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ELEMENTAL COMPOSITION AND NUTRIENT EFFECT ON THE UPTAKE AND METABOLISM OF DISSOLVED ORGANIC CARBON BY BACTERIA FROM A TEMPERATE REGION RIVER

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A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science at Virginia Commonwealth University.

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List of Abbreviations

OC: Organic Carbon

SAV: Submerged Aquatic Vegetation

DOM: Dissolved Organic Matter

DOC: Dissolved Organic Carbon

C: Carbon

N: Nitrogen

DI: Deionized

HCl: Hydrochloric

Abstract

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By Anne Townshend Stuart, Master of Science

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Rivers are arteries that connect land and sea, and provide a conduit and reactor for allochthonous and autochthonous organic carbon sources (OC) delivered to the coastal ocean. In comparison to marine waters, inland waters quantitatively represent only a fraction of the marine system; however, their importance to global C cycling maybe disproportional to its actual size. Inland systems are subject to multiple sources of OC (autochthonous and allochthonous) that vary individually in space and time with respect to

their concentration and potential bacterial bioavailability. This study investigates the impact of high and ambient inorganic nutrient concentrations on the bacterial bioavailability of potential exogenous and internal organic C sources to bacterial decomposition in the Chickahominy River using a long term incubation approach. In addition the elemental composition of each organic C substrate is investigated as a predictor of OC source bioavailability. The results of sole source incubations showed that autochthonous SAV sources were the most labile whereas soil derived OC was the least bioavailable, irrespective of nutrients. However, leaf litter sources showed relatively high bioavailability. The C:N ratios of SAV, *Peltandra virginica, Botryococcus braunii*, leaf litter, and soil (19.6, 12.4, 15, 29.7, 8.4 respectively) oppose historically accepted theory that autochthonous OC sources with low C:N ratios are a more bioavailable substrate for bacteria than allochthonous OC substrates with higher C:N ratios. The results of this study should provide a better of understanding of the interaction between inorganic nutrients and OC decomposition from allochthonous and autochthonous sources as well and potentially allow model prediction of OC lability based on its elemental signature.

Introduction

The role of inland waters has often been underestimated in the past regarding its potential significance to regional and global carbon budgets (Cole et al. 2007; Battin et al. 2008). Inland waters do not make up a substantial area as compared to marine waters; however, the importance of inland waters to global C cycling is thought to be disproportional to its actual size (Cole et al. 2007). Carbon budgets show a disconnect from terrestrial OC inputs to the OC in the oceans. The fate of riverine transported terrestrial derived OC and *in situ* derived OC is impacted by abiotic and biotic factors such as microbial metabolism.

Research on both catabolic (respiration) and anabolic (growth) reactions involved in metabolism are needed to fully understand organic carbon (OC) cycling in aquatic systems. Investigating the lability of singular OC sources within a system may potentially lead to a better understanding of bacterial partitioning of OC to catabolic and anabolic processes. A predictive knowledge of carbon cycling through inland waters can be improved upon through further investigations of sole OC source (autochthonous and allochthonous) lability and the inherent and environmental factors promoting consumption, such as inherent dissolved organic matter (DOM) nutrients and the duration of dissolved organic carbon (DOC) lability (Apple and del Giorgio 2007). This may provide insight into the export and fate of the more recalcitrant carbon sources that enter riverine, estuarine and coastal systems (Hedges et al. 1997).

Metabolic balance (net heterotrophy and autotrophy) is an influencing factor on the fate of OC, such as CO_2 efflux out of riverine systems (del Giorgio and Williams 2005). A

system that is net heterotrophic, resulting in CO_2 efflux, is a subsidized system that has allochthonous organic carbon (OC) input that is subsequently metabolized (respired) by bacteria. A net autotrophic system has greater production than primary respiration (McCallister and del Giorgio 2008). As inland water systems are not closed off from the surrounding landscape, multiple sources of organic carbon (autochthonous and allochthonous) contribute to these systems and in varying concentration, lability, and composition for bacterial metabolism. Autochthonous sources include algal and primary producers within the water system while allochthonous sources include leaf litter, soil, run off, and other land derived vegetation as examples. It is important to know the different carbon sources and how they are susceptible to bacterial metabolism within the aquatic system in order to develop a predictive understanding of how different OC sources will be processed prior to export to the coastal ocean. Understanding of how ecosystems will react to environmental changes will be gained.

Determining sole OC sources from bulk water studies has been difficult since there is a mixture of OC that cannot be separated into individual source constituents (Zweifel 1999; McDonald et al. 2007). Highly labile OC, collected within bulk water samples, is potentially lost during the time between collection and processing due to its rapid cycling, leaving only the semi-labile or recalcitrant OC (del Giorgio and Pace 2008). The diversity of DOM *in situ* limits the ability to predict autochthonous versus allochthonous DOC source and quality and consequently there has been no empirical determination of the explicit and varying factors which may predict individual OC source lability as well as bulk DOC lability (Findlay and Sinsabaugh 1999; Zweifel 1999; McDonald et al. 2007).

Sole OC source investigation will potentially add to the understanding gained from bulk water studies that will further our understanding of ecosystem processing of DOC and DOM.

Lability has been measured through many methods such as, elemental composition, land use, relative concentrations of potential sources, UV-light, vegetation, and temperature (Baines and Pace 1991; Kirchman et al. 1991; Zweifel et al. 1993; Kirchman 1994; Amon and Benner 1996; Sun et al. 1997; Hopkinson et al. 1998; Bertilsson and Tranvik 2000; Hunt et al. 2000). A proxy for DOM lability, proposed by Sun et al. (1997), uses the elemental composition (specifically C:N ratios in this study) of leachates and compares aliphatic compounds present relative to aromatic compounds in DOM as a means of interpreting lability of different OC sources. Nutrient availability is also an important factor for bacterial growth and respiration (Zweifel et al. 1993). As nutrients increase the potential for more DOC consumption and bacterial growth is improved (Zweifel et al. 1993; Kirchman 1994; Zweifel et al. 1995). Currently, research has not clearly determined how to correlated organic carbon, either through intrinsic characteristics or environmental parameters, with lability for bacterial uptake (Vallino et al. 1996).

The understanding for a need to better understand the composition bioavailability of DOC has been known since the 1980's (Thurman 1985; Meyer et al 1987). Even with advancing technology and extensive research the lability of DOC has frequently been attribute to its substrate quality and yet the absolute parameters defining "quality" have not been identified. The potential for defining the parameters associated with OC source lability would benefit from investigation of sole source DOM elemental characterization

coupled with lability studies. Objectives and goals of this study were to investigate five organic carbon sources from the Chickahominy River and to analyze the lability of these sources. Sources included in this study were submerged aquatic vegetation (SAV), Botryococcus *braunii*, freshwater algae, *Peltandra virginica*, an emergent macrophyte, leaf litter from bottomland hardwoods, and soil from an undisturbed location within the Chickahominy River basin. The lability of these sources was measured through DOC loss and decay rate constants (k). Utilizing leachates and soil desorbed C for sole source research will be supplemented by elemental composition (C and N) analysis and inorganic nutrient addition studies to better understand the potential fate and transport through the Chickahominy River. It was hypothesized that SAV, specifically, would be an isotopically unique carbon source that was highly labile and easily consumed by the river's natural bacterial community.

Methods

Site Description

The Chickahominy River was selected as a sampling location for several reasons: (1) its connection to the James River, which has high nutrients, (2) presence of multiple autochthonous OC sources, including SAV and (3) its ultimate delivery of OC to the Chesapeake Bay (Fig.1). The Chickahominy River is an oligohaline, tidally influenced system that has an approximately 470 square mile water shed (Blankenship 1997). The river had abundant SAV beds comprised of 3 main species, *Ceratophyllum demersum, Hydrilla verticillata, Najas minor*, and emergent marsh vegetation, *Peltandra virginica* (Moore 2008). The surrounding forested areas along the Chickahominy include a mix of bottomland hardwoods (Fleming et al. 2006; Department of Forestry 2009). This river is also protected from point source discharges that might originate at such places as municipal waste water treatment plants (Blankenship 1996). This is in direct contrast to the James River, which is influenced by nutrient additions from industrial sites such as, Hopewell, Virginia (Haley 2008). Sampling was coordinated to included a high flow (April) and low flow (July, October) period in 2008 (http://water.usgs.gov).

Organic Carbon Sources

Organic carbon sources used included various autochthonous and allochthonous OC sources from the Chickahominy River watershed and drainage basin and were used for

lability assays. The primary autochthonous sources of OC investigated in this system were SAV (* refer to results for non-specific species reference), *B. braunii*, and *Peltandra virginica*. Potential importance is that SAV may be a significant source of autochthonous OC to the Chickahominy River as they cover approximately 282 hectares within the river system (Orth et al 2006). *P. virginia* was included as an autochthonous source due to its direct connection with the river and its similarity to aquatic plants. Allochthonous sources examined include both leaf litter and soil OC desorbed from the soil matrix.

Leachate Preparation

SAV, *P. virginica,* and leaf litter were collected to create leachates for lability assays. Leaf litter was collected in early autumn while SAV and *P. virginica* were collected at high growth in late summer (2008). SAV was collected from within the river and *P. virginica* from the marsh lands adjoining the Chickahominy River. Surface soil was collected in an undisturbed area from the Chickahominy drainage basin in spring (2009). The SAV, *P. virginica*, and leaf litter were rinsed with deionized (DI) water, segmented using scissors, and distributed into 8 liters of DI water, and incubated in the dark at 20°C for 3-4 days (Maie et al. 2006). Filtered leachates (Whatman GF/F filters, combusted at 525°C for 4 hours) were analyzed for DOC concentration (mg L^{-1}) and stored at 4°C until use (see below for analytical techniques).

Multiple unsuccessful attempts and complications occurred while trying to isolate a pure *in situ* algae sample from the Chickahominy River. *B. braunii* (hereafter, algae), a freshwater algal species, was obtained from the Department of Oceanography at Old Dominion University. Algae were gently pelleted using an Eppendorf Centrifuge 5810R

(15 amp version at 4°C for 1.5 hours) and the remaining water decanted to remove residual inorganic nutrients that might be present. The algal pellets were rinsed with deionized (DI) water and then added to 2 liters of artificial sea water (ASW; 1ppt) and leached in the dark at room temperature (20°C) for 1-2 days. Leachate was analyzed for DOC concentration (see analytical techniques below) and stored at 4°C until use. Lability of OC leachates from sources found in the Chickahominy River were measured using DOC loss as percent labile OC.

Desorption

Soil was collected from an undisturbed area within the Chickahominy River basin. Leaf litter and other debris were removed from the area and soil within the top 2 inches was collected, using a hand shovel, and taken back to the lab. Roots and other plant material were removed from the soil before it was weighed and dried (60°C). Dried soil was ground using a mortar and pestle and added to 5 liters of DI water for C desorption. After 2 days, the water was filtered (Whatman GF/F filters, combusted at 525°C for 4 hours) and stored at 4°C until use. Lability of desorbed C from soil was measured using DOC loss as percent labile OC.

Organic Carbon Lability of *in situ* SAV Species Comparison

The lability of the 3 SAV species was determined in triplicate bioavailability incubations under non-limiting nutrient conditions to discern whether there were major differences in lability between SAV species. In addition, NaN_3, sodium azide, was used as a bacteriostat in a control set to estimate the portion of labile OC consumed immediately by bacteria during the leaching phase (Maie et al. 2006). Incubations were carried out in

the dark at room temperature (20°C) for 28 days. Samples for DOC concentration were collected over a 21 day time course (see analytical techniques for protocol).

Elemental composition of OC sources

Leachates and desorbed soil C were placed in a drying oven at 60°C, on combusted (525°C for 4 hours) glass plates, until all liquid had evaporated. This was repeated until residue on glass accrued between 1-10mg C. Residues were scraped from glass surface and weighed using a PerkinElmer AD 6 Autobalance and Autobalance controller with a range of 20mg. The dried leachate was then analyzed for C and nitrogen (N) composition on a Costech ECS4010 CHNS/O analyzer at the University of South Carolina, Belle Baruch Institute for Marine and Coastal Sciences to determine C:N ratios of dissolved organic matter (DOM).

Process Study: Nutrient Treatment affects on Lability

Each of the leachates and desorbed soil C lability was tested against non-limiting nutrient concentrations (NH_4, NO_3, and PO_4) and ambient *in situ* nutrient concentrations to assess the potential amount of OC that would be consumed within the Chickahominy River and mimic the fate of the C, should it all be exported to the nutrient rich James River (Table 1). High nutrient concentrations were determined using non-limiting N and P concentrations for bacterial carbon consumption and were estimated from bacteria C:N:P ratio (Zweifel et al. 1993). Approximate ambient nutrient concentrations were determined using 3 years of monitoring data for the Chickahominy River (www.chesapeakebay.net). Initial leachate concentration was standardized to 300μM C, which falls within the range of DOC measured in the Chickahominy River. Leachates and desorbed soil C (triplicate sets)

were incubated with an *in situ* inoculum (GF/F filtered water; 1% vol:vol) in the dark at room temperature (20°C) and were sampled for changes in DOC over a 21 day time period (Fig. 2).

Analytical techniques

DOC

Samples for DOC were collected in 40ml glass vials combusted at 525°C for 4 hours. Samples (25ml) were acidified with 100µL of concentrated HCl acid and stored at 4°C until measured on a TOC-V CSN Shimadzu analyzer (Wickland et al. 2007). To minimize potential equipment error, samples were kept at 4°C until the time series was complete and samples could be run simultaneously (Raymond and Bauer 2000). DOC loss measured using the following equation, was used as a measurement of lability.

(1) X_I-X_F

Statistical Techniques

Data were imported into Origin Pro8 for statistical analyses. 1st order exponential decay rates were calculated using the following formula:

(2) $y = y_0 + Ae^{-kt}$

where y_0 is the remaining DOC concentration, A is the amount of consumed (labile) DOC, k is the decay constant, and t is the time.

This was utilized as a second measure of DOC lability. A two-factor ANOVA was used to test for statistically significant differences based on nutrient treatments and variable organic carbon sources. When the two-factor ANOVA were significant, one-factor ANOVAs were used with the post hoc Tukey's Honestly Significant Difference (HSD) test

and Levene's test for equal variance. Only one-way ANOVA significant values are reported (significance level was set at $\alpha = 0.05$). Descriptive statistics for the standard deviation of the data was included in the output from Origin Pro8.

Results

Organic Carbon Sources: Solubility

Approximately the same amount of particulate source material was used for leaching and desorption, however, the amount of water soluble OC varied between sources (Fig. 3; Table 2). Data for algal water soluble OC was not available due to unknown initial algal concentration of culture processed at ODU (see Methods for leaching process details). Terrestrial derived water soluble OC varied from 0.8% mobilized from soil desorption to 81% produced by leaf litter leaching. Marsh and SAV derived OC were intermediary with values of 49% and 22%, respectively (Fig. 3).

Organic Carbon Sources: Lability

In order to better assess the fate of OC sources along a river continuum lability incubations for each of the five OC sources were exposed to both ambient and high nutrient treatments (N and P) to better mimic the transformation of OM sources from the oligotrophic Chickahominy River to the eutrophic James River. Lability was assessed by two measures; DOC loss and DOC decay rate constants. The three predominant SAV species collected from the Chickahominy River showed no DOC lability difference between species (Fig. 4 inset). This resulted in no further intra species investigation and

therefore references the three species singularly as SAV. Consequently, for all further lability experiments, SAV refers to a mix of 3 prevalent species.

Ambient Nutrient Treatment

Allochthonous derived OC was consistently less labile than autochthonous (Fig. 4) with OC lability derived from leaf litter (44.1 ± 2.3) being approximately double that of soil-derived lability (22.8 ± 1.6) at ambient nutrient concentrations. In comparison the lability of autochthonous-derived OC were invariant between sources; SAV and algae had 57.4±1.4 and 59.7 ± 3.1 respectively and *P. virginica* was 59.4 ± 3.3 (Fig. 4). The strongest statistical differences were observed between the autochthonous sources lability in comparison to soil-derived OC lability ($p< 0.0001$; Fig. 4). Overall the lability of allochthonous (leaf litter and soil) sources was significantly less than autochthonous OC sources ($p<0.05$).

The averaged (triplicates) DOC concentrations over a 21 day incubation time course were plotted using a first order exponential decay function (Table 3, Fig. 5, panel A). The SAV decay rate with ambient nutrients was higher and significantly different than both of the allochthonous OC sources. Comparison of SAV- versus soil-derived OC decay rate showed the strongest statistical difference ($p< 0.00005$), followed by leaf litter with $p< 0.005$. The SAV decay rate was double that of the leaf litter-derived OC and 5 times greater than that of soil-derived OC (Table 3). In comparison, the algal decay rate was higher and significantly different at the $\alpha = 0.05$ level when compared the decay rates of soil-derived OC but was lower and statistically different than leaf litter-derived OC

decay rate. *P. virginica* decay rate was higher and statistically different than soil-derived OC, however, it was not significantly different than leaf litter-derived OC decay rate.

Nutrient Addition Influence on Lability

The lability of allochthonous and autochthonous OC sources showed large variations and followed a similar pattern as the ambient nutrient treatment with respect to lability of allochthonous derived OC versus autochthonous OC (Fig. 4). In contrast to ambient nutrient concentrations the addition of nutrients at non-limiting concentrations revealed significant differences between all of the OC sources. SAV- derived DOC showed higher and statistically significant lability relative to all other sources when compared to allochthonous sources ($p<0.05$). SAV derived labile DOC (82.6 ± 0.4) was 4 times greater than soil-derived DOC lability (19.6 ± 0.3) and approximately 1.5 times greater than the leaf litter-derived DOC (49.9 ± 1.0; Fig.4). The *P. virginica* and algal derived labile OC (77.7 ± 2.4 and 70.6 ± 2.8 respectively) were statistically different than leaf litter-derived OC and the less labile and least mobilized soil-derived OC ($p <0.05$).

The decay rates calculated for each OC source were statistically analyzed using one-way ANOVA comparing autochthonous versus allochthonous rates (Table 3; Fig.5; panel B). The decay rate for SAV was significantly different than both of the allochthonous sources and had the strongest statistical difference in comparison to soil-derived OC ($p < 0.00005$). SAV-derived OC decay rate was approximately 3 times greater than leaf litter and 12 times greater than the decay rate for soil-derived OC. Decay rates showed a trend similar to that of the lability discussed above. Algal decay rates were significantly different ($p < 0.05$) than that of the leaf litter-derived OC and soil-derived OC

decay rates. *P. virginica* only showed statistical significance against one allochthonous source (leaf litter-derived OC) which is in contrast to ambient nutrient concentrations where *P. virginica* was statistically significantly different when compared to both allochthonous sources.

Nutrient Treatment Effect on Individual OC Sources

Of the three autochthonous OC sources, SAV-derived OC lability had the most significant change with addition of nutrients ($p < 0.00005$). This was mirrored by the decay rate as it was also significantly different between the high and ambient nutrient treatment. *P. virginica* showed a similar pattern, the lability was statistically significant from high nutrient treatment to ambient nutrient treatment along with the rates of decay ($p< 0.05$). In contrast, algae lability and decay rates were not statistically different between nutrient treatments.

The allochthonous sources lability was less varied between high and ambient nutrient treatments as compared to the autochthonous sources. Leaf litter- and soil derived DOC lability of each was used invariably with respect to increased nutrient concentrations. The decay rates of the terrestrial-derived DOC did not change significantly ($p>0.05$) between high and ambient nutrient concentrations.

Elemental Composition Effect on Individual OC Source Decomposition

The C:N ratios of potential OC source leachates varied from 8.4 to 29.7 (Table 2). C desorbed from the soil had the lowest C:N ratio (8.4) and leaf litter had an approximately 3.5 times greater ratio (29.7). The 3 autochthonous OC source had C:N ratios greater than Redfield and varied from 12.6 to 19.4 (Table 2).

Treating leaf litter-derived OC as an outlier, the C:N ratio showed an increasing trend with lability (Fig. 6). The treatment with high nutrients had a stronger correlation than the ambient treatment (r^2 = 0.83 and 0.64 respectively). The decay rates compared to C:N of OC substrate ratio demonstrates a relatively strong coupling than between C:N ratio and lability with high and ambient nutrient concentrations (r^2 = 0.83 and 0.84 respectively; Fig. 7). Leaf litter-derived OC was again treated as an outlier for C:N and lability analysis.

Discussion

Importance of Sole Source Studies

The potential quantitative importance of inland waters to the global C cycle has recently been suggested (Cole et al. 2007; Battin et al. 2008). However, inland waters are extremely diverse with variable biogeochemical and hydrological aspects so there is still a need for further understanding of the processes affecting C cycling within these systems (McCallister et al 2004). There has been extensive research using bulk water studies working towards an understanding of the cycling of DOM (Findlay et al. 1986; Sun et al. 1997; Hunt et al. 2000). This method potentially underestimates the total labile DOM pool as DOC can be consumed rapidly and may have been degraded *in situ* before the start of the incubation. Consequently, bioassays may only capture the semi-labile and refractory DOM pools (Williams 2000). The sole OC source approach used in this study for each allochthonous and autochthonous source allows a more representative suite of compounds to be assessed as the material has not been previously degraded and showed labile compounds often missed in bulk water studies. Further, when heterogeneous mixtures of bulk water are used for bioassays, it is difficult to attribute an individual OM source to the DOC consumed.

Using lability within this study, the high lability of certain sources was evident. DOC loss focused more specifically on respired C not C incorporated into biomass. Singular OC sources investigated in this research has the potential to fill in the gaps where bulk water studies miss these highly labile DOM sources. For example, Findlay et al.

(1998) was also unable to detect an SAV-derived OC signature in bulk water DOC when sampling during peak production and directly over SAV beds. The exceedingly high decay state of SAV found in this study suggests that bulk water sampling techniques potentially miss this highly labile source of DOC as it is consumed before it can be collected and measured (Findlay et al. 1998; Williams 2000). The slower decay rates and lability measured for the leaf litter- and soil-derived DOM suggest that these sources may be major components of bulk water in certain inland ecosystems (Kirchman et al. 1991).

What also needs to be highlighted is that the composition of the particulate or initial OC sources may not be represented in the water soluble portion released through leaching and desorption (McArthur and Richardson 2002; Williams 2000). For instance, OC sources that contain compounds such as humic acids and aromatics in the particulate form do not contain the same proportion in the dissolved form as found during leaching and desorption processes (Williams 2000). It has also been shown that the freshness of leaf litter will affect the amount and rate of DOM lost to leaching (McArthur et al 2002). Similarly, different soil types have been shown to yield varying amounts of labile DOC with comparable decay rates (Wickland et al. 2007). DOC derived from poorly drained soils, such as the one used in this study from the Chickahominy River watershed, have been shown to have low biodegradability (Wickland et al. 2007).

Why Nutrients Matter

Nutrient influences on bacterial carbon uptake is an important parameter in elucidating the bacterial impact on the fate (respiration, transformation, export) of individual OC sources temporally and spatially within and between various rivers. The

importance of inorganic nutrients for bacterial growth has previously been investigated, however in contrast to this study they used bulk water samples containing a mix of OC sources of variable freshness (Lønborg and Søndergaard 2009). In the sole source incubations the SAV-derived DOM was rapidly consumed in comparison with the leaf litter-and soil-derived DOM under ambient nutrient concentrations. In contrast, leaf litter-derived DOC loss in this study did not change with the input of greater inorganic nutrients. Typically it has been accepted that terrestrially derived OM is a poor substrate given its high C:N ratio and consequently it has frequently been suggested that the addition of nutrients will not increase the bioavailability of this material. Similarly, a lake bulk water study looking at bioavailable DOC showed that inorganic nutrient additions did not increase degradation rates of bulk DOC (with the exception of a glucose addition experiment; Sondergaard et al. 2000). It is also conjectured that the leaf litter-derived OC had such a small bacterially available pool of DOC that it was all consumed under ambient nutrient concentrations thus increased nutrients did not stimulate the usage of the remaining refractory DOM.

Elemental Analysis

The chemical composition and elemental analysis of DOM has been measured as an indicator of DOM bioavailability. Sun et al. (1997) and Hunt et al. (2000) both utilize C, N, O, and H to elucidate bioavailability of DOM. Results from the previously mentioned studies emphasized the importance of C:N ratios in determining bioavailability when the DOC source is of terrestrial origin. Elemental ratios were also analyzed to determine aliphatic and aromatic content of DOC where the lower the N:C ratio the more

aliphatic, and labile, the source (Sun et al 1997). These studies have found conflicting results based on C:N values and may be partially a result of bulk water with mixed OC sources. Non-linear regression used to compare single OC source C:N ratios with decay rates and lability from this study, showed a positive and significant correlation with both nutrient treatments. Leaf-litter derived OC did not fit this model and may result from varying C:N ratios from the leaf litter composed of different deciduous tree species. Several studies have shown that leaf litter leachate from different species had varying C:N ratios and different bioavailability (McArthur et al. 2002; Cleveland et al. 2004). Even with varied ratios there was still a significant fraction of the leached material that contributed a labile C source utilizable for bacterial consumption (McArthur et al. 2002; Cleveland et al. 2004).

Fate of OC

The transport of OC is impacted by many factors such as, flow rates and retention time, the size of the water body and watershed, OC sources, and consumption rate of bacteria. Using estimated dimensions (ArcGIS Explorer) and flow rates from USGS (http://waterdata.usgs.gov/nwis/measurements), the transit time for OC through the study site in the Chickahominy River and from the entrance into the James River to the Chesapeake Bay was projected. Figure 4 & 5 show the different DOC loss and decay rates for the various allochthonous and autochthonous OM sources to the Chickahominy River. Consequently, the OM sources may be degraded *in situ* or exported to the James River depending on their decay rate, residence time and potential ambient nutrient concentration. Soil desorbed C showed little change with the addition of nutrients; potentially making the

Chickahominy River more of a transport pipe to adjacent systems than an active transformer for this source. OC sources with low decay rates are potentially transported similarly through the "pipe" during high flow seasons with short water retention and then during low flow seasons OC will more likely be consumed within the original system. Rudimentary calculations of water transit time were made to estimate consumption or export of individual OC sources (Table 4), however, variations in microbial communities, temperature effects on bacterial production rates, photochemical reactions, and additional inputs along the river were not taken into account. These calculations were based on the amount of OC that was consumed and decay rates resulting from the ambient nutrient concentration treatment discussed above (Table 5).

The winter season, having the shortest retention time (approximately 19 hours; Table 5), will export soil-derived OC from the Chickahominy River to the Chesapeake Bay. As this chromophoric material exits the Chickahominy River en route to the Chesapeake Bay the suspended load of the system decreases allowing increased UV penetration and potentially provide an alterative mechanism for soil-derived DOC consumption (McCallister et al 2006). Given calculated water transit timing, leaf litter-derived OC will also be exported from the Chickahominy River but in contrast to soil-derived OC with lower decay rates, it will likely be consumed prior to reaching the Chesapeake Bay. During the winter and early spring season, SAV and *P. virginica* are not prominent and therefore were not used in the model for those seasons. When algal production begins the decay rates suggest that this OC will be used *in situ* and consumed within the system; furthermore the labile fraction of soil- and leaf litter-derived OC should

also be consumed. Summer and autumn seasons have minimal flow rates compared to that of winter, thus allowing lower retention times of OC such that this OC will most likely be consumed within the system. Given the *in situ* metabolism of allochthonous OC substrates, these results suggest that at times of the year the Chickahominy should be net heterotrophic and potentially provide a source of CO_2 to the atmosphere.

Literature Cited

Literature Cited

Amon, R. M. W. and R. Benner (1996). "Bacterial utilization of different size classes of dissolved organic matter." Limnology and Oceanography **41**(1): 41-51.

Apple, J. K. and P. del Giorgio (2007). "Organic substrate quality as the link between bacterioplankton carbon demand and growth efficiency in a temperate salt-marsh estuary." Isme Journal **1**(8): 729-742.

Arnosti, C. (2004). Speed bumps and barricades in the carbon cycle: substrate structural effects on carbon cycling, Elsevier Science Bv.

Baines, S. B. and M. L. Pace (1991). "The production of dissolved organic-matter by phytoplankton and its importance to bacteria - Patterns across marine and fresh water systems." Limnology and Oceanography **36**(6): 1078-1090.

Battin, T. J., L. A. Kaplan, et al. (2008). "Biophysical controls on organic carbon fluxes in fluvial networks." Nature Geoscience **1**(2): 95-100.

Bertilsson, S. and J. Jones (2003). Supply of dissolved organic matter to aquatic ecosystems: Autochthonous sources. Aquatic Ecosystems: Interactivity of dissolved organic matter. S. Findlay and R. L. Sinsabaugh. San Diego, Academic Press**:** 496.

Bertilsson, S. and L. J. Tranvik (2000). "Photochemical transformation of dissolved organic matter in lakes." Limnology and Oceanography **45**(4): 753-762.

Blankenship, K. (1996). "The Chickahominy and the Chesapeake Bay." 2009.

Blankenship, K. (1997) Chickahominy a study of landscape and landuse. Bay Journal.

Brugger, A. b., B. Wett, et al. (2001). "Immobilization and bacterial utilization of dissolved organic carbon entering the riparian zone of the alpine Enns River, Austria." Aquatic Microbial Ecology **24**: 129-142.

Cherrier, J., J. E. Bauer, et al. (1996). "Utilization and turnover of labile dissolved organic matter by bacterial heterotrophs in eastern North Pacific surface waters." Marine Ecology Progress Series **139**: 267-279.

Cimbleris, A. C. P. and J. Kalff (1998). "Planktonic bacterial respiration as a function of C : N : P ratios across temperate lakes." Hydrobiologia **384**: 89-100.

Coffin, R. B., B. Fry, et al. (1989). "Carbon isotopic compositions of estuarine bacteria." Limnology and Oceanography **34**(7): 1305-1310.

Coffin, R. B., J. P. Connolly, et al. (1993). "Availability of dissolved organic-carbon to bacterioplankton examined by oxygen utilization." Marine Ecology-Progress Series **101**(1-2): 9-22.

Cole, J. J., Y. T. Prairie, et al. (2007). "Plumbing the global carbon cycle: Integrating inland waters into the terrestrial carbon budget." Ecosystems **10**(1): 171-184.

del Giorgio, P. A. and J. J. Cole (1998). "Bacterial growth efficiency in natural aquatic systems." Annual Review Ecology Systems **29**: 503-541.

del Giorgio, P. A. and P. J. l. B. Williams (2005). Respiration in Aquatic Ecosystems. Oxford, Oxford University Press.

del Giorgio, P. A. and M. L. Pace (2008). "Relative independence of dissolved organic carbon transport and procesing in a large temperate river: The Hudson River as both a pipe and reactor." Limnology and Oceanography **53**(1): 185-197.

Department of Forestry, V. (2009). "Virginia's state forests." Retrieved April 15, 2009, from http://www.dof.virginia.gov/stforest/index.shtml#Crawfords.

Findlay, S., L. Carlough, et al. (1986). "Bacterial-growth on macrophyte leachate and fate of bacterial production." Limnology and Oceanography **31**(6): 1335-1341.

Findlay, S., M. L. Pace, et al. (1992). "Bacterial metabolism of organic carbon in the tidal freshwater Hudson estuary." Marine Ecology Progress Series **89**: 147-153.

Findlay, S. and R. L. Sinsabaugh (1999). "Unraveling the sources and bioavailability of dissolved organic matter in lotic aquatic ecosystems." Marine and Freshwater Research **50**(8): 781-790.

Findlay, S., R. L. Sinsabaugh, et al. (2003). "Metabolic and structural response of hyporheic microbial communities to variations in supply of dissolved organic matter." Limnology and Oceanography **48**(4): 1608-1617.

Fleming, G. P., P. P. Coulling, et al. (2006). "The natural communities of Virginia: classification of ecological community groups: Second approximation. Version 2.2." 2009, from <http://www.dcr.virginia.gov/natural_heritage/ncintro.shtml.>

Freese, H. M., S. Goers, et al. (2007). "Dissolved inorganic nutrients and organic substrates in the River Warnow (North-Eastern Germany) - Utilisation by bacterioplankton." Limnologica **37**(3): 264-277.

Haley, M. A. (2008). "Wastewater treatment." 2009, from

http://hopewellva.gov/data/publish/article_104.shtml.

Hedges, J. I., R. G. Keil, et al. (1997). "What happens to terrestrial organic matter in the ocean?" Organic Geochemistry **27**(5/6): 195-212.

Hoch, M. P. and D. Kirchman (1993). "Seasonal and inter-annual variability in bacterial production and biomass in a temperate estuary." Marine Ecology-Progress Series **98**: 283-295.

Hopkinson, C. S., I. Buffam, et al. (1998). "Terrestrial inputs of organic matter to coastal ecosystems: an intercomparison of chemical characteristics and bioavailability." Biogeochemistry **43**: 211-234.

Hullar, M. A. J., B. Fry, et al. (1996). "Microbial utilization of estuarine dissolved organic carbon: a stable isotope tracer approach tested by mass balance." Applied and Environmental Microbiology **62**(7): 2489-2494.

Hunt, A. P., J. D. Parry, et al. (2000). "Further evidence of elemental composition as an indicator of the bioavailability of humic substances to bacteria." Limnology and Oceanography **45**(1): 237-241.

Kaplan, L. A. and J. D. Newbold (2003). The role of monomers in stream ecosystem metabolism. Aquatic Ecology. S. Findlay and R. L. Sinsabaugh. San Diego, Academic Press**:** 496.

Karlsson, J., J. Ask, et al. (2008). "Winter respiration of allochthonous and autochthonous organic carbon in a subarctic clear-water lake." Limnology and Oceanography **53**(3): 948-954.

Killops, S. and V. Killops (2005). Introduction to Organic Geochemistry. Malden, Blackwell Publishing Ltd.

Kirchman, D., E. K'Nees, et al. (1985). "Leucine incorporation and its potential as a measure of protein synthesis by bacteria in natural aquatic systems." Applied and Environmental Microbiology **49**(3): 599-607.

Kirchman, D., Y. Suzuki, et al. (1991). "High turnover rates of dissolved organic carbon during a spring phytoplankton bloom." Nature **352**: 612-614.

Kirchman, D. L. (1994). "The uptake of inorganic nutrients by heterotrophic bacteria." Microbial Ecology **28**(2): 255-271.

Kirchman, D. (2001). Measuring bacterial biomass production and growth rates from leucine incorporation in natural aquatic environments. Methods in Microbiology, Vol 30. San Diego, Academic Press Inc. **30:** 227-237.

Kritzberg, E. S., J. J. Cole, et al. (2004). "Autochthonous versus allochthonous carbon sources of bacteria: results from whole lake ^{13}C addition experiments." Limnology and Oceanography **49**(2): 588-596.

Kritzberg, E. S., J. J. Cole, et al. (2005). "Does autochthonous primary production drive variability in bacterial metabolism and growth efficiency in lakes dominated by terrestrial C inputs?" Aquatic Microbial Ecology **38**: 103-111.

Kritzberg, E. S., J. J. Cole, et al. (2006). "Bacterial growth on allochthonous carbon in humic and nutrient-enriched lakes: results from whole-lake ^{13}C addition experiments." Ecosystems **9**: 489-499.

Kroer, N. (1993). "Bacterial-growth efficiency on natural dissolved organica matter."

Limnology and Oceanography **38**(6): 1282-1290.

Lønborg, C. and M. Søndergaard (2009). "Microbial availability and degradation of dissolved organic carbon and nitrogen in two coastal areas." Estuarine Coastal and Shelf Science **81**(4): 513-520.

McCallister, S. L., J. E. Bauer, et al. (2004). "Assessing sources and ages of organic matter supporting river and estuarine bacterial production: A multiple-isotope (Δ^{14}, $\delta^{13}C$, and $\delta^{15}N$) approach." Limnology and Oceanography **49**(5): 1687-1702.

McCallister, S. L., J. E. Bauer, et al. (2006). "Bioreactivity of estuarine dissolved organic matter: A combined geochemical and microbiological approach." Limnology and Oceanography **51**(1): 94-100.

McCallister, S. L. and P. A. del Giorgio (2008). "Direct measurement of the $\delta^{13}C$ signature of carbon respired by bacteria in lakes: Linkages to potential carbon sources, ecosystem baseline metabolism and CO_2 fluxes." Limnology and Oceanography **53**(4): 1204-1216.

McDonald, S., J. M. Pringle, et al. (2007). "Bioavailability of dissolved organic carbon and fulvic acid from an Australian floodplain river and billabong." Marine and Freshwater Research **58**(2): 222-231.

Maie, N., R. Jaffe, et al. (2006). "Quantitative and qualitative aspects of dissolved organic carbon leached from senescent plants in an oligotrophic wetland." Biogeochemistry **78**: 285-314.

Middelboe, M. and M. Søndergaard (1993). "Bacterioplankton growth yield: seasonal

variations and coupling to substrate lability and ß-Glucosidase activity." Applied and Environmental Microbiology **59**(11): 3916-3921.

Moore, K. "Virginia Estuarine & Coastal Observing System." 2008, from http://www2.vims.edu/vecos/default.aspx.

Moran, M. A. and R. E. Hodson (1990). "Bacterial production on humic and nonhumic components of dissolved organic carbon." Limnology and Oceanography **35**(8): 1744-1756.

Mulholland, P. J. (2003). Large-scale pattersn in dissolved organic carbon concentration, flux, ad sources. Aquatic Ecosystems: Interactivity of dissolved organic matter. S. Findlay and R. L. Sinsabaugh. San Diego, Academic Press**:** 496.

Orth, R. J., D. J. Wilcox, et al. (2006). 2005 distribution of submerged aquatic vegetation in Chesapeake Bay. Special Scientific Report Gloucester Point, Virginia Institution of Marine Science.

Ostrofsky, M. L. (1997). "Relationship between chemical characteristics of autumn-shed leaves and aquatic processing rates." Journal of the North American Benthological Society **16**(4): 750-759.

Pace, M. L., P. A. delGiorgio, et al. (2004). "Estimates of bacterial production using the leucine incorporation method are influenced by differences in protein retention of microcentrifuge tubes." Limnology and Oceanography: Methods **2**: 55-61.

Rao, S. K., H. Fukayama, et al. (2006). "Identification of C-4 responsive genes in the facultative C-4 plant Hydrilla verticillata." Photosynthesis Research **88**(2): 173 183.

Raymond, P. A. and J. E. Bauer (2000). "Bacterial consumption of DOC during transport through a temperate estuary." Aquatic Microbial Ecology **22**: 1-12.

Raymond, P. A. and J. E. Bauer (2001). "DOC cycling in a temperate estuary: A mass balance approach using natural ^{14}C and ^{13}C isotopes." Limnology and Oceanography **46**(3): 655-667.

Reitner, B., A. Herzig, et al. (1999). "Dynamics in bacterioplankton production in a shallow, temperate lake (Lake Neusiedl, Austria): evidence for dependence on macrophyte production rather than on phytoplankton." Aquatic Microbial Ecology **19**(3): 245-254.

Smith, D. and F. Azam (1992). "A simple and economical way for measuring bacterial protein synthesis rates in seawater using 3H-leucine." Marine Microbial Food Webs **6**: 107-114.

Smith, E. M. and Y. T. Prairie (2004). "Bacterial metabolism and growth efficiency in lakes: The importance of phosphorus availability." Limnology and Oceanography **49**(1): 137-147.

Smith, E. M. and R. Benner (2005). "Photochemical transformations of riverine dissolved organic matter: effects on estuarine bacterial metabolism and nutrient demand." Aquatic Microbial Ecology **40**: 37-50.

Sun, L., E. M. Perdue, et al. (1997). "Use of elemental composition to predict bioavailability of dissolved organic matter in a Georgia river." Limnology and Oceanography **42**(4): 714-721.

Touratier, F., L. Legendre, et al. (1999). "Model of bacterial growth influenced by substrate C : N ratio and concentration." Aquatic Microbial Ecology **19**(2): 105-118.

Turner, R. E. (1993). "Carbon, nitrogen, and phosphorus leaching rates from spartina alterniflora salt marshes." Marine Ecology-Progress Series **92**(1-2): 135-140.

USGS. (2009, March 4 2009). "National Water Information System: Web Interface." 2008, from http://water.usgs.gov/.

Vallino, J. J., C. S. Hopkinson, et al. (1996). "Modeling bacterial utilization of dissolved organic matter: Optimization replaces Monod growth kinetics." Limnology and Oceanography **41**(8): 1591-1609.

Wickland, K. P., J. C. Neff, et al. (2007). "Dissolved organic carbon in Alaskan boreal forest: sources, chemical characteristics, and biodegradability." ecosystems **10**: 1323-1340.

Wicks, R. J. and R. D. Robarts (1988). "Ethanol extraction requirement for purification of protein labeled with [^{3}H] leucine in aquatic bacterial production studies." Applied and Environmental Microbiology **54**(12): 3191-3193.

Williams, P. J. l. B. (2000). Hetertrophic bacteria and the dynamics of dissolved organic matter. Microbial Ecology of the Oceans. D. Kirchman. New YOrk, Wiley-Liss**:** 153-200.

Yamashita, Y. and E. Tanoue (2003). "Chemical characterization of protein-like fluorophores in DOM in relation to aromatic amino acids." Marine Chemistry **82**(3 4): 255-271.

Yamashita, Y. and E. Tanoue (2004). "Chemical characteristics of amino acid-containing

dissolved organic matter in seawater." Organic Geochemistry **35**(6): 679-692.

Yamashita, Y., R. Jaffe, et al. (2008). "Assessing the dynamics of dissolved organic matter (DOM) in coastal environments by excitation emission matrix fluorescence and parallel factor analysis (EEM-PARAFAC)." Limnology and Oceanography **53**(5): 1900-1908.

Zweifel, U. L., B. Norrman, et al. (1993). "Consumption of dissolved organic carbon by marine bacteria and demand for inorganic nutrients." Marine Ecology Progressu Series **101**: 23-32.

Zweifel, U. L., J. Wikner, et al. (1995). "Dynamics of dissolved organic carbon in a coastal ecosystem." Limnology and Oceanography **40**(2): 299-305.

Zweifel, U. L. (1999). "Factors controlling accumulation of labile dissolved organic carbon in the Gulf of Riga." Estuarine Coastal and Shelf Science **48**(3): 357-370.

Table 1 Nitrogen and phosphorus concentrations needed for high and ambient nutrient concentration treatments. High nutrient concentration was based on of C:N of bacterial biomass while ambient nutrient concentration were calculated based on nutrient values over a 3 year period in the river. Initial leachate carbon concentrations were 300μM.

Nutrient	**High Nutrient Concentrations**	**Ambient *In Situ* Nutrient Concentrations**
NH_4 – Ammonium	50μM	2.41 μM
NO_3 – Nitrate	5 μM	0.34 μM
PO_4 – Phosphate	6 μM	0.42 μM

Table 2 Organic carbon sources from the Chickahominy River and watershed were leached or desorbed for analysis.

OC Source	Material (g)	[DOC] (mg L^{-1})	Water Soluble (%)	C:N
SAV	300	82.4	22	19.4
*Algae	1 liter	8.5	--	12.6
P. virginica	298	183.4	49	15.0
Leaf litter	260	262.7	81	29.7
Soil	300	5.0	0.8	8.4

* Different leaching procedure used due to method of collection. -- 1 liter was volume of water algae was contained in; actual algal POC unknown.

Table 3 Each treatments 1st order exponential decay rate calculated using Originpro8. Formula for 1st order exponential decay is: $y = y_0 + Ae^{-x/t}$. Decay rate constants (k) are from high and ambient nutrient treatments. r^2 values represent strength of curve fitting.

DOC source	k (d^{-1}) (high nutrients)	r^2	k (d^{-1}) (ambient nutrients)	r^2
SAV	-0.99±0.13	0.98	-0.61±0.12	0.97
Algae	-0.66±0.17	0.80	-0.16±0.04	0.99
P. virginica	-0.52±0.18	0.90	-0.28±0.06	0.96
Leaf litter	-0.31±0.11	0.85	-0.30±0.05	0.97
Soil	-0.08±0.004	0.90	-0.12±0.0072	0.99

Table 4 Transit time of water (ft^3/s) for the Chickahominy River transect and the James River from the Chickahominy to the Chesapeake Bay was roughly calculated. Seasons are defined as Winter (December, January, February), Spring (March, April, May), Summer (June, July, August), Autumn (September, October, November).

River	Winter	Spring	Summer	Fall
Chickahominy River	0.82	10.44	43.74	18.20
James River	36.73	31.31	79.35	77.83

Table 5 Using estimated dimensions, DOC transport from the Chickahominy River to the James River was calculated. OC exported into the James River is represented as **JR**, OC exported to the Chesapeake Bay is represented with **CB**, and OC that does not leave the Chickahominy River is represented with **CR**. NA is for OC source not considered during that season.

Species	**Decay Rate (d^{-1})**	**DOC Consumed (mg L^{-1})**	**[†]Winter**	**[‡]Spring**	**[§]Summer**	**[**]Autumn**
SAV	-0.61	1.870	NA	NA	CR	CR
Algae	-0.16	1.552	NA	CR	CR	CR
P. virginica	-0.28	2.118	NA	NA	CR	CR
Leaf litter	-0.30	1.525	JR	CR	CR	CR
Soil	-0.12	0.787	CB	CR	CR	CR

[†] Winter OC sources considered were leaf litter and soil as the SAV, *P. virginica*, and Algae are not growing at this point.
[‡] Spring included only the leaf litter -, soil-, and algae-derived OC sources.
[§] Summer and Autumn included all OC sources for potential export out of the Chickahominy River.
[**] Summer and Autumn included all OC sources for potential export out of the Chickahominy River.

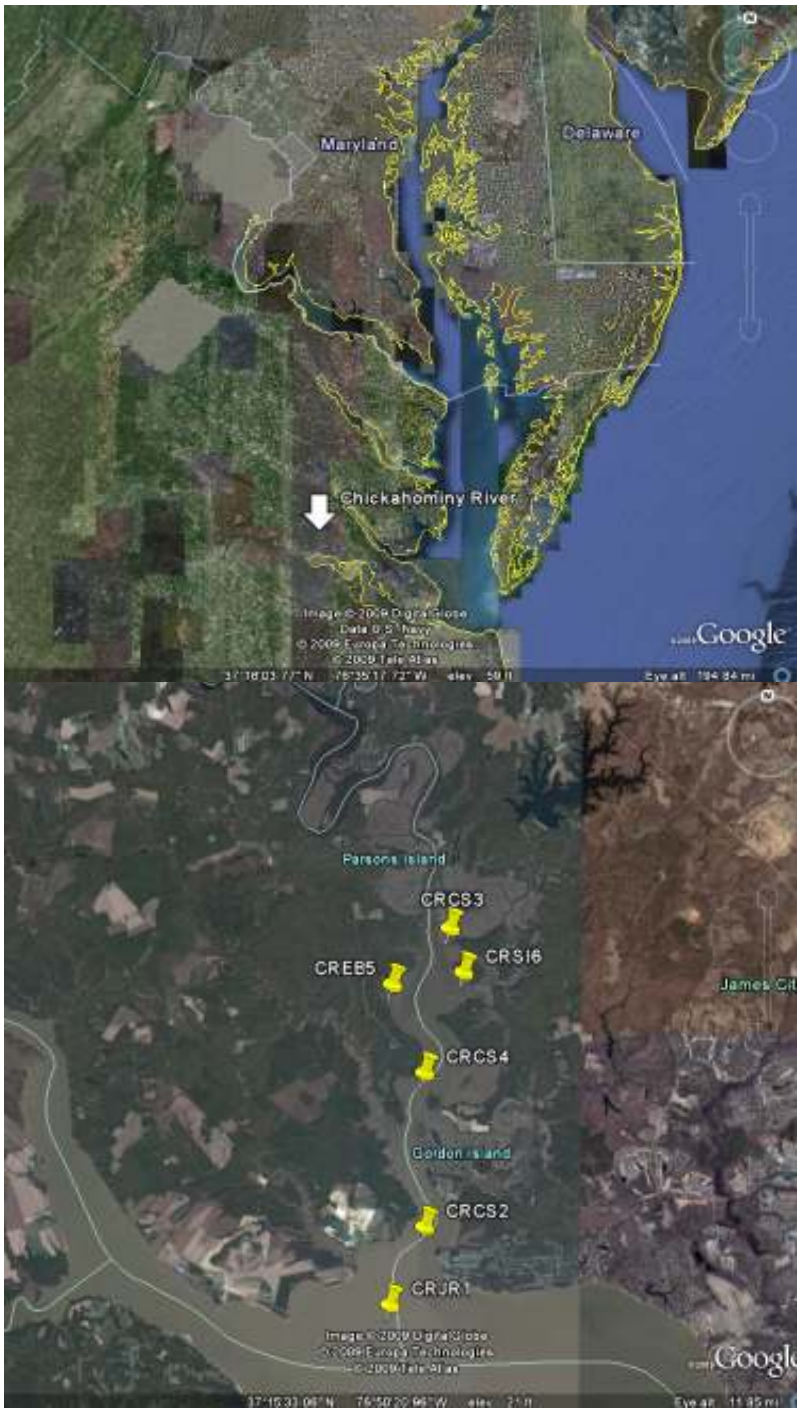


Figure 1 Upper panel shows the Chickahominy River location in relation to the Chesapeake Bay. The bottom panel indicates site location with pins where *in situ* water measurements were taken.

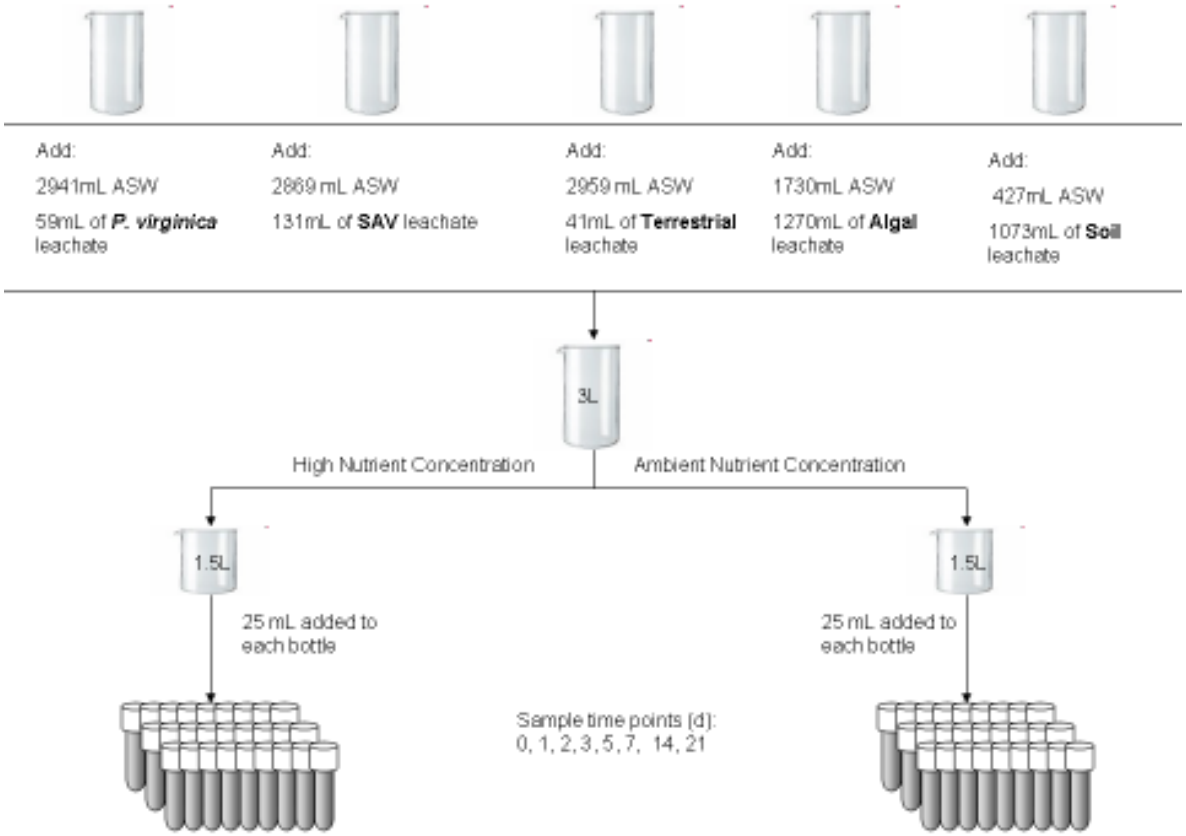


Figure 2 Diagram representing high and ambient nutrient treatment experimental design. Nutrient concentration treatments were distributed equally among the leachates and desorbed C. Soil leachate treatment was done in half the volume due to leachate concentration; nutrients, inoculum, and leachate volumes were adjusted accordingly.

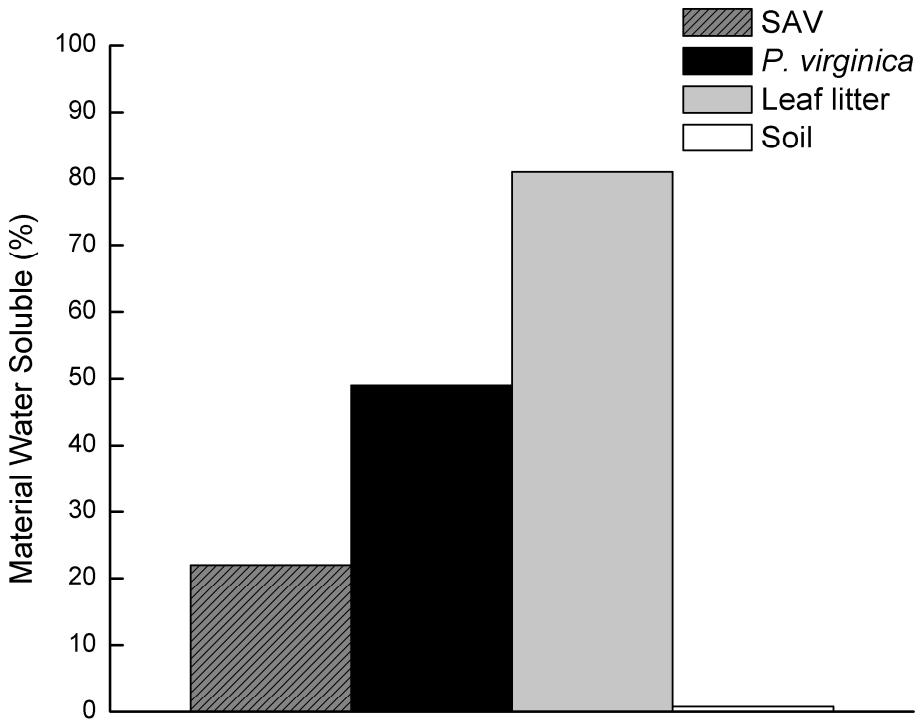


Figure 3 The amount of water soluble material was calculated using the amount of material leached or desorbed over 3-4 days. *Algal soluble material is not shown due to the exact volume of material in water was unknown

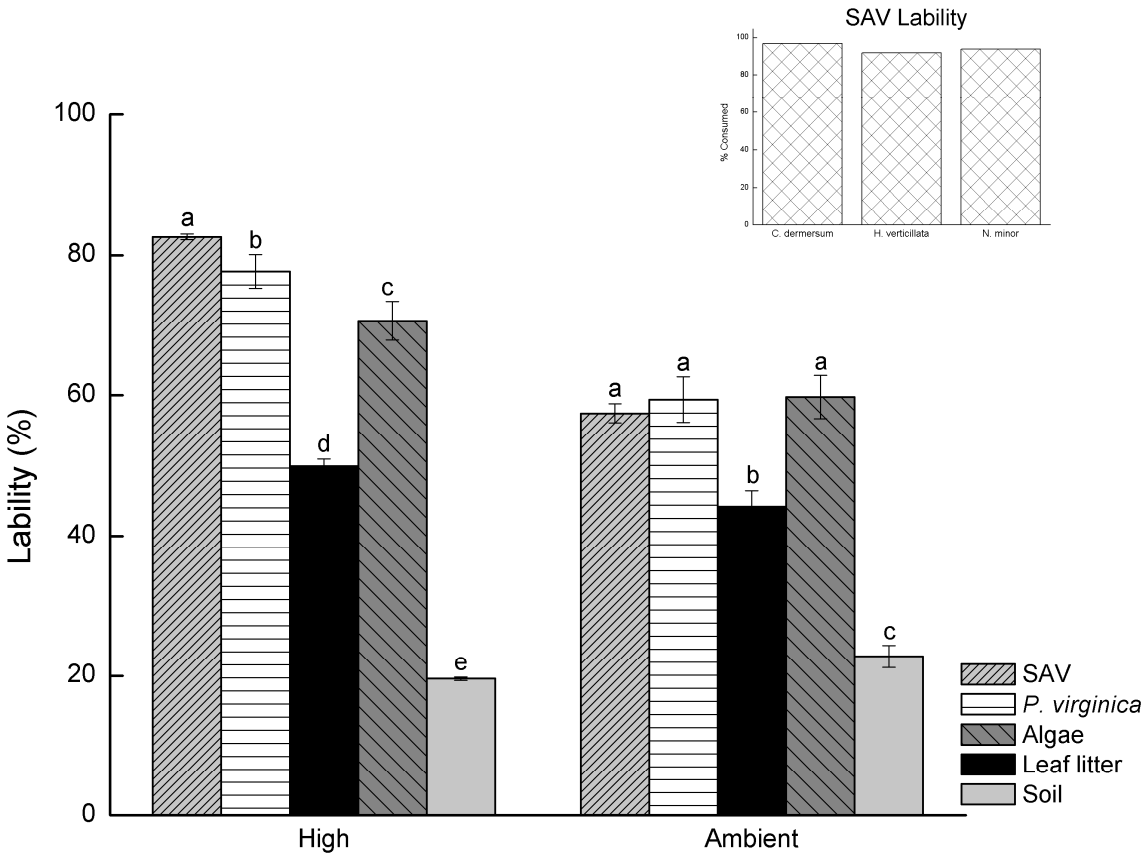


Figure 4 Five OC source leachates tested for lability under two nutrient conditions. DOC consumed is average of triplicate incubations. Autochthonous sources indicated by patterned bars and allochthonous sources indicated by solid bars. Inset graph shows the three SAV species lability. Error bars represent 1 standard deviation of the mean. Lower case letters indicate statistically significant differences.

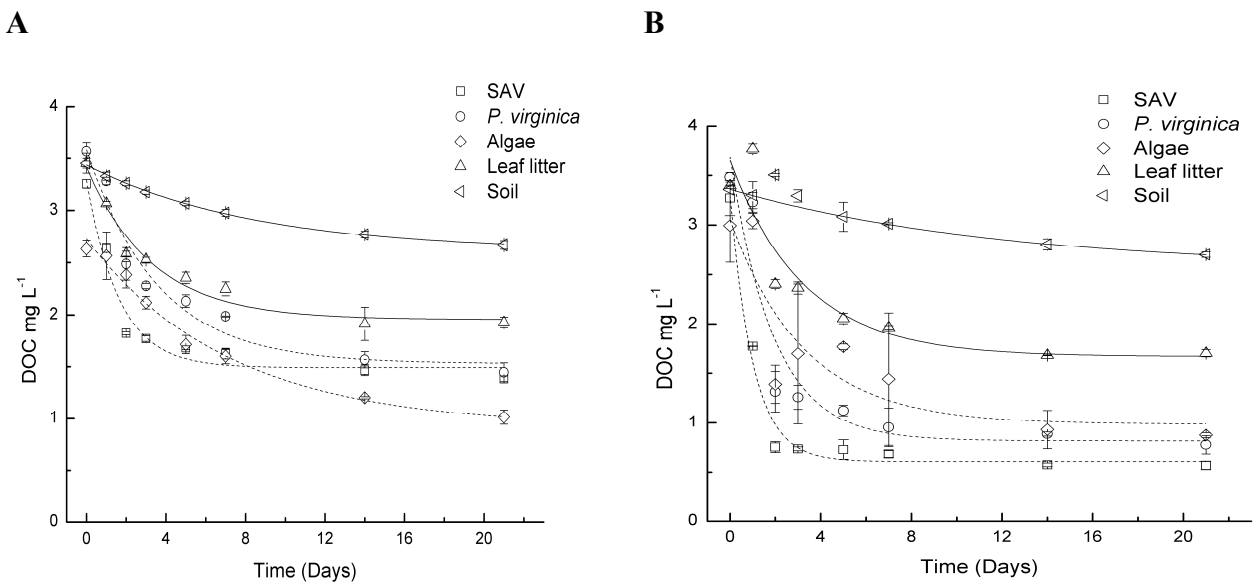


Figure 5 Non-linear, first order exponential curves were fitted to high and ambient nutrient concentrations for each OC source DOC loss over 21 day time course. Autochthonous sources are distinguished with dashed lines and allochthonous sources with a solid line. Ambient nutrient concentrations treatment are in panel A and high nutrient concentrations treatment are in panel B. Error bars represent 1 standard deviation of the mean.

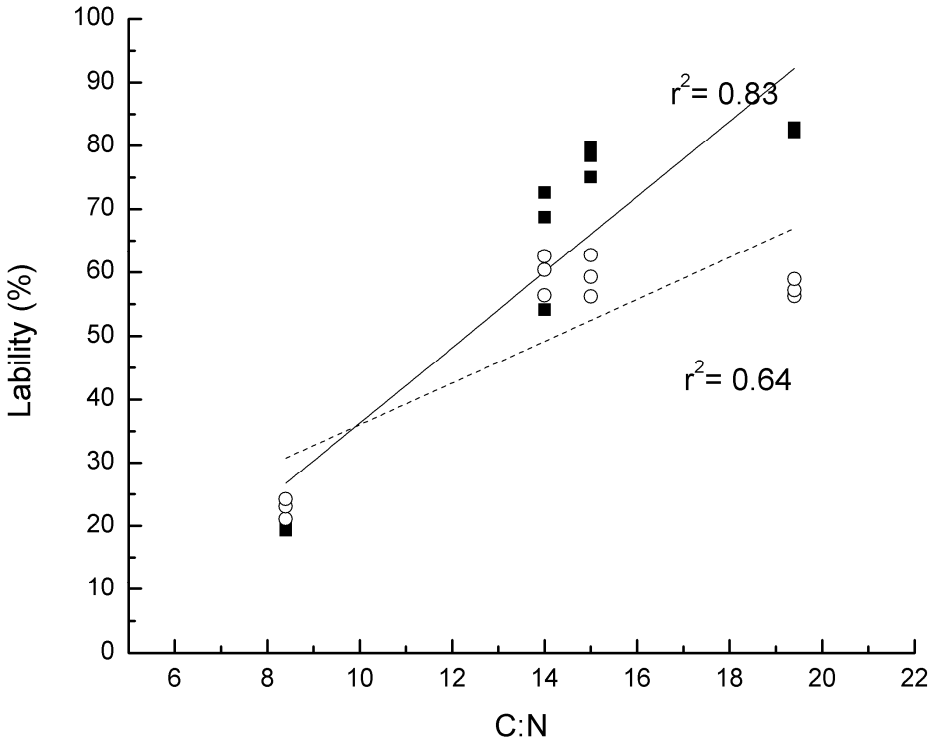


Figure 6 OC leachates C:N vs lability were compared using a linear fit. Solid line and symbol (■) represent high nutrient treatment; hollow symbols **(**○**)** and dashed line represent ambient nutrient treatment. Refer to table 2 for OC source C:N ratios.

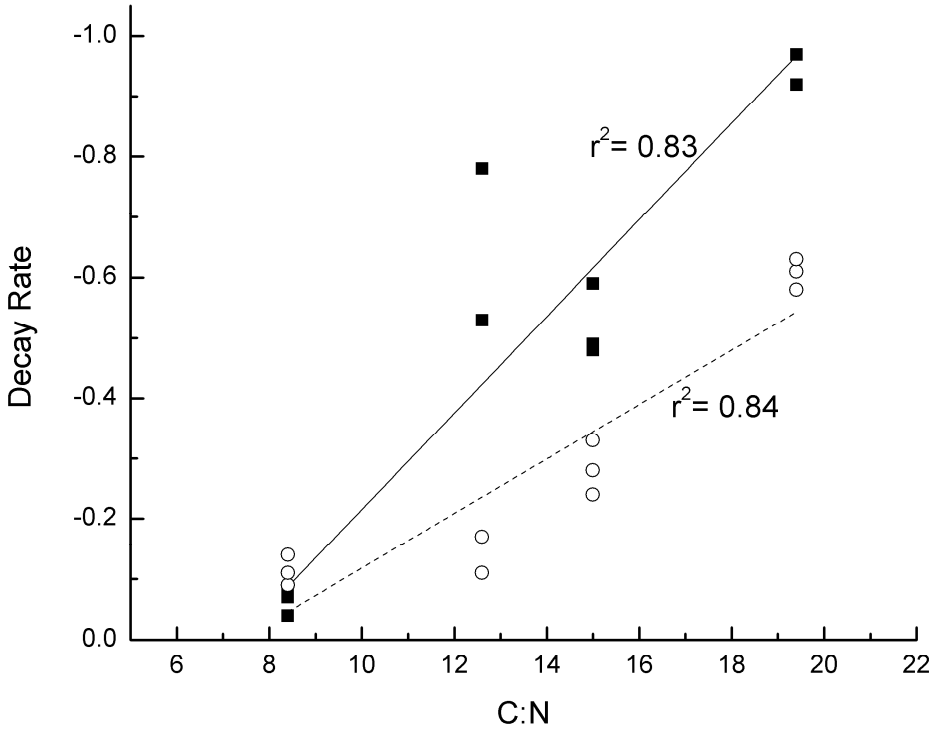


Figure 7 OC leachates C:N and decay rates were compared using a linear fit. Solid line and symbol (■) represent high nutrient treatment; hollow symbols **(**○**)** and dashed line represent ambient nutrient treatment. Refer to table 2 for OC source C:N ratios.

APPENDIX A

Part of this research project was to look at different autochthonous and allochthonous carbon sources in dissolved form (DOC) spatially and temporally in the Chickahominy River. Parameters investigated included DOC, BP, excitation emission matrices (EEM), and stable isotopes. The results were not robust enough alone to include in the study reported above. Below are the results collected as a result of this portion of the research.

Methods

Excitation Emission Matrix

Samples for excitation emission matrix (EEM) analysis were collected in 40 ml glass vials that had been muffled at 525°C for 4 hours. Samples were stored at 4°C until analyzed at Florida International University (Yamashita and Tanoue 2003; Yamashita and Tanoue 2004). Protocol for EEM analysis can be found in Yamashita et al. 2008. Samples include OM leachates and initial and final results from process studies.

Re-growth of bacteria from two mixed OC sources

DOC Lability

Processes studies were conducted for potential discernment of OC source preference by the bacterial community representative of the Chickahominy River. SAV, *P. virginica*, and leaf litter-derived OC source leachates were combined with one other source in equal concentrations (150µmol of each leachate, in duplicate, stored in the dark at 20°C), and were combined in artificial seawater (~1 ppt salinity). These leachates were chosen as they represent what has been typically seen in the literature as labile (SAV) and refractory (terrestrial) sources along with an intermediary OC source (macrophytes) between the terrestrial and aquatic interface. Leachates were filtered through a 0.2µm Nuclepore filter to remove bacteria and an *in situ* inoculum (1% vol:vol; 0.7µm Whatman GF/F filter muffled at 525°C for 4 hours) was added. DOC and bacterial productions were sampled from incubations over a 21 day period (see below for analytical techniques).

Bacterial Biomass

Re- growth incubation OC treatments (as described above) were replicated for bacterial biomass collection. After 48 hours of dark incubation the water was filtered through a 0.3µm GF/F filter (muffled at 525° C for 4 hours), a 0.2µm Nuclepore filter and then again through a 0.3µm filter to create a blank (Coffin et al. 1989; McCallister et al. 2006). The filters were dried (60°C), acid fumed with concentrated HCl acid, dried and sent to G. G. Hatch Stable Isotope Laboratory, Ottawa, Canada, for C concentration (mg) and isotopic analysis ($\delta^{13}C$).

Analytical techniques

DOC

Samples for DOC were collected in 40ml glass vials combusted at 525°C for 4 hours. Samples (25ml) were acidified with 100µL of concentrated HCl acid and stored at 4°C until measured on a TOC-V CSN Shimadzu analyzer (Wickland et al. 2007). To minimize potential equipment error, samples were kept at 4°C until the time series was complete and samples could be run simultaneously (Raymond and Bauer 2000). DOC loss was used as a measurement of lability.

Stable Isotopes

SAV, *P. virginica,* and leaf litter leachate and bacterial biomass collected on GF/F filters (baked at 525°C for 4 hours) were sent to the G.G. Hatch Stable Isotope Laboratory, Ottawa, Canada for carbon ($\delta^{13}C$) isotopic analysis. Samples were measured using an OI Analytical "TIC-TOC" analyzer model 1010. Results include ppm C organic/inorganic concentration and $\delta^{13}C$ signatures. Analytical techniques have an error of 0.2‰ as reported by the laboratory. Isotope values could then be used to aid in

elucidating DOC lability of OC sources (Eq. 1; see results and discussion for further details).

$$\delta^{13}C_{Biomass} = f_1\delta^{13}C_{OM1} + f_2\ \delta^{13}C_{OM2}$$
$$f_1 + f_2 = 1$$

(Eq.1)

Bacterial production

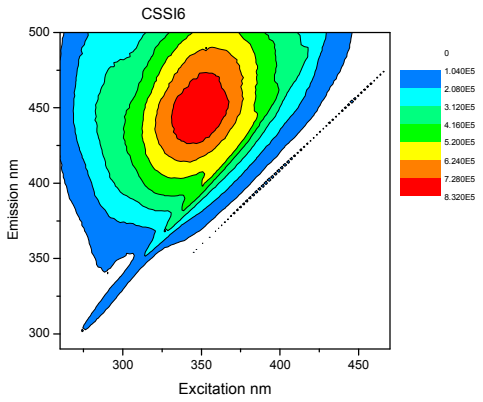
During the re-growth experiment, samples were collected for bacterial production measurement. Bacterial production methodology was modified from Smith & Azam (1992). This method used [^{3}H] Leucine as the substrate for measuring increases in protein bacterial biomass (Kirchman et al. 1985; Wicks and Robarts 1988; Smith and Azam 1992; Kirchman 2001; Pace et al. 2004). This technique was used in conjunction with isotopic analysis for the elucidating OC preference by bacteria.

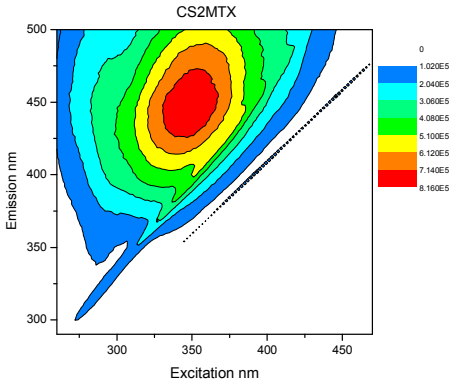
Table 1 Parameters were measured using a YSI sonde. Surface, middle and bottom depth were approximately 0.5, 1.5, and 4 m respectively (variation depended on depth the channel).

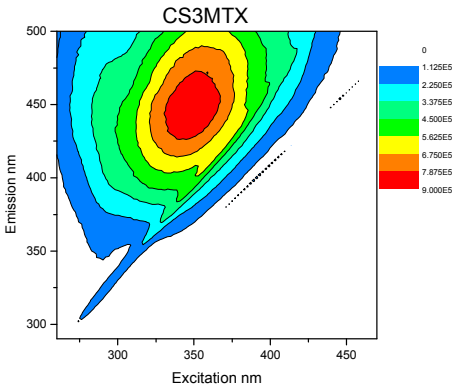
April Sampling	**Surface**					
Parameters	**CRJR1**	**CRCS2**	**CRCS4**	**CREB5**	**CRSI6**	**CRCS3**
Temperature (°C)	na	na	na	19.22	18.52	18.88
Conductivity (ms/cm)	na	na	na	0.349	0.326	0.347
DO (%)	na	na	na	127	115.2	101.7
Turbidity (NTU)	na	na	na	13.8	26.5	9.8
Chl a (µg/L)	na	na	na	12.2	13.1	8.4
Alkalinity	44.5	na	na	34.0	36.5	na
pH	7.3	na	na	7.6	7.7	na
July Sampling	**Surface**					
Parameters	**CRJR1**	**CRCS2**	**CRCS4**	**CREB5**	**CRSI6**	**CRCS3**
Temperature (°C)	29.31	29.29	29.63	29.19	29.61	29.12
Conductivity (ms/cm)	5.665	5.469	5.111	4.921	4.591	4.763
DO (%)	115.3	111.8	117.8	147.9	110.2	102.3
Turbidity (NTU)	12.7	8.5	4.5	22.8	5.6	4.1
Chl a (µg/L)	8.3	6.5	7.8	18.3	7.7	8.9
Alkalinity	50	47.5	49	38	48	44.5
pH	7.19	7.23	7.01	6.90	6.88	7.01
	Middle					
Parameters	**CRJR1**	**CRCS2**	**CRCS4**	**CREB5**	**CRSI6**	**CRCS3**
Temperature (°C)	29.7	29.12	28.75	---	28.88	28.8
Conductivity (ms/cm)	5.648	5.498	5.055	---	4.596	4.817
DO (%)	107.5	107.7	101.2	---	107.3	95.3
Turbidity (NTU)	16.2	13.6	26.5	---	7.2	6.6
Chl a (µg/L)	7.6	8.3	10.4	---	9.4	8.9
	Bottom					
Parameters	**CRJR1**	**CRCS2**	**CRCS4**	**CREB5**	**CRSI6**	**CRCS3**
Temperature (°C)	29.06	29.07	28.75	---	28.7	28.19
Conductivity (ms/cm)	5.732	5.519	5.091	---	4.642	4.724
DO (%)	104.7	104.8	99.2	---	106.2	85.7
Turbidity (NTU)	43.1	21.6	74.2	---	13.4	25.7
Chl a (µg/L)	9.2	8.4	10.2	---	10.3	9.1

Table 2 Stable isotope values for bacterial biomass collected on GF/F baked filters after incubation. End-member (%) calculated from Eq.1 were $\delta^{13}C$ values were used to determine the amount of C incorporated into biomass.

Treatment	$\delta^{13}C$	SAV End Member (%)	Leaf Litter End Member (%)	*P. virginica* End Member (%)
SAV + *P. virginica*	-24.8±0.02	60.8±0.4	-	39.2±0.4
SAV + Leaf litter	-23.7±0.1	68±2.8	32±2.8	-
Leaf litter + *P. virginica*	-28.6±0.1	-	66±8.5	34±8.5


CSSI6
Emission nm
Excitation nm
300
350
400
450
500
0
1.040E5
2.080E5
3.120E5
4.160E5
5.200E5
6.240E5
7.280E5
8.320E5


CS2MTX
Emission nm
Excitation nm
300
350
400
450
500
0
1.020E5
2.040E5
3.060E5
4.080E5
5.100E5
6.120E5
7.140E5
8.160E5


CS3MTX
Emission nm
Excitation nm
300
350
400
450
500
0
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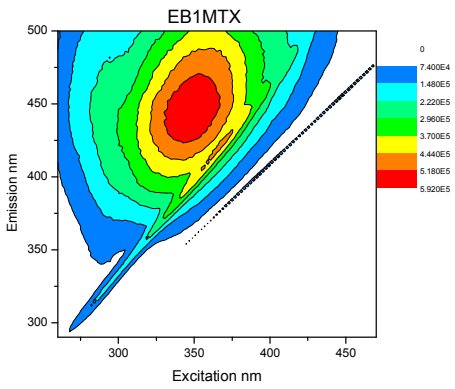
5.625E5
6.750E5
7.875E5
9.000E5


EB1MTX
Emission nm
Excitation nm
300
350
400
450
500
0
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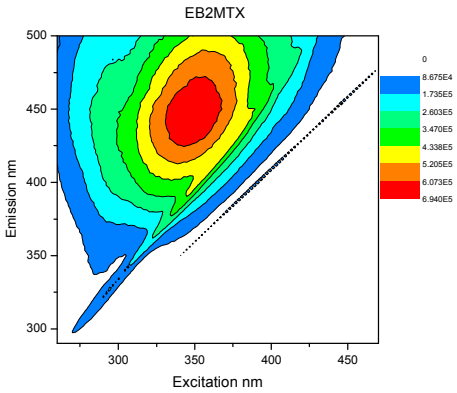
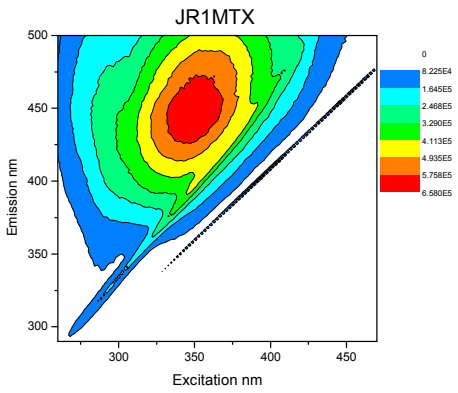
1.480E5
2.220E5
2.960E5
3.700E5
4.440E5
5.180E5
5.920E5


EB2MTX
Emission nm
Excitation nm
300
350
400
450
500
0
8.675E4
1.735E5
2.603E5
3.470E5
4.338E5
5.205E5
6.073E5
6.940E5


JR1MTX
Emission nm
Excitation nm
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350
400
450
500
0
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1.645E5
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4.113E5
4.935E5
5.758E5
6.580E5

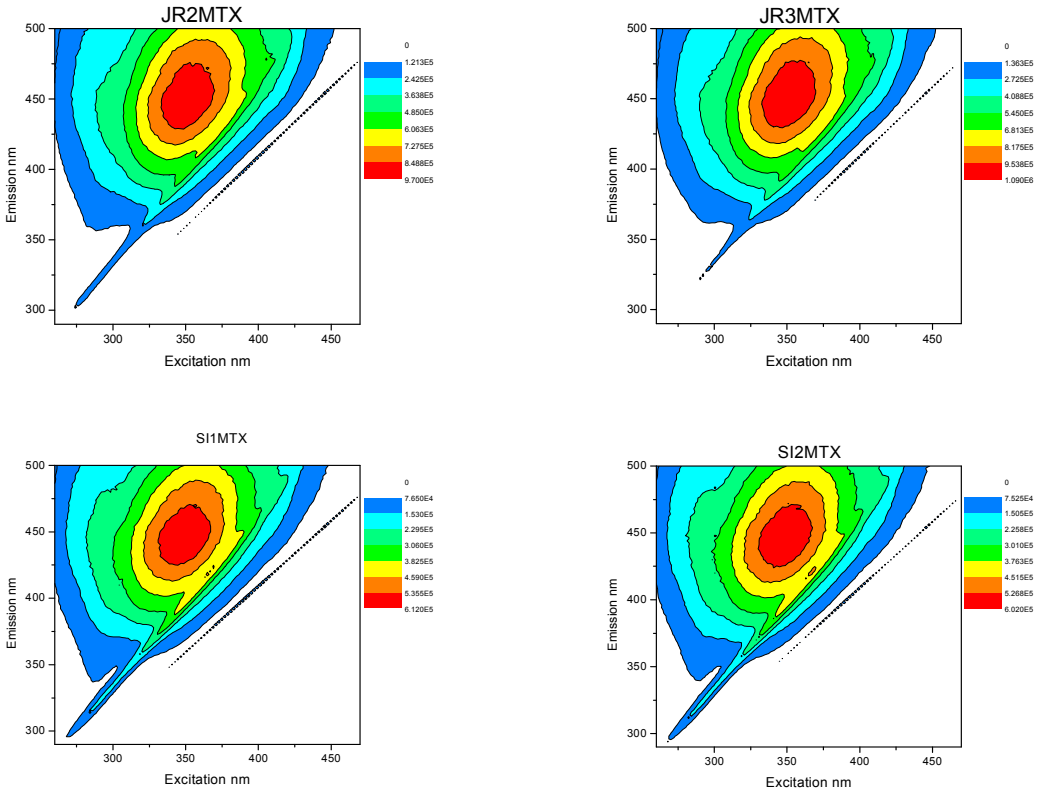
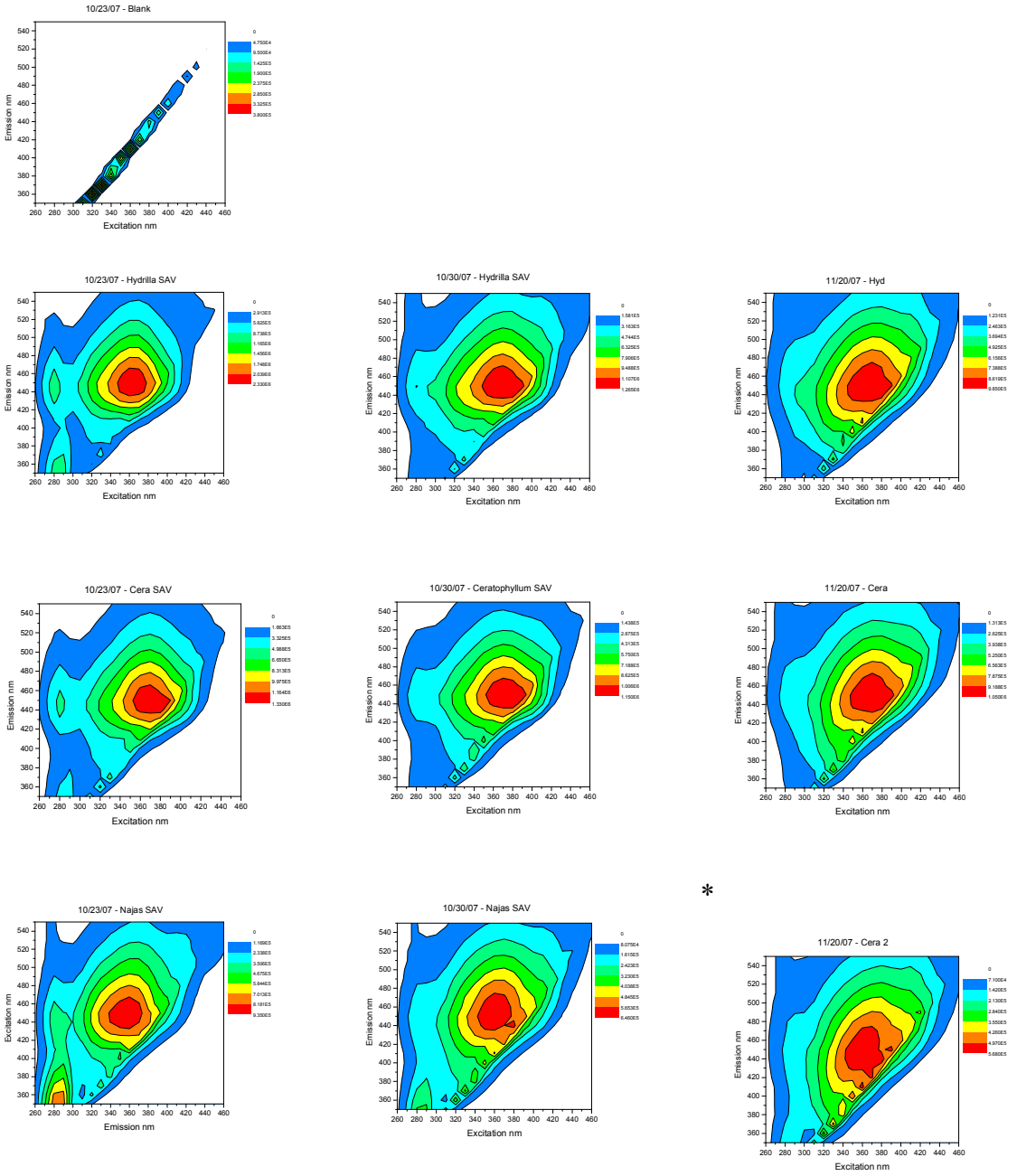


Figure 1: Bulk water EEM florescence results from *in situ* sampling in April 2008 from the Chickahominy River and the James River. Similarity of EEM's potentially due to large amounts of precipitation occurring prior to sampling.

T=0 T=7 T=11



*

Figure 2: SAV leachate EEM florescence analysis collected from 0, 7, and 11 days. *Miss labeled and is actually *Najas minor*.

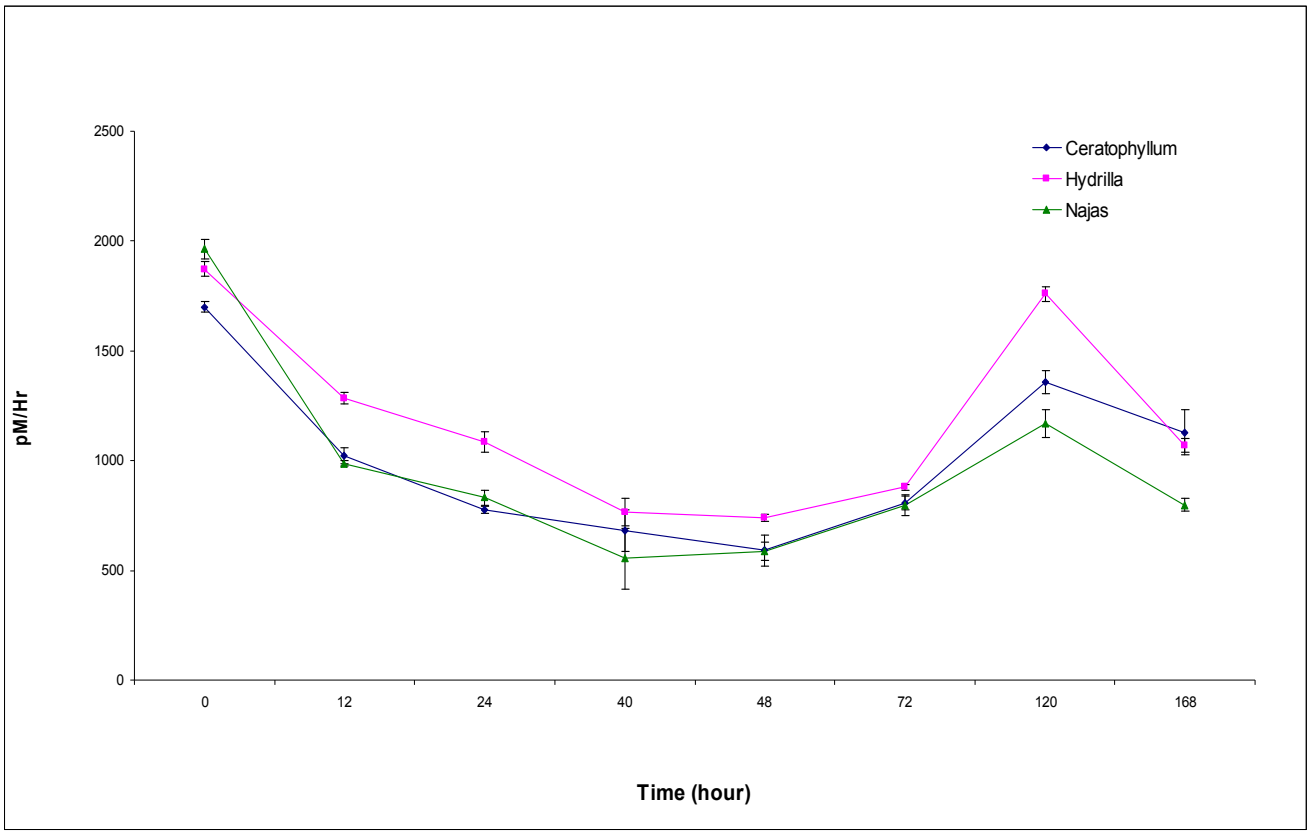


Figure 3: SAV species, Hydrilla verticillata, Ceratophyllum demersum, and Najas minor bacterial production rates. Error bars represent 1 standard error of the mean.

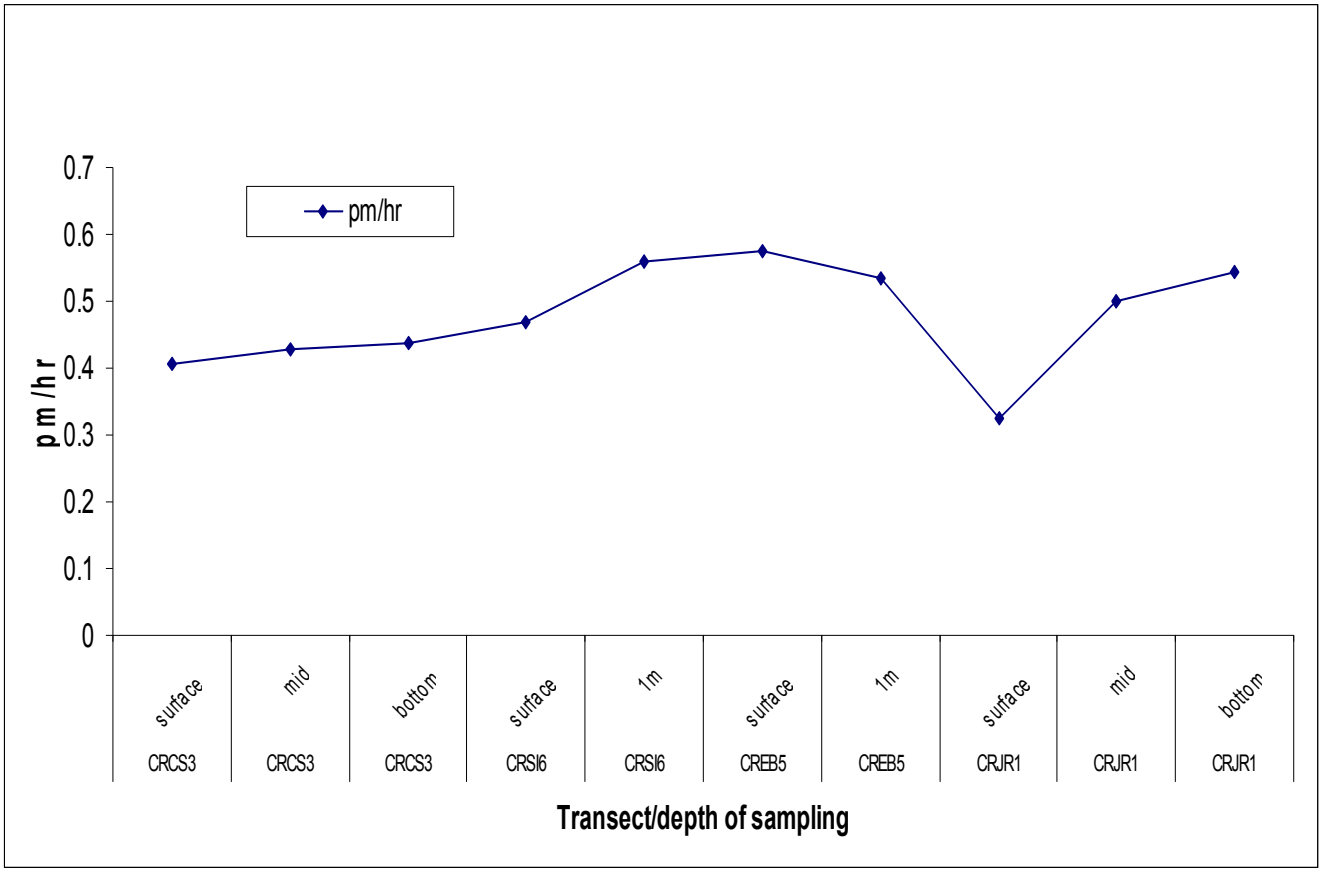
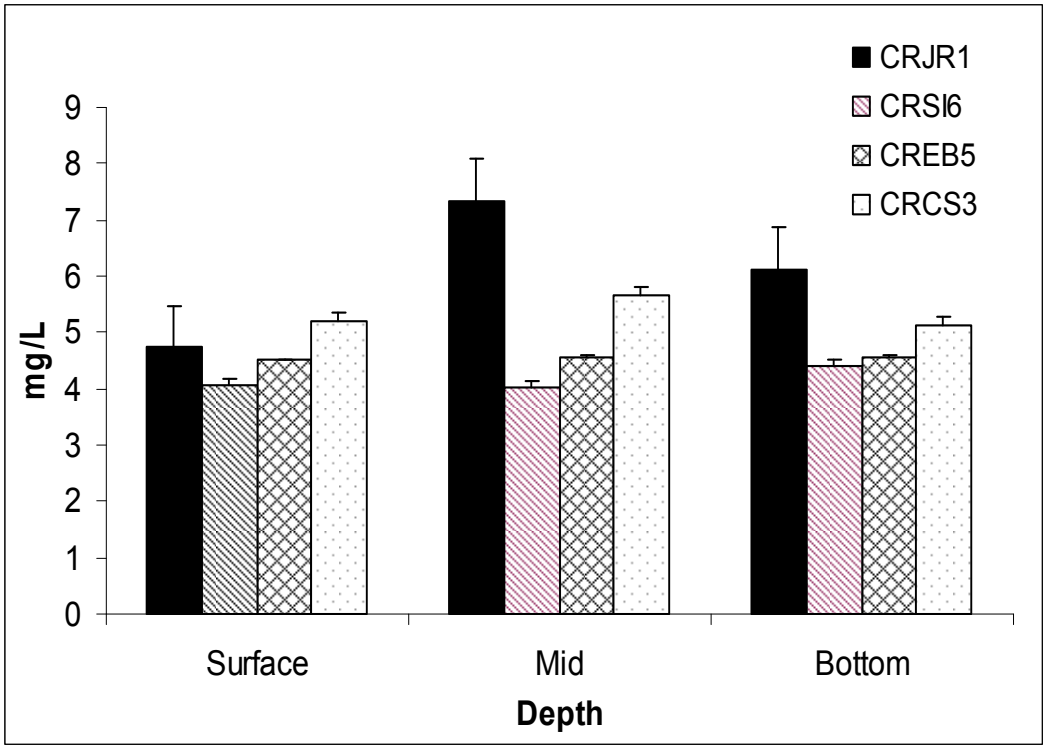


Figure 4: Bulk water *in situ* bacterial production measurements conducted in spring 2008.



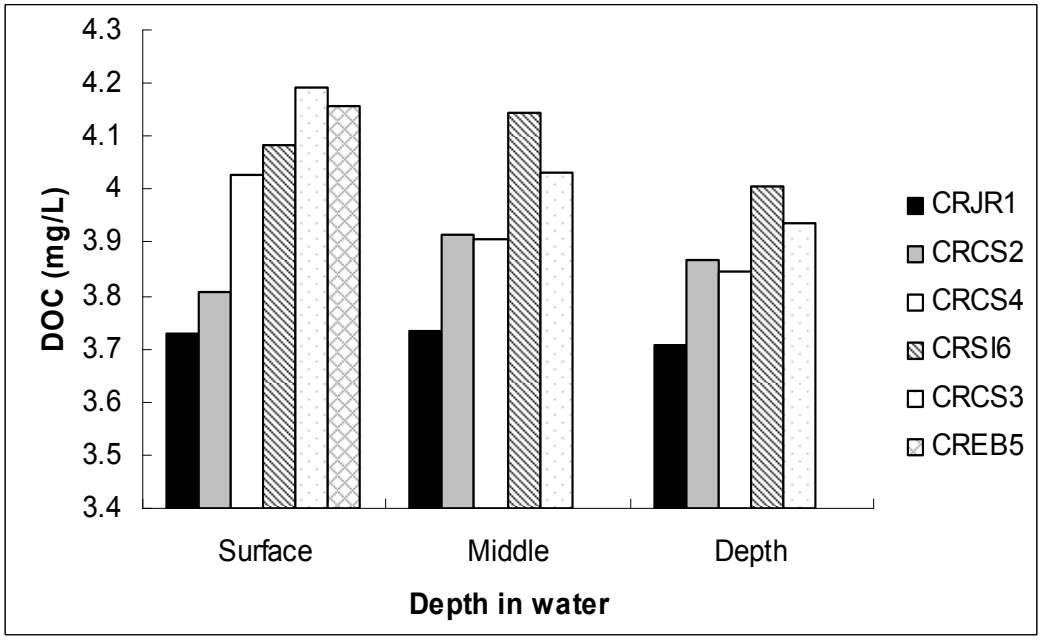


Figure 5: In situ bulk water DOC concentrations over spatial and temporal scale (April and July 2008). Error bars represent 1 standard deviation of the mean.

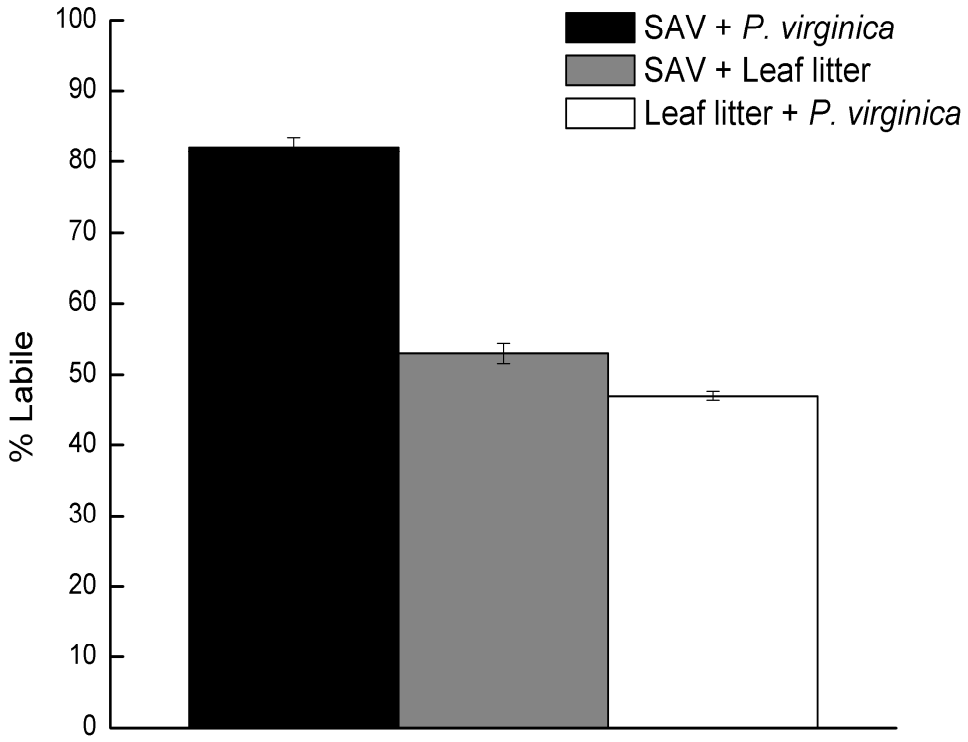


Figure 6 Lability (%) of treatments with 2 DOC sources of equal concentrations. Error bars representing I standard deviation of the mean.

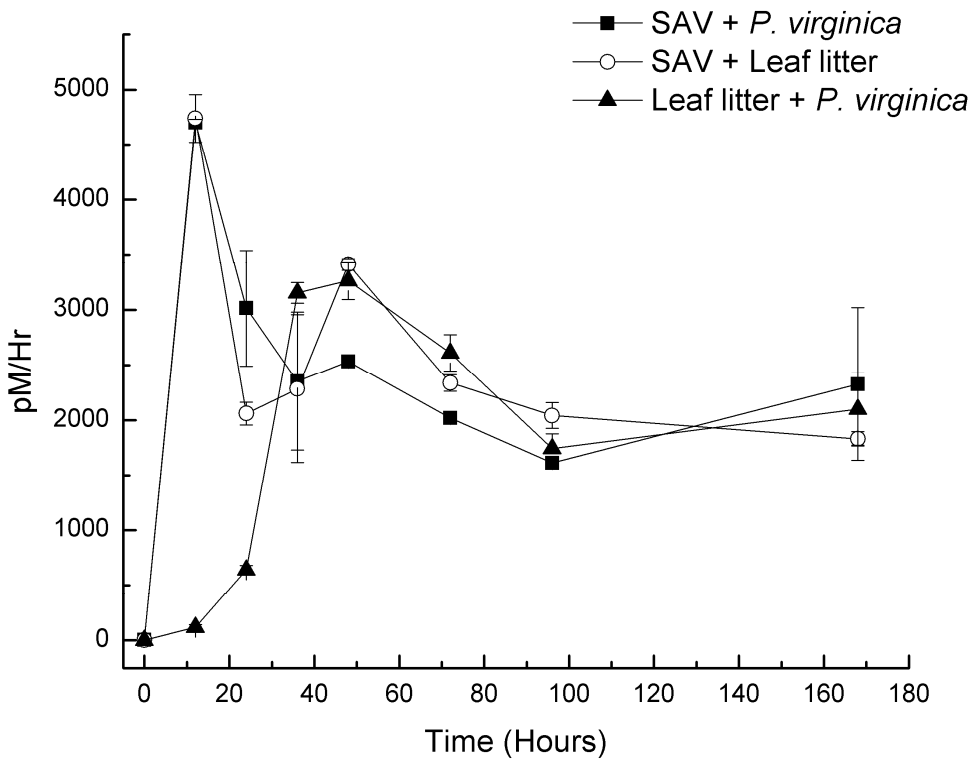


Figure 7 BP for each treatment of the course of a week. Error bars represent 1 standard deviation of the mean.

VITA

Anne Townshend Stuart was born on February 20, 1984, and is native to Richmond, Virginia. Anne's pre-college education was non-tradition as she was home schooled throughout most of elementary school and up through high school. In 2006, she graduated, *Cum Laude*, with a Bachelor of Science in Environmental Science degree at Lynchburg College, Lynchburg Virginia. Immediately following graduation she began work towards her Masters of Science at Virginia Commonwealth University. Other work experience obtained during her educational pursuits included work as a GIS intern with Worldview Solutions, Inc. and as a research assistant at Virginia Commonwealth University. Each opportunity has led to valuable experiences and training, applicable for future work and educational application.