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MICROBIAL RESPONSES TO VARIED CONCENTRATIONS OF DISSOLVED ORGANIC CARBON IN GROUNDWATER

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Abstract. An investigation of groundwater from several different aquifers was undertaken to evaluate the potential relationship between lithology and dissolved organic carbon (DOC) concentration. The wells tested in this study were from throughout Dutchess County, New York, representing a wide range of different aquifer types. Differences in the DOC concentration between the various well water sources were not statistically significant. However, some evidence suggests that DOC concentration varied between bedrock and non-bedrock aquifer sources with values that range from 0.20 to 0.23 mg/L for bedrock sources and 0.34 to 0.46 mg/L for non-bedrock sources. This investigation also included an evaluation of the effect of DOC concentrations on microbial populations. Microbial responses varied during respiration and bacterial production trials, but yielded very different results. The bacterial production showed slight differences, but did not demonstrate any significant relationship to DOC concentration. However, the respiration rates varied significantly between samples, but no direct correlation to DOC concentrations has yet been found. The analysis of the types of carbon utilized by microbes may hold the key to understanding the respiration results and the potential link between lithological characteristics and the DOC concentration.

INTRODUCTION

Carbon is an essential element found in rocks and minerals and used by biologically diverse life forms as a source of energy. Carbon is complex, appearing in many forms and in a variety of environments. One specific type of carbon is dissolved organic carbon (DOC). DOC is a term used to represent organic carbon that is less than 0.45 micrometers in diameter (Thurman, 1985). DOC is commonly found in lakes, river systems, the hyporheic zone (zone of surface water/groundwater interaction), and groundwater systems. However, the research in these four areas is very different; much more work has been done on DOC in relation to lakes, rivers, and the hyporheic zone, whereas groundwater studies are limited and often have neglected deep groundwater. Deep groundwater is difficult to study due to its location and limited accessibility. However, it is important to study groundwater because it is an integral part of river systems as well as a source of drinking water for many regions.

This research focuses on three main questions. The first question is to determine how lithology influences the concentration of DOC in groundwater.

The second is to investigate how differences in the amount of DOC available influence microbial communities by looking at respiration and production. The third question is to study what types of carbon microbes are utilizing in these processes. This investigation is a multi-disciplinary effort to examine possible links between groundwater, DOC concentration and microbial responses.

BACKGROUND

Dissolved Organic Carbon

The study of DOC is very important on both a large and a small scale. On the larger scale, DOC has been linked to essential ecological processes such as denitrification because carbon is a necessary energy source for bacteria and other microbes responsible for the completion of this process. Denitrification is a bacterial process where nitrate (NO_3) is converted to nitrogen (N_2) and released into the atmosphere (Campbell et al., 1999). According to Paramasivam et al. (1999) denitrification capacity increases with an increase in DOC content. Statistical analysis also showed a high correlation between denitrification capacity and DOC ($r^2 = 0.96 - 0.97$) for samples collected both at the soil/groundwater interface and in groundwater (Paramasivam et al., 1999). This suggests that DOC plays an essential role in many ecological systems and environments. On a smaller scale, DOC influences the ability of microorganisms to conduct their everyday functions. For example, bacteria play a pivotal role in ecosystems; they are responsible for processes including nitrification and the mineralization of organic matter (Gilbert et al. 1994). Thus, determining the influence of lithology on DOC concentration can give a better understanding of the interaction between geology and microbial processes.

In examining the availability of carbon for organisms, the source of the carbon and the link to lithology is often of interest. Several investigations have tried to understand where the source of carbon is in water systems. Ellis and Stanford (1988) attempted to link hydrogeomorphic processes in alluvial aquifers to the temporal and spatial distribution of carbon and other nutrients. Work completed on the variability of DOC concentration in the natural environment, have focused on surface water and groundwater systems by looking at factors such as soils, etc. The cause of these differences is still under investigation, with much of the literature presenting different hypotheses. The concentration of DOC in streams has been related to the dynamic hydrological, physical-chemical, and biological interactions that water undergoes in the riparian zone, water column, hyporheic zone, and at the bedrock interface (Jones and Mulholland, 2000). Paramasivam et al. (1999) measured differences in DOC content as found in different monitoring wells in addition to variations in concentration occurring with depth in a single well. In addition, soil type and soil attributes have been found to be influential in determining DOC concentration according to Jones and Mulholland (2000) where clay content is a major factor in the amount of DOC present. In general, the greater the clay content the larger the DOC concentration since clays typically have a higher than average carbon content. In their study, two Australian watersheds with similar climates, vegetation and land use, but different soils, have DOC concentrations that varied by 2.5-fold. Another study found that the organic content of different soils vary considerably with peat containing up to 30% organic matter by mass, whereas tundra and desert soils contain only 0.1% organic matter by mass (Stolp 1988). Thus, factors other than just lithology influence the amount of DOC in water sources. In fact, lithology is a minor factor in most cases, but could been significant when looking at groundwater systems especially those that are deep or have long flow paths. This needs to be taken into account when working in any complex environment.

The study of groundwater is essential. The ability of deep groundwater to provide habitat suitable for organisms is considered to be a critical part of biogeochemical cycling (Jones and Mulholland 2000). The significance of groundwater is increased with its role in transporting DOC from terrestrial sources to stream ecosystems in which it is then used as a source of carbon and energy for heterotrophic organisms (Jones and Mulholland 2000) and in the process of denitrification.

The specific link between DOC concentration and microbial response has been previously studied in two different manners; by looking at respiration and by looking at bacterial production. A study done by Pusch and Schwoerbel (1994) found that variability in DOC concentration affects hyporheic respiration. Further work in this area completed by Baker et al. (1999) found that microbial respiration in the hyporheic zone is limited by labile DOC availability. It is understood that labile DOC is the DOC that microbes are able to break down and use. The work of Findlay et al. (1993) indicated that variability in DOC was also shown to affect sediment bacterial activity. For example, many studies have demonstrated a positive correlation between bacterial growth and the concentration of dissolved organic matter in lakes (Overbeck and Chrost 1990). These different investigations provide the foundation for the research proposed in this study.

General Geology

The highly varied geology in the state of New York is divided easily into provinces, each one showing unique geology and physiography (Van Diver 1985). Several major geological events have occurred in the New York area to create the topography seen today. Some of these events include the collision of North America with Africa and its subsequent separation, the Taconic orogeny and other local and regional orogenies, and the glaciation of almost the entire state of New York (Van Diver 1985).

Dutchess County Geology

The general geological characteristics of New York are reflected in the highly variable characteristics of Dutchess County. Dutchess County is located in the southeastern part of New York and occupies an area of 800 square miles (Figure 1) (Simmons et al. 1961).

The topography in the county is an alternation of hills and valleys (Simmons et al. 1961). The original rocks in this area range from Precambrian to Ordovician in age. These rocks have since experienced many orogenic events that converted sandstone, limestone, and shale to quartzite, marble, phyllite, and schists in the south and east sections of the county with the southeast section of the county having undergone the highest degree of metamorphism (Simmons et al., 1961). After the orogenic events in this area had concluded, erosion took place. Following the periods of uplift and erosion, glaciation occurred, resulting in much of the Pleistocene deposits of till, sand and gravel, which was followed by the deposition of recent alluvial deposits.

According to Simmons et al. (1961), groundwater occurs in all the geological formations of Dutchess County with the most important sources of water coming from two main groups of rocks: (1.) Consolidated rocks that range in age from Precambrian to Ordovician (Figure 2) and (2.) Unconsolidated deposits from the Pleistocene to recent times (Figure 3). Dutchess County relies on groundwater as the principal water source for farms, rural homes and summer camps. In addition, the use of groundwater as a source of water for industrial and public supply has increased over time (Simmons et al., 1961). Within the county, the major valleys are known to have large supplies of groundwater suitable for municipal and industrial needs from stratified deposits of sand and gravel (Simmons et al., 1961). Most of the local cities and towns in Dutchess County currently rely on groundwater for at least some portion of their water supply. The present study has made use of the existing municipal wells.

Study Site Lithology

Five different municipal water supply wells from different areas in the county were selected. Each of the water supplies utilizes different aquifers as their primary source of groundwater. Most information is known about two sites—Pine Plains and Millerton—located in the northern part of Dutchess County. Pine Plains is located in the north central to northeast section of the county. Pine Plains relies mainly on one six-inch diameter well, although two other wells are also owned by the Pine Plains Water Company. (Simmons et al. 1961). In this area, the Stockbridge Limestone is known as the major water bearing formation (Simmons et al. 1961). The Stockbridge Limestone is Cambrian to Ordovician in age and consists of white, blue, and gray limestone with some marble (metamorphosed dolostone) in the east (Simmons et al. 1961). This formation is also known as the most productive bedrock formation, yielding an average of 22 gallons per minute (GPM) (Simmons et al. 1961). The well is approximately 235ft. deep with the water-bearing zone located beneath 15ft. of sand and 86ft. of silt and clay (Spohr 2000). This description most likely corresponds to Dutchess County well Du 680.

Millerton is the other well located in the northern part of Dutchess County, located in the northeast corner of the county. Millerton has two wells, both of which are 10 inches in diameter and approximately 50ft. deep (DCWWA, 1992). In both wells the water-bearing zone located in unconsolidated sand and gravel (DCWWA, 1992; Simmons et al. 1961). Additional information on the aquifer indicates that is bounded on three sides by low permeability till or bedrock (DCWWA 1992). It is difficult to tell the two wells in Millerton apart since they have the same diameter and virtually the same depth. However, stratigraphic information for well Du 167 is representative, if not exactly, the environment from which the sample was taken.

The final study sites were located in the southern section of the county (Figures 2 and 3). Pawling is the southeastern most site and has a very different lithology than all of the other sites. Pawling is located in the area that has undergone extensive metamorphism (Simmons et al. 1961). In addition to having experienced high degrees of metamorphism, the southern part of the county is known for its relatively abundant faults, which control the extent of bedrock formations (Simmons et al. 1961). Pawling is characterized as a non-specific bedrock (garnet-biotite-quartz-feldspar gneiss) aquifer located on a thrust fault (DCWWA 1992). This well is probably the one that the least amount is known about due to the faulting and metamorphism in the area. No information has been found about the characterization of the aquifer.

The LaGrange site is in the south central part of Dutchess County. The LaGrange area is typical of a glaciated area with a number of swamp deposits in the region due to the creation of differential drainage (Simmons et al. 1961). The local aquifers are overlain by glacial till of varying thickness, which may include some swamp deposits (DCWWA 1992). It is not clear which well was sampled—one is 180 ft. deep, while the other one is 354 ft. deep—however, both wells are located within a shale bedrock aquifer of the extensively faulted Walloomsac formation (DCWWA 1992).

The final site is slightly southwest of LaGrange in the town of East Fishkill. This site is unique in that it is a shallow water table aquifer (DCWWA 1992). The aquifer is 26ft. thick with no confining layer. The well is located in sand and gravel, which is most likely an outwash deposit (DCWWA 1992). The aquifer is known to have a static water level 4ft. below land surface (DCWWA 1992). In addition, the well operator for East Fishkill noted that this specific well location was directly under a swamp (Witsen 2000). The underlying bedrock is a part of the Wappinger Group, but in this case, it will play a lesser role in terms of dissolved organic carbon content. A summary of this well/aquifer information for all five sites is provided in Table 1.

MATERIALS AND METHODS

Site Selection

Five municipal wells were selected as groundwater sources for this study. The wells were chosen based on aquifer characteristics and local geology. Surficial and bedrock information was used to survey a variety of different geological environments. These wells represent the two main types of aquifers—bedrock (BR) and nonbedrock (NON). Information about aquifer characteristics, including class and code, were also used in site selection. Five study sites were selected for sampling of the wells (Table 2 and Figures 2 and 3) to perform the water chemical tests.

In addition, two other water samples were used as low- and high-level controls. The low-level control used was deionized water and the high-level control was stream water collected in the Wappinger Creek, Millbrook, New York. For each water source, 70L of water was collected and stored at 0°C.

Laboratory Configuration

Gravel substrates were set-up inside 12” tall PVC piping to allow for microbial settling and reproduction. These substrates are also referred to as sediment cores. The PVC pipe was stoppered at both ends, but allowed for the flow of water through the sediment core via tubing both above and below the system. Samples were run in triplicate with three sediment cores per sample water source (Figure 4). Sediment cores were run for 20 days with a flow rate of approximately 25mL/hr to approximate the natural groundwater flow environment. Flow rates were checked at least once daily and adjusted accordingly.

DOC, Nitrate, and Phosphate Concentration

Water samples were collected from the head tanks and below the sediment cores several times during the experiment. From these samples, DOC, nitrate and phosphate concentrations were measured and recorded. DOC concentration was analyzed using a Shimadzu 5000 TOC Analyzer that estimates DOC concentration after high temperature combustion to CO_2. This is then detected with an infa-red gas analyzer. Nitrate and phosphate concentrations were measured via automated wet chemistry using an ALPKEM Auto Analyzer.

Respiration

Respiration was determined through the measurement of dissolved oxygen concentrations in the head tanks and below the sediment cores. Oxygen concentrations were measured using a YSI model 57-oxygen probe.

The probe was calibrated to the ambient air temperature before used. Flow rates in each of the cores were also calculated by measuring the volume of water that traveled through the core over a set time. Actual respiration rates are calculated through the use of an Excel spreadsheet.

Bacterial Production

Bacterial production was measured through a method of Thymidine incorporation as outlined in the Handbook of Methods in Aquatic Microbial Ecology by Kemp et al (1993). This procedure is used to estimate the sediment bacterial production.

Enzyme Analysis

The enzyme analysis was conducted according to the Microplate Enzyme Assays- Basic Activity Determination as presented by the University of Toledo (1994). A copy of this procedure can be found in Appendix 1.

RESULTS

DOC concentrations varied significantly ($p < 0.0001$) when comparing all seven water sources (figure 6). When viewing only the five groundwater sites, the variation is still apparent, but is not statistically significant. Groundwater DOC concentrations were on the range of 0.20 to 0.46 mg/L. In comparison, the DOC concentration in the stream water site was 2.47 mg/L. Viewed in this manner, groundwater DOC concentrations are very low in comparison to that of stream water concentrations. In further evaluating DOC levels, the five groundwater sites were broken up into two categories, bedrock and DOC concentrations for bedrock aquifers range from 0.20 to 0.23 mg/L while DOC concentrations for non-bedrock aquifers range from 0.34 to 0.46 mg/L. These values, however, are not statistically significant ($p < 0.125$). Respiration results were highly varied, as shown in Figure 7, with no particular pattern evident respiration values. The differences in respiration rates between sites are statistically significant, with a p-value less than 0.000001. In this data set there is no distinction between bedrock and non-bedrock aquifers.

The results for bacterial production show a general trend of increasing production from left to right (Figure 8). The low level control deionized water (DI) showed the lowest values for respiration, but the high level control Wappinger Creek (WC), otherwise known as stream water, did not show the highest. In general, this shows lower values for deionized water and bedrock aquifers and slightly higher values for non-bedrock aquifers and stream water. However, there are obvious exceptions to this statement. Site PAW, for instance, is a bedrock aquifer, but has a higher production rate than MIL - a non-bedrock aquifer. In addition, EFK has an extremely high amount of variability, but has a generally higher production rate than WC (stream water).

Shown here (Figure 9) is one of enzymes used in the analysis of the types of carbon utilized by microbes. The amount of activity that took place varies significantly between sites ($p<0.05$). This graph shows that there are differing amounts of carbon that the microbes are able to utilize for each of the seven water sources. This result is indicative that the composition of DOC at each of the various sites might be different.

DISCUSSION

It is important to address differences in DOC concentrations between the study sites and the controls. The level of DOC in deionized water (DI) was near zero in all cases as expected. In addition, the stream water—the high level control—also acted as expected with higher concentrations of DOC than any of the groundwater samples. Therefore, in terms of DOC concentrations, the controls worked very well in bracketing the values of groundwater DOC concentration.

The overall values of groundwater DOC were low (range 0.20-0.46mg/L) and did not exhibit a statistically significant difference. However, there are some trends and differences that can be seen between sites. The three bedrock aquifers showed lower DOC concentrations than the two non-bedrock aquifers. This difference may be due in part to the depth to the aquifer, as it has been seen shallow aquifers are often subject to higher levels of DOC due to higher infiltration rates and contamination from the surface (Malcolm, 1993). However, additional characteristics of each of the sites must be considered. East Fishkill, for example, is unique in the respect that it is a shallow water table aquifer lying beneath a swamp. These characteristics alone could account for some of the difference in DOC concentration since swamps have DOC concentrations on the range of 10-30 mg/L (Thurman, 1985) which is much higher than other sources of subsurface or surface water. The combination of higher infiltration rates and a greater availability of carbon is likely the cause of the elevated DOC concentrations at the East Fishkill site. In addition, the availability of the Millerton well to receive surface infiltration because there is no filtration between the surface and the subsurface could have significantly raised the DOC concentration in the water. The similar concentrations in the three other sites (LAG, PP, and PAW) may be directly associated with the fact that all these wells are fed by bedrock aquifers. Although, the comparison between non-bedrock and bedrock aquifers (Figure 6) does not reveal any statistically significant differences in DOC concentrations, there is an indication that such a relationship could exist. This would require further investigation and a larger number of study sites in order to draw any major conclusions. The trend, however, suggests that in deep aquifers, the bedrock geology is more influential than the overlying strata. For example, LaGrange is a bedrock aquifer that is overlain by swamp deposits; based upon the results at East Fishkill it would be expected that DOC levels at LaGrange would also be high, but they are not. In fact, the DOC concentration at LaGrange is the lowest of all the wells indicating that bedrock is by far more influential that surface deposits for deep aquifers.

It is not surprising to find DOC concentrations on the order of 0.20 to 0.46 mg/L since similar groundwater studies note that average DOC concentrations are typically on the order of 1 mg/L with a range of 0.2-15 mg/L (Malcolm 1993). However, the values in this study are on the low end of the spectrum. A discussion included in Thurman 1985 also addresses why concentrations of DOC in groundwater are so small. First, it is indicated that groundwater has residence times as long as hundreds or thousands of years; at this stage, it is also a source of food for heterotrophic microbes present in groundwater. Secondly, the organic carbon can be adsorbed onto the aquifer materials, reducing groundwater DOC concentrations, where it can be degraded either chemically or biochemically. Finally, aquifers have only a small fraction of organic carbon that can be dissolved thus resulting in low levels of DOC (Thurman 1985).

In addition to the carbon content as a characteristic of lithology, there were also the biochemical components of this research. Results from both the respiration and bacterial production were scattered, showing no specific pattern relating to DOC concentration as was expected. In this situation, this can be explained in a couple of ways. First of all, carbon may not have been the limiting factor in microbial processes. However, this is very difficult to determine since nitrate and phosphate analyses showed similar concentrations in all well water sources. Secondly, based upon the enzyme analysis, it can be suggested that it is not the amount of carbon that is important in this instance, but rather the type of carbon available to microbes. It not only means the type of carbon available, but it also must be in a labile form, or a form that they can utilize. Many different factors must be considered when trying to account for the differences seen in this study. It is, however, nearly impossible to consider all of the variations in each system, but an effort was made in the laboratory to control as many variables as possible.

CONCLUSION

This study indicates that although variation among groundwater DOC concentrations was slight for the range of geological environments represented in this study, they are still present and should be considered in evaluating aquatic ecosystems in which stream water or the hyporheic zone are concerned. These areas can be highly influenced by the infiltration of groundwater especially in summer months when most streams are fed purely by groundwater. Results indicate that there may be a difference in DOC concentration between bedrock and non-bedrock aquifer systems. This potential relationship should be further studied by looking at other works already completed as well as doing additional field studies in a variety of geologically different environments. More work can also be done to evaluate the importance of carbon in controlling microbial responses to variations in carbon in the amount and the type present. The biochemical analysis in this study suggested that the type of carbon available to microbial populations may be more important than the amount of carbon available.

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APPENDIX

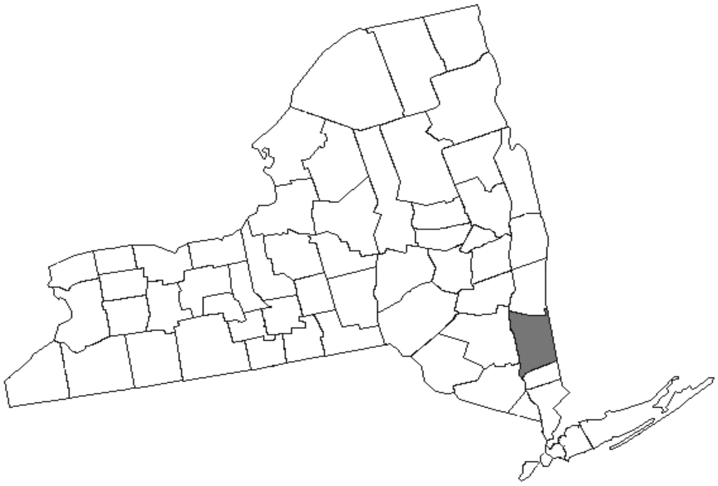

FIGURE 1. Study Location Map. This map shows the state of New York as it is divided into counties. Dutchess County is highlighted and is the location of this study

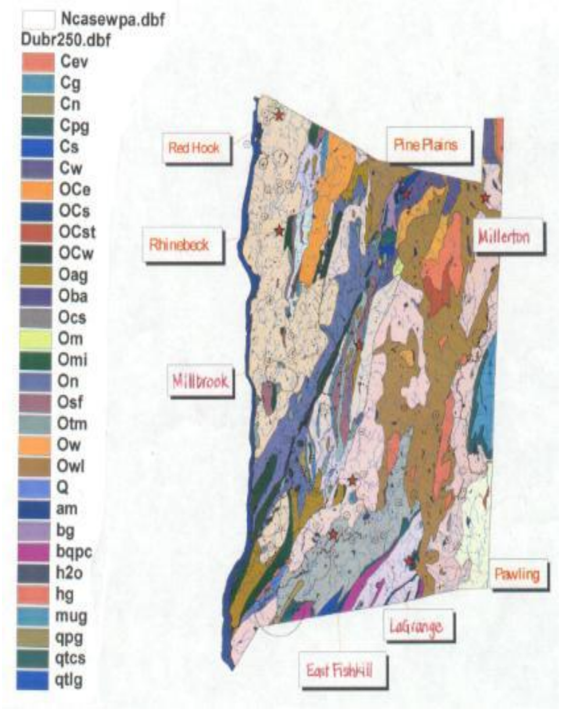

FIGURE 2. Bedrock Geology of Dutchess County

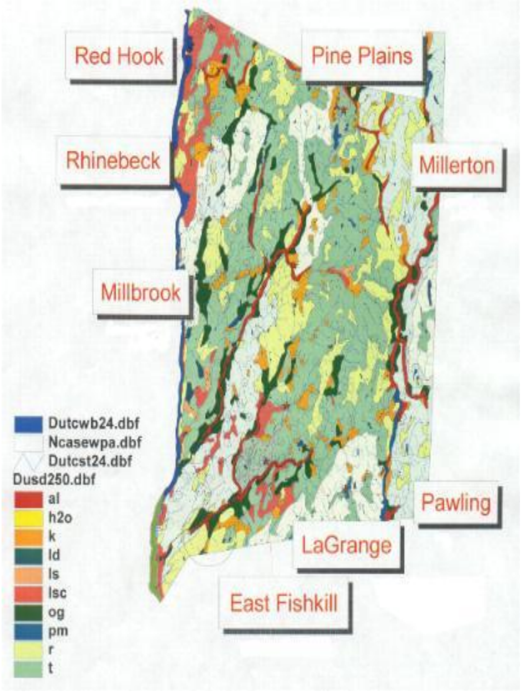

FIGURE 3. Surficial Geology of County, New York. Sites include Pine Plains, Dutchess County, New York. Sites include Millerton, East Fishkill, LaGrange and Pawling. Pine Plains, Millerton, East Fishkill, LaGrange and Pawling.

TABLE 1. Geology Summary Table. A brief summary of the geology at each of the five study locations.

Site	Bedrock Geology	Surficial Geology
LaGrange (LAG)	Walloomsac Formation	Lacusterine/ swamp deposits
Pine Plains (PP)	Balmville Limestone	Outwash gravel
Pawling (PAW)	Gneiss	Till
Millerton (MIL)	Stockbridge Marble	Alluvial deposits (recent)
East Fishkill (EFK)	Wappinger Group	Outwash gravel

TABLE 2. Site Characteristics Summary Table. A brief summary of the aquifer type and key characteristics for each of the five study locations.

Site	Aquifer Type	Key Characteristics
LaGrange (LAG)	Shale Bedrock	Faulted, no filtration
Pine Plains (PP)	Cabronate Bedrock	Beneath 86ft. of clay
Pawling (PAW)	Crystalline Bedrock	Metamorphosed, faulted
Millerton (MIL)	Sand & Gravel	Confined
East Fishkill (EFK)	Shallow Water Table	Swampy environment, no filtration

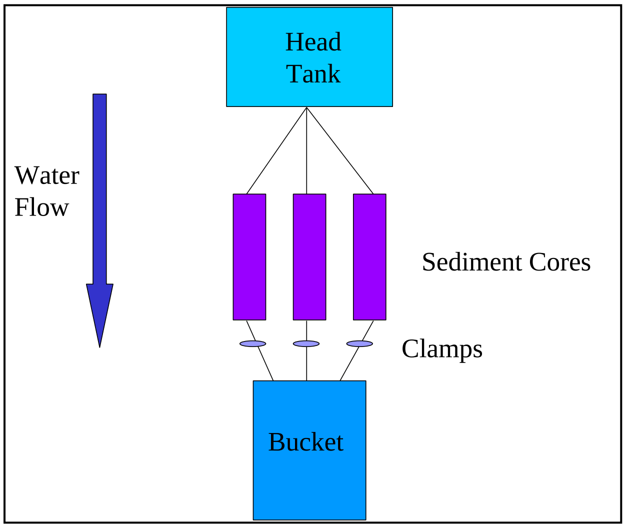

FIGURE 4. Laboratory Configuration. The schematic shows the premise behind the laboratory set-up.

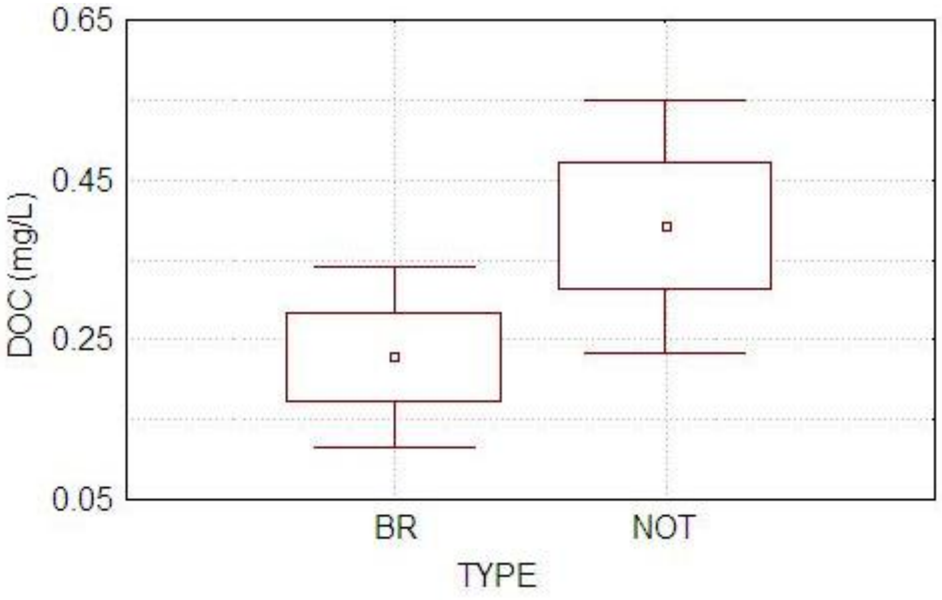

FIGURE 5. DOC concentration by aquifer type. Bedrock aquifers (BR) are LAG, PP, and PP. Non-bedrock aquifers (NOT) are EFK and MIL. For site abbreviations see Figure 5.

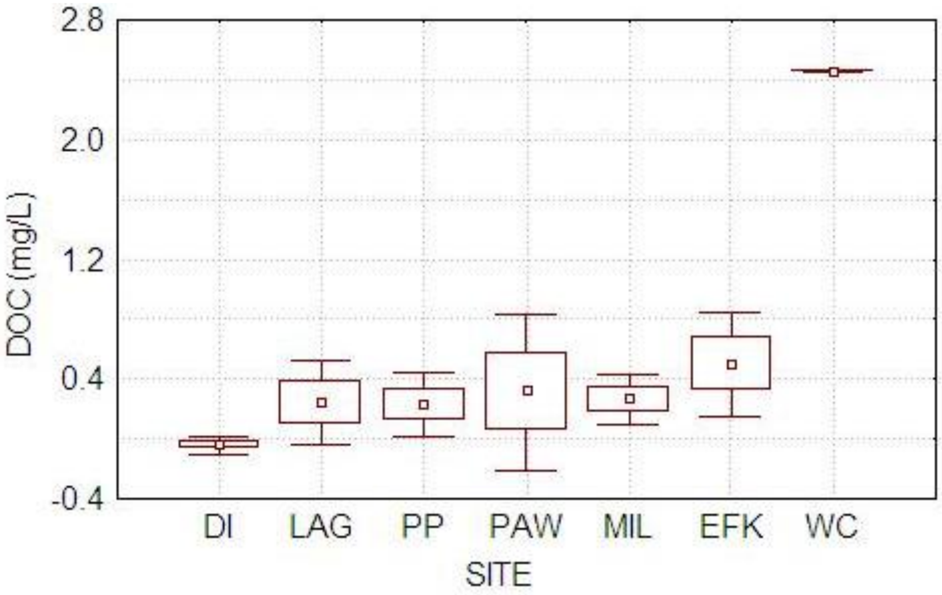

FIGURE 6. Dissolved organic carbon concentration in each of seven water sources. Site abbreviations: DI = deionized water (control); LAG = LaGrange; PP = Pine Plains; PAW = Pawling; EFK = East Fishkill; and WC = Wappinger Creek (stream water/control). For site characteristic information refer to Materials & Methods.

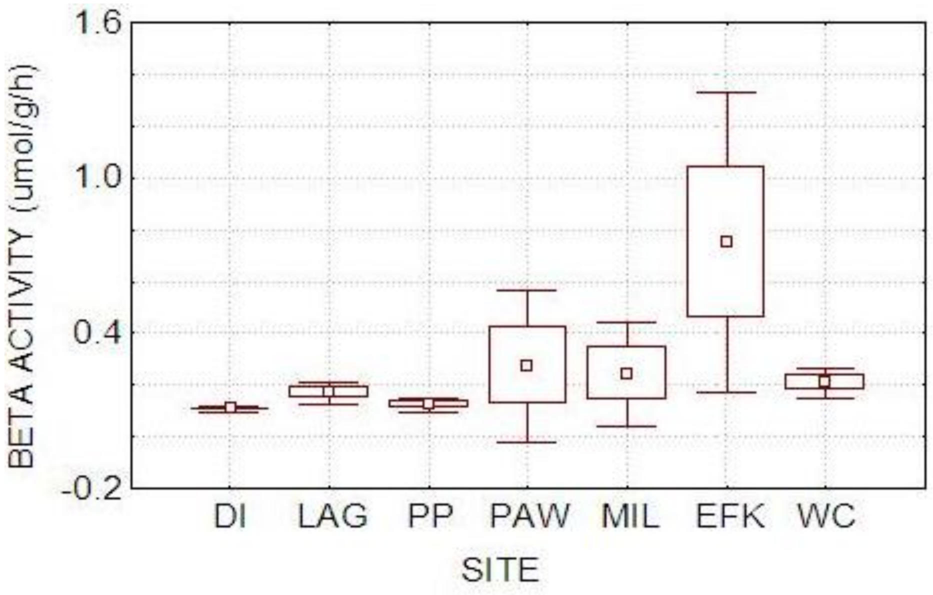

FIGURE 7. Microbial respiration rates measured for the seven water sources. For site abbreviations see Figure 5.

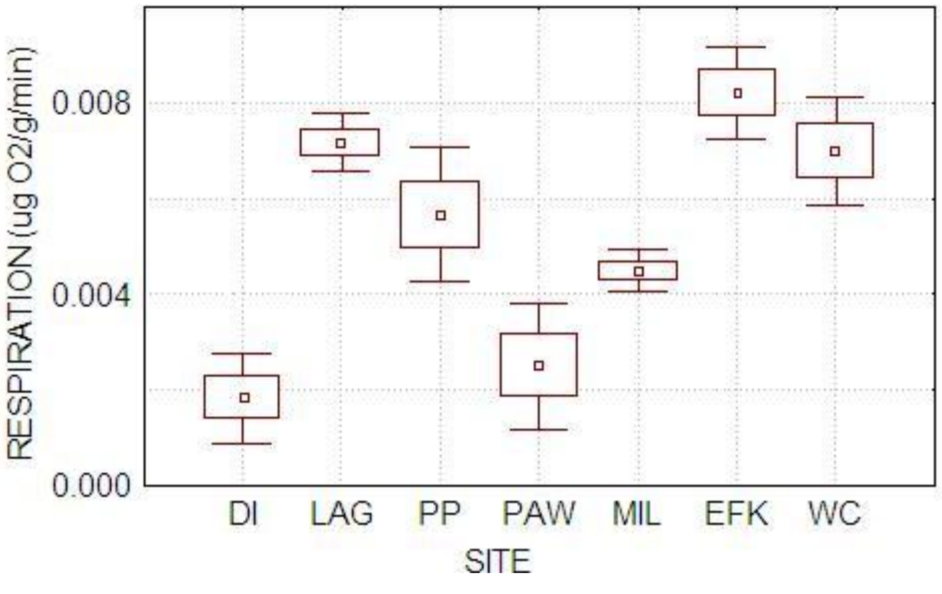

FIGURE 8. Bacterial production rates. For site abbreviations see Figure 5.

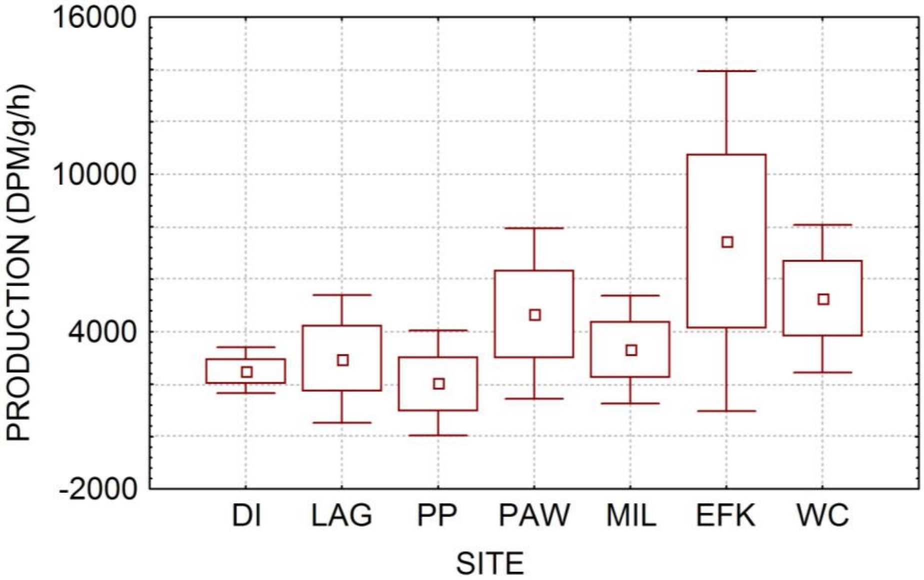

FIGURE 9. One of eight enzyme analyses conducted. This one shows Beta activity as a function of water source. For site abbreviations see Figure 5.