noted by formation of a distinct red band in the media) were detected in the tubes (timeframe of one to four weeks).

Preliminary identification of iron oxidizing bacteria was conducted on the second generation of gradient tubes. Biomass was harvested from the oxidized band in the gradient tubes. Genomic DNA was extracted using FastDNA® SPIN Kit for Soil (MP Biomedical) following manufacturer instructions. The polymerase chain reaction was utilized to amplify portions of the 16S ribosomal DNA gene (8F and 1492R) primers using a Veriti 96 Well Thermal Cycler (Applied Biosystem). Sequences were examined and trimmed using FinchTV (Geospiza). Unaligned sequences were compared to the National Center for Biotechnology Information (NCBI) database using the BLAST search algorithm to find closely related sequences (Table 2).

Microscopic Analysis

Microscope slides were prepared in an aseptic environment during the transfer of isolated iron oxidizing bacteria from incubated tubes into new gradient tubes. Approximately 5 µL of biomass from incubated tubes was transferred into microcentrifuge tubes and diluted 1:5 with deionized water. Samples were evenly mixed and 5 µL of dilute sample was transferred onto a microscope slide, covered, and dried overnight. Images were taken with an EVOS XL microscope (Life Technologies) at 40× magnification.

Results

Water and Oxide Chemistry

In Situ Data. Through five weeks of field sampling, the pH ranged from 6.7–6.8 and 6.3–6.7 in WC and RBC sites, respectively. The average water temperature was 24˚C in all sites. Regular episodic rainfall events were observed throughout the summer, resulting in loss of iron oxide biomass, but these events were followed by rapid regeneration of deposits. The sites with iron oxides (noted by bright orange deposit, as shown in Figure 1) were sites RBC1-3 and the spring site at WC (WC1S).

Abundance, color, texture, and density of the iron oxides throughout sites RBC1-3 and the seep at site WC1S were visually different. Figure 1 shows the bright orange color of RBC3 (Figure 1A), in contrast to the light brown and dark red appearance of site RBC1 (Figure 1D) and RBC2 (Figure 1B, C), respectively. Additionally, deposits in site RBC3 were visually denser than the other sites. In WC, billowy iron oxides formed around and downstream of the groundwater seep before the intersection with the WC2 tributary. Iron oxides were not observed in the second tributary (site WC2), but samples were collected to assess the water chemistry in comparison to sites at WC1.

Solute Concentrations. Trends in metal and DOC elemental concentrations throughout the sampling period at WC and RBC sites are shown in Figures 2A and 2B, respectively. The DOC in RBC averaged 3.82, 3.62, 2.78, and 3.44 mg/L DOC in RBCM, RBC1, RBC2, and RBC3, respectively. In WC, concentrations of DOC were an average of 4.55, 4.40, and 3.90 mg/L DOC in WC1S, WC1M, and WC2, respectively. These average values were reasonably consistent throughout the five weeks of the study.

The elemental analysis in WC revealed the presence of Fe and Mn at smaller concentrations than Ca, K, and Mg. Fe concentrations ranged from 0–0.25 mg/L, whereas Mn concentrations ranged from 0–0.10 mg/L. In contrast, Ca, Mg, and K concentrations ranged from 2.2–5.6, 1.5–3.2, and 2.4–4.2 mg/L, respectively. The concentrations

Table 2. DNA sequencing data from RBC and WC. The National Center for Biotechnology Information (NCBI) Nucleotide BLAST tool was used to identify the closest isolate. Isolates in bold are known or putative iron oxidizing bacteria.

Location	Identified Isolates	Fragment Size (bp)	% Identity	Accession
RBC1	*Propionivibrio dicarboxylicus* strain*	635	94%	NR_026477.1
	Dechlorosoma* sp. AH	870	96%	AY171616.1
	Rhodocyclus sp. HOD 5**	869	96%	AY691423.1
RBC2	***Sideroxydans lithotrophicus* ES-1***	636	89%	CP001965.1
	Propionivibrio sp. Smarlab*	986	94%	AY643079.1
	Propionivibrio dicarboxylicus strain**	793	92%	NR_026477.1
	Rhodocyclus sp. HOD 5**	1017	95%	AY643079.1
RBC3	Beta proteobacterium**	826	96%	AB376632.1
WC1S	*Herbaspirillum huttiense* strain*	673	79%	KF515669.1
	Sideroxydans lithotrophicus* strain ES-1*	717	95%	NR_074731.1
WC1M	*Acholeplasma oculi* strain*	669	90%	NR_025960.1
	Sideroxydans lithotrophicus* strain ES-1*	1221	84%	NR_074731.1
WC2	*Propionivibrio dicarboxylicus* strain*	787	92%	NR_026477.1
	Candidatus Accumulibacter phosphatis clade**	834	96%	CP001715.1
	Herbaspirillum sp. NR 3-04*	472	91%	KM253112.1
	Thiobacter subterraneus strain C55**	588	93%	NR_024834.1

*Sequence from forward primer (8F). **Sequence from reverse primer (1492R), sequences with similar identified isolates are only presented once.

of Ca, Mn, and Fe tended to increase during the sampling period, whereas Mg and K did not clearly trend with time.

Similarly, lower Fe and Mn concentrations in comparison to other elements were also observed in RBC, where Fe, Mn, Ca, Mg, and K were 0–2.38, 0.07–1.55, 6.3–33.4, 3.3–11.7, and 4.0–11.8 mg/L, respectively. Concentrations of Fe and Mn were more variable at sites containing oxide deposits than at the midstream sampling location. Also, it is notable that the concentrations of all elements in WC are lower than that of the corresponding concentration at RBC.

Bacterial Assessment

Gradient Tube Isolation. Tubes inoculated with samples from WC and RBC sites yielded discrete red bands, indicative of the presence of iron oxidizing bacteria (Figure 3). Interestingly, gradient tubes inoculated from sites with no visible iron oxides (WC2 and RBCM) also yielded iron oxidizing bacteria in gradient tubes. Bacterial growth (as judged by the formation of discrete red bands) in the first generation of gradient tubes was relatively slow in comparison to the second generation. Therefore, transfer of oxidizing organisms occurred after four weeks of growth for the first generation and two weeks for the second generation. Furthermore, the first generation of gradient tubes had a less defined growth zone in comparison to the second generation, which had a defined band of oxidation at a specific media depth (cf. Figure 3A and 3B).

A preliminary DNA assessment of the iron



Figure 1. Iron oxides at the Rocky Branch Creek (RBC) sample area. (A) Bright orange and thick iron oxides at site RBC3 (B) Location of sites RBC1 and RBC2 in the bank and adjacent to each other (C) Iron sheen and dark red iron oxides at site RBC2 (D) Billowy light brown iron oxides in site RBC1.

oxidizing bacteria isolation cultures was conducted on the second generation of gradient tubes using 16S rDNA. Sequencing results produced chromatograms (not shown) with overlapping peaks after 472-1221 base pairs (bp), suggesting a mixed culture is present in the tubes.

Microscale Morphological Assessment. Iron oxide structures observed under a microscope showed string-like structures seen throughout all samples, consistent with previously observed morphologies of bacteriogenic iron oxides. Isolated filamentous (sheath-like) and small globular structures, previously noted in optical micrographs of iron oxide biofilms (Duckworth et al. 2009), were also observed in the samples (Figure 4). String-like structures decreased in frequency from the first generation of gradient tubes to the second generation (images not shown).

Discussion

Aqueous Constituents Correlations

A detailed heat map representing the regression coefficients of correlations (R) is shown in Table 1. Significant correlations ($p < 0.1$; Table 1 values in bold italics) were found between Mn-Ca at all WC sites, and between Fe-Mn, Fe-Ca, and Ca-K at WC2. At RBC, significant correlations were established at RBCM between Ca-Mg, at RBC1 between Fe-Mn, at RBC2 between Mn-Mg and Ca-K, and at RBC3 between Fe-Mn, Fe-Mg, Mn-Mg, Ca-Mg, and Mn-K.

In RBC3, more frequent significant correlations between constituents were found compared to the other sites. Based on the unique location on the north side of the creek (as compared to the other RBC sites), it is likely that the groundwater discharge at this site is fed by a different water

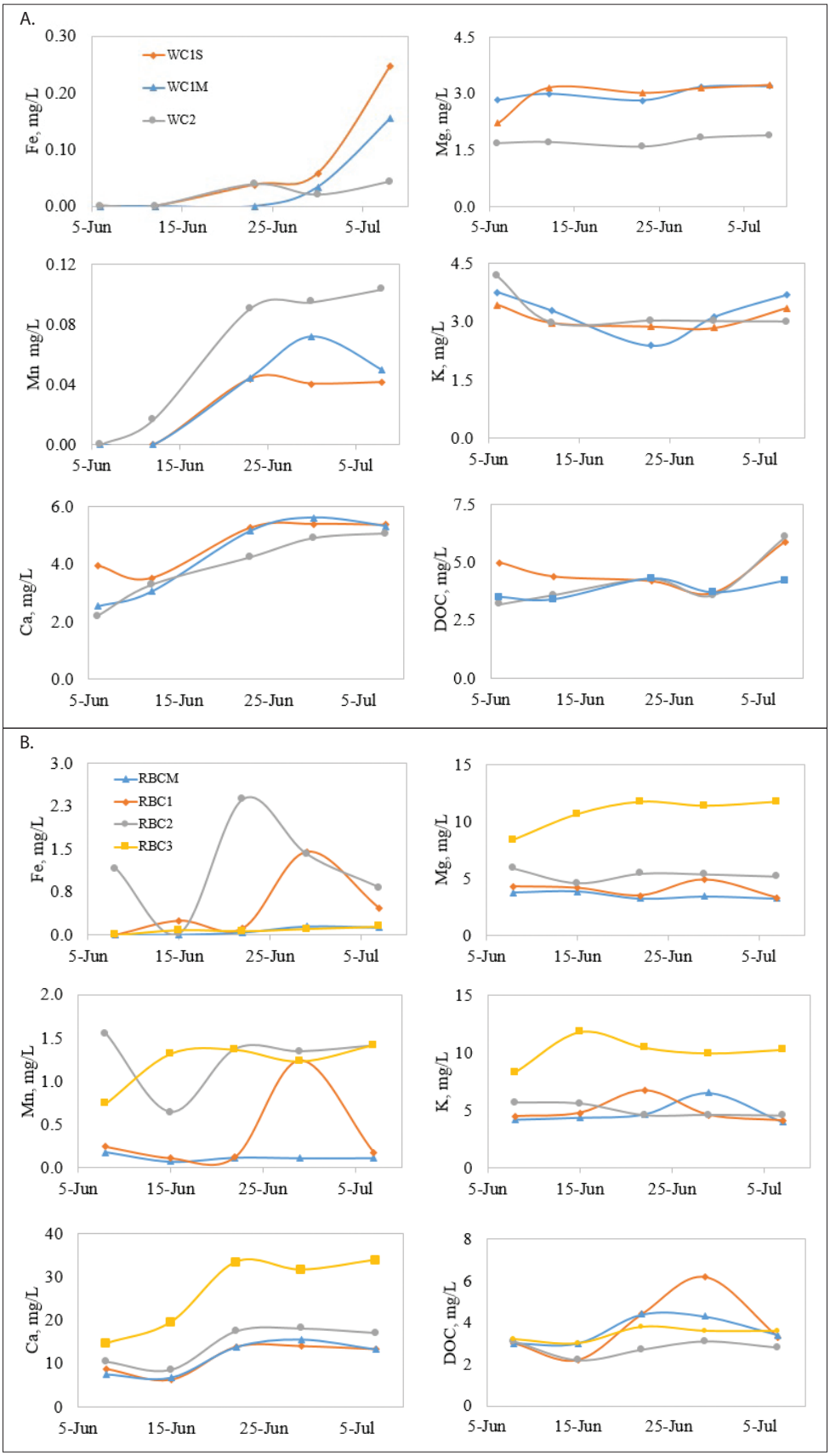

Figure 2. Temporal changes in element concentrations throughout the summer in (A) WC and (B) RBC.

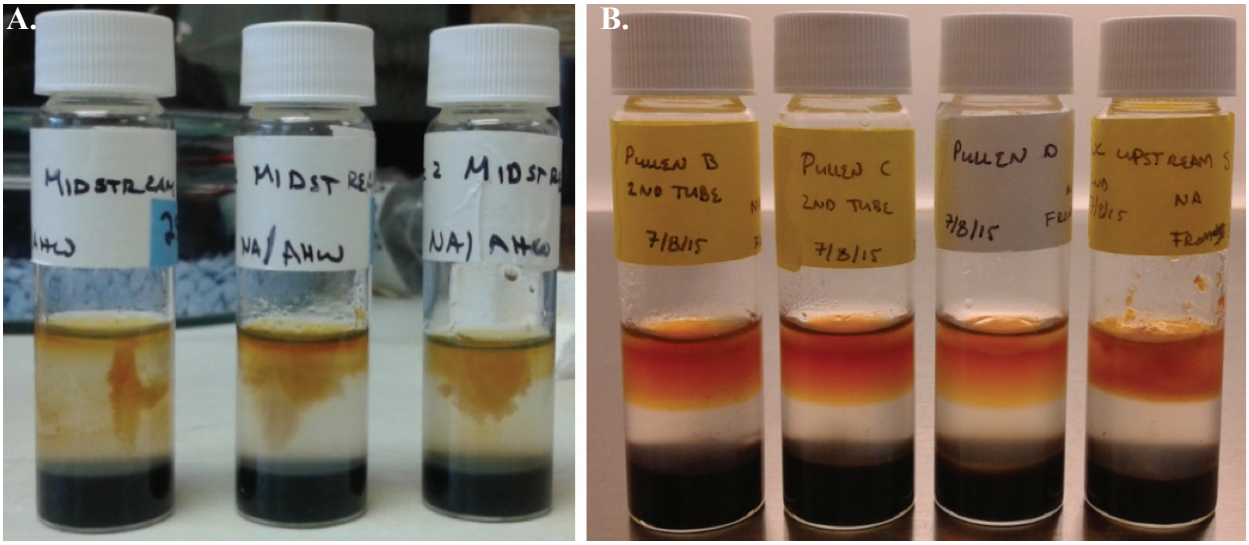

Figure 3. Gradient tubes depicting the distinct growth pattern of the (A) first generation and (B) second generation gradient tubes.

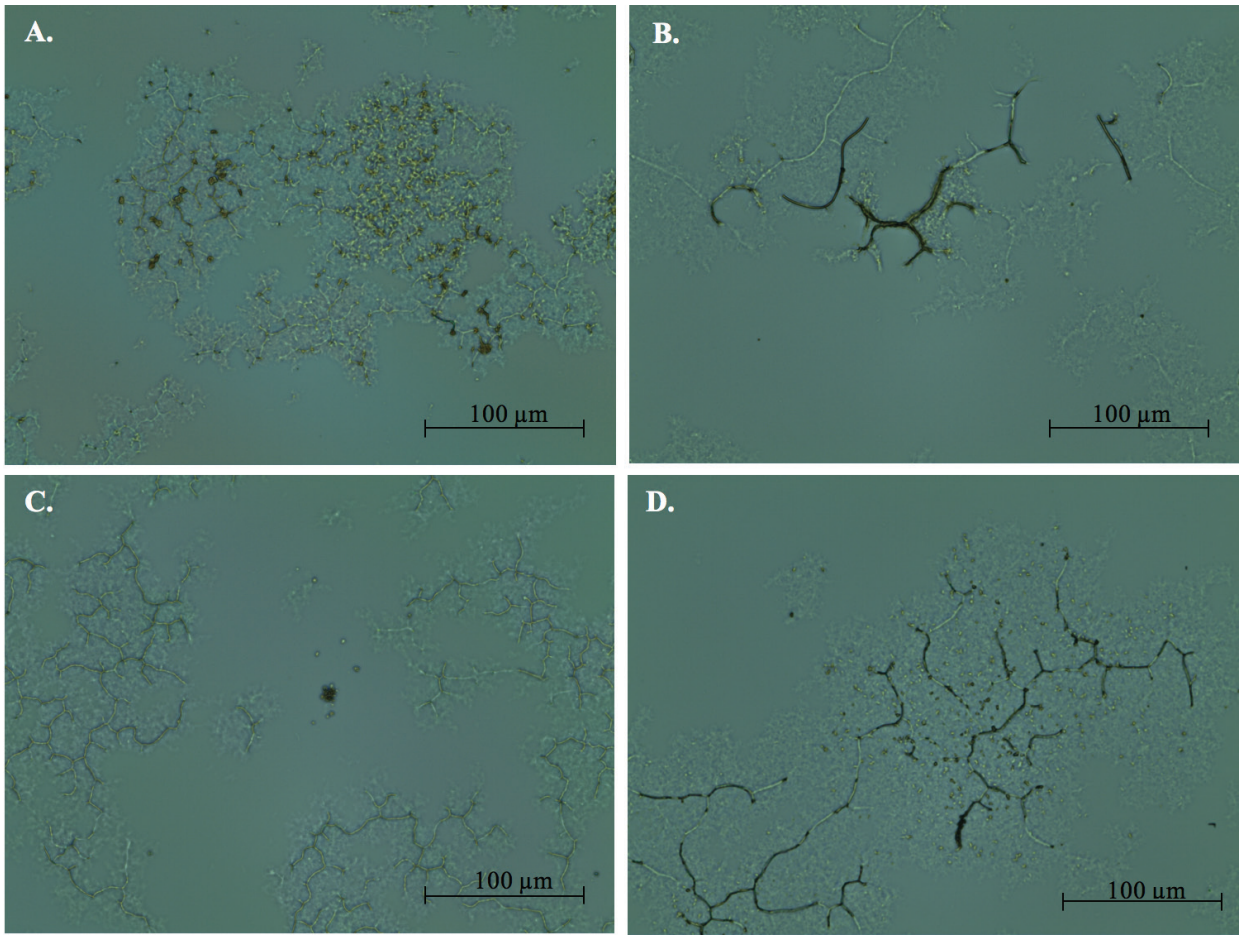

Figure 4. Morphological characteristics of each site's first generation of gradient tubes. (A) WC1M, (B) WC1S, (C) RBC1, and (D) RBC3.

source than RBC1 and RBC2. In addition, these correlations can provide information on coupled chemical processes associated with specific constituents that have similar concentration patterns and significant correlations. Significant correlations between elements that are collocated in similar crystallographic sites in minerals (such as Ca and Mg in clays and carbonates) can be indicative of rock-weathering interactions that contribute to the water's composition (Duckworth et al. 2009), as well as chemical interactions and mineral cycling. We also note that the RBC site is bordered to the north by athletic fields that receive inputs of fertilizer and other chemicals, as well as a greenway and a road to the south. Indeed, Figure 2 shows the relatively higher concentrations of most constituents in RBC compared to WC, with Ca concentrations being almost twice as large, especially in RBC3.

Concentrations of Fe and Mn in all sites were smaller and more variable relative to the other constituents. These observed concentrations are possibly an effect of an assortment of reactions and interactions between the dissolved metals and microorganisms. Precipitation of oxides (governed by low solubility) can result in low concentrations of iron; however, we note that iron concentrations can be highly influenced by the presence of colloidal or chelated iron (Duckworth et al. 2009), and thus be present at concentrations above predicted solubility.

In WC, concentrations of Mg, K, and DOC were consistent throughout the summer. This pattern may result from a higher contribution from groundwater sources and not surface streams. The strong chemical similarities between WC1S and upstream WC1M (Figure 2) was not expected because the spring downstream had water mixing from surface and groundwater sources (where oxides form) that we did not observe upstream.

Establishing the groundwater and surface water contributions to stream water are important in understanding the limiting and driving factors of physical and biological iron oxidation which can be influenced by water chemistry. Based on the formation of iron oxides in gradient tubes (Figure 3) from sites that did not have any on the field (Table 2), it seems likely that the metabolic capability to produce biogenic oxide is widespread in these streams, and thus multiple limiting factors and constantly changing environments play a major role in the formation of iron oxides where FeOB are present.

Microbial Communities

DNA sequences varied in read length with multiple signals interfering in the chromatograms. The poor quality was an indication of the presence of multiple organisms in the gradient tubes. As a result, DNA sequences were not assembled nor aligned and each individual sequence from the 8F and 1492R primers were only BLAST scanned for identification of the most similar isolate (not clone) sequence.

Table 2 shows the closest isolate match to DNA fragments taken from second generation gradient tubes. It should be noted that BLAST results do not necessarily indicate the presence of a bacterium closely related to the organism (due to mixture of DNA sequences in samples), but closest matches do provide some evidence for the presence of related organisms. This mixed nature of DNA is emphasized by the lack of match on forward and reverse sequences of the sample.

Although microbes associated with fermentative metabolisms were frequent (e.g., *Herbaspirillium* and *Propionvibrio* sp.), three tubes contain sequences that are similar to those of the iron oxidizing organisms *Sideroxydans lithotrophicus* and *Dechlorosoma* sp. (Lack et al. 2002; Emerson and Floyd 2005). Interestingly, one tube contained a sequence similar to that of *Thiobacter subterranus*, a bacterium associated with redox cycling of sulfur (Hirayama et al. 2005).

The morphological signatures seen under the microscope can provide additional information on the bacterial communities responsible for the formation of filamentous and globular structures observed under the microscope (Figure 4). The predominant iron oxidizing bacteria identified in environments similar to those described in this study (circumneutral, microaerophilic environment) are *Betaprotobacteria* (Emerson et al. 2010; Maamar et al. 2015) belonging to the *Gallionella*, *Leptothrix,* and *Sideroxydans* genera (Cullimore and McCann 1978; Emerson et al. 2010). The sheath-like filamentous structures (Figure 4) observed under the microscope are

characteristic of *Leptothrix* (Emerson et al. 2010; Fleming et al. 2011; Fleming et al. 2014). These bacteria are neutrophilic, microaerophilic, and grow at ideal temperatures ranging from 20–25° (Cullimore and McCann 1978; Emerson et al. 2010). A study by Fleming et al. (2014) observed the formation of sheath-like structures associated with *Leptothrix ochracea* during the summer and the succession of *Gallionella* in the spring. This study further supports the possible presence and contribution of *Leptothrix* to the environments of our study, which was conducted in warm (24°C daytime temperature) waters during the summer (it should be noted that *Leptothrix ochracea* is currently uncultured, thus not in 16S databases (Fleming et al. 2011)). In addition, the globular structures observed may be a mix of cell matter and precipitates that adhere to iron oxides, or encrusted iron oxides similar to those in the *Sideroxydans* genera (Duckworth et al. 2009).

The observed formation of biogenic iron oxides in gradient tubes inoculated from sites without visible oxide deposits indicates that other factors may limit the accumulation of iron oxides environments. Complex microbial communities can reduce the dominance of one specific organism and reduce microbial mediated biomineral formation. The slow growth of organisms with iron oxide bands in the first generation of gradient tubes compared to the second generation may be an indication of the diversity of microbial communities in that niche that compete for resources and limit the formation of iron oxides.

Conclusion

Understanding the chemistry of diverse environments where iron oxidizing bacteria can harness energy through the precipitation of biominerals (i.e., iron oxides) is key to understanding iron biogeochemical cycling. Through an overall analysis of the water chemistry conducted in this study the following observations were made: 1) the concentrations of measured constituents were significantly higher in RBC compared to WC, yet some microenvironments in both sites sustained constant iron oxide formation; 2) concentrations of Fe and Mn were relatively lower and more variable than other measured constituents; and 3) presence of iron oxidizing bacteria was established through preliminary DNA analysis and microscopic examination of the biofilm collected from these sites, including those with no visible oxides.

Despite the close proximity of some of these sites, iron oxides only form in certain locations due to unknown limiting factors. As noted previously, the microbial community and aqueous chemistry of microsites may affect the formation and accumulation of oxide biofilms. Indeed, the chemistries of sites near each other were observed to be variable, as was our ability to identify iron oxidizing organisms. However, the physical environment at these locations (e.g., hydraulic conditions) may not be favorable for appreciable accumulation of iron oxide bearing biofilms. Oxygen concentrations in these sites are influenced by water depth, gas boundary diffusion, and fluid mixing processes. The shallow and variable stream flow in these sites may result in large fluctuations of oxygen concentration areas, potentially making areas transiently or permanently unsuitable for FeOB that require selective oxygen gradients for metabolic activities. The research presented herein was part of a 10-week research experience for undergraduates which limited the scope of the research to this timeframe. Future work will include further purification of gradient tubes to conduct DNA assessment of the FeOB isolates, and attempts to better constrain groundwater composition.

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