ABSTRACT

Title of Dissertation: MACROINVERTEBRATE COMMUNITY STRUCTURE AND ECOLOGICAL FUNCTION IN MARYLAND COASTAL PLAIN STREAMS

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Abiotic conditions within streams, especially those conditions impacted by human activities, often influence the community structure and ecosystem function. While coastal regions have been strongly impacted by urban development and agriculture, little research has focused on characterizing the biotic community structure and function in these Coastal Plain streams. Such watersheds are characterized by low gradient, blackwater streams with sandy and silty substrate, coupled with large amounts of human disturbance. The objectives of this research were 1) to characterize the macroinvertebrate community and the chemical and physical characteristics of two Coastal Plain watersheds with differing landuse practices, 2) to examine appropriate macroinvertebrate sampling protocol comparing leaf pack and Maryland Biological Stream Sampling (MBSS) methods, 3) to compare these community structure measures with the functional measure

of leaf decomposition, and 4) to investigate potential mechanisms for shifts in decomposition due to elevated nutrients and dissolved organic carbon (DOC) concentrations in mesocosms. Results of three years of monthly sampling showed differences between watersheds in a number of chemical parameters, including nutrient concentrations. However, structural differences between the macroinvertebrate communities, using both three years of leaf pack sampling and a MBSS survey, were only identified for certain taxa and depended on the taxa resolution used. Also, two in situ leaf decomposition experiments using leaf decomposition tubes showed no differences in the macroinvertebrate or microbial contribution to detrital processing. Correlation analyses demonstrated that rates of decomposition were negatively associated with macroinvertebrate predator abundance and positively associated with the abundance of Ephemeroptera, Plecoptera, and Trichoptera (EPT) taxa in the community. Lastly, a laboratory mesocosm experiment illustrated the strong effect of DOC from blackwater streams in reducing rates of leaf decomposition and processing efficiency by a macroinvertebrate shredder. While abiotic measurements of Coastal Plain stream sites varied greatly both spatially and temporally, and the field experiments yielded little consistent pattern with the macroinvertebrate community, the mesocosm experiment demonstrated the strong inhibitory effect of DOC on detrital processing and processing efficiencies of a macroinvertebrate shredder. Thus, while measuring rates of decomposition may not be suitable as a biomonitoring tool to differentiate already

nutrient enriched Coastal Plain streams, it can add to stream assessments by providing a measure of ecosystem function where impacts are less subtle.

MACROINVERTEBRATE COMMUNITY STRUCTURE AND ECOLOGICAL

FUNCTION IN MARYLAND COASTAL PLAIN STREAMS

BY

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Dissertation submitted to the Faculty of the Graduate School of the
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2004

DEDICATION

To Dr. Leslie Baer, for showing me the importance of knowledge and discovery.

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Chapter 1. Patterns of chemical, physical, and biological characteristics in two Coastal Plain watersheds in Maryland and the consequences of non-point source pollution

Introduction:

Doubling of the world's food supply through modern agricultural practices doesn't come without its consequences. There have been dramatic increases in the use of fertilizers, the cultivated land cover, and the amount of irrigated land (Tilman 1999). These changes can lead to alteration in aquatic ecosystems, which are receiving waters to altered landscapes (Cooper et al. 1995, Carpenter et al. 1998, Arbuckle and Downing 2001, Niyogi 2003). It has been suggested that the extinction rate for aquatic fauna is occurring at faster rate than in terrestrial systems (Ricciardi and Rasmussen 1999). Agriculture can have several associated adverse effects on water quality and the biota that inhabit these aquatic ecosystems. Impacts include loss of riparian zone vegetation, increased sediment loads, nutrient enrichment, diversion and loss of water for irrigation, and potential contamination from pesticides, herbicides, and fungicides. Elevated nutrient loads have been documented throughout the world where intensive agriculture is practiced (Malmqvist and Rundle 2002, Donner 2003, Little et al. 2003).

Of particular interest are nutrient enrichment and the changes in the biotic community that result. Biomonitoring can be used as a tool to assess how natural or human derived disturbances can affect aquatic organisms (Karr and Chu 1999). For example, McDougal et al. (1997) showed that increased nutrient concentrations could cause community structure shifts. Other studies have shown changes in the community

composition due to agricultural influences (Stewart et al. 2001, Huryn et al. 2002, Shieh et al. 2003, Davis et al. 2003).

Specifically, studies have shown that percent forest cover and agriculture were important variables influencing both fish and macroinvertebrate communities on a large landscape scale, while embeddedness was a strong reach-scale factor (Stewart et al. 2001). Huryn et al. (2002) focused on the effects of non-point source nutrient loading to streams and how it affects the macroinvertebrate community structure and the rate for detrital processing. Their work showed that shifts in the richness of macroinvertebrate shredders, who function to decompose the terrestrially derived leaf material, explained differences in decomposition rates across different land use areas. They also illustrated different dominant taxa within the shredder functional feeding group changed with the varied landscapes.

Human derived disturbances, such as agriculture, urbanization, and forestry practices have the capacity to alter stream chemical and physical characteristics, which in turn, can have adverse consequences on the aquatic biota dependent. Karr and Chu (1999) illustrate the need to monitor aquatic environments to document biotic changes as indicators of anthropogenic changes to these systems. Long term monitoring to help characterize streams can aid in identifying shifts in community structure (Cairns and Pratt 1993).

The mid-Atlantic Coastal Plain region has been heavily influenced by anthropogenic impacts for hundreds of years due to its temperate climate, fertile alluvial soils and accessible river and coastal areas for trade (Cooper 1995). Agriculture in Maryland's Coastal Plain results in elevated levels of phosphorus (P) and nitrogen (N) in

running waters that eventually impact the Chesapeake Bay (EPA 1999). This nutrient enrichment was responsible for major shifts observed in the microorganisms, macrophytes, benthic fauna, fish, and crab communities within the bay (Davis 1985, Burkholder and Glasgow Jr. 1997, Boesch et al. 2001, Cronin and Vann 2003). Stratigraphic methods have demonstrated the dramatic changes in sediment transport, nutrient enrichment, and anoxia within the Chesapeake Bay. The 1800's saw major sedimentation transport due to up to 80% of the land being cleared, while the post 1940's has seen dramatic rise in nutrient transport due to fertilizers (Cooper and Brush 1991). Best management practices have been implemented in agricultural production systems throughout Maryland to abate some of the problems associated with non-point source pollution and its effect on the State's surface and ground water supplies. Although these practices have assisted in reducing some of the contaminant loads to streams, certain contaminants continue to appear in both the surface water and groundwater (EPA 1999).

The soil conditions and hydrologic influences in this region may contribute to the increased levels of soluble phosphorus in the streams (Bricker et al. 2003). The physical conditions of sandy soils with low clay content and the presence of organic materials, combined with low gradient watersheds with a relatively high water table level, create a reduced chemical condition where the P present in the system may not be readily bound to the soil (Brady and Weil 1999). In these environments, elevated available phosphate levels can be observed.

Additionally, a shallow water table environment allows nitrate from non-point sources to leach into the groundwater and readily enter the streams through groundwater flow. However, there are several natural processes that can attenuate nitrate as it moves

through shallow groundwater like those surficial flows found in Coastal Plain regions with low gradient landscapes. Plant roots that are in contact with this high water table have an opportunity to assimilate nitrates from the groundwater. Also, hydrologic and geologic conditions can facilitate denitrification, commonly mediated by bacteria, through a number of chemical pathways using nitrate as an oxidizing agent (Krantz and Powers 2000). As a consequence, Coastal Plain streams that are impacted by agriculture may be nitrogen-limited due to the relative high abundance of the available phosphorus. These changes in the available nutrient may alter the bottom up controls on the aquatic ecosystem (Miltner and Rankin 1998, Forrester et al 1999, Robinson and Gessner 2000, Barlocher and Corkum 2003).

Other studies have documented the macroinvertebrate community structure unique to swampy Coastal Plain streams (Smock et al. 1985). More recent work has demonstrated community metrics that can help to differentiate impaired stream conditions in Coastal Plain streams (Stribling et al. 1998, Davis et al. 2003). Here, I studied two watersheds, the Nassawango and Nanjemoy, which differed in land use, particularly for agriculture.

The objectives of this study of the Nassawango and Nanjemoy watersheds were to conduct a biohydrological survey to characterize the chemical, physical, hydrological and biological components of streams within two Coastal Plain watersheds, and relate environmental conditions to the biotic community structure observed. I hypothesized that differences in the water chemical and physical conditions would be associated with differences in the macroinvertebrate community structure when we compare an agriculturally impacted watershed with a more pristine watershed.

Study Site

This study was initiated in collaboration with The Nature Conservancy to conduct a biohydrological study on two watersheds in the coastal plains region of Maryland. The Nassawango Creek, south of Salisbury on the Eastern Shore, and the Nanjemoy, in southern Maryland, are the two systems of particular interest (Fig. 1.1). There are a number of rare plants and animals found in the wetland habitats of the Nassawango watershed (The Nature Conservancy 1996), while the Nanjemoy Creek is one of four unique Maryland environments home to the dwarf wedge mussels (*Alasmidonta heterodon*) (The Nature Conservancy 1998). The unique flora and fauna of these two stream systems has spurred efforts to gain more information about these habits. This was the primary reason that the current study was established. The objectives of this study were to provide a greater understanding of the patterns of these two aquatic conditions and to relate these parameters to the biotic communities in these environments.

These two Coastal Plain watersheds represent relatively less impacted watersheds in a landscape of urbanization, agricultural use, forestry practices and preservation efforts. While both of these watersheds have greater than 70% forested land cover, they differ in the remaining 30%. The Nassawango watershed extends from Worcester County to Wicomico County on the Eastern Shore. It is a tributary to the Pocomoke River that flows into the Chesapeake Bay. The Nassawango Creek is located between the towns of Salisbury and Snow Hill. The watershed has woodland, small farms, and residential areas. According to the Maryland Department of Natural Resources (MDNR), the watershed has an area of approximately 177.0 km^2. Based on 1994 landuse surveys, it consists of 2.3% urban, 25.8% agricultural, 71.7% forested, 0.2% wetland, and 0.1% barren land cover. This watershed has 24 % non-forested riparian zones. MDNR also

estimated that the fertilizer application rate is 3.1 kg/acre nitrogen and 0.2 kg/acre phosphorus (MDNR retrieved April 5, 2004). The watershed soils are dominated by sandy and sandy loam soils. These soil surveys characterized the soils as a Lakeland-Klej-Plummer association and a Pocomoke-Mattapex-Elkton association. The Lakeland-Klej-Plummer association spans a wide area, which has both steep gradients with excessively drained soils to very poorly drained sandy and loamy sand soils. The Pocomoke-Mattapex-Elkton association located in the upper watershed area consists of level to near level, very poorly drained to moderately well drained with sandy loam and sandy clay loam subsoils (USDA 1973).

The Nanjemoy Creek is located in Charles County and is a tributary of the Potomac River. The MDNR estimated that the watershed has an area of 188.6 km^2. The 1994 landuse survey shows that the watershed consists of 6.5% urban, 15.5% agricultural, 73.9% forested, 4.0% wetland, and 0.1% barren land cover. The watershed has 8 % non-forested riparian zones. It is also estimated that the fertilizer application rate is 2.0 kg/acre nitrogen and 0.1 kg/acre phosphorus (MDNR retrieved April 5, 2004). The soils within the watershed are a mix of clay, small cobble, silt and sand. The soils are comprised of Bibb-Tidal Marsh-Swamp association in the upper part of the watershed. These areas have a level to moderate slope, a moderately well drained loamy soils, and only moderately deep to a hard dense fragipan. The lower reaches of the watershed are considered a Beltsville-Exum-Wickham association, characterized by a level or near level slope and poorly drained soils on the flood plain (USDA 1974).

Although both watersheds have large amounts of forested lands I considered the Nassawango to have more intensive agricultural practices and overall reduced riparian

buffers as compared to the less disturbed, forested watershed within the Maryland Coastal Plains. Each of the five study sites within each watershed was located on Nature Conservancy lands with readily available access. Sample sites were selected with the cooperation of the Nature Conservancy (Table 1.1). Sample sites were selected in wooded riparian region along the stream corridor and consisted of 2nd to 4th order streams.

Methods:

Chemical, physical, hydrologic, and benthic macroinvertebrate samples were collected from August 1998 to August 2001 in the two watersheds in the Maryland Coastal Plain region: the Nassawango Creek in Worchester and Wicomico Counties, and the Nanjemoy Creek in Charles County.

Chemical and Physical sampling

Water samples were collected monthly for a three year period to determine a suite of chemical and physical characteristics of the watersheds. Table 1.2 provides a list the parameters measured on a monthly basis. Both 500 ml brown glass bottles and 125 ml plastic bottles were used to collect the water samples. Both bottles were soap washed, and rinsed with deionized water prior to sampling. The brown glass bottle, as well as all other glassware used in the water chemistry analyses had an additional 1:1 HCL acid rinse followed by deionized water cleaning to ensure that no phosphorus was bound to the glass. The water in the glass bottle was used to analyze the nutrient concentrations, while the water in the plastic bottle was used to determine the alkalinity, hardness, and turbidity levels. All chemical tests, with the exception of total phosphorus and fluoride,

were tested the same day water samples were collected. The total phosphorus and fluoride tests were performed within a two-day period of water collection.

All water samples were stored on ice until returned to the laboratory. Samples were then stored at 4°C until the remaining chemical measurements were made. The chemical and physical parameters, including nitrate and nitrite-nitrogen, reactive and total phosphorus, alkalinity, hardness, and turbidity were measured from one water sample for the first one and one half years of this study. For the second half of the study three water samples were used in order to get an estimate of variance for each of these parameters.

The nitrate and nitrite-nitrogen, reactive and total phosphorus, and fluoride were analyzed using a Hach DT-890 Colorimeter. This is a spectrophotometric technique to measure color changes that quantify a chemicals relative concentration in solution. The combined nitrate and nitrite-nitrogen were measured using a modification of the cadmium reduction method, which reduces the nitrates (NO_3^-) in the water sample to nitrites (NO_2^-). Under acid conditions nitrite ions then react with sulfanilic acid forming a diazonium salt. This salt in turn creates an amber colored compound when it reacts with gentisic acid. This method replaces 1-naphthylamine with gentisic acid to bring about the color change (Hach 1998). Two cuvettes were rinsed three times with stream water followed by 10 ml of sample stream water being placed in each cuvette. A packet of NitraVer5 chemical pillow was added to one of the cuvettes while the second cuvette was used as a blank to zero the colorimeter. After adding the chemical packet the cuvette was shaken for 1 minute followed by a waiting period of 5 minutes before measuring the nitrate-nitrogen concentration (Hach 1998). The colorimeter was zeroed using the sample blank before each reading.

The soluble reactive phosphorus (SRP) concentration was measured using the ascorbic acid method. Orthophosphates reacted with molybdate under acid conditions to create a phosphomolybdate complex. This complex was then reduced by ascorbic acid that forms a blue solution due the formation of a molybdenum species. 10 ml of water sample was poured into two cuvettes. A PhosVer3 Phosphate reagent powder pillow was added to one of the cuvettes and shaken for 15 seconds. The colorimeter was zeroed using the sample blank before each reading. The cuvette with the chemical reaction was then placed in the spectrophotometer and the phosphorus concentration measured. Each of the chemical tests had a preprogrammed channel on the Hach DR 890 spectrophotometer so no manual wavelength adjustments were necessary between chemical tests (Hach 1998).

In order to measure total phosphorus (TP), an additional acid digestion step was necessary to convert all organic and inorganic phosphates to organophosphate prior to analysis. Using a graduated cylinder, 25 ml of water sample were poured into a 75 ml Erlenmeyer flask. The sample was acidified using 2 ml of a 5.25 N sulfuric acid solution. The sample was then heated to a low boil for 30 minutes while ensuring that approximately 20 ml of sample was maintained. Deionized water was added to the sample as needed to maintain volume. The samples were then cooled to room temperature and neutralized, using 5.0 N sodium hydroxide. The pH was adjusted using dilute sodium hydroxide and sulfuric acid solutions until it was stabilized between pH 7.0 and 8.0. The sample solution was then measured in a graduated cylinder and deionized water added until the total sample volume equaled 25 ml (Hach 1998). This sample was then divided with 10 ml used as a sample blank for the colorimeter and the other 10 ml

used as the test sample, which was then tested using the same reactive phosphorus methods previously described.

Fluoride measurements are used to assess whether there is municipal waters in the stream waters from upstream water treatment plant, sewage treatment, or other sources of community water that was previously fluoridated. Fluoride concentration is determined using a solution of sodium arsenite and red zirconium-dye (SPADNS reagent). The dye bleaches in an amount proportional to the fluoride concentration present. The test was performed by pipetting 10.0 ml of sample water into one cuvette and 10.0 ml of deionized water into a second cuvette. To both cuvettes, 2.0 ml of SPADNS reagent was added and swirled to homogenize the solution. After one minute of reaction time the colorimeter was zeroed using the deionized water solution, followed by the stream water sample being read (Hach 1998).

Conversion to chemical concentrations from absorbance reading using the colorimeter was automatically calculated using the Hach DR 890. This calculation is based on Beer's Law relating spectrophotometric absorbance to the relative concentration of a compound in solution. Beer's Law is expressed as:

$OD = eCL$ where OD is the absorbance, e is the extinction coefficient, C is the chemical concentration, and L is the length the light travels, which is the width of the cuvette in the spectrophotometer (Kegley and Andrews 1998).

Standards were run on the Hach colorimeter early in 1999 and in 2001 to ensure that the machine was reading accurately. Also, each new package of chemical packets was run with deionized water to calculate the reagent blank to be subtracted from the

colorimeter reading. This information was input into the DR 890 directly, so the colorimeter automatically adjusted the output results.

A digital titrator, Hach model 16900, was used to quantify both the alkalinity and hardness of the stream water samples. The alkalinity measures the neutralizing capacity of the water that is predominately due to the presence of bicarbonate and carbonate. The phenolphthalein method converts bicarbonate and carbonate to carbonic acid if acidified to pH 4.5. The Hach method uses a bromocresol green-methyl red color indicator to identify when the reaction is complete. The reaction changes the blue indicator to a pink color. Total alkalinity was measured by pouring 100 ml of the water sample in a 250 ml Erlenmeyer flask. Then one phenolphthalein indicator powder pillow to the sample and a color change identified. Because all the samples did not show a color change the method called for the addition of one bromocresol green-methyl red indicator powder pillow prior to titration. A sulfuric acid 1.6 N solution was digitally titrated into the water sample while swirling flask. The reaction was stopped when a light pink color appeared indicating a pH of 4.5 was reached. The amount of sulfuric acid added was then used to determine the concentration of calcium carbonate present in the original water sample. This was determined by multiplying the number of digits added by 1.0 to yield (mgL^{-1}) total alkalinity as calcium carbonate (Hach 1996).

Hardness measures the concentrations of dissolved minerals, comprised mostly of divalent cations. These are primarily magnesium and calcium, but also include iron, zinc, manganese, and strontium. Water hardness was measured by titrating EDTA into the water sample with calmagite. The Hach method measures calcium hardness, which accounts for the hardness in the water sample due to calcium ions in solution. Following

the Hach procedure 50 ml of water sample were poured into a 250 ml Erlenmeyer flask with an additional 50 ml of deionized water. The addition of 2 ml 8.0 N potassium hydroxide was added to this solution and swirled. One packet of CalVer 2 was added and swirled. This was followed by digitally titrating 0.714 M ethylenedianimetetraacetic acid (EDTA) into the water sample solution with the Hach digital titrator model 16900. The titration was stopped when the color of the water sample changes from pink to blue. This occurred at pH 10.1. The amount of EDTA added was then used to determine the concentration of calcium ions present in the original water sample. This was determined by multiplying the number of digits added by 0.1 to yield (mgL^{-1}) calcium hardness as calcium carbonate (Hach 1996).

A Corning Checkmate II handheld field meter was used to record pH, dissolved oxygen, conductivity, and temperature. Each probe was calibrated in the laboratory prior to each sampling effort. In addition to measuring the parameter of interest all three of the probes recorded temperature. For the purpose of consistency, the temperature used in the analyses was recorded from the pH probe. The pH and conductivity was measured immediately from a sample of stream water collected in a plastic cup. Dissolved Oxygen was measured by placing the probe in the water and allowed to stabilize. Each parameter was measured three times in order to estimate variance.

Turbidity was measured using a visual assessment of particulates in the stream water solution. The first of two modified volumetric cylinders was filled with 50 ml stream water, while the second was filled with 50 ml deionized water. A standard turbidity reagent (LaMotte Company reagent 7520) was added two drops at a time to the deionized water cylinder until the visual clarity of a black dot on the bottom of the

cylinder appeared to be the same. The number of drops added to equalize the visual opacity between the two cylinders was then divided by 2 in order to calculate the Jackson Turbidity Units (JTU's) (LaMotte Co. 1996).

The photosynthetic available radiation (PAR) was measured using a Decagon Sunfleck Ceptometer (Decagon Devices, Inc. 1989). The PAR was measured in three places at each site, in direct open canopy sunlight, along the stream-side riparian area (referred to as the stream bank), and above the in-stream water surface. A mean of 5 PAR measurements was calculated for each of the three places for each site. The proportion of sunlight reaching the stream water surface and the stream bank are then compared across sites and watersheds. Both the stream bank PAR and in-stream PAR were divided by the open canopy PAR to calculate these proportions.

Stream discharge was measured to assess the relative magnitude of hydrologic force each watershed and sites within watershed experience. The flow regime is a crucial environmental parameter that affects the biotic community within the stream. The discharge was calculated by first measuring the cross-sectional width and depth of the stream in order to determine cross-sectional area. The depth measurements were taken every 50 cm and the water velocity was measured, using a Marsh-McBirney flow meter, half way in between each depth measurement (Marsh-McBirney Inc. 1990). In this way the velocity per unit area could be calculated for every 50 cm segment of the cross-section and summed together for the stream discharge. The water velocity was measured at two-thirds depth of the stream water. This is to obtain the average water velocity in the vertical column (Leopold et al. 1992). A standardized discharge was also calculated on a per unit area basis as another way to compare discharge across sites. Additionally,

qualitative sediment characteristics were measured for each of the sample sites using the EPA's Rapid Bioassessment Protocol (Barbour et al. 1999).

Biological Sampling

Artificial leaf packs were used to collect benthic macroinvertebrate samples three times each year; spring, summer, and fall. Red maple (*Acer rubrum*) leaves were selected for my study, because they are commonly found in both watershed study areas and are one of the terrestrially derived food sources that the benthic community readily utilizes. They have also been shown to have a moderate rate of decomposition, with medium range k-values (approximately 0.0075 - 0.0060 day^{-1}) and a relatively small variance (Petersen and Cummins 1974, Webster and Benfield 1986). Five grams of desiccated red maple leaves were bound to a brick and left in the stream for 30 days. Eight replicate leaf pack samples were deployed at each site. The leaf packs were placed across the cross-section of the stream in order to measure the community structure including the organisms that prefer the slow water edge and the ones found in the faster mid-stream region. The leaf packs were then collected, put in plastic bags and stored on ice until returned to the laboratory where they were preserved at 4° C until processed.

To process the samples the leaves were rinsed in a pan and all the leaf material washed and discarded, leaving the macroinvertebrates in the pan of water. The pan contents were then filtered through a 425-micrometer mesh size sieve to collect the macroinvertebrates. Each sample was labeled and preserved in 80% alcohol solution. The macroinvertebrates were sorted and identified to genus level or as far as was taxonomically possible (McCafferty 1983, Merritt and Cummins 1996, Peckarsky et al. 1990, Wiggins 1996, Williams 1972, Mackay 1978). The family Chironomidae was the

exception with individuals identified to the subfamily level. The mean number of taxa, and abundance of individuals were calculated per brick per site. This standardized the data across sites in case there were missing leaf packs at the time when the leaf packs were collected. The taxa present and absent at each site was determined and used to calculate a number of indices including; the number of taxa, the number of Ephemeroptera, Plecoptera, Trichoptera (EPT) taxa, the proportion of Ephemeroptera in the community, the proportion of Tanytarsini in the Chironomidae family, the Beck's sensitive taxa index, the number of macroinvertebrates that are in the functional feeding group 'scrapers', and the proportion of macroinvertebrates considered 'clingers' in the community. The classifications used for sensitive taxa, functional feeding groups, and taxa habit (e.g. clinger) were based on the MDNR classification (Stribling et al. 1998). Maryland's Montgomery County Department of Environmental Protection taxa listings, and Merritt and Cummins (1996) taxa information were used as additional resources when taxa characteristics were missing. These above metrics were then averaged to create an Index of Biological Integrity (benthic IBI) based on the MDNR methods (Stribling et al. 1998). These metrics were selected because the Maryland Biological Stream Survey had analyzed what measures of the community best differentiated impaired streams in the Coastal Plain region (Stribling et al. 1998). Additional metrics including, total abundance, the Shannon diversity index, the number of taxa were considered predators, shredders, collectors, filterers, and scrapers, as well as the percent each of these five functional feeding groups were assessed to incorporate other trophic measurements when evaluating the watersheds (Barbour et al. 1999).

Landscape parameters

A global positioning system (GPS) was used to determine the coordinates of each sample site within the two watersheds. A correction antenna was used to improve the accuracy of the coordinates received. The points were then converted to units that could be recognized by a GIS software program called GISHYDRO developed at the University of Maryland (Moglen and Casey 2000). Using this software the watersheds were delineated for the area above each of the sample points within the two watersheds. The program also provided several landscape characteristics for each of the sites, including, channel slope, land slope, percent agriculture, percent urbanization, percent impervious surface, and percent forest cover.

Data Analysis

A mixed model analysis of variance (ANOVA) was used to determine the significant chemical, physical, and hydrologic parameters that differentiated the two watersheds (SAS Institute Inc. version 8.2). However, it was necessary to log transform some of the explanatory variables prior to the ANOVA to satisfy the assumptions of normality and homogeneity of variance. Several of the parameters were analyzed using a nonparametric Kruskal-Wallis tests when the assumptions of normality were not met (Table 1.3). This method was also used to distinguish sites within each watershed that were significantly different from the majority of sites. The mean per leaf pack per site was calculated to standardize the number of macroinvertebrates found per leaf pack for further biotic community structure comparisons. The community taxa information was then compared across watersheds using the Atchison's log ratio test for the taxa at the order level, and the functional feeding groups. ANOVA and a non-parametric, Kruskal-

Wallis test were used to compare community metrics, including the ones used by MBSS as well as total abundance, the Shannon diversity metric, and the abundance and numbers of taxa in functional feeding groups. Also, rank abundance curves were created to compare the taxa diversity within and across watershed. Multiple regressions, using stepwise selection method, were used to identify the key environmental parameters that can help explain the critter community structure and the variance observed in the sampling of these two watersheds.

Results:

The chemical, physical, and biological database compiled for this study, from August 1998 to September 2001, is available from the author, or Dr. William O. Lamp at the University of Maryland, College Park.

Chemical and physical parameters

There were significant differences between the Nanjemoy Creek and the Nassawango Creek watersheds both chemically and physically. The Nassawango Creek watershed had significantly higher nutrient and ion concentrations, as measured by SRP, TP, nitrate-nitrite nitrogen, alkalinity, hardness, and conductivity parameters (Table 1.3). All but the ratio of stream to direct sun PAR were significantly different between the two watersheds with $p<0.0001$. Both the phosphorus (SRP and TP) and the nitrate-nitrite nitrogen concentrations yielded greater values in the Nassawango Creek than in the Nanjemoy Creek watershed. Fluoride concentrations were also significantly higher in the Nassawango Creek watershed ($p<0.0001$) (Table 1.3). Measurements of overall ion concentrations, both anion and cation followed the same trend with Nassawango Creek

watershed having higher alkalinity, hardness and conductivity concentrations than Nanjemoy Creek watershed (all with $p<0.0001$).

Dissolved oxygen concentrations and turbidity measures were lower, while the pH levels were higher in the Nassawango Creek watershed (all with $p<0.0001$). Because the Nassawango Creek watershed was larger in overall area it is not surprising that this watershed has significantly great discharge ($p<0.0001$). The light availability to the stream and bank areas, as measured using PAR, showed that two watersheds did not differ in stream PAR to open sunlight PAR ratio, while the bank PAR to open sunlight PAR ratio was larger (0.46) in the Nanjemoy Creek watershed ($p<0.0001$).

Reach scale differences in substrate composition between sample sites demonstrated within watershed variability of substrate ranging from clay dominated to sand or gravel dominated in the Nanjemoy Creek and sand, silt or clay dominated sites in the Nassawango watershed (Table 1.4). There were also differences in the percent organic matter present in the substrate. Sites such as MOC, TBC, and HBC in the Nassawango Creek, and PTR in the Nanjemoy Creek had relatively greater organic matter content in the substrate when compared to the other sample sites within each watershed.

Results from the GISHydro2000 analysis for each of the sample sites provided landscape information both on physical attributes and landuse (Table 1.5). The Nanjemoy Creek sample sites ranged in watershed area from 4.9 km^2 in the second order stream to 37.8 km^2 at the highest order sample site (NMS), while the Nassawango Creek had sample sites with contributing watershed areas ranging from 7.8 km^2 for HBC to 113.0 km^2 at RTE12. The overall channel slope was steeper for the Nanjemoy site, with

a mean of 3.4 m/km, than the Nassawango sites averaging a channel slope of 0.6 m/km. Percent urbanization ranged from 6.9% to 14.6% in the Nanjemoy Creek watershed as compared to the 0.0% to 12.6% in the Nassawango Creek. Agricultural landuse was between 10% and 17% in the Nanjemoy watershed compared to a range of 14% to 48% in the Nassawango watershed. The HBC sample site in the Nassawango had the highest agricultural landuse with 48%. The forest cover in the Nanjemoy Creek had a range of 69.6% to 81.3% while the Nassawango Creek sample sites were much more variable, ranging from 31.8% at HBC to 87.9% at STC.

Macroinvertebrate sampling

The macroinvertebrate sampling collected over the 8 sampling dates from fall 1998 to spring 2001 consisted of 10,896 individuals comprising 106 different taxa with the majority identified to the genus level. Mean abundance and the relative percent of each taxa metrics are presented by season for each watershed (Table 1.6). For all seasons and within each watershed Diptera taxa dominated the community structure. Within watershed variation was demonstrated using rank abundance curves for the sample sites (Fig. 1.2 and Fig 1.3). These graphs illustrate that these sites were dominated by a few taxa. It also shows that in both watersheds, the highest order stream furthest downstream in the watershed, RTE12 in the Nassawango Creek and NMS in the Nanjemoy Creek, had the shallowest slope indicating the most even community structure of all the sites sampled.

None of the Maryland Biological Stream Survey (MBSS) taxa metrics differentiated the two watersheds. However, the Shannon diversity index ($p<0.03$) (Fig. 1.4), the number of predator taxa ($p<0.01$), and the abundance of predators ($p<0.001$)

(Fig. 1.5) were found to be significantly different between watersheds (Table 1.7). The abundance of scrapers was marginally significant ($p<0.06$) when comparing the two watersheds. Overall the MANOVA analysis showed that the Nanjemoy and Nassawango Creek watersheds differed in community structure based on order level information ($p<0.0001$) (Table 1.8, Fig. 1.6). The watershed also differed using the FFG partitioning of the community ($p<0.0001$) (Table 1.9, Fig. 1.7).

Multiple Regression Models

As much as 39% of the taxa metrics variance could be explained by the explanatory variable measured. There were a number of the community metric regression models that had similar environmental parameters explaining some of the variation observed (Table 1.11). Dissolved oxygen, pH, temperature and fluoride all contributed to explaining several of the metrics measured. Of particular interest were those models that contained only a few environmental parameters. For instance, 31% of the variation in the number of taxa could be explained by temperature, dissolved oxygen, and watershed area ($p<0.0001$) (Table 1.11). Dissolved oxygen was the only significant explanatory variable that explained the number of EPT taxa present ($p<0.045$). However, the dissolved oxygen only explained 4% of the variation. Although significant, ($p<0.025$), only 9% of the variation in the percent of mayflies, Ephemeroptera, present was explained by pH, conductivity, and temperature. A portion of the variability in the percent of filter feeding, Tanytarsini, (19%), was due to turbidity, pH, and temperature. Dissolved oxygen and watershed area explained 25% of the variance in the number of scrapers ($p<0.0001$). Alkalinity, pH, and conductivity contributed to explaining 19% of the variance of the abundance of predators in the community ($p<0.0006$), while only 8%

of the variability of the abundance of shredders was explained by fluoride, alkalinity and percent forest cover ($p<0.035$). Of particular interest was that fluoride and the percent urbanization accounted for 19% of the variance in the percent shredders in the community ($p<0.0001$). Also the percent filters were influenced by the turbidity ($p<0.051$).

Discussion:

This study showed significant differences in the chemical and physical habitats between the Nanjemoy and Nassawango Creek watersheds. As predicted the Nassawango watershed with its non-point source agriculture influences, had significantly higher nutrient levels (Table 1.3). Both the elevated nitrate and phosphorus concentrations measured over this three-year study are supported by the Maryland Biological Stream Survey (MBSS) performed in 2000. The MBSS study illustrated the higher nutrient concentrations within the Eastern Shore watersheds than in the Nanjemoy watershed (Roth et al 2001).

Streams without anthropogenic influences commonly have phosphorus concentrations of 0.025 mgL^{-1} (Allan 1995). While both watersheds exceeded this level, indicating some human or unique geological derived nutrient influences, the Nassawango Creek had significantly great SRP and TP concentrations (Table 1.3). The elevated phosphorus levels probably have two origins; the intensive agricultural practices of row cropping and poultry farms within the Nassawango watershed, and the underlying geology. The Nassawango watershed has bog-iron ore or swamp ore deposits which can contain up to 10% phosphorus (Singewald 1911). Both phosphorus rich sediments

flowing into the streams from agricultural areas and the leaching of phosphorus from the bog ore under low pH conditions could increase the phosphorus concentrations in the streams.

Although nitrate-nitrite nitrogen concentrations were higher in the Nassawango Creek watershed, they were still relatively low levels (0.48 mgL^{-1}±0.05) for having row crop and poultry farming located in the watershed when compared to other Eastern Shore agricultural areas (US EPA 1999). There may be several reasons for the low nitrogen levels. Coastal Plain stream environments are conducive to denitrification due to the high organic content, and potentially anoxic conditions along the stream bottom or within the groundwater. There is also the potential for riparian vegetation to uptake the nitrogen from the high water table (Krantz and Powars 2000). These conditions create a novel stream condition where nitrogen limitation may occur for the in-stream biotic community. This is uncommon, as stream systems are often found to be phosphorus limiting due to more common elevated nitrogen inputs (Allan 1995).

All measurements of ion concentrations, alkalinity, hardness and conductivity showed significantly greater levels in the Nassawango Creek watershed again supporting my assertion of non-point source influences on the water chemistry. Differences in pH showed that overall the Nanjemoy Creek had lower pH levels than the Nassawango (Table 1.3). Maryland Department of Natural Resources (2001) reported the greatest extent of low pH levels (<pH 6) occurred in both the Nanjemoy Creek during spring 2000 sampling and the Lower Wicomico, a nearby stream to the Nassawango in summer 2000 during their Maryland Biological Stream Survey (MBSS) sampling period (Roth et al

2001). This suggests that although the Nanjemoy and Nassawango Creeks had different pH levels they are both relatively low when compared to other Maryland streams.

There was a lower concentration of dissolved oxygen in the Nassawango, which may be due to lower channel slopes, or differences in the primary production within the streams. However, both watersheds had a wide range of dissolved oxygen concentrations including very low values, which suggest that anaerobic conditions occur within both of these systems. Another interesting finding was the higher turbidity levels measured in the Nanjemoy watershed. Although this is significantly more turbid it should be noted that these values are very close to one another and is still relatively low turbidity. The differences in discharge may be explained by the larger stream size of the Nassawango stream sites with overall larger watershed area contributing to the discharge (Table 1.5). Lastly, the Nanjemoy appears to have greater riparian canopy cover based on comparing bank and direct sunlight photosynthetic available radiation (PAR) between watersheds.

Interestingly, these Coastal Plain streams are dipteran dominated environments. This is most likely due to a combination of tolerance to relatively low dissolved oxygen levels and sandy/silty substrate. Nearly 50-70% of the taxa within each watershed were Diptera (Table 1.6). Similarly, Wright and Smock (2001) found Chironomidae taxa dominated the macroinvertebrate abundance in Coastal Plain watersheds they studied. Significant shifts in the community structure were difficult to show due to the high between site and seasonal variability. This study was conducted from Fall 1998 through Spring 2001, during which time the Mid-Atlantic region suffered from drought conditions, potentially adding to community variability. In addition this study had only a

small number of seasonal replicates, making it difficult to show differences in community structure at the finer seasonal scale.

Also, there were a large number of rare taxa (each constituting less than 1% of community structure) that made up the community structure. It is a challenge to incorporate these taxa into the analysis without lumping taxa into larger taxa, such as family or order level. It becomes difficult to identify environmental constraints that cause shifts in genera when they are pooled into a larger category. Although the taxa metrics used by the Maryland Department of Natural Resources did not differentiate the two watersheds, comparisons of the entire community structure using the Atchison's log ratio multivariate analysis (Atchison 1986) indicated a significant difference between the two watershed communities, when comparing structure based on the taxa Orders, and functional feeding groups (Table 1.8 and Table 1.9). While this method did group all the taxa into the larger taxa Orders, it analyzed the community holistically rather than trying to identify individual taxa changes. The log ratio analysis also illustrated the significant seasonal variation of both the community structure and the FFGs (Table 1.8 and 1.9). A limitation of this method is it does not indicate which taxa are directly responsible for the differences in community structure. Interestingly, the only community metrics that were different between the two watersheds were the Shannon diversity index, abundance of predators and scrapers, and the number of predator taxa. All but the abundance of scrapers was significantly greater in the Nanjemoy watershed.

King and Richardson (2003) developed nutrient-IBI, which were able to identify shift in the community structure based on elevated phosphorus. This technique offers great promise for assessing nutrient enrichment for site comparisons having phosphorus

levels above and below a phosphorus threshold. However, because both the Nassawango and Nanjemoy watersheds had total phosphorus concentrations well above the 12-15 μg/L threshold suggested, this bioassessment tool may be limited in these Coastal Plain watersheds. Other studies show that while EPT taxa and the abundance of Tanytarsini taxa declined with increasing nutrients there were mixed results with other macroinvertebrate taxa and functional feeding groups responding positively to the nutrient enriched environment (Miltner and Rankin 1998).

While few of the community metrics differentiated the two watersheds, patterns are revealed about what influences some of these metrics when multiple regression models are developed. These regressions demonstrated significant models with a number of environmental parameters with a range of 4 to 38% of the variation explained. Temperature, pH, and dissolved oxygen were the environmental explanatory variables used most frequently to describe the variation within metrics. While some of the models contained numerous significant parameters others were limited to only one significant explanatory variable. Of particular note, the percent filter feeding taxa were negatively associated with turbidity (Table 1.11). Other taxa, such as EPT, increased as dissolved oxygen concentrations increased. Still others responded to landscape alterations. The percent shredders were negatively impacted by both fluoride concentrations and percent urbanization. Other FFG metrics such as the percent collectors increased as the amount of bank light availability and percent forest cover declines. This seems to support the River Continuum Concept where it is predicted that community structure will shift to a greater proportion collectors as the river widens and canopy cover is reduced (Vannote et al. 1980).

Overall, the landscape parameters measured, including channel and land slope, percent urban, impervious, and forest cover did not account for the variability in the metrics used by the Maryland Department of Natural Resources to classify impaired Coastal Plain streams. However percent urban and percent forest contributed significantly to the regression models assessing FFG metrics and total abundances (Table 1.10). While the Maryland Biological Stream Survey (MBSS) uses community metrics to differentiate Coastal Plain streams (Stribling 1998), this technique may miss more subtle shifts in the community structure. This study illustrates no difference between watersheds using the MBSS metrics applied to the leaf pack communities measured.

Distinguishing patterns in the biotic community as it changes with chemical and physical parameters can be difficult if the degree of change is subtle. This study was successful at identifying large scale patterns of differences in chemical properties between the two watersheds however the macroinvertebrate community was not directly driven by these nutrient differences. Instead it was secondary effects such as dissolved oxygen as a consequence of enrichment and geomorphology of these stream sites that seemed to influence community structure.

Table 1.1. GPS coordinates of sample sites within two Coastal Plain watersheds.

H2Oshed	Site Name	GPS Coordinates Latitudes	Longitude
Nanjemoy	JPR	38°28.972N	77°13.697W
Nanjemoy	PTR	38°28.380N	77°12.140W
Nanjemoy	RTE6	38°26.556N	77°12.055W
Nanjemoy	LBD	38°26.333N	77°13.388W
Nanjemoy	NMS	38°25.412N	77°12.803W
Nassawango	HBC	38°19.589N	75°28.532W
Nassawango	STC	38°15.694N	75°27.892W
Nassawango	TBC	38°19.243N	75°28.256W
Nassawango	MOC	38°15.784N	75°27.775W
Nassawango	RTE12	38°13.724N	75°28.286W

Table 1.2. Chemical and physical parameters measured monthly from August 1998 to August 2001.

Parameters and the relative units measured monthly
Total Phosphorus (TP) (mgL^{-1})
Soluble Reacted Phosphorus (SRP) (mgL^{-1})
Nitrate and Nitrite-nitrogen(mgL^{-1})
Fluoride (mgL^{-1})
Alkalinity (mgL^{-1})
Hardness (mgL^{-1})
Conductivity (μScm^{-1})
Water temperature (°C)
Dissolved Oxygen (mgL^{-1})
PH
Turbidity (JTU)
Photosynthetic Available Radiation (PAR)
Discharge (m^3sec^{-1})

Table 1.3. Differences in chemical and physical parameters measured monthly over three years, 1998 - 2001, between the Nanjemoy and Nassawango Creek watersheds. ANOVA tests were used to test for significant differences between watersheds. A non-parametric, Kruskal-Wallis, test (denoted by the χ^2 statistical test) was performed when normality was not met. (*) represent statistical results based on Log transformed data in order to meet the assumptions of normality. Bold chemical and physical parameters are significantly different between the Nanjemoy and Nassawango Creek watersheds. 'BD' denotes below instrument detection.

	Nanjemoy Watershed			Nassawango Watershed					
Parameters	Mean (Range)	Median	SE	Mean (Range)	Median	SE	df	p-value	Test statistic
SRP (mgL^{-1})	0.20 (BD-2.56)	0.17	0.01	0.57 (BD-2.03)	0.54	0.02	1	<0.0001	χ^2= 231.29
TP (mgL^{-1})	0.70 (0.02-2.69)	0.65	0.02	1.07 (0.17-2.93)	1.03	0.02	1,37	<0.0001	F =55.96
Nitrate and Nitrite-N (mgL^{-1})	0.02 (BD-0.50)	BD	0.003	0.48 (BD-4.3)	0.10	0.05	1	<0.0001	χ^2= 140.80
Fluoride (mgL^{-1})	0.12 (BD-0.66)	0.11	0.01	0.21 (BD-0.78)	0.21	0.01	1	<0.0001	χ^2= 97.02
Alkalinity (mgL^{-1})	8.4 (BD-49.0)	7.0	0.4	13.6 (1.0-93.0)	12.0	0.6	1,567	<0.0001	F =56.78*
Hardness (mgL^{-1})	0.6 (BD-3.3)	0.5	0.03	0.7 (0.1-7.9)	0.6	0.04	1,577	<0.0001	F =17.87*
Conductivity (μScm^{-1})	75.99 (4.07-318.00)	67.9	1.49	102.54 (12.40-427.00)	91.80	1.81	1,1056	<0.0001	F =157.14*
Water Temperature (^oC)	14.0 (0.1-31.5)	14.6	0.3	13.6 (0.1-29.2)	13.1	0.3	1,1063	0.42	F =0.66
Dissolved Oxygen (mgL^{-1})	5.4 (BD-13.3)	5.2	0.1	4.2 (0.5-11.8)	4.1	0.1	1,1024	<0.0001	F =72.48
PH	6.00 (4.06-7.84)	6.11	0.03	6.28 (4.74-7.85)	6.22	0.03	1,1054	<0.0001	F =47.77*
Turbidity (JTU)	7.4 (BD-90.0)	5.0	0.5	6.0 (BD-110.0)	4.0	0.6	1,575	<0.0001	F =23.84
Discharge (m^3sec^{-1})	0.40 (BD-30.72)	0.04	0.21	0.45 (BD-6.44)	0.18	0.067	1	<0.0001	χ^2= 37.20
Stream Photosynthetic Available Radiation (PAR)	268 (2-2297)	157	12	271 (1-2257)	104	16	1,1689	<0.0001	F =32.87*
Bank Photosynthetic Available Radiation (PAR)	257 (2-2233)	147	11	219 (BD-2422)	65	15	1,1689	<0.0001	F =112.50*
Sun Photosynthetic Available Radiation (PAR)	844 (25-2487)	722	21	719 (6-2432)	540	21	1,1690	<0.0001	F =39.61*
Stream - Direct Sun PAR ratio	0.47 (0.00-3.48)	0.31	0.02	0.51 (0.00-3.65)	0.26	0.02	1	0.81	χ^2= 0.06
Bank - Direct Sun PAR ratio	0.46 (0.00-3.33)	0.31	0.02	0.38 (BD-2.39)	0.17	0.01	1	<0.0001	χ^2= 21.92

Table 1.4. Substrate profile for each sample site measured using EPA's qualitative Rapid Bioassessment Protocol (Barbour et al. 1999).

		Inorganic Components				Organic Components		
Watershed	Sites	Gravel	Sand	Silt	Clay	Detritus	Muck-Mud	Marl
Nanjemoy	JPR		2	8	90		8	
Nanjemoy	PTR		90	5	5	20	5	
Nanjemoy	RTE6		15	5	80		5	
Nanjemoy	LBD	10	30		60	5		
Nanjemoy	NMS	70	30				5	
Nassawango	HBC		80	20		4	16	
Nassawango	STC		98	2		1	1	
Nassawango	TBC		75	20	5	5	15	
Nassawango	MOC		5	95		15	80	
Nassawango	RTE12		10	10	80	8	2	

Table 1.5. Landscape analyses using GISHydro2000 software (Moglen and Casey 2000). Area represents watershed area above sampling site.

Watershed	Site Name	Area (km^2)	Channel Slope ($m*km^{-1}$)	Land Slope ($m*m^{-1}$)	Percent Urban Area(%)	Percent Agric. Area(%)	Percent Impervious Area(%)	Percent Forest Cover(%)
Nanjemoy	JPR	4.9	4.5	0.047	11.2	16.6	3.0	69.6
Nanjemoy	PTR	4.7	3.4	0.039	14.6	14.7	3.9	72.0
Nanjemoy	RTE6	12.4	3.5	0.039	10.2	11.9	2.9	76.3
Nanjemoy	LBD	15.0	2.8	0.039	6.9	11.0	2.1	81.3
Nanjemoy	NMS	37.8	2.8	0.039	7.3	10.1	2.1	81.3
Nassawango	HBC	7.8	0.7	0.005	12.6	48.0	5.9	31.8
Nassawango	STC	13.0	0.8	0.003	0.0	14.2	0.1	87.9
Nassawango	TBC	37.0	0.4	0.005	6.7	38.5	2.4	59.2
Nassawango	MOC	81.6	0.4	0.004	5.7	21.9	2.6	71.9
Nassawango	RTE12	113.0	0.5	0.005	4.7	19.9	2.2	75.6

Table 1.6. Mean abundance and percent of total community structure (in parentheses) for each taxa identified in leafpack samples measured once each season from fall 1998 through spring 2001. Insects were identified to the genus level or the lowest taxonomic level possible, except for Chironomidae, which were identified to sub-family. (*) indicates Class level taxanomic resolution.

			Fall		Spring		Summer	
Order	Family	Taxa	Nanjemoy	Nassawango	Nanjemoy	Nassawango	Nanjemoy	Nassawango
Diptera	Chironomidae	Chironomini	4.97 (33.2)	7.61 (48.7)	6.61 (48.9)	3.66 (32.5)	18.31(66.0)	20.29 (62.6)
		Tanypodinae	2.78 (18.5)	0.96 (6.1)	1.37 (10.2)	0.85 (7.5)	2.29 (8.3)	1.28 (4.0)
		Orthocladiinae	2.56 (17.1)	1.89 (12.1)	0.42 (3.1)	0.59 (5.3)	0.24 (0.9)	0.13 (0.4)
		Tanytarsini	0.23 (1.5)	0.39 (2.5)	1.05 (7.8)	1.26 (11.2)	0.81 (2.9)	0.44 (1.3)
	Ceratopogonidae	*Bezzia*	0.03 (0.2)	–	0.21 (1.5)	0.02 (0.2)	0.29 1.1)	0.01 (0.04)
		Alluaudomyia	–	–	0.01 (0.1)	0.01 (0.1)	–	–
		Probezzia	0.03 (0.2)	–	0.02 (0.1)	0.04 (0.4)	–	–
		Culicoides	0.01 (0.1)	–	0.01 (0.1)	0.02 (0.2)	–	–
		Dasyhelea	–	–	0.01 (0.1)	–	–	–
		Sphaeromias	–	0.01 (0.1)	–	–	–	–
	Tipulidae	*Pilaria*	–	–	–	–	0.03 (0.1)	–
		Tipula	0.03 (0.2)	–	–	–	–	–
		Limnophila	–	0.02 (0.1)	–	–	–	–
		Ormosia	–	–	–	–	–	0.01 (0.04)
		Psuedolimnophila	–	–	–	0.01 (0.1)	–	–
	Simuliidae	*Simulium*	0.05 (0.3)	–	–	–	–	–
		Cnephia	–	0.02 (0.1)	0.17 (1.3)	–	–	–
	Empididae	*Hemerodromia*	0.02 (0.1)	–	0.02 (0.1)	0.01 (0.1)	–	0.05 (0.2)
		Chelifera	0.01 (0.1)	–	–	–	–	–
	Chaoboridae	*Chaoborus*	–	–	–	–	0.01 (0.1)	–
	Tabanidae	*Chrysops*	–	–	–	–	0.03 (0.1)	–
	Syrphidae		–	–	–	0.01 (0.1)	–	–
Coleoptera	Elmidae	*Ancyronyx*	–	–	0.05 (0.4)	0.09 (0.8)	–	0.04 (0.1)

Table 1.6. Continued.

Order	Family	Taxa	Fall		Spring		Summer	
			Nanjemoy	Nassawango	Nanjemoy	Nassawango	Nanjemoy	Nassawango
Coleoptera	Elmidae	*Dubiraphia*	0.03 (0.2)	0.16 (1.0)	0.02 (0.1)	0.03 (0.3)	0.07 (0.2)	0.42 (1.3)
		Stenelmis	–	0.01 (0.1)	0.01 (0.1)	0.02 (0.2)	–	0.05 (0.2)
		Macronychus	–	0.02 (0.1)	–	0.04 (0.4)	0.01 (0.1)	0.01 (0.04)
	Dytiscidae		–	–	–	0.01 (0.1)	–	–
		Hydroporus	0.49 (3.3)	0.45 (2.9)	0.24 (1.8)	0.46 (4.1)	–	0.01 (0.04)
		Oreodytes	–	–	–	0.14 (1.3)	–	–
	Scirtidae	*Scirtes*	–	–	–	0.01 (0.1)	–	–
	Gyrinidae	*Dineutus*	–	–	–	–	0.05 (0.2)	0.01 (0.04)
	Haliplidae	*Peltodytes*	–	–	–	0.01 (0.1)	–	0.01 (0.04)
	Hydrophilidae		–	–	–	–	0.01 (0.1)	–
		Tropisternus	–	0.01 (0.1)	–	–	–	–
	Hydrochidae	*Hydrochus*	–	–	–	0.01 (0.1)	–	0.03 (0.1)
Ephemeroptera			0.03 (0.2)	0.02 (0.1)	0.03 (0.2)	–	0.11 (0.4)	0.03 (0.1)
	Baetidae		0.01 (0.1)	–	–	–	–	–
		Procloeon	0.02 (0.1)	0.01 (0.1)	–	–	–	–
	Leptophlebiidae		0.03 (0.2)	–	–	–	–	–
		Leptophlebia	0.16 (1.1)	0.17 (1.1)	–	–	0.24 (0.9)	–
	Ephemerellidae	*Drunella*	–	–	–	–	–	0.01 (0.04)
		Serratella	0.01 (0.1)	–	–	–	–	–
		Eurylophella	0.47 (3.2)	0.08 (0.5)	–	0.03 (0.3)	–	–
	Heptageniidae	*Stenonema*	0.17 (1.1)	0.04 (0.3)	0.03 (0.3)	–	0.01 (0.1)	–
		Stenacron	–	–	0.02 (0.1)	0.04 (0.4)	–	0.01 (0.04)
Odonata			–	–	–	–	0.03 (0.1)	–
	Coenagrionidae		–	–	–	–	0.23 (0.8)	0.01 (0.04)
		Argia	0.02 (0.1)	0.03 (0.2)	–	0.01 (0.1)	–	–
		Enallagma	–	–	–	0.01 (0.1)	0.19 (0.7)	–

Table 1.6. Continued.

Order	Family	Taxa	Fall		Spring		Summer	
			Nanjemoy	Nassawango	Nanjemoy	Nassawango	Nanjemoy	Nassawango
Odonata	Coenagrionidae	*Ischnura*	–	0.01 (0.1)	–	–	–	–
	Calopterygidae	*Calopteryx*	–	–	0.01 (0.1)	–	–	–
	Libellulidae	*Libellula*	–	–	0.01 (0.1)	–	–	–
		Pachydiplax	–	–	–	0.01 (0.1)	–	–
	Corduliidae	*Somatochlora*	–	–	–	–	0.01 (0.1)	0.01 (0.04)
		Helocordulia	–	–	–	–	0.03 (0.1)	–
Trichoptera			0.01 (0.1)	–	–	–	–	–
	Calamoceratidae	*Heteroplectron*	–	–	0.01 (0.1)	0.05 (0.5)	0.01 (0.1)	–
	Dipseudopsidae	*Phylocentropus*	–	0.01 (0.1)	0.02 (0.1)	0.01 (0.1)	0.04 (0.2)	–
	Hydropsychidae	*Hydropsyche*	0.10 (0.7)	–	0.01 (0.1)	–	0.27 (1.0)	–
		Cheumatopsyche	0.04 (0.3)	0.01 (0.1)	0.01 (0.1)	–	0.28 (1.0)	–
	Leptoceridae	*Triaenodes*	–	–	–	0.02 (0.2)	–	–
		Oecetis	0.01 (0.1)	0.01 (0.1)	–	0.01 (0.1)	0.01 (0.1)	–
		Ceraclea	–	0.02 (0.1)	–	–	–	–
		Mystacides	–	0.02 (0.1)	–	–	–	–
	Hydroptilidae	*Ochrotrichia*	–	–	0.02 (0.1)	–	–	–
		Oxyethira	0.01 (0.1)	–	–	–	–	–
	Limnephilidae	*Pycnopsyche*	0.14 (1.0)	0.02 (0.1)	–	0.06 (0.5)	–	0.04 (0.1)
		Ironoquia	–	–	0.02 (0.1)	0.03 (0.3)	–	–
	Philopotamidae	*Chimarra*	0.03 (0.2)	–	–	0.02 (0.2)	–	–
	Molannidae	*Molanna*	0.03 (0.2)	–	0.01 (0.1)	–	–	–
	Polycentropodidae	*Cyrnellus*	–	–	–	–	0.01 (0.1)	–
		Neureclipsis	–	0.01 (0.1)	–	0.04 (0.4)	0.01 (0.1)	–
		Nyctiophylax	–	0.02 (0.1)	0.02 (0.1)	0.02 (0.2)	–	0.04 (0.1)
		Polycentropus	0.03 (0.2)	0.04 (0.3)	0.03 (0.3)	0.04 (0.4)	0.03 (0.1)	0.10 (0.3)
	Psychomyiidae	*Lype*	–	0.01 (0.1)	–	–	–	–

Table 1.6. Continued.

Order	Family	Taxa	Fall		Spring		Summer	
			Nanjemoy	Nassawango	Nanjemoy	Nassawango	Nanjemoy	Nassawango
Trichoptera	Odontoceridae	*Psilotreta*	0.01 (0.1)	–	–	–	–	–
	Phryganeidae		–	0.01 (0.1)	–	–	–	–
	Lepidostomatidae	*Lepidostoma*	–	–	0.02 (0.1)	0.01 (0.1)	–	–
Plecoptera			–	–	0.01 (0.1)	–	0.01 (0.1)	–
	Perlidae	*Perlesta*	–	–	0.02 (0.1)	–	–	–
	Peltoperlidae		–	–	0.01 (0.1)	–	–	–
		Tallaperla	–	–	0.01 (0.1)	0.01 (0.1)	–	–
	Capniidae	*Allocapnia*	1.00 (6.7)	0.19 (1.2)	–	0.01 (0.1)	–	–
	Nemouridae	*Amphinemura*	0.10 (0.7)	–	0.13 (1.0)	–	–	–
	Taeniopterygidae	*Taeniopteryx*	0.03 (0.2)	0.18 (1.1)	–	–	–	–
Megaloptera	Corydalidae	*Nigronia*	0.01 (0.1)	0.01 (0.1)	0.01 (0.1)	–	0.04 (0.2)	–
		Chauliodes	–	–	–	–	0.03 (0.1)	–
	Sialidae	*Sialis*	0.02 (0.1)	0.01 (0.1)	–	–	0.28 (1.0)	0.12 (0.4)
Lepidoptera			–	–	0.01 (0.1)	–	0.05 (0.2)	–
	Pyralidae	*Munroessa*	0.01 (0.1)	–	–	–	–	–
Oligochaeta*			0.75 (5.0)	2.81 (18.0)	1.23 (9.1)	1.78 (15.8)	0.71 (2.6)	3.35 (10.3)
Isopoda	Asellidae		0.09 (0.6)	0.08 (0.5)	0.70 (5.2)	0.79 (7.0)	0.67 (2.4)	0.94 (2.9)
Amphipoda			0.26 (1.8)	0.03 (0.2)	0.54 (4.0)	0.23 (2.1)	1.47 (5.3)	0.68 (2.1)
Harpacticoida			0.01 (0.1)	–	–	–	0.01 (0.1)	–
Cladocera			0.03 (0.2)	–	–	–	–	–
Rhynchobdellida	Glossiphoniidae		–	0.17 (1.1)	0.02 (0.1)	–	0.12 (0.4)	0.33 (1.0)
Pelecypoda			0.01 (0.1)	–	0.12 (0.9)	0.11 (1.0)	0.20 (0.7)	0.51 (1.6)
Gastropoda			0.06 (0.4)	0.05 (0.3)	0.22 (1.6)	0.39 (3.4)	0.41 (1.5)	3.42 (10.6)
Basommatophora	Ancylidae		–	–	–	–	0.01 (0.1)	0.01 (0.04)
Prostigmata			0.07 (0.4)	0.02 (0.2)	0.01 (0.1)	–	–	0.01 (0.04)
Collembola			0.01 (0.1)	–	–	0.03 (0.3)	0.07 (0.2)	–

Table 1.7. Macroinvertebrate community metrics used to compare the Nanjemoy and Nassawango Creek watersheds. Analyses based on taxa per leafpack data. Bold Community metrics indicate significant differences between the watersheds (α = 0.05).

Community Metric	Nanjemoy Creek	Nassawango Creek	Statistical Test	p-value
Number of Taxa	10.93 (±0.71)	9.95 (±0.61)	F = 1.52	0.228
EPT Taxa	0.85 (±0.18)	0.43 (±0.11)	χ^2 = 2.95	0.086
Percent Ephemeroptera	0.07 (±0.01)	0.04 (±0.01)	χ^2 = 2.45	0.118
Percent Tanytarsini	0.16 (±0.02)	0.19 (±0.03)	χ^2 = 0.47	0.493
Beck's Index	2.08 (±0.31)	1.58 (±0.32)	χ^2 = 1.93	0.165
Number of Scrapers	0.73 (±0.15)	1.10 (±0.17)	χ^2 = 2.80	0.094
Percent Clingers	0.21 (±0.03)	0.22 (±0.02)	F = 0.21	0.650
IBI Score	1.95 (±0.11)	1.89 (±0.09)	F = 0.16	0.688
Abundance	17.51 (±2.27)	18.07 (±2.23)	χ^2 = 0.0004	0.985
Shannon Index	**0.30 (±0.01)**	**0.26 (±0.02)**	**F = 5.13**	**0.026**
Abundance of Predators	**2.94 (±0.35)**	**1.62 (±0.24)**	**F = 11.94**	**0.0009**
Abundance of Shredders	1.29 (±0.36)	0.52 (±0.14)	χ^2 = 2.37	0.124
Abundance of Collectors	11.47 (±1.85)	13.33 (±1.91)	χ^2 = 1.41	0.707
Abundance of Filterers	1.01 (±0.29)	0.8 (±0.21)	χ^2 = 0.35	0.852
Abundance of Scrapers	0.34 (±0.17)	0.36 (±0.08)	χ^2 = 3.64	0.057
# Predator taxa	**3.20 (±0.22)**	**2.28 (±0.21)**	**χ^2 =8.02**	**0.005**
# Shredder taxa	1.20 (±0.20)	0.85 (±0.15)	χ^2 =1.50	0.221
# Collector taxa	3.40 (±0.21)	3.43 (±0.16)	χ^2 =0.084	0.772
# Filterer taxa	1.15 (±0.19)	1.05 (±0.15)	χ^2 =0.003	0.960
# Scraper taxa	0.73 (±0.15)	1.10 (±0.17)	χ^2 = 2.80	0.094

Table 1.8. Testing the difference between watersheds based on the macroinvertebrate taxa Orders, using the Atchison's log ratio MANOVA test. Analysis is performed on the proportional data for each Order in the community. The Wilks' Lambda was used to generate the F-statistic presented.

Effect	df (numerator, denominator)	F-value	p-value
Watershed	(8,67)	3.24	0.004
Season	(16,134)	2.69	0.001
Watershed*Season	(16,134)	1.47	0.120

Table 1.9. Testing the difference between watersheds based on the macroinvertebrate functional feeding groups (FFG), using the Atchison's log ratio MANOVA test. Analysis is performed on the proportional data for each FFG in the community. The Wilks' Lambda was used to generate the F-statistic presented.

Effect	df (numerator, denominator)	F-value	p-value
Watershed	(4,71)	2.75	0.035
Season	(8,142)	2.19	0.031
Watershed*Season	(8,142)	1.43	0.189

Table 1.10. Shaded boxes represent significant explanatory variables (selected as significant in model with p<0.15) that explain the variance in the macroinvertebrate community structure response variables measured using multiple regression analysis.

	NO_3^- NO_2^--N	Reactive P	Total P	Fluoride	Alkalinity	Hardness	Conductivity	pH	Temperature	Dissolved O_2	Turbidity	Discharge	Discharge by Area	Stream PAR Difference	Bank PAR Difference	H_2O Area	Channel Slope	Land Slope	Percent Urban	Percent Impervious	Percent Forest
# Taxa									■	■						■					
EPT Taxa										■											
% Ephem.							■	■	■												
% Tany.								■	■		■										
Beck's			■		■					■						■					
# Scrapers										■						■					
%Clingers						■		■	■	■						■					
IBI score						■		■		■						■					
Abundance				■	■	■		■	■	■		■							■		
Shannon				■				■				■	■								
# Predator					■		■	■													
# Shredder				■	■																■
# Collector	■			■	■		■	■	■	■		■							■		
# Filterers									■		■								■		
% Predator				■						■											■
% Shredder				■															■		
% Collector															■						■
% Filterers											■										
% Scrapers				■				■				■	■				■				

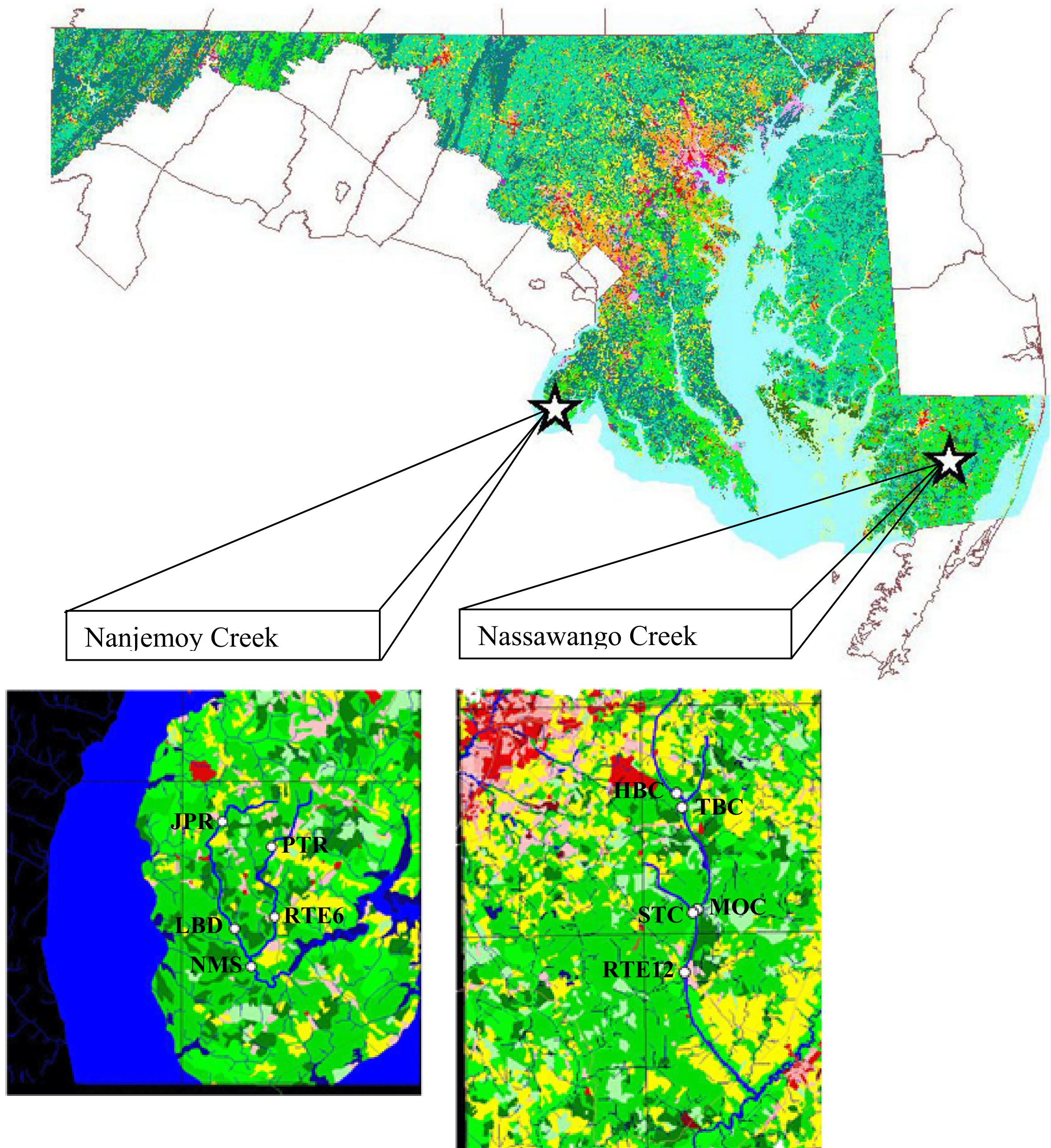
Table 1.11. Multiple regression models selected using stepwise method to identify significant environmental explanatory variables that describe some of the variance found in the community structure response variables measured. Only models with 3 explanatory variables or less are illustrated. Explanatory variable were included in the model if significant with p< 0.15. Abbreviations used are; N = nitrate nitrite-N, TP = total phosphorus, F = fluoride, Alk = alkalinity, Cond = conductivity, Temp = temperature, DO = dissolved oxygen, Q = discharge, Qarea = discharge by watershed area, H_2Oarea = watershed area, ChanSlope=channel slope, %Urban = percent urban area, %Forest = percent forest area.

Response Variable	Regression Models	Adjusted R^2	Test Statistic	Probability
Number of Taxa	#Taxa = 0.06(±1.89) + 0.30(±0.08)Temp + 1.13(±0.27)DO + 0.03(±0.01) H_2Oarea	0.31	F=11.44	<0.0001
EPT Taxa	EPTtaxa = 0.046(±0.23) + 0.11(±0.06)DO	0.04	F=4.14	0.045
Percent Ephemeroptera	%Ephem = -0.11(±0.11) + 0.04(±0.019) pH – 0.001(±0.0003)Cond – 0.0023(±0.0016)Temp	0.09	F=3.33	0.025
Percent Tanytarsini	%Tany = -0.20(±0.23) – 0.01(±0.002)Turb + 0.06(±0.04)pH + 0.01(±0.003)Temp	0.19	F=6.55	0.0006
Number of Scrapers	#Scrapers = -0.32(±0.30) – 0.22(±0.07)DO + 0.01(±0.003)H_2OArea	0.25	F=12.28	<0.0001
Predators Abundance	Pred_Abund. = -3.31(±2.72) – 0.09(±0.03)Alk + 1.26(±0.48)pH – 0.02(±0.01)Cond.	0.19	F=6.53	0.0006
Shredders Abundance	Shred_Abund. = -1.59(±2.72) – 3.99(±1.98)F + 0.06(±0.03)Alk + 0.04(±0.01)%Forest	0.08	F=3.04	0.035
Filters Abundance	Filt_Abund. = -0.05(±0.74) – 0.08(±0.03)Turb + 0.06(±0.04)Temp + 0.08(±0.05)%Urban	0.06	F=2.41	0.075
Scrapers Abundance	Scrap_Abund. = -0.11(±0.11) + 0.05(±0.03)DO + 0.004(±0.001)H_2OArea	0.21	F=10.23	<0.0001
Percent Predators	%Pred = 0.08(±0.06) – 0.46(±0.14)F – 0.01(±0.06)DO + 0.003(±0.001)%Forest	0.22	F=7.35	0.0003
Percent Shredders	%Shred = 0.22(±0.04) – 0.48(±0.12)F – 0.01(±0.003)%Urban	0.19	F=9.32	<0.0001

Table 1.11. Continued.

Response Variable	Regression Models	Adjusted R^2	Test Statistic	Probability
Percent Collectors	%Collect = 1.10(±0.12) – 0.15(±0.07)BankPARDiff – 0.005(±0.001)%Forest	0.12	F=5.63	0.006
Percent Filters	%Filt = 0.08(±0.02) – 0.003(±0.002)Turb	0.04	F=3.96	0.051

Figure 1.1. Map of the Nanjemoy and Nassawango Creek watersheds in the Coastal Plain region of Maryland. Site names for each of the five sample sites are included.



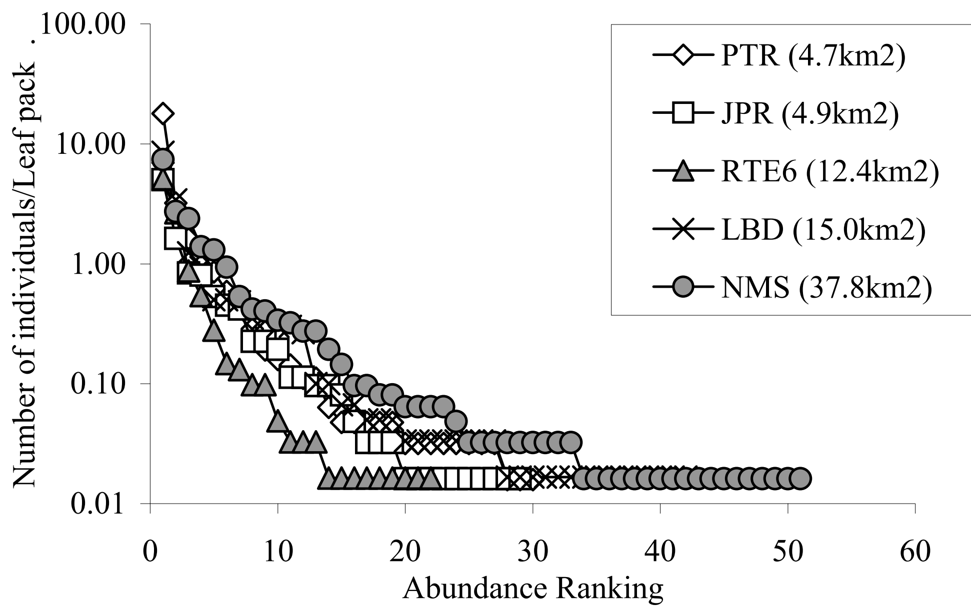


Figure 1.2. Comparing the rank abundance curves for the 5 samples sites within the Nanjemoy Creek watershed, Charles County, MD. Pooled data collected from seasonal benthic sampling using leaf packs from Fall 1998 to Spring 2001.

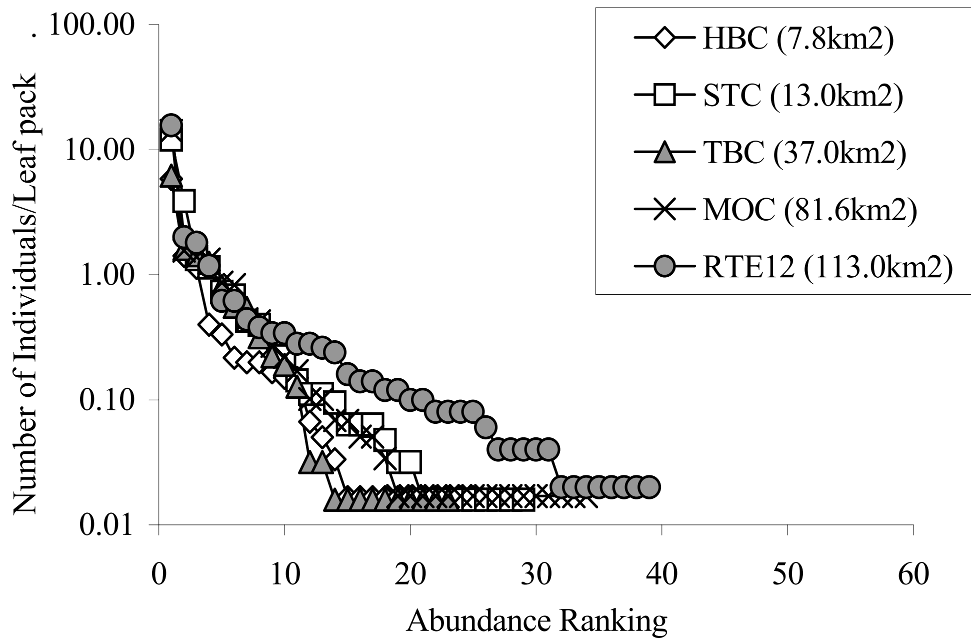


Figure 1.3. Comparing the rank abundance curves for the 5 samples sites within the Nassawango Creek watershed, Wicomico county, MD. Pooled data collected from seasonal benthic sampling using leaf packs from Fall 1998 to Spring 2001.

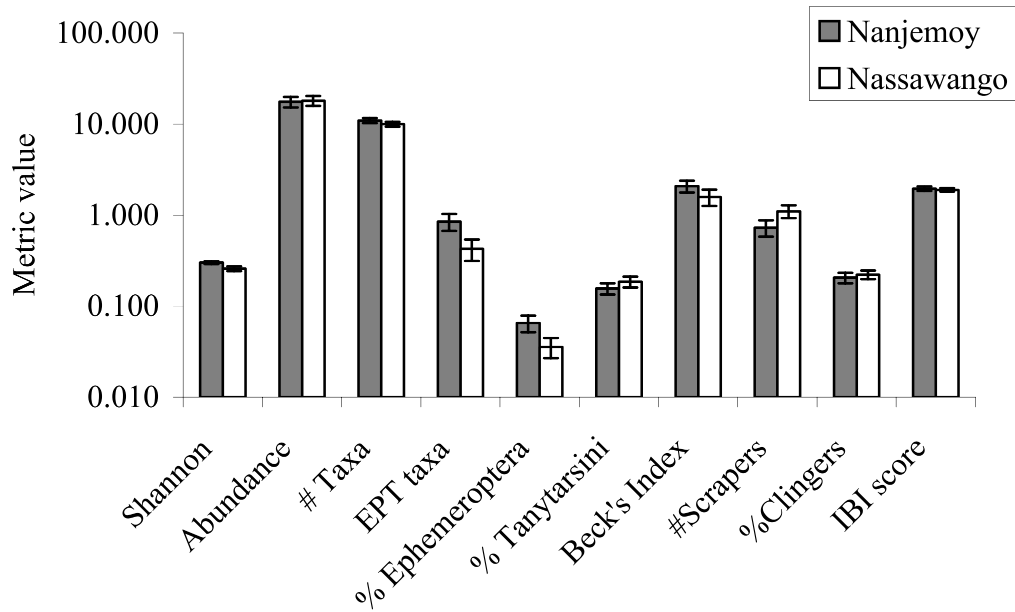


Figure 1.4. Comparison across watersheds of mean macroinvertebrate community metrics (± SEM). Pooled data represents five sample sites within each watershed collected from seasonal benthic sampling using leaf packs from Fall 1998 to Spring 2001.

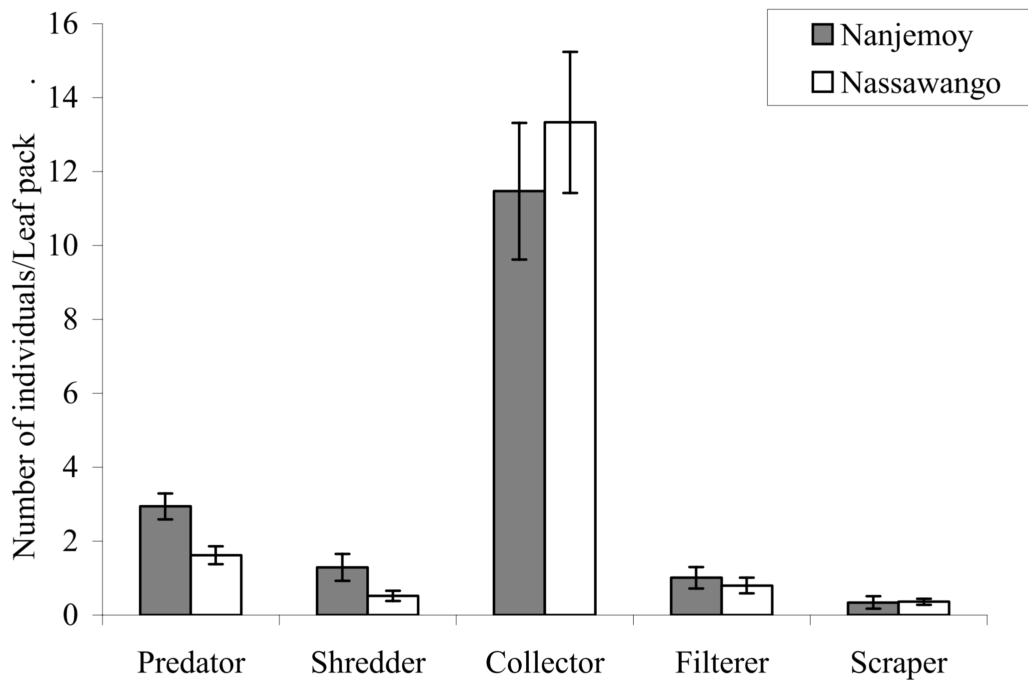


Figure 1.5. Comparison across watersheds of mean macroinvertebrate functional feeding group abundance data (± SEM). Pooled data represents five sample sites within each watershed collected from seasonal benthic sampling using leaf packs from Fall 1998 to Spring 2001.

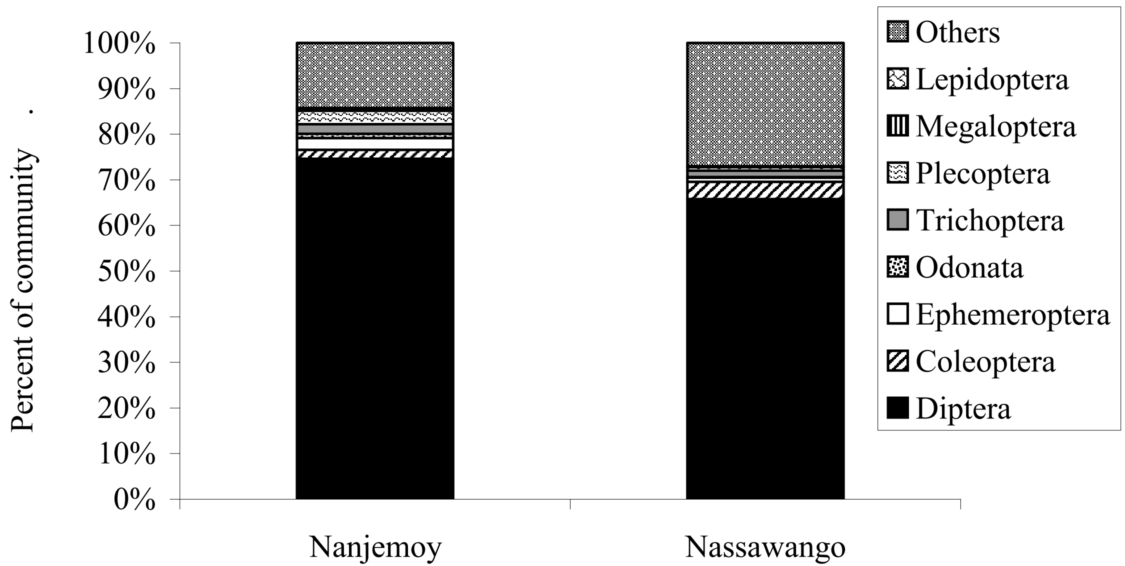


Figure 1.6. The percent of each macroinvertebrate order comprising the community structure within each watershed. Community structure based on pooled data from Spring, Summer, and Fall samples collected from Fall 1998 to Spring 2001.

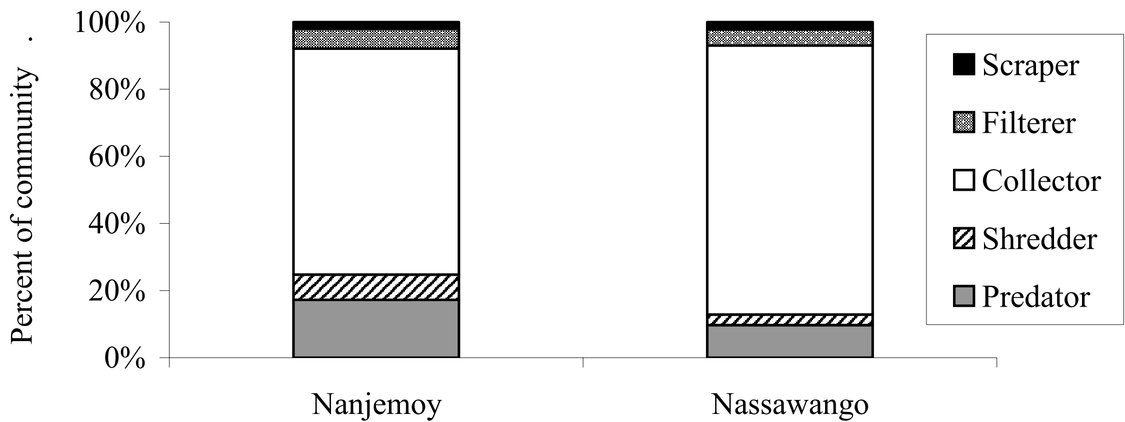


Figure 1.7. The percent of each of the macroinvertebrate functional feeding groups (FFG) comprising the community structure within each watershed. Community structure from pooled data; Spring, Summer, and Fall samples collected Fall 1998 to Spring 2001.

Chapter 2. Development of a new method to measure the biotic contributions to leaf decomposition in Coastal Plain streams

Introduction:

Detrital processing is an integral part of the aquatic foodweb dynamics. This is particularly true for small order forested streams where the majority of the basal energy resources come from terrestrially derived leaf material (Vannote et al. 1980). The input of leaf material starts a cascade of events whereby the microbial community initiates the decomposition process with their enzymatic degradation of lignin and tough cellulose (Sridhar and Barlocher 2000). Shredder macroinvertebrates colonize the leaf material and associated microflora, and further decompose the course particulate organic matter (CPOM) into fine particulate organic matter (FPOM), which other feeding guilds utilize (Webster and Benfield 1986, Wallace and Webster 1996). Leaf decomposition rates provide researchers with an integrated view of stream ecosystem health (Webster et al. 1995).

A number of approaches have been developed to measure functional changes in the biotic community within streams, such as primary production, secondary production, nutrient spiraling, and leaf decomposition. These techniques are an effort by ecologists to assess the community's functional capacity, and how it changes in response to perturbations (Bunn et al. 1999, Sponseller and Benfield 2001). Biotic functions, such as leaf decomposition, are integrally linked with the biodiversity of the community (Kinzig et al 2002). Naeem (2002) illustrates how the diversity of the biota elevates the level of functional capacity of the ecosystem. There has been a recent call by researchers to

expand aquatic efforts to better understand in-stream functional processes and the connection with the biotic community (Giller et al. 2004, Petchey et al. 2004).

Measurement of the rate of leaf decomposition has been widely used to characterize streams and to demonstrate detritivore sensitivity to a variety of impairments, including acid mine drainage (Tuchman 1993, Niyogi et al. 2001), nutrient enrichment (Elwood et al. 1981, Suberkropp 1995, Grattan and Suberkropp 2001, Robinson and Gessner 2000, Chadwick and Huryn 2003), and temperature limitations (Hauer et al. 1986, Maloney and Lamberti 1995). Alterations in this terrestrial leaf material supply have been shown to drastically alter the detrital dynamics (Wallace et al. 1999). Typically, leaf decomposition studies in streams use a known quantity of senesced leaves placed in plastic mesh bags and measured the amount of leaf mass lost through time (Benfield 1996). The rate of leaf decay is then calculated using the classic exponential decay model, $w_t = w_o e^{-kt}$, where w_t is the ending leaf weight at day t, w_o is the starting leaf weight, and k is the rate of leaf decomposition (Petersen and Cummins 1974). In nearly all cases, leaf decomposition in streams has been measured using this method (e.g. Jenkins and Suberkropp 1995, Paul and Meyer 1996, Robinson et al. 1998, Sponseller and Benfield 2001, Jonsson et al. 2001, Royer and Minshall 2001, Chadwick and Huryn 2003). However, this technique quantifies not only the biotic contributions to leaf decomposition, but also the hydraulic forces physically degrading the leaf material from water turbulence. Few studies have focused on reducing the hydrologic impacts on leaf degradation with other decomposition study designs. Niyogi et al. (2001) demonstrated the utility of using a tube with mesh on an upstream and downstream opening to assess the effects of acid mine drainage on the benthic community. This

technique successfully illustrated differences in the rates of leaf decomposition due to zinc concentrations in the impacted stream sites.

The goal of my research was to understand how the biotic community, in Coastal Plain streams, contributes to the leaf decomposition process without the additional hydrologic influences. Coastal Plain streams have inherent constraints that limit the use of functional measurements. These slow moving, blackwater streams in forested riparian areas rely on terrestrially derived leaf material for energy rather than primary production, and are dominated by heterotrophic instead of autotrophic processes. This is due to dense canopy cover and opacity of dark organic carbon rich water reducing light availability to the streams. Several problems exist with using mesh bags to measure the rate of leaf decomposition. First, mesh bags do not readily allow one to differentiate the microbial and macroinvertebrate contributions, and simultaneously maintain similar ambient aquatic conditions. Using fine and coarse mesh bags will measure differences in the microbial and macroinvertebrate contributions to detrital processing, however, the coarse mesh bags will result in very different hydraulic influences acting on the leaf packs. Secondly, Coastal Plain stream substrates are dominated by sand and silt, and in preliminary studies large fluctuations in discharge during rain events often buried mesh bags with substrates. Therefore, I developed a method to minimize hydrologic losses and to reduce siltation, using tubes mounted on bricks (referred to as decomposition tubes through this chapter).

The objectives of this chapter were to compare the performance of the decomposition tubes to the standard mesh bag technique, and test whether there were hydrologic and/or dissolved oxygen concentration differences between the fine and

coarse mesh treatments. I also assessed their relative efficacy of quantifying the microbial and macroinvertebrate contributions to leaf decomposition in Coastal Plain streams. I hypothesized that the decomposition tubes would have slower rates of leaf decomposition when compared to mesh bags and that differences between the coarse and fine mesh treatments would show differences in the biotic communities' contributions to the detrital process.

Methods:

Decomposition tube design

Leaf decomposition tubes were designed using small plastic tubes made from 0.8 mm thick Eastman tenite butyrate having a 3.5 cm diameter and 20 cm length. These tubes were a modified version of similar decomposition tubes used by Niyogi et al. (2001). The tubes were designed with fine or coarse mesh on either end to exclude or include macroinvertebrates, respectively, while allowing for adequate water circulation and aeration. The coarse treatment had a coarse mesh screen, with 4mm openings, attached to the upstream end, and a fine mesh, 165 µm, attached on the downstream tube opening. The fine mesh treatment had fine mesh, 165 µm, on both the upstream and downstream ends of the tube. The mesh was attached to the tubes on both ends using plastic rings that held the mesh securely over the opening. Four holes, approximately 1.25 cm in diameter, were cut on each tube and covered with the fine mesh, 165 µm, to increase water flow through the chambers. Mesh was fused to the tube wall using acetone (Fig. 2.1). Additionally, blank coarse tube treatments were placed adjacent to

each of the coarse mesh tube treatments to measure organic leaf material accumulation within the coarse mesh tubes from upstream drift.

Comparison of methods

A randomized complete block treatment design was used during two independent field experiments to compare the decomposition tube with other leaf decomposition methods. The decomposition tube design was compared to the classic leaf decomposition technique using mesh bags, as well as to a larger PVC tube design (Fig. 2.2).

First, in August 2000, the three methods were compared at the Nanjemoy Creek mainstem site, in Charles County, Maryland (see details in Chapter 1). In addition to the coarse and fine mesh decomposition tubes, I used fine nylon mesh bags (125 µm mesh openings). In addition, I used PVC tubes, 10 cm diameter and 20 cm long. The coarse mesh was attached to the ends using PVC joint couplings. All decomposition tubes and bags were attached on top of a brick to avoid fouling of the mesh openings with bottom substrate.

Senesced red maple (*Acer rubrum*) leaves were leached for 36 hours in aerated deionized water, followed by drying at 60°C for 78 hours. Approximately 2.5 g of leaves were weighed on a Mettler AE50 balance to the nearest mg, and then placed in each tube or mesh bag. Three sets of dried leaves were weighed and set aside in order to calculate the initial percent organic weight. There were 16 replicate blocks randomly placed in a 75 m reach of the stream. Each block contained all three of the methods. The tubes were aligned so that the coarse mesh ends were facing upstream and the fine mesh prevented any downstream organic matter loss. After 37 days, tubes and bags were collected, put in

zip-lock plastic bags, and stored on ice until returning to the laboratory. The samples were then stored in a 4°C refrigerator until processed.

In the laboratory, the samples were rinsed in a sieve with 1 mm mesh size. The remaining leaf material in the sieve was placed into pre-dried and weighed aluminum tins, and dried at 60°C for 48 hours. Samples were then ashed using a Fisher Scientific Muffle Furnace at 550°C for 2 hours. The ashed material was dried at 60°C for 24 hours before reweighing. The ashed material weight represents the inorganic weight, and was therefore subtracted from the dry weight to determine the organic weight of the leaf material. The final ash-free dry mass (AFDM) for the coarse decomposition and PVC tubes were adjusted by subtracting the AFDM determined for the associated blank tubes adjacent to each replicate tube. The total loss of AFDM was then calculated as the difference between the initial and final organic leaf weight. The AFDM was then used to calculate the rate of leaf decomposition, k.

In a second experiment performed in January 2003, the coarse and fine decomposition tubes were tested against the fine and coarse mesh leaf bag method. The coarse mesh bags were constructed of 4mm opening nylon sewn into 20cm x 10cm bags. Twelve replicate blocks, each containing all 4 decomposition method treatments, were randomly placed in the Nassawango Creek, in Wicomico County, Maryland (see details in Chapter 1). Additionally, handling loss was measured using 10 replicates of each of the 4 treatments that were transported to the field and back. Handling loss was then used to adjust future leaf loss in field decomposition studies. The experiment was run for 30 days. The samples were processed as described above.

An analysis of variance was performed to measure differences among the field methods. The mean k-values were calculated for each treatment and analyzed by block using a mixed ANOVA model (SAS 1999). The degrees of freedom were adjusted using Kenward-Rogers to compare among categories. Apriori pairwise mean comparisons were made to assess specific differences between coarse mesh and fine mesh treatments. The probability of a Type I error was set at $\alpha = 0.05$.

Current flow

To determine if there was a difference in water velocities within the coarse and fine decomposition tubes, I measured salt tablet weight loss from the two different mesh treatments. This study was performed three times, once in a Piedmont stream and twice in Coastal Plain streams. The first trial used rock salt while the second and third trials used water softening salt pellets. The change in salt medium was an attempt to reduce size variability of the dissolving rates of the different sized rock salt pieces. In all trials the coarse and fine mesh decomposition tubes described above were paired together, and 12 replicates were randomly placed within the stream.

The first trial was performed by placing pre-weighed 10.0 (±0.1) g of rock salt in each of the tubes, then submerging the paired mesh treatments and evenly distributing them across the Paint Branch stream, located in College Park, MD, in an effort to account for both near shore and mid-stream flow differences. The tubes were left in for 10 minutes and then taken out. The tubes were then transported back to the laboratory where they were immediately placed in a drying oven at 60°C. The salt was dried inside the tube for 24 hours before being placed in dried and pre-weighed aluminum tins. Salt

loss was calculated and a paired t-test was performed to compare the differences between coarse and fine mesh effects on the rate of salt loss.

The second and third trials were conducted at the Nassawango Creek, Mount Olive Church sample site (see Baer chapter 1 for details), to ensure flow regimes were representative of Coastal Plain streams. In the second trial, 12 replicate blocks of both coarse and fine mesh decomposition tube treatments had 19.0 (±0.1) g of water softening sodium chloride salt tablets placed inside each tube, and were then distributed in the stream, in an effort to account for both near shore and mid-stream flow differences and left for 10 minutes. The tubes were retrieved and the salt removed and replaced into individual aluminum tins. The tins were then placed in the drying oven at 60°C for 24 hours and processed using the same protocol followed in the first trial.

In the third trial, tubes were conditioned in the stream for 90 days prior to the experiment to allow the mesh screens to collect debris that may occlude the opening before testing for salt loss differences. Because the salt loss experiments are short in duration, conditioning the tubes may have affected hydrologic flow after the tubes had been submerged for an extended period of time. There were 12 replicate blocks of both coarse and fine mesh decomposition tubes randomly placed in the stream. After conditioning, tubes were collected and 19.0 (±0.1) g of salt tablets were inserted into the tubes from the downstream end in an effort to reduce film build-up on the upstream end of the tubes. The tubes were then placed in stream in areas representative of the near shore and mid-stream velocities for 10 minutes, before removing and processing the salt as described above in the previous two trials.

Dissolved oxygen concentration

To test whether the mesh treatments caused changes in the dissolved oxygen concentration within the tubes, a modified decomposition tube design was used, which had a 22 mm hole, the diameter of a dissolved oxygen probe (YSI Model 55, YSI Incorporated, Yellow Springs, Ohio) bored out in the middle of the tube length. The hole was plugged with a rubber stopper, and 12 replicates were submerged in area representative of the near-shore and mid-stream velocities of the Nassawango Creek, Mount Olive Church site. After 30 days, the stopper was removed and the dissolved oxygen concentration was measured using the dissolved oxygen probe. A paired t-test was performed to compare the differences between coarse and fine mesh tubes in dissolved oxygen concentrations.

Macroinvertebrate abundances

To determine if the fine mesh treatment excluded macroinvertebrates and coarse mesh treatment allowed for colonization, a field experiment was performed. One hundred paired fine and coarse mesh tubes were randomly placed in 10 sites with two watersheds, the Nanjemoy Creek and the Nassawango Creek (see Chapter 1). The tubes were collected after 36 days and stored on ice until returning to the laboratory where they were kept at $4^{o}C$ until processed. The macroinvertebrates were sorted from the leaf material using stacked 2000 and 500 μm sieves. The tube contents were put in the 2000 μm sieve and washed with water. The coarse material from the 2000 μm sieve was placed in one sorting metal tray while the fine material from the 500 μm sieve was place in a second sorting metal tray. The macroinvertebrates were collected and stored in 80% alcohol until identified. Taxa were identified to genus level or the lowest taxa possible,

with the exception of specimens in the family, Chironomidae, which were identified to family level. A Wilcoxon signed rank test was performed to determine the differences between the total abundance between the two mesh treatments using 70 randomly selected pairs of coarse and fine mesh tubes. Only 70 pairs were subsampled from the original 100 pairs for time efficiency and due to the obvious difference between the two treatments.

Results:

Comparison of Methods

Although the rate of leaf decomposition differed when comparing all 4 field methods ($F_{3,44} = 2.38$, $P = 0.083$, Fig. 2.3), only the fine mesh tube method had a significantly slower rate of decomposition. The fine mesh tube had a 13% reduction in leaf decomposition (0.014 ±0.001 d^{-1}) relative to the next slowest treatment, the fine mesh bag with a decomposition rate of 0.016 ±0.001 d^{-1}. Also, methods with similar mesh size had similar k values. The percent of leaf loss ranged from 39 to 46 percent over the 37 day study. Only the fine mesh tube treatment was significantly different from both the large and small coarse mesh treatments ($P = 0.026$ and $P = 0.025$, respectively, Fig. 2.3). When comparing the fine and coarse mesh bags and tubes in the second experiment, there was a significant interaction between method, and mesh size ($F=16.79$, $P=0.0003$). This was explained by only the coarse mesh bag treatment having a significantly faster rate of leaf decomposition relative to the other treatments (Table 2.1, Fig. 2.4). While the k values ranged from 0.0018 ± 0.0003 to 0.0041 ± 0.0003 d^{-1} (mean ± 1 SEM), the amount of leaf material loss only ranged from 6.2 ± 0.2 % to 15.2 ± 1.2 %.

Current flow

Both the first and second trials showed no difference in the salt loss between the coarse and fine mesh treatments ($P = 0.80$ and $P = 0.15$, respectively) (Table 2.2 and 2.3). However, the third trial added the additional consideration of fouling of the mesh through time. In this test, there was an 8 % greater salt loss in the coarse mesh tubes (8.8 ± 1.2 g) compared to the fine mesh tubes (8.1 ± 1.1 g) ($T = 2.43$, $P = 0.033$, Table 2.4).

Dissolved oxygen concentration

Dissolved oxygen concentrations were 3% greater in the coarse mesh tubes (9.98 ± 0.53 mgL^{-1}) compared to the fine mesh decomposition tubes (9.69 ± 0.43 mgL^{-1}) after the tubes had been conditioned in stream for 30 days ($T = 3.58$, $P < 0.005$, Table 2.5).

Macroinvertebrate abundances

The number of individual macroinvertebrates was over 3 times greater in the coarse mesh tube (3.9 ± 0.5 individuals/tube) than in the fine mesh tubes (0.8 ± 0.1 individuals/tube) ($\chi^2 = 32.7$, $P < 0.001$, Table 2.6). Chironomid larvae comprised 52% of the individuals in the fine mesh tubes, while only 31% of this same taxa represented the individuals found in the coarse mesh tubes. A total of 20 taxa were collected from the coarse mesh tubes compared to 15 taxa in the fine mesh tubes. The Ephemeroptera, Plecoptera, Trichoptera (EPT) taxa represented 39% of the individuals found in the coarse mesh tubes, compared to only 17% of the taxa in the fine mesh tubes.

Discussion:

The new leaf decomposition tube method was designed to measure the microbial and macroinvertebrate contributions to leaf decomposition in Coastal Plain streams while

reducing the physical leaf breakdown by hydrologic forces. Under Coastal Plain summer conditions, the rate of leaf decomposition was similar for all three methods in the first experiment. Only the decomposition rate within the fine mesh tube was significantly slower (0.014±0.001 d^{-1}) than the coarse mesh tube treatments (0.017±0.001 d^{-1}) (Fig.2.3). The decomposition rate in the fine mesh bag treatment (0.016±0.001 d^{-1}) was similar to the coarse mesh treatment, which could have been a result of the greater hydrologic forces accelerating the rate of leaf breakdown. Although the PVC tube method yielded similar results (0.016±0.001 d^{-1}) to the smaller coarse mesh tube method, it was considerably more cumbersome to deploy and vulnerable to displacement during flood events**.** The second experiment comparing the decomposition tubes to the leaf bags method showed an increase in the rate of leaf decomposition for the coarse leaf bag treatment compared to both the coarse decomposition tube as well as both fine mesh treatments. However unlike the first trial, no difference was observed between the fine and coarse tube treatments. Perhaps, this was the result of the short duration and the seasonal constraints reducing the overall amount of leaf mass lost during the trial. This second trial was performed in the winter months when the lower temperatures can slow the biotic contributions to leaf decomposition (Gonzalez and Graca 2003). The study was terminated after 30 days and all the decomposition tubes were collected because of heavy flooding and the potential of the loss of remaining tubes. Results of these first two field tests suggest that the tube method with different mesh sizes is able to show differences between microbial and macroinvertebrate contributions to leaf decomposition.

Of the three salt loss experiments performed, two indicated that there was no significant differences in water flow effects on salt loss. However, in the third

experiment flow differences were significant between the two mesh treatments. In this third trial I conditioned the tubes for 90 days within the stream to allow for fouling of the mesh due to debris accumulation. These flow differences represent the effect of long time duration in the stream. Shorter duration studies may not have adverse flow effects due to mesh differences.

Although the coarse mesh tubes had significantly higher dissolved oxygen concentrations (9.98 $mgL^{-1} \pm 0.15$) than the fine mesh tube (9.69 $mgL^{-1} \pm 0.12$), they were not biologically significant. Both mesh treatments had dissolved oxygen concentrations well above 5 mgL^{-1}, which is what the United States Environmental Protection Agency (US EPA) considers to be the threshold below which there may be concern for certain aquatic organisms (US EPA 1986).

As hypothesized, there were differences in macroinvertebrates collected from the two mesh treatments. The macroinvertebrates fauna found in the fine mesh tubes was dominated by early instar larvae and was significantly less than the coarse mesh treatment. On average there were nearly 4 macroinvertebrates in each of the coarse mesh tubes compared to fewer than one in every fine mesh tube. The coarse mesh tubes also contained greater number of EPT taxa, while the fine mesh tubes predominantly contained chironomid larvae.

Using the tube method may provide finer resolution in measuring the biotic contributions to leaf decomposition. However, results also suggest that there are limitations due to mesh fouling altering flow and dissolved oxygen concentrations during long duration studies. These differences may not be of concern for short-term studies or in ecosystems where the conditions are already oxygen limited or have little flow. For

example, in streams with little to no stream flow the differences in dissolved oxygen concentrations may not exist between the two mesh treatments. Using this method during summer months may cause dissolved oxygen to drop below the 5 mgL^{-1} threshold, if the environmental conditions are oxygen limited. This method may not cause appreciable differences in stream systems that are at the extremes of highly oxygenated or approaching anoxic such as fast moving well oxygenated systems, cold water or winter conditions with high oxygen concentration capacity, or low gradient and slow flows rates of Coastal Plain streams in summer. It is the intermediate conditions where the tube method may cause oxygen limitations if the tubes have accumulated appreciable film on the mesh.

This study illustrates the utility of the tube method as a means to reduce the hydrologic degradation of leaf material and allow the researcher to focus on the biotic community contributions. It also demonstrates the need to consider appropriate times to use this technique. This method may work best when there is ample dissolved oxygen concentration in streams, or when the concentration is approaching anoxic conditions. In both cases, the difference between the oxygen concentrations within mesh treatments may be minimal. Alternatively, the method could be modified so that the upstream mesh would always be fine, and the downstream end would have the coarse or fine mesh treatments. This might help extend the use of this decomposition tube method to accommodate all dissolved oxygen conditions. In low order streams where dense canopy cover makes allochthonous litter inputs a dominant energy resource for the aquatic biota, these decomposition tubes offer an opportunity for scientist to measure contributions by different trophic levels to detrital processing. Davis et al. (2003) illustrate the need to

continue to develop biomonitoring tools that can help differentiate the impact of agriculture on Coastal Plain streams.

In such stream systems where sand and silt substrates can foul the traditional leaf decomposition measurement bags, decomposition tubes can offer an alternative method. Functional measurement tools, such as these decomposition tubes, developed for the unique conditions of Coastal Plain streams will help to improve our ability to monitor changes in ecosystem processes.

Table 2.1. A Mixed model ANOVA comparing the rates of leaf decomposition using two field methods (tubes versus bags), and two mesh sizes (coarse versus fine) to evaluate decomposition tubes as an alternative method of measuring biotic contributions to detrital processing. Degrees of freedom represent numerator and denominator.

Treatment	df	F-value	P-value
Method	1,33	24.13	<0.0001
Mesh size	1,33	17.94	0.0002
Method * Mesh Size	1,33	16.79	0.0003

Table 2.2. Testing flow effects on the percent salt loss between the two mesh size tube treatments in April 2001 in the Paint Branch Stream, Prince Georges County, Maryland. T-test is for the differences between treatment means of percent mass loss.

Mesh Size	Mean	Standard Error	N	t-test	P-value
Coarse	93.14	2.08	12	0.26	0.799
Fine	93.72	1.65	12		

Table 2.3. Testing flow effects on the percent salt loss between the two treatments in February 2002 in the Nassawango Creek, Wicomico County, Maryland. T-test is for the differences between treatment means of percent salt loss.

Mesh Size	Mean	Standard Error	N	t-test	P-value
Coarse	31.78	0.68	12	1.54	0.153
Fine	30.81	0.69	12		

Table 2.4. Testing flow effects on the percent salt loss between the two mesh size tube treatments in October 2002 in the Nassawango Creek, Wicomico County, Maryland. T-test is for the differences between treatment means of percent mass loss.

Mesh Size	Mean	Standard Error	N	t-test	P-value
Coarse	46.78	2.10	12	2.43	0.033
Fine	43.17	2.02	12		

Table 2.5. Paired t-test comparing the differences in dissolved oxygen between the coarse and fine mesh tube treatments.

Mesh Size	Mean (mgL^{-1})	Standard Error	N	t-test	P-value
Coarse	9.98	0.15	12	3.58	<0.005
Fine	9.69	0.12	12		

Table 2.6. Chi-squared Wilcoxon rank sums test comparing differences in the number of individual macroinvertebrates between the coarse and fine mesh tube treatments.

Mesh Size	N	χ^2	P-value
Coarse	70	32.7	<0.001
Fine	70		

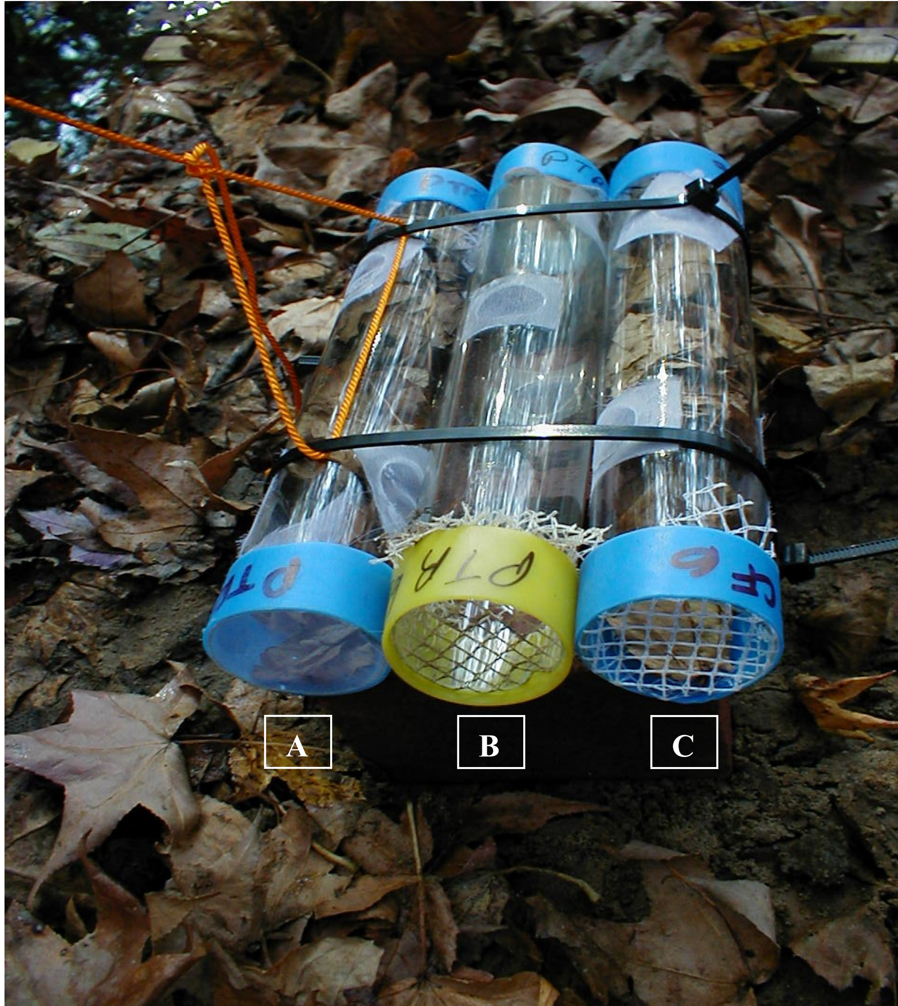


Figure 2.1. Small decomposition tubes developed to measure biotic contributions to leaf loss while reducing hydrologic leaf abrasion and breakdown. The far left tube (A) represents the fine mesh treatment measuring the microbial contributions to leaf decomposition, while the far left tube (C) illustrates the coarse mesh treatment measuring the additional effect of including macroinvertebrates to colonize the leaf material. The middle tube (B) shows the blank coarse tube that was used to measure organic material accumulated from upstream flow.

Figure 2.2. Methods comparison using large PVC decomposition tubes (A), smaller decomposition tubes (B), and mesh bags.

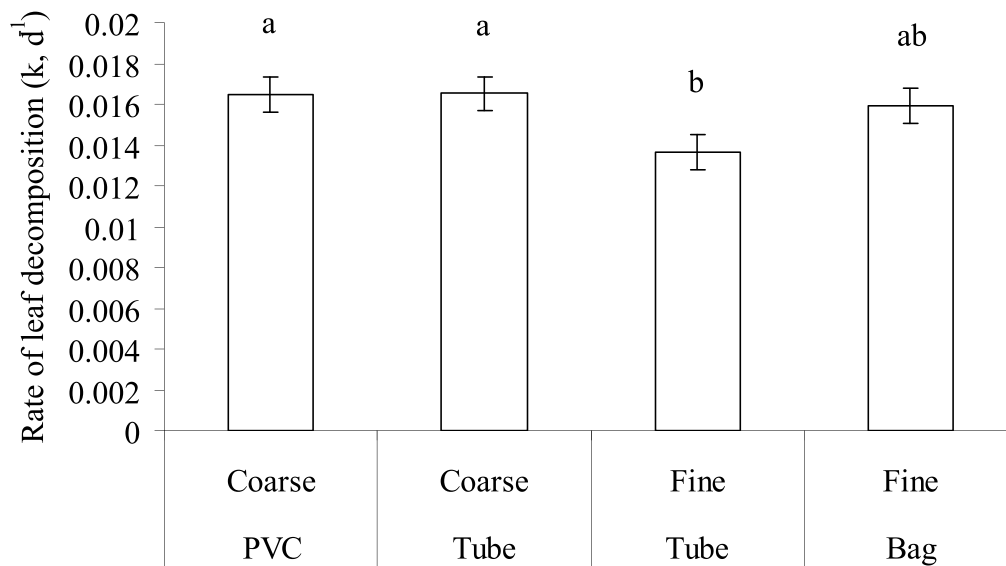


Figure 2.3. The mean rates of leaf decomposition, k, (±SEM) for 4 treatments tested in Nanjemoy Creek in Charles County during the summer 2000. Treatments with differing letter values (a and b) represent significant differences measured using ANOVA with α = 0.05.

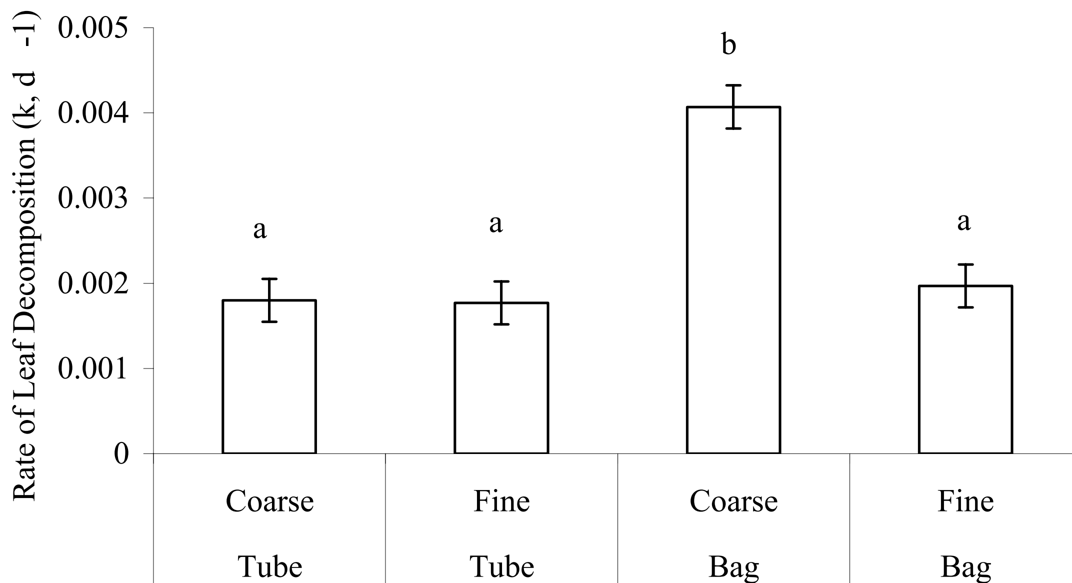


Figure 2.4. The mean rates of leaf decomposition, k, (±SEM) comparing tube and bag field designs with coarse and fine mesh conducted in the Nassawango Creek in Wicomico county, Maryland during the winter 2003. Treatments with differing letter values (a and b) represent significant differences with α=0.05.

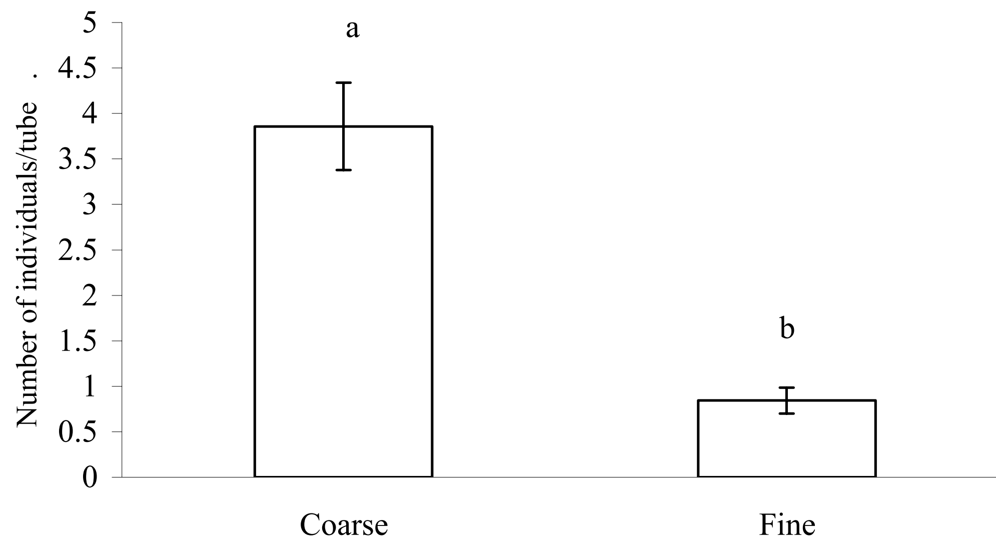


Figure 2.5. The mean number of individuals (±SEM) within each coarse and fine mesh tube used in field leaf decomposition studies. Mean values and standard errors based on n=70. Treatments with differing letter values (a and b) represent significant differences with α=0.05.

Chapter 3. A comparison of structural indices to leaf decomposition

Introduction:

Understanding the role of community structure on ecosystem processes, such as primary production, nutrient spiralling, leaf decomposition, and secondary production, is fundamental for our understanding of how disturbances can alter aquatic ecosystems (Elmqvist et al. 2003). Recent reviews of research on biodiversity and ecosystem function have demonstrated that the majority of theoretical and empirical research has come from terrestrial systems (Kinzig et al. 2001, Naeem and Wright 2003). These reviews suggest that community structure influences the contribution of assemblages of organisms toward ecosystem functions (Chapin et al. 1995, Tilman 1996, Naeem 2002, Kinzig et al. 2001). However, other researchers suggest that aquatic environments behave differently than terrestrial systems, since they are characterized as having, for example, a more fluid medium without strong boundaries between aquatic media and substrate, rapid recruitment of species after disturbances, and a highly heterogeneous environment (Palmer et al. 1997, Covich et al. 1999, Palmer et al. 2000, Cardinale et al. 2002, Naeem and Wright 2003, Giller et al. 2004, Kobayashi and Kagaya 2004). These authors conclude that aquatic environments need further exploration to understand the role natural and anthropogenic impacts have on the community structure and function. Additionally, research should expand to include larger scale examinations of in situ community dynamics and measures of ecosystem services since much of the current

theory is based on manipulated model communities (Naeem and Wright 2003, Gessner et al. 2004, Giller et al. 2004).

An extension of the biodiversity-ecosystem function theory suggests that the degree of resilience in ecosystem processes subsequent to disturbance events may depend on the biotic community structure as well as redundancy in functional contributions by the various species (Walker 1992, Wellnitz and Poff 2001). The challenge remains to assess whether functional measures can reflect the changes in community structure, or whether redundancy in the community structure masks any functional shifts, leaving the measured ecosystem process to proceed similarly, even under altered structure. Previous aquatic research has demonstrated structural shifts in the community following disturbance events (Harding et al. 1998, Huryn et al. 2002, Collier and Quinn 2003). Furthermore, a number of aquatic studies illustrate changes in taxa abundance or structure, leading to changes in the foodweb dynamics and functional processes as a whole (Poff and Allan 1995, Robison and Gessner 2000, Jonsson and Malmqvist 2000, Cardinale et al. 2002, Gessner and Chauvet 2002, Petchey et al 2004, Dangles et al. 2004). Quantifying these links between the biotic community and the ecological processes, provides a temporal scale in the monitoring of these systems. Integrating functional measurements into structure-based biomonitoring programs can provide additional assessment tools to evaluate the health of aquatic ecosystems for resource managers.

Historically, the use of biological monitoring has emphasized the use of structural community measurements over functional measurements as a gauge of environmental degradation in streams and rivers. In part, the use of structural measurements has been

favored because of the ease of data collection and processing (Rosenberg and Resh 1993). Biotic indices typically incorporate multiple structural metrics into an overall score describing the ecosystems' health (Karr 1991). Karr and Chu (1999) suggest expanding the scope of the biological monitoring tools by incorporating several metrics that together provide a greater picture of the stream, including taxa richness, functional feeding group, population attributes, and tolerance levels. For example, the Maryland Department of Natural Resources established the Maryland Biological Stream Survey (MBSS), and a region specific index of biological integrity (IBI), to assess water quality and identify probable environmental impacts across the State of Maryland (Kazyak 1997). The MBSS uses different community metrics to create IBI specific to different ecoregions of Maryland, thus distinguishing healthy and impaired waters using site-specific data (Stribling 1998). The strength of the IBI is its combination of multiple metrics in order to develop a broader picture of the stream or river condition (Karr and Chu 1999). However, the IBI provides a static view of the aquatic conditions by focusing on structural components. Although these indices use functional feeding groups to introduce the use of ecosystem function (Barbour et al. 1999). Researchers have recently suggested that broader ecosystem functional measurements may provide a better measure of environmental impacts (Naeem and Wright 2003).

The impact of non-point source pollution on aquatic ecosystems can be difficult to assess due to its diffuse and potentially subtle effects. For example, the US Environmental Protection Agency (US EPA), in conjunction with State agencies, worked to establish nutrient criteria for rivers and streams throughout the United States to identify the level at which biotic impairments occur (US EPA 2000). These nutrient

criteria are based on chemical and chlorophyll a content as a functional measure with the rivers and streams. This measure of primary production quantifies the amount of nutrients and light energy assimilated by in-stream algae (Steinman and Lamberti 1996). However, in forested, low order streams, dense canopy cover limits the sunlight reaching the water. Under these conditions, the primary energy source for the biotic community within the stream is dominated by the processing of detrital materials (Benfield 1996). Thus, other functional measurements may be needed to assess water quality impairments where primary production is not significant. For example, while research has demonstrated structural and functional shifts in both the microbial (Jenkins and Suberkropp 1995, Ramirez et al. 2003, Gulis and Suberkropp 2003) and the macroinvertebrate (Lenat and Crawford 1994, Robison and Gessner 2000) communities due to disturbance events, relatively few studies have focused on Coastal Plain stream dynamics where black water, low gradient streams have unique characteristics which may limit functional assessment techniques (Meyer et al. 1987, Fuss and Smock 1996, Qualls and Richardson 2000, Grattan and Suberkropp 2001, Wright and Smock 2001, Davis et al. 2003). In these low available light conditions, leaf decomposition and nutrient spiraling may provide stronger measures of impairment.

A number of factors have been shown to affect decomposition rates of terrestrially derived leaf inputs (Webster et al. 1995). For example, decomposition has been shown to respond to changes ambient nutrient concentration (Howarth and Fisher 1976, Triska and Sedell 1976, Elwood et al. 1981, Newbold et al. 1983, Mulholland et al. 1985, Qualls and Richardson 2000). Soluble nutrients in the water column are a key component in fungal and bacterial growth on the leaves (Grattan and Suberkropp 2001, Koetsier et al. 1997,

Suberkropp and Chauvet 1995). Grattan and Suberkropp (2001) showed that both phosphorus alone and the combination of phosphorus and nitrate amendments could stimulate higher rates of leaf decomposition and the fungal biomass. Nutrient enriched environments can lead to changes in detrital processing rates, caused by shifts in microbial enzymatic activity as the lignin to nitrogen ratios change (Carreiro et al. 2000). Macroinvertebrates have also been shown to enhance decomposition under elevated nutrient conditions (Robinson and Gessner 2000). Higher fungal enzymatic activity for leaf breakdown was associated with higher pH streams (Jenkins and Suberkropp 1995). Other abiotic factors can affect the detrital process. Heterogeneity spatially and temporally (Fuss and Smock 1996), including the leaf species diversity within leaf packs can affect the rates of leaf decomposition (Swan and Palmer 2004). Spatial scales, such as at the landscape (Niyogi 2003), or smaller (Sponseller and Benfield 2001) have been associated with changes in litter processing rates. Temperature has been shown to influence the rate of decomposition (Hauer et al. 1986, Maloney and Lamberti 1995). Stream discharge influenced both the nitrogen and phosphorus affects on decomposition (Creed and Band 1998, Meyer and Likens 1979). Thus, leaf decomposition rates reflect a wide variety of biotic and abiotic factors within the ecosystem, aiding our assessment of the integrated ecosystem capacity of running waters.

The first objective of this study was to compare two structural measurements for bioassessment of Coastal Plain streams. One structural measurement was the MBSS approach, which uses a semi-quantitative approach to sample taxa from different in-stream habitats (Kazyak 1997). In this study I compared this technique with an alternative leaf pack sampling method that quantifies the macroinvertebrate taxa closely

associated with terrestrially derived leaf material. In contrast to the MBSS approach, leaf pack sampling provides a way to collect the specific biota that utilize the leaf material as a food resource or as a refuge. It also provides an integrated look at the community structure by allowing the samples to remain in the stream for extended periods of time. It is expected that the leaf pack sample will be a subset of the taxa the MBSS survey would collect, however I hypothesize that the overall community assessment will be similar between the two techniques.

The second objective is to compare the rates of leaf decomposition in two Coastal Plain watersheds to identify differences in both the macroinvertebrate and microbial contributions to detrital processing. The third objective is to examine the patterns of the rates of leaf decomposition in the context of patterns of the structural measurements.

Methods:

Study System

Two watersheds were selected as study sites because they represent an agriculture-intensive watershed (along the Nassawango River) and a more pristine, forested watershed (along the Nanjemoy River) within the Maryland Coastal Plains. Each of these sites is located on Nature Conservancy lands with readily available access. Both of these watersheds are considered relatively less impacted as compared to the surrounding landscape (The Nature Conservancy 1996, 1998). They both have a wooded riparian region along the stream corridor. 5 sites were selected within each watershed ranging in size from 2nd to 4th order streams. The watershed area contributing to each sample site ranged from 5-38 km^2 along the Nanjemoy Creek and 7-113 km^2 along the Nassawango

Creek. Monitoring of selected stream sites in the Nassawango watershed on the Eastern Shore and in the Nanjemoy watershed of southern Maryland has identified chemical, physical, and structural differences between the two environments (Baer Chapter 1).

Community structure measurements

Leaf pack sampling

Artificial leaf packs were used to collect benthic macroinvertebrate samples three times each year; spring, summer. Samples were collected at each of the 5 sample sites within each of the two Coastal Plain watersheds from Fall 1998 through Spring 2001. For this comparison with the MBSS survey only spring samples were used. Red maple (*Acer rubrum*) leaves were selected because they are commonly found in both watershed study areas. They have also been shown to have a moderate rate of decomposition, with medium range k-values (approximately 0.0075 - 0.0060 day^{-1}) and a relatively small variance (Petersen and Cummins 1974, Webster and Benfield 1986). Five grams of desiccated red maple leaves were bound to a brick. Eight leaf packs were deployed at each site. The leaf packs were placed across the cross-section of the stream to measure the community structure, including the organisms that prefer the slow water edge and the ones found in the faster mid-stream region. After 30 days, the leaf packs were collected, put in plastic bags, and stored on ice until returned to the laboratory where they were maintained at 4° C until processed.

To process the samples, the leaves were rinsed in a pan and all the leaf material was washed and discarded, leaving the macroinvertebrates in the pan of water. The pan contents were then filtered through a 425-micrometer mesh size sieve to collect the macroinvertebrates. Each sample was labeled and preserved in 80% alcohol solution.

For each leaf pack, the macroinvertebrates were sorted and identified to genus level or as far as was taxonomically possible (McCafferty 1983, Merritt and Cummins 1996, Peckarsky et al. 1990, Wiggins 1996, Williams 1972, Mackay 1978). The family Chironomidae was the exception with individuals identified to the subfamily level. The mean number of taxa, and abundance of individuals were calculated per leaf pack per site. This standardized the data across sites in case where there were missing leaf packs at the time of collection. Several indices were then calculated including: the number of taxa; the number of Ephemeroptera, Plecoptera, Trichoptera (EPT) taxa; the proportion of Ephemeroptera in the community; the proportion of Tanytarsini in Chironomidae; the Beck's sensitive taxa index; the number of macroinvertebrates that are in the functional feeding group 'scrapers'; and the proportion of macroinvertebrates considered 'clingers' in the community. The classifications used for sensitive taxa, functional feeding groups, and taxa habit (e.g. clinger) were based on the Maryland Department of Natural Resources classification (Stribling et al. 1998). Maryland's Montgomery County Department of Environmental Protection taxa listings, and Merritt and Cummins (1996) taxa information were used as additional resources when taxa characteristics were missing. These above metrics were then averaged to create an Index of Biological Integrity (benthic IBI) based on the Maryland Department of Natural Resources methods (Stribling et al. 1998). These metrics were selected because the MBSS had analyzed the measures of the community that best differentiated impaired streams in the Coastal Plain region (Stribling et al. 1998). Additional metrics were calculated including: total abundance; the Shannon diversity index; the number of taxa that were considered predators, shredders, collectors, filterers, and scrapers; and the percent each of these five

functional feeding groups represented in the macroinvertebrate community. Since the leaf pack samples were taken in Spring 1999, 2000, and 2001, while the MBSS survey was performed in Spring 2002, the leaf pack samples were pooled to yield a mean spring leaf pack sample.

Maryland Biological Stream Survey (MBSS) sampling

MBSS sampling for macroinvertebrates was performed in Spring 2002 following the protocol established by the Maryland Department of Natural Resources (Kazyak 1997). Briefly, a D-frame net with 425 μm mesh was used at each site to cover 1.86 m^2, which consisted of 20 - 0.09 m^2 grabs (Kazyak 1997). The net samples were rinsed into a 23 liter bucket, then large woody debris and leaves were removed, and the sample strained through a 500 μm sieve. The contents of the sieve were then transferred into large 4 liter plastic containers and preserved with 100% ethanol until sorted. The pure ethanol was used to prevent over dilution of the preservative by stream water retained the sample debris. To sort, the contents of the sample were placed in a 23 liter bucket and approximately 18 liters of water were added. The sample was then stirred and subsampled using a 0.5 liter cup with a mesh,165 μm, bottom. This subsample was then placed into a white plastic pan that had square quadrants marked on the tray. Each quadrant was inspected under a dissecting scope and all macroinvertebrates were collected and set aside. The subsampling was complete when the quadrant containing the 100th individual was reached. All macroinvertebrates were collected from this last quadrant containing the 100th individual. These organisms were then identified as described above and the same metrics were calculated. Taxa were also assigned

tolerance values based on the Maryland Department of Natural Resources (Stribling et al. 1998), Montgomery County Department of Environmental Protection and taxa information provided by Merrit and Cummins (1996). If they did not have a classification for a taxa, then the taxa was assigned the family level tolerance value.

Leaf decomposition measurement

Leaf decomposition rates were determined for two consecutive years. A modified field design was used based on Benfield's leaf pack method (1996). Leaf decomposition was measured in small Eastman tenite butyrate plastic tubes of 0.8mm thickness, 3.5 cm diameter, and 20 cm length (see details in Baer Chapter 2). This method was based on similar design used by Niyogi et al. (2001). There were four holes, approximately 1.25 cm in diameter, on the tube sides covered with the fine 165 μm mesh to increase water flow through the chambers. There were both coarse mesh and fine mesh treatments used for the decomposition tubes. For the coarse mesh treatment, screens with 4mm openings, were attached to the upstream end, and a fine mesh of 165 μm was attached on the downstream tube opening. For the fine mesh treatment, I used 2.5 grams of preleached (for 36 hours in aerated distilled water) and desiccated (at 60°C for 48 hours) red maple (*Acer rubrum*) leaves in each tube. Samples of these leaves were set aside and used to determine the initial proportion of leaf organic matter.

The decomposition tubes were attached to a brick in sets of three; the coarse and fine mesh tube treatments, as well as a coarse tube blank which was used to account for organic matter drift into the coarse mesh treatment tube from upstream. For each of the two decomposition experiments, these blocks of 3 tubes were randomly placed within 75

m stream reaches at each site. The bricks were secured with string to stream-side trees for easy location during retrieval.

The first decomposition experiment, performed in fall 2000, consisted of 10 replicates placed at each site. The tubes were collected after 36 days, placed in plastic bags, returned to the laboratory on ice, and stored at 4°C until processed. The leaves were gently rinsed and macroinvertebrates were collected and stored in 80% ethanol for identification. The leaf samples were dried for 48 hours at 60°C and weighed. A portion of the dry leaves were ashed at 550°C using a muffle furnace for 2 hours to determine the inorganic components of the leaves. The weight of the ash was then subtracted from the dry mass to determine the ash-free dry mass (AFDM) of the leaves. The final ash-free dry mass (AFDM) for the coarse decomposition was adjusted by subtracting the AFDM determined for the associated blank tubes adjacent to each replicate tube. The total loss of AFDM was then calculated as the difference between the initial and final organic leaf weight. The AFDM was then used to calculate the percentage of organic matter loss due to decomposition within the stream environment was determined. The rate of leaf decomposition, k, was calculated using the exponential decay model $w_t = w_o e^{-kt}$, where w_t is the ending leaf weight at day t, w_o is the starting leaf weight, and k is the rate of leaf decomposition (Petersen and Cummins 1974).

In summer 2001, the second field experiment was performed with 12 replicates randomly placed in the 10 stream sites. Sets of three replicates were collected at day 14, 38, and 121, and samples were processed similarly as described above. Due to loss of replicate samples from a flooding event a fourth sampling date was omitted.

Statistical Analysis

For community structure measurements, Atchison's log ratio analysis (Atchison 1986) was performed using SAS version 8.2 (SAS 1999) to test for differences in leaf pack and MBSS sampling techniques and differences between watersheds. This analysis was also used to compare the macroinvertebrate community using FFG in the sampling technique and between watersheds. An analysis of variance (ANOVA) compared differences in community taxa metrics across watersheds and sampling methods. A Spearman correlation was use to compare the leaf pack and MBSS sampling methods. For community function measurements, differences between watersheds and treatment (fine versus coarse decomposition tubes), were measured using mean rates of leaf decomposition in a mixed model ANOVA (SAS 1999). The probability of a Type I error was set at $\alpha = 0.05$. Lastly, the rate of decomposition was correlated, using a Spearman correlation, with the macroinvertebrate structural metrics across the 10 sites.

Results:

Community structure

The taxa abundance as well as the percent each taxa represent of the community are listed in Table 3.1. I considered taxa to be rare when they were below the threshold of 1% in the community. Thus, 33 of 45 (73%) of taxa collected in leaf pack samples and 36 of 49 (73%) of taxa samples using the MBSS method were rare. Similarly, in the Nassawango watershed 34 of 46 (74%) of taxa collected using leaf packs and 27 of 41 (66%) of taxa using MBSS survey technique were rare. Dipteran taxa dominated samples from both watersheds. Other abundant taxa included Oligochaetes ranging from 9-16%

of the community using the leaf pack sampling to only 2-4% with the MBSS sampling method. Isopods, family Asellidae, ranged from 1-7 % of the community, while amphipods made up 2-9% of the structure. *Leptophlebia* were absent from leaf pack samples, while contributing 9-37% to the community structure measured with the MBSS technique.

At the watershed scale, there was a significant difference in the community structure using the two sampling methods when a multiple analysis of variance (MANOVA) was performed using order level resolution to classify the taxa ($p<0.01$) (Table 3.2, Fig. 3.1). However, there was no order level differences between the two watersheds. There was no difference in the community composition of functional feeding groups between watersheds or when evaluating the two sampling methods (Table 3.3, Fig. 3.2).

The community metrics were also similar in the two watersheds using either the leaf pack or MBSS techniques (Table 3.4, Fig. 3.3, Fig. 3.4). As expected, I observed that all but the Shannon diversity index, percent Tanytarsini, and the number of scraper taxa were significantly higher in the MBSS samples compared to the leaf pack samples. This is predominantly due to greater number of individuals collected using MBSS. Only the Shannon diversity index showed a significant interaction of watershed*method effect, showing that the two sampling methods predicted opposite trends between the watersheds ($p<0.01$) (Table 3.4). The leaf pack sampling did not measure a difference between the two watersheds due to a significant year effect ($F=5.20$, $p<0.02$) from the pooled data over the 3 years of spring samples. The MBSS samples demonstrate a difference

between the two watersheds ($F=14.62$, $p<0.01$) with the Nassawango watershed having greater taxa diversity.

At the sub-watershed scale, correlations illustrated significant associations when comparing the two sampling methods using the Shannon diversity index, the Beck's index, the number of scrapers, the percent clingers, the index of biological integrity, abundance of filterers, and the proportion of scrapers within the community (Table 3.5). Interestingly, while the other metrics were positively related illustrating similar measurement trends by both sampling method, the Shannon index ($p<0.03$) and the abundance of filterers ($p<0.04$) both had negative correlation coefficients indicating an inverse taxa response measured. Specifically, the leaf pack sampling demonstrated a decline in the Shannon index when comparing the Nanjemoy watershed with the Nassawango watershed, from 0.31 (±0.01) to 0.25 (±0.03), while the MBSS measured an increase, from 0.16 (±0.04) to 0.33 (±0.02) indicating great diversity in the Nassawango watershed (Table 3.4). While both sampling methods demonstrated a similar pattern of decline in the IBI score across sites (correlation coefficient $p<0.05$), when comparing the Nanjemoy watershed with the Nassawango watershed it was not significant, (ANOVA, $p<0.08$).

Community function

The leaf decomposition experiment, performed in fall 2000, showed a significant difference in leaf decomposition rate between coarse and fine mesh tube treatments ($p<0.0001$), but no difference between the Nanjemoy and Nassawango watershed (Table 3.6, Fig. 3.5). The microbial contribution to detrital processing was 0.0060 d^{-1} (±0.0004), in both the Nanjemoy and Nassawango creeks, while the macroinvertebrate and microbial

contribution, represented by the coarse mesh treatment, was 0.0068 d^{-1} (±0.0004) in the Nanjemoy Creek and 0.0067 d^{-1} (±0.0004) in the Nassawango Creek. The summer 2001 decomposition experiment illustrated similar significant differences in the rate of leaf loss ($p < 0.0001$) when comparing the fine versus coarse mesh treatments (Table 3.7, Fig. 3.6). Again, there was no difference ($p < 0.17$) in the rate of leaf decomposition between watersheds. The microbial contribution to detrital processing represented by the fine mesh treatment was 0.0105 d^{-1} (±0.0008) in the Nanjemoy Creek and 0.0125 d^{-1} (±0.0008) in the Nassawango Creek, while the macroinvertebrate and microbial contribution, represented by the coarse mesh treatment, was 0.0124 d^{-1} (±0.0008) and 0.0135 d^{-1} (±0.0008) in the Nanjemoy and Nassawango Creeks, respectively. Summer rates of leaf decomposition were twice as rapid as the rates of decomposition during the fall study.

Correlations demonstrated several interesting associations of rates of leaf decomposition with community structure and chemical and physical parameters. Both the fine and coarse mesh treatments were positively related (correlation coefficient=0.80, $p<0.01$) during the fall 2000 experiment and similarly related (correlation coefficient=0.81, $p<0.01$) during the summer 2001 experiment. The fall 2000 experiment showed positive associations of EPT taxa ($p<0.07$) and percent Ephemeroptera ($p<0.05$) with increasing rates of leaf decomposition in the coarse tubes, and similarly positive associations of EPT taxa ($p<0.06$) and percent Ephemeroptera ($p<0.07$) with increasing rates of leaf decomposition in the fine tubes. However, no chemical, physical, or landscape parameters were significantly related to leaf decomposition rates. The fine mesh decomposition tubes responded similarly to the coarse mesh tubes with regard to

taxa influences. No abiotic factors were found to be associated with the trends in the microbial contributions.

The results of the summer 2001 decomposition experiment differed from those of the 2000 experiment. The rate of decomposition was negatively related to the predator abundance for both the coarse mesh treatment ($p<0.01$) and the fine mesh treatment ($p<0.05$). Also, as the proportion of predators in the community increased, and there was an associated decrease in to the rates of leaf decomposition in coarse mesh tables ($p<0.02$). Other community FFG groups, such as filterers, were also negatively related to the decomposition rates for both coarse and fine mesh treatments ($p<0.05$ and $p<0.07$, respectively). The number of scraper taxa ($p<0.03$), abundance of predator taxa ($p<0.04$), filterer taxa ($p< 0.07$), and the proportion of scrapers ($p<0.01$) in the community were positively related to the rates of leaf decomposition in the fine mesh treatment. The abundance of predator taxa ($p<0.01$) and the abundance of filterer taxa ($p<0.03$) were inversely associated with rates of decomposition in the coarse mesh treatment.

The summer decomposition experiment also showed an association of chemical and physical parameters with changes in the rates of leaf decomposition. Hardness ($p<0.03$), pH ($p<0.07$), and conductivity ($p<0.03$) were related leaf decomposition in the coarse mesh treatment, while reactive phosphorus ($p<0.03$), pH ($p<0.05$), conductivity ($p<0.04$), and temperature ($p<0.03$) were positively related to increases in decomposition in the fine mesh treatments.

Discussion:

Both sampling techniques illustrated the same strong dominance of the dipteran taxa in the community, and a large proportion of the community represented by rare (less than 1%) taxa. This is supported by previous work done in Coastal Plain streams by Wright and Smock (2001) that showed a large Chironomidae community in these blackwater streams. While differences in several taxa metrics were observed between leaf pack and MBSS sampling (Table 3.4), only the Shannon index differentiated the two watersheds when measured from the MBSS sampling method. Large within watershed variation contributed to the lack of significance for these community metrics. The two sampling techniques showed similar trends in predicting Beck's Index, number of scrapers, percent clingers, IBI score, abundance of filterers, and proportion of scrapers. Thus, although the leaf pack sample represents a smaller sample of the community relative to the MBSS sampling technique, it does predict similar overall trends for several metrics including the overall IBI score. Although these techniques followed similar patterns of measurement, the scales were quite different. Specifically, the number of taxa collected by each method differed, with the MBSS technique assessing the community structure based on approximately 100 individuals per sample while the leaf pack sampling only had on average 10.9±1.4 individuals per leaf pack in the Nassawango watershed, and 13.4±2.8 individuals per leaf pack in the Nanjemoy watershed on which to measure community metrics.

Although the functional feeding group analysis did not show a difference between watersheds, there was a difference in the composition of communities by invertebrate orders using Atchison's statistical technique. Even though individual taxa groups were

not distinguished as different between the two watersheds, this technique demonstrated that there were shifts within the community as a whole. Doberstein et al. (2000) suggest that a limitation of fixed count techniques is an underestimate of distinguishable stream classes. This implies that MBSS sampling technique may underestimate differences in community structure between streams. My comparison of the leaf pack sampling with the MBSS technique demonstrates that these techniques illustrated similar patterns in several metrics overall, while both had little success in differentiating the watersheds. If indeed fixed counts using 100 individuals misrepresents the community structure, leaf pack samples suffer from the same shortcoming due to their low number of individuals sampled. However, the leaf pack samples provide a direct measure of those macroinvertebrates utilizing the leaf material resource. This subset of taxa can then be assessed as to their influence on detrital processes. Leaf packs may also miss taxa that utilize the leaf material due to limited temporal or spatial use of the leaf resource. Another study by Davis et al. (2003) illustrated similar challenges that Coastal Plain systems can cause for biomonitoring evaluations. Their work focused on intermittent streams and found agriculturally impacted areas with high dipteran abundances. While this taxa group differentiated their sites, I found no such differentiation between the Nanjemoy and Nassawango watersheds.

The two watersheds did not differ in macroinvertebrate or microbial contributions to the rate of leaf decomposition. The results from the fall 2000 study suggest that EPT taxa and percent Ephemeroptera in the community are associated with rates of leaf decomposition. These taxa are commonly in the shredder functional and scraper

functional feeding groups (Merrit and Cummins 1996), and may be key detritivores in these Coastal Plain communities.

Other studies demonstrate the ease and utility of using EPT taxa as a good biomonitoring measurement of changes in community structure and function (Wallace et al. 1996). Wallace et al. (1996) showed that changes in the EPT index corresponded to changes in a number of functional measurements, including leaf decomposition, fine particulate organic matter production, and secondary production.

Functional shifts from bottom-up controls have been demonstrated extensively using nutrient additions to elicit an increased rate of detrital processing by both the microbial community (Suberkropp and Chauvet 1995, Suberkropp 2003) and the macroinvertebrates (Niyogi et al. 2003). Although there has been some attention to top-down controls on detrital processing (Forrester et al. 1999, Ponsard et al. 2000, Ruetz et al. 2002), little attention has focused on macroinvertebrate predators as a control. Peckarsky (1985) demonstrated top-down mediated controls by a predaceous stonefly on mayfly grazers. Wallace et al. (1997) showed a strong relationship between predator invertebrates and non-predator production. In the two leaf decomposition experiments the correlations found between predator abundance and percent of community structure could have strong implications on the ability of macroinvertebrate detritivores to contribute to leaf decomposition as predation pressures change. If there is a negative relation between the predator abundance and the amount of leaf decay, perhaps the predators are putting a greater pressure on the detritivores, thereby forcing detritivore to spend less time consuming leaves or reducing detritivore numbers. The results of the summer 2001 decomposition study suggest that there may be top-down mediated controls

from macroinvertebrate predators. Since this relationship was not observed during the Fall 2000 experiment, the influence of predatory taxa on leaf decomposition may not be important during this time of abundant leaf litter that can act not only as a food source, but also as a refuge.

Additionally, the lack of differences across watersheds in the rates of leaf decomposition by the macroinvertebrate community may be explained by the redundancy in the shredder taxa contributing to detrital processing. Huryn et al. (2002) illustrated that, while rates of leaf decomposition did not change significantly due to land use changes, they did change due to shredder richness and biomass. It is possible that there was redundancy in the community structure within the two Coastal Plain watersheds, whereby shifts in taxa groups still contributed similarly to detrital processing.

While my study did not show significant differences between watersheds in shredder abundance or percent of shredders in the community, other studies have demonstrated a significantly greater leaf decomposition rate as shredder richness increases (Robison and Gessner 2000, Jonsson et al. 2001). This suggests that perhaps there was too much variability within the watersheds to differentiate the two, or that there were a number of rare shredders in both communities that were equal in abundance and species richness. Alternatively, it may be that using the sub-family level resolution for the Chironomidae omitted potentially important dipteran shredders. In these dipteran dominated communities shredding chironomids may play an important role in detrital processing.

The microbial community contributions to leaf decomposition were also not significantly different across watersheds. Grattan and Suberkropp (2001) showed that

while there was low diversity of fungi in a Coastal Plain stream, functional shifts were still measured with increased nutrients. Ramirez et al. (2003) demonstrated elevated respiration rates of the microbial community due to phosphorus enrichment. While these Coastal Plain watersheds were nitrogen limited due to the high phosphorus levels, other factors may have made differentiation difficult.

The rates of decomposition between the two experiments were considerably faster for the summer 2001 experiment when compared with the fall 2000 data. Increased metabolic processes in the stream can be explained by higher temperature. Gonzalez and Graca (2003) showed increased consumption rates as temperature increases to an optimum ranging from 13.7-16.7^{o}C.

Looking at the larger watershed level, this study demonstrated differences in the community structure based on broad scale whole community assessment with the Atchison's log ratio test. Finer scale resolution did not show significant differences between the watersheds using two separate sampling techniques, leaf pack and MBSS sampling. This suggests that either the communities were similar in structure, or that the within watershed variability masked any between watershed differences at a finer taxa resolution.

In addition, there was no difference at the watershed scale between rates of leaf decomposition based on both macroinvertebrate and microbial contribution. This makes drawing conclusions about patterns of structure and function difficult when the study only consisted of two watersheds. However, site level analyses demonstrate patterns in community structure and function. For example, EPT taxa were positively correlated while the predator abundance was negatively correlated with rates of leaf decomposition.

This finding has exciting implications since most of the previous work measuring leaf decomposition has focused on the bottom-up effects.

In summary, this study suggests that rates of leaf decomposition may not serve as a biomonitoring tool for Coastal Plain streams. However, the two watersheds, although differing in current agriculture use, have relatively enriched water quality. The taxa currently present in these watersheds may be influenced by the long-term press disturbance of elevated nutrients. Studies suggest that past landscape patterns can influence current community taxa (Harding et al. 1998), and their response to disturbance (Collier and Quinn 2003). Using leaf decomposition tubes as a measurement tool of community function may be better suited for distinguishing differences due to larger disturbances.

Table 3.1. Macroinvertebrate taxa abundance and percent of the community structure (in parentheses) measured within two Coastal Plain watersheds in Maryland using two sampling techniques; Leaf Pack and MBSS. (*) indicates Class level taxanomic resolution.

			Nanjemoy		Nassawango	
Order	Family	Taxa	Leaf Pack	MBSS	Leaf Pack	MBSS
Diptera	Chironomidae	*Chironomini*	6.61(48.92)	7.60(6.31)	3.66(32.53)	29.00(26.03)
		Tanypodinae	1.37(10.17)	15.80(13.12)	0.85(7.52)	11.80(10.59)
		Orthocladiinae	0.42(3.09)	5.20(4.32)	0.59(5.26)	18.00(16.16)
		Tanytarsini	1.05(7.79)	2.40(1.99)	1.26(11.15)	2.80(2.51)
	Ceratopogonidae	*Bezzia*	0.21(1.54)	0.80(0.66)	0.02(0.18)	1.00(0.90)
		Alluaudomyia	0.01(0.06)	-	0.01(0.09)	-
		Probezzia	0.02(0.13)	0.40(0.33)	0.04(0.36)	0.20(0.18)
		Culicoides	0.01(0.06)	-	0.02(0.18)	-
		Dasyhelea	0.01(0.06)	-	-	-
	Tipulidae	*Tipula*	-	0.20(0.17)	-	0.20(0.18)
		Psuedolimnophila	-	-	0.01(0.09)	-
		Hexatoma	-	0.40(0.33)	-	0.20(0.18)
	Simuliidae	*Prosimulium*	-	3.60(2.99)	-	-
		Cnephia	0.17(1.29)	0.20(0.17)	-	-
	Empididae	*Hemerodromia*	0.02(0.13)	-	0.01(0.09)	-
	Chaoboridae	*Chaoborus*	-	0.40(0.33)	-	0.40(0.36)
	Tabanidae	*Chrysops*	-	0.40(0.33)	-	-
	Syrphidae		-	-	0.01(0.09)	-
	Ptychopteridae		-	0.20(0.17)	-	-
	Culicidae		-	0.20(0.17)	-	-
Coleoptera	Elmidae	*Ancyronyx*	0.05(0.39)	0.20(0.17)	0.09(0.82)	-
		Dubiraphia	0.02(0.13)	0.20(0.17)	0.03(0.27)	3.80(3.41)
		Stenelmis	0.01(0.06)	-	0.02(0.18)	0.20(0.18)
	Dytiscidae		-	-	0.01(0.09)	-

Continue Table 3.1.

Order	Family	Taxa	Nanjemoy		Nassawango	
			Leaf Pack	MBSS	Leaf Pack	MBSS
Coleoptera	Dytiscidae	*Hydroporus*	0.24(1.80)	1.60(1.33)	0.46(4.08)	8.40(7.54)
		Copelatus	-	-	-	0.20(0.18)
		Macronychus	-	-	0.04(0.36)	-
		Oreodytes	-	-	0.14(1.27)	-
	Scirtidae	*Scirtes*	-	-	0.01(0.09)	0.20(0.18)
	Gyrinidae	*Dineutus*	-	0.40(0.33)	-	-
	Haliplidae	*Peltodytes*	-	-	0.01(0.09)	0.20(0.18)
	Hydrochidae	*Hydrochus*	-	-	0.01(0.09)	-
Ephemeroptera			0.03(0.19)	-	-	-
	Baetidae		-	1.60(1.33)	-	1.20(1.08)
	Metretopodidae	*Siphloplecton*	-	0.40(0.33)	-	-
	Leptophlebiidae	*Leptophlebia*	-	37.60(31.23)	-	10.00(8.98)
	Ephemerellidae	*Eurylophella*	-	1.00(0.83)	0.03(0.27)	2.60(2.33)
	Heptageniidae	*Stenonema*	0.03(0.26)	1.00(0.83)	-	-
		Stenacron	0.02(0.13)	0.40(0.33)	0.04(0.36)	0.40(0.36)
	Caenidae	*Caenis*	-	-	-	0.20(0.18)
Odonata	Coenagrionidae	*Argia*	-	0.20(0.17)	0.01(0.09)	-
		Enallagma	-	0.20(0.17)	0.01(0.09)	0.40(0.36)
		Ischnura	-	0.40(0.33)	-	0.20(0.18)
		Chromagrion	-	0.20(0.17)	-	-
	Calopterygidae	*Calopteryx*	0.01(0.06)	-	-	-
	Lestidae	*Lestes*	-	0.20(0.17)	-	-
	Libellulidae		-	-	-	0.40(0.36)
		Libellula	0.01(0.06)	-	-	0.20(0.18)
		Pachydiplax	-	0.40(0.33)	0.01(0.09)	0.80(0.72)
	Corduliidae	*Macromia*	-	-	-	0.20(0.18)
		Somatochlora	-	0.20(0.17)	-	0.80(0.72)

Continue Table 3.1.

Order	Family	Taxa	Nanjemoy		Nassawango	
			Leaf Pack	MBSS	Leaf Pack	MBSS
Odonata	Aeshnidae	*Boyeria*	-	0.20(0.17)	-	-
Trichoptera	Calamoceratidae	*Heteroplectron*	0.01(0.06)	-	0.05(0.45)	-
	Dipseudopsidae	*Phylocentropus*	0.02(0.13)	-	0.01(0.09)	-
	Hydropsychidae	*Hydropsyche*	0.01(0.06)	-	-	-
		Cheumatopsyche	0.01(0.06)	0.40(0.33)	-	-
	Leptoceridae	*Triaenodes*	-	-	0.02(0.18)	0.80(0.72)
		Oecetis	-	-	0.01(0.09)	-
	Hydroptilidae	*Ochrotrichia*	0.02(0.13)	-	-	-
	Limnephilidae	*Pycnopsyche*	-	0.40(0.33)	0.06(0.54)	0.60(0.54)
		Ironoquia	0.02(0.13)	-	0.03(0.27)	-
	Philopotamidae	*Chimarra*	-	-	0.02(0.18)	-
	Molannidae	*Molanna*	0.01(0.06)	-	-	-
	Polycentropodidae	*Neureclipsis*	-	-	0.04(0.36)	-
		Nyctiophylax	0.02(0.13)	-	0.02(0.18)	-
		Lype	-	-	-	-
		Polycentropus	0.03(0.26)	0.20(0.17)	0.04(0.37)	0.40(0.36)
	Odontoceridae	*Psilotreta*	-	0.40(0.33)	-	-
	Lepidostomatidae	*Lepidostoma*	0.02(0.13)	0.20(0.17)	0.01(0.09)	-
Plecoptera			0.01(0.06)	-	-	-
	Perlidae	*Perlesta*	0.02(0.13)	-	-	-
	Peltoperlidae		0.01(0.06)	-	-	-
		Tallaperla	0.01(0.06)	-	0.01(0.09)	-
		Allocapnia	-	1.00(0.83)	0.01(0.09)	-
	Nemouridae	*Amphinemura*	0.13(0.97)	-	-	-
Megaloptera	Corydalidae	*Nigronia*	0.01(0.06)	-	-	-
	Sialidae	*Sialis*	-	0.40(0.33)	-	0.60(0.54)
Lepidoptera			0.01(0.06)	-	-	0.20(0.18)

Continue Table 3.1.

Order	Family	Taxa	Nanjemoy		Nassawango	
			Leaf Pack	MBSS	Leaf Pack	MBSS
Oligochaeta*			1.23(9.14)	4.60(3.82)	1.78(15.84)	2.20(1.97)
	Asellidae		0.70(5.15)	1.40(1.16)	0.79(6.98)	3.20(2.87)
Amphipoda			0.54(3.99)	11.40(9.47)	0.23(2.08)	3.40(3.05)
Podocopida			-	0.40(0.33)	-	-
	Paloemonidae		-	-	-	0.60(0.54)
Cladocera			-	9.60(7.97)	-	0.20(0.18)
Rhynchobdellida	Glossiphoniidae		0.02(0.13)	0.40(0.33)	-	-
Pelecypoda			0.12(0.90)	0.80(0.66)	0.11(1.00)	2.40(2.15)
Gastropoda			0.22(1.61)	1.00(0.83)	0.39(3.44)	1.80(1.62)
Copepoda			-	3.20(2.66)	-	0.40(0.36)
Decapoda			-	-	-	0.60(0.54)
Hemiptera			-	0.40(0.33)	-	-
Prostigmata			0.01(0.06)	-	-	-
Collembola			-	-	0.03(0.27)	-

Table 3.2. MANOVA using Atchison's log ratio test to test for differences in taxa, using Order level data, comparing sampling method and watershed.

Effect	df (numerator, denominator)	F-value	p-value
Method	(8,9)	6.97	0.01
Watershed	(8,9)	2.49	0.10
Method*Watershed	(8,9)	1.40	0.31

Table 3.3. MANOVA using Atchison's log ratio test to test for differences in taxa, using FFG data, comparing sampling method and watershed.

Effect	df (numerator, denominator)	F-value	p-value
Method	(4,13)	0.99	0.45
Watershed	(4,13)	1.86	0.18
Method*Watershed	(4,13)	2.02	0.15

Table 3.4. Comparison of mean community metrics (± SEM) to compare watersheds, sampling method and the interaction using a Mixed model ANOVA. A non-parametric, Kruskal-Wallis, test (denoted by the χ^2 statistical test) was performed when normality was not met. (*) represent statistical results based on Log transformed data in order to meet the assumptions of normality. Bold p-values indicate significant differences.

Watershed	Sample Method	Abund.	Shannon Index	# of Taxa	EPT Taxa	% Ephem.	% Tany.	Beck's Index	# of Scrapers	% of Clingers	IBI Score
Nanjemoy	Leaf Pack	13.4 (±2.8)	0.31 (±0.01)	10.3 (±1.2)	0.4 (±0.2)	2.5 (±1.1)	16.1 (±3.6)	1.6 (±0.5)	0.8 (±0.3)	14.6 (±4.2)	1.8 (±0.2)
	MBSS	120.4 (±15.3)	0.16 (±0.04)	19.0 (±1.8)	5.0 (±1.5)	16.1 (±2.6)	25. 0(±0.0)	6.4 (±2.0)	1.6 (±0.7)	33.3 (±9.8)	3.3 (±0.4)
Nassawango	Leaf Pack	10.9 (±1.4)	0.25 (±0.03)	9.4 (±0.9)	0.2 (±0.1)	1.8 (±1.0)	20.6 (±4.9)	1.4 (±0.4)	0.8 (±0.3)	16.9 (±4.0)	1.7 (±0.1)
	MBSS	111.4 (±4.1)	0.33 (±0.02)	18.0 (±0.6)	2.8 (±0.7)	10.0 (±2.0)	15.0 (±6.1)	3.8 (±1.2)	1.4 (±0.5)	21.4 (±4.8)	2.5 (±0.2)
Test Statistic											
Watershed	Df	1,32	1,28	1,33	1	1	1	1,35	1	1,35	1,29
	Statistic	F=0.13	F=2.50	F=0.43	χ^2 =0.401	χ^2 =0.418	χ^2 =0.122	F=0.56	χ^2 =0.0008	F=0.66	F=3.48
	p-value	0.726*	0.1255	0.5188	0.5266	0.5181	0.7268	0.4603*	0.9769	0.4222	0.0726
Method	Df	1,32	1,28	1,33	1	1	1	1,35	1	1,35	1,29
	Statistic	F=158.90	F=1.01	F=36.70	χ^2 =24.61	χ^2 =19.435	χ^2 = 0.005	F=14.67	χ^2 = 3.316	F=3.90	F=26.07
	p-value	**<.0001***	0.3235	**<.0001**	**<.0001**	**<.0001**	0.9464	**0.0005***	0.0686	**0.0564**	**<.0001**
Watershed Method*	Df	1,32	1,28	1,33	N/A	N/A	N/A	1,35	N/A	1,35	1,29
	Statistic	F=0.00	F=11.29	F=0.00	N/A	N/A	N/A	F=0.53	N/A	F= 1.44	F= 2.37
	p-value	0.9469*	**0.0023**	0.9631	N/A	N/A	N/A	0.4715*	N/A	0.2376	0.1346

Table 3.5. Association between community measurements derived from Leaf Pack and MBSS sampling techniques. Bold metrics were those found to have significant relations to one another.

Taxa Metric	Correlation Coefficient	Probability
Abundance	-0.13415	0.7118
Shannon Index	**-0.71125**	**0.0211**
Number of Taxa	-0.28311	0.4280
EPT taxa	0.59861	0.0675
Percent Ephemeroptera	0.53762	0.1090
Percent Tanytarsini	-0.08839	0.8082
Beck's Index	**0.62964**	**0.0511**
Number of Scraper Taxa	**0.70372**	**0.0231**
Percent Clingers	**0.69301**	**0.0263**
IBI	**0.65245**	**0.0409**
Abundance of Predators	-0.24848	0.4888
Abundance of Shredders	-0.12925	0.7219
Abundance of Collectors	0.59575	0.0692
Abundance of Filterers	**-0.67073**	**0.0338**
Proportion Predators	-0.05455	0.8810
Proportion Shredders	0.23636	0.5109
Proportion Collectors	-0.01818	0.9602
Proportion Filterers	-0.52280	0.1210
Proportion Scrapers	**0.75696**	**0.0112**

Table 3.6. A Mixed model ANOVA test for differences in the rates of leaf decomposition between the Nanjemoy and Nassawango Creeks using coarse and fine mesh tubes as treatments for the Fall 2000 field study. Analysis based on log transformed data.

Effect	df (numerator, denominator)	F-value	p-value
Watershed	(1,8)	0.02	0.89
Treatment	(1,94)	19.49	<0.0001
Watershed*Treatment	(1,94)	0.00	0.98

Table 3.7. A Mixed model ANOVA test for differences in the rates of leaf decomposition between the Nanjemoy and Nassawango Creeks using coarse and fine mesh tubes as treatments for the Summer 2001 field study.

Effect	df (numerator, denominator)	F-value	p-value
Watershed	(1,83)	1.95	0.17
Treatment	(1,83)	31.37	<0.0001
Watershed*Treatment	(1,83)	2.96	0.09

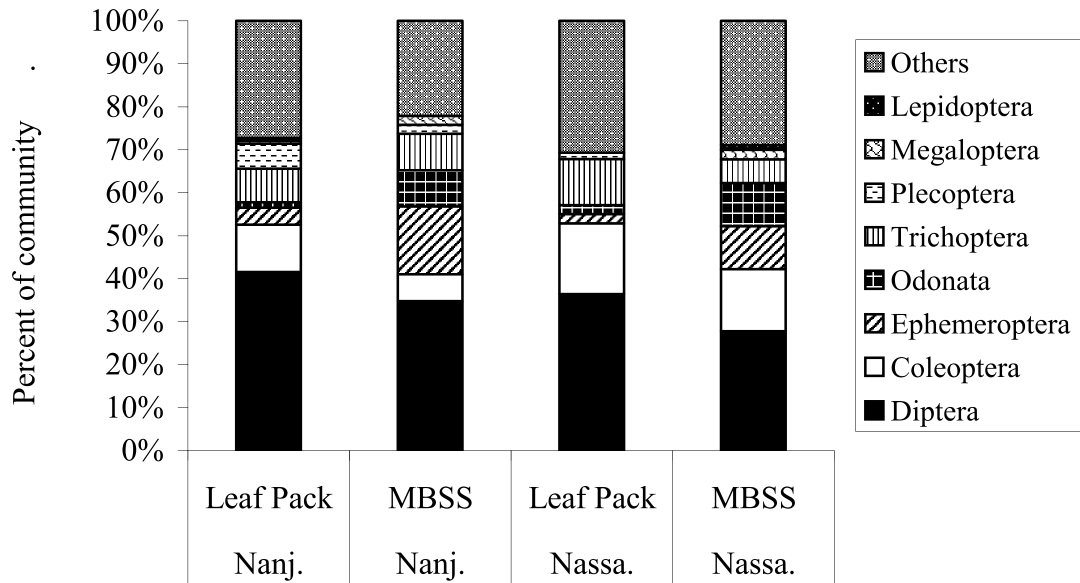


Figure 3.1. Comparing of macroinvertebrate community structure using two field sampling methods; leaf pack and MBSS techniques.

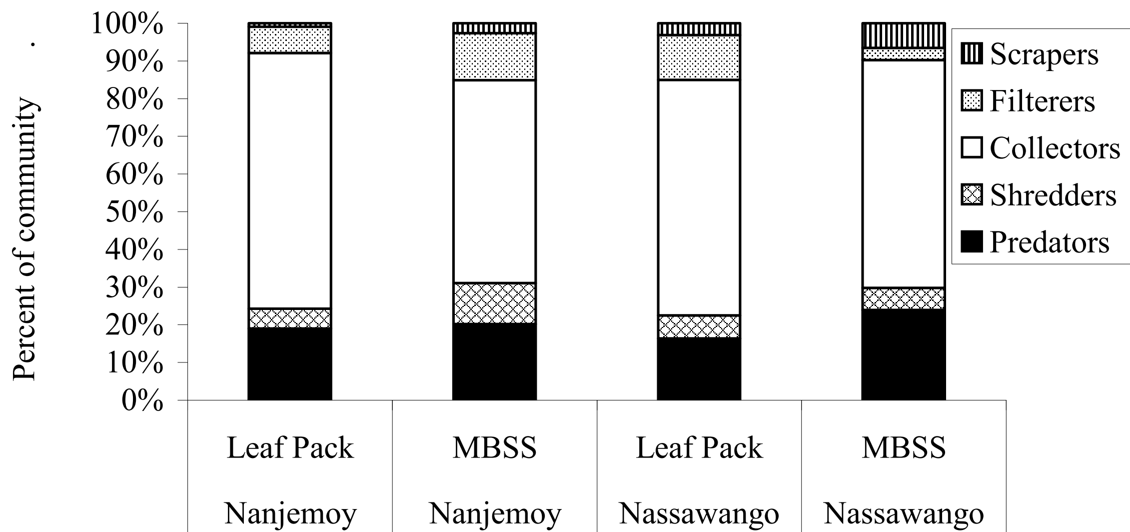


Figure 3.2. Comparing functional feeding groups for each watershed using two different sampling techniques; leaf pack and MBSS.

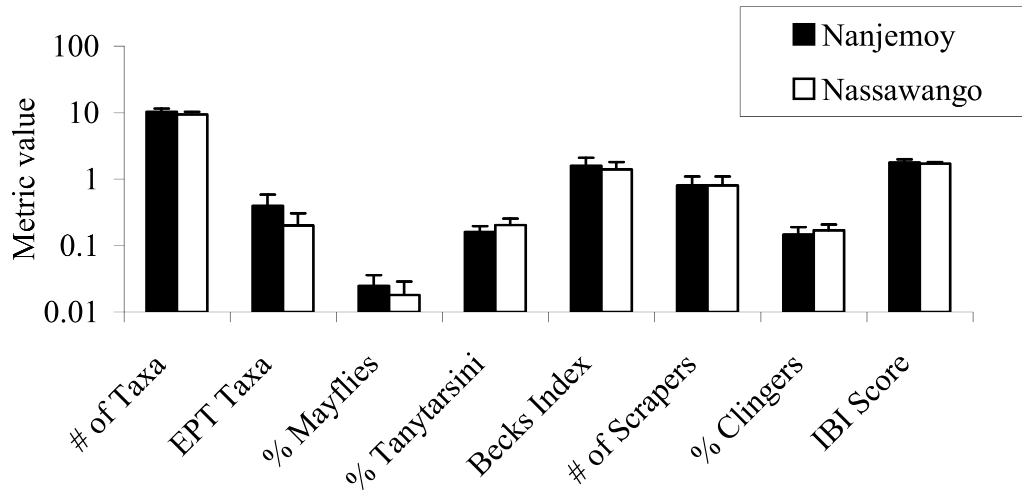


Figure 3.3. Leaf pack sampling community metrics and IBI score from spring macroinvertebrate collections. Pooled data represents five sample sites within each watershed collected from spring benthic sampling 1999 to 2001.

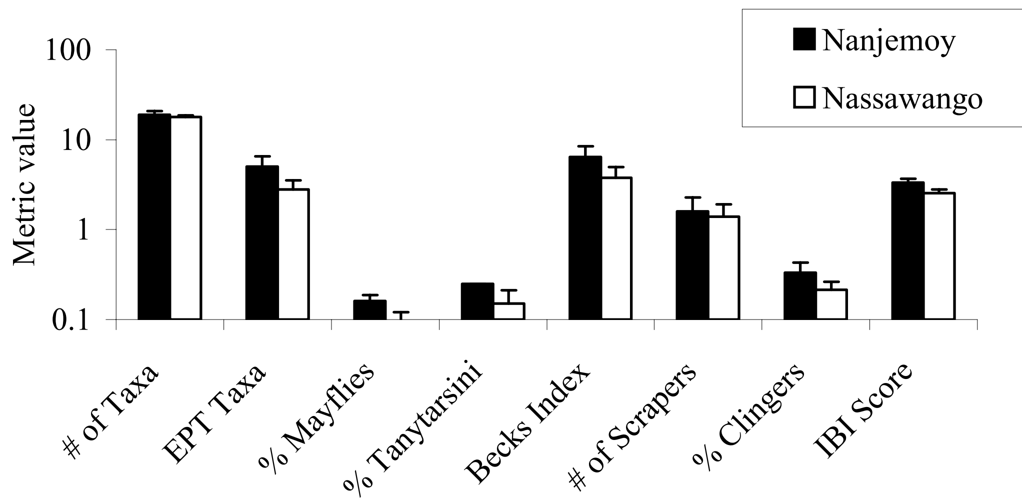


Figure 3.4. MBSS community metrics and IBI score for spring macroinvertebrate collection. Pooled data represents five sample sites within each watershed collected from a spring benthic sampling 2002.

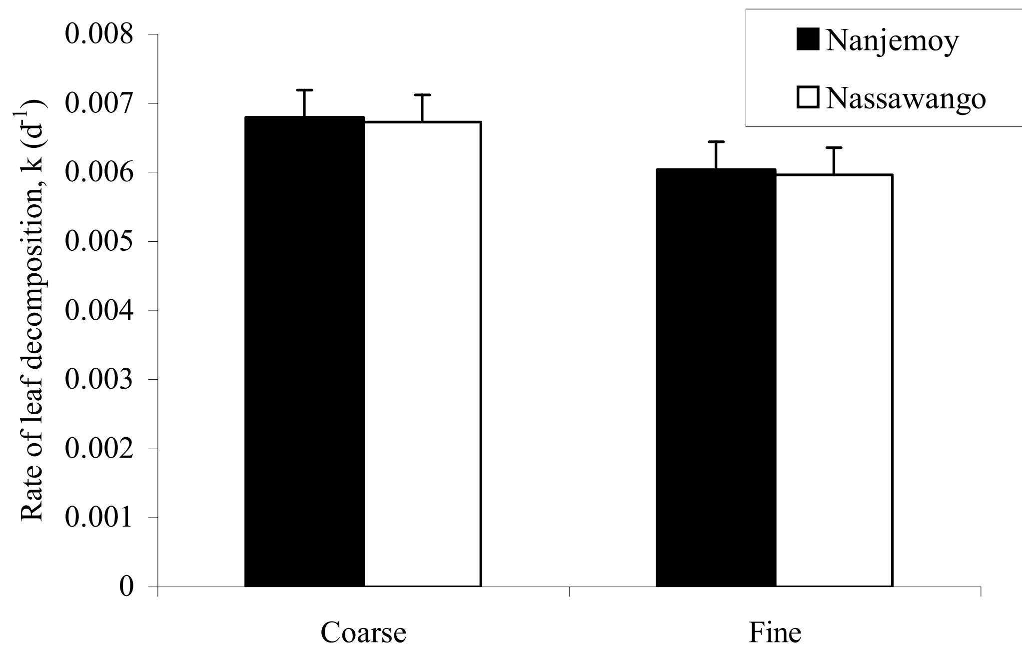


Figure 3.5. A comparison across watersheds of the macroinvertebrate and microbial community (represented by the coarse treatment) and the microbial community (represented by the fine mesh treatment) contributions to the mean (+1 SE) rate of leaf decomposition. Pooled data is from five sample sites within each watershed during a fall 2000 field experiment.

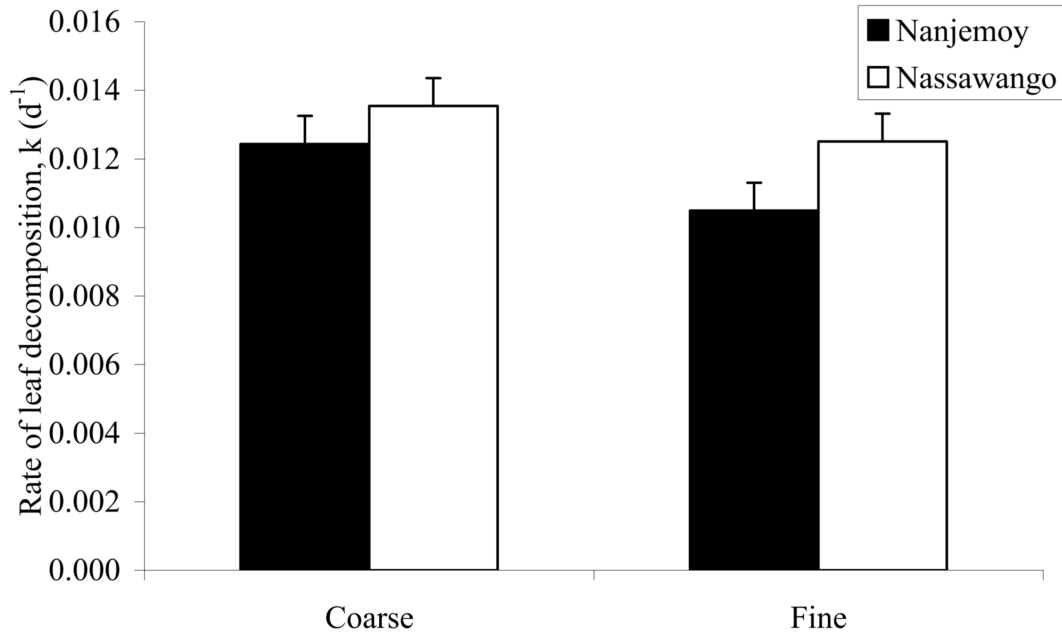


Figure 3.6. A comparison across watersheds of the macroinvertebrate and microbial community (represented by the coarse treatment) and the microbial community (represented by the fine mesh treatment) contributions to the mean (+1 SE) rate of leaf decomposition. Pooled data is from five sample sites within each watershed during a summer 2001 field experiment.

Chapter 4. Effects of nutrient and dissolved organic carbon levels on leaf litter decomposition rates by microbes and macroinvertebrates in coastal plain streams

Introduction:

Terrestrially derived leaf material provides a crucial energy source to the biotic community in forested stream systems (Fisher and Likens 1973, Webster and Benfield 1986). Because forested streams have reduced photosynthetic light available and therefore reduced primary production, it is the senesced leaf material, with its high carbon and low nutrient content that provides the primary energy source for the basal trophic organisms of these stream foodwebs (Cummins et al. 1973). This food resource contributes to the performance of species at multiple trophic levels, ranging from the bacteria and fungi to the macroinvertebrate biota (Cummins et al. 1973, Suberkropp and Klug 1976, Wallace et al. 1982, Webster and Benfield 1986, Gessner and Chauvet 1994**,** Jenkins and Suberkropp 1995, Wallace et al. 1997, Wallace et al. 1999). The interaction of these trophic levels function to convert this coarse particulate organic matter (CPOM) leaf material to a more refractory fine particulate organic matter (FPOM), and is then used by subsequent feeding guilds. Bacteria and fungi first colonize the surface of newly fallen leaves creating a biofilm layer on the leaves, and begin the leaf decomposition process (Cummins 1974). The microbial enzymes allow for the breakdown of the tough cellular leaf lignin in leaves (Jenkins and Suberkropp 1995). The macroinvertebrate shredders then further breakdown this CPOM to FPOM by shredding the leaves. Macroinvertebrates gain much of their nutritional needs from the biofilm layer,

comprised of microbes, on the surface of the leaves (Cummins 1974, Webster and Benfield 1986).

Disturbance events, both on landscape and local scales, can cause shifts in the detrital foodweb dynamics (Hall and Meyer 1998, Wallace et al. 1999). Agriculture can affect aquatic habitats by altering riparian vegetation, buffering capacity, allochthonous litter inputs, nutrient enrichment, sediment fluxes, available stream light and water temperature (e.g., Cooper and Brush 1991). A consequence of these changes in stream conditions can lead to shifts in the structure and function of the biotic community (Huryn et al. 2002, Grubaugh and Wallace 1995). Elevated nutrients can alter the biotic community structure and function (Howarth and Fisher 1976, Triska and Sedell 1976, Elwood et al. 1981, Mulholland et al. 1985, Newbold et al. 1983, Lenat and Crawford 1994, Fuss and Smock 1996, Suberkropp 1998, Carpenter et al. 1998, Carreiro et al. 2000, Qualls and Richardson 2000, Niyogi et al. 2003). The rate of leaf decomposition has been shown to change as a consequence of changes in water quality. For example, studies show increased biotic activity and leaf decomposition due nutrient enrichment affecting the microbial community (Qualls and Richardson 2000), the fungi (Suberkropp and Chauvet 1995, Sridhar and Barlocher 2000, Gulis and Suberkropp 2003), and the macroinvertebrate community (Grubaugh and Wallace 1995, Robinson and Gessner 2000, Huryn et al. 2002). However, there does not appear to be consistent patterns of detrital processing by the biotic community under nutrient enrichment conditions.

Several of these studies show N and N + P limitations (Howarth and Fisher 1976, Suberkropp and Chauvet 1995, Robinson and Gessner 2000), while others illustrate that N additions did not stimulate greater rates of leaf decomposition (Triska and Sedell 1976,

Chadwick and Huryn 2003). Still others demonstrate that P is the limiting nutrient to increase detrital processing rates (Elwood et al. 1981, Newbold et al. 1983, Qualls and Richardson 2000, Grattan and Suberkropp 2001). For example, Grattan and Suberkropp (2001) showed that both P alone and the combination of N + P amendments could stimulate higher rates of leaf decomposition and increase the fungal biomass. These results demonstrate that the biotic community is generally stimulated by nutrient additions, however the specific limiting nutrient often varies between stream systems studied.

Elevated levels of DOC can also alter the rate of leaf decomposition. A number of studies have shown the ability of the microbial community to assimilate DOC as an alternative labile carbon source (Meyer 1987, Findlay et al. 1993, O'Connell et al. 2000, Battin et al. 2003, Romani et al. 2003). The availability of DOC can lead to structural and functional shifts in the microbial community (Koetsier et al. 1997, Strauss and Lamberti 2000, Bernhardt and Likens 2002). Elevated DOC is often associated with low gradient, blackwater streams (Meyer 1986). In a laboratory study, Meyer et al. (1987) demonstrated that elevated blackwater DOC increases the bacterial production. Further studies have shown that the microbial community preferentially uses the low molecular weight DOC and increases activity as DOC is added to mesocosms (Kaplan and Bott 1983, Meyer et al. 1987). Elevated DOC has been shown to cause shifts in the carbon to nitrogen (C/N) ratio in the aquatic environment precipitating a competition between the heterotrophic and autotrophic bacteria for limited N resources (Strauss and Lamberti 2000, Bernhardt and Likens 2002). These changes in the basal foodweb may cascade up, affecting higher trophic levels in the detrital foodweb.

Although a number of studies link increases in DOC to changes in the microbial function, little is known about how nutrient enrichment will interact with elevated DOC to affect the microbial community and the next trophic level up, the macroinvertebrate shredders. My study focused on understanding how DOC interacts with elevated nutrients and alters both the microbial and the macroinvertebrate contributions to the rate of leaf decomposition.

Coastal Plain streams have unique characteristics that may contribute to the interaction of DOC and nutrient enrichment on the detrital foodweb. In addition to being low gradient streams with slow moving water, these watersheds have high water tables in contact with upper soil horizons. The sandy soil conditions can lead to poor soil sorption capacity, resulting in the organic compounds being available in the groundwater (Brady and Weil 1999). Groundwater DOC concentrations increase due to the leaching from the organic horizons of the surrounding soils into the groundwater. These conditions result in predominantly groundwater fed streams with 'black' or 'brown' tea-colored water. Meyer (1986) measured DOC concentrations in Southeastern Coastal Plain streams ranging from 12-32 mgL^{-1}. In addition, it has been shown that up to 30% of the stream DOC originates from in-stream leaf litter leaching (Meyer et al. 1998).

Historically, the Coastal Plain region of Maryland's Eastern Shore has been heavily influenced by agriculture, due to its rich alluvial soils and accessibility to rivers and coastal areas for commerce and trade (EPA 1999). Agricultural practices have altered the Coastal Plain landscape not only by increasing nutrient loading from fertilizers, but also by altering hydrologic flow patterns due to tile drainage used to expand row-cropping fields. Three years of water chemistry sampling in two watersheds

has shown significantly elevated N and P levels in the Nassawango Creek watershed located in Wicomico and Worchester counties of Maryland, where poultry farms and row cropping are present (Baer Chapter 1). The combination of these unique Coastal Plain stream conditions, coupled with the effects of non-point source pollution, is of particular interest in my investigation.

The objectives of this study was to conduct a laboratory experiment with a factorial treatment design measuring the effects of elevated N, P, and DOC concentrations on the microbial and macroinvertebrate contributions to the rate of leaf decomposition. This study measured the biotic response to shifts in DOC and nutrient concentrations to understand the underlying mechanisms for changes in detrital foodweb dynamics. The purpose of my study was to compare the functional and physiological performance of the microbial community and a macroinvertebrate shredder, *Caecidotea communis* (Order Isopoda, Family Asellidae), while exposed to different concentrations of N, P and DOC.

Methods:

I hypothesized that the rate of leaf decomposition would increase based on the addition of nutrient limiting components to the water. Because there was an excess of carbon due to high DOC, I expected increases in N and P concentrations to accelerate the rate of leaf decomposition. I also hypothesized that increased DOC would reduce leaf decomposition by the microbial community and the isopod due to the presence of a labile carbon source. Artificial stream environments were constructed in a temperature-controlled chamber. Microbes and isopods were reared in stream water samples varying

in N, P and DOC concentrations. Respiration rates, decomposition rates, and processing rates were measured to compare treatments.

A factorial treatment design was created to study 3 levels of DOC, 2 levels of nitrate, and 2 levels of phosphate in the presence and absence of an isopod shredder on detrital leaf processing.

Water Chemistry

Water was collected from the Nassawango Creek in Worchester County, Maryland in October 2002, with the intent to use it as the ambient Coastal Plain stream water. Approximately 200 liters were stored in five gallon buckets and stored at $4^{o}C$ until needed. All the water was filtered twice; once through a Buchner funnel with 0.7 μm glass fiber filter, and the second time through a high-pressure filtration system using a 0.22 μm glass fiber filter. The high-pressure filter used between 10–20 psi compressed nitrogen gas to force the water through the filter. This procedure was performed to obtain water with only the dissolved chemical constituents. The filtered water was used as stock solution of high DOC concentration. Next, this water was characterized to determine ion and DOC concentrations using an ion chromatographer and a total carbon analyzer, respectively (Table 4.1). The carbon analyzer was a Shimadzu TOC-500A Total Organic Carbon Analyzer with the ASI-500A Auto Sampler.

The 12 different water chemistry treatments were created as all possible combinations of nutrient and DOC concentrations. Two serial dilutions of the ambient water were performed to create three different DOC concentrations; 18.2 mgL^{-1}, 1.82 mgL^{-1}, and 0.18 mgL^{-1} for the ambient high DOC, medium DOC, and low DOC concentrations, respectively. These dilutions of the stock water DOC concentrations

were achieved by maintaining the mM equivalents for each of the selected major ions. I created a solution from deionized water and the major selected ion constituents highlighted in Table 4.1. The chemicals used were selected in order to balance both the anion and cation concentrations. Table 4.2 shows the chemical constituents used to maintain background ion concentrations. The ions were added to the deionized water and bubbled for 30 minutes to ensure homogenization of solution. Other background ions were intentionally omitted due to the potential for pH shifts and potential interactions with the DOC.

Nutrient treatments were then added to the individual 19 liter buckets that called for elevated N, P, or both. The nutrient enrichment treatments increased the ambient N from 1.20 mgL^{-1} to 12.00 mgL^{-1} and the P from 0.13 mgL^{-1} to 1.30 mgL^{-1} using sodium nitrate and sodium phosphate monobasic monohydrate, respectively (Table 4.3). The buckets were capped and shaken prior to every water change. These buckets containing the 12 different water treatments plus the additional salt treatment were stored in the same temperature controlled room in which the experiment was performed.

An additional salt treatment was created to test whether there was a difference in leaf decomposition due to salt additions, specifically sodium. When N and P anions were added, sodium cations were added. The salt treatment used NaCl concentrations equivalent to the sodium concentrations associated with the maximum nutrient additions, or at 0.94 mM (0.055 mgL^{-1}).

Experimental Setup

Erlenmeyer flasks (250 ml) were used as experimental mesocosms. Each flask was secured to a plywood platform by being seated within a petri-dish, which was glued

to the plywood. Each plywood sheet held 24 flasks. Double holed rubber stoppers sealed the top of the flasks, with one hole left open for ventilation and the second used for the aeration tube which acted as a bubbler maintaining high levels of dissolved oxygen and water current in the mesocosms (Fig. 4.1). The aeration system was run from the building air supply and connected to each flask using tubing. Using air pressure valves, aeration was adjusted to be approximately the same for each flask.

Senesced red maple (*Acer rubrum*) leaves were collected in the fall of 2002. These leaves were pre-leached for 12 days until no visible DOC remained. The leaves were then dried at 60°C for 48 hours, and the petioles removed. For each flask, approximately 300 gm of leaves were stored in aluminum tins (30 mm high by 70 mm diameter) until use. The leaves were softened prior to placing them in the flasks, by filling the aluminum tins with deionized water two hours.

Additional leaves from the Nassawango Creek were collected in stream water and brought back to the laboratory to be used as a microbial inoculant for the experiment. These leaves were stored at 15°C and aerated for two weeks prior to the experiment. This leached the DOC from these leaves while rearing a microbial community. Approximately 500 mg of fresh leaves were placed in fine mesh bags (mesh size of 65 μm) and suspended in each flask for the first 6 days of the experiment. They were attached with a rubberband to the aeration tube just below the surface of the water.

The isopod, *C. communis*, was used as the macroinvertebrate shredder because it is found throughout Maryland and is easily collected in large numbers. All the isopods were collected from a first order stream located on the Central Maryland Research and Education Center, near Clarksville, MD. Three isopods of similar size were weighed

fresh and then placed in each of the treatment flasks designated to be a shredder treatment. The isopods were collected using an eye-dropper, placed briefly on absorbent tissue paper, then placed in a pre-weighed aluminum tin and weighed. To estimate the initial biomass, another 20 isopods were used to quantify a fresh to dry weight regression. The dry weight conversion was then used to determine the weight gained by the isopods during the 30 day experiment.

The treatments were randomly assigned their placement on the platform to ensure that there was not a bias due to potential temperature or light gradients within the temperature controlled room. This experiment was conducted in a temperature and light controlled room. The temperature was kept at 15°C and the light schedule of 8 continuous hours of light and 16 continuous of dark was maintained for each 24-hour period. The light source was one 122 cm 40 watt fluorescent bulb. The low light was intended to maintain the diel cycle for the invertebrates while reducing the amount of primary production contributing to respiration rates. Approximately 200 ml of treatment water was added to each flask and the water was changed every 3 days for 30 days. Two replicates were run at one time and there were a total of 6 replicates performed.

Observations

Fine particulate organic matter (FPOM) was collected during each water change. A 1 mm nylon screen was held over the top of the flask to prevent loss of coarse leaf material and isopods. The water was poured through a pre-weighed 45 mm GF/A filter, with a porosity of 1.6 µm, using a Buchner funnel attached to a filter flask. The filters were then dried at 60°C for 24 hours, and reweighed to calculate the amount of FPOM accumulation.

After 30 days, the experiment was terminated and the respiration rate of the microbial community was measured using a piece of leaf material from each flask. A Strathkelvin 928 6-Channel Oxygen System, version 2.2, was used with four separate chambers and electrodes. Prior to running the samples, the dissolved oxygen electrodes were calibrated using deionized water at 15°C. The samples were measured using the protocol outlined in the Strathkelvin manual (Strathkelvin 2001). A piece of leaf material from each treatment was placed into a separate chamber with 4 ml of water from the same flask where the leaf was taken. The respiration rates were measured for 30 minutes. The leaf material was then placed in a pre-weighed tin and dried at 60°C for 24 hours, and then weighed to standardize the respiration rates.

The remaining leaves were collected in pre-weighed tins and dried at 60°C for 48 hours. The remaining leaf weight plus the weight of the leaf material used to measure respiration comprised the total leaf weight, which was then subtracted from the starting leaf weight to calculate the amount of leaf loss. Lastly, the isopods were collected, counted, and weighed before being placed in pre-weighed tins and dried for 24 hours. They were then weighed to measure the change in biomass over the duration of the experiment. A fresh to dry weight regression was calculated.

Statistical analysis

The rate of leaf decomposition was calculated using the exponential decay model $w_t = w_o e^{-kt}$, where w_t is the ending leaf weight at day t, w_o is the starting leaf weight, and k is the rate of leaf decomposition (Petersen and Cummins 1974). Processing Efficiency (AE) of *C. communis* was determined as: $PE= ((C – F)/C)*100$ where C is the amount of

food consumed and F is the amount of food excreted (modified from the assimilation efficiency equation presented in Wootton 1998).

For each of the endpoints, differences among treatments were analyzed using a two way mixed ANOVA model. The assumptions of normality and homogeneity of variance were checked. Significant differences between treatments were measured with an $\alpha = 0.05$. Apriori means comparisons were used to test for significance of endpoints measured between DOC treatments at the ambient nutrient treatment level, and comparing each of the nutrient enrichment treatments to the ambient nutrient treatment level.

Results:

The assumption of normality and homogeneity of variance were met for each of the endpoints. One outlier was removed from one replicate of the lowest DOC concentration treatment with ambient water chemistry both with and without isopod. One other replicate, from the middle DOC concentration level, 1.82 mgL^{-1}, was removed, as it was an order of magnitude higher than any other response measured. There were no significant differences in rate of decomposition between salt and ambient treatments indicating that further results regarding the nutrient additions were due to the N and/or P component of the nutrient salt additions and not the sodium salt component (Table 4.4). The initial isopod dry mass was determined using a fresh to dry mass relation calculated from a linear regression model based on 20 randomly selected isopods at the start of the study (R^2= 0.86) (Fig. 4.2).

The percent of dry leaf mass remaining ranged from 64.0% to 84.1% across the different water chemistry treatments, and with or without the isopod present. Nutrient additions affected the response depending on the level of DOC present. The analysis of variance showed a significant three-way interaction with DOC, P, and the presence of the isopod (Table 4.5). There were significant two-way interactions with DOC levels and N or P concentration. Also, there were highly significant main effects of the presence of the isopod ($p<0.0001$) and N concentration ($p<0.0005$). First considering only the DOC effect on the percent of dry leaf remaining, there is a significant increase in the percent of dry leaf mass remaining with increasing DOC concentrations when the isopod was present (apriori means comparison, $t=-2.77$, $p<0.007$). However, no significant change in the percent of dry leaf mass remaining was observed without the isopod present (apriori means comparison, $t=-0.27$, $p>0.05$) (Fig. 4.3). It increased from 74% to 82% leaf dry mass remaining from the low to high DOC concentration, respectively (Fig. 4.3).

When the isopod was present, no significant changes in the percent of dry leaf mass remaining at low DOC concentrations were observed across nutrient addition treatments. However, the presence of the isopod was associated with a significant decrease in the percent of dry leaf mass remaining at high DOC concentrations for all three nutrient addition treatments, compared with the ambient water treatment. The N addition decreased the percent of leaf mass remaining by 11% ($p<0.004$), while the P addition decreased it by 7% ($p=0.05$). The N + P addition magnified the depression in the percent of leaf mass 22% from the ambient treatment results ($p<0.0001$) (Fig. 4.4). Without the isopod present, no significant changes in the percent of leaf material remaining were found across DOC and nutrient treatments (Fig. 4.5).

The rate of leaf decomposition, k, mirrored the leaf mass remaining response for both the treatments with and without the isopod present (Table 4.6). In particular, the analysis of variance of the k values showed a significant main effect of N ($p<0.001$) and the presence of the isopod ($p<0.0001$). There were significant interactions of both DOC with N ($p<0.01$) and with P ($p<0.05$). There was one significant three-way interaction of DOC, P, and the isopod ($p<0.05$). The DOC concentration had a significant impact on the rate of leaf decomposition for treatments with isopods present. The k value significantly decreased from a mean value of 0.010 d^{-1} at the low DOC concentration, to 0.007 d^{-1} when there was a high DOC treatment concentration, representing a 30 % decline (Fig 4.6). However, without the isopod, there were no significant differences observed in k values as DOC concentrations increased (Fig. 4.7). Nutrient additions followed a similar response as the percent of dry leaf mass remaining. While there were no significant differences in the k values at low DOC concentrations when comparing the ambient treatment to each of the nutrient treatments, there were significant increases at the high DOC concentration (Fig. 4.6). The nutrient additions increased the rate of leaf decomposition from $0.0066 \pm 0.0010\ d^{-1}$, under ambient water chemistry, to $0.009 \pm 0.0010\ d^{-1}$ with elevated P, $0.0105 \pm 0.0010\ d^{-1}$ with elevated N, and $0.0151 \pm 0.0010\ d^{-1}$ with elevated N + P, respectively. This shows an increase in k values by 35%, 59%, and 127% respectively.

A significant decline in the microbial respiration rate was measured between the low DOC and mid-DOC treatment concentrations within the N + P nutrient treatment when the isopod was present ($p<0.02$). However, no significant trends in respiration rates for both the isopod and without isopod treatment was likely due to the high degree of

variance in measuring this endpoint (Fig. 4.8 and 4.9). Overall, only the three-way interaction of DOC, N, and isopod was significant ($p<0.02$) (Table 4.7).

In the presence of the isopod there was a 110% increase in the production of FPOM when comparing the N + P treatment to the ambient nutrient treatment at the high DOC concentration. No difference in production was measured when either of the two nutrients was added independently at any of the DOC concentrations (Table 4.8, Fig. 4.10). Although there were no significant differences between any of the treatments when the FPOM production was adjusted by the final weight of isopods within each treatment (Table 4.9, Fig. 4.11), a significant increase in the FPOM production was observed when adjusted for the number of isopod days within treatments (Table 4.10, Fig. 4.12). The pattern was the same as when the FPOM production was not adjusted. The N + P treatment increased FPOM production by 80% at the high DOC concentration when compared with the ambient treatment ($p<0.0001$). The microbial community in the absence of the isopod only increased FPOM production with the addition of P (Fig. 4.13). At the lowest DOC concentration, the P addition treatment increased the microbial community production of FPOM from 0.0093±0.0016 gm/30d to 0.0141± 0.0015 gm/30d, when compared with the ambient treatment ($p<0.04$), representing a 50% increase in FPOM production.

Lastly, the processing efficiency of the isopod was significantly reduced as DOC concentrations increased ($p<0001$) (Table 4.11). Under ambient water chemistry conditions, the isopod processing efficiency declined 11% from the low DOC to high DOC concentrations. Additionally, processing efficiency for the elevated N + P treatment decreased by 16% as DOC concentration increased ($p<0.02$) (Fig. 4.15).

Discussion:

The increase in DOC concentrations had a strong negative effect on the rate of leaf decomposition under ambient nutrient conditions (Fig. 4.6). Nutrient additions only showed a significant increase in the amount of leaf material processed by the isopod under the high DOC treatment. This result suggests that the isopod shifted its feeding activity in response to a change in the food quality. For example, the isopod may obtain a greater food quality from the biofilm layer on the leaf as the microbial biomass increased in response to the high DOC concentration, in comparison to the more recalcitrant carbon bound in the leaf material. Meyer et al. (1987) showed increases in the bacteria biomass when exposed to low molecular weight DOC sources. Thus, the isopods may either shift from shredding to more scraping to obtain food and consume less leaf material due to improved food quality per gram leaf material, or they may passively acquire additional microbes and carbon from the water column. The processing efficiency results do not necessarily support this hypothesized shift from shredding to scraping the leaf surface. While one would expect improved processing efficiency for scrapers as they are not consuming as much carbon rich leaf material as shredders, the results illustrated a decline in processing efficiency (Fig. 4.14). The results do suggest that the isopod may be acquiring excess carbon passively by ingesting DOC. This scenario would elicit the observed response of greater amounts of carbon egested, reducing the processing efficiencies at the high DOC concentration.

An alternative reason for the shift in the rate of leaf decomposition could be due to the elevated DOC causing an inhibitory response by the isopod or the microbial

community to leaf litter consumption. While no positive or negative microbial response to DOC was measured due to high variability, other research does not support this inhibitory hypothesis, and in fact has demonstrated the opposite for autumn senesced leaves (Koetsier et al. 1997). Numerous studies show that bacteria use DOC both from naturally occurring stream DOC (Meyer et al. 1987, Findlay et al. 1993, and Koetsier et al. 1997) and from other labile carbon sources, such as acetate (Bernhardt and Likens 2002). Researchers suggest that the microbial community shifts to a more labile carbon source in the water column rather than the more recalcitrant leaf carbon (Strauss and Lamberti 2000). Bernhardt and Likens (2002) showed that heterotrophic bacteria will out-compete other autotrophs, thereby providing additional microbial activity to decompose leaf material. Although my study did not see any significant increase or decline in the microbial respiration as DOC concentration increased, the variation was too high to effectively gauge these changes. Previous research has shown that there are bacterial shifts when a labile DOC source is made available (Bernhardt and Likens 2002, Strauss and Lamberti 2000).

The interaction of the elevated nutrient and DOC treatments on leaf decomposition illustrates that nutrient limitation is dependent on not only on the relative ratio of N to P, but also the amount of available carbon (Redfield 1958). While N, P, and N + P treatments illustrate limiting resources under high DOC concentrations, there is no nutrient addition effect at low DOC concentrations when the isopods are present. This result is coupled with the observation of significant declines of the percent of dry leaf mass remaining only at the high DOC concentration, suggesting nutrient limitations at high DOC. This is further supported by a greater than additive effect of N and P

additions to depress the leaf mass remaining. An associated acceleration in the rate of leaf decomposition, k, was shown as N and P were added together at high DOC. In both cases, the isopod had strong measured responses.

N and P are often limiting factors in the rate of detrital processing by both the microbial community (Suberkropp 1998, Grattan and Suberkropp 2001) and the macroinvertebrate contributions (Robinson and Gessner 2001, Niyogi et al. 2003). While my study was unable to demonstrate changes in the microbial activity, several other studies show the importance of N and P to both the fungal and bacterial communities colonizing and conditioning the leaves (Carreiro et al. 2000, Sridhar and Barlocher 2000, Grattan and Suberkropp 2001, Gulis and Suberkropp 2003). Other studies have shown a co-limitation of N and P on decomposition of leaf material by microbes (Grattan and Suberkropp 2001). Other researchers demonstrate microbial assemblage shifts due to DOC from leaf leachate associated with low and high order stream riparian vegetation (Koetsier et al. 1997). Their work demonstrates that microbial community structure may shift from generalist to specialist depending on the DOC quality and riparian leaf inputs. Shifts in the rate of leaf decomposition observed in my study may be due to reductions of DOC dependent microbial populations in low DOC treatments, thereby reducing the overall processing rate. While a number of studies have investigated the effects of DOC on the basal trophic level of the detrital foodweb, little work has demonstrated macroinvertebrate responses to shifts in DOC concentration and their corresponding functional responses. Additional work may help to quantify the effects of nutrient enrichment coupled with elevated DOC on the microbial contributions to detrital processes.

The FPOM production increased only when both N and P was added in the treatments containing the isopod (Fig. 4.10). While similar results were found when normalizing the FPOM production by the number of isopod days (Fig. 4.12), no significant increase was measured when normalizing isopod biomass (Fig. 4.11). Adjusting FPOM production by the number of days that isopods were present seems like an improved measure, because the correction made with end weights does not account for contribution to FPOM production by isopods that died prior to the end of the experiment. The processing rate by the isopod declined as DOC increased regardless of nutrient amendments (Fig. 4.14). This suggests that the isopod had to process a greater amount of carbon to acquire the necessary nutrients. It also shows that the elevated DOC masked the benefit increased available nutrients for the isopod. DOC may contribute to reducing the rate of leaf decomposition via providing the microbial community with an alternate carbon source, as well as disrupt the macroinvertebrate shredders from efficiently utilizing the leaf litter for energy.

Other conditions associated with Coastal Plain systems may contribute to slower leaf decomposition rates. The low gradient, slow moving stream water reduces the physical breakdown of the leaf material due to hydrologic forces, as well as reduces the water turbulence, thereby reducing the available dissolved oxygen for the biotic community (Davis et al. 2002). O'Connell et al. (2000) showed that shifts in dissolved oxygen can lead to changes from a fungal dominated biofilm on decaying leaves in aerobic conditions, to a bacterial dominated microbial community in anaerobic conditions which in turn lead to differences in DOC utilization. Furthermore, Gulis and Suberkropp (2003) showed that even under aerobic conditions and nutrient additions bacterial

dominated biofilm contributes a relatively small amount to leaf decomposition when compared to the fungal biota. Coastal Plain blackwater streams may have slower rates of decomposition than upland streams due to abiotic and biotic factors. For example, Grattan and Suberkropp (2001) found similar results as Suberkropp and Chauvet (1995), illustrating the low species diversity of fungal communities in Coastal Plain streams. This may in turn affect the rate of leaf decomposition.

Further studies are needed to develop a mechanism for shifts in detrital processing by the microbial and macroinvertebrate communities. Although my study managed to identify changes in the shredders' ability to assimilate detritus under variable DOC concentrations, as well as nutrient co-limitation as a mechanism for stimulating leaf decomposition in blackwater streams, it is still uncertain whether this is due to community structure shifts in the microbial community, or if the shredders are responding to an additional carbon source. Hall and Meyer (1998) suggests that even shredding macroinvertebrates acquire some of their carbon from exopolymers formed on amorphous detrital particles on the benthic substrate rather than on the decaying leaves. This suggests that perhaps a shredder will select a food source among various substrates. As my laboratory study only had the leaf material present, further in situ studies could provide additional information.

This study adds to our understanding of how the detrital foodweb responds to water quality perturbations. The implications of this work coupled with other research suggests that Coastal Plain blackwater streams with elevated DOC concentrations can induce very different rates of leaf decomposition due to an alternate carbon source being present. Naturally high DOC creates a greater limitation of nutrients thereby slowing

down the loss of leaf material. Nutrient enrichment, which is common in these agricultural Coastal Plain areas (EPA 1999), can lead to increases in the rate of leaf decomposition. Thus, while previous research has demonstrated the effects of nutrient enrichment and elevated levels of DOC independently, this study suggests that interactions between these two water constituents can lead to different functional responses than expected. DOC and nutrients antagonistically affected the rate of leaf decomposition for an isopod shredder. Future work examining the mechanism behind this shift in the shredders functional response can contribute to our understanding of how Coastal Plain ecosystems respond to eutrophication and other human induced disturbances.

Table 4.1. Ion concentrations characterizing the Nassawango Creek water used as the experiments stock solution. Shaded rows indicate the ion concentrations that were kept constant for the serial dilutions to dilute DOC concentrations.

Ions	Concentrations		Concentrations used in experiment
	mg/L	mM	mM/L
Na	8.856	0.39	0.39
Si	8.732	0.31	
Ca	5.774	0.14	0.14
K	5.27	0.13	0.13
Mg	2.652	0.11	0.11
Fe	1.406	0.03	
P	0.127	0.004	0.004
Al	0.45	0.02	
Zn	0.086	0.00	
Mn	0.076	0.00	
Cu	0.022	0.00	
B	0.02	0.00	
Cr	0.005	0.00	
Cl	14.07	0.40	0.4
SO_4^{-2}	13.27	0.41	0.41
NO_3^-	1.2	0.09	0.09
F^-	0.02	0.00	
Total mM equivalents		2.03	1.67

Table 4.2. Amount of chemicals added to maintain background ion concentrations in diluted DOC treatments.

Maintaining background ions for DOC dilution treatments	
Chemicals added	Amount (mM)
$NaCl$	0.39
K_2SO_3	0.14
$CaSO_4$	0.13
$MgSO_4\ 7H_2O$	0.11
$NaNO_3$	0.09
$NaH_2PO_4\ H_2O$	0.004

Table 4.3. Amount chemicals added for elevated nutrient and salt treatments.

Nutrient and salt treatments	
Chemicals added	Amount (mM)
$NaNO_3$	0.9
$NaH_2PO_4\ H_2O$	0.04
$NaCl$	0.94

Table 4.4. Satterthwaite T-test showing no significant difference between salt and ambient treatments.

Variable	df	t-value	p-value
Leaf loss	22	-0.09	0.93
FPOM Production	22	-0.46	0.65
Respiration rate	16	-0.10	0.92
k-value	22	-0.08	0.93

Table 4.5. The analysis of variance table for the percent of dry leaf mass remaining shows the significant main effects and interactions.

Effect	df (numerator, denominator)	F-value	p-value
DOC	(2,116)	2.90	0.0589
N	(1,116)	13.42	0.0004
DOC*N	(2,116)	5.33	0.0061
P	(1,116)	1.53	0.2183
DOC*P	(2,116)	3.56	0.0315
N*P	(1,116)	1.97	0.1636
DOC*N*P	(2,116)	0.34	0.7097
Isopod	(1,116)	124.23	<0.0001
DOC*Isopod	(2,116)	0.12	0.8878
N*Isopod	(1,116)	1.87	0.1736
DOC*N*Isopod	(2,116)	2.38	0.0973
P*Isopod	(1,116)	0.05	0.8243
DOC*P*Isopod	(2,116)	4.34	0.0152
N*P*Isopod	(1,116)	0.47	0.4935
DOC*N*P*Isopod	(2,116)	0.10	0.9037

Table 4.6. The analysis of variance table for the rate of leaf decomposition (k value) shows the significant main effects and interactions.

Effect	df (numerator, denominator)	F-value	p-value
DOC	(2,116)	1.94	0.1477
N	(1,116)	12.18	0.0007
DOC*N	(2,116)	5.44	0.0055
P	(1,116)	1.05	0.3077
DOC*P	(2,116)	3.52	0.0328
N*P	(1,116)	2.13	0.1468
DOC*N*P	(2,116)	0.43	0.6531
Isopod	(1,116)	111.43	<0.0001
DOC*Isopod	(2,116)	0.12	0.8842
N*Isopod	(1,116)	2.27	0.1347
DOC*N*Isopod	(2,116)	2.71	0.0707
P*Isopod	(1,116)	0.06	0.8064
DOC*P*Isopod	(2,116)	4.57	0.0123
N*P*Isopod	(1,116)	0.17	0.6788
DOC*N*P*Isopod	(2,116)	0.18	0.8377

Table 4.7. The analysis of variance table shows the significant main effects and interactions for the respiration rates of the microbial community on the leaves.

Effect	df (numerator, denominator)	F-value	p-value
DOC	(2,117)	1.83	0.165
N	(1,117)	0.09	0.762
DOC*N	(2,117)	0.24	0.790
P	(1,117)	0.00	0.952
DOC*P	(2,117)	1.58	0.209
N*P	(1,117)	0.06	0.800
DOC*N*P	(2,117)	1.39	0.254
Isopod	(1,117)	0.96	0.329
DOC*Isopod	(2,117)	0.30	0.743
N*Isopod	(1,117)	1.65	0.201
DOC*N*Isopod	(2,117)	4.36	0.015
P*Isopod	(1,117)	0.65	0.423
DOC*P*Isopod	(2,117)	0.60	0.552
N*P*Isopod	(1,117)	0.83	0.365
DOC*N*P*Isopod	(2,117)	0.92	0.401

Table 4.8. The analysis of variance table shows the significant main effects and interactions for the total cumulative FPOM production summed at the termination of the experiment.

Effect	df (numerator, denominator)	F-value	p-value
DOC	(2,116)	4.28	0.016
N	(1,116)	2.65	0.106
DOC*N	(2,116)	3.12	0.048
P	(1,116)	0.56	0.455
DOC*P	(2,116)	2.44	0.091
N*P	(1,116)	0.09	0.767
DOC*N*P	(2,116)	1.61	0.205
Isopod	(1,116)	127.39	<0.0001
DOC*Isopod	(2,116)	3.53	0.033
N*Isopod	(1,116)	7.49	0.007
DOC*N*Isopod	(2,116)	1.45	0.238
P*Isopod	(1,116)	0.09	0.770
DOC*P*Isopod	(2,116)	7.04	0.001
N*P*Isopod	(1,116)	3.63	0.059
DOC*N*P*Isopod	(2,116)	0.81	0.447

Table 4.9. The analysis of variance table shows the significant main effects and interactions for the adjusted total cumulative FPOM with *C. communis* present. The FPOM was standardized by the final weight of the isopods at the end of the experiment .

Effect	df (numerator, denominator)	F-value	p-value
DOC	(2,52)	1.93	0.156
N	(1,52)	0.64	0.428
DOC*N	(2,52)	2.93	0.062
P	(1,52)	0.12	0.727
DOC*P	(2,52)	0.59	0.559
N*P	(1,52)	0.05	0.830
DOC*N*P	(2,52)	0.51	0.604

Table 4.10. The analysis of variance table shows the significant main effects and interactions for the adjusted total cumulative FPOM with *C. communis* present. The FPOM was standardized by the number of days isopods were in contact with the leaves in each experimental unit.

Effect	df (numerator, denominator)	F-value	p-value
DOC	(2,57)	7.15	0.002
N	(1,57)	10.08	0.002
DOC*N	(2,57)	5.06	0.010
P	(1,57)	2.17	0.146
DOC*P	(2,57)	2.15	0.126
N*P	(1,57)	2.04	0.158
DOC*N*P	(2,57)	2.69	0.077

Table 4.11. The analysis of variance table shows the significant main effects and interactions for the processing efficiency of *C. communis*.

Effect	df (numerator, denominator)	F-value	p-value
DOC	(2,58)	17.74	<0.0001
N	(1,58)	0.14	0.707
DOC*N	(2,58)	0.21	0.814
P	(1,58)	0.52	0.473
DOC*P	(2,58)	2.36	0.103
N*P	(1,58)	0.91	0.344
DOC*N*P	(2,58)	1.00	0.373

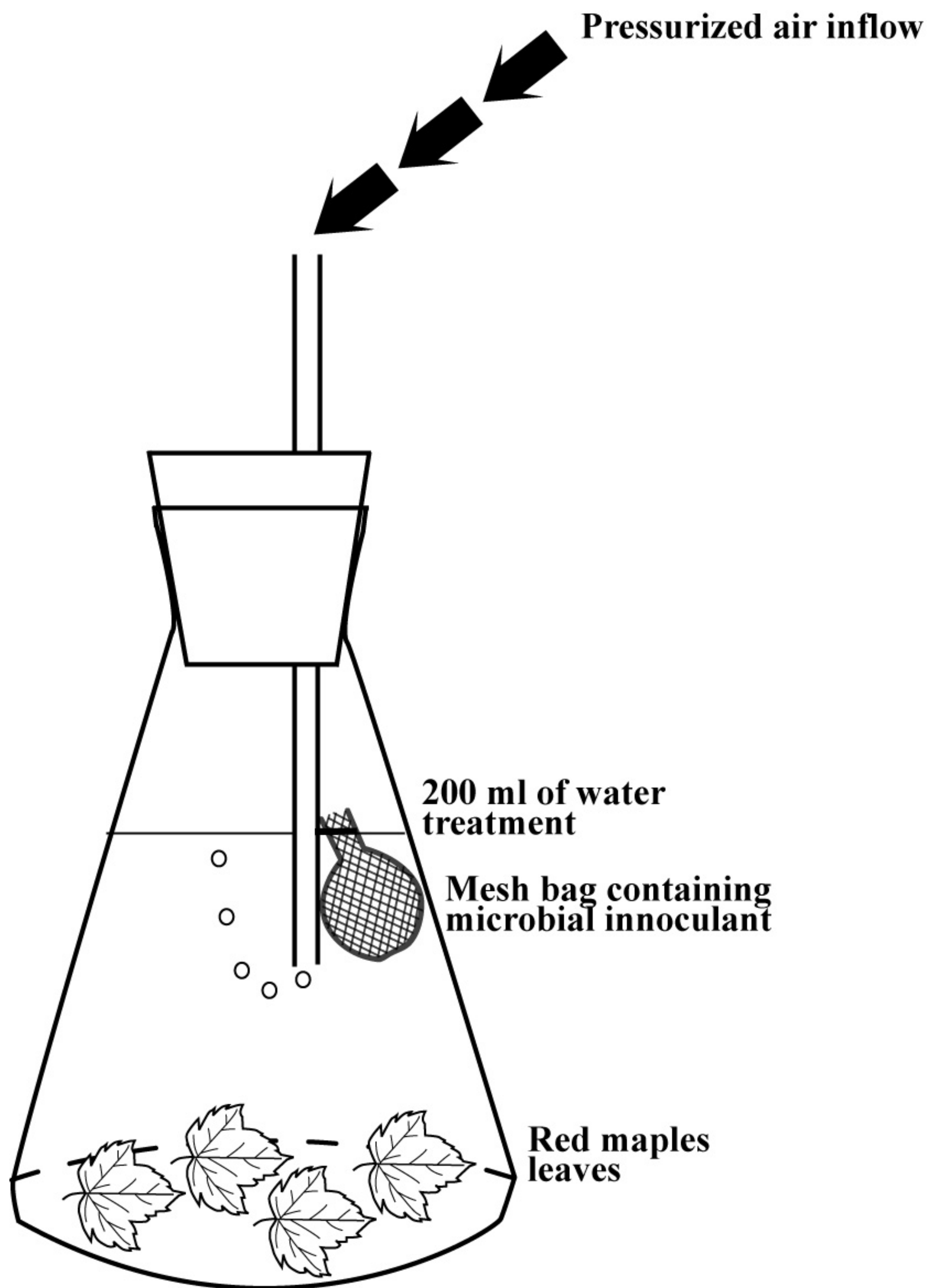


Figure 4.1. Flask mesocosm design representing one experimental unit.

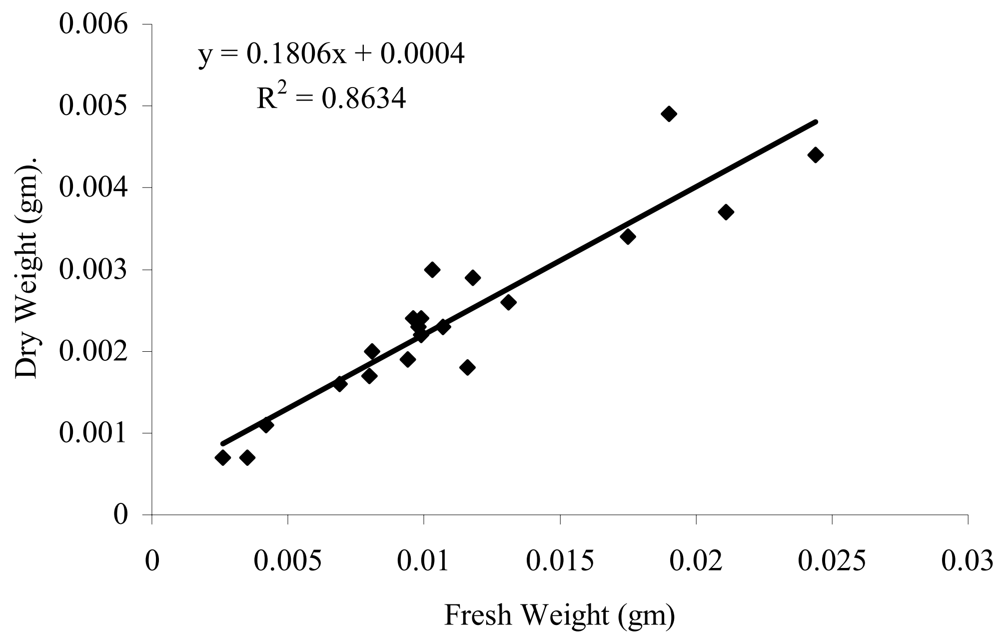


Figure 4.2. Regression of the fresh to dry weight conversion for the isopod to calculate for the starting biomass.

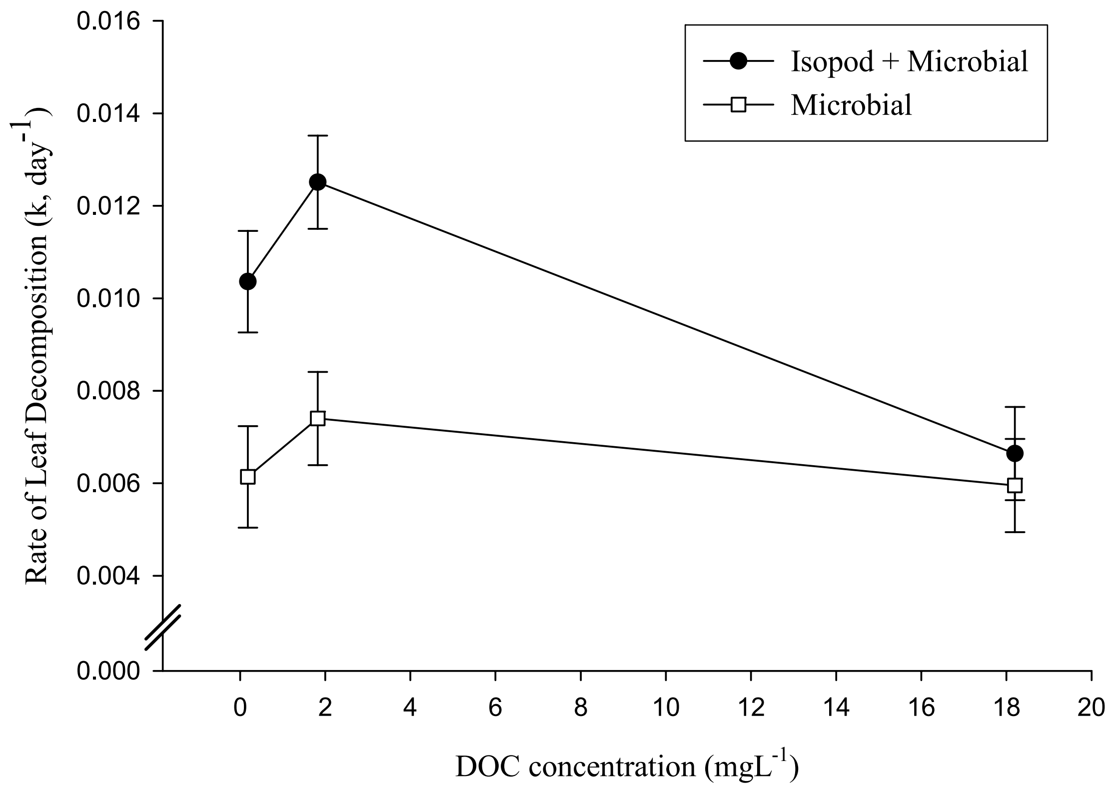


Figure 4.3. The rate of leaf decomposition, k, for the microbial and isopod shredder, *C. communis*, treatments under 4 different water nutrient regimes; ambient, elevated nitrate-N, elevated phosphorus, and elevated nitrate-N and phosphorus concentrations.

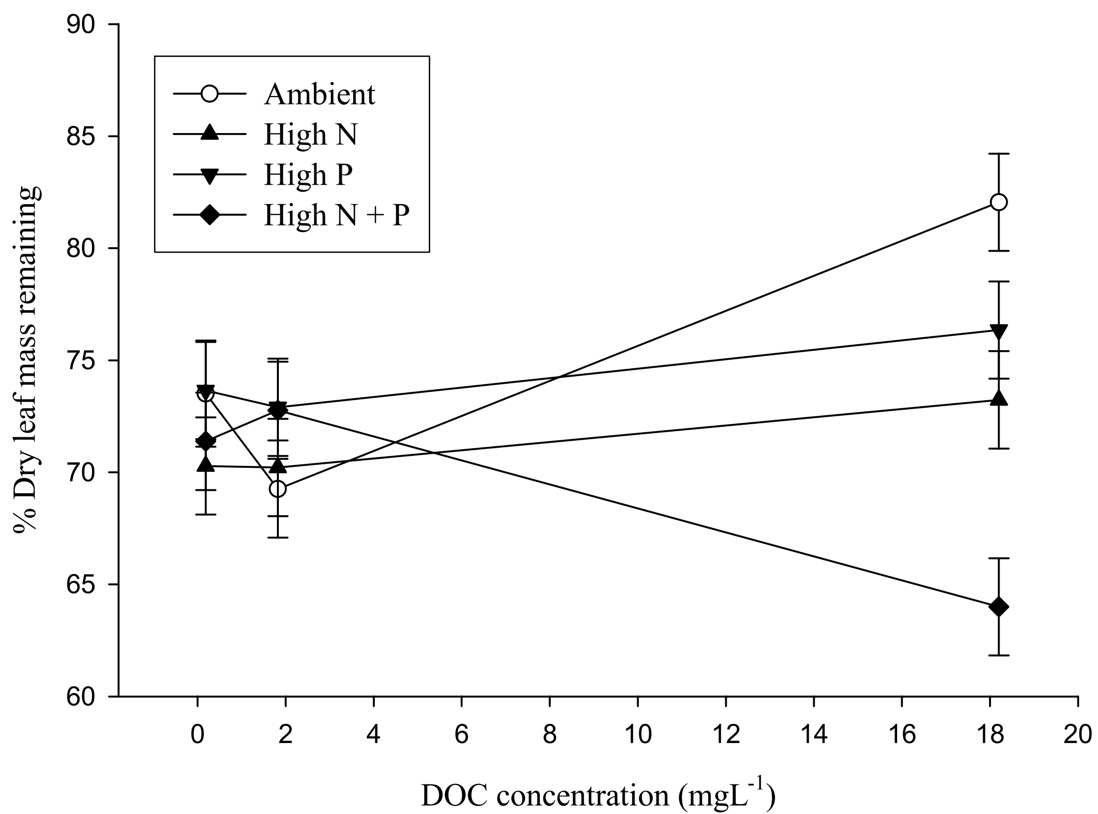


Figure 4.4. Relationship of remaining dry leaf mass (%) across DOC concentrations after 30 d with the isopod present.

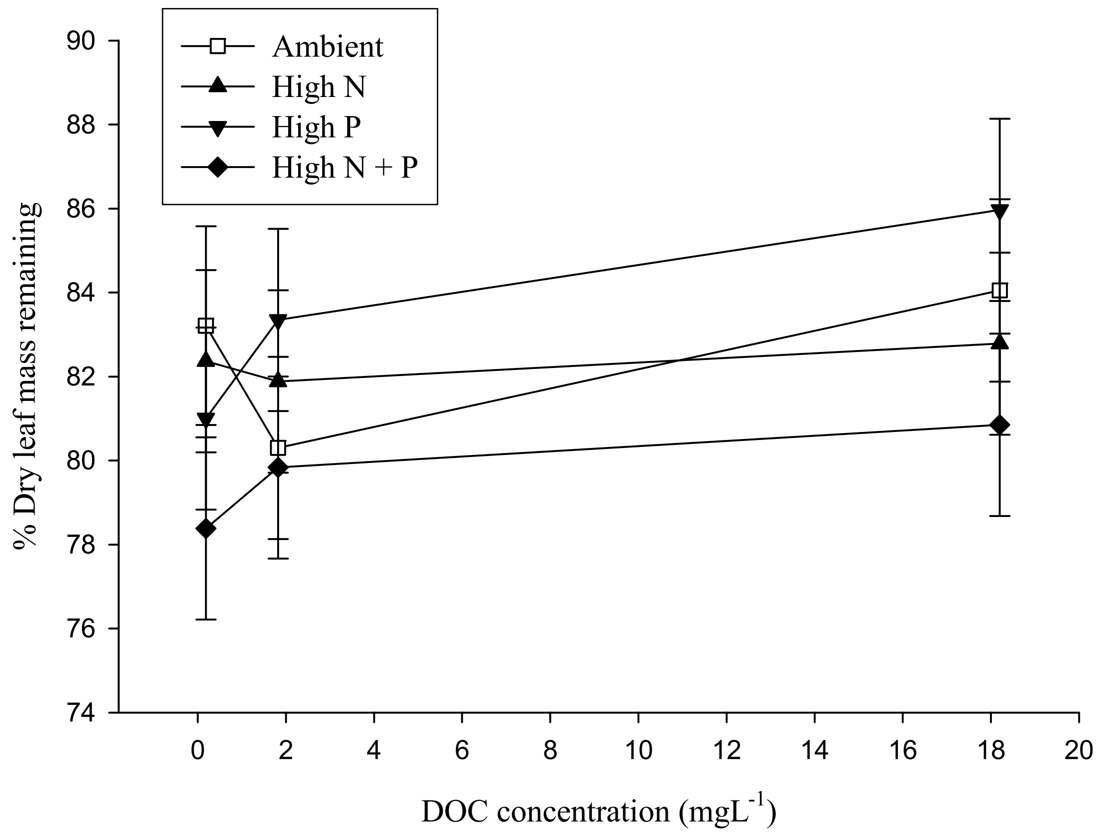


Figure 4.5. Relationship of remaining dry leaf mass (%) across DOC concentrations after 30 d without the isopod present.

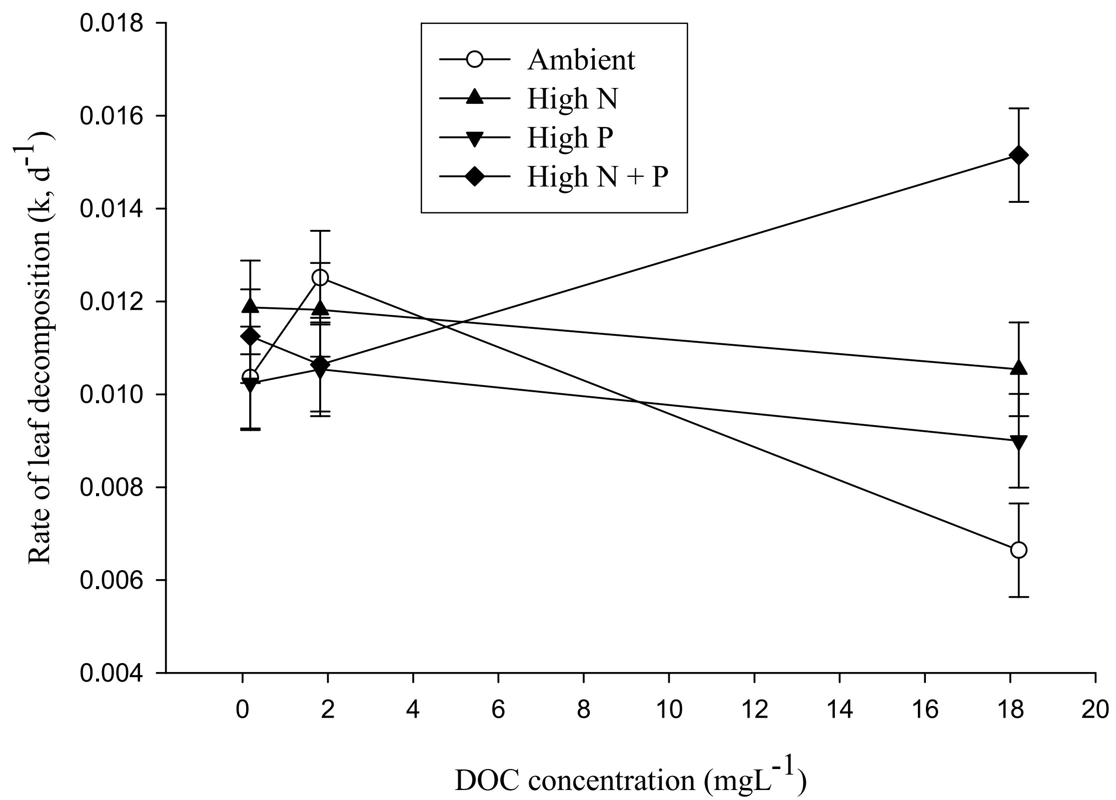


Figure 4.6. The rate of leaf decomposition, k, with the isopod present.

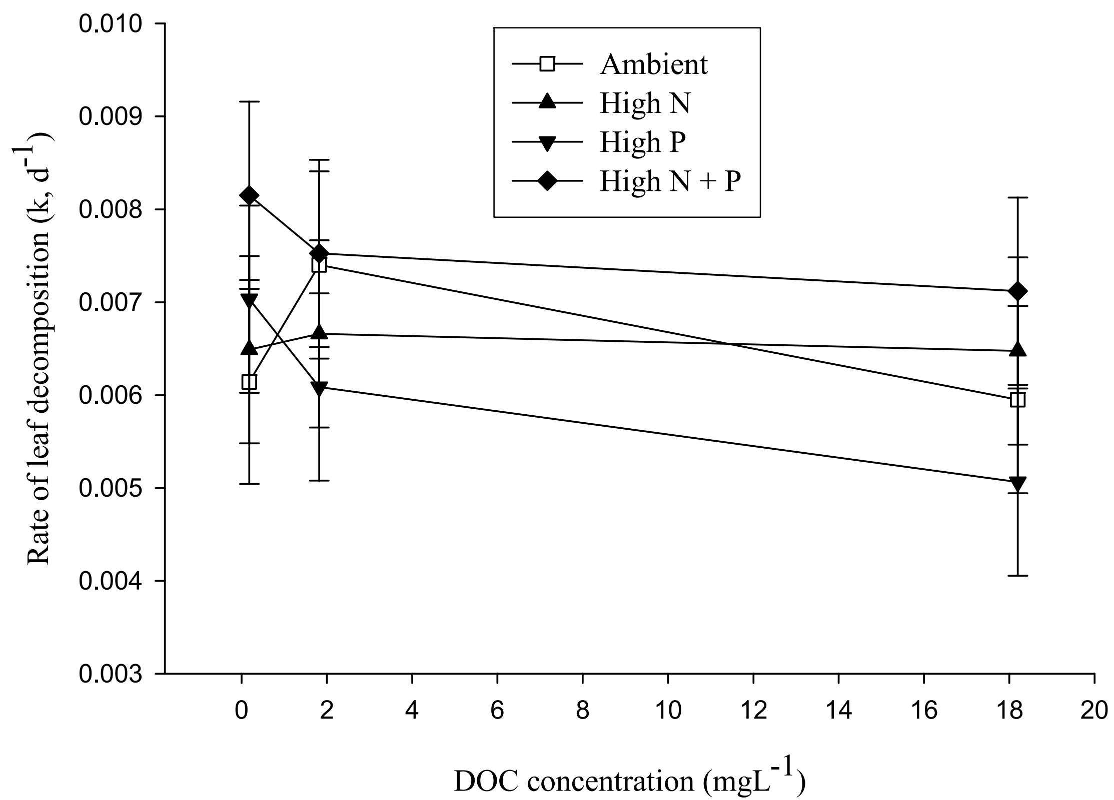


Figure 4.7. Rate of leaf decomposition, k, without the isopod present.

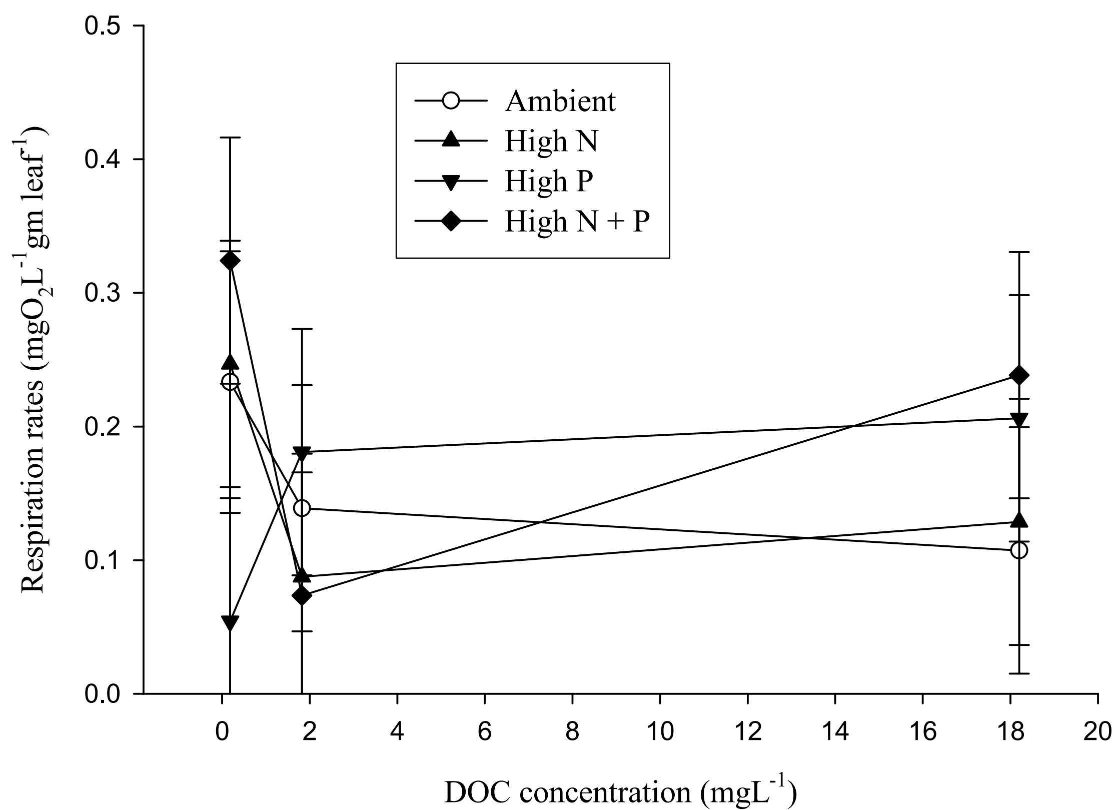


Figure 4.8. Respiration rates for the microbial community measured from treatment samples where the isopod was present.

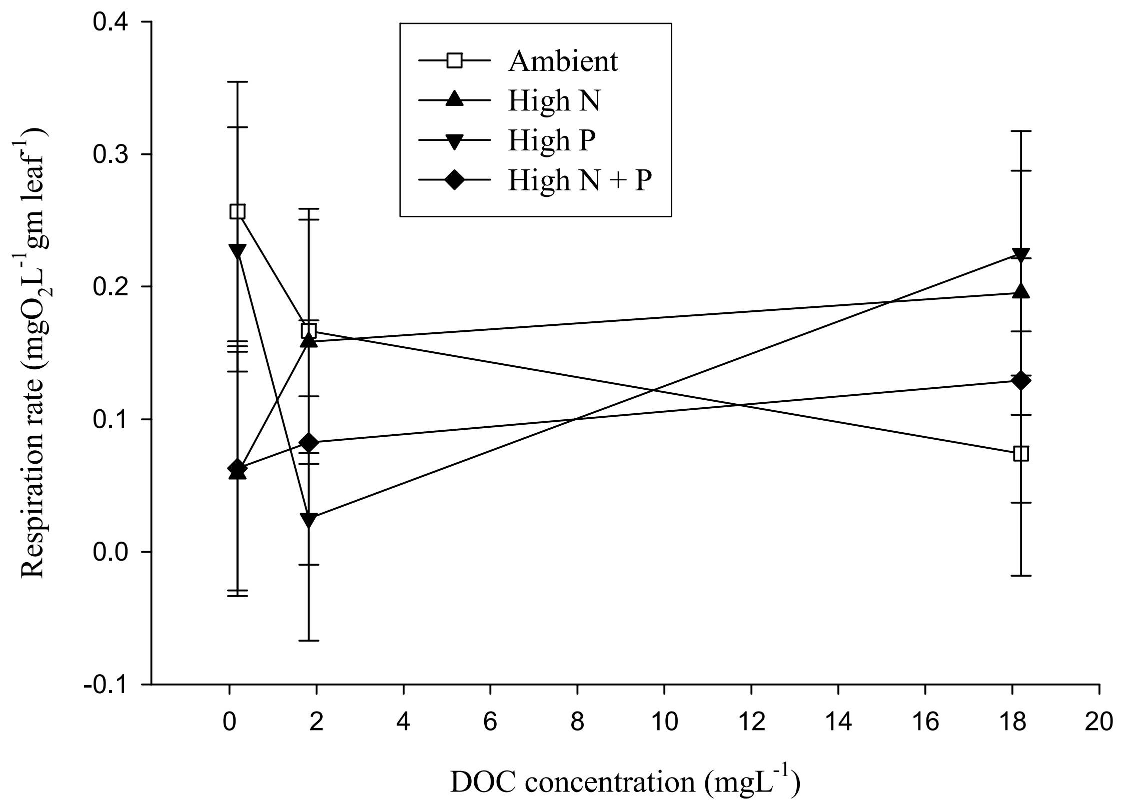


Figure 4.9. Respiration rates for the microbial community measured from treatment samples where the isopod was absent.

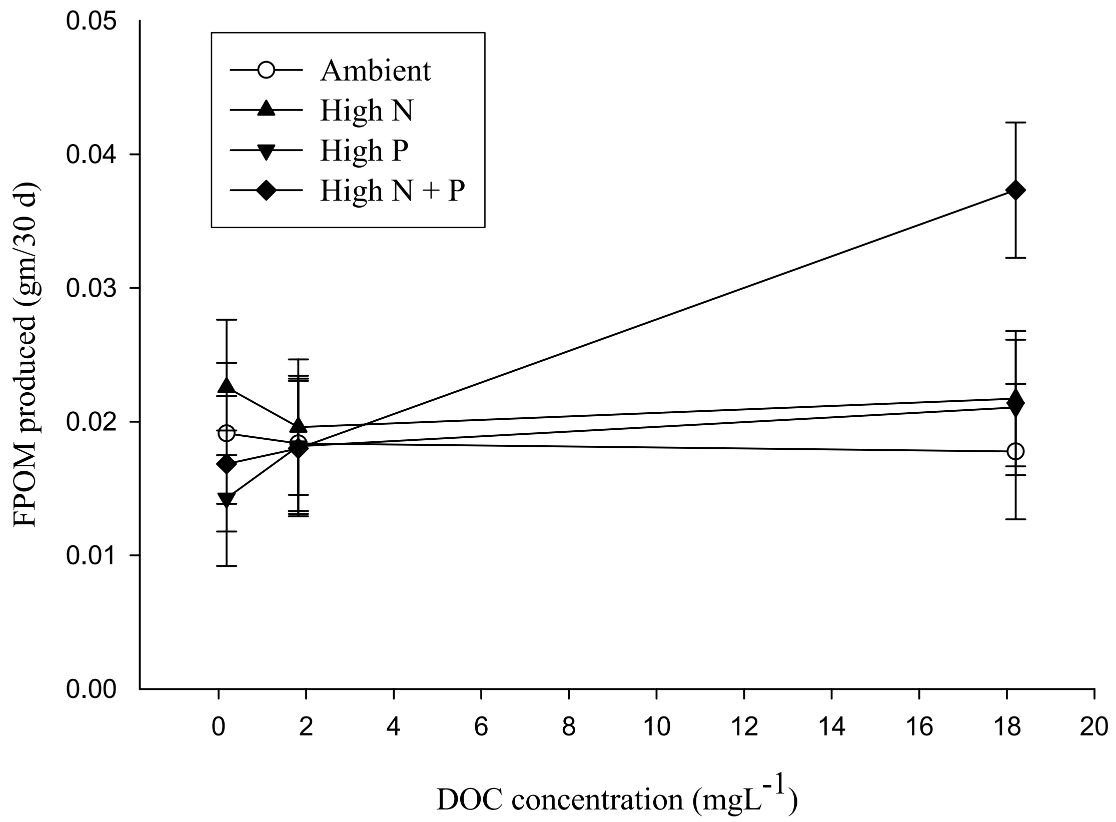


Figure 4.10. FPOM produced with the isopod present.

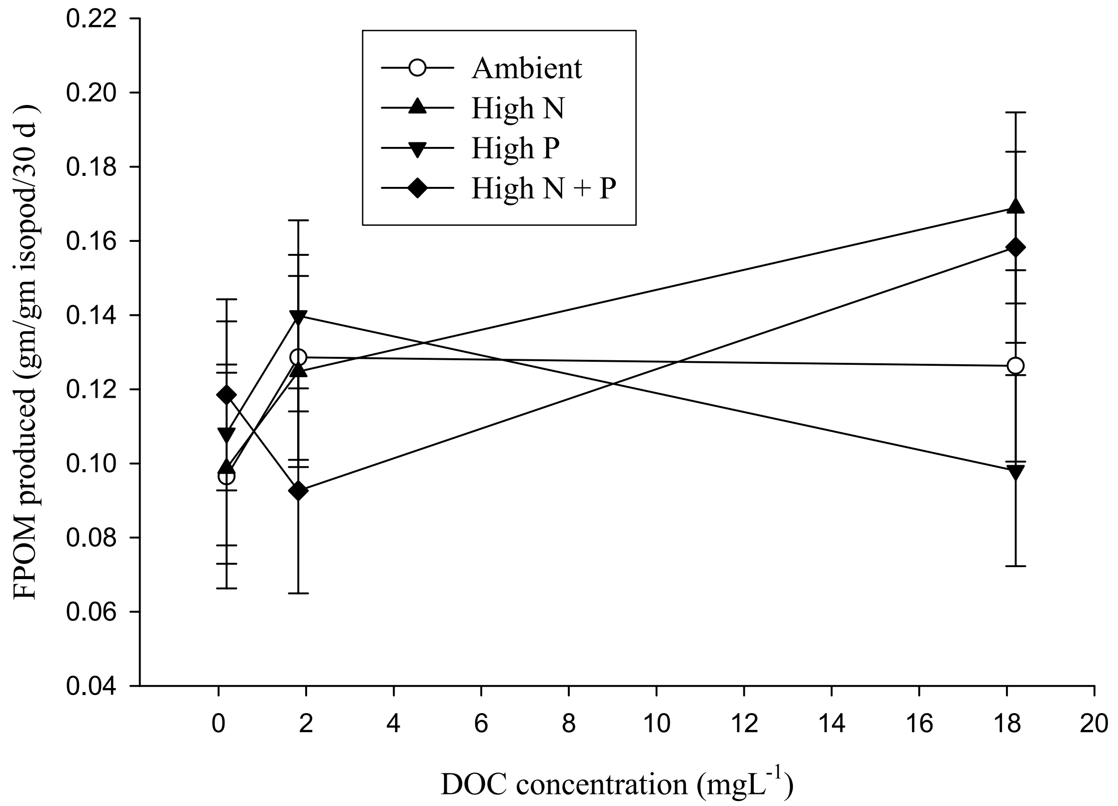


Figure 4.11. FPOM produced with isopod present and standardized for the final isopod weight at the end of 30 days.

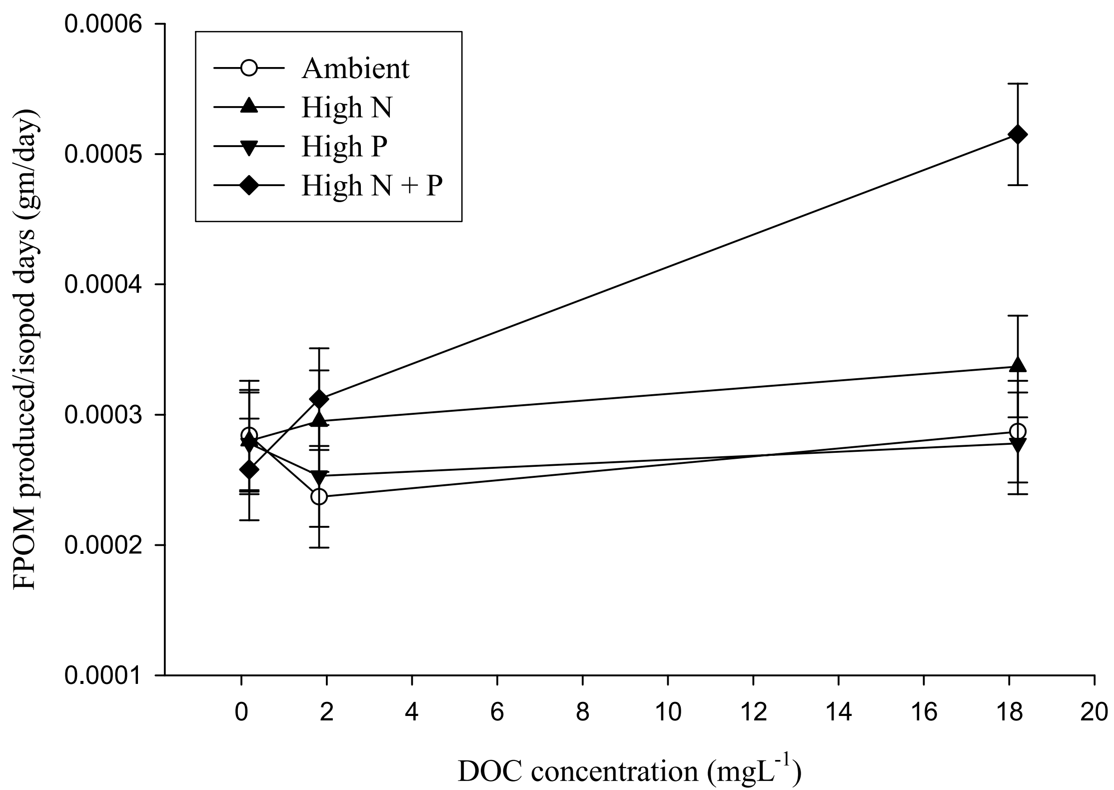


Figure 4.12. FPOM produced with isopod present and standardized by the number of days the isopods were presents.

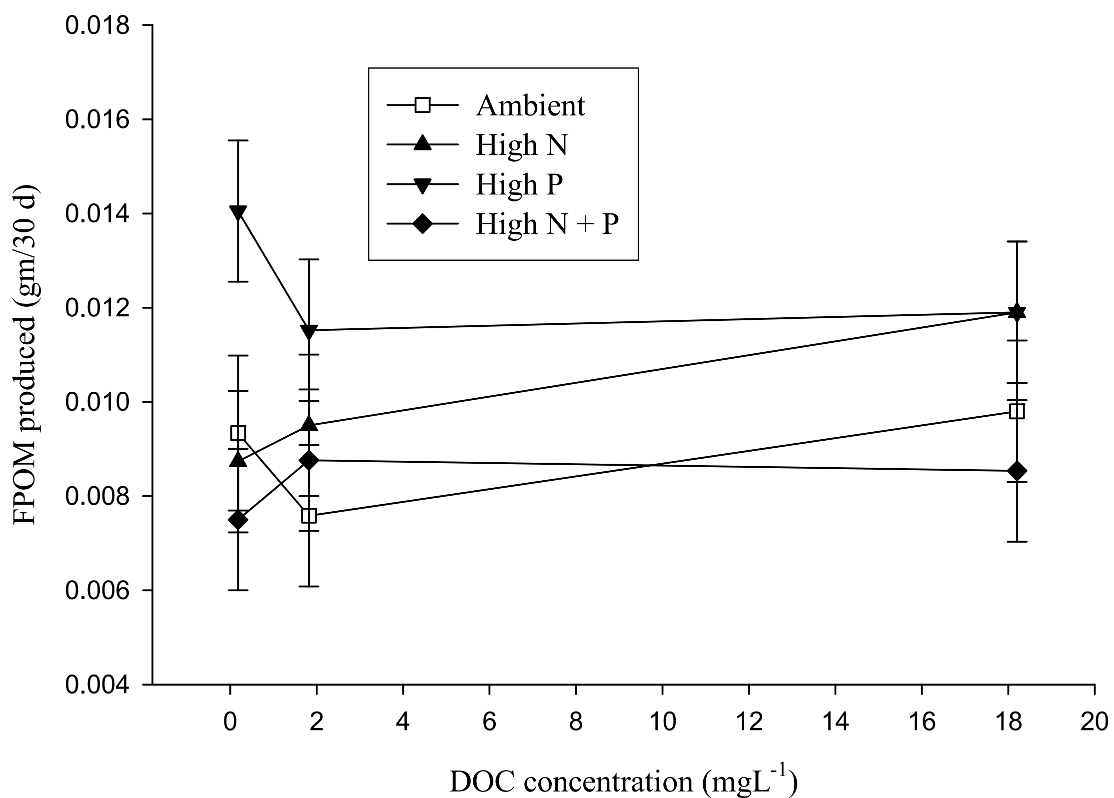


Figure 4.13. FPOM produced without the isopod present.

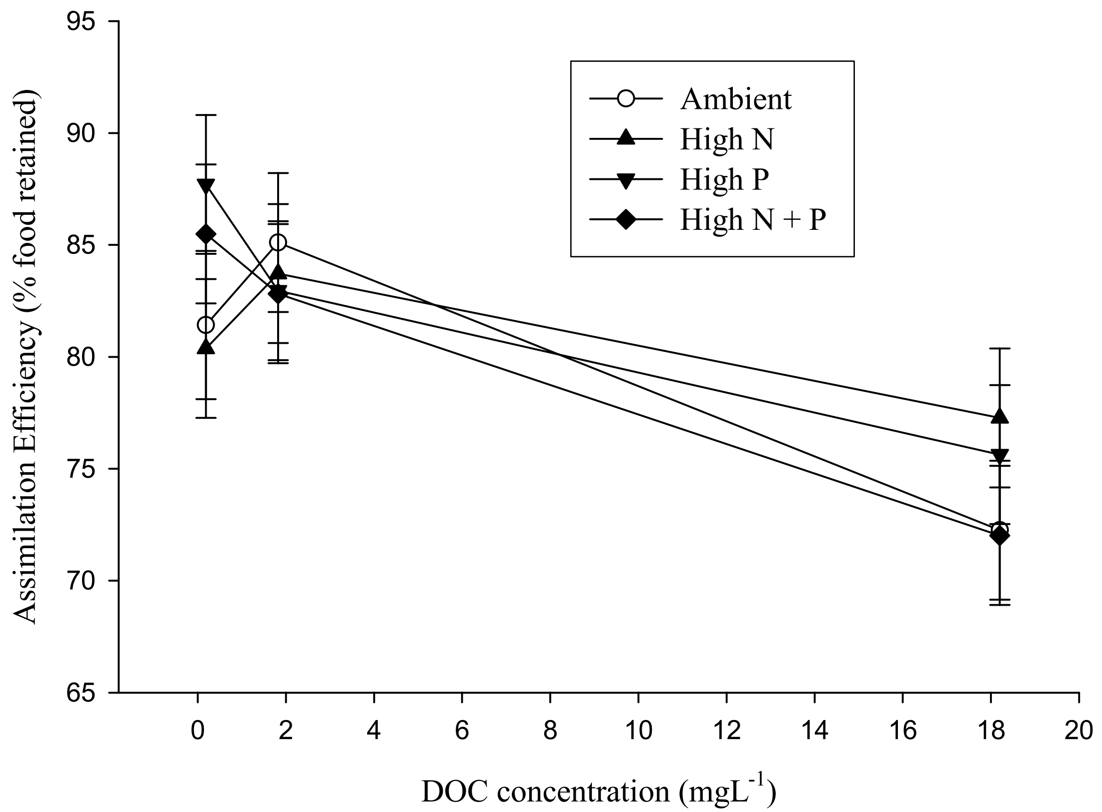


Figure 4.14. The processing efficiency of the isopod under different water treatment regimes over the 30 day experiment.

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