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Conveyance Structures

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Abstract: Regenerative streamwater conveyances (RSCs) are a relatively new stream restoration best management practice (BMP) being extensively implemented in degraded perennial streams and stormwater drainages throughout the mid-Atlantic. Although there is currently a great deal of interest in this type of BMP as a means of reducing nutrient and sediment export from disturbed catchments, little is known of its pollutant reduction capabilities and whether there are unintended ecological consequences associated with these structures. For example, dense accumulations of flocculate from iron-oxidizing bacteria (FeOB) have been observed at numerous RSC sites, yet it is unknown whether this flocculate is predominately natural, a consequence of leached iron (Fe) from the materials used in the RSC construction, or because of mobilized Fe from catchment soils influenced by higher groundwater levels that may occur after construction. We analyzed Fe and other solute concentrations in groundwater and perennial streams at RSC and control sites located in the Coastal Plain and Piedmont physiographic provinces of MD and DC, and conducted leaching experiments using RSC soils and construction materials. Iron flocculate from FeOB in RSCs is commonly localized and depends on several factors including a source of reduced Fe, the availability of dissolved organic carbon (DOC) responsible for leaching this Fe, and specific hydrological (i.e., low flow) and physical (i.e., warmer temperatures) conditions. Sources of reduced Fe are derived from both construction materials and catchment soils, which leach Fe in the presence of DOC originating from natural organic matter (OM) deposits and OM incorporated into the RSCs (i.e., wood chips, leaves, logs). Using construction materials that have relatively low % Fe content in areas strongly influenced by OM will likely decrease the spatial and temporal presence of dense mats of Fe flocculate thereby improving RSC habitat, performance and sustainability.

Sources of Iron (Fe) and Factors Regulating the Development of Flocculate from Fe-Oxidizing Bacteria in Regenerative Streamwater Conveyance Structures

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ABSTRACT (291 words)

Regenerative streamwater conveyances (RSCs) are a relatively new stream restoration best management practice (BMP) being extensively implemented in degraded perennial streams and stormwater drainages throughout the mid-Atlantic. Although there is currently a great deal of interest in this type of BMP as a means of reducing nutrient and sediment export from disturbed catchments, little is known of its pollutant reduction capabilities and whether there are unintended ecological consequences associated with these structures. For example, dense accumulations of flocculate from iron-oxidizing bacteria (FeOB) have been observed at numerous RSC sites, yet it is unknown whether this flocculate is predominately natural, a consequence of leached iron (Fe) from the materials used in the RSC construction, or because of mobilized Fe from catchment soils influenced by higher groundwater levels that may occur after construction. We analyzed Fe and other solute concentrations in groundwater and perennial streams at RSC and control sites located in the Coastal Plain and Piedmont physiographic provinces of MD and DC, and conducted leaching experiments using RSC soils and construction materials. Iron flocculate from FeOB in RSCs is commonly localized and depends on several factors including a source of reduced Fe, the availability of dissolved organic carbon (DOC) responsible for leaching this Fe, and specific hydrological (i.e., low flow) and physical (i.e., warmer temperatures) conditions. Sources of reduced Fe are derived from both construction materials and catchment soils, which leach Fe in the presence of DOC originating from natural organic matter (OM) deposits and OM incorporated into the RSCs (i.e., wood chips, leaves, logs). Using construction materials that have relatively low % Fe content in areas strongly influenced by OM will likely decrease the spatial and temporal presence of dense mats of Fe flocculate thereby improving RSC habitat, performance and sustainability.

KEYWORDS

Best Management Practice (BMP), Coastal Plain, Flocculate, Iron-Oxidizing Bacteria (FeOB), Piedmont, Regenerative Streamwater Conveyance (RSC), Stream Restoration

INTRODUCTION

Natural disturbances and landscape development in watersheds commonly degrade streams and the ecosystem services they provide (Doyle et al. 2000, Sweeney et al. 2004, Grimm et al. 2005, Meyer et al. 2005, Poff and Zimmerman 2010, Vietz et al. 2012). The specific mechanisms that lead to the degradation of streams and the loss of ecosystem services vary according to landscape settings and types of stressors (Walsh et al. 2005, Wenger et al. 2009), and the extent of the degradation is often reflected in streams because water quality integrates various biogeochemical processes affected by natural and human disturbances (Likens et al. 1970, Likens and Bormann 1995, Williams and Melack 1997a & b, Williams et al. 2005).

Stream restoration and stormwater best management practices (BMPs) are increasingly implemented with the expectation that they will restore hydrochemical regimes and improve water quality, habitat, and biodiversity (FISRWG 1998). Because only modest improvements with regard to water quality and sediment transport commonly occur with these types of implementations (Selvakumar et al. 2010, Filoso and Palmer 2011, Palmer et al. 2014), environmental engineers have attempted to improve nutrient and sediment reduction efficiencies (i.e., their ability to reduce these constituents in stormwater runoff) by creating novel designs, such as stream-wetland complexes (Filoso et al. 2015) and regenerative streamwater (or stormwater) conveyances (RSCs). In particular, RSCs implemented in headwater channels are designed to reduce erosion (Brown et al. 2010), increase stormwater retention and the capacity to

48 trap and retain suspended materials, and increase rates of biogeochemical processes such as
49 denitrification (Brown 2000).

50 Despite RSCs having been implemented throughout Maryland and Washington DC for over a
51 decade, there is a paucity of information available concerning their nutrient and sediment
52 reduction capabilities and sustainability (Palmer et al. 2014). It has been argued that RSCs
53 improve water quality (Berg and Underwood 2007; Bowen 2012; Brown et al. 2010), but peer-
54 reviewed studies addressing their performance are lacking. Moreover, there may be deleterious
55 ecological effects associated with RSCs implemented as either reconfigured streams or
56 stormwater BMPs. For example, it is unknown whether the occurrence of large quantities of iron
57 (Fe) flocculate sometimes observed within these structures is natural or an artifact of the
58 structure itself, and whether this flocculate is negatively impacting water quality or stream
59 habitat. Although Fe flocculate in streams is commonly associated with acid mine drainage that
60 has high Fe concentrations and low pH, flocculate is also found in streams with relatively low Fe
61 concentrations (<1 mg/L) and circumneutral pH (Hayer et al. 2013, Fleming et al. 2014).

62 Possible deleterious ecological impacts of dense Fe flocculate include a reduction of benthic
63 substrate that can be used by aquatic macroinvertebrates (Wellnitz et al. 1994), decreases in
64 water exchange due to plugging of sediment pores (Ghiorse 1986), changes in the redox potential
65 of stream sediments, and others (Rasmussen and Lindegaard 1988, Gerhardt 1992).

66 Fe flocculation is driven by Fe oxidation and reduction, and is often mediated by several
67 types of bacteria (Emerson et al. 2010, Melton et al. 2014). Anoxic conditions with ample
68 mineral Fe and labile DOC create an environment where Fe-respiring microbes (FeRM) utilize
69 alternative electron acceptors thereby causing the reduction and dissolution of Fe (i.e., Fe(III) to
70 Fe(II)); manganese (Mn) is affected similarly (i.e., Mn(IV) to Mn(II); Lovely 1986). Some

fraction of these reduced metals will commonly persist in solution as cations (Brannon et al. 1984). The role of DOC in this process is poorly understood, but certain bacteria use humics and quinones as electron acceptors and these chemicals, once reduced, abiotically reduce Fe (Lovley 2000). This process may allow FeRM to access oxidizing agents that would otherwise be occluded, and some DOC in solution may be able to chelate Fe(III) thereby increasing its availability to FeRM (Lovley 2000).

Groundwater can become enriched with reduced Fe through a combination of these processes. In areas where such Fe-rich groundwater percolates to the surface, some is oxidized and abiotically precipitates as Fe hydroxides at circumneutral pH, or is oxidized by Fe-oxidizing bacteria (FeOB) as part of their metabolism (Emerson and Revsbech 1994a, Johnson et al. 2014). Where flocculate develops around these groundwater seeps, as much as 80% of the Fe oxidation can be microbially mediated (Emerson 2000, Emerson and Revsbech 1994b).

Fe flocculation is a natural process that can be seen in many streams throughout the Mid-Atlantic region (Robbins 1998, Robbins and Norden 1994) and elsewhere (Emerson 1994a & b, Schieber and Glamoclija 2007). Maryland is underlain by widespread Fe deposits (Singewald 1911) that influence groundwater chemistry (Robbins and Norden 1994). Nevertheless, some construction materials used in RSCs, such as ironstone (i.e., Bog Iron), have high Fe content, and organic matter (OM) incorporated into RSC structures (commonly about <20% by volume, Bowen 2012) can be a significant source of dissolved organic carbon (DOC). Degradable OM is broken down aerobically and anaerobically to produce low molecular weight sugars and organic acids such as acetate that are common substrates for Fe-reduction (Lovley 2000). Therefore, while Fe flocculate observed at RSC sites could be a natural occurrence at some streams, it is possible that mat formation is enhanced by 1) elevated Fe concentrations in stream water

influenced by construction materials, 2) the mobilization of Fe from catchment soils outside of RSC construction areas that become inundated by elevated groundwater tables or 3) a combination of the two.

Herein we determine the factors responsible for regulating the development of Fe flocculate at RSC sites in streams of the Coastal Plain and Piedmont physiographic provinces of MD and DC. We identify the sources of Fe potentially contributing to the development of Fe flocculate by conducting total Fe analyses and leaching experiments on soils extracted from our study sites and construction materials used in RSCs. Moreover, we evaluate differences in the hydrochemistry of riparian and hyporheic groundwater, and stream water among RSC and degraded control sites. The OM content and physical habitat metrics of the study sites are also compared. Pre- and post-RSC construction Fe and DOC concentrations in groundwater wells and stream water (baseflow and stormflow) at one intensively monitored site are examined. These data were used to determine whether RSCs commonly have more persistent and dense mats of flocculate from FeOB compared to control sites and, if so, whether Fe leached from construction materials or mobilized from elevated groundwater tables in catchment soils are factors contributing to flocculate development. The ecological implications of dense flocculate in RSCs and potential remedial measures are discussed.

STUDY SITES

The RSC and control sites (11 total) monitored in this study are located in Washington DC, and Montgomery and Anne Arundel Counties of MD (Figure 1). All of the Anne Arundel sites and 2 DC sites are located in the Coastal Plain, whereas the other DC sites and the Montgomery County site are in the Piedmont. Site names and abbreviations are: Spring Valley (SV – control), Linnean Ave. (LA – Figure 2), Milkhouse Run (MR), Bingham Ford (BF), and Garrett Park (GP) in the Piedmont (www.montgomeryparks.org/pdd/documents/Waverly-Schuykill%20award%20012610%20final.pdf), and Saltworks Creek Sand Seepage (SS), Saltworks Creek End-of-Pipe (EP), and Aurora Ave. (AR) in the Coastal Plain. Control sites in the Coastal Plain are located at 33rd St. SE (33), Texas Ave. (TA), and a creek at the confluence of the AR site (Aurora Control - AC).

Figure 1: Map of study sites located in the Coastal Plain and Piedmont physiographic provinces of MD and DC.

Other than the GP site that used granite, all other RSC sites were constructed using various types of ironstone substrates (i.e., small and large conglomerate and sandstone boulders and loose sand) to create weirs at the downstream end of each ponding basin (Table 1).

Figure 2: Land use and catchment delineation for Linnean Ave. (LA); well transects are shown as perpendicular lines to the stream reach.

Table 1: Site characteristics obtained from the Natural Resources Conservation Service (NRCS). A more comprehensive list of soils and other attributes of the study sites can be obtained by entering the latitude and longitude in the NRCS soil survey link (<http://websoilsurvey.nrcs.usda.gov/app/WebSoilSurvey.aspx>). The incidence of flocculate is based on a subjective assessment of its spatial and temporal extent observed during site visits.

Site	Latitude, Longitude	Map Unit Name	Slopes (%)	Major: Minor Map Symbols	Boulder Type	Incidence of Flocculate
<i>Coastal Plain Physiographic Province</i>						
Saltworks Sand Seepage (SS)	38.99655°, -76.54810°	Widewater and Issue soils, often flooded	0 to 2	WBA: CkA, AsC, AsE, AsF	ironstone	Severe
Saltworks End-of-Pipe (EP)	38.99200°, -76.54982°	Udorthents, loamy, sulfidic substratum	0 to 5	CkA: AsE	ironstone	Moderate
Aurora Ave. (AR)	39.08997°, -76.64683°	Sassafras and Croom soils	15 to 25	SME: DvD	ironstone	Severe
Aurora control (AC)	39.09033°, -76.64607°	Zekiah and Issue soils, often flooded	0 to 2	ZBA		None
33rd St. SE control (33)	38.86734°, -76.95722°	Muirkirk variant complex	15 to 40	MvD: U8		Minor
Texas Ave. control (TA)	38.86731°, -76.96646°	Iuka sandy loam	15 to 40	Ik: MvD		Moderate
<i>Piedmont Physiographic Province</i>						
Garret Park (GP)	39.03929°, -77.09270°	Glenelg silt loam	8 to 15	2C: 2UC, 16D	granite	Very Minor
Bingham Ford (BF)	38.96789°, -77.05277°	Manor loam	8 to 15	MbC: MbD	ironstone	Minor
Milkhouse Run (MR)	38.96509°, -77.05273°	Glenelg variant silt loam	0 to 8	GIB: MbC	ironstone	Severe
Linnean Ave. (LA)	38.95582°, -77.06514°	Udorthents, loamy	0 to 8	U4: Ck, NeD	ironstone	Minor
Spring Valley control (SV)	38.93757°, -77.09796°	Manor-Urban land complex	15 to 40	MdD: GhC, GhD, MdB		Very Minor
Breewood Rd. (prominent seep)	39.03504°, -77.02796°	Glenelg silt loam	3 to 8	2B	granite	Moderate

145

146 *RSC construction*

147 RSCs are commonly implemented in incised headwater channels or stormwater drainage

148 ditches using sand, wood-chips and riparian soils as bottom substrates (Brown et al. 2010).

149 Subsequently, boulder weirs oftentimes made from ironstone and silica-cobble riffles are

150 installed to create a series of step pools (Figure 3). Alternatively, granite boulders are used (i.e.,

151 GP site). Incised stream reaches or drainage ditches are thus transformed into a structure that

152 enhances water retention and percolation to the groundwater table, thereby decreasing the

153 magnitude of peak stormwater runoff to receiving waters during precipitation events. Large

Figure 3: Schematic of RSC showing weirs constructed of silica cobble and ironstone boulders that separate step pools designed to decrease the magnitude of peak flow and partially retain stormwater runoff.

woody debris such as tree trunks, branches, and root wads are often placed within the channel to serve as habitat and as an additional organic carbon substrate. Generally, few trees are felled during construction and the area disturbed by construction is not much wider than the incised stream channel or drainage ditch.

The rationale for the use of ironstone in RSCs is that it may promote the water quality conditions found in wetland environments (e.g., lower pH) and help to establish native plant communities while limiting invasive species. Ironstone deposits are natural in the Coastal Plain and the stone occurs in a variety of forms including relatively dense and more loosely-consolidated sandstone and conglomerate. All are relatively porous and at several sites the surfaces of the boulders support mosses and small vascular plants without an overlying soil layer. Ironstone is typically more expensive than alternative substrates such as granite, but some stream restoration practitioners indicate that ironstone is easier to work with because it often occurs in the form of large blocks that can be stacked on one another. By contrast, the use of OM in the fill material is meant to quickly re-establish detritus in streams that facilitate the re-colonization of macroinvertebrates and the biogeochemical processes that are present in healthy stream environments (i.e., denitrification, etc.). As mentioned previously, the amount of OM integrated into the fill material is <20% by volume (for example, MR and LA were 6% and 17%, respectively; note that the same % of ironstone substrates were used). It is estimated that the OM in the form of felled tree trunks, branches and root masses incorporated into the RSC ponding basins can more than double this percentage, albeit much of this material likely decomposes slowly. During the fall, leaves in residential communities transported in runoff or from overlying forest canopy are commonly retained in RSC ponding basins and behind debris dams for extended periods thereby contributing to elevated DOC levels in stream water. Leaves from

the surrounding residential community were also incorporated into the RSC ponding basins to provide additional carbon at the LA site.

MATERIAL AND METHODS

Analytical Methods

Stream and shallow groundwater concentrations of Fe, Mn, nitrate (NO_3) and ammonium (NH_4), phosphate (PO_4), total dissolved P (TDP), DOC, chloride (Cl), and sulfate (SO_4) were analyzed in this study. Most of our chemical analyses were done at the Nutrient Analytical Services Laboratory (NASL) at the Chesapeake Biological Laboratory (CBL). Methods and the detection limits for each analytical procedure used in this study can be found on the NASL webpage (<http://nasl.cbl.umces.edu>). Chemical constituents were analyzed using the following equipment: Fe and Mn - Ion Coupled Plasma-Optical Emission Spectrometer (ICP-OES), DOC - Shimadzu 5000A, all P fractions and NH_4 - Aquakem 250. Nitrate, Cl, and SO_4 were analyzed using a Dionex ion chromatograph (IC 1000) in a separate lab at CBL. Subsamples of Fe flocculate and the materials used in RSC construction (pre-slurry) were sent to A&L Eastern Laboratories (al-labs-eastern.com) to determine the total % of Fe. The total % of the metals cadmium, copper, Fe, lead and zinc were also determined on samples of dried flocculate at A&L Eastern Laboratories. Other analyses done were particulate P (PP) on the dried flocculate and total dissolved nitrogen (TDN) for interstitial water, the latter using an Exeter Analytical, Inc. Model CE-440 Elemental Analyzer; particulate N and C were measured with a Perkin Elmer 2400 CHN elemental analyzer.

Precipitation, Stream and Groundwater Monitoring

Pre- and post-construction stream and groundwater were monitored at the LA site to determine both changes in Fe and DOC concentrations, and possible groundwater recharge and associated increases in baseflow expected in the post-construction period. The pre-construction sampling period was from January 2013 to May 2014, and the post-construction sampling period

used in this study is from August 2014 through July 2015. Precipitation volume was collected using a tipping bucket rain gauge (Hobo RG3-M). Baseflow was collected approximately monthly, whereas stormflow was collected using Isco automated water samplers (Isco model 6712) approximately twice per season. Stormflow samples were generally collected every 20 minutes (3 aliquots per 1 L bottle) and volume-integrated using discharge measurements obtained from a 0.76 m H flume where stage was measured using a pressure transducer (Onset HOBO water level logger; model U20-001-04-Ti) recording at 5-min intervals. In the post-construction period, discharge was estimated using a rating curve for a culvert with a 1.04 m inner diameter.

Groundwater wells (n=12) at the LA site were positioned at four separate transects of 2 to 4 wells each set perpendicular to the stream at approximately 0.6 to 1.5 m intervals from the stream bank. Each well was made of schedule 40 PVC with a 6.4 cm inner diameter and had a 0.254 mm slotted well screen at its base. Wells were generally about 2.4 to 3.6 m deep in order to continuously record groundwater fluctuations even in the late summer/ early fall when groundwater table levels were lowest. Well water was sampled using a PVC bailer, and each well was purged prior to sampling. Groundwater level fluctuations in each well were recorded using Hobo pressure transducers. A separate pressure transducer was located in the Isco fiberglass housing to record barometric data at the same time interval, and these data were subtracted from the stream stage and well data.

All other sites had one riparian and one in-stream (or in-pool) hyporheic monitoring well installed to depths ranging from about 1 to 2 m. Water samples were collected 7 times from November 2013 to September 2014, and the two wells and stream water at each site were sampled 3 times per site per visit. The sites were sampled more intensively during June through

mid-July 2014 (2-4 times each). Samples were collected during baseflow, no sooner than 24 hours after a precipitation event.

Water samples were kept on ice and filtered within 24 hours using polycarbonate filters (0.4 μm nominal pore size) into one 60 mL and two 30 mL subsamples. No DOC contamination was apparent in filter blanks. The 30 mL subsamples were prepared and analyzed for Fe, Mn, and DOC, whereas the 60 mL subsamples were frozen and later analyzed for PO_4 and NH_4 . The remainder of the sample was analyzed for NO_3 , Cl, and SO_4 . Rapid biological assessment metrics were recorded once in July 2014, including trash rating, instream habitat, epifaunal substrate, velocity/depth diversity, pool/glide/eddy quality, riffle/run quality, % embeddedness (i.e., how much the streambed is enveloped by flocculate), and % shading (Stranko et al. 2014).

One longitudinal transect was conducted at the AR site. Water samples were collected from flowing water at the riffles between the RSC pools. All samples were analyzed for nutrients, Cl, SO_4 , DOC, and metals to determine if gradients exist within the RSC system and if FeOB thrive at particular overlapping gradients, as can be seen in many natural bacterial communities (Wimpenny 1989). Bivariate analyses, one-tailed t-tests, and analysis of variance (ANOVA) were done with SigmaPlot 12.

Leaching Experiment

The effect of DOC on Fe and Mn dissolution from RSC construction materials and riparian soils was tested using a modified method to study redox systems in sediments (Brannon et al. 1984). Substrate samples consisted of 9 soils excavated from riparian wells (installed using a soil auger) and 5 different construction materials collected from RSC implementation sites. Construction materials were quartz sand, mixed soil-OM fill (with activated carbon and wood-

chips), ironstone conglomerate and sandstone, and granite. Substrates were air-dried for three days prior to use and ground to pass a 2 mm sieve. Oven-dry equivalent masses were determined by drying subsamples at 105°C for 9 hours and calculating the gravimetric water content of each substrate. Substrate/water slurries were prepared (two 1:10 g/mL) for each material in question by adding 405 mL of extractant solution to 40.5 g of substrate (oven-dry equivalent). The two extractant solutions added were two different treatments applied to each substrate. One extractant solution consisted of stream water collected from Woodland Branch in Calvert County, MD (latitude, longitude: 38.4517899, -76.4785633); Fe flocculate was observed at this site. The second extractant solution was collected from the same site on the same day but prepared by placing 0.8 kg of wood chips and 0.7 kg of forest leaf litter (both collected from RSC sites) into an HDPE container and filling to 5 gallons with the stream water. This mixture was capped loosely and allowed to soak for 4 days in order to provide a high-DOC treatment in order to ensure that DOC concentrations would not be rapidly exhausted during the experiment. Both treatments were vacuum filtered through glass-fiber filters (0.7 µm pore size) before being added to the substrates in order to reduce the influence of any particulate organic carbon. Slurries were placed in 500 mL glass canning jars with screw-on metal lids and sparged with N₂ gas for 10 seconds to displace O₂ and speed the development of anoxia. Three replicates were prepared for each treatment and substrate for a total of 84 slurries. The slurries were incubated in the dark at room temperature for 8 weeks, occasionally (i.e., several times before each sampling) swirling them for 10 seconds to suspend the sediments.

The incubation jars were sampled after 1 and 8 weeks of leaching. A syringe was inserted just below the surface of the solution to extract 100 mL of sample from each jar which was then filtered through a polycarbonate filter (0.4 µm pore size). The first replicate was filtered into

three sets of 30 mL subsamples. The first set of these subsamples was stored in HDPE bottles with 20 μ L of trace metal-grade nitric acid and refrigerated until analyses for Fe and Mn. The second set of subsamples were stored in 30 mL Teflon bottles and frozen until they were analyzed for DOC, and the third set was frozen in HDPE bottles until analyzed for NO₃, Cl, and SO₄. The remaining two replicates were only analyzed for Fe and Mn. Statistical differences among treatments were determined using the tests mentioned previously.

Bacterial Mats

Mat accumulation from FeOB was measured at 9 sites in July 2014 (not including LA and SV sites since there was no apparent mat formation in the pre-construction period), and longitudinally at the AR site, using a modified method originally intended to measure periphyton biomass (Steinman et al. 2006). Samples were taken from sites of maximum apparent mat accumulation in flowing water near where the monitoring wells were installed. Mat thickness and water depth to substrate were recorded. Samples were collected by lowering a 119 cm² wooden frame into the mat and using a metal bar to scrape the substrate and vigorously agitate the contents inside the frame. A syringe was then used to extract 280 mL of the resultant slurry. The slurry was stored on ice until it could be returned to the laboratory and frozen. Samples were thawed two days later, agitated, and subsamples of known volume were filtered using pre-weighed, pre-combusted, glass-fiber filters (0.7 μ m nominal pore size). The filtered residue was dried at 105°C for 4 hours, weighed and then combusted at 450°C for 4 hours. The combusted material was then weighed to determine ash-free dry weight (AFDW) and total volatile solids (TVS). The % Fe flocculate by weight was calculated as: (AFDW/dried flocculate)*(100).

293 Thick mats of flocculate (Figures 4a & b) were sampled at the SS and MR sites to analyze
294 interstitial water and dried material. These sites were selected because they commonly have
295 stream reaches with dense mats of Fe flocculate that could be easily sampled. Samples were
296 collected in 1-L Isco bottles and refrigerated for 48 hours at which time aliquots were filtered to

Figures 4a & b: 3a – left photo) Flocculate from FeOB at the lower reaches of the Milkhouse Run (MR) RSC site. The white surface film in the foreground is commonly associated with flocculate production. 3b – right photo). Abundant flocculate from FeOB downstream of a weir constructed of ironstone substrates at the Saltworks Creek Sand Seepage (SS) RSC site. Iron flocculate collected from these sites was dried and analyzed for various constituents.

297 obtain interstitial water (as described previously) and the remaining flocculate dried in Pyrex
298 trays for several days. A portion of the dried material was sent to A&L Laboratories for total
299 metals analysis (i.e., Cu, Cd, Fe, Pb, and Zn), and to NASL for PP analysis. The interstitial
300 water was analyzed for various solutes using the same techniques described previously.

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302
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RESULTS

Stream and Groundwater Monitoring

A pre- and post-construction comparison at the LA site indicated that RSC implementation increased Fe and DOC concentrations in both stream water and groundwater near the stream channel. Sampling began in January of 2013 and continued for 1 year after the RSC was implemented in June – July 2014 (i.e., post-construction period from August 2014 through July 2015). Volume-weighted mean (VWM) concentrations of Fe at the LA site increased more than an order of magnitude from 0.04 to 0.40 mg/L and 0.09 to 1.01 mg/L from the pre- to the post-construction period in baseflow and stormflow, respectively. By contrast, VWM concentrations in the pre- and post-construction periods at the SV control site were only slightly higher in baseflow (0.008 and 0.03 mg/L, respectively) and stormflow (0.08 and 0.09 mg/L, respectively). The EPA criterion for Fe concentrations in natural freshwater systems is 1 mg/L (www.epa.gov/wqc/national-recommended-water-quality-criteria-aquatic-life-criteria-table). Annual precipitation volume at the LA site in the pre-construction period of May 1, 2013 through April 30, 2014 was about 6% lower than in the post-construction period of August 1, 2014 to July 31, 2015 (1167 mm vs 1248 mm, respectively).

The network of well transects in the LA catchment (Figure 2) showed a rapid increase in the water table during construction due to several rain events >10 mm that occurred during the beginning of this period. The average increase in the water table was about 0.76 m (Figure 5), and individual wells ranged from 0.15 to 1.2 m. By contrast, the water table decreased in the control catchment (SV) during the LA site recharge period by 0.3 to 1 m, with an average of about 0.5 m.

Concentrations of Fe and DOC in the groundwater wells were monitored in the pre- and post-

Figure 5: Groundwater level below soil surface (m) $\pm$ SE at the Linnean Ave. (LA) RSC and Spring Valley (SV) control sites indicating how RSC construction at LA substantially increased the water table via groundwater recharge. Well data were collected at 5-min intervals and the graph represents 10-day minimum groundwater levels.

Figure 6: Mean concentrations ($\pm$ SE) of DOC and Fe in groundwater wells located at distances of <1 m (close) and ≥ 1 m (far) from the RSC construction area of the LA site.

construction periods. Both Fe and DOC

concentrations were higher in the wells located

in closer proximity (<1 m) to the LA RSC

construction area than in stream water and the

331

332 wells located >1 m from the stream (Figure 6).

333 An inter-site comparison of stream water, and riparian and hyporheic well water in the 12

334 RSC and control sites indicate that streamwater Fe concentrations were generally much lower

335 than those in groundwater (Figure 7). Differences in Fe concentrations between RSC and control

336 sites for the three types of water monitored were insignificant in each category in the Coastal

337 Plain physiographic province (i.e., stream water, and riparian and hyporheic well water), whereas

Figure 7: Mean concentrations of Fe in riparian and hyporheic wells, and stream water in the Coastal Plain (CP) and Piedmont (P) physiographic provinces. Streamwater concentrations are a factor of 10 times higher than actual values to facilitate comparisons among study sites.

338 those in the Piedmont were significantly

339 different. Using an unpaired, one-tailed t-test,

340 Fe concentrations between Piedmont RSCs

341 (MR, BF, GP) and control sites (LA – pre construction, SV) were significantly higher in the

342 RSCs ($p < 0.05$). The significant difference improved with GP removed from this test and only

343 ironstone-containing RSCs were compared with the control sites ($p < 0.01$). Concentrations of

344 DOC and Fe at MR were as high as 28 and 210 mg/L in hyporheic water. Other constituents

measured varied more by site than physiographic province or type of water. For example, NO_3 was significantly ($p < 0.05$) higher in the stream water than in well water of every site (not including sites EP, SS and MR), as was PO_4 (not including sites 33, TA, SS and BF). Riparian well water was significantly ($p < 0.05$) higher in DOC concentrations than hyporheic or stream water (not including sites BF, MR and GP).

The RSC sites scored significantly higher (i.e., better) than control sites on several physical habitat metrics including trash rating, instream habitat, epifaunal substrate, and significantly

Figure 8: Mean values ($\pm$ SE) of physical habitat metrics for RSC and control sites. Data were obtained using Maryland Biological Stream Survey (MBSS) methods. Acronyms are the following: Trash Rating (TR), Instream Habitat (IH), Epifaunal Substrate (ES), Velocity/Depth Diversity (VDD), Pool/Glide/Eddy Quality (PGEQ), Riffle/ Run Quality (RRQ), Embeddedness (EM), and Shading (S).

lower (i.e., worse) on embeddedness ($p < 0.05$;

Figure 8). The RSC sites scored higher (i.e., better) than control sites on velocity/depth diversity and pool/glide/eddy quality, but significant differences were moderate ($0.05 < p \leq 0.1$). Differences in riffle/run quality or shading were insignificant.

Fe flocculate accumulation did not differ significantly in terms of dry mass per unit area between RSCs and control streams, even when site GP (granitic construction material) was removed from the calculation. However, when a two-sample t-test was run between the 5 RSCs constructed with ironstone and the 4 sites without ironstone added (i.e., 3 control sites and GP), the increase in AFDM of the flocculate was moderately significant ($p = 0.07$). The percentage of volatile solids (%VS) of the Fe flocculate was also significantly higher at RSCs than the control sites ($p = 0.001$).

Figure 9: Mean % Fe values ($\pm$ SE) derived from the soils and construction materials extracted from the study sites. The SS site is shown independently from other RSC sites because of its high % Fe value.

Leaching Experiment

Total Fe (%) was generally higher in the

366 ironstone varieties than the soils extracted from riparian and hyporheic wells, and the fill
 367 materials. Granite and RSC fill were the lowest and soils from the SS site were the highest in Fe
 368 content of the substrates analyzed (Figure 9). No relationships were determined between total Fe
 369 of the substrates and Fe or DOC concentrations in the pure streamwater treatment ($r^2=0.06$ and
 370 0.16, respectively; not shown). The supernatant of soils and construction materials with and
 371 without a DOC addition was sampled from each jar after 1 and 8 weeks. The samples with a
 372 DOC addition had higher Fe concentrations than those without for all substrates tested, and Fe
 373 concentrations increased over time (Figure 10). A paired two-sample t-test using the average Fe
 374 concentrations across the replicates and the effect of the DOC addition was significant ($n=14$,

375 $p<0.05$). Only a small increase in concentration

(maximum of 2.5 mg/L) was observed for Mn

Figure 10: Mean concentrations of Fe ($\pm$ SE) from soils and construction materials used at the RSC sites after 1 and 8 weeks of leaching. Soils and construction materials were leached in filtered stream water at room temperature in the dark after sparging with N_2 . Treatments with DOC additions are labeled accordingly.

377 after one week of leaching ($n=14$, $p<0.05$); samples collected after 8 weeks were not analyzed
 378 for Mn. By contrast, DOC concentrations decreased over time in all the DOC treatments (Figure
 379 11).

Figure 11: Mean concentrations of DOC ($\pm$ SE) from soils and construction materials used at the RSC sites after 1 and 8 weeks of leaching. Soils and construction materials were leached in filtered stream water at room temperature in the dark after sparging with N_2 . Treatments with DOC additions are labeled accordingly.

381

382 *Longitudinal transect*

383 Solute concentrations in water samples collected from the longitudinal transect conducted in
 384 July 2014 at the AR site both increased and decreased from up- to down-stream sampling
 385 stations 1 to 8 (Figure 12). Because Cl is considered a conservative solute and concentrations
 386 gradually decreased along the transect, this decrease was attributed in part to the lateral influx of
 387 more Cl-dilute groundwater. The effect
 388 of groundwater dilution on solute
 389 concentrations along the transect was

Figure 12: Solute concentrations modified by a Cl dilution factor to partially remove the effects of dilution on trends; resulting values are unitless. Stations 1 to 8 (up- to down-stream transect) at the AR site were sampled in July 2014.

partially removed by calculating a dilution factor for each station based on Cl (Williams et al. 2004). Applying the dilution factor creates an invariant unitless value for Cl to which all other solutes can be compared. For example, the trend for SO_4 was similar to Cl, whereas that of DOC increased steadily throughout transect. Similarly, Fe concentrations increased relative to Cl, but exhibited more variability compared to DOC. By contrast, NO_3 steadily decreased relative to Cl along the transect.

Throughout the AR transect, Fe flocculate showed a trend of increasing dry mass (represented as % weight of flocculate in Figure 13) and AFDM from the up- to down-stream sampling stations. The % VS of the Fe flocculate oscillated from 75% to 30% (Wessel and Williams 2014).

Figure 13: Fe flocculate by weight shown as the % of total dry matter at the sampling stations of the AR longitudinal transect.

401

Chemical composition of bacterial mats

Thick mats of Fe flocculate from FeOB were collected at the MR and SS sites in September 2014 to determine the chemical composition of both interstitial water and dried flocculate. The composition of the dried material was analyzed for total Fe (%), particulate phosphorus (%), and other constituents (Table 2). Total Fe ranged from 37 to 42%, and PP from 0.2 to 0.6%. The trace metals of Cu, Cd, Pb and Zn had much higher concentrations than those analyzed in stream water, indicating that these bacterial mats adsorb and concentrate these metals. Solute

concentrations of the interstitial water were generally higher than those in stream water for Fe, DOC, and the N fractions, not including NH_4 .

Table 2: Measurements from Fe flocculate samples - %P, %Fe, and interstitial water. Criteria concentrations in natural freshwaters for Cd, Cu, Pb and Zn are 0.72, NA, 2.5 and 120 ug/L, respectively (www.epa.gov/wqc/national-recommended-water-quality-criteria-aquatic-life-criteria-table).

Dried Flocculate						Total Trace Metal				
-----%-----						------(mg/kg)-----				
Site	Fe	PP	volatiles	PN	PC	Cu	Zn	Cd	Pb	
MR	41.5	0.58	17.0	0.75	5.47	<5	66	5	19	
SS	36.8	0.24	18.3	0.50	5.13	11	89	6	23	

Interstitial Fluid										
Concentration										
-----mg/L-----										
Site	Fe	DOC	PO_4	TDP	NH_4	$\text{NO}_3\text{-N}$	TDN	DON	Cl	SO_4
MR	15.8	10.4	0.001	0.006	0.37	<0.01	1.28	0.91	159.5	8.6
SS	1.2	5.9	0.001	0.009	0.16	0.02	0.57	0.39	25.6	3.2

DISCUSSION

Sources of DOC and Fe in RSCs

It is well known that anaerobic microbes use alternative electron acceptors to maintain metabolic activity (Emerson 2000, Emerson et al. 2010, Melton et al. 2014). For example, as OM decomposes, FeRM use the DOC derived from this OM as substrate to reduce Fe derived from Fe-bearing minerals (Brannon et al. 1984, Lovely 1986). In RSCs, because relatively fresh OM (i.e., wood chips, leaves, tree trunks and branches) is added and boulder weirs retain stream water thereby raising the groundwater table (Figure 5), submerged OM leaches DOC into solution, analogous to the preparation of the DOC treatment in our leaching experiments. Oxygen is quickly exhausted in water-saturated soils and FeRM reduce Fe to continue utilizing the DOC that remains in solution. Groundwater flowing through riparian soils and hyporheic zones where construction materials are located in the plume of concentrated DOC leachates from

OM either embedded into RSCs or naturally occurring then emerges as stream bank and hyporheic seepage thereby contributing reduced Fe to stream water.

The inverse relationship of increasing Fe and decreasing DOC concentrations over time confirm that these processes occurred in our leaching experiments. Moreover, these experiments indicate that the elevated Fe concentrations in the stream water and groundwater wells at RSC sites are derived from Fe minerals not only in riparian soils, but also in the ironstone varieties used as construction materials. Every substrate leached more Fe under anoxic conditions with added DOC, but ironstone conglomerate and sandstone leached more than most of the other substrates tested (Wessel and Williams 2014). By contrast, quartz sand and the GP boulders leached relatively small amounts of Fe into solution compared to other substrates.

No relationship was observed between the Fe content of a substrate and the amount of Fe leached to solution, likely because naturally occurring Fe minerals occur in a variety of crystalline forms. This difference accounts for some Fe minerals being less available to microbial reduction thereby slowing the rate at which they leach (Lovley 2000). Furthermore, the surface-area-to-volume ratio of each substrate would have had some influence on the amount of Fe leached into solution. Thus, ironstone substrates (i.e., sand and conglomerate) that have smaller surface areas by weight than riparian soils, would likely have leached more Fe if their surface areas were larger (i.e., similar to the surface area of fine-textured riparian soils).

As in our leaching experiments, the surface area of ironstone substrates used in prominent flowpath areas of RSCs should have a large impact on the amount of Fe leaching that occurs. Large, relatively pure ironstone boulders that have low surface-area-to-volume ratios likely leach lower amounts of Fe into stream water than areas that are largely composed of ironstone sand and smaller, loosely-consolidated ironstone substrates. Indeed, MR and SS are sites that have a

high incidence of flocculate below weirs that appear to have a larger proportion of loosely-consolidated ironstone rock and sand than at upstream weirs of the RSCs that use larger, more consolidated ironstone boulders. Some of the loosely-consolidated ironstone used in these weirs can be broken apart by hand and their surfaces easily gouged with a knife. At the SS site (note that the name “sand seepage – SS” was given to this site because of the large amount of loosely-consolidated ironstone material used in the construction of the weirs), Fe concentrations in stream water increased from 0.6 to 5.6 mg/L immediately above and below the weir composed of this loosely-consolidated ironstone, and the reach downstream of the weir commonly had very dense mats of flocculate (Figure 4b). As mentioned previously, concentrations of Fe in hyporheic water at the MR site were as high as 210 mg/L, and this was located in the stream reach that utilized smaller sized and loosely-consolidated ironstone substrates.

We observed relatively small riparian areas at several study sites where Fe flocculate forms at groundwater seeps that appear during wetter-than-average periods. The flocculate at these sites typically leaves an Fe-rich residue as the groundwater table subsides and the seeps dry out. However, observations from a RSC site located in the Piedmont not sampled as part of this study (i.e., Breewood Rd.) are of interest because although only low-Fe content substrates were used (i.e., granite boulders and low-Fe content sand), moderate amounts of flocculate developed in the stream channel. The flocculate at this site resulted in large part because of a hydrological connection that was made between the stream and a large deposit of OM in the upper soil horizons (i.e., litter, O and A horizons) that became saturated, likely because of recharge of the groundwater table that occurred after construction. This area was several meters from the RSC reach and exhibited Fe flocculate in tannic, organic-rich water. Water trickling from the saturated seep area to the stream created blooms of FeOB in several ponding basins months after

construction. The following year the RSC reach in proximity to the seep exhibited some dense mats of Fe flocculate, and Fe staining of the silica cobble and light coatings of Fe flocculate on strands of filamentous algae in the riffles downstream of the seep area. The RSC reach above the seep area remained completely clear of Fe flocculate and had no Fe staining of the silica cobble. We surmise that this hydrological connection was formed with recharge of the groundwater table after construction and that the influence of this seep is therefore largely dependent on the height of the water table. If so, then the influence of the seep on flocculate conditions in the RSC will likely diminish as the groundwater table subsides throughout the summer and early fall, as measured at the LA site (Figure 5).

Fe flocculate in an intensively-monitored RSC

Flocculate associated with FeOB was never observed at the intensively-monitored LA site in the pre-construction period. However, rust-colored stream water in the ponding basins was observed in the fall (2014) and winter (2015) of the post-construction period and, by the summer of 2015, thick mats of FeOB were observed at several locations during periods with very little storm activity. The appearance of Fe flocculate in the post- construction period coincided with an increase in Fe concentrations in baseflow and stormflow of 39 to 515 and 93 to 333 ug/L, respectively. Despite a 7% decrease in total runoff compared to the pre-construction period, higher Fe concentrations in the post-construction period resulted in a 13-, 3- and 5-fold increase in the annual area yields of Fe in baseflow, stormflow, and total flow, respectively,. Area yields of Fe for the pre- and post-construction periods were 466 and 2,112 g ha⁻¹ yr⁻¹, respectively. Concentrations of DOC in stream water in the pre-construction period are unavailable, but had a

significant ($p < 0.05$) increasing trend in baseflow from 3 mg/L at the beginning of the post-construction period to 5 mg/L toward the end of this period.

Iron flocculate manifested in many of the RSC ponding basins that were filled with leaves from the overlying tree canopy in the fall, and the flocculate appeared more as colloidal material or accumulations of small particles < 5 mm in diameter in rust-colored water rather than as the mats normally observed in the growing season. Because Fe flocculate can also occur spontaneously through the oxidation of reduced Fe in stream water (Emerson et al. 2010), we speculate that the formation of this flocculate could be more of an abiotic process than a biotically-mediated process by FeOB because of its configuration as colloids and particulates, not as mats. Moreover, particulate flocculate was sometimes observed in the ponding basins during periods when streamwater temperatures were $< 5^{\circ}\text{C}$ (Williams and Filoso 2016). Bacterial growth is limited during very cold periods since metabolic activity at low temperatures ceases, with 5°C commonly being referred to as biologic zero (Rabenhorst 2005). Fleming et al. (2014) similarly observed that there were no bacterial mats of flocculate in a ME stream during the winter, and FeOB exhibited a clear seasonal succession coupled with niche separation between the helical stalk-forming Gallionellales (i.e., *Gallionella ferruginea*, which is apparent in the early spring) and tubular sheath-forming *Leptothrix ochracea* (prevalent in the late spring through the fall).

The increase of Fe and DOC concentrations in stream water coincided with an increase in the concentrations of these solutes in well water, and these increases are more pronounced in wells located < 1 m from the RSC construction area than those located further away (Figure 6). Thus, groundwater data confirm that the plume of more concentrated DOC groundwater originating from the OM embedded into the RSC construction materials is restricted to the area in close

proximity to the stream itself. Because the results of our leaching experiments indicate that Fe concentrations in groundwater are enhanced in the presence of DOC, the wells located further away from the RSC that are uninfluenced by DOC leachates remained low in Fe concentrations.

Hydrological pathways involving lateral flow to water through or over surface soil layers often have relatively high concentrations of DOC (Kaplan and Newbold 1993). By contrast, water flowpaths that pass slowly through lower soil layers usually have low DOC concentrations because these soil layers (B and C horizons) often contain Fe and aluminum oxides that effectively sorb and immobilize DOC (McDowell and Wood 1984). Given that increases in Fe and DOC concentrations did not occur in the wells >1 m away from the RSC construction area, this suggests that in most instances a rise in the groundwater table that does not penetrate organic-rich upper soils horizons will unlikely contribute much leached Fe to groundwater and the stream.

Consequently, at the LA site there is little Fe mobilized from existing soil stocks that contributed to the increase in Fe concentrations in the stream other than from those soils influenced by the plume of more concentrated DOC water originating from the OM embedded into the RSC construction area itself. Thus, once groundwater levels increased after the RSC construction, this water inundated the OM built into the RSC and began to leach concentrated DOC. As in our leaching experiments, this DOC likely promoted the leaching of Fe from both the soils and construction materials in the RSC area thereby contributing to the development of Fe flocculate at various locations throughout the structure.

Solute dynamics in a RSC transect

The analysis of samples collected in a longitudinal transect at the AR RSC study site indicates that several key solutes exhibit very different concentration dynamics as they flow through this structure. Partially removing the effects of streamwater dilution by using a Cl modification factor (Williams et al. 2004) shows that DOC consistently increases along the transect relative to Cl, likely because of residual OM buried in the RSC and natural sources. Even though some DOC is removed as Fe is reduced and leached into solution, as observed in our leaching experiments that used a finite amount of DOC in a closed environment, the OM in proximity to this RSC is constantly leaching DOC to the stream. Concentrations of DOC likely increased throughout the transect because the downstream sampling sites had proportionally larger influence from DOC in groundwater than the upstream sites.

Iron concentrations increased relative to the Cl baseline, albeit with higher variability than with DOC. Decreases in Fe concentration at several locations along the transect suggest that communities of FeOB were effectively scavenging Fe from stream water. As indicated previously, large and dense mats from FeOB were often observed in the water trickling through ironstone boulder weirs (Figure 13). Thus some of the reduced Fe in stream water flowing through the riffles of some boulder weirs was being utilized by FeOB to create flocculate.

Decreases in NO₃ concentrations are commonly associated with uptake (e.g., by algae and periphyton that convert NO₃ to organic N) or loss (e.g., denitrification in riparian soils and hyporheic zones). It has also been hypothesized that NO₃ removal is associated with the oxidation of Fe, where denitrifying bacteria utilize reduced Fe as an electron donor (Burgin and Hamilton 2007). At the LA site, there was considerably more uptake and/or loss of NO₃ after RSC construction compared to before. The annual area yield of NO₃ for the pre-construction

period was significantly higher ($p < 0.05$) than in the post-construction period (4.9 versus 0.9 kg $\text{ha}^{-1} \text{yr}^{-1}$, respectively), although part of this difference is a result of a decrease in total runoff in the post-construction period (7%). The decreasing trend in NO_3 concentration modified by the dilution factor along the AR site transect suggests that N uptake and loss are also prevalent in this RSC.

Effects of RSC implementation on health-based stream habitat metrics and metals

One of the main objectives of restoring degraded stream and stormwater drainage ditches is to improve water quality and physical habitat characteristics in order to promote the recovery of the biota and their ecological functions. Because habitat and substrate diversity are designed into RSCs, it is not surprising that several habitat metrics rate significantly higher (i.e., better) than the control sites (Figure 8). However, the significantly lower embeddedness rating (worse), in large part from Fe flocculate, suggests that many aquatic macroinvertebrates and other species may be restricted from colonizing new substrate (Wellnitz et al. 1994). Nevertheless, frogs and tadpoles were abundant at RSC sites, as were snails, which were commonly found on the surface FeOB mats. Fish were only observed at several sites (i.e., AR, AC and Breewood Rd.), but their occurrence is likely more regulated by other factors. For example, discontinuities in the stream network might prohibit fish migration to RSC reaches. Adult dragonflies were common at RSC sites, though larvae were only observed at the LA and GP sites, which exhibited minor to very minor amounts of Fe flocculate (Table 1).

Mats of FeOB have been shown to accumulate arsenic and may also accumulate other toxic metals (Morin et al. 2003). Indeed, the trace metal content of the dried flocculate material collected at two of our sites indicate that there is a concentrating effect of these constituents in

flocculate that are much higher than criteria levels for natural fresh waters (Table 2). Although RSCs are accumulating heavy metals in these bacterial mats, the concentrating effect is not likely biologically harmful because periodic flushing of the system by precipitation events removes established mats of FeOB and dilutes accumulated metals in stormflow runoff.

Factors regulating the development of Fe flocculate

Iron concentrations in the streams of Coastal Plain RSCs were not significantly different than those of the control streams. By contrast, Fe concentrations in the Piedmont RSCs constructed using ironstone were significantly higher than those of the controls (Figure 7). Only relatively small areas with surface coatings of Fe flocculate were observed at our Piedmont control sites over the period of study. Similarly, the flocculate observed at Coastal Plain control sites commonly occurred in small areas or as a ubiquitous light coating on stream sediments (once at the TA site). However, observations from a number of other sites in the MD and DC areas suggest that the flocculate associated with FeOB is relatively ubiquitous in Coastal Plain streams compared to those in the Piedmont. In both physiographic provinces, there tends to be relatively more and denser flocculate in RSCs than in control streams, particularly during summer and low-flow periods between storms. Thus, streamwater temperature (Fleming et al. 2014) and discharge are two important factors regulating the development of flocculate from FeOB.

While Fe flocculate was observed at both control and RSC sites, there were striking differences in terms of its total abundance, composition and distribution among sites. For example, Fe flocculate at RSC sites tended to have higher percentages of volatile material than the control sites (Wessel and Williams 2014), and the densest accumulations were commonly observed in the water trickling through ironstone boulder weirs and rock riffles. Moreover, most

of the flocculate that does occur is relatively localized and likely depends on certain hydrological conditions. For example, there is a tendency for dense flocculate to develop at the ponding basins and riffles located in more downstream areas than upstream, probably because there is more constant groundwater saturation of soils in these areas and therefore an environment that is more conducive to the leaching of DOC from OM, which enhances the leaching of reduced Fe.

The growth of FeOB is also regulated by sources of Fe derived from riparian zones and their soil composition can vary substantially among sites (e.g., SS site, [Figure 9](#)). Moreover, it is likely that the disturbance of riparian soils during construction contribute to higher Fe concentrations in stream and well water until the system stabilizes. We also observed at the Breewood site that establishing hydrological connections between RSC reaches and natural stocks of OM in the floodplain is another mechanism whereby DOC and reduced Fe inputs to the stream can enhance flocculate.

It is important to recognize that not all RSCs develop what can be considered a serious flocculate problem. For example, our most intensively monitored RSC study site (i.e., LA) has experienced occasional blooms of FeOB in the ponding basins and developed flocculate mats at several locations, but none constitute what could be considered as serious stream impairments. Nevertheless, despite the limited amount of flocculate observed at the LA site and the variability in flocculate among the RSC and control sites in both physiographic provinces, our data show that dense accumulations of Fe flocculate can be an unintended consequence of RSC implementation. This consequence is not surprising given the increases in labile Fe and DOC concentrations that occur in stream water after RSC construction and the riffles and ponding basins of RSCs that create ideal low-flow environments where FeOB can thrive. Our leaching experiments confirm that the mineralization of Fe from Fe-containing substrates is enhanced by

DOC in anoxic environments. Because these conditions are created in RSCs and ironstone substrates have some of the highest % Fe values of any construction material or riparian soil at our study sites (Figure 9), these substrates have the potential to contribute relatively high concentrations of Fe to stream water, particularly in some Piedmont streams (such as those in this study) where background concentrations are commonly below the lower limit needed for FeOB to grow and proliferate (<0.1 mg/L). At concentrations of Fe higher than this threshold, DO concentrations >0.3 mg/L are needed for Fe bacteria to carry out oxidation (Andrews et al. 2012).

These findings support our contention that the particles and dense mats of Fe flocculate sometimes observed at several of our RSC study sites are in large part regulated by DOC inputs originating from OM incorporated into the RSCs in conjunction with naturally occurring OM stocks that leach Fe from construction materials with relatively high Fe content (i.e., ironstone substrates) and natural soils. Accordingly, localized soil characteristics (i.e., Fe and OM content) and catchment topography should be evaluated when deciding how much and where to utilize OM, as well as what types of construction materials to use and their position in the restoration reach.

CONCLUSIONS

Results of this study indicate that the implementation of RSCs in degraded urban streams can decrease nutrient and sediment loads and improve many physical habitat metrics. However, although flocculate was apparent in both RSC and control streams, more pervasive accumulations were commonly observed in RSCs in the late spring through early fall. The formation of Fe flocculate varies spatially and temporally, but the sometimes complete

655 embeddedness of benthic substrate in these areas prohibits their use by many aquatic organisms
656 (e.g., benthic macroinvertebrates) and may result in a range of other limitations that affect
657 normal biogeochemical processes (e.g., denitrification in hyporheic zones) and decrease RSC
658 performance (i.e., a reduction in nutrient loads) and sustainability.

659 Based on a combination of data, extensive observations, and information available from other
660 studies, there are several regulating factors that contribute to excessive flocculate formation in
661 RSCs. Conditions that promote the growth of FeOB include low-flow environments, warmer
662 temperatures, and concentrations of Fe and DO >0.1 and 0.3 mg/L, respectively. The regulating
663 factors that can contribute to the development of flocculate under these conditions include Fe
664 leached from construction materials with relatively high Fe content (e.g., loosely-consolidated
665 ironstone substrates sand, small rocks and boulders) located in areas influenced by DOC derived
666 from OM embedded in RSCs and OM deposits in riparian zones or floodplains that become
667 linked hydrologically to the stream by groundwater recharge. Hence, in instances where
668 relatively low quantities of OM are used in construction and hydrological connections with
669 natural deposits of OM in the catchment are avoided, using relatively high Fe content
670 construction materials such as ironstone will not leach much Fe into groundwater that can
671 potentially augment the production of Fe flocculate. Alternatively, in cases where hydrological
672 connections are made with the stream to natural seeps or deposits of OM in or in close proximity
673 to the RSC reach, then it is likely that the use of construction materials with relatively high Fe
674 content will enhance the development of Fe flocculate. In such instances, the use of ironstone
675 substrates should be avoided, particularly sand and small rocks derived from ironstone deposits
676 and ironstone boulders that are loosely consolidated and more subject to higher rates of
677 biogeochemical weathering.

Although RSCs work well to serve some of their intended functions (i.e., create stream habitat, decrease the magnitude of stormwater runoff and erosion, and recharge groundwater), dense accumulations of flocculate from FeOB can degrade stream habitat and aesthetics. Because flocculate is regulated in part by inherent soil characteristics, the type and quantity of construction materials used, and the unique configuration and hydrology of each RSC, utilizing this knowledge during construction should help to alleviate a range of possible impairments stemming from Fe flocculate.

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