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THE EFFECTS OF LAND USE AND NOVEL STREAM POLLUTANTS ON EXTRACELLULAR ENZYME ACTIVITY IN BALTIMORE COUNTY, MARYLAND URBAN STREAMS

By

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ABSTRACT

Urban streams are characterized by their impervious surface cover, lack of riparian vegetation, and pollutant loading. Studies have shown that urban streams uniquely cycle nutrients and pollutants leading to degradation of water quality throughout the watershed. The goal of my study was to understand the differences in aquatic ecosystem health and resilience between rural and urban streams in order to develop more effective ecological restoration practices.

Previous studies within Baltimore County, Maryland, have found pollutants such as the antimicrobial triclocarban and the poly-aromatic hydrocarbons (PAH) phenanthrene, fluoranthene, and benzo(a)pyrene in the urban streams. Microbes assimilate dissolved organic matter (DOM) using extracellular enzymes, which have been studied as an indicator of microbial function. I measured extracellular enzyme activity (EEA) in the water of two rural and two urban streams focusing on the EEA of three enzymes, phosphatase (PHOS), beta-glucosidase (BG), and leucine amino peptidase (LAP) to account for phosphorus, carbon and nitrogen substrates, respectively, in the DOM. For each site I measured EEA under ambient conditions and with four pollutant treatments at low and high doses.

Overall, the EEA for the untreated water did not show consistent similarities or differences between the rural and urban sites, which were categorized by amount of impervious surface cover in the watershed. This suggests that additional site characteristics, such as hydrologic base flow, temperature or density of septic systems, might be important for determining land use effects on water quality and microbial function.

With the addition of pollutant treatments, I hypothesized that urban sites would have higher EEA than rural sites because the microbial communities would have adapted to the degraded conditions; but that all sites would decrease in enzyme activity as dose of the treatment increased. However, the results showed the rural sites generally had higher activity than the urban sites, and that neither the urban nor the rural sites consistently declined in EEA with an increase in treatment dose. This suggests that greater microbial biodiversity and competition in the rural sites may be as important as the assimilation period of the microbial community in the more degraded sites. In contrast to the PHOS and BG enzyme activity, LAP activity was not significantly affected by any of the treatments or doses, suggesting that the nitrogen sources in these sites may primarily be found in the soil and sediment rather than dissolved as gases in water. These results demonstrate that EEA is an important, but complex tool for understanding microbial function in an ecosystem.

INTRODUCTION

Urbanization in America

The global landscape has significantly changed since the industrial revolution in the late 19th century owing to the combustion of fossil fuels, immigration, increased population, and the building of major metropolises (Foley *et al.* 2005; Walsh *et al.* 2005). Urban areas only account for 0.5% of Earth's land-surface, but represent more than half of the world's seven billion population (Sexton *et al.* 2012). Even with such a small percent of land cover, cities are responsible for more than 78% of total global greenhouse gases (Paul and Meyer 2001; IPCC 2007). We now understand that greenhouse gases are related to climate change and global warming (Watson 1990), and so it is imperative that we understand the role cities and urban land cover have in global pollution.

In the 1790 census, only 5% of the U.S population lived in urban areas, which are defined as places with greater than 2500 residents. Once the industrial revolution began a century later, over 35% of the U.S. population lived in urban areas. As of 1990, 75% of the U.S. population lived in urban areas and this is growing with the phenomenon of "urban sprawl." Urban sprawl refers to the expansion of metropolitans into rural areas to develop suburbs with extensive roads, housing, shopping malls, and places for employment. Living on the periphery of a city in a suburban area requires residents to drive everywhere. Today, Americans drive 90% of the time, walk 9%, and bike 1% for trips compared to European countries that are much smaller and have more public transportation opportunities; e.g. the Dutch drive 52% of the time, walk 18% and

bike 30%. An increase in automobile use, the construction of major roads and highways contribute to major issues associated with impervious surface cover (World Resources Institute 1997; Frumkin 2002; Brown *et al.* 2005).

Impervious Surface Cover

Impervious surface cover (ISC) (see appendix supplemental Table 1 for list of abbreviations) is defined as material and medium through which precipitation cannot permeate, such as: parking lots, buildings, sidewalks, roads, and some other built structures (Sexton *et al.* 2012). Impervious surface cover particularly affects urban stream ecosystems in the United States where over 130,000 km of streams and waterways do not cycle nutrients properly because of urbanization (Paul and Meyer 2001; Brown *et al.* 2005, Kaushal and Belt 2012). There is an "urban stream syndrome" that plagues the waterways with hydrological alteration, increased pollutant loading, varying temperature, removal of riparian vegetation and wetlands, and storm water drainage (Walsh *et al.* 2005; Brown *et al.* 2005; Paul and Meyer 2001). The heavy automobile use in United States creates a large demand for parking lots. Parking in cities is often a major complaint and concern among commuters, and so urban planners and politicians often oversupply parking spaces whenever possible (Davis *et al.* 2010). Parking lots are particularly linked to urban degradation because they take up so much space and are considered to be biologically inert with a biodiversity value of zero because they do not support any biological organisms (Davis *et al.* 2010). Parking lots and other impervious surfaces are considered "hydrologically active,"

which means that they increase runoff and contaminant loading into freshwater systems (Barnes *et al* 2002; Davis *et al.* 2010). Without soil and sediment absorbing precipitation, areas with impervious surfaces have greater storm water runoff and the potential for flash floods and high peak stream discharges (Barnes *et al.* 2002; Brown *et al.*2005; Barron *et al.*2012). Impervious surface cover is a commonly used predictor of urbanization and urban stream health. In a normally functioning stream (in temperate areas) with 0% ISC, there is typically 40% evapotranspiration, 10% runoff, 25% shallow infiltration, and 25% deep infiltration of precipitation into the groundwater. Around 10-20% ISC, stream health begins to noticeably decline and there is 38% evapotranspiration, runoff 20%, 21% shallow infiltration, and deep infiltration 21%. In 35-50% ISC, there is 35% evapotranspiration, 30% runoff 30%, 20% shallow infiltration, and 15% deep infiltration. Finally, with 75-100% ISC there is only 30% evapotranspiration, 55% runoff, 10% shallow infiltration, and 5% deep infiltration (Paul and Meyer 2001).

In general, as ISC increases, surface runoff increases and lag time decreases. Lag time can be thought of as the amount of time surface water takes to enter the stream. Impervious surface cover disturbs the hydrologic cycle and, in particular, evapotranspiration, which is responsible for transporting water and water vapor. It is essential for maintaining soil and plant health. With decreased evapotranspiration, the plants around urban streams are more susceptible to degradation. Impervious surface cover also increases the likelihood of flash flooding events. Flood discharges are at least 2.5 times higher in urban streams

than in forested streams. Finally, increased runoff as a result of ISC affects the unit water yield. A large percentage of precipitation that enters urban streams leaves as surface runoff, rather than deep filtration through to the groundwater. This diminishes groundwater recharge and consequently, reduces base-flow discharge in urban streams (Paul and Meyer 2001; Barron *et al.* 2012).

Urban Stream Ecology

Urban streams are complex and difficult to study in traditional stream ecological terms since they have both natural and engineered components. Kaushal and Belt (2012) created a framework entitled "urban watershed continuum" to illustrate how urbanized watersheds link to larger order streams, and how these hydrologic connections affect the *transportation* and *transformation* of energy, organic carbon, organisms, and nutrients. The urban watershed continuum has four dimensions that revolutionize the ways in which urban stream ecologists conduct their research because it urges them to consider the urban infrastructure as part of the system. For example, according to this method, an urban stream should be called a 2^{nd} or 3^{rd} order stream (not the originating stream) because of storm drains and engineered drainage structures.

Natural headwaters serve as principal sources of organic matter like debris, wood, and leaves to lotic food webs downstream and are considered crucial in classical stream ecological theory (Vannote 1980). On the other hand, urbanized headwaters are very different from their natural state because of engineered and man-made drainage efforts. Drainage features are still important

sources of organic matter in urban streams because they bring leaves, eroded soils, gutters, and curbs. While urban, engineered headwaters still supply organic matter. These headwaters also decrease organic matter processing and retention because of lower residence times, flashier hydrology, simpler channels, and fewer nearby riparian zones than natural streams (Walsh *et al.* 2005; Kaushal and Belt 2012). These characteristics of urban streams are symptoms of the "urban stream syndrome" (Walsh *et al.* 2005).

While there is extensive hydrologic connectivity within an urban watershed, there is also reduced connectivity between the stream and nearby floodplains, wetlands, and riparian zones. Channelization, incision, stream bank protection of property from flooding, and impervious surfaces often separate urban streams from their surrounding riparian zones (Walsh *et al.* 2005; Kaushal and Belt 2012). In a natural watershed, streams are connected directly to adjacent riparian areas through flood pulses and vegetation. In these forested headwater streams, the ecological function of the stream is determined by the surrounding forested areas that are responsible for shade, regulating temperature and heat budgets, and supplying organic matter to the lotic food webs (Allan 2004; Kaushal and Belt 2012). Riparian vegetation not only protects and stabilizes streams from erosion, but also absorbs both nutrient (excess nitrogen and phosphorus) and non-nutrient (heavy metals and toxins) pollutants from the soil (Dosskey *et al.* 2010). The plant tissues in riparian vegetation are able to transform and degrade organic compounds such as benzene (Dosskey *et al.* 2010). Without the connection to nearby vegetation and flood plains, urban

streams are more likely to accumulate, store, and transport materials downstream at a faster- rate than natural streams (Kaushal and Belt 2012).

In addition to surface runoff, groundwater in urban areas does not behave the same way that it would in a natural/non-engineered area. Effluence from septic tanks in rural areas can potentially leach nitrogen into groundwater, and when the tanks begin to degrade, there is a greater chance that contaminants can become more wide spread throughout the watershed (Kaushal and Belt 2012; Barron *et al.* 2013). Furthermore, wastewater treatment plant effluent and sewer overflows can have significant effects on stream health, independent of the amount of impervious surface cover (Walsh *et al.* 2005).

Nutrient Cycling in Urban Streams

Dissolved organic matter (DOM) is released from soil organic matter when there are high flows and discharges in a stream and it serves as a food and energy supply to benthic bacteria and microbial communities (Burns and Ryder 2001). Dissolved organic matter includes all dissolved organic compounds such as carbon, nitrogen, and phosphorus. Understanding the cycling of carbon, nitrogen and phosphorus can provide insight into how these components of DOM affect stream health and microbial communities.

Organic Carbon

The most important aquatic ecosystem process is the biogeochemical cycling of organic carbon (C). The carbon cycle drives the biogeochemical

cycling of other nutrients and the biological processes in aquatic ecosystems (Harbott and Grace 2005). Organic carbon loads from storm drains, ditches, and leaky sewers are transported through an urbanized watershed (Paul and Meyer 2001; Kaushal and Belt 2012). Carbon is a part of cellular respiration and photosynthesis and it exists in the atmosphere as carbon dioxide (CO_2), along with methane (CH_4) and chlorofluorocarbons (CFCs). Carbon dioxide is the most widespread greenhouse gas. Another form of carbon is dissolved organic carbon (DOC), which serves as a food/energy supply to benthic bacteria and other microbial communities.

Reactive Nitrogen

It is an important function for stream ecosystems to remove dissolved inorganic nitrogen by assimilating it into organic compounds (plant, microbial biomass). Nitrogen is a major component of amino acids and is therefore necessary in order for organisms to make enzymes and proteins. In plants, nitrogen is especially important because it makes up a large portion of chlorophyll. Nitrogen gas in its inert, diatomic (N_2) form makes up close to 80% of the earth's atmosphere. Nitrogen also exists in reactive gaseous forms such as ammonia (NH_3) and nitric oxides (NO_x). These reactive forms exist in small proportions in the atmosphere and enter terrestrial ecosystems through nitrogen deposition. N_2 gas is generally not bioavailable for plants until it is converted through nitrogen fixation. Enzymes produced by certain bacterial species are able to convert biologically unavailable N_2 into ammonia (NH_3), which is then

converted into amino acids and ammonium (NH_4^+) by plants, and nitrate (NO_3^-) by soil microorganisms.

Nitrogen is a limiting nutrient because even though it is critical to have nitrogen for amino acids and proteins, too much nitrogen can cause major ecological damage. If inorganic nitrogen, especially in the form of nitrate, is not removed from the watershed, it may cause eutrophication in coastal areas and pollute drinking water supplies (Groffman *et al.* 2005). To prevent pollution and eutrophication, catchment scale strategies are implemented to reduce sources of nitrogen entering streams in the form of fertilizer or sewage (Groffman *et al.* 2005). Another method to inhibit nitrogen-loading pollution is to increase nitrogen sinks, which are processes and areas that prevent inorganic nitrogen from entering streams. Examples of nitrogen sinks are denitrification, the anaerobic conversion of nitrate into inert, diatomic nitrogen gas, and uptake of nitrogen by autotrophs (plants) (Groffman *et al.* 2005). Different parts of streams function as either sinks or sources of nitrogen. When these stream functions are altered by natural and human disturbance, engineered infrastructure, and environmental variation, nitrogen processing can change in a stream (Paul and Meyer 2001).

Nitrogen cycling is greatly influenced by carbon fluxes. Since carbon acts as an energy source, it can alter the ways in which microorganisms process nitrogen in an ecosystem (Groffman *et al.* 2005). An increase in organic carbon loading in urban streams also impacts the biogeochemical cycling of nitrogen such as denitrification and microbial uptake (Kaushal and Belt 2012).

Denitrification can occur when organic matter and debris dam urban streams, creating a denitrification "hot spot" (Walsh *et al.* 2005).

There is an observed relationship between DOM and nitrate concentrations in urban watersheds, particularly in the Gwynns Falls watershed studied by the Baltimore Ecosystem Study (BES) Long Term Ecological Research (LTER) site (Kaushal and Belt 2012). Scientists at the BES LTER found that areas with higher nitrate levels had increased denitrification rates of organic matter debris (Groffman *et al.* 2005). This finding suggests that the anaerobic (low oxygen) process of denitrification was fueled by the DOM in urban streams, which increased rates of microbial respiration (Newcomer *et al.* 2012; Kaushal and Belt 2012). The microbes essentially use all of the available oxygen and eventually create anaerobic stream environments that facilitate denitrification. This complex process creates "hot spots" of denitrification and is a positive feedback-loop that illustrates the problem of nitrate loading, especially in urban areas. During and after storms when more organic carbon matter is transported through the urban watershed, oxygen deficits associated with high biological oxygen demand are common (Paul and Meyer 2001).

Phosphorus

Urban catchments tend to have generally higher levels of phosphorus, in the form of phosphate (PO_4) (Paul and Meyer 2001; Duan *et al.* 2012). Wastewater effluent and fertilizers contain high levels of PO_4 and enter the urban streams during storm events (Duan *et al.* 2012). Without sufficient riparian

vegetation, urban streams are more vulnerable to soil erosion during storm events than natural storms events because there is not the root system or vegetation structure to hold the sediment in place (Paul and Meyer 2001; Kaushal and Belt 2012). When phosphorus enters streams in the form of fertilizers as a result of soil erosion after a storm event, this is called a "chemical time bomb" because it can lead to severe eutrophication and has cascading effects on stream health (Paul and Meyer 2001). In homes that contain septic systems with leach fields, phosphorus can enter the stream through the groundwater (Paul and Meyer 2001, Kaushal and Belt 2012).

Microbial Extracellular Enzyme Activity

Microbial enzymes, called extracellular enzymes or ecoenzymes, control the flow of stream nutrients (i.e. C, N, & P) (Sinsabaugh and Shah 2011). Extracellular enzyme activity (is related to both environmental resource availability and microbial function and metabolism. Extracellular enzyme activity rates are used as an ecological indicator of urban stream health (Harbott and Grace 2005). Streams rely heavily on heterotrophic bacteria to produce enzymes and hydrolyze DOM (Burns and Ryder 2001). The majority of DOM present in streams is made up of high molecular weight compounds that require extracellular enzymatic processes to break down (Findlay *et al.* 2010). When DOM composition changes, for example, from plant polysaccharides or proteinaceous compounds to a different form, the bacterial extracellular enzyme response shifts (Burns and Ryder 2001; Sinsabaugh and Shah 2011; Harbott and Grace 2005).

This relationship between DOM and EEA has been used in research as a way to predict lotic food web responses to changes in stream flow and discharge (Burns and Ryder 2001). Bacterial counts, especially bacteria associated with sewage discharge like coliform and nitrifying bacteria, are usually higher in urban streams than in rural streams (Harbott and Grace 2005). In contrast, in urban streams with high sediment metal concentrations the microbial enzyme activity decreases (Harbott and Grace 2005).

Properties of EEA

Extracellular enzyme activity kinetic measures can be used to represent resource supply because bacteria use EEA to breakdown carbon and other nutrients. Important properties of enzyme function and microbial metabolism are: kinetics (how an enzyme reaction takes place), [S] (the substrate concentration), V (the reaction rate), K_m (the amount of available substrate, or the half saturation constant), and V_{max} (the catalytic capacity, or maximum velocity of reaction measured as substrate saturation). The Michaelis-Menton model describes the enzymatic kinetics in the following equation: $V=-K_m (V/[S]) +1/V_{max.}$

Understanding microbial metabolism is important because it illustrates the biological rate of transforming and transporting organic matter. The metabolic scaling theory (MST) is based on the fact that individual microbes and organisms will process organic matter differently based on their body size. Metabolic scaling theory assumes that the flow of resources into an ecosystem is proportional to the metabolic rate of an individual organism. In streams, it is

difficult to use MST since it is challenging to measure the available resources in an open system. In order to follow the metabolic scaling theory in streams, we should combine enzyme kinetic measures (Sinsabaugh and Shah 2011).

Types of EEA

The stoichiometry (the relationship or balance of chemical components) of enzymes relates to the availability of nutrients in a given ecosystem (Sinsabaugh and Shah 2011; Kaushal and Belt 2012). Different types of EEA correspond with particular DOM substrates, such as beta-glucosidase with carbon, leucine amino peptidase with nitrogen, and phosphatase with phosphorus. Since DOM requires certain enzymes to break it down, researchers are able to examine EEA activity to find an identifying fingerprint of available DOM nutrients in the streams (Findlay *et al.* 2010).

Beta-glucosidase (BG)

Beta-glucosidase is considered an exocellulase that breaks down low molecular-weight cellulose substrates in urban streams. Beta-glucosidase is the last enzyme in a cellulose de-polymerization reaction to change the cellobiose to glucose (a usable sugar and energy source for microorganisms. Cellulose is a polysaccharide and is important for plant structure. It gives rigidity to cells and the bonds between each cellulose molecule are very strong. Cellulose is found in every plant cell. Alpha-glucosidase differs from BG in that it breaks down higher

molecular weight substrates like starch compounds from vascular plants and unicellular green algae (Harbott and Grace 2005; Sinsabaugh and Shah 2011).

Some studies have found significant positive correlations between BG activity and total DOC concentration in riverine ecosystems. Shortly after flooding and high flows, BG activity levels generally increase around the same time that DOC loads peak. The same study also found that leucine amino peptidase activity increases well after the high flows have dissipated. Burns and Ryder (2001) explain that the short pulses (24 hours long) of high flows might be enough to stimulate BG activity to start breaking down and assimilating carbon sources. The authors also rationalize that the lag time between high BG activity and high leucine amino peptidase activity could be explained by hydrolysis of polymerized long hydrocarbon chains, fatty acids, and proteins needing a longer wetting time (Burns and Ryder 2001).

Leucine amino peptidase (LAP)

Leucine amino peptidase (LAP) hydrolyzes leucine from the N terminus of the peptide. The N-terminus is the nitrogen end of the polypeptide hydrocarbon chain and LAP hydrolyzes (decomposes a compound in the presence of H_20) the ester linkages to carbon. High activity levels of LAP in urban streams indicate that these components of DOM are susceptible to decomposition by peptides. This also implies that peptides are an important carbon source of heterotrophic bacteria since the bacteria are using LAP to breakdown and assimilate the DOC for food and energy. High in-stream processing of filamentous algae can dominate urban

streams as an organic matter biomass and this would explain the use of LAP by heterotrophic bacteria. In less-urbanized/more rural streams, LAP is generally low because peptides are not as much of an important carbon source as they are in urbanized streams (Harbott and Grace 2005, Sinsabaugh and Shah 2011).

Phosphatase (PHOS)

Phosphatase is another hydrolytic enzyme that breaks down organic matter through a process called dephosphorylation (removing phosphate groups) (Burns and Ryder 2001; Sinsabaugh *et al.* 1997). Besides heterotrophic bacteria in urban streams, plant roots can also directly produce and release extracellular phosphatase for the purpose of acquiring phosphorus (Gartner *et al.* 2012). It is important for plants and microorganisms to receive phosphorus as a necessary nutrient. Even though cellulose is in plant cell (as previously discussed), it does not contain any nitrogen or phosphorus and so microorganisms and plants have to produce enzymes in order to be able to acquire and assimilate those nutrients (Sinsabaugh and Shah 2011). Phospholipids and phosphosaccharides are main organic sources of phosphorus for microorganisms (Sinsabaugh and Shah 2011). Phosphatases dephosphorylate these molecules so that they are biologically available for uptake and assimilation (Sinsabaugh and Shah 2011).

Anthropogenic Stressors

Antimicrobial: Triclocarban

Anthropogenic stressors in wastewater disturb the lotic food web and the cycling of nutrients (Kolpin *et al.* 2002). Antimicrobials are considered to be organic wastewater contaminants (OWCs) that come from personal care products, meaning that they enter the stream primarily after they are used by humans, and processed by wastewater treatment plants or domestic septic systems (Kolpin *et al.* 2002). Wastewater treatment plants are often not designed to remove these types of complex OWCs from the water and so this component of household chemicals, personal care products, and pharmaceuticals are not removed from the wastewater and will pass through the treatment plant and enter the environment (Kolpin *et al.* 2002; Halden and Paull 2005). The potential synergistic or antagonistic effects of multiple OWCs are largely unknown since the quantities are not regulated (Kolpin *et al.* 2002). The more-studied, legacy novel pollutants like pesticides and industrial byproducts have regulations, whereas consumable products do not (Kolpin *et al.* 2002). When antimicrobials enter the streams they may attack microbes and decrease the breakdown of DOM.

Triclocarban (TCC) is an antimicrobial additive found in personal care products such as face washes and scrubs, cosmetics, and detergents in the United States since 1957. Though the environmental fate of TCC is largely unknown, it is ecologically persistent, toxic, and potentially bioaccumulative because it builds up in organisms through a food web. It is a polychlorinated, binuclear aromatic urea pesticide that kills the bacteria on a person's skin that breaks down proteins. The

potential human health related concerns associated with TCC are abnormal physiological development, reproductive difficulties, increase in cancer incidence, increased toxicity of chemical mixtures, and the creation of antibiotic-resistant bacteria. Since antibiotics and antimicrobials like TCC are designed specifically to decrease bacterial populations in animals, even a very small concentration in the environment could greatly affect microorganism population and cause the rise in antibiotic resistant bacteria (Kolpin *et al.* 2002; Halden and Paull 2005).

Poly-Aromatic Hydrocarbons (PAHs)

The annual release of tens of thousands of tons of poly-aromatic hydrocarbons (PAHs) into the atmosphere each year in the United States leads to soil, air, and water contamination (Burchiel and Luster 2001). Poly-aromatic hydrocarbons are a class of environmental xenobiotics found in tobacco smoke, diesel exhaust, and other combustion-related particles (Burchiel and Luster 2001). Poly-aromatic hydrocarbons are lipophilic meaning that they are stored in fatty tissues in humans and are not very soluble in water. There are many types of PAHs and some of the most common of which are benzo(a)pyrene, phenanthrene, and fluoranthene. Structurally, they are made up of benzene rings (six-membered carbon rings) that have aromatic properties. Aromaticity refers to the way in which a conjugated carbon ring delocalizes charge and has unsaturated bonds (double or triple carbon bonds), lone pairs of electrons, and empty p-orbitals to allow for stabilization of the charge. Aromaticity is an important property because

it separates PAHs from other inorganic stream contaminants, making them more stable and more likely to bio-accumulate.

Poly-aromatic hydrocarbons can affect the human immune system by changing antigen and mitogen receptor signaling pathways, causing the lymphoids and lymphocytes (immune system cells) to be suppressed making them vulnerable to apoptosis (cell death) (Burchiel and Luster 2001). Poly-aromatic hydrocarbons are also carcinogenic and have endocrine disrupting properties that can cause reproductive diseases in humans and other animals (Yang *et al.* 2010). The Environmental Protection Agency and the Occupation Safety and Health Administration set PAH exposure levels were to 0.2 ppb for water (MCL, maximum contamination level for water set by the EPA in 1980) and 0.2 mg/m^3 for air (PEL, permissible exposure level set by OSHA) (Agency for Toxic Substances and Disease Registry 2008).

Poly-aromatic hydrocarbons enter urban streams through storm water runoff in parking lots, from paved roads, and from industries that burn fossil fuels (Scoggins *et al.* 2007 and Yang *et al.* 2010). Most PAHs are insoluble in water and usually settle in the sediment (Scoggins *et al.* 2007). However, PAHs can still be measured in significant quantities in the urban stream water, and the concentrations of PAHs in the water are concerning because they can then be transported throughout the watershed (Kolpin *et al.* 2007; Kaushal and Belt 2012). Pavement sealants are designed to create a protective barrier between the pavement and any damage caused by weather or chemicals (Scoggins *et al.* 2007; Davis *et al.* 2010). These sealants are reapplied every 3-5 years and so they are

continually entering the stream through impervious surface runoff. Poly-aromatic hydrocarbons come in two primary emulsion types: first type with 35% coal tar and the second type containing 35% asphalt (Scoggins *et al.* 2007). Coal tar comes from coking coal and is more than 50% PAH by mass, where as asphalt comes from refining petroleum and is less than 1% PAH by mass (Scoggins *et al.* 2007).

Rathbone *et al.* (1998) addressed the effects of PAHs on microbial communities in soils and found that PAHs can only be broken down via complete mineralization, co-metabolic degradation, and radical oxidation. Since PAHs are lipophilic and are not water-soluble, they settle in the sediment and so most papers and research about PAHs study the microbial breakdown in soils. However, the processes are pertinent for this study and might contribute to understanding the processing of PAHs in water. Complete mineralization requires the microbial community to totally breakdown the PAH into two components: water and carbon dioxide. Complete mineralization is unrealistic and only two-thirds of the PAH is actually mineralized and the rest is taken up as biomass by the microbial cells. Radical oxidation occurs when and organic compound chemically transforms because of extracellular enzymes produced by the microbes.

Resistance and Resilience

Antimicrobials and PAHs have the potential to alter the function of the stream (Kolpin *et al.* 2002; Scoggins *et al.* 2007). Depending on the land use

around the stream, some of the microorganisms may be resistant to the antimicrobials and PAHs because of previous exposure and tolerance. "Resistance" in an ecosystem to a disturbance, such as the introduction of impermeable surfaces, storm water, and novel carbon sources and stressors, is a measure of how much the system changes (Sudgen 2001). "Resilience" is the extent to which an ecosystem can recover after the disturbance is removed (Sudgen 2001). The more degraded, urbanized streams' microbes might be more resistant to novel stressors (like an immune-response) due to prolonged exposure than rural, forested streams. It is difficult to measure a microbial community's resilience to PAHs or antimicrobials since these compounds are likely to bioaccumulate or, as is generally the case in PAHs, store in sediment on the bottom of the stream.

Microbial Biodiversity, Acclimation Period, and Competition

Microbial biodiversity can mean variation between species, communities, and individual microorganisms (Benayas *et al.* 2005). In general, degraded ecosystems only have 51% of the biodiversity of natural ecosystems (Benayas *et al.* 2005). Decreased microbial biodiversity in aquatic ecosystems has been linked to a decrease in ecosystem functioning (Linkfield *et al.* 1989; Rathbone *et al.* 1998; Bell *et al.* 2005). Soil microbial communities that have low biodiversity are ultimately unable to respond to environmental changes (Bohme *et al.* 2005). If a pollutant or stressor enters an ecosystem that has low biodiversity, the ecosystem is more vulnerable, less resilient, and less likely to recover. However, if a

pollutant enters a biologically diverse community, it is more likely to survive and have a better recovery since the entire population would not be devastated (Benayas *et al.* 2005).

Although natural, rural ecosystems have greater biodiversity than degraded, urban ecosystems, they do not necessarily function at a higher capacity because of microbial acclimation period. The acclimation period of a microbial community serves an important role in the way that microorganisms respond to environmental stressors. The acclimation period is the time during which microbial communities grow and induce enzymes in response to substrates, which could range from nutrients or novel stressors and pollutants. In general, microbial communities that have pre-existing contaminated soils can metabolize the PAHs at greater rates than microbial communities that have never been exposed to PAHs. (Rathbone *et al.* 1998; Bell *et al.* 2005).

Different from acclimation period, competition between microorganisms occurs with or without the presence of environmental stressors. The most common interaction between microorganisms is the competition for nutrients. When microbial communities compete for a limiting nutrient, they operate under a "competitive exclusion principle" where the result of competition brings either a total demise or only one species survives (Ajbar 2012; McMeekin *et al. 2013*).

Research in Baltimore County, Maryland

The Baltimore Ecosystem Study (BES) and the University of Maryland, College Park have been monitoring the urban watersheds in Maryland in order to

investigate the effects of urbanization on the environment (e.g. Duan *et al.* 2012; Kaushal and Belt 2012; Groffman *et al.* 2005; Halden and Paull 2005). Past studies have tested for levels of PAHs and antimicrobials to identify nonpoint sources in urban watersheds and found within a range of 0.03-24.0 μg/L concentration of these novel stressors in the Gwynns Falls watershed (Kolpin *et al.* 2002; Halden and Paull 2005; Scoggins *et al.* 2007, Yang *et al.* 2010). My study examines how microbial communities respond to these novel, anthropogenic stressors, thereby going a step further than past studies by examining the significance of these contaminants and how they affect ecosystem health resistance and resilience.

In July of 2012, we collected water samples in two urban, degraded sites and two forested, rural sites in the Gwynns Falls watershed, Baltimore County Maryland. Using the concentrations previously found in Baltimore County streams by Halden and Paull 2005, and Kolpin *et al.* 2002, we amended these samples with an antimicrobial, triclocarban, and three PAHs: benzo(a)pyrene, fluoranthene, and phenanthrene. We measured extracellular enzyme activity to investigate stream ecosystem function in response to these novel anthropogenic stressors, which are common in wastewater and storm water. Past studies have mostly examined microbial enzyme activity in sediment. This study focused on the activity in water in order to understand the effect of novel stressors on transformations of organic matter along flow paths. The main question for this experiment is: What is the effect of urban land use and novel stressors derived from wastewater and storm water on the stream ecological function?

Questions and Hypotheses:

1. What is the effect of land use on microbial communities?

Hypothesis 1: Rural streams will have different microbial communities from urban, degraded streams, as seen through extracellular enzyme activity.

Null hypothesis 1: Rural, forested streams will not show significant differences in enzyme activity from degraded streams.

Rationale: Rural and urban streams have different nutrient fluxes because of impervious surface cover and thus have different microbial communities in order to break down and assimilate nutrients.

2. What is the effect of antimicrobials and polycyclic aromatic hydrocarbons (PAHs) on microbial communities? Are microbial communities in urban streams better adapted to these novel stressors than rural, forested streams?

Hypothesis 2: Antimicrobials and PAHs will have a negative effect on the microbial communities. These treatments will cause an overall decrease in extracellular enzyme activity across all four sites as the dose increases, but the urban sites will have higher enzyme activity at the low dose than the rural sites.

Null hypothesis 2: Antimicrobials and PAHs will not have an effect on the microbial community and we will not see a change in extracellular enzyme activity.

Rationale: Previous studies have documented elevated levels of antimicrobials (Halden and Paull 2005) and PAHs in urban streams of the Gwynn's Falls

Watershed. Comparing the response to antimicrobial/PAH addition among study sites, we can determine whether there is a difference in response to additional stressors along this particular gradient from forested to highly degraded streams. If the microbial community of degraded streams with lots of exposure to antimicrobials and PAHs have adapted to these conditions, we expect to see a lower response to antimicrobial/PAH addition, compared to the less degraded, forested site.

METHODS

Site Descriptions

This study had four sites that are all located within Baltimore County, Maryland. Two of the sites were rural (Pond Branch and Baisman Run) and two were urban (Dead Run and Gwynns Run), based on the amount ISC in the watershed determined by the Baltimore City Department of Public Works. I chose these four sites trying to find as many similar characteristics as possible between the two rural sites and the two urban sites, including impervious surface cover and land use (Table 1). The two urban sites, Dead Run and Gwynns Run have 31% and 61.2% ISC respectively and the two rural sites Pond Branch and Baisman Run have 0% and 0.3% respectively (Serchan and Jones 2009). The two urban sites, Dead Run and Gwynns Run are within the Gwynns Falls watershed and the two rural sites, Pond Branch and Baisman Run are in separate subwatersheds. On July 18th, 2012 I collected water chemistry measurements from each of these sites including water temperature, pH, conductivity, dissolved oxygen (DO), and flow (Supplemental Table 2).

Pond Branch

Pond Branch is located (39°28'49"N latitude, 76°41'16"W longitude) in the small Pond Branch watershed (Table 1, Figure 1). This site is 100% forested and takes up an area of 41 hectares located in Oregan Ridge State Park (Serchan and Jones 2009; Duan *et al.* 2012). Pond Branch is a part of the BES LTER study and has been used as a reference site for comparison with urbanized systems. Continuous discharge readings and stream chemistry are recorded on a weekly

basis at Pond Branch. On July 18th, 2012 the water temperature was 22.1ºC, the pH was 7.73, the conductivity was 303 µS, the DO was 7.88 mg/L, and the flow discharge rate was 0.071 ft^3/s (Supplemental Table 2). When sampled, Pond Branch had a lot of vegetation cover and no visible trash.

Baisman Run

Baisman Run is located (39°28'46.1"N latitude, 76°40'40.9"W longitude) in the Baisman Run watershed, close to Pond Branch in Oregan Ridge Park at Ivy Hill Road. (Table 1, Figure 1). This site is 71% forested, 27% developed, 2% agriculture and has 0.3% impervious surface cover (ICS) (Table 1 and Figure 1) (Serchan and Jones 2009; BES 2010; Duan *et al.* 2012). Baisman Run drains 381 hectares of forested landscape with homes in the headwaters that have septic tanks instead of sewers. Furthermore, there is a USGS gauge at a concrete broad crested weir located on the Ivy Hill Road Bridge and samples were taken at this weir. On July 18th, 2012 the water temperature was 22.7ºC, the pH was 7.70, the conductivity was 167.5 µS, the DO was 7.88 mg/L, and the flow discharge rate was 0.56 ft^3/s (Supplemental Table 2). When sampled, Baisman Run had no distinct odors or trash and appeared forested other than the nearby Ivy Hill Road Bridge.

Dead Run

Dead Run is located (39°18'40.4"N latitude, 76°42'59.9"W longitude) within the Gwynns Falls watershed (Table 1, Figure 1). The monitoring site at

Dead Run is located downstream of the Kernan Drive bridge and has a drainage basin of 5.52 sq. miles (Serchan and Jones 2009; Duan *et al.* 2012). Since the 1950s, this site has significantly changed and urbanized and now has 31% ICS, is 93% developed, has 2% agriculture, and is only 5% forested. This urbanization has led to flashier hydrology after short-term rain events. On July 18th, 2012 the water temperature was 25.3ºC, the pH was 7.83, the conductivity was 801 µS, the DO was 5.66 mg/L, and the flow discharge rate was 0.68 ft^3/s (Supplemental Table 2). While taking samples, I did not notice any trash in the stream and the water was visibly clear.

Gwynns Run

Gwynns Run is also known as Carroll Park and is located (39°16' 41.3"N latitude, 76°39' 07.2"W longitude) in the Gwynns Falls watershed (Table 1, Figure 1). The monitoring site is located within the Carroll Park Municipal Golf Course in Baltimore City, which borders Washington Boulevard near exit 51 on Interstate 95 (Serchan and Jones 2009; Duan *et al.* 2012). The site has watershed drainage coming from 16,000 surrounding hectares. The site has 61.2% ICS, is 85% developed, 15=4%forested, and 1% agricultural (BES 2010). The neighborhood surrounding the monitoring site is considered 79% developed and 21% undeveloped (Serchan and Jones 2009). Gwynns Run is highly contaminated with sewage and a sewage leak was identified and repaired in 2004 (Duan *et al.* 2012). On July 18th, 2012 the water temperature was 20.7ºC, the pH was 7.75, the conductivity was 736 µS, the DO was 7.75 mg/L, and the flow discharge rate was

0.14 ft^3/s (Supplemental Table 2). While taking samples on this day, I noticed a distinct change in flow in some parts of the stream, debris and trash throughout the stream, a pungent odor, and what appeared to be untreated sewage.

Experimental Design

I collected one-liter grab samples of water in wide-mouth Nalgene ® bottles from the four sites during summer base-flow conditions on July 18th, 2012 within 1.5 hours of each other. Each bottle was filled at the USGS gauge about 1-2 inches below the surface. I allowed the water to flow into the bottle and washed the bottle in the stream three times and then filled the container after the third wash. I then brought these water samples back to Sujay Kaushal's biogeochemistry lab at the University of Maryland College Park to incubate them. Using 250mL regular mouth Ball Mason ® Jars (after they have been acid washed and ashed so that they were ready for analytical, laboratory usage), we incubated the water samples for 24 hours on an Eberbach ® shaker table inside a Labconco® protector laboratory hood at a constant shaking speed and temperature. The Mason jars were shaken at a medium pace in order to mix water and PAHs without spilling.

These mason jars were prepared beforehand with treatments and each site had nine jars: 1 untreated water sample and 4 treated water samples, each with two doses. There were 36 total Mason jars and the four treatments (triclocarban and the three PAHs) were measured out as dry powder on a Mettler Toledo® analytical balance since the needed masses were 50 μg for the low dose and

200µg for the high dose. I did not replicate treatments in mason jars. Each Mason jar had 100mL aliquots of sample water and 5 mL of deionized water from the Milipore® MilliQ Purified Water System at 18 Ω. The concentration of the additions in the treated mason jars was thus 50µg/mL and 200µg/mL. These concentrations were based on measured amounts of triclocarban and of the three PAHs within the Gwynns Falls watershed that ranged from 0.03-24.0 µg/L (Kolpin *et al.* 2002; Halden and Paull 2005). These tiny amounts are difficult to measure, even on a microbalance, and so we increased the doses across all of the treatments to 50 µg/ L and 200µg/L.

After the incubation, we started our enzyme assay using p-nitrophenyl linked substrates and methods described by Sinsabaugh (1994). P-nitrophenyl (pNP) linked substrates are useful because when the specific enzyme comes and breaks down the pNP substrate causing the leaving group to leave, the product of the catalytic reaction is colored. The colored product can then have its absorbance measured on a UV-1800 Shimadzu UV spectrophotometer and this absorbance reading can be converted into activity using the equation:

Activity (µmol/h*mL sample)= OD/[(1.590/µmol)(incubation, hr)(1 mL sample in test tube) where OD=Sample Absorbance- [Substrate control Absorbance + Sample Control Absorbance] and the 1.590 is the micromolecular extinction coefficient for p-Nitrophenol under conditions in this assay. In 10 mL test tubes, 0.5 mL of the sample from the incubated mason jars and 0.5 mL of the substrate (in solution) are combined and swirled. Each enzyme had specific incubation times after all of the test tubes begin the enzyme assay with

phosphatase (PHOS) only required 30 minutes, leucine amino peptidase (LAP) requiring 3 hours, and beta-glucosidase (BG) requiring 4 hours. After these time periods of incubation were completed, the enzyme reactions were terminated using 0.1 mL of NaOH. Once the reaction stopped, the test tubes had 5.1 mL total: 0.5 mL sample water, 0.5 mL substrate solution, 0.1 mL NaOH, and 4.0 mL deionized water. The contents of this test tube were shaken and then measured for absorbance on the spectrophotometer with three replicates. These replicate absorbance measurements were averaged and used to calculate the OD in the Activity equation from Sinsabaugh (1994). The final measurement's units for average activity are mmol/h*mL where the "h=hours incubated with substrate."

Statistical Analysis

A one-way ANOVA was performed for the first hypothesis that examined the effect of site on enzyme activity. Instead of using "enzyme" as a factor, I split the SPSS file into three different one-way ANOVAs (IBM 1989). Significance was measured at the $p<0.01$ level and error bars were created with ±1 SE. If there was a significant difference in enzyme activity due to site, I followed up with a Tukey HSD post-hoc test to see the differences between sites. A series of two-way ANOVAs were performed for the second hypothesis that examined the effect of site, dose, and the interaction between site and dose on enzyme activity. Instead of including the untreated water as a fifth treatment, I designated the results for the untreated water as the "zero" dose in the three doses. Again, I split the file into different enzymes and different treatments, so that there were actually twelve different ANOVA results. After examining parallel dot plots and residual vs. fit

plots, I discovered that the beta-glucosidase (BG) activity for phenanthrene did not meet the normality assumptions of the ANOVA and required a data transformation. A square-root transformation and this fit the assumptions of the model, and this changed the units for BG activity of water treated with phenanthrene to (√mmol/((h*mL))). If the factors site and dose had significant effects on enzymes by themselves, then a Tukey HSD post-hoc test was performed.

RESULTS

The three variables investigated in this experiment were site, treatment, and the interaction between the two. The four sites were Pond Branch (rural), Baisman Run (rural), Dead Run (urban), and Gwynns Run (urban). The four treatments were triclocarban, PAH phenanthrene, PAH fluoranthene, and PAH benzo(a)pyrene, with three doses of no dose from untreated water, low dose of 50µg/100mL, and a high dose of 200µg/100mL. The three enzymes used to measure microbial function and activity with these treatments and doses were phosphatase (PHOS), beta-glucosidase (BG), and leucine amino peptidase (LAP) to assess phosphorus, carbon and nitrogen substrates, respectively.

Effect of land use on enzyme activity

The first hypothesis tested the effect of land use on enzyme activity in microbial communities, and proposed that the rural, forested streams (Pond Branch and Baisman Run) would have different enzyme activities from degraded, urban streams (Dead Run and Gwynns Run). The results of a one-way ANOVA supported the first hypothesis and showed that land-use had a significant effect ($p<0.01$ level) on PHOS and BG activity, but not on LAP activity (Table 4). Overall, the untreated water had average extracellular enzyme activity (EEA) (n=12) ranging from 0.700-1.400 mmol/h*mL for PHOS, 0.000-0.133 mmol/h*mL for BG activity, and 19.5-20.9 mmol/h*mL for LAP (Table 2, Figure 1, Figure 2).

The Tukey HSD post-hoc test results for PHOS activity showed that all sites were significantly different from each other ($p<0.01$) except for the two rural sites (Table 8, Figure 2a). This finding partially supported the hypothesis, in that the two rural sites, with Pond Branch at 0.966 mmol/h*mL and Baisman Run at 0.900 mmol/h*mL PHOS activity, did not have significantly different PHOS activity from each other, but they did have significantly different PHOS activity from the two urban sites, with Dead Run having 0.700 mmol/h*mL and Gwynns Run having 1.400 mmol/h*mL. On the other hand, the two urban sites are significantly different from each other ($p<0.01$) and so this partially supported the first hypothesis that relied on grouping of the two rural and two urban sites.

The Tukey HSD post-hoc test results for BG activity showed almost an opposite pattern from the PHOS Tukey HSD post-hoc test results (Table 8, Figure2b). The two rural sites were statistically ($p<0.01$) different from each other with Pond Branch at 0.133 mmol/h*mL and Basiman Run at 0.033 mmol/h*mL, and the two urban sites were not statistically different from each other, with Dead Run and Gwynns Run both at 0.000 mmol/h*mL. Surprisingly, the BG activity in the rural site Baisman Run was not significantly different from any of the sites, including the urban sites but this might be due to the fact that both of the urban sites had BG activities of zero mmol/h*mL.

Although the differences were not statistically significant ($p>0.01$), the LAP activity for ambient, untreated water across all four sites was higher than either the PHOS or the BG activities by at least one order of magnitude (Table 2, Table 4). For all three enzymes, the urban site Dead Run had the lowest enzyme

activity as compared to the other three sites. Even though the main effect of site on LAP was insignificant, the rural site Baisman Run had the highest activity at 21.4 mmol/h*mL, followed by the second rural site Pond Branch at 20.9 mmol/h*mL, then the urban sites Gwynns Run with 20.5 mmol/h*mL, and Dead Run with 19.6 mmol/h*mL. No follow up pair wise comparisons were performed. No Tukey HSD post-hoc test was performed since I did not find any significant difference due to site ($p<0.01$).

Effect of treatment and dose on enzyme activity

The second hypothesis tested the effect of adding an antimicrobial, triclocarban, or three different polycyclic aromatic hydrocarbons (PAH) on the extracellular enzyme activity of the microorganisms in each of the four sites. The dose increased from zero (same as untreated or ambient water samples), 50µg/100mL for the low dose, and 200µg/100mL for the high dose. More specifically, the second hypothesis states that with the low dose of the additions the urban sites would have higher microbial enzyme activity than the rural sites. Additionally, with the high dose I hypothesized a decline in enzyme activity close to 0.000 mmol/h*mL across all four sites and all four treatments.

Effect of triclocarban on enzyme activity

Overall, the water treated with triclocarban did not have a significant ($p<0.01$) effect on any of the enzyme activities for site or dose, but the interaction

between site and dose did have a significant ($p<0.01$) effect on PHOS and BG activities (Tables 3a, 5, 7, Figure 3, 4, 5).

Since triclocarban did not have a significant ($p<0.01$) effect on the PHOS activity in any of the sites or for either of the doses, I did not perform any Tukey HSD post-hoc tests for sites or doses (Table 3a, 5, 7, Figure 3). However, there was a significant difference ($p<0.01$) in PHOS activity due to the interaction between the site and dose factors. Opposite to what I predicted, the urban site Gwynns Run actually had the lowest PHOS activity at the low dose with 0.440 mmol/h*mL and the rural site Baisman Run had the highest activity at 1.237 mmol/h*mL. Supporting the second hypothesis, the PHOS activity for the rural site Pond Branch decreased at the low dose from 0.967 in the untreated water to 0.776 mmol/h*mL. Higher than the rural site, the PHOS activity for the urban site Dead Run was 1.006 mmol/h*mL, which was an increase in activity from the untreated water at 0.700 mmol/h*mL. Across all four sites, I hypothesized a decrease to zero enzyme activity with the highest dose. However, the rural site Pond Branch increased in PHOS activity from the low dose to the high dose to 1.216 mmol/h*mL, 0.5 more mmol/h*mL than the activity in the low dose. The urban site Gwynns Run also increased in PHOS activity at the high dose with 0.545 mmol/h*mL. The urban site Dead Run and the rural site Baisman Run partially supported the hypothesis in that they both decreased in PHOS activity at the high dose with 0.964 mmol/h*mL and 0.745 mmol/h*mL respectively. Surprisingly, none of the sites had zero activity level at the high dose.

Similar to PHOS activity, triclocarban had no significant effect ($p<0.01$) on BG activity in any of the sites or for either of the doses and so I did not perform any Tukey HSD post-hoc tests (Table 3a, 5, 7, Figure 4). The interaction between site and dose BG enzyme activity is significant ($p=0.000$). Just like PHOS, the rural site Baisman Run had the highest activity at 0.136 mmol/h*mL and the other rural site Pond Branch had the lowest activity at 0.000 mmol/h*mL. Contrary the second hypothesis, the effect of triclocarban on both PHOS and BG activity in Baisman Run actually increased the activity at the low dose so that it was higher than any other site. Also contrary to the second hypothesis, the activity in the two urban sites, Gwynns Run and Dead Run, increased from 0.000 to 0.084 and 0.016 mmol/h*mL respectively. Instead of decreasing at the highest dose, the activity in the rural site Pond Branch actually increased from 0.000 to 0.052 mmol/h*mL and in the urban site Dead Run from 0.016 to 0.058 mmol/h*mL. Baisman Run was the only site whose activity dropped to 0.000 mmol/h*mL at the highest dose. Gwynns Run activity drops as well, but only slightly from 0.084 to 0.063 mmol/h*mL.

The effect of triclocarban on LAP is insignificant ($p<0.01$) for site, dose, and the interaction between the two (Table 3a, 5, 7, Figure 5). LAP activity with the low dose of triclocarban was greatest in the rural site Baisman Run with 20.83 mmol/h*mL and smallest in the other rural site Pond Branch with 15.70 mmol/h*mL. The two urban sites Dead Run and Gwynns Run varied only slightly from untreated water, the low dose, and the high dose. Dead Run's activity varied approximately 1.00 mmol/h*mL from untreated water to high dose with 19.65,

20.00, to 20.67 mmol/h*mL, again opposite to what I predicted the activity slightly increased with the highest dose of the antimicrobial. Similarly, Gwynns Run's activity varied less than 1.00 mmol/h*mL across the doses with 20.0, 19.14, 19 .48 mmol/h*mL with a small increase in activity between the low and high doses.

Even though the effect on LAP was insignificant, there was a trend seen through each of the three enzymes that Baisman Run had the highest activity among the four sites at the low dose (Table 3a, Figures 3, 4, 5). Additionally, the second rural site Pond Branch generally did not follow the same pattern as Baisman Run when the treatment was added, and in both LAP and PHOS Baisman Run has the highest activity after the first dose and Pond Branch has the lowest activity after the second dose. I thought that the rural sites would show similar trends and have the smallest activity levels in both the low and the high doses; however, the observed trends did not support this.

Effect of PAH phenanthrene on enzyme activity

In addition to Triclocarban, I also examined the effect of the PAH phenanthrene on the enzyme activity in all four sites and at the three doses. Similar to the triclocarban results, only PHOS and BG activity levels were significantly ($p<0.01$) affected in water treated with phenanthrene (Tables 3b, 5, 7, 9, Figures 6a, 6b, 7, 8). Overall, there were only three significant ($p<0.01$) effects on enzyme activity from phenanthrene treated water from the interaction

between site and dose on PHOS and BG activity, as well as the effect of site by itself on PHOS activity.

The ANOVA results showed a significant difference ($p<0.01$) in PHOS activity by site and by the interaction between site and dose, but not by dose alone (Table 3b, Figures 6a, 6b). At the low dose of phenanthrene, PHOS activity was greatest in the rural site Pond Branch with 1.384 mmol/h*mL. Instead of decreasing in activity between the untreated water and the first dose of the PAH, the activity actually increased in Pond Branch, which does not support the hypothesis. The other three sites' responses support the hypothesis at the low dose in that they all decrease in activity with the addition of phenanthrene with the rural site Baisman Run decreasing from 0.900 to 0.692 mmol/h*mL, the urban site Dead Run decreasing from 0.700 to 0.168 mmol/h*mL, and the second urban site Gwynns Run decreasing from 1.400 to 1.258 mmol/h*mL. I hypothesized a further decline in activity from the low dose to the high dose and Dead Run's response fully supports this hypothesis by decreasing from 0.700 to 0.168 to 0.084 mmol/h*mL from untreated water to the high dose. Pond Branch also supports the hypothesis by decreasing in activity from the low dose to the high dose with 1.300 mmol/h*mL. However, Baisman Run's response does not support the hypothesis and the activity actually increased from the low dose to the high dose to an activity close to what was measured for untreated water with 0.900 to 0.692 to 0.881 mmol/h*mL; a 0.019 mmol/h*mL difference between the untreated water samples and the high dose. Also, Gwynns Run's response does

not support the hypothesis because its activity remained the same from the low dose to the high dose with 1.258 mmol/h*mL.

The post-hoc Tukey test for comparing sites shows an opposite pattern to what I hypothesized (Table 9, Figure 6b). Instead of having the two rural sites and the two urban sites grouped together, there is actually a significant difference in PHOS activity ($p<0.01$) between rural sites and between urban sites. Also, the activity in the rural site Pond Branch and the urban site Gwynns Run are not significantly different from each other.

BG activity for water treated with phenanthrene required a square-root transformation in order to fit the assumptions of the ANOVA. The interaction between the factors site and dose had a significant effect ($p<0.01$) on the BG activity, where as site and dose by themselves had no significant effect (Tables 3b, 5, 7, Figure 7). The activity for the rural sites, Pond Branch and Baisman Run, supports the hypothesis because they both decreased with the addition of phenanthrene at the low dose with Pond Branch's activity dropping from 0.133 to 0.005 √mmol/h*mL and Baisman Run's activity dropping from 0.033 to 0.000 √mmol/h*mL. However, the response by the urban sites to the low dose of phenanthrene did not support the hypothesis with the activity of Dead Run increasing from 0.000 to 0.262 √mmol/h*mL and Gwynn's Run increasing from 0.000 to 0.351 √mmol/h*mL. With the high dose I hypothesized activity in all of the sites to drop to zero or at least lower than the activity with the low dose. Dead Run's activity returned to 0.000 √mmol/h*mL with the high dose and appears to spike, or peak at the low dose after going from 0.000 to 0.262 and then back down

to 0.000 √mmol/h*mL with the high dose. Gwynns Run also decreases in activity at the high dose and drops down to 0.126 √mmol/h*mL from 0.351 √mmol/h*mL. The rural site Pond Branch does not necessarily support the hypothesis since its activity level does not change from the low dose to the high dose and it remains at 0.005 √mmol/h*mL. Baisman Run's response is the opposite of what I predicted as it increases, although slightly, in activity from the low to the high dose with 0.000 to 0.010 √mmol/h*mL.

There was no significant ($p>0.01$) difference in LAP activity of water treated with phenanthrene by site, dose, or the interaction between site and dose (Tables 3b, 5, Figure 8). Although insignificant, the response of the rural site, Baisman Run supports the hypothesis with each addition of phenanthrene as it continually decreases in activity from 21.49 to 20.02 and then 19.48 mmol/h*mL. The activity of the other rural site, Pond Branch, also decreases with the low dose from 20.90 to 19.81 mmol/h*mL but then responds opposite to the hypothesis with the high dose and increases to 21.03 mmol/h*mL, higher than the activity of the untreated water. Similarly, Dead Run and Gwynns Run do not support the hypothesis at the low dose since in both cases the activity increases from 19.65 to 22.33 mmol/h*mL in Dead Run and 20.00 to 21.54 mmol/h*mL in Gwynns Run. With the highest dose, both urban sites decrease in activity and support the hypothesis with Dead Run decreases to 19.54 mmol/h*mL only 0.11 mmol/h*mL below the activity level of the untreated water, and Gwynns Run's activity dropping to 18.25 mmol/h*mL. The overall pattern of the LAP activity resembles

BG for phenanthrene with the two urban sites spiking in activity at the low dose and the two rural sites dropping in activity at the low dose (Figure 7, 8).

Effect of PAH fluoranthene on enzyme activity

Along with phenanthrene, I also amended water samples with the PAH fluoranthene and tested the effects of site and dose on enzyme activity. Similar to triclocarban and phenanthrene, the only significant ($p<0.01$) results for water treated with fluoranthene were from the effect of the interaction between site and dose on PHOS and BG activities (Tables 3c, 5, 7, Figures 9, 10, 11).

Specifically, I found that site and dose by themselves had no significant ($p<0.01$) effect on PHOS activity, but that the interaction between site and dose was significant (Tables 3c, 5, 7, Figure 9). I hypothesized a steady decline in activity level with each dose, but the only site that followed that pattern was the urban site Gwynns Run where the activity went from 1.400 to 1.300 and then dropped in half to 0.587 mmol/h*mL at the high dose. The rural site Pond Branch showed the opposite pattern as it steadily increased in activity from the untreated, "zero dose" to the high dose with 0.967 to 1.195 and then 1.279 mmol/h*mL. The pattern for Baisman Run supported the hypothesis at the low dose as it decreased slightly in activity, but at the high dose it increased to have an activity higher than the untreated water with 0.900 to 0.088 and then 1.237 mmol/h*mL. Similarly, the urban site Dead Run did not support the hypothesis at the low dose since it increased in activity from 0.700 to1.258 mmol/h*mL. The activity decreased at the high dose for Dead Run to 1.111 mmol/h*mL; however, this only partially

supports the hypothesis since overall it was still higher than the untreated activity level.

Similar to PHOS, water treated with fluoranthene only showed a significant difference ($p<0.01$) in BG activity due to the interaction between site and dose rather than just site or dose (Table 3c, 5, 7, 10). The rural site Pond Branch supported the hypothesis as it decreased from 0.133 to 0.000 and 0.000 mmol/h*mL from the untreated water to the low and high doses. Additionally, the other rural site Baisman run supported the hypothesis as it dropped in activity from the untreated water to the low dose of fluoranthene from 0.033 to 0.016 mmol/h*mL, cutting the activity in half. However, Baisman Run's response only partially supported the hypothesis because it actually increased in activity from the low to the high dose as it went from 0.016 to 0.042 mmol/h*mL. The two urban sites, Dead Run and Gwynns Run, did not support the hypothesis with the low dose because they both increased from 0.000 to 0.079 mmol/h*mL in Dead Run and 0.000 to 0.142 mmol/h*mL in Gwynns Run. Dead Run peaked in activity at the low dose and then returned to 0.000 mmol/h*mL activity at the high dose. Gwynns Run also decreased at the high dose but still had an activity higher than the activity of the untreated water with 0.073 mmol/h*mL.

There was no significant difference ($p<0.01$) in the LAP activity of water treated with fluoranthene due to site, dose, or the interaction between site and dose (Tables 3c, 3b, 4b, Figure 11). Overall, the activity in rural sites was higher than in urban sites. The urban site Gwynns Run and the rural site Baisman Run supported the hypothesis across the three concentrations of fluoranthene with the

activity going from 20.00 mmol/h*mL to 18.87 mmol/h*mL at the low dose, and 18.48 mmol/h*mL at the high dose for Gwynns Run, and 21.40 mmol/h*mL to 21.02mmol/h*mL, and then 20.98 mmol/h*mL at the high dose for Baisman Run. Pond Branch and Dead Run actually increased in LAP when fluoranthene was added at the low dose from 20.90 to 21.61 mmol/h*mL for Pond Branch and 19.65 to 20.33 mmol/h*mL for Dead Run. However, with the high dose Pond Branch and Dead Run's response supported the hypothesis and dropped to 18.19 mmol/h*mL in Pond Branch and 18.46 mmol/h*mL in Dead Run. Rather than grouping the two rural sites and the two urban sites together, Baisman Run and Gwynns Run and then Pond Branch Dead Run seemed to pair together. This pattern was opposite to what I observed with the PHOS and the BG where the two urban sites generally followed each other.

Effect of PAH benzo(a)pyrene on enzyme activity

In addition to fluoranthene, I examined the effect of benzo(a)pyrene on enzyme activity. Unlike the other three treatments, water treated with benzo(a)pyrene did not have a significant effect on PHOS with site, dose, or the interaction between site and dose. Benzo(a)pyrene treated water also did not follow the trends in the other treatments in that site, dose, and the interaction between site and dose all had a significant ($p<0.01$) effect on the BG activity (Tables 3d, 5, 7, 9, Figures 12, 13abc, 14).

More specifically, I found that site, dose, and the interaction of site and dose did not have a significant effect ($p<0.01$) on PHOS activity in water treated

with benzo(a)pyrene (Tables 3d, 5, 7, Figure 12). Although insignificant ($p>0.01$), there were groupings between the two rural sites and the two urban sites at the low dose. Baisman Run and Pond Branch did not support the hypothesis at the low dose because they both increased in activity and went from 0.900 to 1.132 mmol/h*mL in Baisman Run and 0.967 to 1.090 mmol/h*mL in Pond Branch. At the high dose, the two rural sites went in opposite directions where Pond Branch continued to increase in activity to1.740 mmol/h*mL and Baisman Run dropped to 0.922 mmol/h*mL, close to the activity of untreated water. The two urban sites supported the hypothesis at the low dose and both decreased from 0.700 to 0.503 mmol/h*mL in Dead Run and 1.400 to 0.922 mmol/h*mL in Gwynns Run. However, at the high dose, the two urban sites increased in activity and did not support the hypothesis as Dead Run goes up to 1.126 mmol/h*mL and Gwynns Run goes up to 1.342 mmol/h*mL. The urban sites seemed to dip down at the low dose and then increased at a similar rate in the high dose.

Unlike PHOS activity, there was a significant effect ($p<0.01$) on BG activity of water treated with fluoranthene by site, dose, and the interaction between site and dose (Tables 3d, 5, 7, 9, Figures 13a, 13b, 13c). At the low dose of fluoranthene, only the activity in the rural site Pond Branch supported the hypothesis as it decreased from 0.133 to 0.047 mmol/h*mL. Baisman Run and Gwynns Run did not support the hypothesis at the low dose and they both increased from 0.033 to 0.058 mmol/h*mL in Baisman Run and 0.000 to 0.194 mmol/h*mL in Gwynns Run. The activity in Gwynns Run continued to increase in the high dose to 0.300 mmol/h*mL. Dead Run's activity was 0.000 for both the

untreated water and the low dose, and then it surprisingly increased in the high dose to 0.031 mmol/h*mL. Pond Branch also did not support the hypothesis at the high dose as its activity slightly more than quadrupled from 0.047 to 0.189 mmol/h*mL. Baisman Run was the only site that decreased in activity at the high dose and dropped down to 0.000 mmol/h*mL.

The Tukey HSD post-hoc test results between sites did not necessarily support the hypothesis that the sites would group according with land use, rural: Pond Branch and Baisman Run and urban: Dead Run and Gwynns Run (Table 9, Figure 13b). Instead, Pond Branch and Baisman Run, as well as Dead Run and Gwynns Run were significantly different from one another ($p<0.01$). Surprisingly, there was no significant difference between the urban site Gwynns Run and the rural site Pond Branch (Table 5b, Figure 13b). However, there was a significant difference ($p<0.01$) between the other sites, rural Baisman Run and urban Dead Run.

Similarly, Tukey HSD post-hoc test results did not fully support the hypothesis that there would be a significant difference between the untreated water, the low dose, and the high dose (Table 9, Figure 13c). Surprisingly, the overall average activity across all four sites increased as dose increased. Instead, I hypothesized an inverse relationship: as the dose increased, the microbial activity would decrease. The Tukey HSD post-hoc test results supported the hypothesis in that both the untreated water and the low dose were significantly different ($p<0.01$) from the high dose. However, the results did not support the hypothesis because the effect of the low dose of benzo(a)pyrene on activity was not

significantly different ($p<0.01$) from the effect of the untreated water, even though I thought they would be dissimilar.

There was no significant difference ($p<0.01$) in the LAP activity of water treated with benzo(a)pyrene due to site, dose, or the interaction between site and dose (Tables 3d, 5, 7, Figure 14). However, there was a significant difference at the $p<0.05$ level for interaction and this was the only slightly significant results for LAP across all other treatments, sites, or doses. Although insignificant ($p<0.01$), Gwynns Run, Baisman Run, and Pond Branch all decreased in activity at the low dose of benzo(a)pyrene and supported the hypothesis with Gwynns Run dropping from 20.00 to 19.11 mmol/h*mL, Baisman Run dropping from 21.40 to 18. 94 mmol/h*mL, and Pond Branch dropping from 20.90 to 20.78 mmol/h*mL. However, the urban site Dead Run did not support the hypothesis because it actually spiked in activity at the low dose, going from 19.65 mmol/h*mL to 21.90 mmol/h*mL. At the high dose, Dead Run dropped back down below its activity of untreated water to 18.73 mmol/h*mL. The other three sites actually increased in activity at the high dose and did not support the hypothesis because Gwynns Run increased to 20.16 mmol/h*mL, Baisman Run increased to 19.66 mmol/h*mL, and Dead Run increased from 21.60 mmol/h*mL. Overall, the pattern of LAP across sites at the low and high dose switched with Dead Run at one end and then Gwynns Run, Baisman Run, and Pond Branch at the other.

DISCUSSION

Understanding microbial function in urban stream water is a critical step in assessing the overall stream ecosystem health and the ability for a degraded stream to recover (Kolpin *et al.* 2002 and Scoggins *et al.* 2007). Microbes assimilate dissolved organic matter using extracellular enzymes and these have been measured and studied as an indicator of microbial function in stream sediments (Burns and Ryder 2001; Harbott and Grace 2005; Sinsabaugh and Shah 2011; Sinsabaugh and Shah 2011; Gartner *et al.* 2012). However, this study intentionally examined water samples instead of sediment samples in order to assess the importance of microbial transformations of organic matter during transport (Kaushal and Belt 2012). I used doses of triclocarban and PAHs that were based on concentrations previously found in the Gwynns Falls watershed (Kolpin *et al.* 2002; Halden and Paull 2005). This study attempted to evaluate the functionality of microbes in urban vs. rural streams and treated the water with known, novel stressors to see how the microbial communities would respond. In order to get a fuller picture, I chose three enzymes that correspond with three main substrates and nutrients: phosphatase (PHOS) to examine phosphorus assimilation, beta-glucosidase (BG) to examine glucose and carbon assimilation, and leucine amino peptidase (LAP) to examine nitrogen assimilation. Carbon, Nitrogen, and Phosphorus have very different cycles in the ecosystem and are measured often to assess stream health.

One of the main assumptions of my experiment is that I predicted the two rural sites would have similar responses and be different from the two urban sites,

which would be similar to each other. I tested this hypothesis by examining ambient, untreated water in all four sites. I also hypothesized the rural streams would have lower resistance to the stressors and thus have lower enzyme activity than the urban streams. Conversely, I predicted that the urban streams would have greater resistance to the additions and have more resilience since they would have adapted to environments that commonly have PAHs and antimicrobials due to urbanization. My second hypothesis addressed how factors of site, dose of treatment, and the interaction between site and dose affected enzyme activity.

I hypothesized that for all of treatments at the low dose (50µg/100mL), the enzyme activity at all four sites would decrease, but that the urban sites would have higher activity than the rural sites. I also predicted that at the high dose (200µg/100mL) all of the sites would have no activity, but if there was a detected activity, then the urban sites' enzyme activity would still be higher than the rural sites. I hypothesized this general pattern across all enzymes and all treatments. However, the only case where I actually observed this pattern is the phosphatase activity in the urban site Dead Run (and slightly in Gwynns Run) with phenanthrene treated water (Table 3b, Figure 6a). All other treatments showed patterns that did not fully support my hypotheses. Without the predicted grouping of rural and urban sites of land use type, it is difficult to evaluate the second hypothesis about site effects, dose effects, and interaction between site and dose since the characteristics of site largely depend on land use.

Effect of untreated, ambient water from the four sites on enzyme activity

The untreated, ambient water had a significant effect on PHOS activity (Tables 2, 4, Figure 2a). However, the sites did not group together as I hypothesized and instead the two urban sites were significantly different from each other, but the rural site Baisman Run and the urban site Dead Run were not significantly different from each other (Table 8, Figure 2a). The untreated water also had a significant effect on BG activity and, again, the sites did not group together as I hypothesized and instead Pond Branch was significantly ($p<0.01$) different from the other sites but the other sites were not significantly different from each other (Table 8, Figure 2a). These results indicate that impervious surface cover is not the only or the best way to categorize urban and rural (Table 1, Figure 1).

Some of the water chemistry results showed differences between sites that supported the rural and urban categorization by impervious surface cover (Supplemental Table 2). The DO concentration was the same for the two rural sites Pond Branch and Baisman Run and was very similar for the two urban sites Dead Run and Gwynns Run. Likewise, the conductivity levels for the two rural sites were similar and the two urban sites were similar. The DO and conductivity results support my first hypothesis of grouping the rural and urban sites. However, some of the water chemistry data results, such as water temperature and flow, did not follow the rural and urban categorization. Water temperature was lowest in the rural site Pond Branch and the urban site Gwynns Run and highest in the rural site Baisman Run and the urban site Dead Run. Similarly, flow was the smallest

in Pond Branch and Gwynns Run and the greatest in Baisman Run and Dead Run. These results group Pond Branch and Gwynns Run together and Baisman Run and Dead Run together.

Leucine amino-peptidase (LAP) enzyme activity

Overall, LAP activity was not significantly ($p<0.01$) affected by any of the treatments, doses, sites, or interactions between site and dose. This finding was surprising since nitrogen substrates are readily available in the water as dissolved gases, and thus I thought that the microbial communities would have used LAP to breakdown peptides and assimilate nutrients (Harbott and Grace 2005; Sinsabaugh and Shah 2011). However, since we found no significant results with LAP activity in this experiment, perhaps the nitrogen sources in these sites are primarily found in the soil and sediment rather than dissolved as gases in water.

Triclocarban treatment

The antimicrobial triclocarban is considered a broad-spectrum sterilizer that kills both bacteria and fungi (Halden and Paull 2005). It cannot be treated by current wastewater treatment technologies and so it enters the watershed, virtually unchanged (Kolpin *et al.* 2002; Halden and Paull 2005). In this study, the water treated with triclocarban only had a significant effect on enzyme activity due to the interaction between site and dose of triclocarban. Additionally, only PHOS and BG were significantly ($p<0.01$) affected by this interaction. The results of the

PHOS and BG activities were not similar, other than the result that Baisman Run had the highest activity in both cases at the low dose (Table 3a, Figures 3, 4).

Since triclocarban is a sterilizing agent, it is meant to kill all bacteria and fungi. However, we still measured enzyme activity even at doses that are about ten times greater than concentrations measured in the watershed (Halden and Paull 2005). I hypothesized that the urban sites would have higher enzyme activity than the rural sites because they are more likely to have been previously exposed to triclocarban and thus more able to break it down. However, the urban sites had higher enzyme activity only sometimes: in PHOS at the high dose Dead Run had a higher activity than Baisman Run, in BG at the low dose both urban sites had a higher activity than Pond Branch, and in BG at the high dose the two urban sites were both slightly higher than Pond Branch. Triclocarban might have affected parts of the microbial community in each of the sites differently where a part of the community was stressed and another part was unaffected (Linkfield *et al.* 1989; Rathbone *et al.* 1998; Bell *et al.* 2005).

Rural, natural sites generally have greater biodiversity than degraded sites (Benayas *et al.* 2005) and so the part of the rural microbial community that was unaffected by triclocarban may have produced the enzyme activity; whereas the urban sites did not have as diverse of a community, and so a larger portion may have been affected resulting in lower enzyme activities in the urban sites with the same treatments. A dramatic increase in activity at the high dose does not necessarily indicate that the micro-organisms in Pond Branch, which increased both in PHOS and BG activity at the high dose, are stimulated. Rather, it might

indicate that the stress of one part of the microbial community allowed the unaffected portion of the community to out-compete the others and increase in enzyme activity.

Poly-aromatic hydrocarbons (PAHs) treatments

Poly-aromatic hydrocarbons are made up of aromatic carbon rings and are highly insoluble in water. The three PAHs in this study were structurally very similar in that the only difference among them was in the number of benzene rings where phenanthrene has three benzene rings, fluoranthene has four rings, and benzo(a)pyrene has five benzene rings. Even though they are structurally similar, I chose three different PAHs in order to see if there were any major differences in the way they were each broken down. Overall, the three PAHs affected PHOS activity very differently across sites and doses and there was no clear pattern (Tables 3b-d, Figures 6a, 9,12). However, the three PAHs affect BG activity somewhat similarly and the pattern is most obvious between phenanthrene and fluoranthene. The only major difference between the two PAHs with BG activity, besides actual mean values, was that Baisman Run had the lowest activity at the low dose of phenanthrene and then Pond branch had the lowest activity at the low dose of fluoranthene (Tables 3b-c, Figures 7, 10). In this instance, perhaps the addition of a benzene ring in fluoranthene did not necessarily affect the ways in which the microbial community processed PAHs by using BG.

The enzymatic biodegradation processes of substrates are all affected by the existing pollutant concentration of the sites. The pre-existence of the pollutant affects the ability of microbes to break down PAHs because the bioaccumulation and increased residence times of these PAHs makes it difficult for the microbes to access and break apart hydrocarbons in PAHs (Linkfield *et al.* 1989; Rathbone *et al.* 1998; Bell *et al.* 2005). This is because of a necessary "acclimation period" during which microbial communities grow and induce enzymes (Rathbone *et al.* 1998; Bell *et al.* 2005).

Rathbone *et al.* 1998 partially supports my hypothesis in that the urban sites should breakdown PAHs at higher rates than the rural sites because they are pre-exposed and thus have gone through the "acclimation period" (Kolpin *et al.* 2002; Halden and Paull 2005). This was observed in this study for: the PHOS activity at the high dose of phenanthrene at Gwynns Run, the BG activity at the low dose of phenanthrene at Gwynns Run and Dead Run, the BG activity at the high dose of phenanthrene at Gwynns Run, the PHOS activity at the low dose of fluoranthene at Gwynns Run and Dead Run, the BG activity at the low dose of fluoranthene at Gwynns Run and Dead Run, the BG activity at the high dose of fluoranthene at Gwynns Run, and the BG activity at the low and the high dose of benzo(a)pyrene at Gwynns Run (Tables 3b-3d, Figures 6a, 7, 9, 10, 13a). The sites that had "partially" higher enzyme activities weren't the highest but instead were at least higher than one of the rural sites at that given dose.

However, Rathbone *et al.* 1998 also explains that greater biodiversity within the microbial community is important and necessary for the breakdown of

PAHs. Even though urban sites have the "acclimation period," perhaps the factor of biodiversity within the microbial community is more important in the breakdown of PAHs and that the more biodiverse, rural sites are able to function better and have higher enzyme activity. This trend was observed for: the PHOS activity at the low dose of phenanthrene at Pond Branch (Table 3b, Figure 6a), the PHOS activity at the high dose of phenanthrene at Pond Branch and partially at Baisman Run, the BG activity at the high dose of phenanthrene partially at Pond Branch and Baisman Run (Table 3b, Figure 7), the PHOS activity at the high dose of fluoranthene at Pond Branch and Baisman Run (Table 3c, Figure 9), the BG activity at the high dose of fluoranthene partially at Baisman Run (Table 3c, Figure 10), the PHOS activity at the low dose of benzo(a)pyrene at Pond Branch and Baisman Run (Table 3d, Figure 12), the PHOS activity at the high dose of benzo(a)pyrene at Pond Branch, and the BG activity at the low and high doses of benzo(a)pyrene partially at Pond Branch and Baisman Run (Tables 3d, Figure 13a). The sites that had partially higher enzyme activities weren't the highest, but instead were at least higher than one of the urban sites at that given dose.

Tukey HSD post-hoc tests were only performed for BG activity of sites in phenanthrene treated water and for sites and doses benzo(a)pyrene treated water (Table 9, Figures 6b, 13b, 13c). The results for phenanthrene and benzo(a)pyrene both showed that the effects all sites on BG activity were significantly different from each other except for the urban site Gwynns Run and the rural site Pond Branch. This was opposite to what I hypothesized, that the urban sites would have higher EEA than the rural sites and it suggests that the microbial communities in

these two sites functioned similarly. Perhaps the level of biodiversity in Pond Branch allowed the microbial community to function similarly to the microbial community in Gwynns Run that had a greater assimilation period to grow and produce enzymes to breakdown phenanthrene and benzo(a)pyrene. In addition to sites, the dose of benzo(a)pyrene had a significant effect on BG activity. The results showed that the untreated water/no dose was significantly different ($p<0.01$) from the low dose, but was not different from the high dose. This result suggests that part of the microbial community was unaffected by the high dose and thus outcompetes the stressed part of the community and was able to function and produce enzymes.

In general, each treatment partially supports and partially opposes my hypothesis that the urban sites would function better and have higher enzyme activities than the rural sites. This finding demonstrates that the ecosystem is dynamic and the ability for the enzyme communities to breakdown these recalcitrant PAHs does not depend only on assimilation time or biodiversity but a combination of both of those factors, as well as other factors not considered in this study.

Limitations of the study

There are a few limitations to this study that might have affected the data and results. Firstly, I only examined enzyme activity in water whereas other studies focused on enzyme activity in soils, where different methods are used to capture larger microbial communities, i.e. using tiles to have a biofilm as in Sobczak and Findlay (2002). Secondly, I collected grab samples of water from

one day in a three-hour period and this only showed a snapshot of the ecosystem, which might not be replicated if the experiment were repeated over different seasons and flow conditions. Thirdly, the temperature in the lab was not comparable to the temperature in the water or the air temperature at the site, which might have affected the metabolic rates of the enzymes (Sinsabaugh and Shah 2011). I assayed and incubated the water at a standard temperature in the lab and this facilitated comparisons between treatments and sites, but it also obscured the effect of the ambient temperature fluctuations on enzyme activity.

Future Studies

Future studies should consider testing and measuring microbial biomass, examining biofilms, and taxonomic makeup of the microbial communities in order to assess the parts of the community that are functioning. Also, it would be useful to take samples over a longer period of time and more frequently in order to understand the temporal scale of microbial community activity. The results further suggest that I should have considered other ways of grouping sites by rural and urban other than impervious surface cover. The water chemistry data showed that water temperature and flow results did not follow the rural and urban categorization that I used to separate the sites (Supplemental Table 2). Overall, these varying results came from one day of sampling and would need to be repeated if considering re-categorizing sites based off of another factor other than impervious surface cover. Some other ways to group sites might have been by wastewater treatment and the presence of septic systems or sewage plants, land-

use type such as agriculture or development, or amount of forestation in the watershed.

CONCLUSION

This study examined the effects of land use and novel stressors on extracellular enzyme activity (EEA) in order to assess microbial function in urban and rural streams. The results varied among sites, doses of treatments, and the interaction between the two. In general, there were no clear, consistent patterns across enzymes, sites, or treatments, which highlights the dynamic nature of microbial communities. The four sites in this study, Pond Branch, Baisman Run, Dead Run, and Gwynns Run, were categorized into rural or urban based on impervious surface cover (Table 1, Figure 1). Overall, LAP activity was not significantly ($p<0.01$) affected by any of the treatments, doses, sites, or interactions between sites, suggesting that the nitrogen sources in these sites may primarily be found in the soil and sediment rather than dissolved as gases in water.

The first hypothesis tested the water from each of the four sites under untreated, ambient conditions. Even though site did have a significant ($p<0.01$) effect on enzyme activity, the results did not fully support the land use grouping of rural and urban based on impervious surface cover. Instead, the PHOS activity showed the two rural sites Pond Branch, Baisman Run, and the urban site Dead Run grouped together and the BG activity showed the two urban sites Dead Run and Gwynns Run and the rural site Baisman Run grouped together. These groupings suggest that impervious surface cover is not the most distinguishing feature between the rural and urban sites and that different factors might be more informative, such as waste water treatment, flow, and water temperature.

The second hypothesis tested the effects of treated water on enzyme activity by site, dose of treatment, and the interaction between site and dose. Overall, I hypothesized that all of the treated sites would decrease in EEA, but that the urban sites would have comparatively higher enzyme activity than rural site because the microbial communities would have adapted to the degraded conditions. The PHOS and BG activity of triclocarban treated water was significantly ($p<0.01$) affected by the interaction between site and dose. However, the results actually showed the rural sites generally having higher activity than the urban sites. This indicates that the biodiversity of the rural sites might be more important than the assimilation period of the microbial community (Rathbone *et al.* 1998; Benayas 2009). Also, since the rural sites have greater biodiversity the measurable enzyme activity might be due to parts of the microbial community outcompeting other parts.

The three PAHs had significant effects on PHOS and BG activity due to the interaction between site and dose. Instead of decreasing in activity with increasing dose, the activity often increased. This further supports the idea that competition within the microbial community might play a large role in microbial function. Also, the hypothesis was not always supported in that sometimes the urban sites had higher activity than the rural sites and sometimes the rural sites had higher activity than the urban sites.

BG activity was also significantly affected by site with phenanthrene treated water, and by site and by dose with benzo(a)pyrene treated water. In both cases, the BG activity was significantly ($p<0.01$) affected across all sites

differently except that the urban site Gwynns Run and the rural site Pond Branch did not have significantly different effects. This result depicts the complexity of enzyme activity as a tool for microbial function. The similar effects on BG activity from seemingly different sites shows that the biodiversity and competition within the microbial community in the rural site might yield comparable enzyme activity to the assimilation period in the urban site.

Overall, extracellular enzyme activity is an important and useful tool for understanding microbial function in an ecosystem. In an urban ecosystem that is characterized by degradation, lake of riparian vegetation, and low biodiversity, it is especially important to quantify the ways in which microbial communities are able to biodegrade and assimilate nutrients and novel stressors. Urban streams transport organic matter and pollutants like pipes (Paul and Meyer 2001; Kaushal and Belt 2012) and so understanding the ways in which the organisms in these "pipes" function is an important step towards possible bioremediation and ecological restoration.

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FIGURES AND TABLES

Table 1. Site Descriptions. Refer to supplementary table 2 for physical and chemical characteristics of sites at the time of sampling.

*Data from Baltimore City Department of Public Works (DPW).
**Data from Baltimore Ecosystem Study LTER 2010 research renewal proposal.

Site	Location	Impermeable surface cover %	Land use %
Pond Branch** (rural)	39°28'49"N 76°41'16"W	0**	Forest: 100** Developed: 0** Agriculture: 0**
Baisman Run** (rural)	39°28'46.1"N 76°40'40.9"W	0.25**	Forest: 71** Developed: 27** Agriculture: 2**
Dead Run** (urban)	39°18'40.4"N 76°42'59.9"W	31.0**	Forest: 5** Developed: 93** Agriculture: 2 **
Gwynn's Run (Carroll Park)* (urban)	39°16'41.3"N 76°39'07.2"W	61.2*	Forest: 14** Developed: 85** Agriculture: 1*

Table 2. Extracellular enzyme activity (EEA) (n=12) for each site's ambient water (hypothesis 1).

Site	Enzyme	Mean EEA (mmol/h*mL)	Standard Deviation
Pond Branch	Phophatase	0.966	.0178
Baisman Run		0.900	0.086
Dead Run		0.700	0.226
Gwynns Run		1.400	0.256
Pond Branch	Beta-glucosidase	0.133	0.049
Baisman Run		0.033	0.049
Dead Run		0.000	0.000
Gwynns Run		0.000	0.000
Pond Branch	Leucine Amino- Peptidase	20.9	1.048
Baisman Run		21.4	1.436
Dead Run		19.6	1.361
Gwynns Run		20.5	1.594

Table 3a. Extracellular enzyme activity (EEA) (n=3) for water from each site treated with triclocarban (hypothesis 2).

Enzyme	Site	Dose (µg/100mL)	Mean EEA (mmol/h*mL)	Standard Deviation
Phophatase	Pond Branch	0	0.967	0.208
		50	0.776	0.073
		200	1.216	0.290
	Baisman Run	0	0.900	0.100
		50	1.237	0.072
		200	0.745	0.227
	Dead Run	0	0.700	0.264
		50	1.006	0.000
		200	0.964	0.726
	Gwynns Run	0	1.400	0.300
		50	0.440	0.333
		200	0.545	0.604
Beta-glucosidase	Pond Branch	0	0.133	0.058
		50	0.000	0.000
		200	0.052	0.045
	Baisman Run	0	0.033	0.058
		50	0.136	0.018
		200	0.000	0.000
	Dead Run	0	0.000	0.000
		50	0.016	0.027
		200	0.058	0.018
	Gwynns Run	0	0.000	0.000
		50	0.084	0.079
		200	0.063	0.031
Leucine Amino-Peptidase	Pond Branch	0	20.90	1.229
		50	15.70	1.041
		200	18.22	4.020
	Baisman Run	0	21.40	1.680
		50	20.83	0.198
		200	18.93	0.880
	Dead Run	0	19.65	1.600
		50	20.00	0.143
		200	20.67	0.872
	Gwynns Run	0	20.0	2.255
		50	19.14	1.439
		200	19.48	0.885

Table 3b. Extracellular enzyme activity (EEA) (n=3) for water from each site treated with PAH phenanthrene (hypothesis 2).

Enzyme	Site	Dose (μg/100mL)	Mean EEA (mmol/h*mL)	Standard Deviation
Phophatase	Pond Branch	0	0.967	0.208
		50	1.384	0.000
		200	1.300	0.192
	Baisman Run	0	0.900	0.100
		50	0.692	0.126
		200	0.881	0.126
	Dead Run	0	0.700	0.265
		50	0.168	0.238
		200	0.084	0.073
	Gwynns Run	0	1.400	0.300
		50	1.258	0.252
		200	1.258	0.126
Beta-glucosidase	Pond Branch	0	0.133	0.058
		50	0.005	0.009
		200	0.005	0.009
	Baisman Run	0	0.033	0.578
		50	0.000	0.000
		200	0.010	0.009
	Dead Run	0	0.000	0.000
		50	0.262	0.127
		200	0.000	0.000
	Gwynns Run	0	0.000	0.000
		50	0.351	0.323
		200	0.126	0.110
Leucine Amino-Peptidase	Pond Branch	0	20.90	1.229
		50	19.81	1.927
		200	21.03	0.106
	Baisman Run	0	21.49	1.682
		50	20.02	0.833
		200	19.48	1.122
	Dead Run	0	19.65	1.600
		50	22.33	1.732
		200	19.54	4.382
	Gwynns Run	0	20.00	2.255
		50	21.54	1.200
		200	18.25	0.860

Table 3c. Extracellular enzyme activity (EEA)(n=3) water from each site treated with PAH fluoranthene (hypothesis 2).

Enzyme	Site	Dose (µg/100mL)	Mean EEA (mmol/h*mL)	Standard Deviation
Phophatase	Pond Branch	0	0.967	0.208
		50	1.195	0.333
		200	1.279	0.262
	Baisman Run	0	0.900	0.100
		50	0.880	0.436
		200	1.237	0.073
	Dead Run	0	0.700	0.264
		50	1.258	0.252
		200	1.111	0.073
	Gwynns Run	0	1.400	0.300
		50	1.300	0.262
		200	0.587	0.073
Beta-glucosidase	Pond Branch	0	0.133	0.058
		50	0.000	0.000
		200	0.000	0.000
	Baisman Run	0	0.033	0.058
		50	0.016	0.027
		200	0.042	0.048
	Dead Run	0	0.000	0.000
		50	0.079	0.000
		200	0.000	0.000
	Gwynns Run	0	0.000	0.000
		50	0.142	0.054
		200	0.073	0.064
Leucine Amino-Peptidase	Pond Branch	0	20.90	1.229
		50	21.61	0.998
		200	18.19	0.604
	Baisman Run	0	21.40	1.682
		50	21.02	1.011
		200	20.98	0.475
	Dead Run	0	19.65	1.600
		50	20.33	1.294
		200	18.46	1.080
	Gwynns Run	0	20.00	2.255
		50	18.87	0.784
		200	18.48	2.211

Table 3d. Extracellular enzyme activity (EEA) (n=3) with water from each treated with PAH benzo(a)pyrene (hypothesis 2).

Enzyme	Site	Dose (μg/100mL)	Mean EEA (mmol/h*mL)	Standard Deviation
Phophatase	Pond Branch	0	0.967	0.208
		50	1.090	0.508
		200	1.740	0.073
	Baisman Run	0	0.900	0.100
		50	1.132	0.218
		200	0.922	0.192
	Dead Run	0	0.700	0.264
		50	0.503	0.126
		200	1.126	0.073
	Gwynns Run	0	1.400	0.300
		50	0.922	0.800
		200	1.342	0.290
Beta-glucosidase	Pond Branch	0	0.133	0.058
		50	0.047	0.031
		200	0.189	0.000
	Baisman Run	0	0.033	0.058
		50	0.058	0.036
		200	0.000	0.000
	Dead Run	0	0.000	0.000
		50	0.000	0.000
		200	0.031	0.272
	Gwynns Run	0	0.000	0.000
		50	0.194	0.101
		200	0.300	0.031
Leucine Amino-Peptidase	Pond Branch	0	20.90	1.229
		50	20.78	0.981
		200	21.63	1.392
	Baisman Run	0	21.40	1.682
		50	18.94	1.309
		200	19.66	0.151
	Dead Run	0	19.65	1.596
		50	21.90	1.337
		200	18.73	0.357
	Gwynns Run	0	20.00	2.255
		50	19.11	0.181
		200	20.16	0.494

Table 4. F-statistics for main dependent variables from two-way ANOVA tests for hypothesis 1 (* p<0.10, **p<0.05, ***p<0.01).

Dependent Variable	Site Main Effect
Ambient water and Phosphatase	26.898*** df =3,44
Ambient water and Beta-glucosidase	39.417*** df=3,44
Ambient water and Leucine amino peptidase	3.628 df=3,44

Table 5. F-statistics for main dependent variables from two way-ANOVA tests for hypothesis 2 (* p<0.10 **p<0.05 ***p<0.01).

Dependent Variable (treatment and enzyme)	Site Main Effect	Dose Main Effect	Site by Dose Interaction Effect
Triclocarban and Phosphatase	0.939 df=3,24	0.899 df=2,24	5.647*** df=6,24
Triclocarban and Beta-glucosidase	1.702 df=3,24	0.758 df=2,24	8.275*** df=6,24
Triclocarban and Leucine amino peptidase	2.976 df=3,24	2.077 df=2,24	2.414 df=6,24
Phenanthrene and Phosphatase	51.828*** df=3,24	1.472 df=2,24	4.624*** df=6,24
Phenanthrene and Beta-glucosidase	2.988* df=3,24	4.653** df=2,24	3.642*** df=6,24
Phenanthrene and Leucine amino peptidase	0.219 df=3,24	1.569 df=2,24	1.247 df=6,24
Fluoranthene and Phosphatase	0.634 df=3,24	1.399 df=2,24	5.226*** df=6,24
Fluoranthene and Beta-glucosidase	2.722* df=3,24	1.970 df=2,24	8.798*** df=6,24
Fluoranthene and Leucine amino peptidase	3.789** df=3,24	4.357** df=2,24	1.067 df=6,24
Benzo(a)pyrene and Phosphatase	3.805** df=3,24	4.764** df=2,24	2.020 df=6,24
Benzo(a)pyrene and Beta-glucosidase	27.927*** df=3,24	13.617*** df=2,24	12.223*** df=6,24
Benzo(a)pyrene and Leucine amino peptidase	2.038 df=3,24	0.374 df=2,24	2.924** df=6,24

Table 6. Summary of all statistically significant ANOVA results (at the $p<0.01$ level) for ambient, untreated water (hypothesis 1).

Enzyme	Factors	P-value
Phosphatase	Site	0.000
Beta-glucosidase	Site	0.000

Table 7. Summary of all statistically significant ANOVA results (at the $p<0.01$ level) for water from each site treated with an antimicrobial or a PAH (hypothesis 2).

Treatment	Enzyme	Factors	P-value
Triclocarban	Phosphatase	Interaction site by dose	0.001
	Beta-glucosidase	Interaction site by dose	0.000
Phenanthrene	Phosphatase	Interaction site by dose	0.003
	Phosphatase	Site	0.000
	Beta-glucosidase	Interaction site by dose	0.010
Fluoranthene	Phosphatase	Interaction site by dose	0.001
	Beta-glucosidase	Interaction site by dose	0.000
Benzo(a)pyrene	Beta-glucosidase	Site	0.000
	Beta-glucosidase	Dose	0.000
	Beta-glucosidase	Interaction site by dose	0.000

Table 8. Summary of all statistically significant ($p<0.01$) pair-wise comparisons of untreated water from sites from Tukey HSD post-hoc test (hypothesis 1).

Enzyme	Combination	Significant value at the $p<0.01$ level
Phosphatase	Pond Branch and Dead Run	0.010
	Pond Branch and Gwynns Run	0.000
	Baisman Run and Gwynns Run	0.000
	Dead Run and Gwynns Run	0.000
Beta-Glucosidase	Pond Branch and Baisman Run	0.000
	Pond Branch and Dead Run	0.000
	Pond Branch and Gwynns Run	0.000

Table 9. Summary of all statistically significant ($p<0.01$) pair-wise comparisons from Tukey HSD post-hoc test (hypothesis 2).

Treatment	Enzyme	Combination	Significant value at the $p<0.01$ level
Phenanthrene	Phosphatase	Pond Branch and Baisman Run	0.001
		Pond Branch and Dead Run	0.000
		Baisman Run and Dead Run	0.000
		Baisman Run and Gwynns Run	0.000
		Dead Run and Gwynns Run	0.000
Benzo(a)pyrene	Beta-Glucosidase	Pond Branch and Baisman Run	0.000
		Pond Branch and Dead Run	0.000
		Baisman Run and Gwynns Run	0.000
		Dead Run and Gwynns Run	0.000
		No dose and high dose	0.000
		Low dose and high dose	0.010

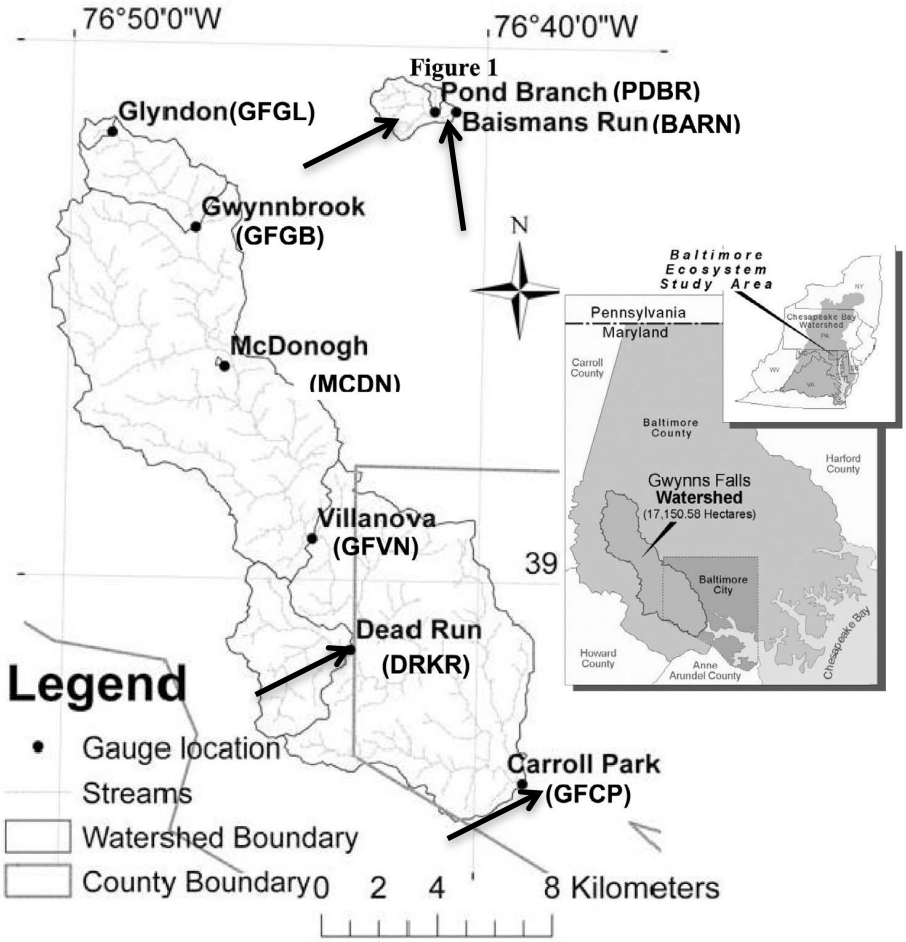
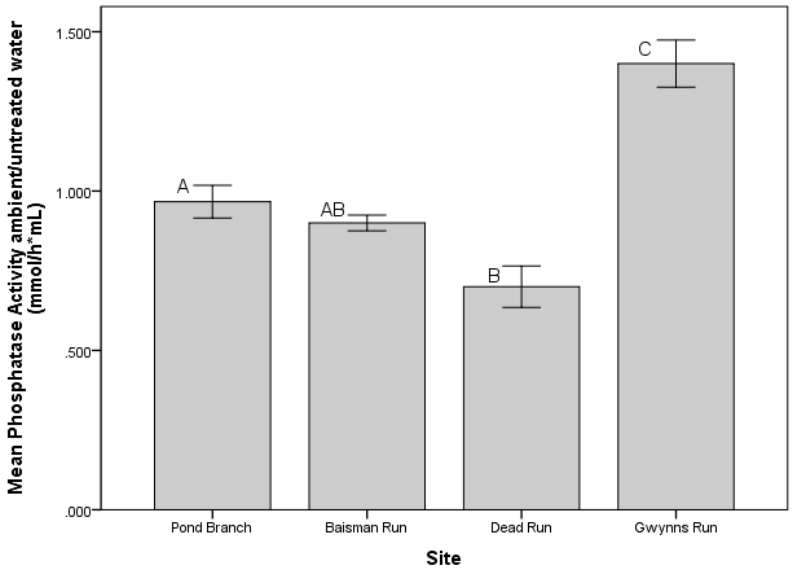


Figure1. Map of Gwynns Run and Baisman Run watersheds, indicating study sites with arrows. Data from Duan *et al. (*2012).

a.



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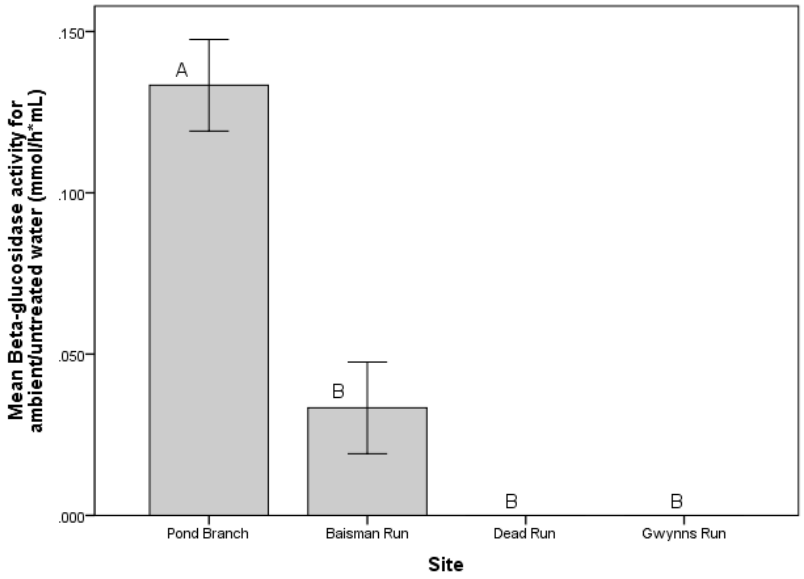


Figure 2. Mean (a) phosphatase activity and (b) beta glucosidase activity (±1 SE) of untreated water from each site (hypothesis 1). Sites that have no letters in common are significantly different at $p<0.01$ (see Table 8 for Tukey HSD post-hoc test results).

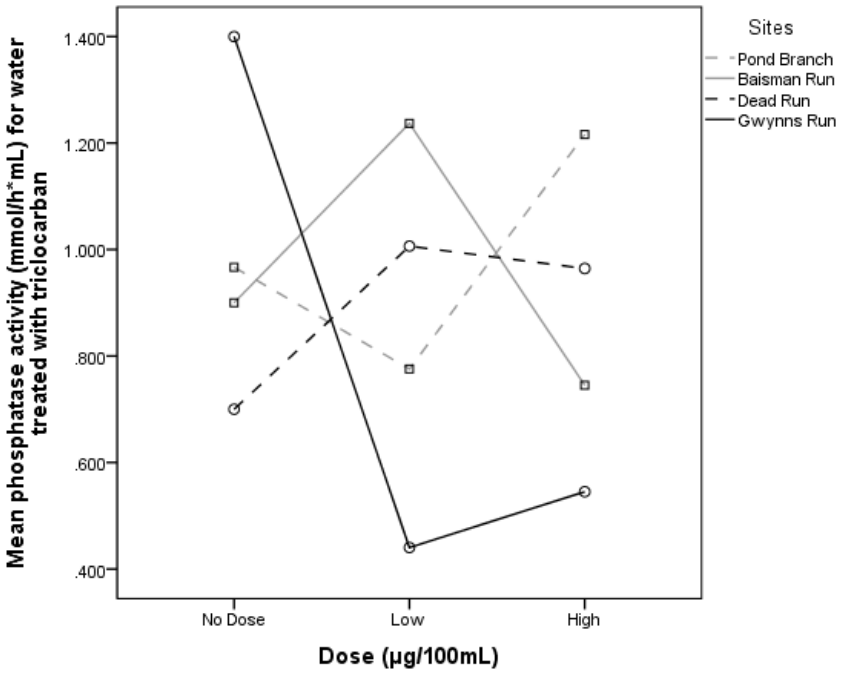


Figure 3. Site by dose interaction for phosphatase activity of water treated with triclocarban. Significant ($p<0.01$) interaction among all four sites (grey symbols for rural sites and black symbols for urban sites) and three doses (untreated water/no dose, 50 µg/100mL, and 200µg/100mL) (see Table 3a for exact values, Table 5 for p-values, Table 7 for F-statistics).

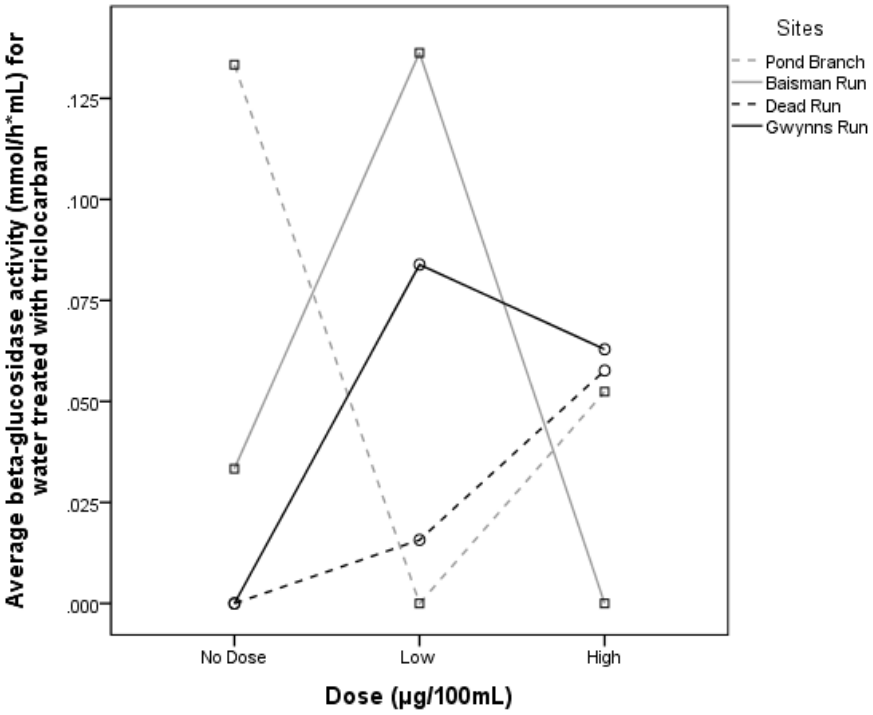


Figure 4. Site by dose interaction for beta-glucosidase activity of water treated with triclocarban. Significant ($p<0.01$) interaction among all four sites (grey symbols for rural sites and black symbols for urban sites) and three doses (untreated water/no dose, 50 µg/100mL, and 200µg/100mL) (see Table 3a for exact values, Table 5 for p-values, Table 7 for F-statistics).

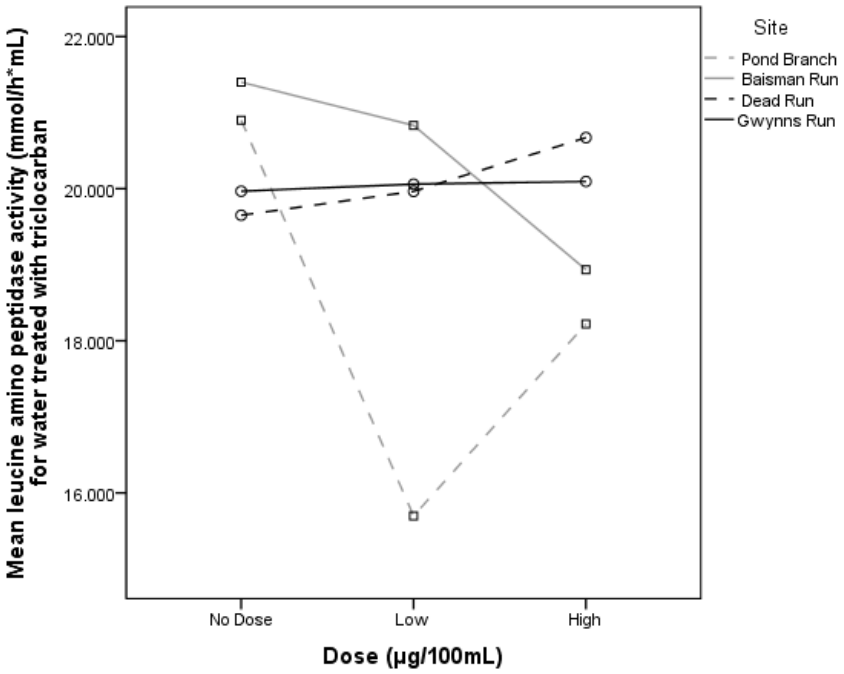


Figure 5. Site by dose interaction for leucine amino peptidase activity of water treated with triclocarban. No significant ($p>0.01$) interaction among all four sites (grey symbols for rural sites and black symbols for urban sites) and three doses (untreated water/no dose, 50 µg/100mL, and 200µg/100mL) (see Table 3a for exact values, Table 5 for p-values, Table 7 for F-statistics).

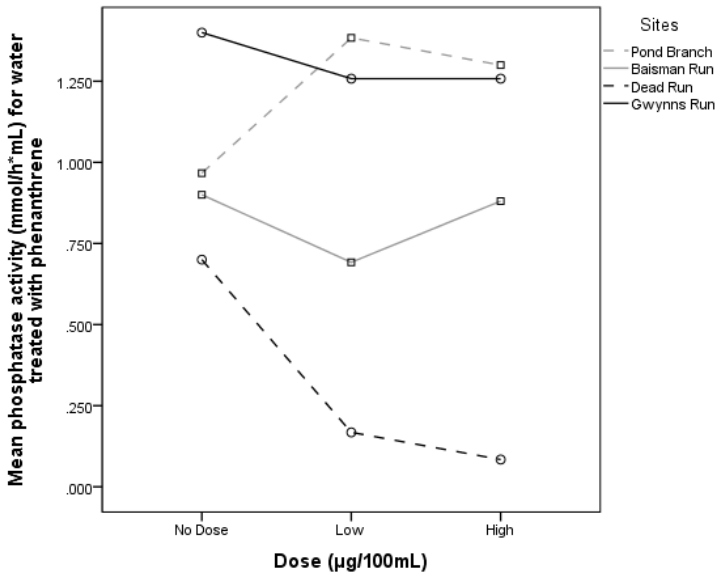


Figure 6a. Site by dose interaction for phosphatase activity of water treated with phenanthrene. Significant ($p<0.01$) interaction among all four sites (grey symbols for rural sites and black symbols for urban sites) and three doses (untreated water/no dose, 50 µg/100mL, and 200µg/100mL) (see Table 3b for exact values, Table 5 for p-values, Table 7 for F-statistics).

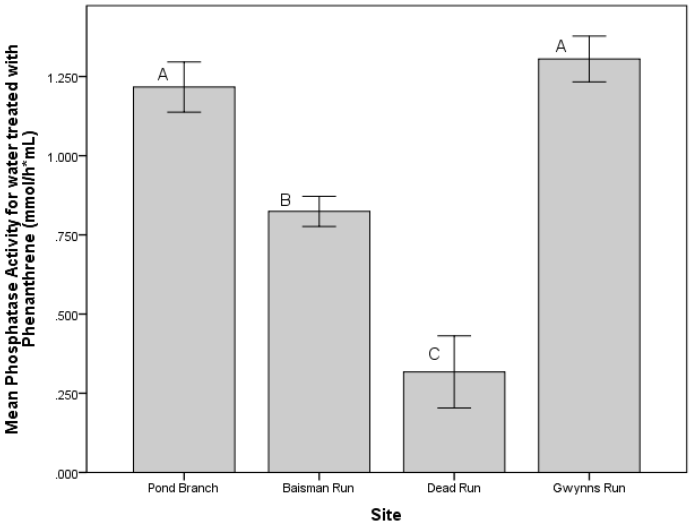


Figure 6b. Mean phosphatase activity (±1 SE) of water treated with phenanthrene per site. Sites that have no letters in common are significantly different at $p<0.01$ (see Table 9 for Tukey HSD post-hoc test results).

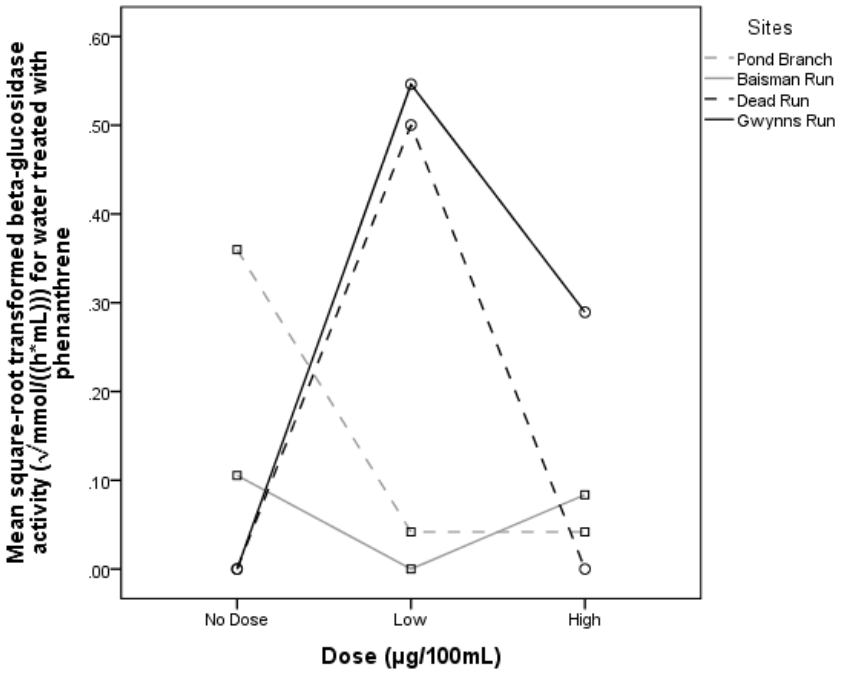


Figure 7. Site by dose interaction for square-root transformed beta-glucosidase activity for water treated with phenanthrene. Significant ($p<0.01$) interaction among all four sites (grey symbols for rural sites and black symbols for urban sites) and three doses (untreated water/no dose, 50 µg/100mL, and 200µg/100mL) (see Table 3b for exact values, Table 5 for p-values, Table 7 for F-statistics).

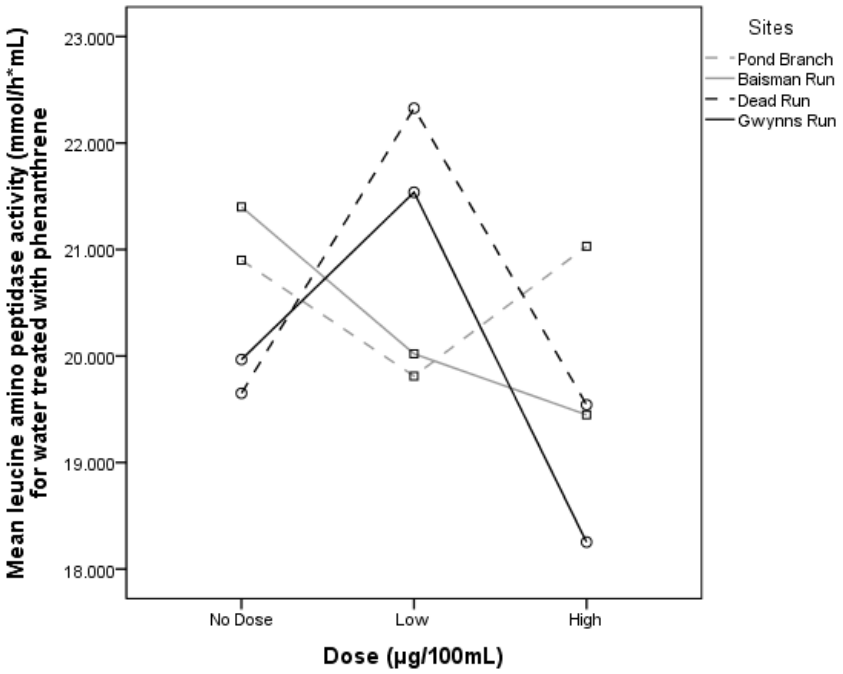


Figure 8. Site by dose interaction for leucine amino peptidase activity of water treated with phenanthrene. No significant ($p>0.01$) interaction among the four sites (grey symbols for rural sites and black symbols for urban sites) and the three doses (untreated/no dose, 50 µg/100mL, and 200µg/100mL) (see Table 3b for exact values, Table 5 for p-values, Table 7 for F-statistics).

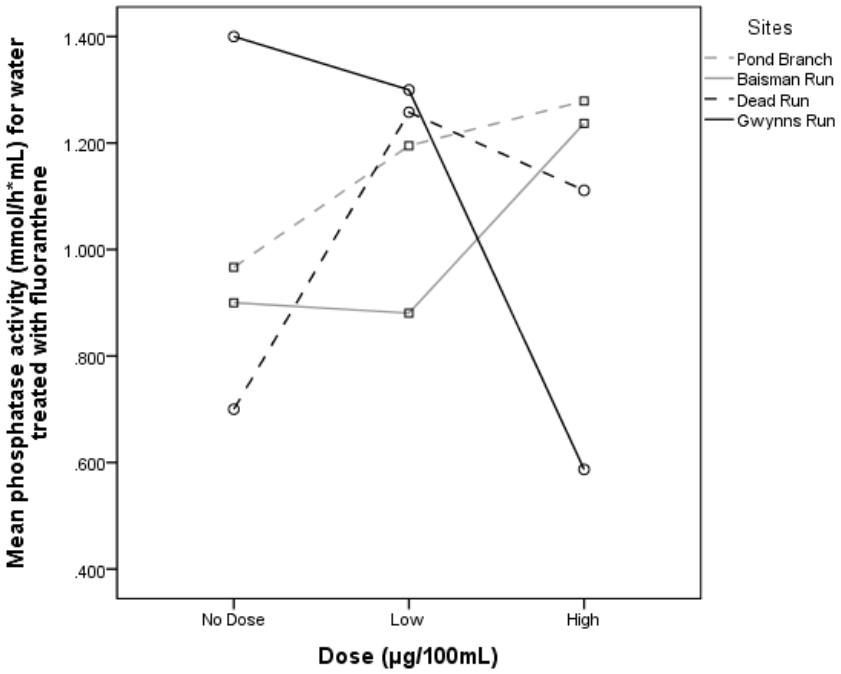


Figure 9. Site by dose interaction for phosphatase activity of water treated with fluoranthene. Significant ($p<0.01$) interaction among all four sites (grey symbols for rural sites and black symbols for urban sites) and three doses (untreated water /no dose, 50 µg/100mL, and 200µg/100mL) (see Table 3c for exact values, Table 5 for p-values, Table 7 for F-statistics).

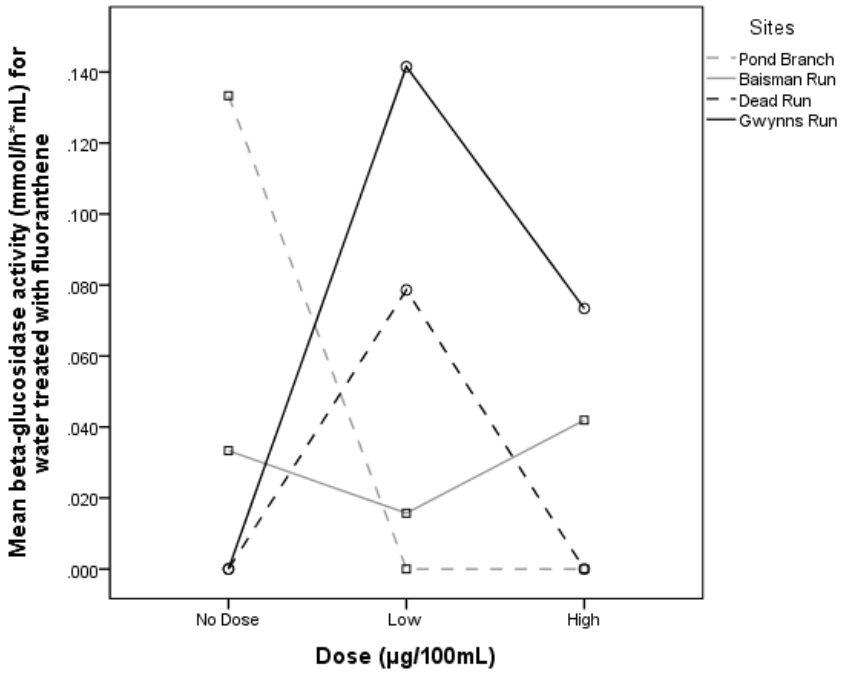


Figure 10. Site by dose interaction for beta-glucosidase activity of water treated with fluoranthene. Significant ($p<0.01$) interaction among all four sites (grey symbols for rural sites and black symbols for urban sites) and three doses (untreated water/no dose, 50 µg/100mL, and 200µg/100mL) (see Table 3c for exact values, Table 5 for p-values, Table 7 for F-statistics).

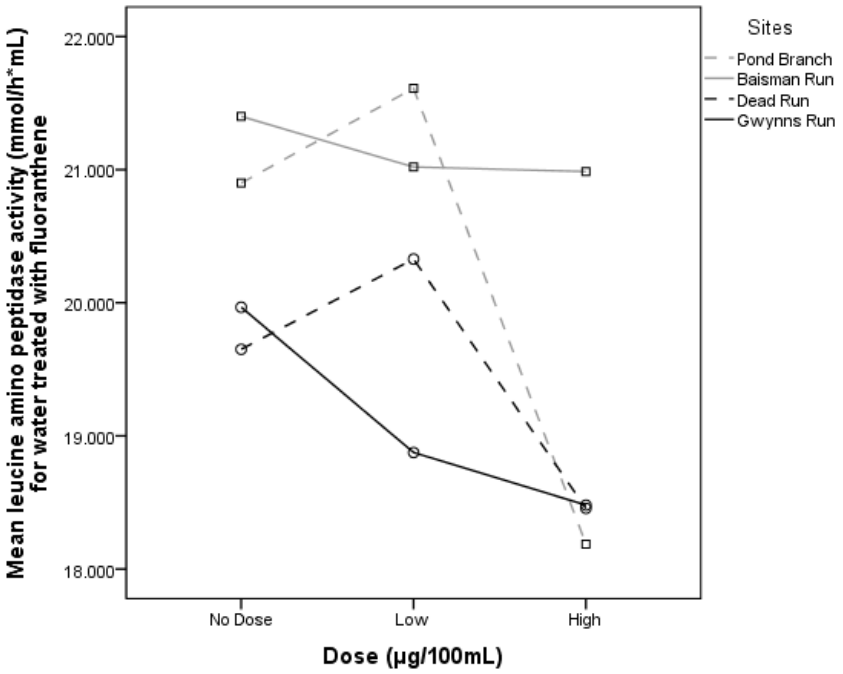


Figure 11. Site by dose interaction for leucine amino peptidase activity of water treated with fluoranthene. No significant ($p>0.01$) interaction among the four sites (grey symbols for rural sites and black symbols for urban sites) and the three doses (untreated water /no dose, 50 µg/100mL, and 200µg/100mL) (see Table 3c for exact values, Table 5 for p-values, Table 7 for F-statistics).

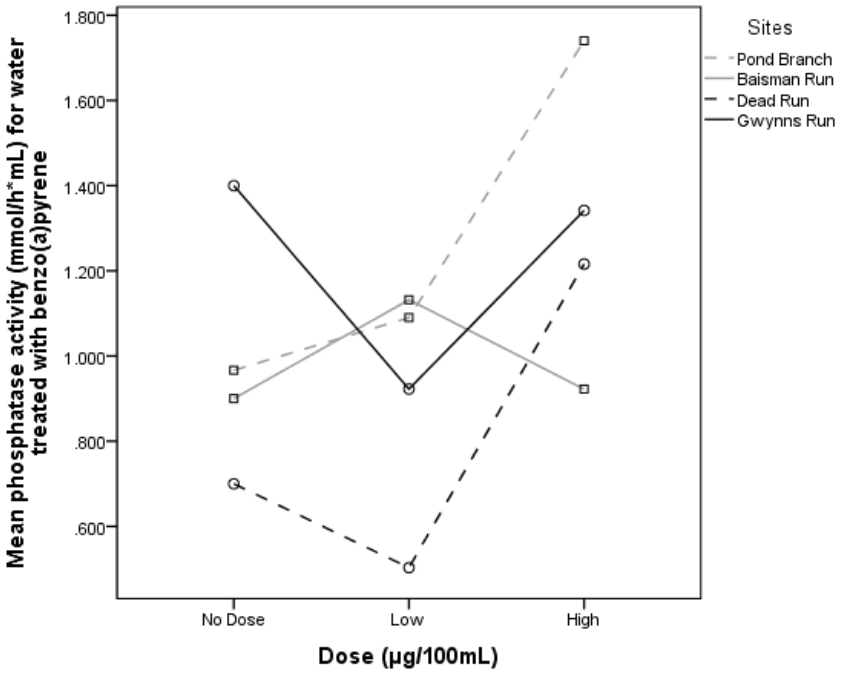


Figure 12. Site by dose interaction for phosphatase activity of water treated with benzo(a)pyrene. No significant ($p>0.01$) interaction among the four sites (grey symbols for rural sites and black symbols for urban sites) and the three doses (untreated water/no dose, 50 µg/100mL, and 200µg/100mL) (see Table 3d for exact values, Table 5 for p-values, Table 7 for F-statistics).

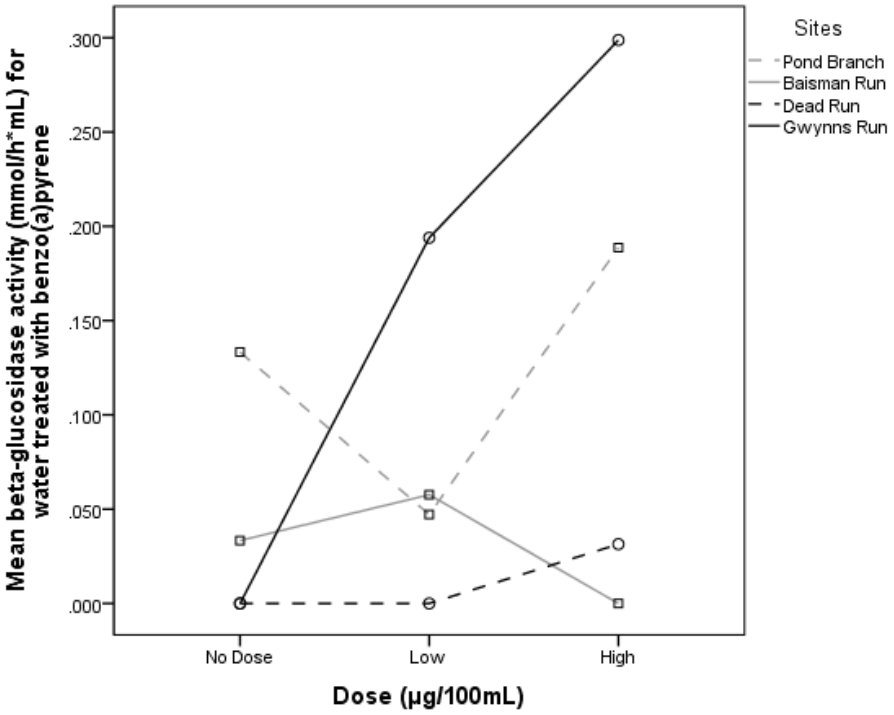
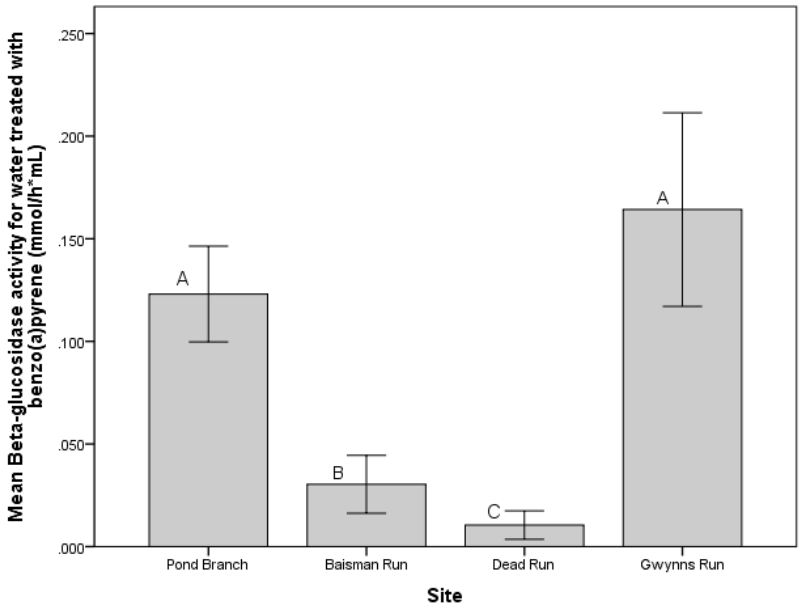


Figure 13a. Site by dose interaction for beta-glucosidase activity of water treated with benzo(a)pyrene. Significant ($p<0.01$) interaction among all four sites (grey symbols for rural sites and black symbols for urban sites) and three doses (untreated water/no dose, 50 µg/100mL, and 200µg/100mL) (see Table 3d for exact values, Table 5 for p-values, Table 7 for F-statistics).

b.



c.

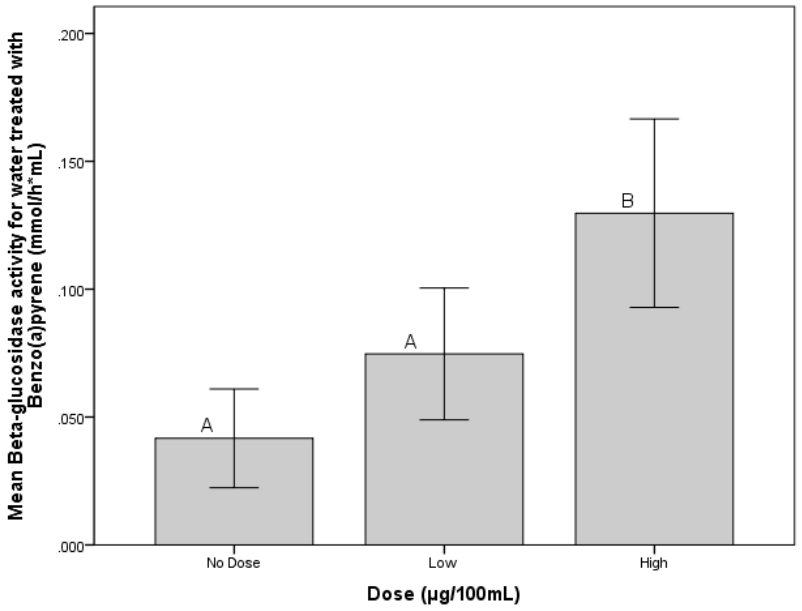


Figure 13b and 13c. Mean beta-glucosidase activity (±1 SE) of water treated with benzo(a)pyrene per site (b) and per dose (c). Sites and doses that have no letters in common are significantly different at $p<0.01$ (see Table 9 for Tukey HSD post-hoc test results).

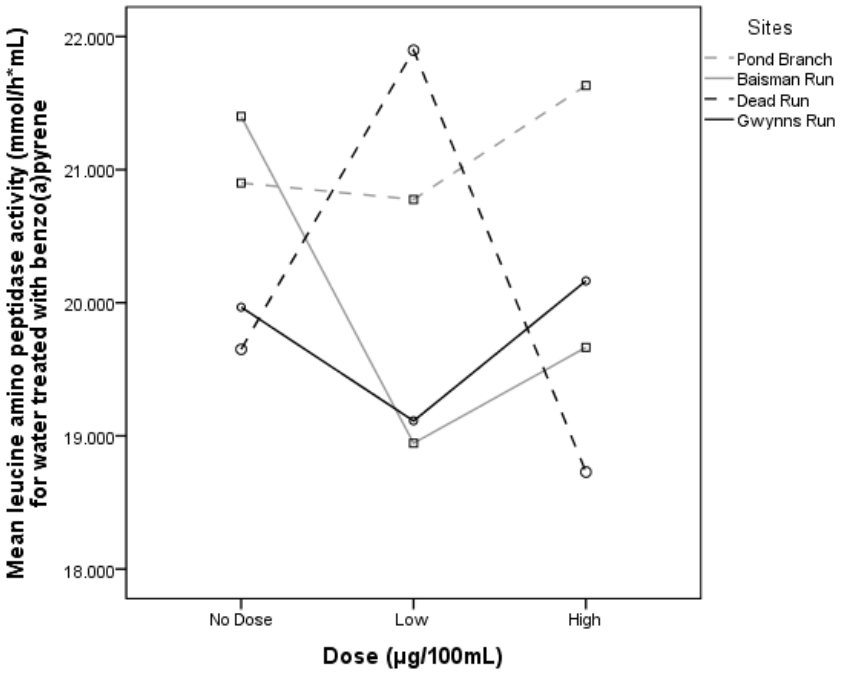


Figure 14. Site by dose interaction for leucine amino peptidase activity of water treated with benzo(a)pyrene. No significant ($p>0.01$) interaction among the four sites (grey symbols for rural sites and black symbols for urban sites) and the three doses (untreated water/no dose, 50 µg/100mL, and 200µg/100mL) (see Table 3d for exact values, Table 5 for p-values, Table 7 for F-statistics).

APPENDIX

Supplemental Table 1: List of frequently used terms and abbreviations

Term	Abbreviation
Impervious surface cover	ISC
Dissolved Organic Carbon	DOC
Dissolved Organic Matter	DOM
Extracellular Enzyme Activity	EEA
Phosphatase	PHOS
Beta-glucosidase	BG
Leucine amino peptidase	LAP
Triclocarban	TCC
Poly-aromatic hydrocarbon	PAH
Organic wastewater contaminant	OWC
United States Geological Survey	USGS
Baltimore Ecosystem Study	BES
Long term ecological research	LTER
Metabolic scaling theory	MST

Supplemental Table 2: Water chemistry data from all four sites taken on 7/18/12

Site	Time	Water Temp (ºC)	pH	Conductivity (µS)	DO (mg/L)	Flow (ft^3/s)
Pond Branch (rural)	10:30	22.1	7.73	303	7.88	0.071
Baisman Run** (rural)	10:30	22.7	7.7	167.5	7.88	0.56
Dead Run** (urban)	09:35	25.3	7.83	801	5.66	0.68
Gwynn's Run (Carroll Park)* (urban)	09:00	20.7	7.75	736	5.11	0.14