

**The Role of Alpine Wetlands as Hot Spots of Dissolved Organic Carbon in the East
River, Colorado**

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Abstract:

Dissolved organic carbon (DOC) is a critical chemical attribute of freshwater systems, affecting nutrient availability, toxicity and solubility of metals, and biological activity via the absorption of light and microbial consumption of O₂ during DOC mineralization. Although DOC contributions to streams are distributed across the landscape in the shallow subsurface, many studies have demonstrated area-outsized contributions from riparian zones with high biological productivity and low subsurface O₂ concentrations. In the East River, CO, a high-elevation watershed located in the central Rocky Mountains, initial observations show that DOC concentrations of two tributaries, Rock Creek and Gothic Creek, are elevated by 2.5-8.9 times compared to concentrations in the main East River and its other tributaries. These elevated concentrations are qualitatively linked to the unique presence of large wetlands in the headwaters of Rock and Gothic creeks, which due to potential anoxic conditions, experience reduced rates of organic matter decomposition and serve as an elevated source of DOC. In this study we quantify the cycling of organic matter in these wetlands and their area-outsized contributions to East River DOC fluxes. We present concentration profiles of DOC along stream reaches and along subsurface flowpaths that span the transition from hillslope to wetland coupled with high-resolution mapping of chronically-saturated zones and calculate area-weighted fluxes of DOC from wetlands to Rock and Gothic creeks at multiple times through the 2016 growing season. Additionally, soil and groundwater DOC fluxes are compared with depth-resolved organic carbon content from soil cores and soil surface CO₂ fluxes to evaluate organic carbon budgets in the hillslope and wetland areas feeding Rock Creek. The characterization of these hotspots of DOC generation and transport in the East River is vital to the ability to predict nutrient cycling changes into the future.

Introduction:

Dissolved organic carbon (DOC) acts as a critical chemical attribute of freshwater systems, affecting the physical, chemical, and biological makeup of water sources (Gergel, 1999; McKnight, et al., 1993). Coming from organic matter, DOC affects nutrient availability, changes the toxicity and solubility of metals in the water, and absorbs light, thus altering biological activities and organism growth (McGlynn, 2003). DOC concentration also impacts the greater carbon cycle in terms of terrestrial carbon export fluxes as water moves from soil to stream (Agren et al., 2010). Understanding the source and transport of DOC through catchments is crucial to the greater understanding of the carbon cycle throughout such areas, as well as in quantifying the environmental impacts of projected changes in hydrology and climate.

It has been widely noted that alpine wetlands are significant contributors of DOC to stream waters (Agren et al., 2010; Fiebig et al., 1990). These regions have high levels of organic material that is flushed into streams as water flows through the soil (Agren et al., 2010). Indeed, an astonishing amount of the total DOC in catchments can be traced back to relatively small amounts of wetland and peat areas. Dosskey and Bertsch (1994), found that riparian peat zones provided 93% of the DOC in their studied stream, despite making up only 6% of the total catchment area. Wetland areas, even small ones, have a significant impact on the total DOC concentration of a catchment (Inglett et al., 2006). The DOC that enters the stream system comes from water that flows through upper soil horizons, picking up organic material that has built up in soils and flushing it into the

stream (Fiebig et al., 1990). Therefore, the streambed and boggy areas around the stream, as well as the uphill water flows, are critical regions for DOC concentrations in a water source.

Concentrations of DOC through soil and stream waters are controlled in part by climate and precipitation conditions. Importantly, DOC concentrations shift with changes in snowmelt intensity and shifting winter weather intensity (Agren et al., 2010). Snowmelt is responsible for the seasonal flushing of DOC from soils, as the increased water flow reaches more shallow depths, picking up and depositing DOC in streams as it moves along (Hornberger et al., 1994; Boyer et al., 2000). Clearly, the understanding of the relationship between DOC and soil waters is crucial to the understanding of greater physical, chemical, and biological properties within downstream water catchments.

The East River Watershed has nine annually flowing tributaries, sourced by snowmelt and summer monsoon storms. Specifically, this project focused on the Rock Creek tributary, which notably runs through multiple alpine wetlands before joining the greater East River Watershed. The marshy areas have high levels of soil water, leading to an anoxic environment that favors DOC buildup as opposed to microbial consumption of organic matter (Agren et al., 2010; McGlynn et al., 2003). This project built an understanding of the catchments' DOC cycling as it flowed into and out of the upstream wetland areas. Preliminary data suggested that the major source of East River Watershed DOC comes from the Rock Creek and Gothic tributaries (Williams, Maher Unpublished), which both go through wetland regions. This work built upon this data in order to create a larger picture of the DOC cycling, starting with upslope soil waters and moving down to the Rock Creek stream itself. This picture of DOC cycling in the East River Watershed is a crucial first step in understanding the physical, chemical, and biological dynamics that control transport of DOC from soils to streams.

This project aimed to quantify the impact of small, wetland riparian zones on the overall organic Carbon chemistry of the East River Watershed catchment system. This includes 1) measuring DOC contribution to overall stream organic fluxes, 2) characterizing the subsurface flow path gradients as water travels downhill towards streams, and 3) noting how DOC changes throughout the season within the Rock Creek catchment study area. Together, these three specific goals built a picture of DOC movement along the entire catchment water flow over time, both underground and in the streams themselves.

Methods:

The goal of this study was to build a picture of DOC cycling throughout the catchment. As such, multiple different data sets were collected. For all of these methodologies, DOC was collected using .2um filters. Waters were pulled into a 60 mL syringe that was flushed out with test waters before collection. Waters were filtered into amber glass vials in the field and refrigerated until testing in a Elementar Vario TOC select TOC/TNb Analyzer, when water samples were cleared of Dissolved inorganic carbon by adding .25 mL of 1M HCl, leaving the DOC as the only testable substance in the water samples.

Stream DOC inputs and discharge at a watershed scale were tested over the course of the summer. Stream reach samples were collected at key points along Rock Creek Stream. We measured at the top of Rock Creek as well as the top, center, and

bottom of the mapped wetlands. DOC concentrations of the samples were measured. Measurements were taken four times over the course of the summer to create a cohesive picture of DOC discharge throughout the season. Using the Trimble system, saturated areas were mapped out manually to determine where stream waters should be collected and to calculate wetland area.

Instrumentation was installed to support specific catchment area measurements for lateral gradient monitoring on a plot scale. Tension lysimeter water collectors were installed at depths varying from 50-90 cm and placed along the hillslope of Rock Creek leading down to the stream. The vertical shift between each consecutive lysimeter as they approach the wetland and stream allowed for flow path gradients of solutes in the subsurface water to be recorded, and the lateral changes in soil water DOC to be collected. This information was consistently recorded once a week to build up a picture of the extent of DOC zones in the Rock Creek tributary. Often, measurements were limited due to lysimeter design and dryness of study soils.

DOC concentrations were monitored in specific locations in the wetlands over the season. The lysimeters placed in the wetland areas were consistently visited to collect soil waters. These waters, collected once a week, were analyzed for DOC concentration and their measurements were compared over the entire season to mark changes in DOC with changing snowmelt and storm conditions.

Gas Fluxes and water chemistry were measured to create a picture of the surrounding aquatic environment. Sample waters from within the watershed were analyzed for dissolved gases to give an idea of the level of anoxia in the environment, which could be related to DOC levels. Highly anoxic zones give way to methane production, a powerful greenhouse gas. Water samples were measured for potential methane and sulfur isotope concentrations along the lysimeter flow path to trace how anoxic the surrounding environment is. Respiration rates of the soil were also measured in order to understand the anoxic environments along a lateral shift.

Statistical and spatial analyses were run using both R script and ArcGIS. Specifically, upstream area was calculated using ArcGIS Hydrology software from a 10m² Digital Elevation Model (DEM) of the land surrounding the tributary. This upstream area was then compared to DOC concentration data. ArcGIS was also used to measure the location of water samples taken along the stream reach, also later compared to DOC concentration data. Finally, DOC flux measurements from each East River Tributary output were put into a multivariable regression analysis run on a DEM with 10m² resolution of the East River Valley (DOC data sourced from Ken Williams). Analysis was run in comparison to percent vegetation cover, forest cover, elevation, ground roughness, and hillslope less than 5% in order to quantify the DOC trends in the area. This analysis was based off of the theory of cryptic wetlands located in low-slope and highly vegetated areas explained by Creed (2003).

Results:

Statistical findings

Multivariable analyses resulted in no statistical significance between vegetation cover, forest cover, elevation, ground roughness, or hillslope as compared to DOC flux measurements from tributary outputs. The statistical findings were run both including and excluding Rock and Gothic tributaries, which were found to have significantly increased

DOC outputs as compared to the other East River tributaries. P values for the analyses ranged from 0.1283 to 0.8702, and R squared values ranged from 0.004823 to 0.4438. No statistical significance was found in any of the analyses. See Appendix 1a for a complete accounting of the multivariate analyses run and the p-value and R squared outcomes.

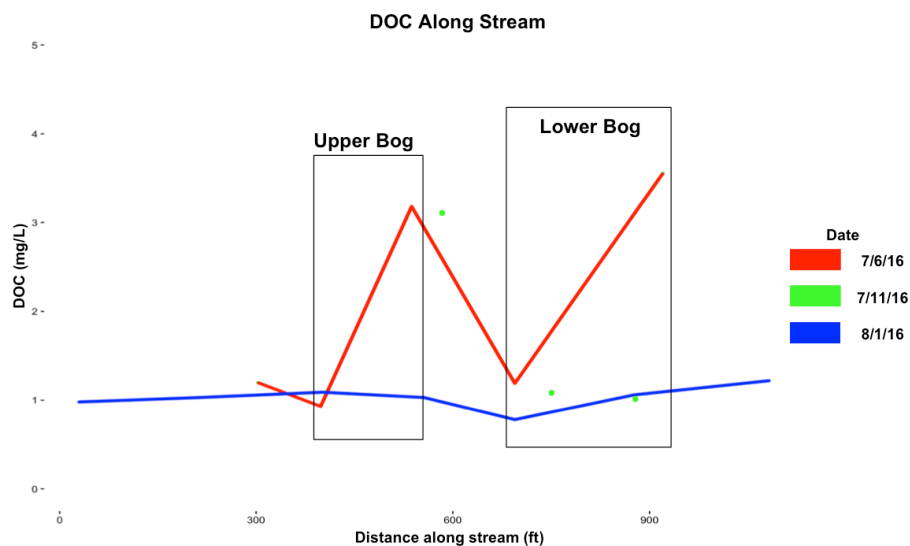
DOC vs. Upstream Area

DOC sampling along Rock Creek yielded evidence of a drop in DOC from the first date of sampling to the last, after a period of dryness in the East River area (Appendix 1b). There were only two significant periods of rain between the July 6th and August 1st sampling periods. Those storms landed between the periods of July 18th to 21st and the 23rd to the 25th, and are represented as vertical red lines in Appendix 1d.

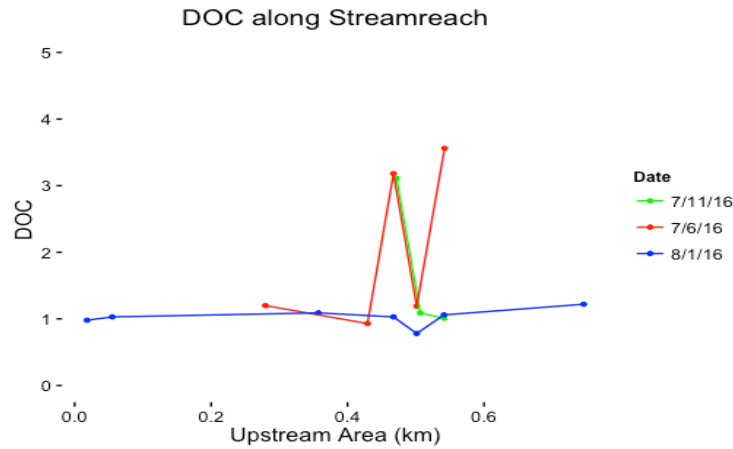
DOC concentrations sampled from the stream were compared to both sample distance along the stream and upstream area. The wetlands input a significant amount of DOC on July 6th, early in the sampling season. Water samples taken from the top and bottom of the upper and lower wetlands on this date followed a trend of increasing DOC concentrations in stream waters through the wetland. Concentrations went from $.93 \pm 0.022$ to 3.18 ± 0.052 mg/L from the top to the bottom of the upper wetland and from $1.19 \pm .047$ to 3.56 ± 0.045 in the lower wetland. A slight drop in stream DOC concentration was noted in the lower wetland on July 11th (with the top measuring 1.09 ± 0.026 and the outlet measuring 1.01 ± 0.053 mg/L). By August 1st, little to no increase in DOC stream concentration was noted as waters moved through the wetland ($1.09 \pm .044$ above the upper wetland to 1.03 ± 0.034 mg/L below the upper wetland; $.78 \pm 0.019$ above the lower wetland to 1.06 ± 0.010 mg/L below the lower wetland). The heightened input of DOC from the wetlands into the streams had leveled out by the final August 1st test date.

Similar trends were seen when DOC concentration (mg/L) was compared to upstream area (Appendix 1c). The decrease in stream DOC concentration between the wetlands is still evident despite the upstream area calculation accounting more accurately for dilution of DOC in the stream reach.

Appendix 1b.



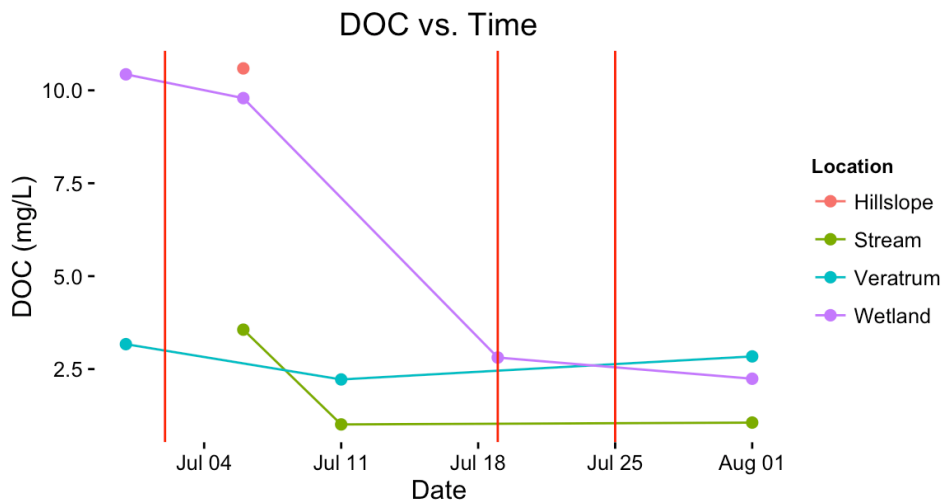
Appendix 1c.



Soil water changes in Rock

In comparing DOC concentrations in the soil and stream of Rock Creek Wetlands, a distinct pattern of decreasing concentration through time appears in the stream samples and 90 cm deep lysimeter located in the bog (Appendix 1d). The hillslope lysimeter was measured at 100cm, while the lysimeter located in the *Veratrum tenuipetalum* meadow was 80 cm deep. The peak DOC in the wetland came at the start of the seasonal measurement, and concentrations fell for the rest of the summer, a trend that was accompanied by a considerable drop in DOC in the stream reach, which peaked at 3.56 ± 0.045 mg/L and then settled to 1.01 ± 0.053 mg/L and ended at 1.22 ± 0.025 mg/L (Appendix 1d, 'Stream'). However, the hillslope lysimeter stopped pulling water after the early season, leading to only one point of study at a 10.59 ± 0.045 mg/L. This is in part due to the significant drying that took place over the month of July. The *Veratrum* lysimeter saw an opposite trend, first losing DOC in the mid season and then regaining some DOC by the end of the sampling period to reach a concentration of 2.84 ± 0.035 mg/L, a concentration higher than that of the wetland lysimeter at the same time.

Appendix 1d:



Respiration in the bog vs. other areas of Rock

Previous studies by Hsiao-Tieh Hsu at Stanford University have found the soil organic Carbon (SOC) in the Rock Wetland to be up to 10 times higher than that of similar low-slope areas in the valley such as the Bradley Creek site. By hand-mapping the wetland areas using a Trimble system, the area of the instrumented Rock Creek wetland was calculated, and the percent carbon content of soils at particular depths in the wetland were multiplied by the outer ranges of bulk soil density ($1.10\text{--}1.60\text{ g/cm}^3$, Argonne National Laboratory). The calculated SOC content by volume was then multiplied by the total area of the wetland to find the total weight of SOC in the study wetland. In total, it was calculated that Rock Creek Wetland contained between 3.3×10^6 and 5.2×10^6 kg of SOC, making it an enormous carbon source for the area.

Despite this area-outsized carbon store, average wetland respiration rates were found to be only $0.9731\text{ gCO}_2/\text{m}^2\text{hr}$ as opposed to 0.7807 in the willows, 0.6886 in the *Veratrum* meadow, and $0.4213\text{ gCO}_2/\text{m}^2\text{hr}$ on the hill slope of the instrumented Rock Creek site. Rock wetland microbes are only respiring at a rate between 1.24 and 2.31 times that of the surrounding vegetated areas, an indicator of anoxia when compared to the 10x increase of DOC in the wetland. Respiration is controlled by the equation $R=KC$, where R is respiration, C is the SOC concentration and K is the intrinsic rate constant. In this particular case, C has increased tenfold, yet the wetland respiration increased at most 2.31 times that of surrounding areas. Therefore, K must be significantly reduced in this case, a reduction most likely due to a high degree of anoxia present in the saturated bog, limiting respiration.

Discussion:

Statistical Tracking of DOC Concentration

Previous studies have found a high accuracy in DOC flux models that use vegetation coverage and areas of low slope within the watershed as determining factors for DOC flux measurements at stream outputs (Winn et al., 2009; Creed, 2003). Cryptic wetlands, or areas of low slope (0-5%) that are inundated below the soil surface, have been found to increase DOC inputs into streams (Eimers, M.C., 2008). These statistical models, when applied to the DOC fluxes at the outputs of each East River tributary, found no statistical significance (DOC data acquired from Ken Williams, 2016). Tests run considering vegetation, forest cover, elevation, and slopes of 0-5% relayed no significant pattern as compared to DOC outputs. This could imply that the cryptic wetlands and vegetation growth that so often define DOC systems are not as important in the East River Watershed. This could be due to the area-outsized DOC flux coming from Rock and Gothic creeks, a flux defined by the hanging valley wetlands at the upper reaches of these tributaries. It is also possible that the 10m^2 resolution of the DEM used in this study may be too large to accurately read the low slope of the study wetland. Future studies working on LiDAR data will support this finding with more detailed tests.

Although no statistical significance could be applied to these watershed inputs, previous research on cryptic and visible wetlands finds that DOC inputs are less significant in areas of low runoff, implying that the shallow-sloped wetlands responsible for the build-up in DOC along the river must have a steady flow into the stream network in times of high DOC runoff, or else soil DOC concentrations would not be influencing the rivers to such an immense extent (Creed, 2003).

Stream Inputs by Upstream Area: Wetland Influence

The earliest test of DOC in the streams above and below the Rock Creek wetlands yielded a significant increase in DOC concentration through each consecutive wetland (Appendix 1b, Appendix 1e). These spikes in DOC through the wetlands support the theory that the anoxic, saturated conditions in the wetland do indeed lead to a large buildup of DOC that is flushed into streams. DOC concentrations increase through the upper bog ($.93 \pm 0.022$ to 3.18 ± 0.052 mg/L), and then drop significantly (from 3.18 ± 0.052 to 1.19 ± 0.047 mg/L) on July 6th. All three stream-reach tests (upstream area, DOC flux, and distance down stream) noted a similar drop in concentration in the stretch between the two wetlands. Drops in DOC concentration can be attributed to distance along stream reaches and dilution from incoming waters as water moves downstream (Kothawala et al., 2006). This drop can potentially be attributed to an influx of water from a seep that springs up between the upper and lower wetlands. It is possible that the seep is draining a larger area than what can be deduced by surface data used to calculate upstream area, potentially draining via complex subsurface flow dynamics. It is also possible that the drop in DOC is due to the volatilization of organic materials between the two bogs, specifically along a waterfall that exists between the two sites (McLachlan, et al., 1990).

(Appendix 1c). The preferential flow into the seep could mean that more water is flowing into the system than is accounted for in models, as each 10m square is granted equal weight in calculations. Therefore, the drop in DOC remains evident even when compared to upstream area and stream distance. The data supports previous research that peatlands hold a highly concentrated pool of DOC, and preferential flow through such regions flushes out the high concentrations in soil pores and moves DOC into stream waters (Moore et al., 2003; Schiff et al., 1998).

A 2006 study tested waters in a northern Sweden headwater bog as compared to the same tributary outlet and found a significant decrease in DOC concentration along the stream reach (Kothawala). Compared to 2015 outlet data, assuming such data is representative of current fluxes, DOC concentrations in Rock Creek have been only slightly diluted at the end of the tributary as compared to the waters exiting the study wetland. The wetlands are contributing a significant portion of DOC, enough to elevate Rock Creek above the rest of the tributaries besides Gothic Creek. Some DOC is possibly lost through the volatilization of organic materials along streams and waterfalls, as well as through microbial loss in the stream as organic materials are consumed (McLachlan, et al., 1990). However, despite this potential loss of DOC, the input of the Rock Creek wetlands are potentially enough to elevate Rock and Gothic above the other tributaries in terms of DOC input.

Stream and Soil Inputs: Temporal comparisons

Time comparisons of soil and stream DOC concentrations paint an interesting picture in the wetland (Appendix 1d). As the season wore on, the stream outlet and wetland lysimeter recorded decreasing DOC concentrations, while the *Veratrum* meadow lysimeters recorded an initial decrease followed by a slight increase in DOC concentrations (from July 6th to August 1st). This study measured concentration, not total DOC, so the amount of water in the stream is not taken into account. Importantly, an

early season influx of snowmelt leads to an increase in flow through the wetland and stream that was qualitatively noted in the field. The measurements of higher DOC concentrations early in the season thus imply a much higher total mass of DOC exported into the stream than can be accounted for in concentration measurements. The later drop in DOC concentration (Appendix 1d, August 1st) occurred after a prolonged dry period, which could suggest that early summer saturation near the surface flushed out upper soil horizon DOC sources that were not picked up in low flow conditions seen later in the summer.

These trends are consistent with previous research regarding upslope and wetland DOC flushing and build-up. DOC is flushed out of the wetland early in the season, and as the season becomes drier, DOC concentrations drop significantly in the stream as it exits the lower Rock Wetland. The coinciding drop in DOC in the wetland and stream waters suggests that the main input of DOC into the streams is coming from the wetlands, supporting the original hypothesis of this study. Steeper areas that drain quickly and have ample oxygen availability produce lower levels of DOC, as microbial consumption of organic materials outweighs the decomposition of organic matter (Winn et al., 2009; Inglett et al., 2006). Regions of lower slope and inundated soils with slower flow produce higher levels of DOC, as saturated soils create anoxic conditions that favor reduced microbial consumption (Winn et al., 2009; Inglett, et al., 2006; Sutfin et al., 2016). The anoxic soils see lower microbial consumption, which in turn allows for increased DOC flow into streams. Wetter soils, such as those in inundated wetlands, flush DOC more frequently than upslope soils, meaning wetlands both build up more DOC and more efficiently flush out the DOC that is built up (Boyer, et al., 1997). In Rock Creek, DOC flushing events are most significantly influenced by snowmelt, which peaks early in the summer. Wetlands were saturated at the start of the season, but by the August 1st sampling date soils had dried significantly as snowmelt decreased. A 1997 study found that DOC concentrations in wetlands decrease readily with storm events as the DOC stores are flushed out (Hinton et al., 1997). The findings of the East River study note a synchronous decrease in DOC concentrations in both wetland soils and streams in the tributary, which corroborates previous research suggesting DOC builds up in wetlands before being flushed out into the stream, thus decreasing the soil DOC concentrations.

The movement of DOC in the upper Rock Creek soils is consistent with the previous understanding of upland soil horizons, as the DOC concentration fluctuates at low levels throughout the season. DOC concentrations decrease between June 30th and July 11th (going from 3.17 ± 0.038 to 2.22 ± 0.052 mg/L) and then increase between July 11th and August 1st (from 2.22 ± 0.052 mg/L to 2.84 ± 0.035 mg/L). The soil DOC concentration in the *Veratrum* meadow above the study site fluctuated at low levels throughout the season. DOC stores were potentially flushed out of upland soils early in the season as snowmelt increased flow through the B-horizon (Cooper et al., 2005, Boyer et al., 1997). It is also possible that the dry upland soils never built up the same DOC concentrations as the wetlands, leading to consistently low measurements throughout the season.

Conclusion:

The findings of this study support the initial hypothesis that the area-outsized DOC contribution of Rock and Gothic Creeks is due to the impact of alpine wetland soils.

Importantly, it is clear that DOC is favorably donated into the streams from these highly concentrated riparian zones in peak early-season flushing that removes a significant amount of the DOC from the anoxic wetland soils. Dilution from sources such as seeps are also apparent in the upper wetland. Findings indicate that the heightened DOC concentrations seen at the outlet of Rock Creek are due to the influx of organic materials from wetlands found along the stream. Additionally, the low concentrations of upslope soils potentially indicate either an early-season flush of DOC or a lack of organic matter building up in upper soil horizons. In comparison, wetland soils are flushed of remaining DOC as water levels remain high throughout the summer, leading to a larger flush that continues throughout the season, and mirrors the decrease of DOC concentrations in the stream. Also, slightly higher respiration rates despite significantly higher SOC in the wetland support the hypothesis of respiration rates being inhibited by anoxia. This, in turn, explains the high DOC concentrations in the bog as microbes consume organic matter far slower than it is broken down in the soil. In summation, this study finds that tributary wetlands represent hot spots of organic carbon cycling. Driven by snowmelt and summer monsoon season, these wetlands may be far more sensitive to changes in climate, a dynamic that will be studied in greater detail in future publications.

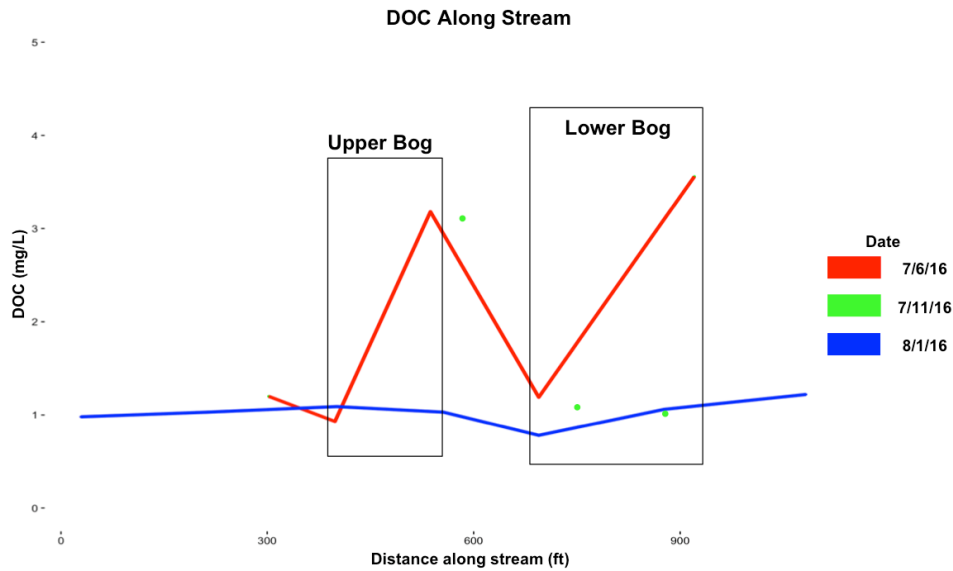
Appendix 1: Tables and Figures

1a.

Analysis Run Against DOC Fluxes from Stream Reaches	p-value	R squared
Vegetation + Slope	0.4438	0.1283
Vegetation	0.1452	0.2773
Slope	0.1273	0.3114
Vegetation + Slope (no Rock or Gothic)	0.4077	0.27
Slope (no Rock or Gothic)	0.004823	0.8702
Vegetation (no Rock or Gothic)	0.2854	0.8702
Forest Cover + Slope	0.2669	0.3374
Forest Cover	0.2591	0.1329
Forest Cover (no Rock or Gothic)	0.08062	0.4956
Forest Cover + Slope (no Rock or Gothic)	0.0846	0.8017
Elevation	0.03881	0.5854
Elevation (no Rock or Gothic)	0.06026	0.5579
Elevation + Percent Roughness	0.1302	0.6138
Elevation + Percent Roughness (no Rock or Gothic)	0.06115	0.8541

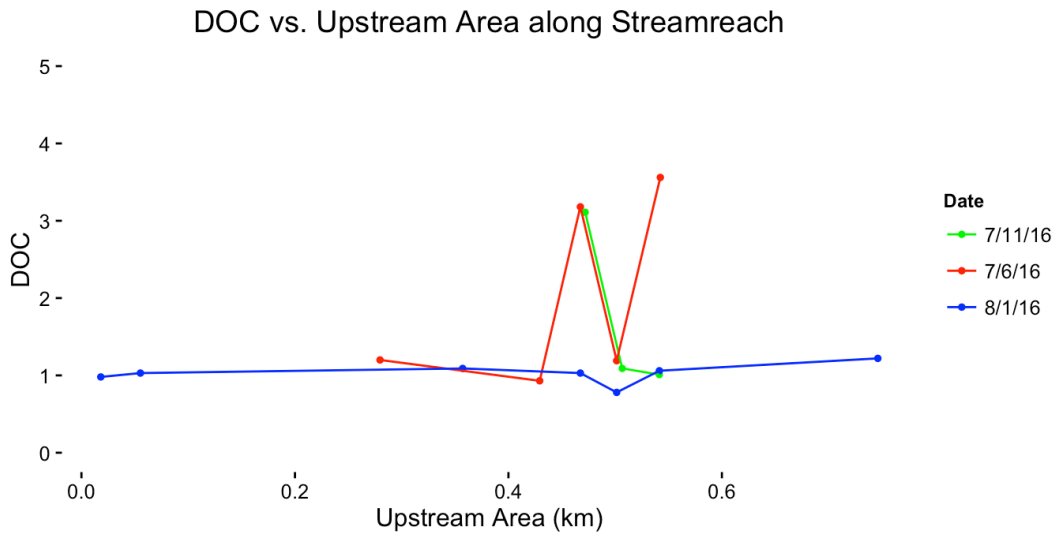
DOC concentrations sourced from Ken Williams. Analysis was run in an R script, covering percent vegetation cover, slope between 0 and 5%, forest cover, and roughness.

1b.



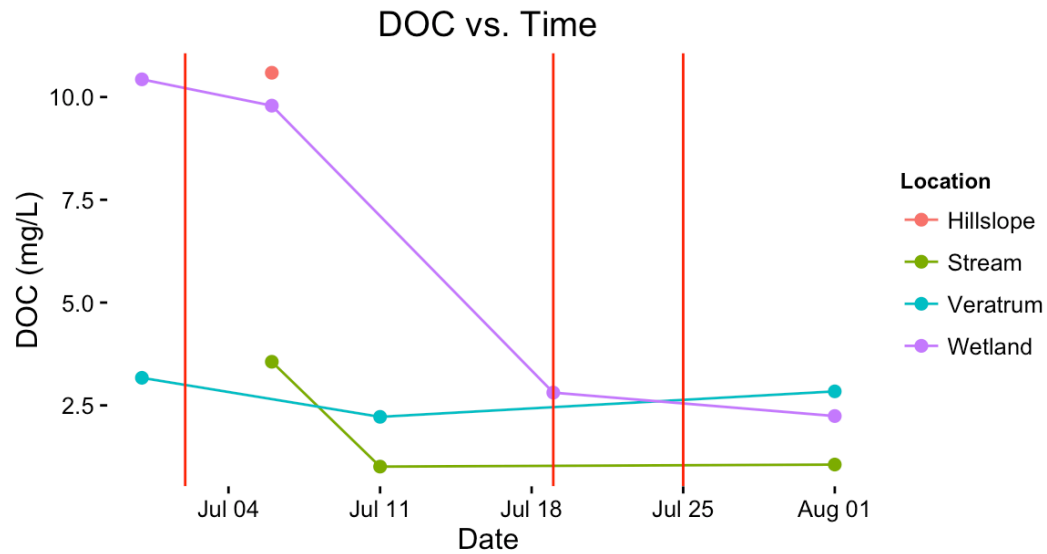
DOC concentrations from water samples taken from Rock Creek Stream by date, profiled along the stream reach by distance. Note the early-season spike in DOC before an eventual baseline on August 1st.

1c.



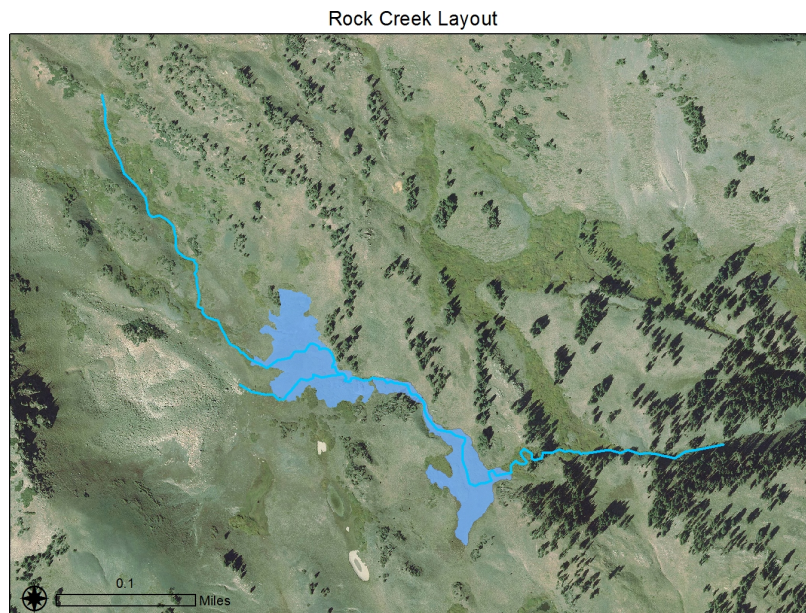
DOC concentrations from water samples taken from Rock Creek Stream by date, profiled along the stream reach by upstream area. Note the early-season spike in DOC before an eventual base-line on August 1st, as well as the continual increase in DOC along the stream in August besides the seep region at approximately .5km². Also note the drop in DOC in the second bog on July 11th, evidence of either a flushing of the bog or a shift in flow away from the lower bog.

1d.



DOC concentrations from water samples taken from Rock Creek stream and surrounding soils, graphed by source location and profiled by date along the x-axis. Stream samples were taken at the outlet of the study bog. Vertical red lines represent precipitation events (billy barr's Weather Station, www.gothicwx.org). Note the corresponding decrease in stream and wetland samples starting in early July.

1e.



The upper reaches of Rock Creek mapped in ArcGIS; note the upper and lower bogs directly beneath the streams' emergence in the upper reaches of the stream.

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