EFFECTS OF DILUTED WASTEWATER AMENDMENTS ON DOM DYNAMICS, BACTERIA, AND PHYTOPLANKTON OF THE HUDSON RIVER

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Abstract. Wastewater continues to be a large anthropogenic input into the Hudson River. The effects of large amounts of wastewater have been well documented, but current effects of more dilute wastewater on river functioning have not been considered. Therefore, this study focused on examining how varying concentrations of wastewater affects the bacteria and phytoplankton communities in the Hudson River. To do this, Hudson River water samples were amended with amounts of wastewater spanning the wastewater inputs observed in the system. To quantify the bacterial response, we measured oxygen consumption, dissolved organic carbon (DOC) concentrations, nutrient concentrations, and the optical properties of colored dissolved organic matter (CDOM) over time. The phytoplankton response was determined through the accumulation of chlorophyll *a*. Overall, the addition of wastewater added nutrients and dissolved organic matter, but it did not drastically affect processes connected to bacteria and phytoplankton communities in the Hudson River. This could be the case for a variety of reasons. One being that wastewater treatment plants are doing a good job of treating wastewater before releasing it into our waterways. Another being that the Hudson River already receives a fair amount of treated wastewater so additional wastewater inputs does not have a large impact on the system. More work needs to be done to fully understand the effects wastewater has on biological and biogeochemical processes in the Hudson River.

INTRODUCTION

In 2002, the US Census Bureau reported that approximately 5 million people live in the Hudson River watershed with the majority of these people living in the New York City metropolitan area. As a result, humans have had a substantial impact on the Hudson River. One of the largest anthropogenic impacts on this system has been the discharge of wastewater into the waterway. Historically, wastewater did not undergo treatment and went directly into the Hudson River through sewer systems (Brosnan et al. 2006, Howarth and Marino et al. 2006). The untreated wastewater caused the water quality of the system to decline rapidly and resulted in many of the following consequences: pathogenic microorganisms closed shellfish beds and beaches, extremely low dissolved oxygen (DO) concentrations as a result of increased breakdown of organic matter, increased suspended solids causing higher levels of turbidity, and the presence of a variety of floatables resulting in the closing of beaches, an increase of wildlife entanglements, and an increase in navigational problems (Suszkowski 1990, Bronson and O'Shea 2000, Brosnan et al. 2006).

The first primary sewage treatment plant was built in the 1930s (Brosnan et al. 2006, Howarth and Marino et al. 2006). However, primary treatment did not make as much of an improvement as expected because primary treatment of sewage only removed 30% of biological oxygen demand (BOD) and total suspended solids (TSS) loads (Brosnan et al. 2006). This continued poor water quality helped to pass the Clean Water

Act in 1972, which required all of the wastewater treatment plants having to be upgraded to secondary treatment systems (Brosnan et al. 2006, Howarth and Marino et al. 2006). Secondary treatment typically removes 85% of BOD and TSS loads and resulted in a great improvement in the Hudson River water quality.

However, even with all of the improvements to the sewage treatment process, the estuary continues to receive a substantial amount of wastewater, which continues to affect the ecology of the Hudson River. Currently, the estuary receives approximately 3.4 million cubic meters of treated sewage on a daily basis (Howarth and Marino et al. 2006). As a result, many studies have been focused on understanding the ecological effects that this treated wastewater can have on the system. Previous studies have found two main consequences for disposing the treated wastewater into the natural waterways.

One consequence is an increase in nutrients concentration. Today, wastewater contributes a large proportion of the nitrogen and phosphorus seen in the Hudson River estuary. It has been estimated that wastewater inputs contributes 24 thousand metric tons of nitrogen and 3.7 thousand metric tons of phosphorus to the system, which is 53% of the nitrogen and 77% of the phosphorus seen in the Hudson River (Howarth and Marino et al. 2006). In the past, the untreated wastewater used to contribute much higher concentrations of nutrients to the Hudson River. The implementation of wastewater treatment systems has decreased the amount of nitrogen and phosphorus to the amounts seen today.

However, there is still an over-enrichment of nutrients, causing the Hudson River to remain classified as moderately eutrophic with some areas possibly classified as hypereutrophic (Howarth and Marino et al. 2006). Hypoxia in the river is no longer a widespread problem with only some areas reporting values below 4 mg/L (Howarth and Marino et al. 2006). As a result, management now needs to focus on other ecological consequences of eutrophication. Studies have shown that eutrophication can lead to a decrease in biodiversity, more frequent as well as longer algal blooms, and shifts in lower level communities creating a bottom-up effect on the system (Howarth and Anderson et al. 2000, Howarth and Marino et al. 2006). Therefore, there have been efforts to add a nutrient removal system to the current wastewater system in order to decrease anthropogenic over-enrichment.

The other consequence is alterations to the quality and amount of dissolved organic matter (DOM) in the river. DOM is a complex mixture of soluble organic compounds, defined operationally as the material passing through a filter with a pore size diameter of 0.22-0.45 μm (Thacker et al. 2005, Fellman et al. 2010). Overall, DOM is one of the most important sources of bioavailable carbon in aquatic systems resulting in it being a huge part of the local and global carbon cycles (Weltz 1992, Battin et al. 2009, Fellman et al. 2010). In addition, DOM supplies nitrogen (Keil and Kirchman 1991, Fellman et al. 2010), influences metal speciation, alters the pH in waterways (Thacker et al. 2005, Hudson et al. 2007), and causes shifts in the abundance of bacterial groups (Kirchman et al. 2004). The chemical structure and composition of DOM determines its photo-reactivity, bioavailability, and ecological role.

Studies have determined that the structure and composition of DOM from wastewater varies depending on the treatment type (Imai et al. 2002) and is different from DOM found in natural waterways (Imai et al. 2002, Hudson et al. 2007, Fellman et al. 2010). Previous studies reported that wastewater DOM is more hydrophilic as well as of smaller molecular weight (Imai et al 2002). Measurements of the fluorescence signature studies have shown that wastewater DOM has a strong tryptophan-like peak that can be used to monitor sewage pollution in aquatic systems (Baker and Inverarity et al. 2003, Baker and Ward et al. 2004, Hudson et al. 2007, Fellman et al. 2010, Tzortziou et al. 2015, Choi et al. 2017). Therefore, such changes

to the structure and composition of DOM induced by wastewater can cause drastic bottom-up effects to the ecosystem due to the many ecological roles performed by DOM.

Because of all of these years of research, one could argue that there is a good understanding about the effects of wastewater. However, all of the studies have failed to address another issue, which is what happens when the wastewater is diluted? So far, everyone has only looked at the drastic effects when there is a large concentration of wastewater, which is only valid for areas close to the wastewater outlet. Therefore, the purpose of this study is to understand how diluted concentrations of wastewater affects the system to better model areas further away from the sewage outlet. In addition, the study will address whether there is a certain dilution where we consider the ecological effects negligible. The knowledge gained will help answer the important question of whether society make more improvements to the wastewater treatment process and plants.

MATERIALS AND METHODS

Wastewater effluent was collected just downstream of the outfall from the Millbrook Village Water Department located in Millbrook, NY (Figure 1A). Hudson River water was collected from the Hudson River near Poughkeepsie, NY (Figure 1B). Both water samples were collected on July 6, 2017 and refrigerated until processing. After that, both the wastewater and river water was filtered using a Whatman glass fiber filter 0.7 μm (GFF) to separate the DOM and most of the bacteria in the sample from the particulate matter. This filtrate was used to assess the impact of wastewater on microbial processes in the system by conducting bacterial bioassay experiments that ran for 7 days. The bacterial bioassays were set up to represent 6 different concentrations of wastewater (0, 3.125, 6.25, 12.5, 25, and 50 percent by volume). A time series incubation experiment was conducted for each of the 6 concentrations for a period of one week with samples analyzed at 0, 2, 5, and 7 days. Each concentration and sampling time had 3 replicates. During the experiment, the samples were stored in the dark at room temperature and were stirred once a day. After collection, the bottles were stored in the refrigerator for less than 5 days until further optical and chemical analysis.

The YSI Handheld meter was used to measure the concentration of dissolved oxygen (DO) on the sample day as well as 5 days later after some of the sample was stored in the dark in sealed BOD bottles. Biological oxygen demand was determined by loss in DO concentration between the two days DO was measured. A Shimadzu TOC analyzer was used to measure dissolved organic carbon (DOC) concentrations. The nitrate concentrations were measured using an Ocean Instruments SUNA. The Analytical Lab at the Cary Institute of Ecosystem Studies used automated wet chemistry to determine total dissolved phosphorus concentrations.

The remaining water in each sample was transported down to the City College of New York, where the optical properties of the DOM were characterized. The absorbance of CDOM was measured by a Cary 300 UV-Vis spectrophotometer using a 1 cm quartz cuvette and milliQ water as the blank. These data allowed us to calculate the magnitude and spectral shape of CDOM absorption using the methods in Tzortziou et al. (2008). Absorption spectral slopes in the 275-295 nm ($S_{275-295}$) and 350-400 nm ($S_{350-450}$) were estimated using nonlinear regression of log-transformed absorption values, and the ratio of these slopes ($S_R=S_{275-295}/S_{350-400}$) was also estimated. Fluorescence emission and excitation matrices (EEMs) were measured for each sample using an AquaLog 800 C fluorometer with a 1 cm quartz cuvette and deionized water as the blank (Tzortziou et al. 2015). Parallel factor analysis (PARAFAC) was used to analyze the fluorescence EEMs and resolve the DOM fluorescence components in our samples.

In addition to the bacterial bioassay experiment, a phytoplankton bioassay experiment was performed in the same manner as the bacterial bioassays described above to understand the wastewater effects on phytoplankton growth. The 6 wastewater concentrations (0, 3.125, 6.25, 12.5, 25, and 50 percent by volume) were collected at 4 sampling times (0, 3, 9, and 14 days) with 2 replicates for each concentration and time. All samples were stored at room temperature and received 12 hours of light at 17 $\mu E\ m^{-2}\ s^{-1}$. On the designated sample day, the specified 12 containers were filtered using a 25 mm Whatman glass fiber filter (GFF) and then the filter was stored in the freezer. Once the experiment was completed, all of the filters were taken out of the freezer and covered with 5 mL of basic methanol at room temperature overnight to extract the chlorophyll. A 1 to 10 dilution of all of the samples was performed and a Shimadzu UV 160 fluorometer was used to measure the UV-Visible absorbance of the diluted samples. The fluorometer readings were used to calculate the concentration of chlorophyll *a* as a way to quantify the growth of phytoplankton.

A two-way ANOVA analysis was performed for the data from each measurement, where a p-value less than 0.05 was considered significant. This statistical test helped to understand the significant differences in all of the measurements with respect to time and wastewater concentration.

RESULTS AND DISCUSSION

Bacterial Oxygen Consumption

Dissolved oxygen (DO) concentration and biological oxygen demand (BOD) were measured to understand how the bacteria's oxygen consumption would respond to various amounts of wastewater. Over the course of the incubation, time had a significant effect on the DO concentration (p-value < 0.05). Across all wastewater concentrations, DO in the bioassays decreased, by approximately 9% over the first two days and continued to decrease at a slower rate towards the end of the experiment following a negative exponential curve and showing an overal change of approximately 11% (Figure 2A). Wastewater concentration had a statistically significant effect on DO concentration (p-value < 0.05), shown as a slight decrease in DO with the addition of wastewater seen on Day 5 of the incubation (Figure 2A). One the other hand, time had a significant effect on BOD (p-value < 0.05), while wastewater concentration did not (p-value=0.14). Overall, BOD decreases slightly over time and does not follow a trend or consistent pattern with the addition of wastewater (Figure 2B). These results suggest that wasterwater did not stimulate bacterial oxygen consumption because the increase in wastewater concentration had no statistically significant effect on BOD and a relatively small effect on the concentration of dissolved oxygen.

Nutrient Concentrations

To quantify how wastewater would affect the amount of nutrients in the system, the nitrate concentration and phosphorus concentration were measured. Time and wastewater concentration had a significant effect on nitrate concentration (p-value < 0.05 for both). Across all wastewater concentrations, the nitrate concentration decreased by approximately 14% over time, where most of the nitrate was used by the bacteria towards the end of the incubation (Figure 3). The addition of wastewater increased the concentration of nitrate, with the 50% wastewater sample showing approximately 50% higher nitrate concentrations compared to the 0% wastewater sample consistently over the course of the incubation (Figure 3). The concentration of phosphorus was also significantly affected by time and wastewater (p-value < 0.05 for both). Overall, the phosphorus concentration significantly decreased by approximately

35% from the beginning to the end of the incubation (Figure 4). Increasing the wastewater concentration resulted in a significant increase in the concentration of phosphorus, as shown at Day 0 of the experiment (Figure 4). These results showed that wastewater increases the concentration of nutrients in the system since both the nitrate concentration and phosphorus concentration increased with the addition of wastewater.

DOC Concentration

The concentration of dissolved organic carbon (DOC) was used as a quick measurement to understand how the amount of DOM changed with the addition of wastewater over time. Both time and wastewater concentration had a significant effect on DOC (p-value < 0.05 for both). The concentration of DOC increased with the concentration of wastewater, with the 50% wastewater and 100% wastewater samples showing approximately 6% and 4% increase in DOC respectively, relative to the 3.2 mg/L DOC concentration in the 0% wastewater sample (Figure 5). DOC decreased over time for all wastewater concentrations with the largest percent losses measured for the 50% wastewater (at around 8% loss in DOC) and 100% wastewater (at approximately 4% loss in DOC) samples. Samples with smaller wastewater concentrations showed a smaller loss in DOC over the course of the incubation, so that overall DOC seemed to converge around 3.2 to 3.3 mg/L on Day 7 (Figure 5). Our results suggest that addition of wastewater added a small amount of DOC to the system; however, the bacteria quickly removed this.

DOM UV-Visible Absorbance Analysis

One way to characterize how the composition of DOM changed with the addition of wastewater is to measure the CDOM UV-visible absorbance spectra. These UV-visible absorbance spectra can then be used to calculate optical parameters that have been previously found to correlate strongly with different chemical properties of DOM. For example, the absorption spectral slope $S_{275\text{-}295}$ has been suggested as a good proxy of degree of CDOM photo-bleaching, while both $S_{275\text{-}295}$ and $S_{350\text{-}400}$ have been previously shown to increase (in absolute value) with an increase in the molecular weight of DOM (Helms et al. 2008, Maizel and Remucal 2017; Tzortziou et al. 2008).

The slopes $S_{275\text{-}295}$ and $S_{350\text{-}400}$ was significantly affected by both time and wastewater concentration (p-value < 0.05 for both). Across all wastewater concentrations, both slopes changed slightly and became steeper over the course of the incubation (Figures 6A and 6B). The addition of more wastewater also resulted in a slightly steeper slopes, which is especially evident on Day 0 of the incubation (Figure 6A and 6B). In agreement with previous studies (Imai et al. 2002), our results indicate that wastewater added DOM of somewhat lower molecular weight, as suggested by the slight increase in the steepness of the slopes with increase in wastewater concentration. Our results also suggest that the molecular weight of DOM slighlty decreased over the course of the incubation, as suggested by the increase in the steepness of the slopes with time.

In addition to the $S_{275\text{-}295}$ and $S_{350\text{-}400}$ slopes, we also looked at their ratio S_R (Figure 6C), as this optical quantity has been suggested to be a good indicator of photochemical versus microbial degradation of CDOM, increasing during photochemical degradation and decreasing (or staying almost the same) during microbial degradation (Helms et al. 2008). Our measurements did not show a statistically significant change in S_R with increase in the concentration of wastewater in our samples. We also did not find a significant change in S_R over the course of the incubation, consistent with the fact that our samples were not exposed to light during our experiments.

In addition to the spectral shape of CDOM absorption, we also looked at the magnitude of CDOM absorption at 300 nm to understand the changes in the amount of absorbing DOM. From the data, both time and wastewater concentration had a significant effect on the absorbance at 300 nm (p-value < 0.05 for both). All samples exhibited the same dynamics in CDOM absorbance over time, with CDOM slightly increasing in the beginning of the incubation and then decreasing towards the end resulting in an overall decrease in absorbance by 6% (Figure 6D). Increasing the wastewater concentration decreased the absorbance at 300 nm from 17 m^{-1} (0% wastewater concentration) to 15 m^{-1} (100% wastewater concentration) (Figure 6D). Therefore, wastewater added non-colored DOM to the system since the addition of wastewater decreased CDOM absorption, while increasing the DOC concentration (Figure 5).

This also indicates that the DOC-specific CDOM absorption (which is the ratio of CDOM absorption versus DOC concentration) decreased with the addition of wastewater (Figure 5 and 6D). The DOC-specific CDOM absorption is also a good indicator of CDOM molecular weight, where it decreases with decreasing molecular weight (Chin et al. 1994). Our findings are consistent with the observed increase in the steepness of the CDOM absorption spectral slopes $S_{275-290}$ and $S_{350-400}$, both indicating the addition of lower molecular weight DOM with the addition of more wastewater in our samples.

DOM Fluorescence Analysis

Another way to characterize how the composition of DOM changed with the addition of wastewater is to measure the CDOM fluorescence. With the fluorescence data, a PARAFAC analysis was performed to identify the major DOM fluorescent components in our samples and understand how these components changed with the addition of wastewater and over time. Based on the data, PARAFAC identified 4 different DOM fluorescent components. Analyzing the intensities and locations of the peaks, component 1 (Figure 7A) was identified as a UVA humic-like DOM that is known to have many aromatic compounds and have a high molecular weight (Fellman et al. 2010). This type of DOM is common in natural waters and is typically less bioavailable (Fellman et al. 2010). Component 2 (Figure 7B) was determined to be humic-like DOM that is common in wastewater and agricultural catchments and is typically less bioavailable (Fellman et al. 2010). Component 3 (Figure 7C) was found to be another UVA humic-like DOM that is largely composed of fulvic-like DOM making it less bioavailable (Fellman et al. 2010, Coble et al. 2017). Lastly, component 4 (Figure 7D) was identified as a tryptophan-like DOM, which is composed of intact proteins and less degraded peptide material making it more bioavailable (Fellman et al. 2010, Coble et al. 2017).

An analysis of how the fluorescence of the components changed with the addition of wastewater over time was performed. Overall, we did not see a large change in fluorescence of component 1 (or UVA humic-like DOM) with the addition of wastewater, or over the course of the incubation. Observed changes were less than ± 2% with the addition of wastewater, shown at Day 0 of the experiment and ranged from 0.36 to 0.42 RU over the course of the incubation (Figure 8A).

However, time and wastewater concentration seemed to have a larger and statistically significant effect (p-value < 0.05 for both) on component 2 (or humic-like DOM). Component 2 increased with increasing wastewater concentration from 0.49 RU at 0% wastewater to 0.55 RU at 100% wastewater (which is approximately a 12% change) (Figure 8B). While, the fluorescence of component 2 increases slightly in the beginning, then decreases until Day 5, and then increases again at the end giving a small change of approximately 3% from the beginning to the end of the incubation (Figure 8B). This overall trend is seen in all of the wastewater concentrations measured.

Component 3 (or the other UVA humic-like DOM) was not significantly affected by time or wastewater concentration (p-value = 0.62 and p-value = 0.19 respectively). No clear trend was observed between the different wastewater concentrations or the different sampling times (Figure 8C).

Time had a statistically significant effect on the fluorescence of component 4 (p-value < 0.05), but wastewater concentration did not (p-value = 0.12). This is clearly shown by the fact that all of the wastewater concentrations follow the same trend over time, where there is a slight increase in the beginning, then a slight decrease, and then another increase at the end to where the fluorescence at Day 7 is about the same fluorescence on Day 0 (Figure 8D).

Overall, the fluorescence of the different components did not change much with the addition of wastewater. The largest increases in fluorescence with increase in wastewater concentration was found for component 2, in agreement with previous studies suggesting that this component is common in wastewater and agricultural catchments (Fellman et al 2010). However, the changes we observed in CDOM fluorescence are relatively small compared to other DOM degradation processes. In agreement with our absorption measurements, our fluorescence results suggest that the wastewater addition did not drastically alter the quality and bioavailability of the colored component of DOM in the system.

With both the slope ratio and fluorescence analysis concluding that wastewater does not change the quality and bioavailability of the DOM, it is reasonable to say that the wastewater did not change the composition of the absorbing and fluorescent DOM pool. So what DOM did the bacteria consume at the beginning of the incubation that resulted in a decrease of DOC? Well, it is hypothesized that the non-colored DOM added to the system by the wastewater is the subset of DOM that was more bioavailable to the bacteria and was thus removed from the system at the beginning of the incubation. Further characterization of the composition and quality of this non-colored DOM component would be needed to understand better the impact of wastewater inputs on the ecology of the Hudson River system.

Chlorophyll a Concentration

All of the measurements previously discussed give information about how the bacteria responded to the addition of wastewater. However, the goal was to look at how increasing wastewater concentrations would affect both the bacteria and phytoplankton communities. As a result, chlorophyll *a* was measured as an indicator of phytoplankton growth. Based on the data, time had a significant effect on chlorophyll *a* (p-value < 0.05), but wastewater concentration did not (p-value = 0.06). All wastewater concentrations followed the same dynamics over time, where the chlorophyll *a* concentration increased exponentially (Figure 9). Therefore, the addition of wastewater did not stimulate the growth of phytoplankton.

DISCUSSION

Through this study, it was determined that the addition of wastewater: (1) does not stimulate bacterial oxygen consumption, (2) adds nutrients and DOC to the system, (3) adds CDOM of lower molecular weight, (4) adds non-colored DOM to the system that has relatively high bioavailability, (5) does not change the quality or bioavailability of colored DOM, (6) results in the bacteria favoring the consumption of non-colored DOM added to the system, and (7) does not stimulate the growth of phytoplankton. Therefore, the addition of wastewater did not drastically affect the bacterial and phytoplankton communities in the Hudson River. Overall, the wastewater had either no effect or a relatively small effect on the system. This can

indicate a couple different conclusions about the Hudson River's wastewater situation. One being that the wastewater treatment plants are doing a great job at treating human waste. Society has improved to the point where wastewater is no longer drastically changing the Hudson water system. Another being that the Hudson River already contains enough wastewater that the addition of more wastewater does not affect the system as much as the first introduction of wastewater into the system.

Based on the conclusions made and previous studies performed, it is more likely that the small response observed is due to ambient levels of wastewater in the Hudson River. Both Gücker et al. (2006) and Huo et al. (2017) compared water samples from upstream and downstream of a wastewater plant using many of the parameters measured in this study. Overall, the studies found that the measurements resulted either in no change or in small significant changes between the two samples (Gücker et al. 2006, Huo et al. 2017). Therefore, the similarities in measurements were attributed to the fact that the water upstream of the wastewater treatment plant already contained wastewater inputs and pollution from other non-point sources (Gücker et al. 2006, Huo et al. 2017). In addition, many studies have indicated that wastewater changes the composition of DOM by adding a tryptophan-like DOM component (Baker and Inverarity et al. 2003, Baker and Ward et al. 2004, Hudson et al. 2007, Fellman et al. 2010, Choi et al. 2017). Therefore, it is very plausible to state that the wastewater sample did add tryptophan-like DOM to all of the samples, so why did we not see a significant increase in the tryptophan-like DOM with the addition of more wastewater? This is probably due to the fact that Hudson River DOM contained similar amounts of tryptophan-like DOM and thus indicates that the Hudson River water sample was already altered by other wastewater systems found upstream. Therefore, it would also be beneficial to look at how the addition of wastewater affects a Hudson River water sample from the pristine headwaters in upstate New York. That way it can be determined if the wastewater does not affect the river because the wastewater has no effect or because any effect is masked by ambient levels of wastewater.

It should also be noted that an additional water collection from the East Branch of Wappinger Creek downstream of the Village of Millbrook's Water Treatment Plant on June 28, 2017 showed a very different optical signature from the DOM in the wastewater sample used in the experiment. It had a much higher absorption signal and a fluorescence signature that exhibited a higher relative contribution from the tryptophan- and tyrosine- protein like fluorescent components. Thus, it would also be useful to assess the influence of this highly variable wastewater source on the Hudson River by performing additional measurements and by increasing the frequency of monitoring the system's water quality and biogeochemical properties.

Lastly, it would be helpful to analyze the effect that wastewater has on other parts of the system not analyzed in this study. For example, wastewater can contain other material like pharmaceuticals, household chemicals, and indicator microbes that are affecting other parts of the Hudson River system. Overall, wastewater will continue to be an input into natural waterways so fully understanding the effects it has on the aquatic environment is essential to improving our wastewater management.

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APPENDIX

FIGURE 1. Maps of the sites where the water samples used in the bioassays were collected, which are represented as yellow stars on both maps. Both maps were obtained using Google Maps, 2017. (A) The wastewater sample was collected from the East Branch of Wappinger Creek (a tributary of the Hudson River) just downstream of the Village of Millbrook's Water Treatment Plant. (B) The Hudson River water sample was collected using a pump located on the Marist College campus in Poughkeepsie, NY.

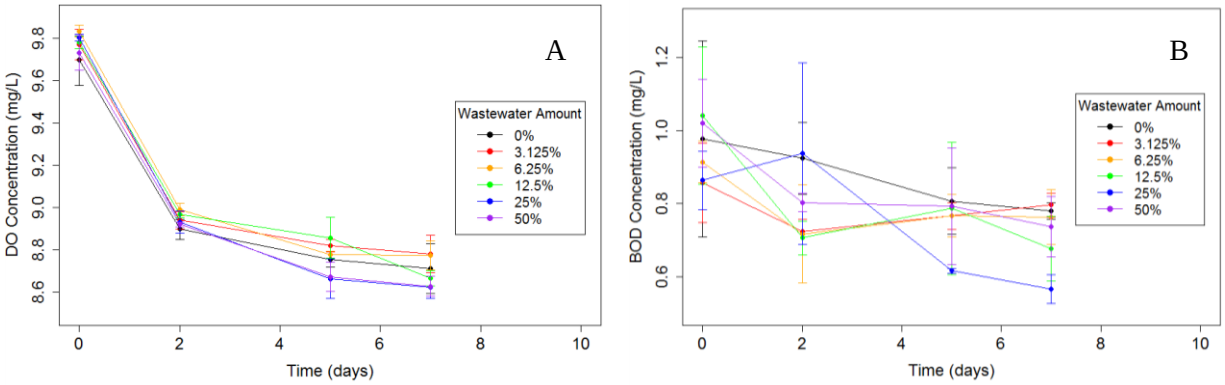

FIGURE 2. Response of bacterial oxygen consumption to varying amounts of wastewater. (A) Dissolved oxygen (DO) concentration over time for all wastewater concentrations measured. (B) Biological oxygen demand (BOD) over time for all wastewater concentrations measured. In both figures, each line represents the average time series for the specific wastewater concentration indicated in the legend. Also, error bars in both figures represent 95% confidence intervals calculated using the three replicate samples.

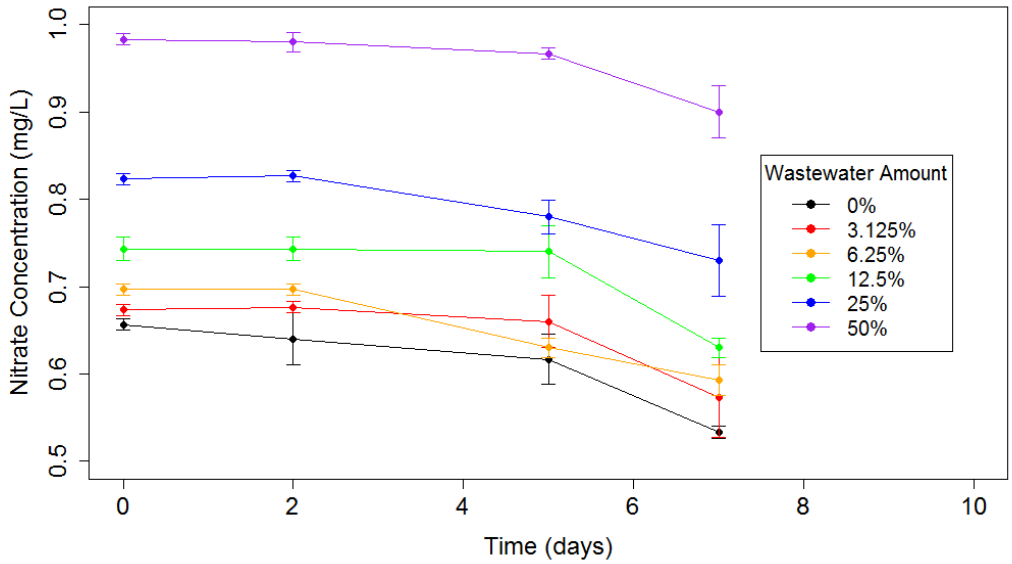

FIGURE 3. Concentration of nitrate over time for all wastewater concentrations measured. Each line represents the time series of nitrate for the designated wastewater concentration shown in the legend. In addition, the error bars in the figure represent 95% confidence intervals.

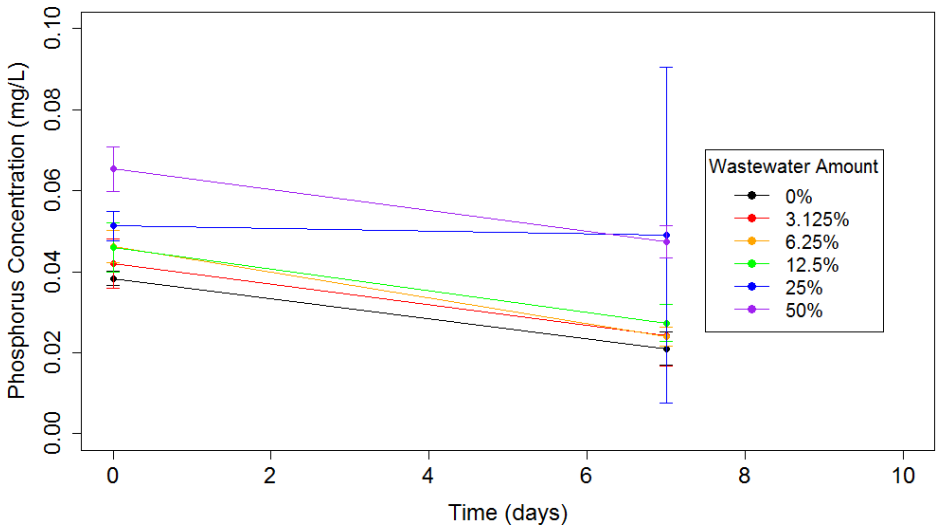

FIGURE 4. Concentration of phosphorus at the beginning and end of the incubation for all wastewater concentrations measured. In the figure, each line represents the linear change from the beginning to the end of the experiment for the designated wastewater concentration indicated in the legend while the error bars represent 95% confidence intervals.

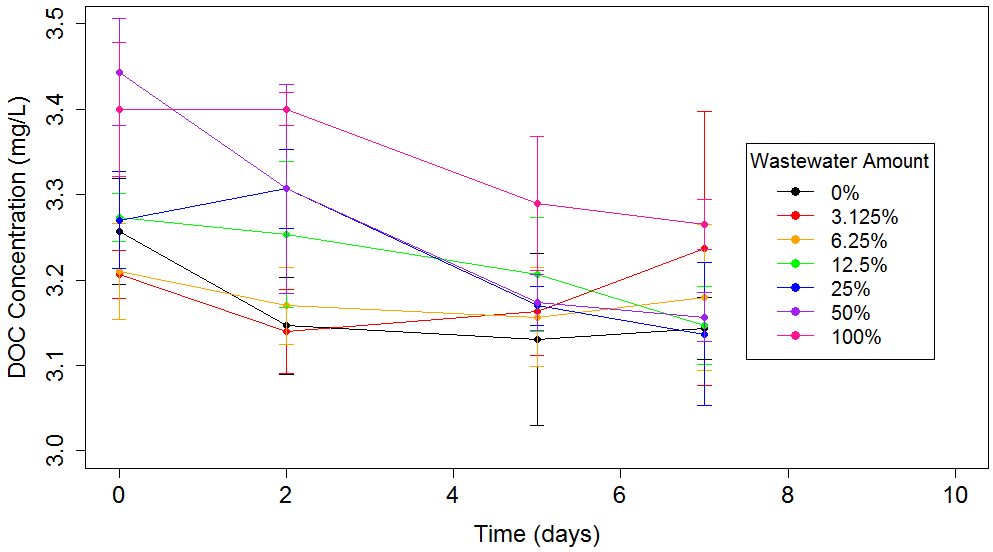

FIGURE 5. Dissolved organic carbon (DOC) concentration over time for all wastewater concentrations measured. The lines represent the time series for one of the wastewater concentrations indicated in the legend. In addition, all of the error bars are 95% confidence intervals calculated using the three replicate samples.

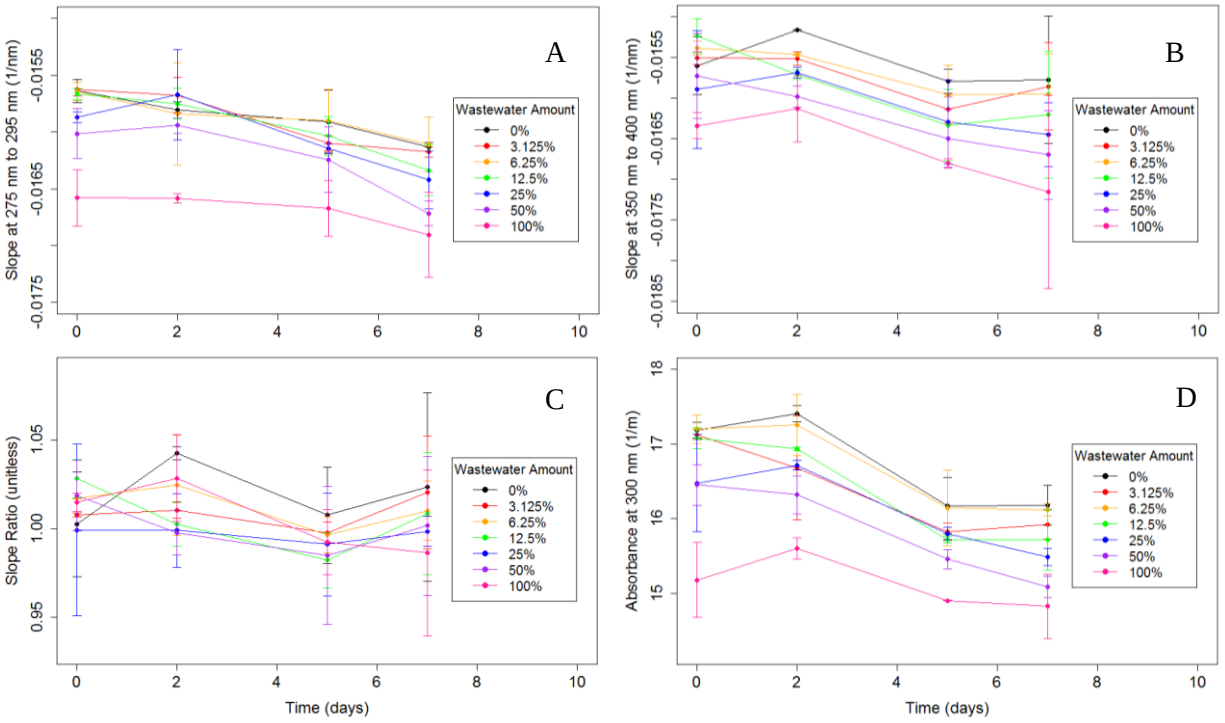

FIGURE 6. Dissolved organic matter (DOM) UV-visible absorbance response to varying amounts of wastewater. (A) Changes in the slope at 275 nm to 295 nm of exponential fit absorbance curve ($S_{275\text{-}295}$) over time for all wastewater concentrations measured. (B) The time series graph of slope at 350 nm to 400 nm of the exponential fit absorbance curve ($S_{350\text{-}400}$) for all of the wastewater concentrations. (C) Slope ratio (ratio of slope at 275-295 nm to slope at 350-400 nm, S_R) over time for all of the concentrations of wastewater. (D) Absorbance at 300 nm of the exponential fit absorbance curve time series graph for all of the concentrations of wastewater measured. In all of the graphs, each line represents the changes across time for a specific wastewater concentration that is color coded accoording to the legend. In addition, all of the error bars in the figure represent 95% confidence intervals.

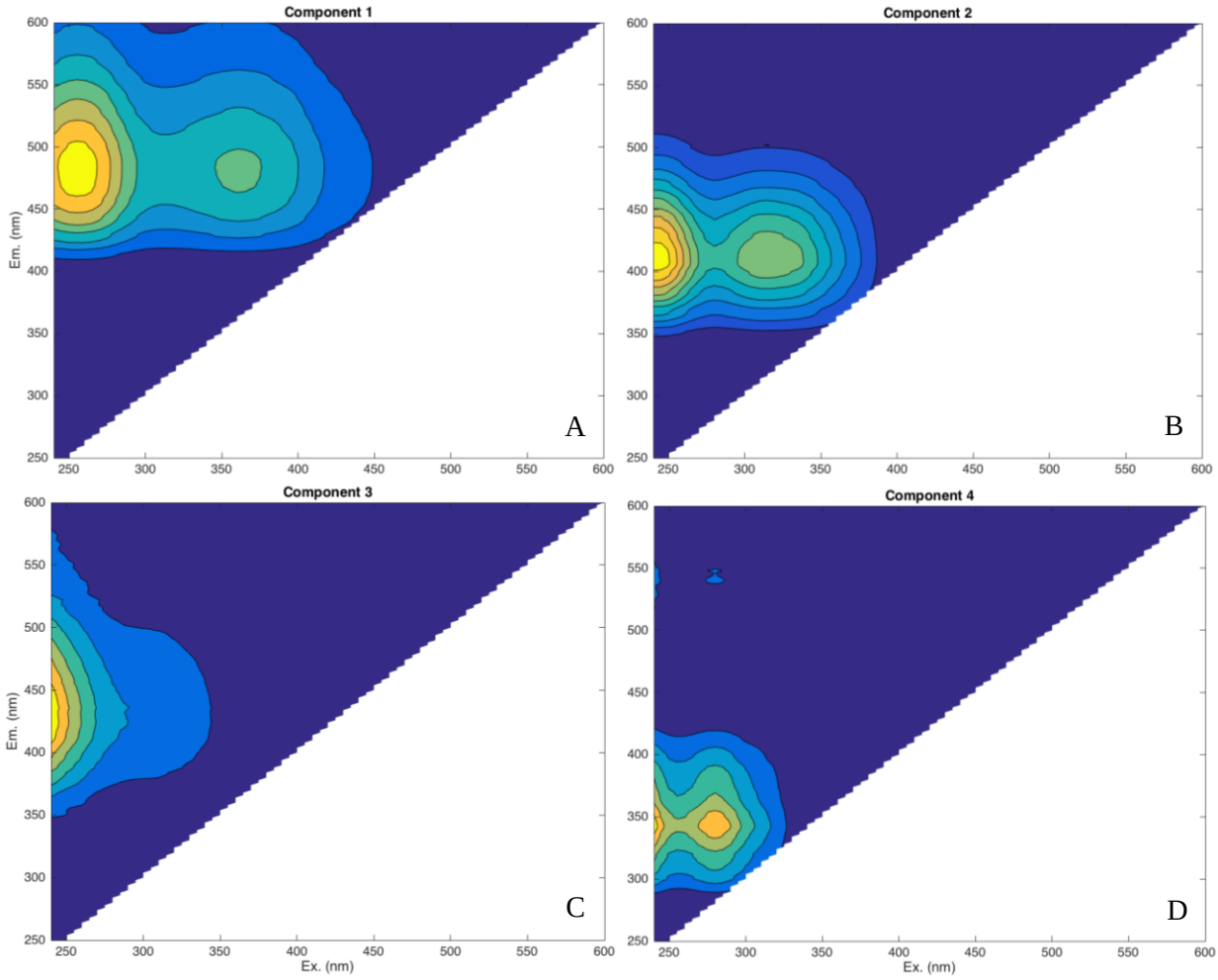

FIGURE 7. Typical excitation-emission matrices for components of DOM identified by PARAFAC analysis. (A) Component 1 excitation-emission matrix (identified as UVA humic-like DOM). (B) Component 2 excitation-emission matrix (identified as Humic-like DOM). (C) Component 3 excitation-emission matrix (identified as UVA-humic like). (D) Component 4 excitation-emission matrix (identified as Tryptophan-like DOM).

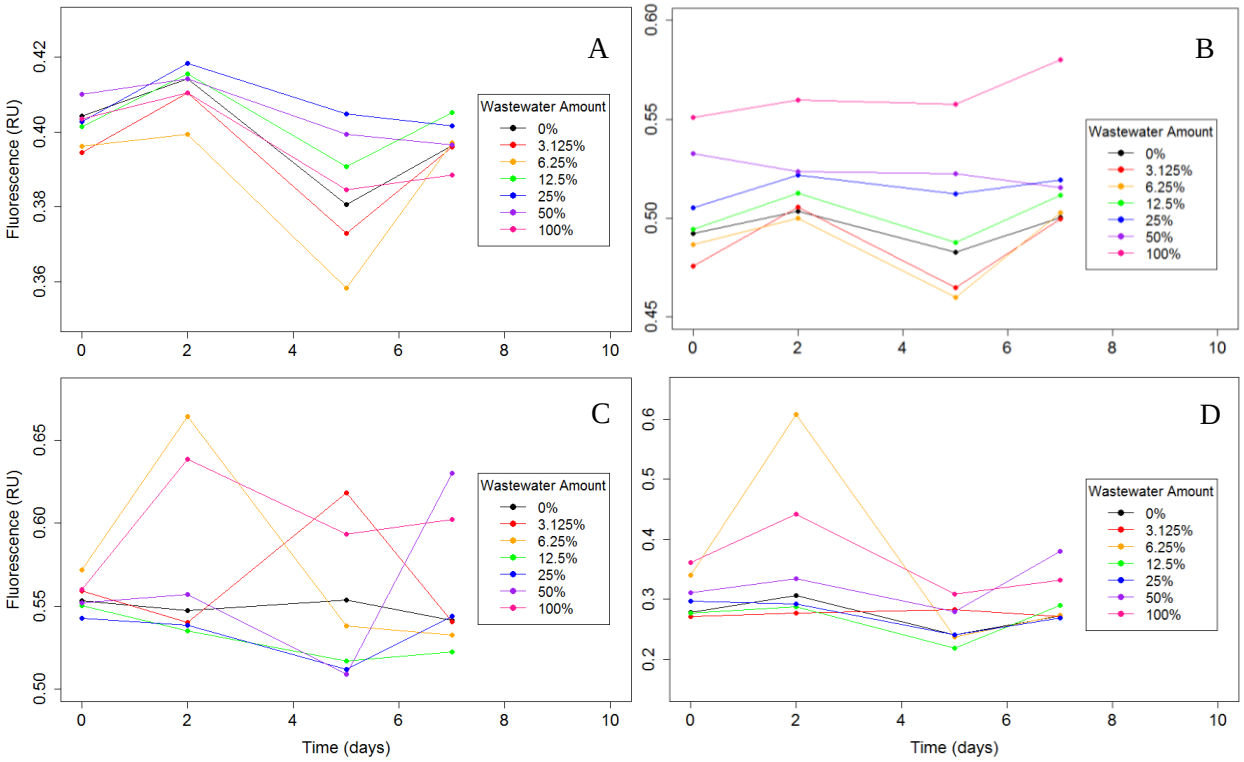

FIGURE 8. Changes in fluorescence over time for each of the PARAFAC components. (A) Component 1 (UVA humic-like DOM) fluorescence time series graph for all of the wastewater concentrations measured. (B) Time series graph of component 2 (Humic-like DOM) fluorescence for all of the wastewater concentrations. (C) Changes in the fluorescence of component 3 (UVA humic-like DOM) over time for each of the concentrations of wastewater measured. (D) Component 4 (Tryptophan-like DOM) fluorescence for all of the concentrations of wastewater over the course of the incubation. In all of the graphs, each line represents the time series for one wastewater concentration as indicated on the legend.

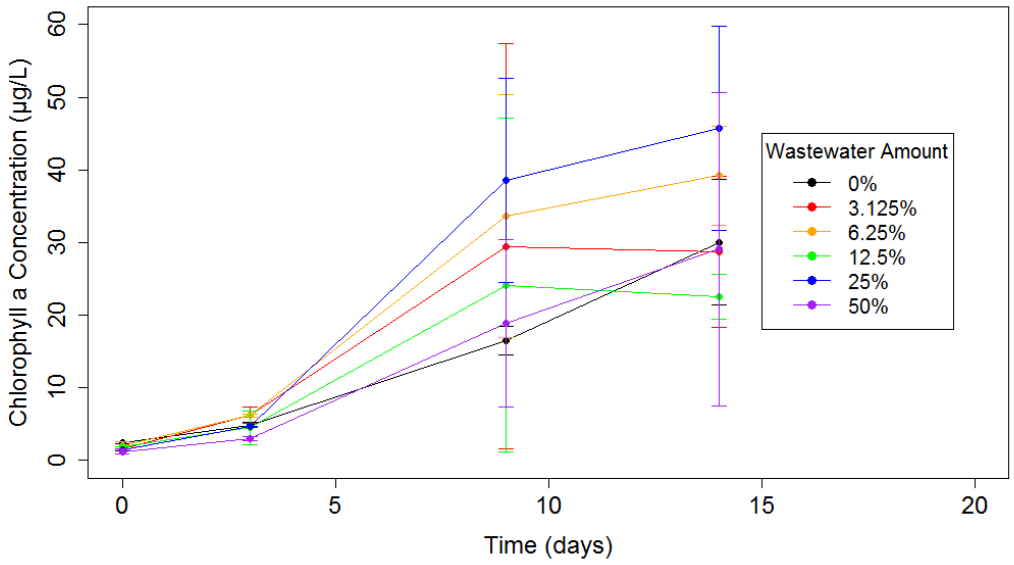

FIGURE 9. Changes in the concentration of chlorophyll *a* over time for all wastewater concentrations measured. In the figure, each line corresponds to a specific wastewater concentration shown in the legend, while the error bars are 95% confidence intervals calculated using the three sample replicates.