Summer 2020

# Composition and Bioavailability of Effluent Dissolved Organic Nitrogen

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# Composition and Bioavailability of Effluent Dissolved Organic Nitrogen

A Thesis

Presented to

The Faculty of the School of Marine Science

The College of William & Mary

In Partial Fulfillment

of the Requirements for the Degree of

Master of Science

by

Quinn N. Roberts

August 2020

# APPROVAL PAGE

This thesis is submitted in partial fulfillment of

the requirements for the degree of

Master of Science

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# ACKNOWLEDGEMENTS

I would like to thank my co-advisor, Deborah Bronk for teaching me everything I know about nitrogen and for introducing me to the world of wastewater treatment. I would also like to thank my co-advisor Iris Anderson for her guidance in Debbie's absence. Additionally, I would like to thank the rest of my committee, Rachel Sipler for helping with the whole process from sampling to writing, and Mike Unger for being an amazing teacher. I would also like to thank Charles Bott, Kevin Parker, and the staff at the Hampton Roads Sanitation District for the design and maintenance of the sequencing batch reactors.

This work would not have been possible without the help of Valerie Johnson, Brianna Stanley, Jenna Spackeen, Lynn Killberg-Thoreson, and Xiaolong Yao. Their assistance in collecting, filtering, and analyzing samples made this project a success.

Lastly I would like to thank my family, specifically my wonderfully understanding and forgiving husband JP, and my friends for their love and support throughout the journey to get my degree.

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# ABSTRACT PAGE

Cultural eutrophication, the overproduction of phytoplankton biomass in response to increased nutrient inputs directly associated with human activities, is a major threat to the health of Chesapeake Bay. Strict regulations, which require a reduction in nutrient loading from all sources, have been a key component to restoration efforts. Water reclamation facilities (WRFs), which discharge effluent containing nitrogen (N) and other nutrients into receiving waters, have implemented upgrades in an effort to comply with regulations. These improvements have decreased the concentration of highly labile dissolved inorganic N (DIN), leaving behind significant concentrations of dissolved organic N (DON) whose bioavailability, and therefore its contribution to eutrophication, remains unclear. The concentration and composition of the N forms in effluent depend upon the characteristics of the influent entering the facility, the processes used to treat the wastewater, and the disinfection procedures employed prior to discharge.

To investigate how these factors affect the composition of the effluent, samples were taken from four bench scale sequencing batch reactors designed to mimic commonly used treatment processes: nitrification only (NO), nitrogen removal (NR), biological nitrogen and phosphorus removal (BNPR), and biological nitrogen and phosphorus removal with additional chemical phosphorus removal (BNCPR). Effluent from each treatment process was also subjected to three disinfection procedures: no disinfection, ultraviolet radiation, and chlorination. To assess bioavailability, effluent from each of the treatment-disinfection combinations was added to natural water samples collected in the York River, VA. Results showed that total dissolved nitrogen (TDN) removal efficiencies of the treatments varied significantly from 12 to 98% and followed the trend NO < NR < BNPR < BNCPR. NO and NR produced effluent composed primarily of nitrate, while BNPR and BNCPR produced effluent composed primarily of DON. Bioassays showed that effluent from NO and NR stimulated phytoplankton growth, and that between 17 and 48% of effluent DON (EDON) was labile. Effluent from BNPR and BNCPR generally stunted or impeded phytoplankton growth and between 4 and 14% of EDON was labile. Overall, disinfection procedures had minor effects on effluent composition and bioavailability, indicating that the largest impacts on cultural eutrophication are made at the initial treatment level.

This study provides results aimed at characterizing the composition of effluent resulting from both treatment and disinfection processes, eliminating influent as a variable. The data show that the discharge of NO and NR effluents would likely lead to eutrophication in both N and P limited receiving waters due to their high inorganic nutrient content and labile EDON. In contrast, the discharge of BNPR and BNCPR effluents, due to their low inorganic nutrient and relatively refractory DON concentrations, is less likely to contribute to eutrophication.

# Composition and Bioavailability of Effluent Dissolved Organic Nitrogen

# CHAPTER 1: PROJECT OVERVIEW

The declining health of Chesapeake Bay, as a result of cultural eutrophication, has been a primary concern for decades (Kemp et al. 2005). Excess nutrients fuel rapid phytoplankton growth leading to harmful algal blooms (Smayda 1990), decreased water clarity (Gallegos and Jordan 2002), depleted oxygen in bottom waters (Hagy et al. 2004), and reduced seagrass concentrations (Kemp 1983). These nutrients are delivered to the Bay by its tributaries, which receive input from multiple sources including urban runoff, farms, and water reclamation facilities (WRF). In 2010, the Total Maximum Daily Load (TMDL) was established to help restore clean water to the Chesapeake Bay's streams, creeks, and rivers. One of the goals of the TMDL is a 25% reduction in total nitrogen (N) loading from all sources by the year 2025 (Chesapeake Bay TMDL 2010). Nitrogen, which is a major constituent of WRF effluent, is often a limiting nutrient in both estuarine and marine systems and has, thus, become the target for removal in Chesapeake Bay. Improvements at WRFs have reduced their overall contribution to the N load from 28%, in 1985, to 16% in 2015 (EPA 2016). Despite these significant reductions, many urbanized coastal systems are continuing to experience eutrophication (Suter et al 2014; Kemp et al 2009). This has led to the question – what if the effluent from upgraded WRFs is still a good source of N for phytoplankton growth because the N has been converted from one usable form to another?

There are numerous processes that WRFs employ to treat wastewater, which vary in efficiency and are chosen based on factors like influent sources, treatment capacity, cost-effectiveness and the availability of space. In the past, the focus of WRFs was to remove solids and reduce a portion of the nutrients. With the implementation of stricter

N loading regulations, WRFs have shifted to design strategies like biological nutrient removal (BNR), to further enhance the removal of inorganic nutrients. The most common BNR systems use coupled nitrification-denitrification, which can remove a large percentage of the dissolved inorganic N (DIN), the highly labile fraction that is known to contribute to eutrophication; however, they are less effective at removing all of the dissolved organic N (DON) (Grady et al. 1999). As a result, a substantial fraction of residual effluent N is organic, up to 85% (Pehlivanoglu and Sedlak 2004), and concentrations typically range from 36 to 129 μmol N $L^{-1}$ (Pagilla et al. 2008; Liu et al. 2011).

Currently, TMDLs use the total amount of N as a regulatory parameter with an assumption that all forms of N have the same effect on phytoplankton growth (Pehlivanoglu-Mantas and Sedlak 2006). However, the case has been made on at least one permit application that effluent DON (EDON) is not bioavailable and so should not count against the amount of N that was discharged from the facility (Mulholland et al. 2007). This case was based on the belief that DON is inert and not a source of N to phytoplankton (Bronk et al. 2007).

Historically, DON has been assumed to be refractory in the environment and, therefore, not biologically available (reviewed in Sipler and Bronk 2015). The root of this assumption is the observation that relatively high DON concentrations persist in aquatic systems where phytoplankton production is known to be limited by the amount of available N. However, development of techniques that can isolate DON from seawater for use in $^{15}N$ tracer studies in the 1990s (Bronk and Glibert 1991) showed that rates of DON production by microbes were quite high in aquatic systems (e.g. Bronk et al. 1994),

and that uptake rates of recently produced DON could be equally high (Bronk and Glibert 1993). These observations indicate that there is a tight coupling between the production and consumption of a pool of bioavailable DON that represents an important source of N to the phytoplankton and bacteria in the environment.

Numerous studies have reported that phytoplankton can use DON as a N source either indirectly or directly (Urgun-Demirtas et al. 2008; Berman 1997; Berman and Chava 1999; Bronk et al. 2007). Bacteria can help breakdown DON via extracellular enzymes for subsequent algal uptake (Pehlivanoglu and Sedlak 2004). Some phytoplankton can release enzymes to break down large DON polymers into smaller molecules for easier utilization or take up DON molecules as a whole through pinocytosis and phagocytosis processes (Bronk et al. 2007). The quality of DON available in the system can have an effect on phytoplankton community composition (Anita et al. 1980), and in some cases may even fuel harmful algal blooms (e.g. Bronk et al. 2007; Sipler et al. 2013).

Like marine DON, EDON is thought to be largely of amide functionality (Dignac et al. 2000), and it is likely that a significant fraction is derived from metabolic products generated by microbes within the WRF itself (e.g. Parkin and McCarty 1987a and b). Several studies have also found distinct similarities between DON produced by microbes in the environment and EDON (Aquino and Stuckey 2003; Nam and Amy 2008; Sattayatewa et al. 2009). In this respect, EDON may be very similar in composition to the small labile pool of DON in the ocean, which is also produced largely by microbial processes. If naturally occurring DON and EDON have similar properties, EDON may

also serve as a source of N to the phytoplankton and microbial communities of effluent receiving waters.

A number of studies have found that a portion of EDON is labile both within plants (Khan et al. 2009; Sattayatewa et al. 2009; Sipler et al. in preparation) and in receiving waters (Pehlivanoglu-Mantas and Sedlak 2008; Yao et al. 2019). Bioassay studies using EDON from different treatment plants demonstrated that up to 96% (based on changes in concentrations) of EDON can be used by microbes in the environment on the time scale of days to a week (Bronk et al. 2010; Filippino et al. 2011). This work further showed that even when the concentration of EDON did not significantly decrease, the EDON pools could still undergo chemical and physical transformations that dramatically altered their composition (Mesfioui et al. 2012; Funkey et al. 2015). These data suggest that, if released into the environment, EDON would contribute to eutrophication.

While studies have shown that EDON is directly and indirectly available to aquatic microbial communities, it is unclear whether the environmentally labile component of the non-degradable (within the WRF) EDON enters the facility in the influent or if the microbes present within the WRF produce it during the treatment process, either by chemical modification of influent DON, or through the production of soluble microbial products (SMP). Sipler et al. (in preparation) found that there was variability between BNR plants in both the concentration and composition of DON at the various stages of treatment and that only 24% of the EDON compounds that entered the plant remained unchanged throughout the treatment process. Using Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS) they identified as many as 166

EDON compounds that were produced during the BNR process. One surprising result was that the majority of these compounds (~62%) defy typical dissolved organic matter (DOM) classifications as proteins, carbohydrates, or lipids; thus, evaluating the production of these compounds by typical analytical means is difficult. The compounds that could be identified were largely composed of lipid-like and protein-like compounds.

To investigate if these 'new' compounds were microbial byproducts that were produced when microbes in the WRF used ammonium ($NH_4^+$) or nitrate ($NO_3^-$), sequencing batch reactors (SBRs) were set up and fed with $^{15}N$-labeled $NH_4^+$ to produce $^{15}N$-labeled EDON (Yao et al. 2019). Once produced, this $^{15}N$-labeled EDON would be a valuable tool to quickly and efficiently quantify EDON uptake at a large number of field sites during different seasons. The SBRs had influent feed supplemented with $^{15}N$-labeled $NH_4^+$ and were run for up to three 15-day solids retention times (SRTs). Unexpectedly, only a small fraction < 5% of the final EDON was produced from the $^{15}N$-labeled $NH_4^+$. To confirm these results, the $^{15}N$-enrichment experiments were run three times, two under partial denitrification conditions (simulating a Modified Ludzack-Ettinger process) and the third using a modified Bardenpho method (simulating a 5-stage Bardenpho process with methanol addition). Surprisingly, the atom % of the resulting EDON was very low indicating that little $^{15}N$ had been incorporated regardless of the treatment used (Yao et al. 2019).

Although results from these experiments fell far short of the hypothesized 25 to 50% $^{15}N$-enrichment levels expected, three important things were learned. First, the majority of EDON produced within the plant is from organic N sources, not from interactions with DIN. Second, field bioassays confirmed that the $^{15}N$-labeled EDON,

produced from $^{15}NH_4^+$ within the SBRs, was bioavailable to natural microbial communities. Third, the type of treatment process significantly impacted the production and composition of EDON. For example, more $^{15}NH_4^+$ was incorporated into EDON treated with a modified Bardenpho process relative to EDON treated with partial denitrification.

Disinfection is the final process applied to effluent before it leaves a WRF and aims to kill or inactivate bacteria and viruses. There are two main methods used in WRFs to disinfect effluent – germicidal UV and chlorination (Koivunen and Heinonen-Tanski 2005). In addition to destroying effluent pathogens, germicidal UV and chlorine disinfection can affect the characteristics of EDON. For example, UV radiation can oxidize organic matter, although the low intensity and short duration used for disinfection may be too weak to cause substantial changes (Sattayatewa et al. 2010). In contrast, during chlorine disinfection, EDON can react to generate nitrogenous disinfection byproducts (N-DBPs) such as toxic nitrosamines, halonitroalkanes, and chloramines (e.g. Plumlee et al. 2008; Shah and Mitch 2012; Zhao et al. 2008). Previous work has also shown that low-molecular weight N is released from effluent when it is exposed to germicidal doses of UV and following chlorination (Funkey et al. 2015). However, Chen et al. (2011) showed that EDON bioavailability decreased significantly after chlorination.

The goal of this study was to determine how various treatments and disinfection procedures used in WRFs affect the final discharged effluent. To that end, four bench-scale sequencing batch reactors (SBRs) were set up and fed with the same influent, which removed many of the variables inherent in other studies (e.g. Sipler et al. in preparation). It is understood that no two facilities operate in exactly the same way; therefore, the

usefulness of the data to facility managers is maximized, regardless of the configuration they use, by isolating the treatments. Effluent was sampled in both the winter and summer because the influent and microbial communities within WRFs can vary greatly between these two seasons.

The first objective was to determine how the composition of the effluent changes with respect to the various treatments and disinfection processes used (Chapter 2). Subsamples were collected from the influent and the effluent produced from each of the four different treatments. These subsamples were analyzed using a suite of wet chemical analyses: total dissolved organic N (TDN), $NH_4^+$, $NO_3^-$, nitrite ($NO_2^-$), DON, urea, dissolved primary amines (DPAs), phosphate ($PO_4^{-3}$), and dissolved organic carbon (DOC). Then, the effluents from each of the SBRs were exposed to two post-treatment disinfection procedures – either a germicidal dose of UV (to mimic what occurs within WRFs) or chlorine disinfection. The UV exposure used to disinfect effluent is defined as germicidal to distinguish it from UV exposure via sunlight in the environment. Relative to sunlight, germicidal UV occurs for a much shorter time period (seconds to minutes) and within much narrower wavelengths (~250 to 270 nm). After exposure, the same suite of wet chemical analyses described above was performed.

The second objective was to determine how the treatment and disinfection processes used affect the reactivity of effluent in receiving waters with respect to biological uptake (Chapter 3). The effluents from each of the four SBRs and each of the treatment-disinfection procedure combinations were added to potential receiving water end-members, one fresh water and one brackish water. Bioassays were carried out for a period of up to 9 days in both the winter and summer to encompass a range of differences

observed for microbial communities in nature. Throughout the bioassay, Chlorophyll *a* concentrations were measured to monitor phytoplankton growth. At the beginning and the end of each bioassay, wet chemical analysis was carried out to determine changes in specific compounds or classes known to be biologically available to receiving microbial communities including TDN, $NH_4^+$, $NO_3^-$, $NO_2^-$, DON, urea, DPAs, $PO_4^{-3}$, silicate (Si), and DOC. This approach allowed the linkage of specific treatments and disinfection processes directly to the environmental response.

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# CHAPTER 2: WET CHEMICAL CHARACTERIZATION


## ABSTRACT

Nitrogen, phosphorus, and carbon from water reclamation effluent contribute to eutrophication in receiving waters. The concentration and composition of these nutrients depend upon the characteristics of the influent into the facility, the processes used to treat the wastewater, and the disinfection procedures employed to make the effluent safe prior to discharge. To investigate how these factors affect the composition of the final effluent, samples were taken from four bench-scale sequencing batch reactors designed to mimic commonly used treatment processes: nitrification only (NO), nitrogen removal (NR), biological nitrogen and phosphorus removal (BNPR), and biological nitrogen and phosphorus removal with additional chemical phosphorus removal (BNCPR). Effluent from each process was then subjected to three disinfection procedures: no disinfection, ultraviolet radiation, and chlorination. Total dissolved nitrogen (TDN) removal efficiencies of the various treatments ranged from 12 to 98%. Phosphate removal efficiencies ranged from 25 to 99%. NO was the least efficient process, producing effluent composed primarily of dissolved inorganic nitrogen (DIN) while BNPR and BNCPR processes were the most efficient, producing effluent composed primarily of dissolved organic nitrogen (DON). The two DON sub-components measured, urea and dissolved primary amines (DPA), accounted for less than 2 to 6% in all effluents. Dissolved organic carbon (DOC) removal efficiencies were similar among treatments ranging from 76 to 81%. While disinfection procedures had minor effects on effluent composition, trends varied among treatments. This study provides results aimed at

characterizing variations in effluent composition in response to both treatment and disinfection processes, eliminating influent as a variable.

## INTRODUCTION

Excess nutrients are one of the primary drivers behind eutrophication in Chesapeake Bay. These nutrients are delivered to waterways by a number of sources including water reclamation facilities (WRFs). According to the U.S. Environmental Protection Agency (EPA), WRFs account for 16% of both nitrogen (N) and phosphorus (P) loads to the Chesapeake Bay watershed (EPA 2016). Strict regulations, including the Chesapeake Bay Total Maximum Daily Load (TMDL), aim to further reduce nutrient pollution and restore the Bay (Chesapeake Bay TMDL 2010). Such reductions are made possible by improvements to WRFs including converting facilities from conventional activated sludge to biological nutrient removal (BNR).

The original goal of the conventional treatment process was to remove solids, organic matter, and sometimes nutrients, resulting in effluents with high concentrations of dissolved inorganic N (DIN) and phosphate ($PO_4^{3-}$). On the other hand, the BNR process is specifically designed to reduce inorganic nutrient concentrations. As a result, it produces effluent that contains a high percentage of dissolved organic N (DON). DIN in the forms of ammonium ($NH_4^+$), nitrate ($NO_3^-$), and nitrite ($NO_2^-$) are known to be highly bioavailable in receiving waters and therefore would contribute to eutrophication. DON, especially low-molecular weight (LMW) species like urea and dissolved primary amines (DPAs), are also highly labile and can be used by bacteria and phytoplankton in natural waters (Bronk 2002; Bronk et al. 2007).

Effluent DON (EDON) is a complex mixture of compounds, which remain largely uncharacterized at the compound level (up to 90%; Pehlivanoglu-Mantas and Sedlak 2006). The largest and most bioavailable fraction of EDON appears to be the hydrophilic fraction, producing 0.06-0.63 g chlorophyll per 1 g hydrophilic N in one study (Liu et al. 2011). Huo et al. (2013) found that > 80% of the EDON from two BNR plants was hydrophilic. Zhang et al. (2016) found that 67 to 89% of EDON was hydrophilic, and after a 15 day incubation 17 to 92% was bioavailable for algal growth. Bronk et al. (2010) and Filippino et al. (2011) found that 2 to 23% and 31 to 96% of EDON respectively, were bioavailable to bacterial and phytoplankton communities within Chesapeake Bay sub-estuaries. Taken together, these results support the idea that a potentially significant portion of the N supplied by EDON can support microbial growth and may contribute to downstream eutrophication and degradation of our waterways.

Disinfection is the final process applied to effluent before it leaves a WRF and aims to kill or inactivate bacteria and viruses. There are two main methods used in WRFs to disinfect effluent – germicidal UV and chlorination (Koivunen and Heinonen-Tanski 2005). In addition to destroying effluent pathogens, germicidal UV and chlorine disinfection can affect the characteristics of EDON. For example, UV radiation can oxidize organic matter, although the narrower wavelengths (~250 to 270 nm) and short duration (seconds to minutes) used for disinfection may be too weak to cause substantial changes (Sattayatewa et al. 2010). In contrast, during chlorine disinfection, organic N can react to generate nitrogenous disinfection byproducts (N-DBPs) such as toxic nitrosamines, halonitroalkanes, and haloamides (i.e. Plumlee et al. 2008; Shah and Mitch 2012; Zhao et al. 2008). Previous work also has shown that LMW N is released from

effluent when it is exposed to germicidal doses of UV and following chlorination (Funkey et al. 2015). However, Chen et al. (2011) showed that EDON bioavailability decreased significantly after chlorination.

While influent is an obvious source of bioavailable EDON, DON concentrations during different BNR processes can be highly variable and can even approach 0 μmol N $L^{-1}$ periodically during the treatment process (Sattayatewa et al. 2009; Czerwionka et al. 2012). This variability suggests that at least a portion of EDON is produced during treatment. Sipler et al. (in preparation) found that over half of all DON compounds that exited as final effluent were produced within WRFs. While what enters a plant cannot be controlled, it may be possible to manage what is produced within a plant through manipulation of biological operating conditions, chemical addition, and disinfection.

Wet chemical characterization is labor intensive, and it is cost prohibitive to sample a large number of plants running different treatments. As an alternative, setting up sequencing batch reactors (SBRs) fed with the same influent removes many of the variables inherent in the survey approach used in previous studies (Sipler et al. in preparation). It is understood that no two facilities operate in exactly the same way; therefore, the usefulness of the data to facility managers is maximized, regardless of the configuration they use, by isolating the treatments. The first objective of this study was to characterize the nutrient pools present in the influent and after processing in SBRs performing one of four different treatments: nitrification only, N removal, biological N and phosphorus removal, and biological N and phosphorus removal with additional chemical phosphorus removal. The second objective was to determine how the concentration and composition of nutrients in effluent change following germicidal UV

and chlorination relative to the effluent that receives no disinfection. Effluent was sampled in both the winter and summer because the influent and microbial communities within WRFs can vary greatly between seasons.

## METHODS

### *Description of Treatments within the Sequencing Batch Reactors*

Four bench-scale sequencing batch reactors (SBRs), maintained at the Hampton Roads Sanitation District (HRSD) in Virginia Beach, VA, were run under set experimental conditions in May, June, and December 2016, and January 2017 to assess how temperature variations and differences in influent composition impact treatment and disinfection processes (Figure 1). Each 22 L SBR was designed to mimic a commonly used wastewater treatment process (Supplemental Figure 1). Nitrification only (NO) involved an aerobic cycle with additions of magnesium hydroxide to maintain neutral pH. Nitrogen removal (NR) involved an anoxic and aerobic cycle in sequence and used sodium bicarbonate additions to maintain neutral pH. Biological nitrogen and phosphorus removal (BNPR) and biological nitrogen and phosphorus removal with chemical P removal (BNCPR) involved anaerobic (oxidized forms of N absent), aerobic, anoxic (oxidized forms of N present), aerobic, and anoxic cycles in sequence, with additions of methanol as the carbon electron donor during the second anoxic cycle. BNCPR also utilized the addition of iron chloride ($FeCl_3$) during the final aeration cycle. During the aeration periods, the dissolved oxygen concentration was maintained at 3 to 4 mg $L^{-1}$, with continuous oxygen uptake rate measurements.

The SBRs were operated on three 8-hour cycles per day with a raw sewage feed from the HRSD Chesapeake-Elizabeth Treatment Plant, a 24 million gallons per day facility that receives mostly domestic household wastewater composed of residential, some commercial, and very little industrial waste (Kevin Parker, HRSD, personal communication), a 24-hour hydraulic residence time, and 15-day solids residence time. SBRs were kept at temperatures similar to those present in local plants at the time of influent collection, which was 25°C during the summer and 15°C during the winter. SBRs were maintained for a minimum of three cycles before any effluent was sampled. Approximately 6 L of effluent were drawn off of each SBR at the end of a cycle, filtered sequentially through a pre-combusted (2 h @ 450°C) Whatman GF/F followed by a 0.2 μm Supor membrane filter. Filtered effluent used in the analyses was collected and pooled over a period of 32 hours, which included 4 cycles.

***Effluent Disinfection Procedures***

Effluent from each of the SBRs was divided into three subsamples for disinfection procedures- no disinfection (ND), germicidal UV radiation (UV), and chlorination (CL). The first portion served as a control and did not undergo a disinfection procedure. On the second portion, germicidal UV disinfection was carried out using a Trojan UV Pro 10 point of use UV system (Monitored Class A NSF certified system designed for multiple sources) at a dose of 40 mJ $cm^{-2}$ (flow rate was 3 L $min^{-1}$.). In the third portion, chlorine disinfection was performed by treating with sodium hypochlorite added as Clorox bleach at a dose of 2.5 to 4 mg $L^{-1}$; the volume added was dependent upon chlorine demand. After 30 minutes, sodium sulfite was added to remove total chlorine residuals; the additions were 0.6 to 2 mg $L^{-1}$ and dependent on the chlorine residuals that needed to be

dechlorinated. To ensure successful dechlorination, chlorine residuals were measured after the hypochlorite addition and after the sodium sulfite addition with a diethyl-p-phenylenediamine (DPD) chlorine test kit. All 0.2 µm filtered effluent was transported on ice back to the Virginia Institute of Marine Science, where they were stored at -20°C until further analysis.

***Analytical Methods***

All analyses were performed on two field replicates unless otherwise stated. Analytical replication is reported for each analysis. Concentrations of ammonium ($NH_4^+$) were measured in triplicate using the phenol hypochlorite method (Koroleff 1983), with a detection limit of 0.05 µmol N $L^{-1}$. Urea concentrations were measured in duplicate using the monoxime method (Price and Harrison 1987), with a detection limit of 0.10 µmol N $L^{-1}$. Nitrate ($NO_3^-$), nitrite ($NO_2^-$), and phosphate ($PO_4^{3-}$) were measured in duplicate on a Lachat QuikChem 8500 autoanalyzer (Parsons et al. 1984), with detection limits of 0.03 µmol N $L^{-1}$, 0.03 µmol N $L^{-1}$, and 0.03 µmol P $L^{-1}$, respectively. Concentrations of TDN and DOC were measured via high temperature combustion using a Shimadzu TOC-V TNM analyzer (Hansell et al. 1993; Sharp et al. 2004; Sharp et al. 1993). University of Miami consensus reference materials, deep-sea and low-carbon water, were used as quality control standards. Limits of detection for TDN and DOC are 2 µmol N $L^{-1}$ and 5 µmol C $L^{-1}$, respectively. Concentrations of DON were then calculated as the difference between TDN and the sum of $NH_4^+$ and $NO_x^-$ ($NO_3^-$ plus $NO_2^-$); the standard deviation for DON concentrations was obtained using a propagation of error analysis (Bronk 2000). There were no field replicates for TDN and DOC samples; however, five analytical replicates were taken. Amino acids, as dissolved

primary amines (DPAs) were measured using the fluorometric method (Parsons et al. 1984), with a detection limit of 0.025 μmol N $L^{-1}$. Protein concentrations were measured using the Bradford assay (Bradford 1976), with a detection limit of 0.5 μg BSA, but are not reported here due to potential interference from colloid formation.

***Statistical Analysis***

Using the statistical software R-studio, analysis of variance (ANOVA) and Tukey's Honest Significant Difference test was used to interpret differences between disinfection treatments for all parameters measured. Correlation analysis was used to assess relationships between TDN removal efficiency and influent DON concentration.

## RESULTS

***Influent***

Influents from all four samplings were averaged to incorporate the possible extremes both within and among seasons. Most influent compositions and concentrations showed little variability between samplings, with the exception of $NO_3^-$, DON, and urea (Table 1). TDN and $NH_4^+$ were consistent, and averaged 2636 ± 95 μmol N $L^{-1}$ and 2412 ± 63 μmol N $L^{-1}$ respectively. Over the four samplings, $NH_4^+$ accounted for 85 to 97% and > 99% of TDN and DIN respectively, making it the dominant N species in both pools (Table 2 and 3). In contrast, $NO_3^-$ and $NO_2^-$ were both low, and averaged 1.01 ± 0.80 μmol N $L^{-1}$ and 0.24 ± 0.03 μmol N $L^{-1}$ respectively (Table 1). Taken together, $NO_3^-$ and $NO_2^-$ accounted for < 1% of both TDN and DIN pools (Table 2 and 3). DON was highly variable, averaged 222 ± 147 μmol N $L^{-1}$, ranged from 72 to 413 μmol N $L^{-1}$, and accounted for 3 to 15% of TDN. Although urea was low, it was also highly variable,

averaged 7.0 ± 5.2 μmol N $L^{-1}$, ranged from 3.1 to 14.7 μmol N $L^{-1}$, and accounted for < 1% and 1 to 20% of TDN and DON pools, respectively. DPA concentrations were below detection due to interference in the method from high $NH_4^+$ concentrations. $PO_4^{3-}$ was consistent, averaged 132.8 ± 17.2 μmol P $L^{-1}$ and ranged from 109.9 to 151.7 μmol P $L^{-1}$. DOC was consistent and averaged 3673 ± 386 μmol C $L^{-1}$. DOC:DON ratios were highly variable and above Redfield suggesting C-enrichment, averaged 28 ± 25 and ranged from 8 to 64.

***Effluent Treatment Composition***

Following the same protocol as the influents, effluents from all four samplings within each treatment were averaged to incorporate the possible extremes both within and among summer and winter.

**Nitrification Only (NO)**

Effluent from the NO process was characterized by very high concentrations of TDN (2308 ± 135 μmol N $L^{-1}$) (Table 4). $NH_4^+$ was generally low (< 3.00 μmol N $L^{-1}$) but had a relatively high average (47.1 ± 90.3 μmol N $L^{-1}$) due to failure of the nitrification cycle in the June 2016 sampling. $NO_3^-$ (1986 ± 107 μmol N $L^{-1}$) was the dominant form of DIN (2036 ± 163 μmol N $L^{-1}$). $NO_2^-$ (2.63 ± 1.68 μmol N $L^{-1}$) was low. DON was highly variable (272 ± 159 μmol N $L^{-1}$) and accounted for only 5 to 18% of TDN. Urea and DPA were 4.0 ± 0.7 μmol N $L^{-1}$ and 0.18 ± 0.14 μmol N $L^{-1}$ respectively, and accounted for < 2% of the DON pool combined. DPA concentrations from the June 2016 sampling were below detection due to interference in the method from high $NH_4^+$ concentrations. $PO_4^{3-}$ was high (101.3 ± 36.9 μmol P $L^{-1}$) and variable.

DOC was 684 ± 112 μmol C $L^{-1}$. DIN:$PO_4^{3-}$ and DOC:DON ratios were 22 ± 8 and 3 ± 2 respectively, suggesting N-enrichment.

The NO process was very inefficient, removing only 12 ± 6 % of TDN (Table 5) and in most cases actually producing DON. $PO_4^{3-}$ removal efficiency was also quite low at 25 ± 23%. DOC removal efficiency was very consistent at 81 ± 2%.

**Nitrogen Removal (NR)**

Effluent from the NR process was also characterized by high concentrations of TDN (843 ± 47 μmol N $L^{-1}$) (Table 4). $NH_4^+$ (2.70 ± 0.70 μmol N $L^{-1}$) was low. $NO_3^-$ (708.7 ± 50.7 μmol N $L^{-1}$) was the dominant form of DIN (713.7 ± 51.1 μmol N $L^{-1}$). $NO_2^-$ (2.41 ± 0.29 μmol N $L^{-1}$) was low. DON was highly variable (129 ± 53 μmol N $L^{-1}$) and accounted for only 10 to 22% of TDN. Urea and DPA were 3.5 ± 0.7 μmol N $L^{-1}$ and 0.33 ± 0.12 μmol N $L^{-1}$ respectively, and accounted for < 3% of the DON pool combined. $PO_4^{3-}$ was high (69.3 ± 9.7 μmol P $L^{-1}$ ). DOC was 676 ± 62 μmol C $L^{-1}$. DIN:$PO_4^{3-}$ ratio was 10 ± 1 suggesting P-enrichment while DOC:DON ratio was 6 ± 2, suggesting slight N-enrichment.

The NR process was pretty efficient, removing 68 ± 1% of TDN (Table 5), but only 25 ± 41% of DON (some samplings showed production). $PO_4^{3-}$ removal efficiency was fair at 48 ± 4%. DOC removal efficiency was very consistent at 81 ± 3%.

**Biological Nitrogen and Phosphorus Removal (BNPR)**

Effluent from the BNPR process was characterized by low concentrations of TDN (66 ± 8 μmol N $L^{-1}$) (Table 4 ). $NH_4^+$ (1.13 ± 0.41 μmol N $L^{-1}$), $NO_3^-$ (0.08 ± 0.07 μmol N $L^{-1}$), and $NO_2^-$ (0.16 ± 0.05 μmol N $L^{-1}$), were all very low, making DIN (1.37 ± 0.45 μmol N $L^{-1}$) a small fraction of TDN. DON was variable (65 ± 8 μmol N $L^{-1}$) and

accounted for 97 to 99% of TDN. Urea and DPA were 3.3 ± 1.0 μmol N $L^{-1}$ and 0.31 ± 0.13 μmol N $L^{-1}$ respectively, and accounted for approximately 6% of the DON pool combined. $PO_4^{3-}$ was very low (1.79 ± 0.87 μmol P $L^{-1}$ ). DOC was 877 ± 135 μmol C $L^{-1}$. DIN:$PO_4^{3-}$ ratio was very low 1 ± 0, suggesting P-enrichment while DOC:DON ratio was 14 ± 1, suggesting C-enrichment.

The BNPR process was extremely efficient, removing 97 ± 0 % of TDN (Table 5), but only 57 ± 29 % of DON. $PO_4^{3-}$ removal was very efficient at 99 ± 1%. DOC removal efficiency was very consistent at 76 ± 5%.

**Biological nitrogen and phosphorus and chemical phosphorus removal (BNCPR)**

Effluent from the BNCPR process was also characterized by low concentrations of TDN (52 ± 10 μmol N $L^{-1}$) (Table 4 ). $NH_4^+$ (1.22 ± 0.36 μmol N $L^{-1}$), $NO_3^-$ (1.29 ± 2.39 μmol N $L^{-1}$), and $NO_2^-$ (0.19 ± 0.21 μmol N $L^{-1}$), were all very low making DIN (2.70 ± 2.70 μmol N $L^{-1}$) a small fraction of TDN. DON was variable (50 ± 8 μmol N $L^{-1}$) and accounted for 89 to 98% of TDN. Urea and DPA were 2.8 ± 1.0 μmol N $L^{-1}$ and 0.13 ± 0.06 μmol N $L^{-1}$ respectively, and accounted for approximately 6% of the DON pool combined. $PO_4^{3-}$ was very low (0.77 ± 0.21 μmol P $L^{-1}$ ). DOC was 742 ± 163 μmol C $L^{-1}$. DIN:$PO_4^{3-}$ ratio was low 3 ± 3, suggesting P-enrichment while DOC:DON ratio was 15 ± 1, suggesting C-enrichment.

The BNCPR process was extremely efficient, removing 98 ± 0% of TDN (Table 5), but only 69 ± 20% of DON. $PO_4^{3-}$ removal was very efficient at 99 ± 0%. DOC removal efficiency was very consistent at 79 ± 7%.

Overall, TDN, DON, urea, and $PO_4^{3-}$ concentrations within treatments followed the trend of: NO > NR > BNPR > BNCPR (Figure 2; Table 4). TDN, DIN, DON, and

$PO_4^{3-}$ removal efficiencies followed the trend BNCPR > BNPR > NR > NO (Table 5). Taken together, this demonstrates that increasing the level of treatment has an inverse relationship on the resulting effluent nutrient concentrations. To the contrary, all treatments had similar removal efficiencies for DOC, between 76 to 81%. The addition of chemical P removal reduced $PO_4^{3-}$ concentrations on average by approximately 50% (the difference between BNPR and BNCPR). DIN:$PO_4^{3-}$ ratios show that only the NO process produced effluent enriched in N above Redfield, while the others were enriched in P (Table 4). DOC:DON ratios increased with increasing level of treatment, shifting from N-enrichment in NO and NR to C-enrichment in BNPR and BNCPR.

***Effluent Disinfection Composition***

Continuing with the same protocol as mentioned previously, effluents from each disinfection procedure within each treatment over all four samplings were averaged to incorporate the possible extremes both within and among seasons.

Disinfection methods did not significantly impact concentrations of $NH_4^+$, urea, DPA, and $PO_4^{3-}$, in effluent processed by NO (Table 6) ($p > 0.05$). Although CL caused increased concentrations of TDN, $NO_3^-$, DON, and DOC, differences were not significant ($p > 0.05$). $NO_2^-$ concentrations in CL were significantly lower than in ND and UV ($p < 0.05$).

For effluent processed by NR, $NO_3^-$, urea, DPA, and $PO_4^{3-}$, concentrations were mot significantly different between disinfection methods (Table 7). Although both UV and CL caused decreased concentrations of TDN and DON, differences were not significant ($p > 0.05$). Both $NH_4^+$ and $NO_2^-$ were lower in CL then either ND or UV but

the difference was only significant in $NO_2^-$ ($p < 0.001$). DOC was highest in CL and lowest in UV as compared to ND, but none of the differences were significant ($p > 0.05$).

For effluent processed by BNPR, CL caused increased concentrations of $NH_4^+$, urea, and DPA, but the difference was not significant ($p > 0.05$) (Table 8). TDN and DON were lower in UV than ND and CL treatments, but the difference was not significant ($p > 0.05$). $NO_3^-$ was highest in UV, primarily because of an abnormally high concentration in the May 2016 sampling (3.77 μmol N $L^{-1}$), but the difference was not significant ($p > 0.05$). $NO_2^-$ concentrations, which were all extremely low (< 0.2 μmol N $L^{-1}$) were significantly lower in CL compared to ND ($p < 0.05$). $PO_4^{3-}$ concentrations were slightly higher in UV and CL compared to ND but the difference was not significant ($p > 0.05$). DOC was highest in CL but was not significantly different from ND and UV ($p > 0.05$).

Disinfection method did not significantly impact concentrations of TDN, $NH_4^+$, and DON processed by BNCPR ($p > 0.05$) (Table 9). Although CL caused increased concentrations of urea and DPA, only the difference in DPA was significant ($p < 0.01$). $NO_3^-$ was highest in UV, primarily because of an abnormally high concentration in the May 2016 sampling (10.9 μmol N $L^{-1}$). $NO_2^-$ concentrations were lowest in CL but differences were not significant ($p > 0.05$). $PO_4^{3-}$ was highest in UV, but differences were not significant ($p > 0.05$). DOC was lowest in UV, but differences were not significant ($p > 0.05$).

Overall, there were no significant differences for concentrations of different N forms in disinfection procedures among treatments (Figure 3), except for $NO_2^-$ and DPA listed above. A trend among all treatments was that CL always had the lowest $NO_2^-$ and

the highest DOC of all disinfection methods. CL also appeared to have elevated urea and DPA in all but the NO treatment. The May 2016 sampling showed production of $NO_3^-$ upon UV disinfection (observed as an increase in concentration from ND) in both the BNPR and BNCPR treatments. $PO_4^{3-}$ appeared to be generally unaffected by disinfection procedures.

## DISCUSSION

This study examined the effect of different treatment processes and disinfection procedures on the resulting effluent originating from a single influent source. Because the concentration and composition of nutrients in effluent are a function of influent properties and are modified by treatment processes and disinfection procedures, it is important to understand how these factors will impact the quality of the effluent discharged to receiving waters that are already threatened or impaired by eutrophication.

Results show that our influent was comparable to average domestic wastewater in that the most abundant forms of N were $NH_4^+$ (92%) and DON (8%) and oxidized forms were minimal (WEF 2009). Relatively high amounts of $NH_4^+$ compared to organic N in the influent reflect a longer residence time in the collection system (Metcalf and Eddy 2003) where ammonification converts organic N into $NH_4^+$ and urea is hydrolyzed (WEF 2005). Sattayatewa et al. (2010) found that organic N is inversely related to the amount of $NH_4^+$ in the influent.

Concentrations of influent TDN and DON from this study were within the same ranges of soluble N (1786 to 3000 μmol N $L^{-1}$) and DON (71 to 714 μmol N $L^{-1}$) from a survey of BNR plants reported by Sattayatewa et al. (2010). Although our influent TDN

concentrations overall were fairly consistent, the wide range of influent DON concentrations (222 ± 147 μmol N $L^{-1}$), demonstrates that influent composition can be quite variable. Other studies showed similar trends in fluctuations of influent N over multiple samplings from the same WRF, particularly with respect to organic N (Raptopoulou et al. 2016; Pagilla et al. 2008; Sattayatewa et al. 2010). These differences may be due to a number of factors including variation in flow and loads as well as residence time in transport.

Variations in influent DON from our study did not appear to have a clear effect on effluent DON. Similarly, Sattayatewa et al. (2010) found that despite high influent organic N fluctuations organic N in the resulting effluent was fairly consistent and generally less than 143 μmol N $L^{-1}$. In addition, Raptopoulou et al. (2016) and de la Torre et al. (2013) found that large variations in N loading did not reduce its removal efficiency, which was found to be consistently high (> 80%). While TDN removal efficiency did not appear to be related to influent DON in our study, there was a clear correlation ($R = 0.93$) between influent DON and DON removal efficiency in the BNPR and BNCPR processes, but the correlation was not significant ($p > 0.05$). One contributing factor to the lack of significance could be the small sample size ($n = 4$). Higher influent DON led to higher DON removal efficiencies. This may have been due to very fresh DON in the influent (e.g. proteins and urea) that is easily degradable in high level BNR processes. Similar trends may not have appeared in NO and NR due to production of DON within the treatment process. While it is helpful to understand relationships between influent and effluent DON concentrations it is important to note

that the quality, not just the quantity of DON, will ultimately determine the impact on receiving waters.

The quality of organic matter can be interpreted by examining the DOC:DON ratio. High ratios likely indicate refractory or older organic matter enriched in C (e.g. humic substances) while low ratios indicate more labile or newer organic matter enriched in N (e.g. proteinaceous substances) (Huang et al. 2005, Liu et al. 2011). If refractory substances are present in the influent, degradation of organic matter during the treatment process may be minimal leading to refractory substances in the effluent and vice versa. Sipler et al. (in preparation) found that of masses detected in two BNR effluents using electrospray ionization mass spectrometry (ESI-MS), 50 to 70% were found to be refractory, which means that they entered with the influent and exited the plant unchanged. In the current study, DON removal efficiencies were lowest among NR, BNPR, and BNCPR in the June 2016 sampling when the influent DOC:DON ratio was the highest (64 ± 29). Influent DOC:DON ratios were highly variable from 8 to 64 and resulted in effluent ratios between 3 to 15 depending on the treatment process. It has been reported that lower C:N ratios of 5 to 11 can adversely affect receiving waters (Pagilla et al. 2008). This range of ratios are close to Redfield and are indicative of N-rich proteinaceous substances. Therefore, organic matter from NO and NR effluents with ratios ≤ 6 are more likely to be bioavailable compared to BNPR and BNCPR effluents with ratios ≥ 14.

Overall, effluent nutrient concentrations, proportions of DIN and DON of TDN, and removal efficiencies for the different treatments were fairly consistent with those found in the literature with a few exceptions. TDN concentrations of NO (2308 ± 135

μmol N $L^{-1}$) and NR (843 ± 47 μmol N $L^{-1}$) were much higher than the expected range of 357 to 643 μmol N $L^{-1}$, while TDN concentrations of BNPR (66 ± 8 μmol N $L^{-1}$ ) and BNCPR (52 ± 10 μmol N $L^{-1}$) were lower than the expected range of 71 to 214 μmol N $L^{-1}$ (Metcalf and Eddy 2003). The proportion of TDN that is DON in BNPR (98 ± 1%) and BNCPR (95 ± 4%), was on the high end or above most reported values (56 to 95% Pagilla et. al 2008) but was similar to a previous study by Bronk et al. 2010 (98%). Differences in our values compared to those found in the literature could be due to differences in analytical approaches, plant operating conditions, treatment setups, and inconsistencies comparing bench-scale SBRs to actual full-scale systems (which include dewatering/recycling streams).

As expected, the NO process produced the highest TDN, DIN, and $PO_4^{3-}$ concentrations. This process is a basic form of wastewater treatment, called conventional, used to remove organic matter under aerobic conditions, accompanied by nitrification, transforming the influent $NH_4^+$ into $NO_3^-$. TDN removal efficiency was poor but not surprising since nutrient removal is not the primary goal of this treatment process. It is also characterized by low P removal because P is in the dissolved form (Lee et al. 2015). Due to the high concentrations of both $NO_3^-$ and $PO_4^{3-}$ in this effluent, its discharge into receiving waters would lead to eutrophication in both N and P-limited regions. Interestingly, the DOC:DON ratio of 3 also implies that the DON may be labile. During treatment, protein-like soluble microbial products can be produced by bacteria, as seen by the increase in DON from influent to effluent. These biopolymers and proteinaceous forms of DON typically exhibit a C:N ratio of 3 to 6 (Stepanauskas et al. 1999; Westerhoff and Mash 2002).

The NR process was meant to simulate the widely used Modified Ludzak Ettinger (MLE) process, at times referred to as partial-denitrification or pre-denitrification. The goal of this process is to reduce N through conversion of $NH_4^+$ to $NO_3^-$ through nitrification and $NO_3^-$ to $N_2$ through denitrification. Advantages of this system include greater N removal than the NO process but without the high cost of enhanced BNR systems like BNPR and BNCPR. However, greater DIN removal leads to a higher proportion of DON in the remaining TDN. Eom et al. (2017) found that pre-denitrification systems produced larger amounts of LMW DON, despite lower TDN concentrations, than conventional systems, and that N-based biomass productivity was greater with pre-denitrification effluent than conventional effluent. Although we found no significant differences in urea and DPA concentrations in comparison with the NO process, there could be other LMW DON species present that were not measured in this study (e.g. peptides). This is further supported by the low DOC:DON ratio (6) suggesting N-enrichment. Liu et al. (2011) found that hydrophilic DON had a C:N ratio around 6, and that this portion of DON was highly labile (40 to 85%) in a bioassay. In addition, the low DIN:$PO_4^{3-}$ ratio (10) indicates P-enrichment. Taken together, this effluent is likely to stimulate primary production in both N and P-limited waters.

The highly efficient BNPR and BNCPR processes were meant to simulate the 5-stage Bardenpho process, which is an example of enhanced biological nutrient removal. The goal of this process is to remove both DIN (through nitrification and denitrification) and P through the growth and wasting of P accumulating organisms that are favored under anaerobic conditions (Wu et al. 2009). The benefits of this process are that very little DIN and $PO_4^{3-}$ remain in the effluent. However, disadvantages include a large

physical footprint, because 5 dedicated tanks are required, and higher maintenance and operational costs. Since the DIN and $PO_4^{3-}$ in our study were < 3.00 µmol N $L^{-1}$ and 2.00 µmol P $L^{-1}$ respectively, this effluent should not contribute significantly to eutrophication. However, concerns over the bioavailability of low TDN and high DON effluents have been the focus of numerous studies where up to 96% of DON was shown to be bioavailable to bacteria and phytoplankton on the time scale of days (Urgun-Demirtas et al. 2008; Sattayatewa et al. 2009; Bronk et al. 2010; Filippino et al. 2011). On the positive side, the DOC:DON ratios of the BNPR and BNCPR effluents in this study were 14 ± 1 and 15 ± 1 respectively, indicating more refractory material. Liu et al. (2011) found that hydrophobic N had a C:N ratio of 16 and that it was not bioavailable in the bioassays they conducted. Taken together, this effluent is not likely to pose a threat to receiving waters.

The BNPR and BNCPR processes differ by the addition of $FeCl_3$ for P precipitation in the latter process. This is sometimes necessary in WRFs because the concentration of easily biodegradable organic matter is insufficient in domestic wastewater for biological N and P removal, resulting in competition for C sources between polyphosphate accumulating organisms and traditional heterotrophic denitrification, which may lead to the failure of biological P removal (Mielcarek et al. 2015; He et al. 2016). This was not the case in our study where $PO_4^{3-}$ removal in BNPR (99 ± 1%) was fairly equal to BNCPR (99 ± 0%). This high efficiency may be attributed to the addition of methanol as the carbon electron donor during the second anoxic cycle. DON also appeared to be affected by the $FeCl_3$ addition. Although the difference was insignificant ($p = 0.08$), the DON concentration of BNCPR (50 ± 8 µmol N $L^{-1}$) was less

than BNPR (65 ± 8 μmol N $L^{-1}$). Sattayatewa et al. (2010) surmised that strategies to enhance P removal may be advantageous in effluent organic nitrogen removal as well.

Overall, there were no significant differences for concentrations of the different N forms in disinfection procedures among treatments (Figure 3), except for $NO_2^-$ and DPA listed above. An apparent trend among all treatments was that CL always had the lowest $NO_2^-$ and the highest DOC of the disinfection treatments. CL also appeared to have elevated urea and DPA in all but the NO treatment. The May 2016 sampling showed production of $NO_3^-$ upon UV disinfection (observed as an increase in concentration from ND) in both the BNPR and BNCPR treatments. Another trend was that TDN and DON concentrations from UV disinfections were always lower than ND (except in BNCPR). $PO_4^{3-}$ appeared to be generally unaffected by disinfection procedures.

While the goal of disinfection procedures is to kill or inactivate bacteria and viruses, it can also alter the composition of effluent nutrients. In this study, alteration was only significant with the decrease in $NO_2^-$ upon chlorination. This could be the result of oxidation of $NO_2^-$ to $NO_3^-$ due to the preferred reactivity between free chlorine and $NO_2^-$ (WEF 2010). Because $NO_2^-$ concentrations were already minimal in all effluents (< 3.00 μmol N $L^{-1}$), chlorination would not greatly affect DIN loading to receiving waters. There were no significant differences in concentrations of DON from UV disinfection or chlorination in our study or in the study conducted by Sattayatewa et al. (2010), who postulated that chlorination was simply transforming DOM but not reducing it and that the UV intensity and exposure used by WRFs was too weak to oxidize organic matter. Similarly, using ESI-MS, Sipler et al. (in preparation) found that 5 to 10% of compounds produced during the treatment process were not present in the effluent while 4% of

effluent masses detected were unique to the final effluent and thus appeared to be produced during chlorination. Sipler et al. (in preparation) explained that the proposed results were likely due to the transformative properties of wastewater disinfection and the change to the functionality that occurs with DOM chlorination (Hua and Reckhow 2007; Krasner et al. 2009; Chang et al. 2011; Shah et al. 2012). Based on our findings from this study, it appears that both UV and CL procedures would not reduce the concentration of nutrients in the effluent and thus would not affect bioavailability. However, a major concern that could not be addressed by the analyses conducted in this study is the production of nitrogenous disinfection byproducts (NDBPs) with chlorination. Recalcitrant organic matter such as humic acids and soluble microbial products both present in effluents are highly reactive with chlorine and therefore serve as precursors for NDBPs (Mitch and Sedlak 2004; Pehlivanoglu and Sedlak 2006; Schreiber and Mitch 2006; Zhang et al. 2012). Some chlorinated organic compounds are carcinogenic to humans or toxic to receiving aquatic systems and thus, are especially concerning (Trehy et al. 1986; Bond et al. 2012).

Although WRFs are not the largest contributors of nutrients to the Bay, as point sources they are easier to regulate than non-point sources like agricultural runoff. WRFs have made significant reductions in N and P loading, but eutrophication remains a major problem in Chesapeake Bay. Strict regulations imposed by the Chesapeake Bay TMDL will continue to require some WRFs to upgrade their systems to enhance nutrient removal. Caution should be taken by WRF managers in deciding what strategies to implement, especially when treatment processes produce low TDN but high DON effluents, which do not necessarily equate to a decrease in bioavailability as seen in the

NR effluent. It is also important to note that bioavailability is not simply determined by the characteristics of the effluent itself but it also depends on the microbes in receiving waters, ambient nutrient concentrations, and abiotic factors like hydrology, sunlight, and salinity.

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Table 1. Influent nutrient concentrations and ratios. Concentrations of total dissolved nitrogen (TDN), ammonium ($NH_4^+$), nitrate ($NO_3^-$), nitrite ($NO_2^-$), dissolved organic nitrogen (DON), urea, phosphate ($PO_4^{3-}$), dissolved organic carbon (DOC), DIN: $PO_4^{3-}$ ratio, and DOC:DON ratio from the four samplings. Values are the average plus or minus the standard deviation.

| Sampling | TDN ($\mu$mol N $L^{-1}$) | $NH_4^+$ ($\mu$mol N $L^{-1}$) | $NO_3^-$ ($\mu$mol N $L^{-1}$) | $NO_2^-$ ($\mu$mol N $L^{-1}$) | DON ($\mu$mol N $L^{-1}$) | Urea ($\mu$mol N $L^{-1}$) | $PO_4^{3-}$ ($\mu$mol P $L^{-1}$) | DOC ($\mu$mol C $L^{-1}$) | DIN:$PO_4^{3-}$ | DOC:DON |
|---|---|---|---|---|---|---|---|---|---|---|
| May 2016 | 2683 ± 27 | 2429 ± 18 | 0.03 ± 0.01 | 0.26 ± 0.00 | 253 ± 18 | 3.1 ± 0.1 | 151.7 ± 3.4 | 3678 ± 55 | 16 ± 1 | 15 ± 1 |
| June 2016 | 2515 ± 8 | 2441 ± 33 | 1.93 ± 0.07 | 0.19 ± 0.00 | 72 ± 33 | 14.7 ± 0.3 | 133.9 ± 1.3 | 4162 ± 80 | 18 ± 0 | 64 ± 29 |
| December 2016 | 2610 ± 8 | 2459 ± 26 | 1.29 ± 0.01 | 0.25 ± 0.01 | 150 ± 26 | 5.7 ± 0.1 | 109.9 ± 0.5 | 3632 ± 71 | 22 ± 0 | 25 ± 4 |
| January 2017 | 2734 ± 0 | 2319 ± 14 | 0.78 ± 0.01 | 0.24 ± 0.00 | 413 ± 14 | 4.5 ± 0.1 | 135.8 ± 0.8 | 3220 ± 46 | 17 ± 0 | 8 ± 0 |
| **AVERAGE** | **2636 ± 95** | **2412 ± 63** | **1.01 ± 0.80** | **0.24 ± 0.03** | **222 ± 147** | **7.0 ± 5.2** | **132.8 ± 17.2** | **3673 ± 386** | **18 ± 3** | **28 ± 25** |

Table 2. Percentage of total dissolved nitrogen (TDN) of influent nitrogen forms. Influent ammonium ($NH_4^+$), nitrate ($NO_3^-$), nitrite ($NO_2^-$), dissolved organic nitrogen (DON), and urea as a percentage of TDN in the four samplings.

| Sampling | $NH_4^+$ (%) | $NO_3^-$ (%) | $NO_2^-$ (%) | DON (%) | Urea (%) |
|---|---|---|---|---|---|
| May 2016 | 91 | < 1 | < 1 | 9 | < 1 |
| June 2016 | 97 | < 1 | < 1 | 3 | < 1 |
| December 2016 | 94 | < 1 | < 1 | 6 | < 1 |
| January 2017 | 85 | < 1 | < 1 | 15 | < 1 |
| **AVERAGE** | **92** | **< 1** | **< 1** | **8** | **< 1** |

Table 3. Percentage of dissolved inorganic nitrogen (DIN) or dissolved organic nitrogen (DON) of influent nitrogen forms. Influent ammonium ($NH_4^+$), nitrate ($NO_3^-$), and nitrite ($NO_2^-$), as a percentage of DIN, and urea as a percentage of DON in the four samplings.

| Sampling | $NH_4^+$ (%) | $NO_3^-$ (%) | $NO_2^-$ (%) | Urea (%) |
|---|---|---|---|---|
| May 2016 | > 99 | < 1 | < 1 | 1 |
| June 2016 | > 99 | < 1 | < 1 | 20 |
| December 2016 | > 99 | < 1 | < 1 | 4 |
| January 2017 | > 99 | < 1 | < 1 | 1 |
| **AVERAGE** | **> 99** | **< 1** | **< 1** | **7** |

Table 4. Effluent nutrient concentrations and ratios from all treatment processes. Concentrations of total dissolved nitrogen (TDN), ammonium ($NH_4^+$), nitrate ($NO_3^-$), nitrite ($NO_2^-$), dissolved organic nitrogen (DON), urea, dissolved primary amines (DPA), phosphate ($PO_4^{3-}$), dissolved organic carbon (DOC), DIN: $PO_4^{3-}$ ratio, and DOC:DON ratio in each treatment. Values are the average over four samplings plus or minus the standard deviation. BD indicates below detection.

| **NO** | TDN ($\mu$mol N $L^{-1}$) | $NH_4^+$ ($\mu$mol N $L^{-1}$) | $NO_3^-$ ($\mu$mol N $L^{-1}$) | $NO_2^-$ ($\mu$mol N $L^{-1}$) | DON ($\mu$mol N $L^{-1}$) | Urea ($\mu$mol N $L^{-1}$) | DPA ($\mu$mol N $L^{-1}$) | $PO_4^{3-}$ ($\mu$mol P $L^{-1}$) | DOC ($\mu$mol C $L^{-1}$) | DIN:$PO_4^{3-}$ | DOC:DON |
|---|---|---|---|---|---|---|---|---|---|---|---|
| May 2016 | 2174 ± 5 | 2.69 ± 0.01 | 2049 ± 17 | 3.19 ± 0.03 | 119 ± 0 | 2.9 ± 0.0 | 0.33 ± 0.01 | 120.4 ± 0.5 | 556 ± 8 | 17 ± 0 | 5 ± 0 |
| June 2016 | 2382 ± 27 | 182.5 ± 2.9 | 2048 ± 19 | 0.12 ± 0.00 | 152 ± 22 | 4.6 ± 0.0 | BD | 140.1 ± 0.4 | 816 ± 11 | 16 ± 0 | 5 ± 1 |
| December 2016 | 2215 ± 5 | 1.58 ± 0.44 | 1826 ± 10 | 3.53 ± 0.07 | 383 ± 11 | 4.3 ± 0.1 | 0.17 ± 0.10 | 56.1 ± 3.1 | 727 ± 13 | 33 ± 2 | 2 ± 0 |
| January 2017 | 2459 ± 16 | 1.47 ± 0.01 | 2021 ± 12 | 3.67 ± 0.12 | 433 ± 12 | 4.1 ± 0.0 | 0.23 ± 0.04 | 88.4 ± 3.5 | 636 ± 14 | 23 ± 1 | 1 ± 0 |
| **AVERAGE** | **2308 ± 135** | **47.1 ± 90.3** | **1986 ± 107** | **2.63 ± 1.68** | **272 ± 159** | **4.0 ± 0.7** | **0.18 ± 0.14** | **101.3 ± 36.9** | **684 ± 112** | **22 ± 8** | **3 ± 2** |

| **NR** | TDN ($\mu$mol N $L^{-1}$) | $NH_4^+$ ($\mu$mol N $L^{-1}$) | $NO_3^-$ ($\mu$mol N $L^{-1}$) | $NO_2^-$ ($\mu$mol N $L^{-1}$) | DON ($\mu$mol N $L^{-1}$) | Urea ($\mu$mol N $L^{-1}$) | DPA ($\mu$mol N $L^{-1}$) | $PO_4^{3-}$ ($\mu$mol P $L^{-1}$) | DOC ($\mu$mol C $L^{-1}$) | DIN:$PO_4^{3-}$ | DOC:DON |
|---|---|---|---|---|---|---|---|---|---|---|---|
| May 2016 | 872 ± 7 | 3.22 ± 0.01 | 781.7 ± 2.4 | 2.39 ± 0.04 | 85 ± 2 | 3.1 ± 0.1 | 0.17 ± 0.02 | 78.2 ± 1.7 | 621 ± 17 | 10 ± 0 | 7 ± 0 |
| June 2016 | 787 ± 15 | 2.58 ± 0.03 | 697.3 ± 2.6 | 2.16 ± 0.00 | 85 ± 3 | 2.8 ± 0.0 | 0.39 ± 0.05 | 62.5 ± 0.2 | 626 ± 14 | 11 ± 0 | 7 ± 0 |
| December 2016 | 822 ± 8 | 1.77 ± 0.09 | 664.4 ± 3.7 | 2.82 ± 0.00 | 153 ± 4 | 4.2 ± 0.2 | 0.45 ± 0.16 | 59.4 ± 2.5 | 744 ± 8 | 11 ± 0 | 5 ± 0 |
| January 2017 | 889 ± 5 | 3.25 ± 0.00 | 691.3 ± 2.4 | 2.25 ± 0.21 | 192 ± 3 | 3.9 ± 0.0 | 0.32 ± 0.06 | 76.9 ± 5.7 | 712 ± 8 | 9 ± 1 | 4 ± 0 |
| **AVERAGE** | **843 ± 47** | **2.70 ± 0.70** | **708.7 ± 50.7** | **2.41 ± 0.29** | **129 ± 53** | **3.5 ± 0.7** | **0.33 ± 0.12** | **69.3 ± 9.7** | **676 ± 62** | **10 ± 1** | **6 ± 2** |

| **BNPR** | TDN ($\mu$mol N $L^{-1}$) | $NH_4^+$ ($\mu$mol N $L^{-1}$) | $NO_3^-$ ($\mu$mol N $L^{-1}$) | $NO_2^-$ ($\mu$mol N $L^{-1}$) | DON ($\mu$mol N $L^{-1}$) | Urea ($\mu$mol N $L^{-1}$) | DPA ($\mu$mol N $L^{-1}$) | $PO_4^{3-}$ ($\mu$mol P $L^{-1}$) | DOC ($\mu$mol C $L^{-1}$) | DIN:$PO_4^{3-}$ | DOC:DON |
|---|---|---|---|---|---|---|---|---|---|---|---|
| May 2016 | 63 ± 3 | 1.72 ± 0.04 | 0.08 ± 0.02 | 0.16 ± 0.02 | 61 ± 0 | 2.9 ± 0.0 | 0.42 ± 0.00 | 3.00 ± 0.00 | 752 ± 8 | 1 ± 0 | 12 ± 0 |
| June 2016 | 59 ± 6 | 0.76 ± 0.06 | BD | 0.09 ± 0.01 | 58 ± 0 | 2.2 ± 0.0 | 0.14 ± 0.02 | 0.93 ± 0.07 | 789 ± 32 | 1 ± 0 | 14 ± 0 |
| December 2016 | 78 ± 2 | 1.08 ± 0.14 | 0.06 ± 0.00 | 0.16 ± 0.01 | 77 ± 0 | 4.3 ± 0.0 | 0.41 ± 0.00 | 1.58 ± 0.04 | 1050 ± 9 | 1 ± 0 | 14 ± 0 |
| January 2017 | 64 ± 2 | 0.97 ± 0.00 | 0.16 ± 0.00 | 0.22 ± 0.00 | 63 ± 0 | 3.9 ± 0.0 | 0.25 ± 0.00 | 1.65 ± 0.04 | 919 ± 15 | 1 ± 0 | 15 ± 0 |
| **AVERAGE** | **66 ± 8** | **1.13 ± 0.41** | **0.08 ± 0.07** | **0.16 ± 0.05** | **65 ± 8** | **3.3 ± 1.0** | **0.31 ± 0.13** | **1.79 ± 0.87** | **877 ± 135** | **1 ± 0** | **14 ± 1** |

| **BNCPR** | TDN ($\mu$mol N $L^{-1}$) | $NH_4^+$ ($\mu$mol N $L^{-1}$) | $NO_3^-$ ($\mu$mol N $L^{-1}$) | $NO_2^-$ ($\mu$mol N $L^{-1}$) | DON ($\mu$mol N $L^{-1}$) | Urea ($\mu$mol N $L^{-1}$) | DPA ($\mu$mol N $L^{-1}$) | $PO_4^{3-}$ ($\mu$mol P $L^{-1}$) | DOC ($\mu$mol C $L^{-1}$) | DIN:$PO_4^{3-}$ | DOC:DON |
|---|---|---|---|---|---|---|---|---|---|---|---|
| May 2016 | 47 ± 3 | 1.66 ± 0.03 | 0.11 ± 0.03 | 0.10 ± 0.00 | 45 ± 0 | 2.1 ± 0.1 | 0.12 ± 0.01 | 0.84 ± 0.03 | 663 ± 12 | 2 ± 0 | 15 ± 0 |
| June 2016 | 41 ± 3 | 0.82 ± 0.04 | 0.16 ± 0.00 | 0.06 ± 0.00 | 40 ± 0 | 1.7 ± 0.0 | 0.11 ± 0.04 | 0.45 ± 0.02 | 561 ± 5 | 2 ± 0 | 14 ± 0 |
| December 2016 | 59 ± 2 | 1.07 ± 0.06 | BD | 0.10 ± 0.00 | 58 ± 0 | 3.6 ± 0.1 | 0.22 ± 0.02 | 0.88 ± 0.04 | 815 ± 12 | 1 ± 0 | 14 ± 0 |
| January 2017 | 62 ± 1 | 1.34 ± 0.05 | 4.87 ± 0.01 | 0.51 ± 0.01 | 55 ± 0 | 3.7 ± 0.0 | 0.08 ± 0.04 | 0.88 ± 0.10 | 929 ± 13 | 8 ± 0 | 17 ± 0 |
| **AVERAGE** | **52 ± 10** | **1.22 ± 0.36** | **1.29 ± 2.39** | **0.19 ± 0.21** | **50 ± 8** | **2.8 ± 1.0** | **0.13 ± 0.06** | **0.77 ± 0.21** | **742 ± 163** | **3 ± 3** | **15 ± 1** |

Table 5. Treatment removal efficiencies. Total dissolved nitrogen (TDN), dissolved organic nitrogen (DON), dissolved inorganic nitrogen (DIN), phosphate ($PO_4^{3-}$), and dissolved organic carbon (DOC) removal efficiencies in the four treatments. Negative numbers indicate production. Values are the average of four samplings plus or minus the standard deviation.

| Treatment | TDN (%) | DON (%) | DIN (%) | $PO_4^{3-}$ (%) | DOC (%) |
|---|---|---|---|---|---|
| NO | 12 ± 6 | -55 ± 96 | 16 ± 7 | 25 ± 23 | 81 ± 2 |
| NR | 68 ± 1 | 25 ± 41 | 70 ± 2 | 48 ± 4 | 81 ± 3 |
| BNPR | 97 ± 0 | 57 ± 29 | > 99 ± 0 | 99 ± 1 | 76 ± 5 |
| BNCPR | 98 ± 0 | 69 ± 20 | > 99 ± 0 | 99 ± 0 | 79 ± 7 |

Table 6. Effluent nutrient concentrations and ratios of nitrification only (NO) disinfection procedures. Concentration of total dissolved nitrogen (TDN), ammonium ($NH_4^+$), nitrate ($NO_3^-$), nitrite ($NO_2^-$), dissolved organic nitrogen (DON), urea, dissolved primary amines (DPA), phosphate ($PO_4^{3-}$), dissolved organic carbon (DOC), DIN: $PO_4^{3-}$ ratio, and DOC:DON ratio in the NO process with three disinfection procedures- no disinfection (ND), ultraviolet radiation (UV), and chlorination (CL). Values are the average over four samplings plus or minus the standard deviation. BD indicates below detection.

| **ND** | TDN ($\mu$mol N $L^{-1}$) | $NH_4^+$ ($\mu$mol N $L^{-1}$) | $NO_3^-$ ($\mu$mol N $L^{-1}$) | $NO_2^-$ ($\mu$mol N $L^{-1}$) | DON ($\mu$mol N $L^{-1}$) | Urea ($\mu$mol N $L^{-1}$) | DPA ($\mu$mol N $L^{-1}$) | $PO_4^{3-}$ ($\mu$mol P $L^{-1}$) | DOC ($\mu$mol C $L^{-1}$) | DIN:$PO_4^{3-}$ | DOC:DON |
|---|---|---|---|---|---|---|---|---|---|---|---|
| May 2016 | 2174 ± 5 | 2.69 ± 0.01 | 2049 ± 17 | 3.19 ± 0.03 | 119 ± 0 | 2.9 ± 0.0 | 0.33 ± 0.01 | 120.4 ± 0.5 | 556 ± 8 | 17 ± 0 | 5 ± 0 |
| June 2016 | 2382 ± 27 | 182.5 ± 2.9 | 2048 ± 19 | 0.12 ± 0.00 | 152 ± 22 | 4.6 ± 0.0 | BD | 140.1 ± 0.4 | 816 ± 11 | 16 ± 0 | 5 ± 1 |
| December 2016 | 2215 ± 5 | 1.58 ± 0.44 | 1826 ± 10 | 3.53 ± 0.07 | 383 ± 11 | 4.3 ± 0.1 | 0.17 ± 0.10 | 56.1 ± 3.1 | 727 ± 13 | 33 ± 2 | 2 ± 0 |
| January 2017 | 2459 ± 16 | 1.47 ± 0.01 | 2021 ± 12 | 3.67 ± 0.12 | 433 ± 12 | 4.1 ± 0.0 | 0.23 ± 0.04 | 88.4 ± 3.5 | 636 ± 14 | 23 ± 1 | 1 ± 0 |
| **AVERAGE** | **2308 ± 135** | **47.1 ± 90.3** | **1986 ± 107** | **2.63 ± 1.68** | **272 ± 159** | **4.0 ± 0.7** | **0.18 ± 0.14** | **101.3 ± 36.9** | **684 ± 112** | **22 ± 8** | **3 ± 2** |

| **UV** | TDN ($\mu$mol N $L^{-1}$) | $NH_4^+$ ($\mu$mol N $L^{-1}$) | $NO_3^-$ ($\mu$mol N $L^{-1}$) | $NO_2^-$ ($\mu$mol N $L^{-1}$) | DON ($\mu$mol N $L^{-1}$) | Urea ($\mu$mol N $L^{-1}$) | DPA ($\mu$mol N $L^{-1}$) | $PO_4^{3-}$ ($\mu$mol P $L^{-1}$) | DOC ($\mu$mol C $L^{-1}$) | DIN:$PO_4^{3-}$ | DOC:DON |
|---|---|---|---|---|---|---|---|---|---|---|---|
| May 2016 | 2128 ± 41 | 2.61 ± 0.04 | 2071 ± 3 | 3.15 ± 0.13 | 51 ± 3 | 2.8 ± 0.1 | 0.18 ± 0.00 | 112.0 ± 6.4 | 564 ± 12 | 19 ± 1 | 11 ± 1 |
| June 2016 | 2406 ± 42 | 180.7 ± 3.2 | 2023 ± 29 | 0.11 ± 0.01 | 202 ± 25 | 4.6 ± 0.1 | BD | 139.3 ± 0.2 | 834 ± 16 | 16 ± 0 | 4 ± 1 |
| December 2016 | 2206 ± 7 | 1.43 ± 0.05 | 1826 ± 3 | 3.73 ± 0.02 | 375 ± 3 | 4.4 ± 0.1 | 0.17 ± 0.09 | 52.5 ± 5.9 | 725 ± 18 | 35 ± 4 | 2 ± 0 |
| January 2017 | 2445 ± 13 | 1.58 ± 0.02 | 2007 ± 6 | 3.80 ± 0.01 | 433 ± 6 | 4.1 ± 0.0 | 0.20 ± 0.04 | 86.7 ± 1.4 | 635 ± 7 | 23 ± 1 | 2 ± 0 |
| **AVERAGE** | **2296 ± 153** | **46.6 ± 89.4** | **1982 ± 107** | **2.70 ± 1.75** | **265 ± 173** | **4.0 ± 0.8** | **0.14 ± 0.09** | **97.6 ± 37.0** | **690 ± 117** | **23 ± 8** | **5 ± 4** |

| **CL** | TDN ($\mu$mol N $L^{-1}$) | $NH_4^+$ ($\mu$mol N $L^{-1}$) | $NO_3^-$ ($\mu$mol N $L^{-1}$) | $NO_2^-$ ($\mu$mol N $L^{-1}$) | DON ($\mu$mol N $L^{-1}$) | Urea ($\mu$mol N $L^{-1}$) | DPA ($\mu$mol N $L^{-1}$) | $PO_4^{3-}$ ($\mu$mol P $L^{-1}$) | DOC ($\mu$mol C $L^{-1}$) | DIN:$PO_4^{3-}$ | DOC:DON |
|---|---|---|---|---|---|---|---|---|---|---|---|
| May 2016 | 2397 ± 32 | 1.98 ± 0.08 | 2159 ± 4 | 0.10 ± 0.00 | 236 ± 3 | 3.1 ± 0.2 | 0.21 ± 0.00 | 98.1 ± 3.5 | 658 ± 12 | 22 ± 1 | 3 ± 0 |
| June 2016 | 2325 ± 6 | 180.7 ± 2.1 | 2038 ± 25 | 0.07 ± 0.00 | 107 ± 27 | 4.4 ± 0.1 | BD | 139.7 ± 0.3 | 824 ± 13 | 16 ± 0 | 8 ± 2 |
| December 2016 | 2203 ± 7 | 1.60 ± 0.07 | 1830 ± 5 | 0.08 ± 0.00 | 372 ± 5 | 4.6 ± 0.1 | 0.16 ± 0.04 | 59.3 ± 5.5 | 721 ± 14 | 31 ± 3 | 2 ± 0 |
| January 2017 | 2601 ± 5 | 1.50 ± 0.07 | 2008 ± 3 | 0.08 ± 0.00 | 591 ± 3 | 4.0 ± 0.0 | 0.26 ± 0.06 | 90.6 ± 0.6 | 668 ± 11 | 22 ± 0 | 1 ± 0 |
| **AVERAGE** | **2382 ± 167** | **46.4 ± 89.5** | **2009 ± 136** | **0.08 ± 0.01** | **327 ± 207** | **4.0 ± 0.7** | **0.16 ± 0.11** | **96.9 ± 33.1** | **718 ± 76** | **23 ± 6** | **4 ± 3** |

Table 7. Effluent nutrient concentrations and ratios of nitrogen removal (NR) disinfection procedures. Concentration of total dissolved nitrogen (TDN), ammonium ($NH_4^+$), nitrate ($NO_3^-$), nitrite ($NO_2^-$), dissolved organic nitrogen (DON), urea, dissolved primary amines (DPA), phosphate ($PO_4^{3-}$), dissolved organic carbon (DOC), DIN: $PO_4^{3-}$ ratio, and DOC:DON ratio in the NO process with three disinfection procedures- no disinfection (ND), ultraviolet radiation (UV), and chlorination (CL). Values are the average over four samplings plus or minus the standard deviation.

| **ND** | TDN ($\mu$mol N $L^{-1}$) | $NH_4^+$ ($\mu$mol N $L^{-1}$) | $NO_3^-$ ($\mu$mol N $L^{-1}$) | $NO_2^-$ ($\mu$mol N $L^{-1}$) | DON ($\mu$mol N $L^{-1}$) | Urea ($\mu$mol N $L^{-1}$) | DPA ($\mu$mol N $L^{-1}$) | $PO_4^{3-}$ ($\mu$mol P $L^{-1}$) | DOC ($\mu$mol C $L^{-1}$) | $DIN:PO_4^{3-}$ | DOC:DON |
|---|---|---|---|---|---|---|---|---|---|---|---|
| May 2016 | 872 ± 7 | 3.22 ± 0.01 | 781.7 ± 2.4 | 2.39 ± 0.04 | 85 ± 2 | 3.1 ± 0.1 | 0.17 ± 0.02 | 78.2 ± 1.7 | 621 ± 17 | 10 ± 0 | 7 ± 0 |
| June 2016 | 787 ± 15 | 2.58 ± 0.03 | 697.3 ± 2.6 | 2.16 ± 0.00 | 85 ± 3 | 2.8 ± 0.0 | 0.39 ± 0.05 | 62.5 ± 0.2 | 626 ± 14 | 11 ± 0 | 7 ± 0 |
| December 2016 | 822 ± 8 | 1.77 ± 0.09 | 664.4 ± 3.7 | 2.82 ± 0.00 | 153 ± 4 | 4.2 ± 0.2 | 0.45 ± 0.16 | 59.4 ± 2.5 | 744 ± 8 | 11 ± 0 | 5 ± 0 |
| January 2017 | 889 ± 5 | 3.25 ± 0.00 | 691.3 ± 2.4 | 2.25 ± 0.21 | 192 ± 3 | 3.9 ± 0.0 | 0.32 ± 0.06 | 76.9 ± 5.7 | 712 ± 8 | 9 ± 1 | 4 ± 0 |
| **AVERAGE** | **843 ± 47** | **2.70 ± 0.70** | **708.7 ± 50.7** | **2.41 ± 0.29** | **129 ± 53** | **3.5 ± 0.7** | **0.33 ± 0.12** | **69.3 ± 9.7** | **676 ± 62** | **10 ± 1** | **6 ± 2** |

| **UV** | TDN ($\mu$mol N $L^{-1}$) | $NH_4^+$ ($\mu$mol N $L^{-1}$) | $NO_3^-$ ($\mu$mol N $L^{-1}$) | $NO_2^-$ ($\mu$mol N $L^{-1}$) | DON ($\mu$mol N $L^{-1}$) | Urea ($\mu$mol N $L^{-1}$) | DPA ($\mu$mol N $L^{-1}$) | $PO_4^{3-}$ ($\mu$mol P $L^{-1}$) | DOC ($\mu$mol C $L^{-1}$) | $DIN:PO_4^{3-}$ | DOC:DON |
|---|---|---|---|---|---|---|---|---|---|---|---|
| May 2016 | 793 ± 8 | 3.03 ± 0.01 | 725.4 ± 2.3 | 2.22 ± 0.04 | 63 ± 2 | 2.9 ± 0.1 | 0.27 ± 0.15 | 78.4 ± 4.1 | 577 ± 2 | 9 ± 1 | 9 ± 0 |
| June 2016 | 739 ± 1 | 2.70 ± 0.03 | 697.7 ± 1.1 | 2.21 ± 0.02 | 36 ± 1 | 2.8 ± 0.0 | 0.33 ± 0.05 | 57.0 ± 0.8 | 605 ± 11 | 12 ± 0 | 17 ± 1 |
| December 2016 | 823 ± 3 | 1.88 ± 0.07 | 661.9 ± 4.8 | 2.88 ± 0.09 | 157 ± 5 | 4.3 ± 0.0 | 0.25 ± 0.03 | 56.5 ± 6.7 | 717 ± 12 | 12 ± 1 | 5 ± 0 |
| January 2017 | 879 ± 4 | 3.31 ± 0.06 | 693.5 ± 2.6 | 2.41 ± 0.01 | 180 ± 2 | 3.9 ± 0.0 | 0.32 ± 0.03 | 75.9 ± 0.8 | 722 ± 11 | 9 ± 0 | 4 ± 0 |
| **AVERAGE** | **809 ± 58** | **2.73 ± 0.62** | **694.6 ± 26.0** | **2.43 ± 0.31** | **109 ± 70** | **3.5 ± 0.7** | **0.29 ± 0.04** | **67.0 ± 11.8** | **655 ± 75** | **11 ± 2** | **9 ± 6** |

| **CL** | TDN ($\mu$mol N $L^{-1}$) | $NH_4^+$ ($\mu$mol N $L^{-1}$) | $NO_3^-$ ($\mu$mol N $L^{-1}$) | $NO_2^-$ ($\mu$mol N $L^{-1}$) | DON ($\mu$mol N $L^{-1}$) | Urea ($\mu$mol N $L^{-1}$) | DPA ($\mu$mol N $L^{-1}$) | $PO_4^{3-}$ ($\mu$mol P $L^{-1}$) | DOC ($\mu$mol C $L^{-1}$) | $DIN:PO_4^{3-}$ | DOC:DON |
|---|---|---|---|---|---|---|---|---|---|---|---|
| May 2016 | 900 ± 3 | 1.71 ± 0.05 | 802.9 ± 0.9 | 0.08 ± 0.01 | 96 ± 1 | 3.3 ± 0.1 | 0.31 ± 0.06 | 91.3 ± 4.7 | 738 ± 20 | 9 ± 0 | 8 ± 0 |
| June 2016 | 751 ± 15 | 1.77 ± 0.06 | 694.7 ± 10.5 | 0.07 ± 0.00 | 55 ± 10 | 3.0 ± 0.0 | 0.39 ± 0.04 | 59.4 ± 0.5 | 640 ± 7 | 12 ± 0 | 12 ± 2 |
| December 2016 | 770 ± 7 | 1.58 ± 0.05 | 668.5 ± 2.2 | 0.08 ± 0.00 | 100 ± 2 | 4.1 ± 0.2 | 0.39 ± 0.02 | 63.0 ± 1.5 | 677 ± 14 | 11 ± 0 | 7 ± 0 |
| January 2017 | 865 ± 3 | 2.09 ± 0.02 | 690.1 ± 5.4 | 0.08 ± 0.00 | 173 ± 5 | 3.9 ± 0.0 | 0.39 ± 0.04 | 82.0 ± 8.1 | 737 ± 8 | 9 ± 1 | 4 ± 0 |
| **AVERAGE** | **822 ± 72** | **1.79 ± 0.22** | **714.0 ± 60.3** | **0.08 ± 0.01** | **106 ± 49** | **3.6 ± 0.5** | **0.37 ± 0.04** | **73.9 ± 15.2** | **698 ± 48** | **10 ± 2** | **8 ± 3** |

Table 8. Effluent nutrient concentrations and ratios of biological nitrogen and phosphorus removal (BNPR) disinfection procedures. Concentration of total dissolved nitrogen (TDN), ammonium ($NH_4^+$), nitrate ($NO_3^-$), nitrite ($NO_2^-$), dissolved organic nitrogen (DON), urea, dissolved primary amines (DPA), phosphate ($PO_4^{3-}$), dissolved organic carbon (DOC), DIN: $PO_4^{3-}$ ratio, and DOC:DON ratio in the NO process with three disinfection procedures- no disinfection (ND), ultraviolet radiation (UV), and chlorination (CL). Values are the average over four samplings plus or minus the standard deviation. BD indicates below detection.

| **ND** | TDN ($\mu$mol N $L^{-1}$) | $NH_4^+$ ($\mu$mol N $L^{-1}$) | $NO_3^-$ ($\mu$mol N $L^{-1}$) | $NO_2^-$ ($\mu$mol N $L^{-1}$) | DON ($\mu$mol N $L^{-1}$) | Urea ($\mu$mol N $L^{-1}$) | DPA ($\mu$mol N $L^{-1}$) | $PO_4^{3-}$ ($\mu$mol P $L^{-1}$) | DOC ($\mu$mol C $L^{-1}$) | $DIN:PO_4^{3-}$ | DOC:DON |
|---|---|---|---|---|---|---|---|---|---|---|---|
| May 2016 | 63 ± 3 | 1.72 ± 0.04 | 0.08 ± 0.02 | 0.16 ± 0.02 | 61 ± 0 | 2.9 ± 0.0 | 0.42 ± 0.00 | 3.00 ± 0.00 | 752 ± 8 | 1 ± 0 | 12 ± 0 |
| June 2016 | 59 ± 6 | 0.76 ± 0.06 | BD | 0.09 ± 0.01 | 58 ± 0 | 2.2 ± 0.0 | 0.14 ± 0.02 | 0.93 ± 0.07 | 789 ± 32 | 1 ± 0 | 14 ± 0 |
| December 2016 | 78 ± 2 | 1.08 ± 0.14 | 0.06 ± 0.00 | 0.16 ± 0.01 | 77 ± 0 | 4.3 ± 0.0 | 0.41 ± 0.00 | 1.58 ± 0.04 | 1050 ± 9 | 1 ± 0 | 14 ± 0 |
| January 2017 | 64 ± 2 | 0.97 ± 0.00 | 0.16 ± 0.00 | 0.22 ± 0.00 | 63 ± 0 | 3.9 ± 0.0 | 0.25 ± 0.00 | 1.65 ± 0.04 | 919 ± 15 | 1 ± 0 | 15 ± 0 |
| **AVERAGE** | **66 ± 8** | **1.13 ± 0.41** | **0.08 ± 0.07** | **0.16 ± 0.05** | **65 ± 8** | **3.3 ± 1.0** | **0.31 ± 0.13** | **1.79 ± 0.87** | **877 ± 135** | **1 ± 0** | **14 ± 1** |

| **UV** | TDN ($\mu$mol N $L^{-1}$) | $NH_4^+$ ($\mu$mol N $L^{-1}$) | $NO_3^-$ ($\mu$mol N $L^{-1}$) | $NO_2^-$ ($\mu$mol N $L^{-1}$) | DON ($\mu$mol N $L^{-1}$) | Urea ($\mu$mol N $L^{-1}$) | DPA ($\mu$mol N $L^{-1}$) | $PO_4^{3-}$ ($\mu$mol P $L^{-1}$) | DOC ($\mu$mol C $L^{-1}$) | $DIN:PO_4^{3-}$ | DOC:DON |
|---|---|---|---|---|---|---|---|---|---|---|---|
| May 2016 | 56 ± 1 | 1.62 ± 0.03 | 3.77 ± 0.01 | 0.15 ± 0.00 | 51 ± 0 | 2.9 ± 0.0 | 0.45 ± 0.02 | 3.83 ± 0.03 | 739 ± 13 | 1 ± 0 | 15 ± 0 |
| June 2016 | 45 ± 1 | 0.76 ± 0.01 | 0.04 ± 0.01 | 0.10 ± 0.02 | 44 ± 0 | 2.3 ± 0.0 | 0.10 ± 0.04 | 0.92 ± 0.02 | 809 ± 34 | 1 ± 0 | 18 ± 0 |
| December 2016 | 70 ± 3 | 1.13 ± 0.03 | 0.07 ± 0.00 | 0.17 ± 0.00 | 68 ± 0 | 4.2 ± 0.1 | 0.31 ± 0.03 | 1.34 ± 0.07 | 996 ± 16 | 1 ± 0 | 15 ± 0 |
| January 2017 | 68 ± 3 | 1.04 ± 0.02 | BD | 0.15 ± 0.02 | 67 ± 0 | 3.8 ± 0.1 | 0.35 ± 0.01 | 1.58 ± 0.10 | 918 ± 5 | 1 ± 0 | 14 ± 0 |
| **AVERAGE** | **60 ± 12** | **1.14 ± 0.36** | **0.97 ± 1.87** | **0.14 ± 0.03** | **58 ± 12** | **3.3 ± 0.9** | **0.30 ± 0.15** | **1.92 ± 1.30** | **865 ± 114** | **1 ± 0** | **15 ± 2** |

| **CL** | TDN ($\mu$mol N $L^{-1}$) | $NH_4^+$ ($\mu$mol N $L^{-1}$) | $NO_3^-$ ($\mu$mol N $L^{-1}$) | $NO_2^-$ ($\mu$mol N $L^{-1}$) | DON ($\mu$mol N $L^{-1}$) | Urea ($\mu$mol N $L^{-1}$) | DPA ($\mu$mol N $L^{-1}$) | $PO_4^{3-}$ ($\mu$mol P $L^{-1}$) | DOC ($\mu$mol C $L^{-1}$) | $DIN:PO_4^{3-}$ | DOC:DON |
|---|---|---|---|---|---|---|---|---|---|---|---|
| May 2016 | 74 ± 5 | 1.72 ± 0.05 | 0.37 ± 0.02 | 0.06 ± 0.00 | 72 ± 0 | 3.3 ± 0.0 | 0.50 ± 0.07 | 3.92 ± 0.07 | 830 ± 2 | 1 ± 0 | 12 ± 0 |
| June 2016 | 48 ± 2 | 1.09 ± 0.01 | 0.15 ± 0.00 | 0.05 ± 0.01 | 47 ± 0 | 2.5 ± 0.0 | 0.29 ± 0.01 | 0.89 ± 0.03 | 761 ± 13 | 2 ± 0 | 16 ± 0 |
| December 2016 | 74 ± 1 | 1.57 ± 0.02 | 0.24 ± 0.01 | 0.07 ± 0.00 | 72 ± 0 | 4.3 ± 0.0 | 0.51 ± 0.09 | 1.57 ± 0.14 | 1053 ± 22 | 1 ± 0 | 15 ± 0 |
| January 2017 | 72 ± 2 | 1.34 ± 0.04 | 0.39 ± 0.02 | 0.08 ± 0.01 | 70 ± 0 | 3.9 ± 0.0 | 0.52 ± 0.00 | 1.72 ± 0.05 | 931 ± 7 | 1 ± 0 | 13 ± 0 |
| **AVERAGE** | **67 ± 13** | **1.43 ± 0.28** | **0.29 ± 0.11** | **0.07 ± 0.01** | **65 ± 12** | **3.5 ± 0.8** | **0.46 ± 0.11** | **2.03 ± 1.31** | **894 ± 127** | **1 ± 0** | **14 ± 2** |

Table 9. Effluent nutrient concentrations and ratios of biological nitrogen and phosphorus removal and chemical phosphorus removal (BNCPR) disinfection procedures. Concentration of total dissolved nitrogen (TDN), ammonium ($NH_4^+$), nitrate ($NO_3^-$), nitrite ($NO_2^-$), dissolved organic nitrogen (DON), urea, dissolved primary amines (DPA), phosphate ($PO_4^{3-}$), dissolved organic carbon (DOC), DIN: $PO_4^{3-}$ ratio, and DOC:DON ratio in the NO process with three disinfection procedures- no disinfection (ND), ultraviolet radiation (UV), and chlorination (CL). Values are the average over four samplings plus or minus the standard deviation. BD indicates below detection.

| **ND** | TDN ($\mu$mol N $L^{-1}$) | $NH_4^+$ ($\mu$mol N $L^{-1}$) | $NO_3^-$ ($\mu$mol N $L^{-1}$) | $NO_2^-$ ($\mu$mol N $L^{-1}$) | DON ($\mu$mol N $L^{-1}$) | Urea ($\mu$mol N $L^{-1}$) | DPA ($\mu$mol N $L^{-1}$) | $PO_4^{3-}$ ($\mu$mol P $L^{-1}$) | DOC ($\mu$mol C $L^{-1}$) | DIN:$PO_4^{3-}$ | DOC:DON |
|---|---|---|---|---|---|---|---|---|---|---|---|
| May 2016 | 47 ± 3 | 1.66 ± 0.03 | 0.11 ± 0.03 | 0.10 ± 0.00 | 45 ± 0 | 2.1 ± 0.1 | 0.12 ± 0.01 | 0.84 ± 0.03 | 663 ± 12 | 2 ± 0 | 15 ± 0 |
| June 2016 | 41 ± 3 | 0.82 ± 0.04 | 0.16 ± 0.00 | 0.06 ± 0.00 | 40 ± 0 | 1.7 ± 0.0 | 0.11 ± 0.04 | 0.45 ± 0.02 | 561 ± 5 | 2 ± 0 | 14 ± 0 |
| December 2016 | 59 ± 2 | 1.07 ± 0.06 | BD | 0.10 ± 0.00 | 58 ± 0 | 3.6 ± 0.1 | 0.22 ± 0.02 | 0.88 ± 0.04 | 815 ± 12 | 1 ± 0 | 14 ± 0 |
| January 2017 | 62 ± 1 | 1.34 ± 0.05 | 4.87 ± 0.01 | 0.51 ± 0.01 | 55 ± 0 | 3.7 ± 0.0 | 0.08 ± 0.04 | 0.88 ± 0.10 | 929 ± 13 | 8 ± 0 | 17 ± 0 |
| **AVERAGE** | **52 ± 10** | **1.22 ± 0.36** | **1.29 ± 2.39** | **0.19 ± 0.21** | **50 ± 8** | **2.8 ± 1.0** | **0.13 ± 0.06** | **0.77 ± 0.21** | **742 ± 163** | **3 ± 3** | **15 ± 1** |

| **UV** | TDN ($\mu$mol N $L^{-1}$) | $NH_4^+$ ($\mu$mol N $L^{-1}$) | $NO_3^-$ ($\mu$mol N $L^{-1}$) | $NO_2^-$ ($\mu$mol N $L^{-1}$) | DON ($\mu$mol N $L^{-1}$) | Urea ($\mu$mol N $L^{-1}$) | DPA ($\mu$mol N $L^{-1}$) | $PO_4^{3-}$ ($\mu$mol P $L^{-1}$) | DOC ($\mu$mol C $L^{-1}$) | DIN:$PO_4^{3-}$ | DOC:DON |
|---|---|---|---|---|---|---|---|---|---|---|---|
| May 2016 | 50 ± 3 | 1.62 ± 0.01 | 10.9 ± 0.0 | 0.11 ± 0.00 | 37 ± 0 | 2.0 ± 0.0 | 0.09 ± 0.01 | 1.91 ± 0.01 | 626 ± 9 | 7 ± 0 | 17 ± 0 |
| June 2016 | 41 ± 1 | 0.88 ± 0.04 | 0.06 ± 0.00 | 0.06 ± 0.00 | 40 ± 0 | 1.8 ± 0.0 | 0.12 ± 0.01 | 0.47 ± 0.00 | 566 ± 8 | 2 ± 0 | 14 ± 0 |
| December 2016 | 58 ± 1 | 1.15 ± 0.00 | 0.06 ± 0.01 | 0.11 ± 0.00 | 57 ± 0 | 3.7 ± 0.0 | 0.20 ± 0.03 | 0.74 ± 0.04 | 821 ± 2 | 2 ± 0 | 14 ± 0 |
| January 2017 | 64 ± 1 | 1.35 ± 0.02 | 4.97 ± 0.00 | 0.51 ± 0.00 | 57 ± 0 | 3.8 ± 0.0 | 0.09 ± 0.01 | 0.86 ± 0.03 | 914 ± 11 | 8 ± 0 | 16 ± 0 |
| **AVERAGE** | **53 ± 10** | **1.25 ± 0.31** | **4.00 ± 5.15** | **0.20 ± 0.21** | **48 ± 11** | **2.8 ± 1.1** | **0.13 ± 0.05** | **1.00 ± 0.63** | **732 ± 163** | **5 ± 3** | **15 ± 1** |

| **CL** | TDN ($\mu$mol N $L^{-1}$) | $NH_4^+$ ($\mu$mol N $L^{-1}$) | $NO_3^-$ ($\mu$mol N $L^{-1}$) | $NO_2^-$ ($\mu$mol N $L^{-1}$) | DON ($\mu$mol N $L^{-1}$) | Urea ($\mu$mol N $L^{-1}$) | DPA ($\mu$mol N $L^{-1}$) | $PO_4^{3-}$ ($\mu$mol P $L^{-1}$) | DOC ($\mu$mol C $L^{-1}$) | DIN:$PO_4^{3-}$ | DOC:DON |
|---|---|---|---|---|---|---|---|---|---|---|---|
| May 2016 | 45 ± 3 | 1.66 ± 0.01 | 0.30 ± 0.00 | 0.06 ± 0.01 | 43 ± 0 | 2.5 ± 0.0 | 0.20 ± 0.06 | 1.03 ± 0.07 | 709 ± 22 | 2 ± 0 | 16 ± 0 |
| June 2016 | 44 ± 3 | 1.06 ± 0.08 | 0.14 ± 0.01 | 0.05 ± 0.01 | 42 ± 0 | 2.0 ± 0.1 | 0.31 ± 0.04 | 0.48 ± 0.01 | 564 ± 10 | 3 ± 0 | 13 ± 0 |
| December 2016 | 58 ± 1 | 1.20 ± 0.01 | 0.33 ± 0.00 | 0.06 ± 0.00 | 57 ± 0 | 3.7 ± 0.1 | 0.43 ± 0.01 | 0.87 ± 0.01 | 822 ± 12 | 2 ± 0 | 15 ± 0 |
| January 2017 | 61 ± 1 | 1.11 ± 0.02 | 5.64 ± 0.00 | 0.07 ± 0.01 | 54 ± 0 | 3.8 ± 0.1 | 0.27 ± 0.01 | 0.95 ± 0.00 | 886 ± 8 | 7 ± 0 | 16 ± 0 |
| **AVERAGE** | **52 ± 9** | **1.26 ± 0.27** | **1.60 ± 2.69** | **0.06 ± 0.01** | **49 ± 8** | **3.0 ± 0.9** | **0.30 ± 0.10** | **0.83 ± 0.24** | **745 ± 141** | **3 ± 3** | **15 ± 2** |

## FIGURE LEGENDS

Figure 1. Flowchart of experimental design.

Figure 2. Total dissolved nitrogen (TDN) concentration in the treatment processes. TDN concentration in the four treatment processes averaged over four samplings. Error bars represent standard deviation.

Figure 3. Total dissolved nitrogen (TDN) concentration in the disinfection procedures. Dissolved inorganic nitrogen (DIN) and dissolved organic nitrogen (DON) as a proportion of TDN in disinfection procedures within each treatment averaged over four samplings. ND stands for no disinfection. UV stands for ultraviolet radiation. CL stands for chlorination. Error bars represent standard deviation. Note the difference in scale between the top and bottom graphs.

Supplemental Figure 1. Treatment process cycles. Cycles for each of the four treatment processes: nitrification only (NO), nitrogen removal (NR), biological nitrogen and phosphorus removal (BNPR), and biological nitrogen and phosphorus removal with additional chemical phosphorus removal (BNCPR).

Figure 1.

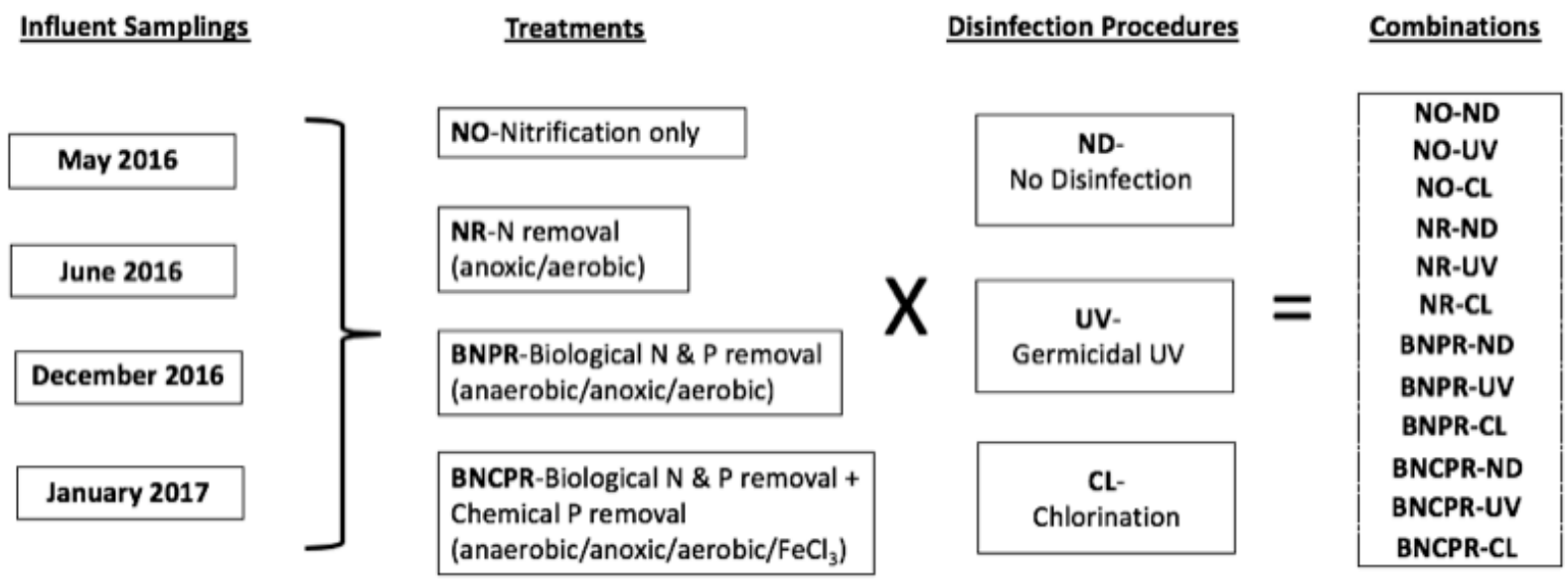

Influent Samplings
Treatments
Disinfection Procedures
Combinations
May 2016
June 2016
December 2016
January 2017
NO-Nitrification only
NR-N removal (anoxic/aerobic)
BNPR-Biological N & P removal (anaerobic/anoxic/aerobic)
BNCPR-Biological N & P removal + Chemical P removal (anaerobic/anoxic/aerobic/FeCl3)
X
ND- No Disinfection
UV- Germicidal UV
CL- Chlorination
=
NO-ND
NO-UV
NO-CL
NR-ND
NR-UV
NR-CL
BNPR-ND
BNPR-UV
BNPR-CL
BNCPR-ND
BNCPR-UV
BNCPR-CL

Figure 2.

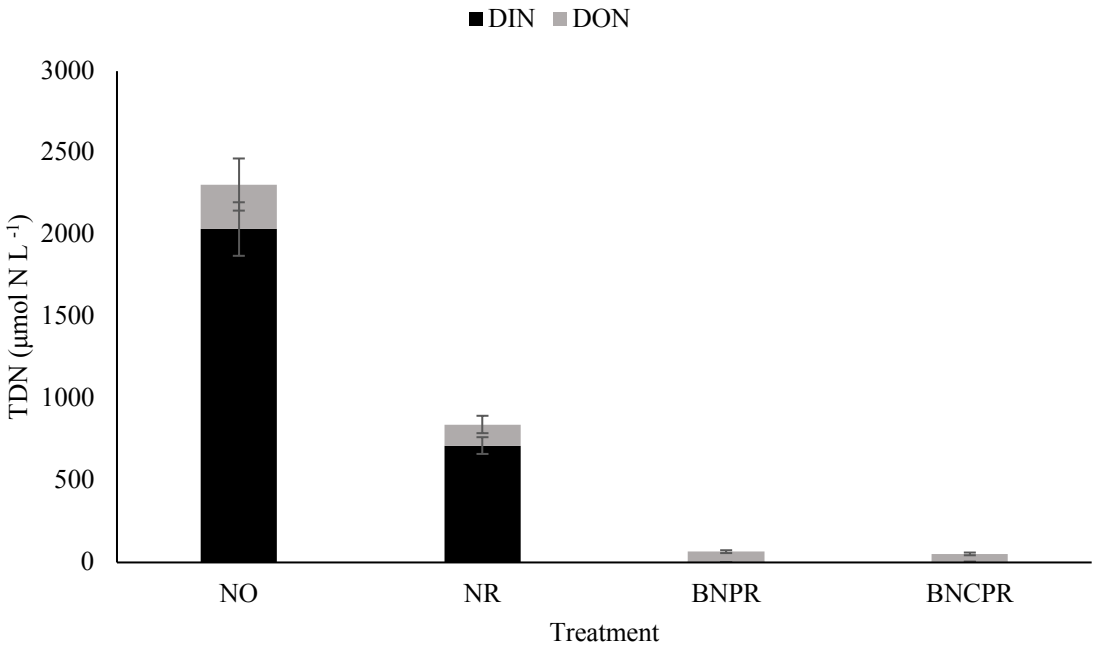
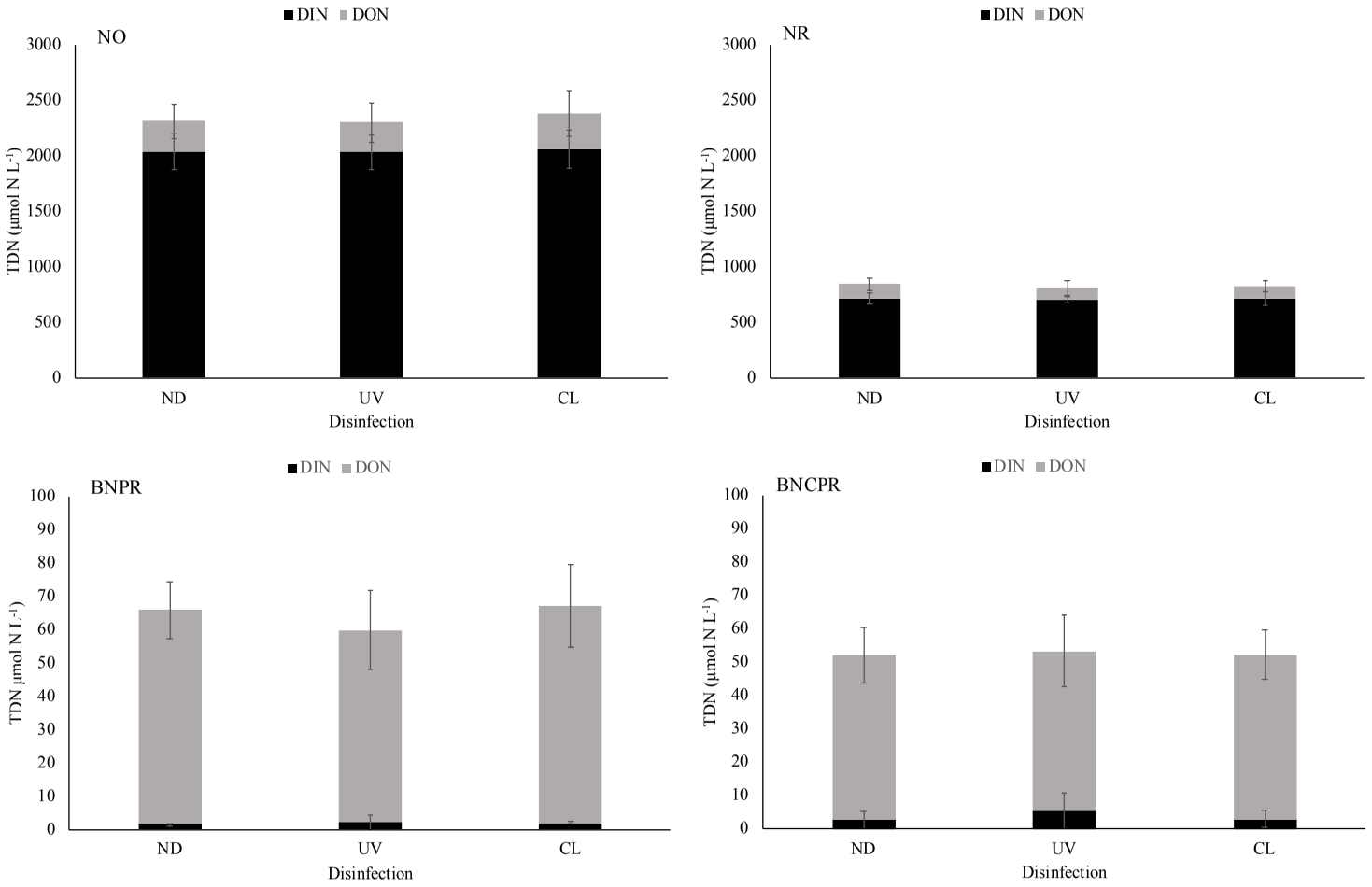
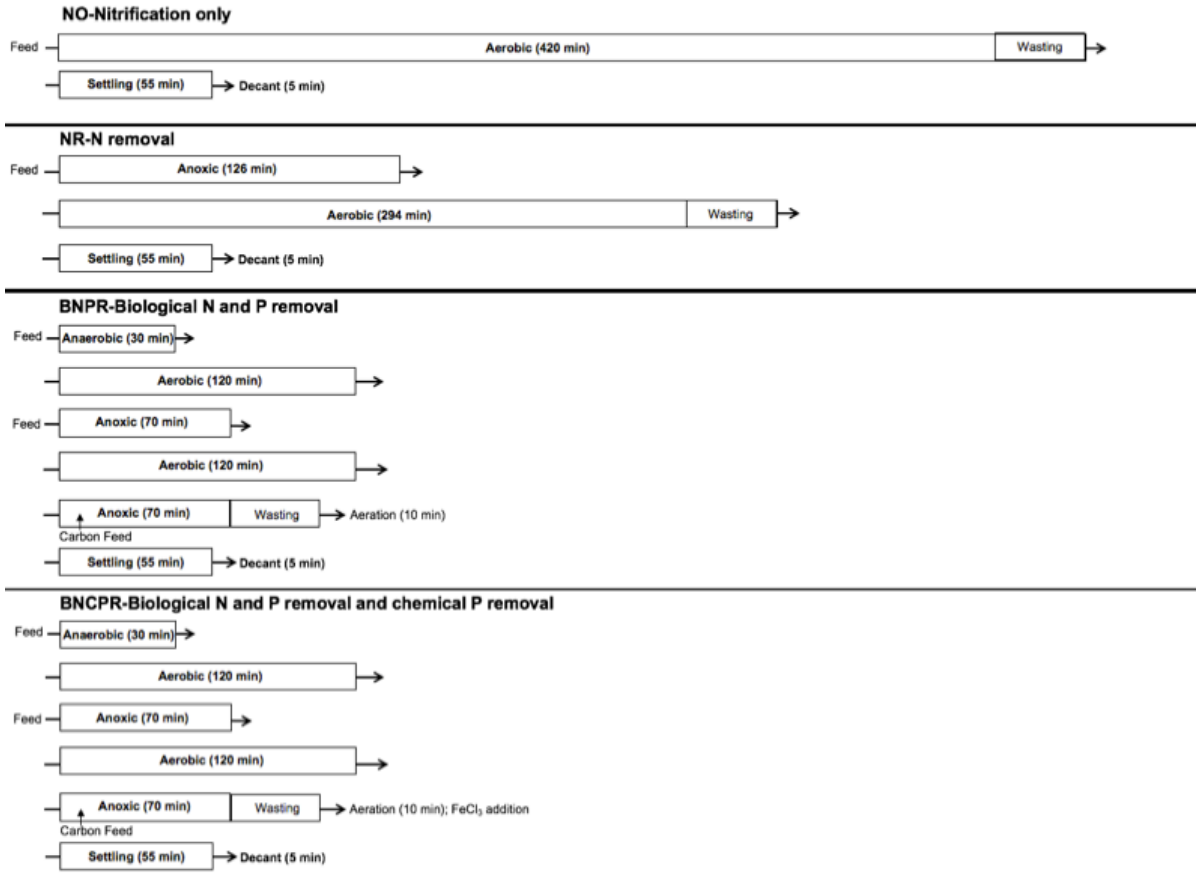

DIN
DON
3000
2500
2000
1500
1000
500
0
TDN (µmol N L -1)
NO
NR
BNPR
BNCPR
Treatment

Figure 3.


DIN
DON
NO
TDN (µmol N L-1)
3000
2500
2000
1500
1000
500
0
ND
UV
CL
Disinfection
NR
BNPR
TDN µmol N L-1
100
90
80
70
60
50
40
30
20
10
BNCPR

Supplemental Figure 1.



# CHAPTER 3: BIOASSAY


## ABSTRACT

Treatment processes and disinfection procedures used by water reclamation facilities (WRFs) affect the concentration and composition of nitrogen (N) species in effluent delivered to receiving waters. With eutrophication being a primary concern in Chesapeake Bay, it's increasingly vital for WRFs to limit N loading that could further increase primary productivity. Technological upgrades of WRFs have decreased the amount of highly labile dissolved inorganic nitrogen (DIN) in effluent, but the bioavailability of the remaining dissolved organic nitrogen (DON), is unclear. Using bioassays, we investigated the impact of effluent dissolved organic nitrogen (EDON), taken from bench-scale sequencing batch reactors designed to mimic commonly used treatment processes and disinfection procedures, on natural water samples collected in the York River, VA. Effluent with high DIN concentrations stimulated phytoplankton growth while effluent with high DON concentrations stunted growth and in some cases even impeded growth, compared to the control; in some cases phosphorus limitation may have led to an underestimation of the lability of EDON. Changes in DON concentrations indicate the removal of some fraction of EDON in the majority of bioassays, and production of DON in others, the degree of which varied between effluents. This study provides a bioassay comparison using the same influent for each of the effluent treatments, and the results provide further evidence that up to 48% of EDON is biologically available to estuarine microbes and so is a contributor to eutrophication in receiving waters.

## INTRODUCTION

According to the U.S. Environmental Protection Agency (EPA), water quality in Chesapeake Bay is so poor that it's on the agency's "dirty waters" list (EPA 2016). Since 1986, the Chesapeake Bay Foundation has published a yearly report card that details various indicators of Chesapeake Bay's overall health. Among those indicators is nitrogen (N), one of the main pollutants responsible for excess algal production or cultural eutrophication, harmful algal bloom formation, coastal hypoxia, and fish kills (Conley et al. 2009; Heisler et al. 2008). Nitrogen enters Chesapeake Bay through various sources including effluent from water reclamation facilities (WRFs), which accounts for 16% of N loading (EPA 2016). In 2010, the Total Maximum Daily Load (TMDL) was established to help restore clean water to Chesapeake Bay's streams, creeks, and rivers. One of the goals of the TMDL is a 25% reduction in N loading by the year 2025 (Chesapeake Bay TMDL 2010). In order to accomplish this goal, WRFs must undergo costly upgrades to decrease the amount of total N discharged in their effluent to receiving waters.

WRF effluent contains both dissolved inorganic N (DIN) and dissolved organic N (DON). While it is well known that DIN stimulates phytoplankton growth, historically DON was considered refractory due to the relatively high concentrations present in N-limited systems (reviewed in Sipler and Bronk 2015). However, numerous studies have shown that a variety of DON compounds are directly bioavailable to natural plankton communities (reviewed in Berman and Bronk 2003 and Mulholland and Lomas 2008). Low-molecular weight (LMW) DON can be taken up by active transport or facilitated diffusion (Mulholland and Lomas 2008) or by phototransformation (Gryzbowski and

Tranvik 2008), while high-molecular weight DON can be broken down by extracellular enzymes (Berges and Mulholland 2008). Dinoflagellates in particular, including some harmful algal bloom species, can utilize DON and may even prefer it to DIN (Bronk et al. 2007; Glibert and Terlizzi 1999; Dyhrman and Anderson 2003). Several studies have found distinct similarities between microbially produced DON and effluent DON (EDON) (Sattayatewa et al. 2009; Aquino and Stuckey 2003; Nam and Amy 2008). Therefore, it follows that EDON is likely to also stimulate growth in receiving waters.

As stricter N load limits in Chesapeake Bay are enforced, WRFs are employing strategies to reduce the amount total dissolved N (TDN) in their effluent. Most of these strategies involve biological nutrient removal (BNR) that targets the removal of DIN through coupled nitrification/denitrification processes. Highly efficient BNR procedures can remove almost all of the DIN, while 71 to 357 μmol N $L^{-1}$ of DON (Pehlivanoglu-Mantas and Sedlak 2006) remains persistent through treatment, leading to high DON:DIN ratios in low TN effluents (Pehlivanoglu and Sedlak 2004; Urgun-Demirtas et al. 2008; Westgate and Park 2010). EDON is a complex mixture of compounds of which up to 90% still remains uncharacterized at the compound level (Pehlivanoglu-Mantas and Sedlak 2006). EDON can contain non-biodegradable natural organic matter (NOM) from the influent, soluble microbial products that are generated by bacteria as a result of substrate metabolism and biomass decay during treatment (Barker and Stuckey 1999), and compounds chemically modified during disinfection (Parkin and McCarty 1981 a, b). The exact composition of EDON can vary based on influent characteristics, treatment processes, and disinfection procedures, all of which can affect EDON's lability. Interestingly, despite significant decreases in N loads though upgrades of WRFs, many

urbanized coastal systems are continuing to experience eutrophication (Suter et al. 2014; Kemp et al. 2009). While multiple factors may be responsible for this paradox (Eom et al. 2017; Suter et al. 2014), the consequences of EDON release to estuarine and coastal waters remain largely unknown.

In the Chesapeake Bay region, the question of whether EDON is bioavailable arose because of tighter restrictions on the amount of N WRFs could release. Citing old oceanographic literature, the case was made on at least one permit application that EDON is not bioavailable and so should not count against the amount of N that was releasable from the facility. A group was convened to investigate the issue with the finding that there was insufficient information to come to any conclusion (Mulholland et al. 2007). This led to a number of bioassay studies that showed that up to 96% of the EDON was biologically available on relatively short (1 to 7 day) time scales (Bronk et al. 2010; Filippino et al. 2011). It was also shown that EDON additions resulted in larger increases in chlorophyll in salt water, relative to additions to freshwater and that the degree of uptake by phytoplankton varied with EDON collected from different treatment facilities.

The objective of this study was to assess the bioavailability of effluent from four commonly used treatment processes and three disinfection procedures used on the same influent. Bioassays were performed on effluent from each of twelve treatment-disinfection combinations added to fresh and saline water collected in the York River to measure biological uptake in receiving waters. At the end of the bioassay, wet chemical analysis was used to determine changes in specific compounds or classes known to be biologically available to microbial communities in receiving waters including total dissolved nitrogen (TDN), ammonium ($NH_4^+$), nitrate ($NO_3^-$), nitrite ($NO_2^-$), dissolved

organic nitrogen (DON), urea, dissolved primary amines (DPAs), phosphate ($PO_4^{-3}$), silicate (Si), and dissolved organic carbon (DOC).

This approach allowed the linkage of specific treatments and disinfection processes directly to the biological response measured in receiving waters. Bioassays were carried out in both the winter and summer because the influent and microbial communities within WRFs as well as the phytoplankton and bacterial assemblages can vary greatly with temperature and time of year.

## METHODS

### *Description of Treatments within the Sequencing Batch Reactors*

Four bench-scale sequencing batch reactors (SBRs), maintained by the Hampton Roads Sanitation District (HRSD) in Virginia Beach, VA, were run under set experimental conditions in May, June, and December 2016, and January 2017 to assess how temperature variations and differences in influent composition impact treatment and disinfection processes (Figure 1). Each 22 L SBR was designed to mimic a commonly used wastewater treatment process. Nitrification only (NO) involved an aerobic cycle with additions of magnesium hydroxide to maintain neutral pH. Nitrogen removal (NR) involved an anoxic and aerobic cycle in sequence and used sodium bicarbonate additions to maintain neutral pH. Biological nitrogen and phosphorus removal (BNPR) and biological nitrogen and phosphorus removal with chemical phosphorus removal (BNCPR) involved anaerobic, aerobic, anoxic, aerobic, and anoxic cycles in sequence, with additions of methanol as the carbon electron donor during the second anoxic cycle. BNCPR also utilized the addition of iron chloride ($FeCl_3$) during the final aeration cycle.

During the aeration periods, the dissolved oxygen concentration was maintained at 3 to 4 mg $L^{-1}$, with continuous oxygen uptake rate measurements.

The SBRs were operated on three 8-hour cycles per day with a raw sewage feed from the HRSD Chesapeake-Elizabeth Treatment Plant, a 24 million gallons per day facility that receives mostly domestic household wastewater, composed of residential, some commercial, very little industrial sewage (Kevin Parker, HRSD, personal communication), a 24-hour hydraulic residence time, and 15-day solids residence time. SBRs were kept at temperatures similar to those present in local plants at the time of influent collection; 25°C during the summer and 15°C during the winter. SBRs were maintained for a minimum of three cycles before any effluent was sampled. Approximately 6 L of effluent were drawn off of each SBR at the end of a cycle, filtered sequentially through a pre-combusted (2 h @ 450°C) Whatman GF/F followed by a 0.2 μm Supor membrane filter. Filtered effluent used in the analyses was collected and pooled over a period of 32 hours, which included 4 cycles.

***Effluent Disinfection Procedures***

Effluent from each of the SBRs was divided into three subsamples for disinfection procedures- no disinfection (ND), germicidal UV radiation (UV), and chlorination (CL). The first portion served as a control and did not undergo a disinfection procedure. The second portion, exposed to germicidal UV disinfection, was treated using a Trojan UV Pro 10 point of use UV system (Monitored Class A NSF certified system designed for multiple sources) at a dose of 40 mJ $cm^{-2}$ (flow rate was 3 L $min^{-1}$). The third portion, subjected to chlorine disinfection, was treated with sodium hypochlorite added as Clorox bleach at a dose of 2.5 to 4 mg $L^{-1}$, dependent upon chlorine demand. After 30 minutes,

sodium sulfite was added to remove total chlorine residuals; the additions were 0.6 to 2 mg $L^{-1}$ and dependent on the chlorine residuals that needed to be dechlorinated. To ensure successful dechlorination, chlorine residuals were measured after the hypochlorite addition and after the sodium sulfite addition with a diethyl-p-phenylenediamine (DPD) chlorine test kit. All 0.2 µm filtered effluents were transported on ice back to the Virginia Institute of Marine Science, where they were stored at -20°C until further analysis.

***Site Water***

Bioassays were conducted using water collected from two sites in the York River, VA, a sub-estuary of Chesapeake Bay (Figure 2). Samples from Site 1 were collected from a pier adjacent to the VIMS campus (37.248022°N, 76.499761°W) in the mainstem York River and had a salinity that ranged from 16 to 23, while Site 2 samples were collected from the Shanghai public pier (37.591994°N, 76.798639°W) for all dates with the exception of March 2017, when it was collected from the Walkerton public pier (37.725101°N, 77.023210°W) in the Mattaponi River (a tributary of the York River) and had a salinity of 0. Salinities were selected to bracket the range where effluent could potentially be discharged. Water was collected on June 3 and July 11, 2016 for summer incubations, and January 23 and March 20, 2017 for winter incubations. All site water was collected from the surface (< 0.5 m) in acid-rinsed carboys and pre-screened through a 150 µm Nitex mesh to exclude any large zooplankton. Initial samples from each site were collected for determination of phytoplankton biomass and wet chemical analyses.

***Effluent Addition***

Effluent collected in May 2016 was added to site water collected in June 2016 (Figure 1). Effluent collected in June 2016 was added to site water collected in July 2016. Effluent collected in December 2016 was added to site water collected in January 2017. Effluent collected in January 2017 was added to site water collected in March 2017. Triplicate 250 mL polyethylene terephthalate glycol (PETG) bottles with water from each site were amended with one of the 12 effluent treatment-disinfection combinations for an estimated 15 μmol N $L^{-1}$ effluent dissolved organic nitrogen (EDON). Bottles without effluent additions were incubated as controls (CONT). An additional $NO_3^-$ control (+N3) with approximately 250 μmol N $L^{-1}$, was set up for each site to address the high $NO_3^-$ concentrations that were present in the NO and NR treatments. High concentrations of $NO_3^-$ in those treatments made it difficult to attribute a biological response to the EDON in those bioassays.

***Incubations***

Bottles were incubated for up to 9 days in a Percival incubator that was programmed to mimic ambient light/dark cycles as well as temperature (25 to 30°C and 8 to 9°C in the summer and winter, respectively). At each of the five timepoints, triplicate bottles of each treatment-disinfection combination were filtered through a pre-combusted (2 h @ 450°C) Advantec GF-75 filter (0.3 μm-nominal pore size); filters were immediately processed and analyzed for Chlorophyll *a* to monitor growth throughout the experiment. At the beginning (T0) and at the end (TF) of the incubation, filtrate was collected for analyses of TDN, $NH_4^+$, $NO_3^-$, $NO_2^-$, DON, urea, DPAs, $PO_4^{-3}$, Si, and DOC.

During the summer (June and July 2016), day 0 samples were not taken for each treatment-disinfection combination in June 2016, so CONT day 0 concentration was used for all treatments. Also in June 2016, BNPR UV, BNPR CL, BNCPR UV, and BNCPR CL bioassays entered senescence after day 3, identified by visual inspection of cells clumped together at the bottom of the incubation bottles, so there were no measurements taken on day 5 for those treatment-disinfection combinations.

***Analytical Methods***

Concentrations of ammonium ($NH_4^+$) were measured in triplicate using the phenol hypochlorite method (Koroleff 1983), with a detection limit of 0.05 µmol N $L^{-1}$. Urea concentrations were measure in duplicate using the monoxime method (Price and Harrison 1987), with a detection limit of 0.10 N $L^{-1}$. Nitrate ($NO_3^-$), nitrite ($NO_2^-$), phosphate ($PO_4^{3-}$), and silicate (Si) were measured in duplicate on a Lachat QuikChem 8500 autoanalyzer (Parsons et al. 1984), with detection limits of 0.03 µmol N $L^{-1}$, 0.03 µmol N $L^{-1}$, 0.03 µmol P $L^{-1}$, and 0.11 µmol Si $L^{-1}$, respectively. Concentrations of TDN and DOC were measured via high temperature combustion using a Shimadzu TOC-V TNM analyzer (Hansell et al. 1993; Sharp et al. 2004; Sharp et al. 1993); University of Miami consensus reference materials, deep-sea and low-carbon water, were used as quality control standards. Limits of detection for TDN and DOC are 2 µmol N $L^{-1}$, and 5 µmol C $L^{-1}$, respectively. Concentrations of DON were then calculated as the difference between TDN and the sum of $NH_4^+$ and $NO_x^-$ ($NO_3^-$ plus $NO_2^-$); the standard deviation for DON concentrations was obtained using a propagation of error analysis (Bronk 2002). Amino acids, as dissolved primary amines (DPAs) were measured using the fluorometric method (Parsons et al. 1984), with a detection limit of 0.025 µmol N $L^{-1}$. Chlorophyll *a*

concentrations were measured fluorometrically on a Turner Design Model 10-AU fluorometer according to Parsons et al. (1984).

***EDON Lability Calculation***

The percentage of EDON that was labile in each treatment (treat) was determined using the following equations:

EDON added (μmol N $L^{-1}$): $DON_{treat\ T0} - DON_{CONT\ T0}$

EDON used (μmol N $L^{-1}$): $(DON_{treat\ T0} - DON_{treat\ TF}) - (DON_{CONT\ T0} - DON_{CONT\ TF})$

EDON labile (%): (EDON used / EDON added) * 100

***Statistical Analysis***

The statistical software R-studio was used to conduct analysis of variance (ANOVA) and Tukey's Honest Significant Difference test to interpret differences in Chlorophyll *a* concentrations between disinfection procedures among treatments.

**RESULTS**

***Effluent Composition***

Effluents from all four samplings within each treatment were averaged to incorporate the possible extremes both within and among summer and winter (Table 1). Effluent from the NO process was characterized by very high concentrations of TDN (2308 ± 135 μmol N $L^{-1}$), primarily in the form of $NO_3^-$ (1986 ± 107 μmol N $L^{-1}$), and high concentrations of $PO_4^{3-}$ (101.3 ± 36.9 μmol P $L^{-1}$). $NH_4^+$ was generally low with the exception of the June 2016 sampling when nitrification failed. Effluent from the NR process was also characterized by high concentrations of TDN (843 ± 47 μmol N $L^{-1}$), primarily in the form of $NO_3^-$ (708.7 ± 50.7 μmol N $L^{-1}$), and high concentrations of

$PO_4^{3-}$ (69.3 ± 9.7 µmol P $L^{-1}$). Effluent from the BNPR process was characterized by low concentrations of TDN (66 ± 8 µmol N $L^{-1}$), primarily in the form of DON (65 ± 8 µmol N $L^{-1}$), and low concentrations of $PO_4^{3-}$ (1.79 ± 0.87 µmol P $L^{-1}$). Effluent from the BNCPR process was also characterized by low concentrations of TDN (52 ± 10 µmol N $L^{-1}$), primarily in the form of DON (50 ± 8 µmol N $L^{-1}$), and low concentrations of $PO_4^{3-}$ (0.77 ± 0.21 µmol P $L^{-1}$). Overall, TDN, DON, urea, and $PO_4^{3-}$ concentrations within treatments followed the trend of: NO > NR > BNPR > BNCPR. This demonstrates that increasing the level of treatment has an inverse relationship on the resulting effluent nutrient concentrations. To the contrary, all treatments had similar concentrations of DOC. DIN:$PO_4^{3-}$ ratios show that only the NO process produced effluent enriched in N above Redfield, while the others were enriched in P. DOC:DON ratios increased with increasing level of treatment, shifting from N-enrichment in NO and NR (3 and 6, respectively) to C-enrichment in BNPR and BNCPR (14 and 15, respectively).

With respect to disinfection procedures, averaged over all samplings, there were no significant differences for concentrations of products in disinfection procedures among treatments, except for $NO_2^-$ and DPA, which both accounted for < 1% of TDN (Supplemental Tables 1-4). A trend among all treatments was that CL always had the lowest $NO_2^-$ and the highest DOC of all disinfections. CL also appeared to have elevated urea and DPA in all but the NO treatment. The May 2016 sampling showed production of $NO_3^-$ upon UV disinfection (observed as an increase in concentration from ND) in both the BNPR and BNCPR treatments. $PO_4^{3-}$ appeared to be generally unaffected by disinfection procedures.

***Site 1***

**Ambient Conditions**

During the summer (June and July 2016), TDN was consistent at 15 ± 0 μmol N $L^{-1}$, $NH_4^+$ ranged from 0.26 to 1.58 μmol N $L^{-1}$, $NO_3^-$ was low (0.22 ± 0.02 μmol N $L^{-1}$), and $NO_2^-$ was below detection (Table 2). DON (14 ± 1 μmol N $L^{-1}$) was the dominant form of fixed N and accounted for 93% of TDN. Urea and DPAs combined contributed < 4% of DON. $PO_4^{3-}$ was low, but Si was consistently high. DOC ranged from 237 to 306 μmol C $L^{-1}$. The DIN:$PO_4^{3-}$ ratio and DOC:DON ratio were 6 ± 1 and 19 ± 2 respectively, both suggesting N-limitation. Chlorophyll *a* concentrations were lower in June (17.7 μg $L^{-1}$) than in July (41.0 μg $L^{-1}$).

During the winter (January and March 2017), TDN was fairly consistent at 16 ± 2 μmol N $L^{-1}$, $NH_4^+$ and $NO_3^-$ were both low (< 0.5 μmol N $L^{-1}$), and $NO_2^-$ was below detection (Table 2). DON (15 ± 3μmol N $L^{-1}$) was again the dominant form of N and accounted for 94% of TDN. Urea and DPAs were again a small fraction of the DON pool (< 4%). $PO_4^{3-}$ was below detection and thus suggested P-limitation. Si was lower compared to the summer and ranged from 6.04 to 16.2 μmol Si $L^{-1}$. DOC was similar to summer. DOC:DON ratio was 19 ± 6, suggesting N-limitation. Chlorophyll *a* concentrations were similar in January (7.8 μg $L^{-1}$) and March (8.7 μg $L^{-1}$), and less than half the concentrations observed during summer.

**Bioassay Chlorophyll *a***

In order to simplify the presentation of results, only ND treatments were compared to one another and then disinfection procedures were compared within treatments.

During the summer (June and July 2016), similar patterns emerged from both samplings (Figure 3). NO and NR were very similar and had the greatest increase in Chlorophyll *a* (ranged from 189 to 212 μg $L^{-1}$) followed by +N3 (ranged from 57 to 147 μg $L^{-1}$). CONT, BNPR, and BNCPR all showed no growth.

With regard to disinfection within treatments, there were no significant differences on the final day of the incubation in either June or July 2016 ($p > 0.05$) (Figure 4). However, on day 3 in July 2016, all disinfections from NO were significantly different from one another, CL > ND > UV ($p < 0.01$).

During the winter (January and March 2017), similar patterns emerged from both samplings (Figures 5). NO and NR were very similar and had the greatest increase in Chlorophyll *a* (ranged from 69 to144 μg $L^{-1}$), followed by +N3 (ranged from 13 to 44 μg $L^{-1}$). In January 2017, there was no growth in CONT, BNPR, and BNCPR, while in March 2017 there was some growth in the first 24 hours in those treatments, followed by a continuous decline.

With regard to disinfection within treatments, the only significant difference on the final day of the incubation was in March 2017 for BNCPR where CL was higher than both ND and UV ($p < 0.01$) (Figure 6). There were also a few other significant differences, in January 2017 in NR on day 4 where CL was higher than both ND and UV ($p < 0.01$), and in March NO on day 7 where CL > UV > ND ($p < 0.001$).

**Bioassay Nutrient Uptake**

In order to simplify the presentation of results, all disinfection procedures within a treatment were grouped together within each season (3 treatment-disinfection combinations x 2 samplings per season = 6 sets) to analyze trends.

During the summer (June and July 2016), TDN was consumed in all treatments except CONT in the June 2016 bioassay (Supplemental Tables 5 and 6). $NO_3^-$ was generally the dominant form of TDN consumed in NO and NR with the exception of the NO treatments in the July 2016 bioassay, where $NH_4^+$ was the dominant form consumed due to unusually high concentrations in the effluent. $NH_4^+$ and DON were the dominant forms consumed in BNPR and BNCPR. DON was not consumed in CONT and a majority of BNCPR (4 out of 6 sets). DON was consumed in a majority of NO and BNPR (5 out of the 6 sets and 4 out of the 6 sets, respectfully). DON was produced in a majority of NR (4 out of the 6 sets). Urea and DPAs constituted a smaller proportion of TDN consumed in NO and NR (0 to 6%) compared to BNPR and BNCPR (8 to 24%). By the last day of the incubation, DIN and $PO_4^{3-}$ were still high in NO and NR ( > 47.0 μmol N $L^{-1}$ and > 7.00 μmol P $L^{-1}$, respectively), while they were very low, and in some cases close to the limit of detection in BNPR and BNCPR (in most cases < 0.21 μmol N $L^{-1}$ and < 0.38 μmol P $L^{-1}$). Si was consumed in nearly all treatments, but to a much greater extent in NO and NR, where concentrations remained above 1.00 μmol Si $L^{-1}$ but may have limited diatom growth when compared to concentrations of other available nutrients.

During the winter (January and March 2017), TDN was consumed in all but one of the treatments, BNPR ND from the January 2017 bioassay, and CONT from the March 2017 bioassay (Supplemental Tables 7 and 8). $NO_3^-$ was the dominant form of TDN consumed in NO and NR. $NH_4^+$ and DON were the dominant forms consumed in BNPR and BNCPR. DON was consumed in CONT from the January 2017 bioassay, all of NO, and the majority of BNPR and BNCPR (4 out of the 6 sets for both). DON was produced

in the CONT from the March 2017 bioassay, and the majority of NR (4 out of the 6 sets). Urea and DPAs constituted a smaller proportion of TDN consumed in NO and NR (< 1%) compared to BNPR and BNCPR (0 to 33%) (Supplemental Table 1 – 4). By the last day of the incubation, DIN and $PO_4^{3-}$ were still available in NO and NR ( > 15.0 µmol N $L^{-1}$ and > 0.80 µmol P $L^{-1}$, respectively), while in BNPR and BNCPR DIN was < 2.05 µmol N $L^{-1}$ and $PO_4^{3-}$ was below the limit of detection. Si was consumed below 1.00 µmol Si $L^{-1}$ in NO and NR.

***Site 2***

**Ambient Conditions**

During the summer (June and July 2016), TDN was fairly consistent at 36 ± 5 µmol N $L^{-1}$, $NH_4^+$ ranged from 0.83 to 1.34 µmol N $L^{-1}$, $NO_3^-$ was high (13.6 ± 0.3 µmol N $L^{-1}$) and $NO_2^-$ was low (0.18 ± 0.05 µmol N $L^{-1}$) (Table 3). DON (21 ± 5 µmol N $L^{-1}$) was the dominant form of fixed N and accounted for 58% of TDN. Urea and DPAs combined contributed less than 5% of DON. $PO_4^{3-}$ was 1.15 ± 0.08 µmol P $L^{-1}$ and Si was consistently high. DOC ranged from 532 to 660 µmol C $L^{-1}$. DIN:$PO_4^{3-}$ ratio and DOC:DON ratio were 13 ± 1 and 29 ± 3 respectively, both suggesting slight N-limitation. Chlorophyll *a* concentrations were lower in June (5.8 µg $L^{-1}$) than in July (47.7 µg $L^{-1}$ ).

During the winter (January and March 2017), TDN was consistent at 48 ± 2 µmol N $L^{-1}$, $NH_4^+$ was 1.70 ± 0.13 µmol N $L^{-1}$, $NO_3^-$ was high and was the dominant form of fixed N (29.0 ± 2.0 µmol N $L^{-1}$), and $NO_2^-$ was low (0.10 ± 0.06 µmol N $L^{-1}$) (Table 3). DON was 17 ± 4 µmol N $L^{-1}$ and accounted for 35% of TDN. Urea and DPAs combined contributed < 4% of DON. $PO_4^{3-}$ was low, but Si was consistently high. DOC ranged

from 311 to 344 μmol C $L^{-1}$. DIN:$PO_4^{3-}$ ratio was 79 ± 6, suggesting P-limitation, while DOC:DON ratio was 20 ± 4, suggesting N-limitation. Chlorophyll *a* concentrations were slightly higher in January (4.5 μg $L^{-1}$) than in March (1.3 μg $L^{-1}$).

**Bioassay Chlorophyll *a***

In order to simplify results, only ND treatments were compared to one another and then disinfection procedures were compared within treatments.

During the summer (June and July 2016), similar patterns emerged from both samplings, although the response was greater in July 2016 (Figure 7). There was growth in all treatments including CONTs. NO showed the greatest response followed by NR and +N3. Growth in the CONT, BNPR, and BNCPR were lower, and those treatments appeared to inhibit growth compared to CONT.

With regards to disinfection within treatments, the only significant difference on the last day of the incubation was in June 2016 in NR, where ND was lower than both UV and CL ($p < 0.05$) (Figure 8). There were also a few other significant differences, in June 2016, in NO on day 3 CL was higher than both ND and UV ($p < 0.01$), and in June 2016, in BNCPR on day 3 CL was higher than both ND and UV ($p < 0.005$).

During the winter (January and March 2017), different patterns emerged from the samplings, but there was growth in all treatments including the CONTs (Figure 9). In January 2017, +N3 and the CONT were nearly identical and had the highest Chlorophyll *a* concentration on the last day of the incubation (~15 μg $L^{-1}$). NR peaked on day 7 while all others peaked on day 9. Surprisingly, NO showed the lowest response and BNPR and BNCPR seemed to inhibit growth compared to CONT. In March 2017, NO had the

highest Chlorophyll *a* by day 9, but NR peaked with a higher concentration at day 7. +N3 was similar to CONT, while BNCPR inhibited growth.

With regards to disinfection within treatments, the only significant difference on the last day of the incubation was in January 2017 in NO, where ND was higher than both UV and CL ($p < 0.01$) (Figure 10).

**Bioassay Nutrient Uptake**

In order to simplify the presentation of results, all disinfection procedures within a treatment were grouped together within each season (3 treatment-disinfection combinations x 2 samplings per season = 6 sets) to analyze trends.

During the summer (June and July 2016), TDN was consumed in all treatments (Supplemental Tables 9 and 10). $NO_3^-$ was generally the dominant form of TDN consumed in all treatments. Exceptions were NO ND and NO CL in the June 2016 bioassay, where DON was consumed slightly more than $NO_3^-$, and the NO ND and NO CL treatments in the July 2016 bioassay, where $NH_4^+$ was the dominant form consumed due to abnormally high concentrations in the effluent. DON was not consumed in CONT from the July 2106 bioassay. DON was consumed in CONT from the June 2016 bioassay and the majority of NO (5 out of the 6 sets), NR (5 out of the 6 sets), and BNPR (4 out of the 6 sets). DON was produced in the majority of BNCPR (4 out of the 6 sets). Urea and DPAs constituted a slightly lower proportion of TDN consumed in NO and NR (2 to 6%) compared to BNPR and BNCPR (3 to 14%). By the last day of the incubation, DIN and $PO_4^{3-}$ were still high in NO and NR ( > 86.0 μmol N $L^{-1}$ and > 9.00 μmol P $L^{-1}$, respectively), while they were very low, and in some cases close to the limit of detection

in BNPR and BNCPR (in most cases < 0.50 μmol N $L^{-1}$ and < 0.40 μmol P $L^{-1}$). Si was consumed in all treatments, but concentrations remained high ( > 60.0 μmol Si $L^{-1}$).

During the winter (January and March 2017), TDN was consumed in all but one of the treatments, NO ND from the January 2017 bioassay (Supplemental Tables 11 and 12) and TDN was produced in NO CL from the January 2017 bioassay. $NO_3^-$ was the dominant form of TDN consumed in all treatments except NO ND, NO CL, and BNCPR CL in the January 2017 bioassay. DON was not consumed in half of BNCPR (3 out of 6 sets). DON was consumed in both CONT, the majority of NR (5 out of 6 sets), half of the BNPR (3 out of 6 sets), and half of the BNCPR (3 out of 6 sets). DON was produced in the majority of NO (4 out of 6 sets). Urea and DPAs constituted a slightly larger proportion of TDN consumed in BNPR and BNCPR (2 to 13%) compared to NO and NR (0 to 9%). By the last day of the incubation, DIN and $PO_4^{3-}$ were high in NO and NR ( > 121.0 μmol N $L^{-1}$ and > 6.00 μmol P $L^{-1}$, respectively), while in BNPR and BNCPR, DIN was still available ( >18.2 μmol N $L^{-1}$), but $PO_4^{3-}$ was low (< 0.34 μmol P $L^{-1}$). Si was hardly consumed (< 5.00 μmol Si $L^{-1}$) or in most cases was not consumed in any treatment.

***EDON Lability – Both Site 1 and 2***

An issue that was identified upon completion of the bioassays was that some EDON additions were less than anticipated (based on the concentrations calculated as the sum of the effluent addition and the ambient concentration in the site water); a few to a degree that resulted in DON concentrations that were less than the DON concentration of CONT. Factors that may have contributed to this issue include colloid formation, matrix

effects, and high DIN:TDN ratios coupled with the dilution factor from the addition of the effluent to the site water and associated propagated error. Therefore, some sets, defined as below detection (EDON added = 0 μmol N $L^{-1}$), may be underestimates of actual lability. For this reason, the following sets were not included in the overall analysis: Site 1 NR CL from the June 2016 sampling, Site 1 NR UV from the July 2016 sampling, Site 1 NR UV from the March 2017 sampling, and Site 2 NR CL from the January 2017 sampling. Clearly an effluent addition was made as seen by an increase in $NO_3^-$, so alternative mechanisms as described above need to be explored in the future to fully constrain the chemical interactions and matrix effects.

Due to the relatively few cases of significant differences in Chlorophyll *a* concentrations among disinfections within a treatment and the lack of significant differences in effluent nutrients among disinfections within a treatment over the four samplings (with the minor exceptions of $NO_2^-$ and DPA, which both accounted for < 1% of TDN), the percentage of EDON that was labile was averaged over all four samplings to give a more realistic range.

In Site 1 bioassays, NO had the highest percent lability with up to 100% of the EDON used and an overall average of 48 ± 35% (Table 4). NR had the second highest percent lability with up to 80% of the EDON used with an overall average of 25 ± 31%. Percent lability was much lower in BNPR and BNCPR with up to 32% and 46% of the EDON used, respectively, and an overall average of 14 ± 13% and 12 ± 15%, respectively.

In Site 2 bioassays, NO had the second highest percent lability with up to 100% of the EDON used and an overall average of 17 ± 34% (Table 5). NR had the highest

percent lability with up to 100% of the EDON used and an overall average of 21 ± 31%. Percent lability was much lower in BNPR and BNCPR with up to 22% and 33% of the EDON used, respectively, and an overall average of 4 ± 7% and 4 ± 10%, respectively.

## DISCUSSION

This study investigated the bioavailability of effluents to potential receiving waters. The effluents examined here were all produced from the same starting influent so that variable has been removed. Characteristics of the effluents varied in response to the different treatment processes and disinfection procedures that they were exposed to and, thus, we anticipated a range of bioavailabilities. Our goal was to provide information to WRF managers to enable them to better identify the treatment and disinfection procedure combinations that could produce effluent that is the least bioavailable to microbes in receiving waters and thereby minimize the contribution of effluent to eutrophication.

This was a challenging study because it targeted sources with complex matrices. As a result, some data sets were not included in the final analysis. Losses of DON with the addition of effluent resulted in bioavailabilities that were below detection. The issues were likely caused by colloid formation that occurred in the effluent itself, as a result of interactions of the effluent and natural organic matter from the site water, and or produced as a result of freezing and thawing samples prior to analysis. Sattayatewa et al. (2010) found that colloidal organic N constituted up to 45% of the total N in effluent. Colloids have a size range between 0.1 and 1.2 μm, and in this study we used 0.2 μm Supor filters for final filtration; it is possible that there were some colloids present in the filtered effluent. Additionally, due to the cation exchange capacities of dissolved

compounds from both the effluent and site water, abiotic reactions could have occurred to create particulates which could have fallen out of solution and thus would not be measured. Matrix effects may have also contributed to losses from the DON pool by releasing N to the DIN pool as seen in a similar study by Filippino et al. (2011). Another possible explanation could be a combination of high DIN:TDN ratios coupled with high dilution factors that occurred as a result of the effluent addition to the site water. Lee and Westerhoff (2005) reported that when the DIN:TDN ratio was higher than 0.6, variance in DIN measurements can be greater than actual DON levels. This makes it difficult to obtain reliable DON values and further emphasizes the need for the development of a method to measure DON directly. Some of these issues may be mitigated in future studies by filtering the effluent through a 0.1 μm filter, avoid freezing samples before analysis, and include abiotic control treatments in the bioassay. Due to the factors mentioned above, EDON lability numbers may be matrix specific and may not be representative for all systems.

Results show that the NO and NR bioassays, which had high initial concentrations of DIN primarily in the form of $NO_3^-$, supported the highest phytoplankton growth in almost all of the samplings at both sites. This growth also exceeded that of the +N3 bioassay in all but one case (January 2017 Site 2), which can likely be attributed to the addition of $PO_4^{3-}$ from the effluent. This result was anticipated as it is well known that $NO_3^-$ is readily utilized by heterotrophic bacteria and phytoplankton (Bronk 2002).

Both NO and NR effluents also contained detectable amounts of DON which was added to the bioassay; characterization of the effluent was reported in Chapter 2. This DON includes protein-like soluble microbial products that are produced by bacteria

during the treatment process. These biopolymers and proteinaceous forms of DON typically exhibit a C:N ratio of 3 to 6 (Stepanauskas et al. 1999; Westerhoff and Mash 2002). The DOC:DON ratios of our effluents from NO and NR fell within that range, and were 3 and 6, respectively. In addition, Liu et al. (2011) found that hydrophilic DON with a C:N ratio around 6, was highly labile (40-85%) in a bioassay. It follows then, that the NO and NR bioassays showed the highest EDON lability of 48 ± 35% and 25 ± 31% in Site 1, and 17 ± 34% and 21 ± 31% in Site 2, respectively.

For the most part, NR effluent supported similar, but generally slightly lower phytoplankton growth in the bioassays compared to NO effluent. However, in some cases, particularly in March 2017 at Site 1, the NR bioassay growth significantly exceeded that of the NO bioassay ($p < 0.05$). Because plenty of DIN and $PO_4^{3-}$ remained to support phytoplankton growth in both bioassays, the difference may be attributed to the bioavailability of the EDON. This is supported by our findings that 15% of the EDON was labile in NO compared to 29% in NR in March 2017 at Site 1. This is further supported by findings that the pre-denitrification process, comparable to NR in this study, was prone to the production of low-molecular weight DON, which stimulated phytoplankton production more than the DON produced during the conventional activated sludge process, comparable to NO in this study, despite having a lower TDN concentration (Eom et al. 2017). On the other hand, there were cases in our study in which EDON from either NO or NR was not labile. Dignac et al. (2000) explained that the presence of complex structures, found in conventional activated sludge effluent may be refractory to microbial degradation since they are concentrated during the treatment of wastewater. In addition, they saw transformations from long aliphatic chains in the

influent to less abundant and more branched structures after biological treatment, which are likely sterically protected from biodegradation.

Phytoplankton growth in BNPR and BNCPR bioassays, both composed almost entirely of DON, generally mimicked the response of CONT for Site 1, and were generally less than CONT for Site 2, showing inhibition of growth. In addition, the EDON lability was much lower for these two advanced processes (4 to 14%) as compared to NO and NR (17 to 48%). This may be the result of greater production of more refractory EDON. Liu et al. (2011) found that hydrophobic EDON, which constitutes a significant portion of total N in WRFs with enhanced nitrogen removal, had little or no effect on algal growth during a 14-day bioassay. This humic-like hydrophobic EDON had a C:N ratio of 16, similar to that in our BNPR and BNCPR effluent, which had DOC:DON ratios of 14 and 15, respectively, as opposed to 6 in the more labile hydrophilic EDON (Liu et. al 2011). Alternatively, the lower lability of the EDON from BNPR and BNCPR could be the result of DON that entered with the influent and was impervious to treatment. In an attempt to produce $^{15}N$-labeled EDON in an advanced nutrient removal process, Yao et al. (2019) found that only a small fraction of the $^{15}N$-labeled $NH_4^+$ ended up in the EDO$^{15}N$ pool leading to the conclusion that most of the EDON must have come from the sewage itself.

It is well known that disinfection procedures can change the composition of effluent nutrients and thus bioavailability. In a 5-day biodegradability/bioavailability study using biologically active sand reactors, Chen et al. (2011) found that bioavailability of EDON decreased from 39% to 3% upon chlorination. They suggested the dominant cause to be the oxidation of proteins and amino acids to organic chloramines, some of

which are relatively stable and have disinfecting capabilities (Donnermair and Blatchley 2003).

However, in this study, alteration of effluent nutrients was minimal. Overall, with a few minor exceptions, disinfection by either UV radiation or chlorination did not significantly effect phytoplankton growth. While EDON % lability for the disinfection procedures within a treatment were not all equal, there were no apparent trends throughout the bioassays. Similarly, in a study conducted by Sattayatewa et al. (2010), they found no significant differences in concentrations of DON from UV disinfection or chlorination and postulated that chlorination was simply transforming DOM but not reducing it and that the UV intensity and exposure used by WRFs was too weak to oxidize organic matter. While our bioassays did not show any apparent harmful trends resulting from disinfection procedures, the production of nitrogenous disinfection byproducts (NDBPs) with chlorination is a major concern because some chlorinated organic compounds are carcinogenic to humans or toxic to receiving aquatic systems (Trehy et al. 1986; Bond et al. 2012).

The lability of EDON from the various treatment-disinfection combinations examined in our study (4 to 47%) seem comparable to those of similar studies reported in the literature. Yao et al. (2019) found that 7 to 16% of EDON, from processes similar to NR and BNPR in this study, was bioavailable to York River, VA microbial communities within 48 hours. Similarly, Bronk et al. (2010) found that 9% to 23% of EDON from two enhanced biological nutrient removal processes, similar to BNPR and BNCPR in this study, was bioavailable to James River, VA microbial communities within 48 hours. Filippino et al. (2011) found a higher and wider range of 31 to 96% of EDON, from two

biological nutrient removal processes, was bioavailable to Elizabeth River, VA microbial communities within 48 hours. Over the past fifteen years, the bioavailability of low TDN and high DON effluents has been the focus of numerous studies, which show that the bioavailability of EDON varies greatly from one WRF to another (e.g. Urgun-Demirtas et al. 2008; Sattayatewa et al. 2009; Qin et al. 2015; Zhang et al. 2016, Fan et al. 2017).

Experiments investigating bioavailability of EDON have their limitations because bioavailability is a subjective term that depends on a number of factors. These factors may explain the wide range of reported labilities of EDON. Bioassays cited in this paper ranged from 2 to 14 days. In some cases, EDON may not be utilized until DIN is consumed first. In the study by Eom et al. (2017), during the initial period of the bioassay (until day 5 to 8), mainly DIN was consumed, regardless of the type of effluent, while removal of low-molecular weight DON occurred more slowly (from day 8 to 12), but the latter consumption lead to a greater increase in phytoplankton biomass. Our bioassays lasted up to 9 days, and many of them, especially with additions of effluent from NO and NR, had high concentrations of DIN remaining that could continue to support growth with minimal consumption of EDON. On the other hand, bioassays with additions of BNPR and BNCPR were likely at or near $PO_4^{3-}$ limitation, which would inhibit further uptake of any N species, perhaps leading to an underestimate of EDON bioavailability. Taken together, this demonstrates that time and the concentration of other available nutrients have a profound impact on assessing bioavailability.

The bacterial and phytoplankton communities present in receiving waters also affect bioavailability. Macromolecular forms of EDON must be transformed before they stimulate algal growth (Pehlivanoglu and Sedlack 2006). Degradation of DON by

bacteria and subsequent release of DIN is typically considered the dominant process by which DON is made bioavailable to algae (Urgun-Demiritas et al. 2008). If certain bacterial species that are able to identify and breakdown the EDON are not present in receiving waters, then the EDON may appear to be refractory. However, some phytoplankton species are capable of utilizing DON directly, without the aid of bacterial breakdown, by using extracellular enzymes (Urgun-Demiritas et al. 2008). Dinoflagellates in particular appear to have a higher affinity for DON than DIN (Glibert and Terlizzi 1999). In this study, at least in the June and July 2016 bioassays at Site 1, diatoms rather than dinoflagellates seemed to dominate as seen by the drawdown of Si.

Seasonality, which is closely linked to other factors mentioned above, also affects bioavailability. Yao et al. (2019) found that DON plays a more important role in N nutrition for microbes during the summer months, when phytoplankton productivity is at its peak and DIN concentrations are lowest. Our study also showed that in most cases, EDON lability was greater in the summer compared to the winter.

Bioassays which consider only changes in bulk concentration should also be viewed with caution. Bioavailability of EDON is determined by the change in DON from the beginning to the end of an experiment (accounting for the same change in the control). However, in addition to phytoplankton uptake, DON can be released during phytoplankton growth, viral lysis, or autolysis of bacteria (Berman and Bronk 2003). Even when the concentration of EDON does not significantly decrease, the EDON pools can still undergo significant compositional transformations as shown by Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR-MS) (Mesfioui et al. 2012). In this study, there were many cases of no uptake of EDON and or production of

DON. Other bioassay studies evaluating EDON bioavailability have reported similar results (Bronk et al. 2010; Zhang et al. 2016; Fan et al. 2017). This could lead to an underestimation of the lability of EDON.

While the reduction of DIN in effluent is still the primary concern for WRFs, the concentration and quality of EDON should not be overlooked. Based on the results of effluent characterization, bioassay Chlorophyll *a* concentrations, and the EDON % lability calculated from DON uptake in bioassays, it is likely that the discharge of NO and NR effluents could lead to eutrophication in both N and P-limited receiving waters due to their high inorganic nutrient content and labile EDON. In contrast, the discharge of BNPR and BNCPR effluents, due to their low inorganic nutrients and relatively refractory DON concentrations, is less likely to contribute to eutrophication.

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Table 1. Effluent nutrient concentrations and ratios from all treatment processes. Concentrations of total dissolved nitrogen (TDN), ammonium ($NH_4^+$), nitrate ($NO_3^-$), nitrite ($NO_2^-$), dissolved organic nitrogen (DON), urea, dissolved primary amines (DPA), phosphate ($PO_4^{3-}$), dissolved organic carbon (DOC), DIN: $PO_4^{3-}$ ratio, and DOC:DON ratio in each treatment. Values are the average over four samplings plus or minus the standard deviation. BD indicates below detection.

| **NO** | TDN ($\mu$mol N $L^{-1}$) | $NH_4^+$ ($\mu$mol N $L^{-1}$) | $NO_3^-$ ($\mu$mol N $L^{-1}$) | $NO_2^-$ ($\mu$mol N $L^{-1}$) | DON ($\mu$mol N $L^{-1}$) | Urea ($\mu$mol N $L^{-1}$) | DPA ($\mu$mol N $L^{-1}$) | $PO_4^{3-}$ ($\mu$mol P $L^{-1}$) | DOC ($\mu$mol C $L^{-1}$) | $DIN:PO_4^{3-}$ | DOC:DON |
|---|---|---|---|---|---|---|---|---|---|---|---|
| May 2016 | 2174 ± 5 | 2.69 ± 0.01 | 2049 ± 17 | 3.19 ± 0.03 | 119 ± 0 | 2.9 ± 0.0 | 0.33 ± 0.01 | 120.4 ± 0.5 | 556 ± 8 | 17 ± 0 | 5 ± 0 |
| June 2016 | 2382 ± 27 | 182.5 ± 2.9 | 2048 ± 19 | 0.12 ± 0.00 | 152 ± 22 | 4.6 ± 0.0 | BD | 140.1 ± 0.4 | 816 ± 11 | 16 ± 0 | 5 ± 1 |
| December 2016 | 2215 ± 5 | 1.58 ± 0.44 | 1826 ± 10 | 3.53 ± 0.07 | 383 ± 11 | 4.3 ± 0.1 | 0.17 ± 0.10 | 56.1 ± 3.1 | 727 ± 13 | 33 ± 2 | 2 ± 0 |
| January 2017 | 2459 ± 16 | 1.47 ± 0.01 | 2021 ± 12 | 3.67 ± 0.12 | 433 ± 12 | 4.1 ± 0.0 | 0.23 ± 0.04 | 88.4 ± 3.5 | 636 ± 14 | 23 ± 1 | 1 ± 0 |
| **AVERAGE** | **2308 ± 135** | **47.1 ± 90.3** | **1986 ± 107** | **2.63 ± 1.68** | **272 ± 159** | **4.0 ± 0.7** | **0.18 ± 0.14** | **101.3 ± 36.9** | **684 ± 112** | **22 ± 8** | **3 ± 2** |

| **NR** | TDN ($\mu$mol N $L^{-1}$) | $NH_4^+$ ($\mu$mol N $L^{-1}$) | $NO_3^-$ ($\mu$mol N $L^{-1}$) | $NO_2^-$ ($\mu$mol N $L^{-1}$) | DON ($\mu$mol N $L^{-1}$) | Urea ($\mu$mol N $L^{-1}$) | DPA ($\mu$mol N $L^{-1}$) | $PO_4^{3-}$ ($\mu$mol P $L^{-1}$) | DOC ($\mu$mol C $L^{-1}$) | $DIN:PO_4^{3-}$ | DOC:DON |
|---|---|---|---|---|---|---|---|---|---|---|---|
| May 2016 | 872 ± 7 | 3.22 ± 0.01 | 781.7 ± 2.4 | 2.39 ± 0.04 | 85 ± 2 | 3.1 ± 0.1 | 0.17 ± 0.02 | 78.2 ± 1.7 | 621 ± 17 | 10 ± 0 | 7 ± 0 |
| June 2016 | 787 ± 15 | 2.58 ± 0.03 | 697.3 ± 2.6 | 2.16 ± 0.00 | 85 ± 3 | 2.8 ± 0.0 | 0.39 ± 0.05 | 62.5 ± 0.2 | 626 ± 14 | 11 ± 0 | 7 ± 0 |
| December 2016 | 822 ± 8 | 1.77 ± 0.09 | 664.4 ± 3.7 | 2.82 ± 0.00 | 153 ± 4 | 4.2 ± 0.2 | 0.45 ± 0.16 | 59.4 ± 2.5 | 744 ± 8 | 11 ± 0 | 5 ± 0 |
| January 2017 | 889 ± 5 | 3.25 ± 0.00 | 691.3 ± 2.4 | 2.25 ± 0.21 | 192 ± 3 | 3.9 ± 0.0 | 0.32 ± 0.06 | 76.9 ± 5.7 | 712 ± 8 | 9 ± 1 | 4 ± 0 |
| **AVERAGE** | **843 ± 47** | **2.70 ± 0.70** | **708.7 ± 50.7** | **2.41 ± 0.29** | **129 ± 53** | **3.5 ± 0.7** | **0.33 ± 0.12** | **69.3 ± 9.7** | **676 ± 62** | **10 ± 1** | **6 ± 2** |

| **BNPR** | TDN ($\mu$mol N $L^{-1}$) | $NH_4^+$ ($\mu$mol N $L^{-1}$) | $NO_3^-$ ($\mu$mol N $L^{-1}$) | $NO_2^-$ ($\mu$mol N $L^{-1}$) | DON ($\mu$mol N $L^{-1}$) | Urea ($\mu$mol N $L^{-1}$) | DPA ($\mu$mol N $L^{-1}$) | $PO_4^{3-}$ ($\mu$mol P $L^{-1}$) | DOC ($\mu$mol C $L^{-1}$) | $DIN:PO_4^{3-}$ | DOC:DON |
|---|---|---|---|---|---|---|---|---|---|---|---|
| May 2016 | 63 ± 3 | 1.72 ± 0.04 | 0.08 ± 0.02 | 0.16 ± 0.02 | 61 ± 0 | 2.9 ± 0.0 | 0.42 ± 0.00 | 3.00 ± 0.00 | 752 ± 8 | 1 ± 0 | 12 ± 0 |
| June 2016 | 59 ± 6 | 0.76 ± 0.06 | BD | 0.09 ± 0.01 | 58 ± 0 | 2.2 ± 0.0 | 0.14 ± 0.02 | 0.93 ± 0.07 | 789 ± 32 | 1 ± 0 | 14 ± 0 |
| December 2016 | 78 ± 2 | 1.08 ± 0.14 | 0.06 ± 0.00 | 0.16 ± 0.01 | 77 ± 0 | 4.3 ± 0.0 | 0.41 ± 0.00 | 1.58 ± 0.04 | 1050 ± 9 | 1 ± 0 | 14 ± 0 |
| January 2017 | 64 ± 2 | 0.97 ± 0.00 | 0.16 ± 0.00 | 0.22 ± 0.00 | 63 ± 0 | 3.9 ± 0.0 | 0.25 ± 0.00 | 1.65 ± 0.04 | 919 ± 15 | 1 ± 0 | 15 ± 0 |
| **AVERAGE** | **66 ± 8** | **1.13 ± 0.41** | **0.08 ± 0.07** | **0.16 ± 0.05** | **65 ± 8** | **3.3 ± 1.0** | **0.31 ± 0.13** | **1.79 ± 0.87** | **877 ± 135** | **1 ± 0** | **14 ± 1** |

| **BNCPR** | TDN ($\mu$mol N $L^{-1}$) | $NH_4^+$ ($\mu$mol N $L^{-1}$) | $NO_3^-$ ($\mu$mol N $L^{-1}$) | $NO_2^-$ ($\mu$mol N $L^{-1}$) | DON ($\mu$mol N $L^{-1}$) | Urea ($\mu$mol N $L^{-1}$) | DPA ($\mu$mol N $L^{-1}$) | $PO_4^{3-}$ ($\mu$mol P $L^{-1}$) | DOC ($\mu$mol C $L^{-1}$) | $DIN:PO_4^{3-}$ | DOC:DON |
|---|---|---|---|---|---|---|---|---|---|---|---|
| May 2016 | 47 ± 3 | 1.66 ± 0.03 | 0.11 ± 0.03 | 0.10 ± 0.00 | 45 ± 0 | 2.1 ± 0.1 | 0.12 ± 0.01 | 0.84 ± 0.03 | 663 ± 12 | 2 ± 0 | 15 ± 0 |
| June 2016 | 41 ± 3 | 0.82 ± 0.04 | 0.16 ± 0.00 | 0.06 ± 0.00 | 40 ± 0 | 1.7 ± 0.0 | 0.11 ± 0.04 | 0.45 ± 0.02 | 561 ± 5 | 2 ± 0 | 14 ± 0 |
| December 2016 | 59 ± 2 | 1.07 ± 0.06 | BD | 0.10 ± 0.00 | 58 ± 0 | 3.6 ± 0.1 | 0.22 ± 0.02 | 0.88 ± 0.04 | 815 ± 12 | 1 ± 0 | 14 ± 0 |
| January 2017 | 62 ± 1 | 1.34 ± 0.05 | 4.87 ± 0.01 | 0.51 ± 0.01 | 55 ± 0 | 3.7 ± 0.0 | 0.08 ± 0.04 | 0.88 ± 0.10 | 929 ± 13 | 8 ± 0 | 17 ± 0 |
| **AVERAGE** | **52 ± 10** | **1.22 ± 0.36** | **1.29 ± 2.39** | **0.19 ± 0.21** | **50 ± 8** | **2.8 ± 1.0** | **0.13 ± 0.06** | **0.77 ± 0.21** | **742 ± 163** | **3 ± 3** | **15 ± 1** |

Table 2. Ambient nutrient concentrations, ratios, and Chlorophyll *a* for Site 1. Ambient concentrations of total dissolved nitrogen (TDN), ammonium ($NH_4^+$), nitrate ($NO_3^-$), nitrite ($NO_2^-$), dissolved organic nitrogen (DON), urea, dissolved primary amines (DPA), phosphate ($PO_4^{3-}$), silica (Si), dissolved organic carbon (DOC), DIN: $PO_4^{3-}$ ratio, DOC:DON ratio, and Chlorophyll *a* (Chl *a*) from Site 1 over the four samplings. Values are the average plus or minus the standard deviation. BD indicates below detection.

| **Sampling** | TDN ($\mu$mol N $L^{-1}$) | $NH_4^+$ ($\mu$mol N $L^{-1}$) | $NO_3^-$ ($\mu$mol N $L^{-1}$) | $NO_2^-$ ($\mu$mol N $L^{-1}$) | DON ($\mu$mol N $L^{-1}$) | Urea ($\mu$mol N $L^{-1}$) | DPA ($\mu$mol N $L^{-1}$) | $PO_4^{3-}$ ($\mu$mol P $L^{-1}$) | Si ($\mu$mol Si $L^{-1}$) | DOC ($\mu$mol C $L^{-1}$) | DIN:$PO_4^{3-}$ | DOC:DON | Chl *a* ($\mu$g $L^{-1}$) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| June 2016 | 15 ± 1 | 0.26 ± 0.04 | 0.23 ± 0.02 | BD | 15 ± 1 | 0.2 ± 0.0 | 0.26 ± 0.02 | 0.07 ± 0.01 | 36.1 ± 0.9 | 306 ± 1 | 7 ± 1 | 20 ± 1 | 17.7 ± 1.2 |
| July 2016 | 15 ± 0 | 1.58 ± 0.00 | 0.20 ± 0.01 | BD | 13 ± 0 | 0.3 ± 0.0 | 0.22 ± 0.05 | 0.33 ± 0.00 | 35.3 ± 0.0 | 237 ± 21 | 5 ± 0 | 18 ± 1 | 41.0 ± 0.6 |
| **AVERAGE** | **15 ± 0** | **0.92 ± 0.93** | **0.22 ± 0.02** | **BD** | **14 ± 1** | **0.3 ± 0.1** | **0.24 ± 0.03** | **0.20 ± 0.18** | **35.7 ± 0.6** | **272 ± 49** | **6 ± 1** | **19 ± 2** | **29.4 ± 16.5** |
| January 2017 | 17 ± 1 | 0.26 ± 0.07 | 0.05 ± 0.01 | BD | 17 ± 1 | 0.3 ± 0.0 | 0.23 ± 0.05 | BD | 16.2 ± 0.0 | 254 ± 17 | 0 ± 0 | 15 ± 2 | 7.8 ± 1.4 |
| March 2017 | 14 ± 0 | 0.48 ± 0.02 | 0.03 ± 0.00 | BD | 13 ± 0 | 0.4 ± 0.0 | 0.22 ± 0.02 | BD | 6.04 ± 0.03 | 298 ± 13 | 0 ± 0 | 23 ± 1 | 8.7 ± 0.1 |
| **AVERAGE** | **16 ± 2** | **0.34 ± 0.20** | **0.04 ± 0.01** | **BD** | **15 ± 3** | **0.4 ± 0.1** | **0.23 ± 0.01** | **BD** | **11.1 ± 7.2** | **276 ± 31** | **0 ± 0** | **19 ± 6** | **8.3 ± 0.6** |

Table 3. Ambient nutrient concentrations, ratios, and Chlorophyll *a* for Site 2. Ambient concentrations of total dissolved nitrogen (TDN), ammonium ($NH_4^+$), nitrate ($NO_3^-$), nitrite ($NO_2^-$), dissolved organic nitrogen (DON), urea, dissolved primary amines (DPA), phosphate ($PO_4^{3-}$), silica (Si), dissolved organic carbon (DOC), DIN: $PO_4^{3-}$ ratio, DOC:DON ratio, and Chlorophyll *a* (Chl *a*) from Site 2 over the four samplings. Values are the average plus or minus the standard deviation. BD indicates below detection.

| **Sampling** | TDN ($\mu$mol N $L^{-1}$) | $NH_4^+$ ($\mu$mol N $L^{-1}$) | $NO_3^-$ ($\mu$mol N $L^{-1}$) | $NO_2^-$ ($\mu$mol N $L^{-1}$) | DON ($\mu$mol N $L^{-1}$) | Urea ($\mu$mol N $L^{-1}$) | DPA ($\mu$mol N $L^{-1}$) | $PO_4^{3-}$ ($\mu$mol P $L^{-1}$) | Si ($\mu$mol Si $L^{-1}$) | DOC ($\mu$mol C $L^{-1}$) | DIN:$PO_4^{3-}$ | DOC:DON | Chl *a* ($\mu$g $L^{-1}$) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| June 2016 | 39 ± 1 | 1.34 ± 0.06 | 13.4 ± 0.2 | 0.21 ± 0.00 | 24 ± 1 | 0.9 ± 0.0 | 0.36 ± 0.00 | 1.09 ± 0.02 | 83.1 ± 3.1 | 660 ± 7 | 14 ± 0 | 28 ± 1 | 5.8 ± 0.4 |
| July 2016 | 32 ± 0 | 0.83 ± 0.17 | 13.8 ± 0.0 | 0.14 ± 0.02 | 17 ± 0 | 0.4 ± 0.0 | BD | 1.20 ± 0.01 | 95.1 ± 1.8 | 532 ± 2 | 12 ± 0 | 31 ± 0 | 47.7 ± 2.4 |
| **AVERAGE** | **36 ± 5** | **1.09 ± 0.36** | **13.6 ± 0.3** | **0.18 ± 0.05** | **21 ± 5** | **0.7 ± 0.4** | **0.18 ± 0.25** | **1.15 ± 0.08** | **89.1 ± 8.5** | **596 ± 91** | **13 ± 1** | **29 ± 3** | **26.8 ± 29.6** |
| January 2017 | 46 ± 1 | 1.79 ± 0.01 | 30.4 ± 0.0 | 0.05 ± 0.01 | 14 ± 1 | 0.6 ± 0.0 | 0.05 ± 0.00 | 0.43 ± 0.05 | 124.4 ± 0.5 | 311 ± 5 | 75 ± 8 | 22 ± 0 | 4.5 ± 0.8 |
| March 2017 | 49 ± 1 | 1.61 ± 0.17 | 27.6 ± 0.1 | 0.14 ± 0.00 | 20 ± 1 | 0.5 ± 0.0 | BD | 0.35 ± 0.00 | 66.5 ± 3.5 | 344 ± 7 | 83 ± 1 | 17 ± 1 | 1.3 ± 0.2 |
| **AVERAGE** | **48 ± 2** | **1.70 ± 0.13** | **29.0 ± 2.0** | **0.10 ± 0.06** | **17 ± 4** | **0.6 ± 0.1** | **0.03 ± 0.04** | **0.39 ± 0.06** | **95.5 ± 40.9** | **328 ± 23** | **79 ± 6** | **20 ± 4** | **2.9 ± 2.3** |

Table 4. Lability of effluent dissolved organic nitrogen (EDON) in Site 1 bioassays. Concentrations of EDON added, EDON used, and the percentage of EDON that was labile, for Site 1 bioassays. *indicates production of EDON. **indicates more DON was used than added from effluent. ***denotes data not included in overall analysis.

| Sampling | Treatment | Disinfection | EDON added (µmol N $L^{-1}$) | EDON used (µmol N $L^{-1}$) | EDON labile (%) |
|---|---|---|---|---|---|
| June 2016 | NO | ND | 30 | 25 | 85 |
| June 2016 | NO | UV | 19 | 18 | 98 |
| June 2016 | NO | CL | 13 | 9 | 69 |
| **AVERAGE** | | | | | **84** |
| July 2016 | NO | ND | 61 | 5 | 8 |
| July 2016 | NO | UV | 23 | 13 | 58 |
| July 2016 | NO | CL | 41 | 0* | 0 |
| **AVERAGE** | | | | | **22** |
| January 2017 | NO | ND | 16 | 2 | 10 |
| January 2017 | NO | UV | 22 | 9 | 42 |
| January 2017 | NO | CL | 62 | 31 | 50 |
| **AVERAGE** | | | | | **34** |
| March 2017 | NO | ND | 52 | 9 | 17 |
| March 2017 | NO | UV | 6 | 2 | 35 |
| March 2017 | NO | CL | 8 | 8** | 100 |
| **AVERAGE** | | | | | **15** |
| ***TOTAL AVERAGE*** | | | | | ***48 ± 35*** |

| Sampling | Treatment | Disinfection | EDON added (µmol N $L^{-1}$) | EDON used (µmol N $L^{-1}$) | EDON labile (%) |
|---|---|---|---|---|---|
| June 2016 | NR | ND | 2 | 0* | 0 |
| June 2016 | NR | UV | 9 | 7 | 80 |
| June 2016 | NR | CL | 0 | 0 | 0*** |
| **AVERAGE** | | | | | **40** |
| July 2016 | NR | ND | 16 | 6 | 36 |
| July 2016 | NR | UV | 0 | 0 | 0*** |
| July 2016 | NR | CL | 7 | 0* | 0 |
| **AVERAGE** | | | | | **18** |
| January 2017 | NR | ND | 7 | 0* | 0 |
| January 2017 | NR | UV | 6 | 0* | 0 |
| January 2017 | NR | CL | 14 | 7 | 49 |
| **AVERAGE** | | | | | **16** |
| March 2017 | NR | ND | 25 | 14 | 58 |
| March 2017 | NR | UV | 0 | 0 | 0*** |
| March 2017 | NR | CL | 1 | 0* | 0 |
| **AVERAGE** | | | | | **29** |
| ***TOTAL AVERAGE*** | | | | | ***25 ± 31*** |

| Sampling | Treatment | Disinfection | EDON added (µmol N $L^{-1}$) | EDON used (µmol N $L^{-1}$) | EDON labile (%) |
|---|---|---|---|---|---|
| June 2016 | BNPR | ND | 11 | 3 | 24 |
| June 2016 | BNPR | UV | 9 | 2 | 24 |
| June 2016 | BNPR | CL | 7 | 0* | 0 |
| **AVERAGE** | | | | | **16** |
| July 2016 | BNPR | ND | 5 | 0 | 8 |
| July 2016 | BNPR | UV | 7 | 0 | 1 |
| July 2016 | BNPR | CL | 7 | 1 | 13 |
| **AVERAGE** | | | | | **8** |
| January 2017 | BNPR | ND | 6 | 0* | 0 |
| January 2017 | BNPR | UV | 9 | 1 | 0 |
| January 2017 | BNPR | CL | 11 | 4 | 31 |
| **AVERAGE** | | | | | **13** |
| March 2017 | BNPR | ND | 7 | 0* | 0 |
| March 2017 | BNPR | UV | 7 | 2 | 31 |
| March 2017 | BNPR | CL | 6 | 2 | 32 |
| **AVERAGE** | | | | | **21** |
| ***TOTAL AVERAGE*** | | | | | ***14 ± 13*** |

| Sampling | Treatment | Disinfection | EDON added (µmol N $L^{-1}$) | EDON used (µmol N $L^{-1}$) | EDON labile (%) |
|---|---|---|---|---|---|
| June 2016 | BNCPR | ND | 7 | 3 | 46 |
| June 2016 | BNCPR | UV | 6 | 1 | 17 |
| June 2016 | BNCPR | CL | 4 | 0 | 9 |
| **AVERAGE** | | | | | **24** |
| July 2016 | BNCPR | ND | 6 | 2 | 28 |
| July 2016 | BNCPR | UV | 5 | 0 | 1 |
| July 2016 | BNCPR | CL | 6 | 0 | 0 |
| **AVERAGE** | | | | | **10** |
| January 2017 | BNCPR | ND | 6 | 0 | 3 |
| January 2017 | BNCPR | UV | 11 | 1 | 5 |
| January 2017 | BNCPR | CL | 8 | 1 | 8 |
| **AVERAGE** | | | | | **6** |
| March 2017 | BNCPR | ND | 4 | 0* | 0 |
| March 2017 | BNCPR | UV | 5 | 0 | 0 |
| March 2017 | BNCPR | CL | 5 | 1 | 28 |
| **AVERAGE** | | | | | **9** |
| ***TOTAL AVERAGE*** | | | | | ***12 ± 15*** |

Table 5. Lability of effluent dissolved organic nitrogen (EDON) in Site 2 bioassays. Concentrations of EDON added, EDON used, and the percentage of EDON that was labile, for Site 2 bioassays. *indicates production of EDON. **indicates more DON was used than added from effluent. ***denotes data not included in overall analysis.

| Sampling | Treatment | Disinfection | EDON added (μmol N $L^{-1}$) | EDON used (μmol N $L^{-1}$) | EDON labile (%) |
|---|---|---|---|---|---|
| June 2016 | NO | ND | 12 | 12** | 100 |
| June 2016 | NO | UV | 5 | 4 | 77 |
| June 2016 | NO | CL | 35 | 4 | 10 |
| **AVERAGE** | | | | | **63** |
| July 2016 | NO | ND | 21 | 1 | 3 |
| July 2016 | NO | UV | 57 | 0* | 0 |
| July 2016 | NO | CL | 31 | 4 | 12 |
| **AVERAGE** | | | | | **5** |
| January 2017 | NO | ND | 41 | 0* | 0 |
| January 2017 | NO | UV | 38 | 0* | 0 |
| January 2017 | NO | CL | 14 | 0* | 0 |
| **AVERAGE** | | | | | **0** |
| March 2017 | NO | ND | 14 | 0* | 0 |
| March 2017 | NO | UV | 13 | 0* | 0 |
| March 2017 | NO | CL | 34 | 1 | 2 |
| **AVERAGE** | | | | | **1** |
| ***TOTAL AVERAGE*** | | | | | ***17 ± 34*** |

| Sampling | Treatment | Disinfection | EDON added (μmol N $L^{-1}$) | EDON used (μmol N $L^{-1}$) | EDON labile (%) |
|---|---|---|---|---|---|
| June 2016 | NR | ND | 4 | 0* | 0 |
| June 2016 | NR | UV | 2 | 2** | 100 |
| June 2016 | NR | CL | 25 | 0* | 0 |
| **AVERAGE** | | | | | **33** |
| July 2016 | NR | ND | 17 | 4 | 27 |
| July 2016 | NR | UV | 21 | 3 | 15 |
| July 2016 | NR | CL | 4 | 1 | 17 |
| **AVERAGE** | | | | | **19** |
| January 2017 | NR | ND | 17 | 0* | 0 |
| January 2017 | NR | UV | 11 | 0* | 0 |
| January 2017 | NR | CL | 0 | 0 | 0*** |
| **AVERAGE** | | | | | **0** |
| March 2017 | NR | ND | 5 | 3 | 53 |
| March 2017 | NR | UV | 5 | 0* | 0 |
| March 2017 | NR | CL | 9 | 1 | 15 |
| **AVERAGE** | | | | | **23** |
| ***TOTAL AVERAGE*** | | | | | ***21 ± 31*** |

| Sampling | Treatment | Disinfection | EDON added (μmol N $L^{-1}$) | EDON used (μmol N $L^{-1}$) | EDON labile (%) |
|---|---|---|---|---|---|
| June 2016 | BNPR | ND | 6 | 0* | 0 |
| June 2016 | BNPR | UV | 7 | 0* | 0 |
| June 2016 | BNPR | CL | 9 | 0* | 0 |
| **AVERAGE** | | | | | **0** |
| July 2016 | BNPR | ND | 10 | 2 | 22 |
| July 2016 | BNPR | UV | 8 | 1 | 13 |
| July 2016 | BNPR | CL | 7 | 0* | 0 |
| **AVERAGE** | | | | | **12** |
| January 2017 | BNPR | ND | 10 | 0* | 0 |
| January 2017 | BNPR | UV | 9 | 0* | 0 |
| January 2017 | BNPR | CL | 4 | 0* | 0 |
| **AVERAGE** | | | | | **0** |
| March 2017 | BNPR | ND | 3 | 0* | 0 |
| March 2017 | BNPR | UV | 2 | 0* | 0 |
| March 2017 | BNPR | CL | 7 | 1 | 12 |
| **AVERAGE** | | | | | **4** |
| ***TOTAL AVERAGE*** | | | | | ***4 ± 7*** |

| Sampling | Treatment | Disinfection | EDON added (μmol N $L^{-1}$) | EDON used (μmol N $L^{-1}$) | EDON labile (%) |
|---|---|---|---|---|---|
| June 2016 | BNCPR | ND | 4 | 0* | 0 |
| June 2016 | BNCPR | UV | 5 | 0* | 0 |
| June 2016 | BNCPR | CL | 4 | 0* | 0 |
| **AVERAGE** | | | | | **0** |
| July 2016 | BNCPR | ND | 5 | 0* | 0 |
| July 2016 | BNCPR | UV | 6 | 0 | 2 |
| July 2016 | BNCPR | CL | 5 | 0* | 0 |
| **AVERAGE** | | | | | **1** |
| January 2017 | BNCPR | ND | 9 | 0* | 0 |
| January 2017 | BNCPR | UV | 7 | 0* | 0 |
| January 2017 | BNCPR | CL | 7 | 2 | 33 |
| **AVERAGE** | | | | | **11** |
| March 2017 | BNCPR | ND | 3 | 0* | 0 |
| March 2017 | BNCPR | UV | 2 | 0* | 0 |
| March 2017 | BNCPR | CL | 5 | 1 | 16 |
| **AVERAGE** | | | | | **5** |
| ***TOTAL AVERAGE*** | | | | | ***4 ± 10*** |

Supplemental Table 1. Effluent nutrient concentrations and ratios of nitrification only (NO) disinfection procedures. Concentration of total dissolved nitrogen (TDN), ammonium ($NH_4^+$), nitrate ($NO_3^-$), nitrite ($NO_2^-$), dissolved organic nitrogen (DON), urea, dissolved primary amines (DPA), phosphate ($PO_4^{3-}$), dissolved organic carbon (DOC), DIN: $PO_4^{3-}$ ratio, and DOC:DON ratio in the NO process with three disinfection procedures- no disinfection (ND), ultraviolet radiation (UV), and chlorination (CL). Values are the average over four samplings plus or minus the standard deviation. BD indicates below detection.

| **ND** | TDN ($\mu$mol N $L^{-1}$) | $NH_4^+$ ($\mu$mol N $L^{-1}$) | $NO_3^-$ ($\mu$mol N $L^{-1}$) | $NO_2^-$ ($\mu$mol N $L^{-1}$) | DON ($\mu$mol N $L^{-1}$) | Urea ($\mu$mol N $L^{-1}$) | DPA ($\mu$mol N $L^{-1}$) | $PO_4^{3-}$ ($\mu$mol P $L^{-1}$) | DOC ($\mu$mol C $L^{-1}$) | DIN:$PO_4^{3-}$ | DOC:DON |
|---|---|---|---|---|---|---|---|---|---|---|---|
| May 2016 | 2174 ± 5 | 2.69 ± 0.01 | 2049 ± 17 | 3.19 ± 0.03 | 119 ± 0 | 2.9 ± 0.0 | 0.33 ± 0.01 | 120.4 ± 0.5 | 556 ± 8 | 17 ± 0 | 5 ± 0 |
| June 2016 | 2382 ± 27 | 182.5 ± 2.9 | 2048 ± 19 | 0.12 ± 0.00 | 152 ± 22 | 4.6 ± 0.0 | BD | 140.1 ± 0.4 | 816 ± 11 | 16 ± 0 | 5 ± 1 |
| December 2016 | 2215 ± 5 | 1.58 ± 0.44 | 1826 ± 10 | 3.53 ± 0.07 | 383 ± 11 | 4.3 ± 0.1 | 0.17 ± 0.10 | 56.1 ± 3.1 | 727 ± 13 | 33 ± 2 | 2 ± 0 |
| January 2017 | 2459 ± 16 | 1.47 ± 0.01 | 2021 ± 12 | 3.67 ± 0.12 | 433 ± 12 | 4.1 ± 0.0 | 0.23 ± 0.04 | 88.4 ± 3.5 | 636 ± 14 | 23 ± 1 | 1 ± 0 |
| **AVERAGE** | **2308 ± 135** | **47.1 ± 90.3** | **1986 ± 107** | **2.63 ± 1.68** | **272 ± 159** | **4.0 ± 0.7** | **0.18 ± 0.14** | **101.3 ± 36.9** | **684 ± 112** | **22 ± 8** | **3 ± 2** |

| **UV** | TDN ($\mu$mol N $L^{-1}$) | $NH_4^+$ ($\mu$mol N $L^{-1}$) | $NO_3^-$ ($\mu$mol N $L^{-1}$) | $NO_2^-$ ($\mu$mol N $L^{-1}$) | DON ($\mu$mol N $L^{-1}$) | Urea ($\mu$mol N $L^{-1}$) | DPA ($\mu$mol N $L^{-1}$) | $PO_4^{3-}$ ($\mu$mol P $L^{-1}$) | DOC ($\mu$mol C $L^{-1}$) | DIN:$PO_4^{3-}$ | DOC:DON |
|---|---|---|---|---|---|---|---|---|---|---|---|
| May 2016 | 2128 ± 41 | 2.61 ± 0.04 | 2071 ± 3 | 3.15 ± 0.13 | 51 ± 3 | 2.8 ± 0.1 | 0.18 ± 0.00 | 112.0 ± 6.4 | 564 ± 12 | 19 ± 1 | 11 ± 1 |
| June 2016 | 2406 ± 42 | 180.7 ± 3.2 | 2023 ± 29 | 0.11 ± 0.01 | 202 ± 25 | 4.6 ± 0.1 | BD | 139.3 ± 0.2 | 834 ± 16 | 16 ± 0 | 4 ± 1 |
| December 2016 | 2206 ± 7 | 1.43 ± 0.05 | 1826 ± 3 | 3.73 ± 0.02 | 375 ± 3 | 4.4 ± 0.1 | 0.17 ± 0.09 | 52.5 ± 5.9 | 725 ± 18 | 35 ± 4 | 2 ± 0 |
| January 2017 | 2445 ± 13 | 1.58 ± 0.02 | 2007 ± 6 | 3.80 ± 0.01 | 433 ± 6 | 4.1 ± 0.0 | 0.20 ± 0.04 | 86.7 ± 1.4 | 635 ± 7 | 23 ± 1 | 2 ± 0 |
| **AVERAGE** | **2296 ± 153** | **46.6 ± 89.4** | **1982 ± 107** | **2.70 ± 1.75** | **265 ± 173** | **4.0 ± 0.8** | **0.14 ± 0.09** | **97.6 ± 37.0** | **690 ± 117** | **23 ± 8** | **5 ± 4** |

| **CL** | TDN ($\mu$mol N $L^{-1}$) | $NH_4^+$ ($\mu$mol N $L^{-1}$) | $NO_3^-$ ($\mu$mol N $L^{-1}$) | $NO_2^-$ ($\mu$mol N $L^{-1}$) | DON ($\mu$mol N $L^{-1}$) | Urea ($\mu$mol N $L^{-1}$) | DPA ($\mu$mol N $L^{-1}$) | $PO_4^{3-}$ ($\mu$mol P $L^{-1}$) | DOC ($\mu$mol C $L^{-1}$) | DIN:$PO_4^{3-}$ | DOC:DON |
|---|---|---|---|---|---|---|---|---|---|---|---|
| May 2016 | 2397 ± 32 | 1.98 ± 0.08 | 2159 ± 4 | 0.10 ± 0.00 | 236 ± 3 | 3.1 ± 0.2 | 0.21 ± 0.00 | 98.1 ± 3.5 | 658 ± 12 | 22 ± 1 | 3 ± 0 |
| June 2016 | 2325 ± 6 | 180.7 ± 2.1 | 2038 ± 25 | 0.07 ± 0.00 | 107 ± 27 | 4.4 ± 0.1 | BD | 139.7 ± 0.3 | 824 ± 13 | 16 ± 0 | 8 ± 2 |
| December 2016 | 2203 ± 7 | 1.60 ± 0.07 | 1830 ± 5 | 0.08 ± 0.00 | 372 ± 5 | 4.6 ± 0.1 | 0.16 ± 0.04 | 59.3 ± 5.5 | 721 ± 14 | 31 ± 3 | 2 ± 0 |
| January 2017 | 2601 ± 5 | 1.50 ± 0.07 | 2008 ± 3 | 0.08 ± 0.00 | 591 ± 3 | 4.0 ± 0.0 | 0.26 ± 0.06 | 90.6 ± 0.6 | 668 ± 11 | 22 ± 0 | 1 ± 0 |
| **AVERAGE** | **2382 ± 167** | **46.4 ± 89.5** | **2009 ± 136** | **0.08 ± 0.01** | **327 ± 207** | **4.0 ± 0.7** | **0.16 ± 0.11** | **96.9 ± 33.1** | **718 ± 76** | **23 ± 6** | **4 ± 3** |

Supplemental Table 2. Effluent nutrient concentrations and ratios of nitrogen removal (NR) disinfection procedures. Concentration of total dissolved nitrogen (TDN), ammonium ($NH_4^+$), nitrate ($NO_3^-$), nitrite ($NO_2^-$), dissolved organic nitrogen (DON), urea, dissolved primary amines (DPA), phosphate ($PO_4^{3-}$), dissolved organic carbon (DOC), DIN: $PO_4^{3-}$ ratio, and DOC:DON ratio in the NO process with three disinfection procedures- no disinfection (ND), ultraviolet radiation (UV), and chlorination (CL). Values are the average over four samplings plus or minus the standard deviation.

| **ND** | TDN ($\mu$mol N $L^{-1}$) | $NH_4^+$ ($\mu$mol N $L^{-1}$) | $NO_3^-$ ($\mu$mol N $L^{-1}$) | $NO_2^-$ ($\mu$mol N $L^{-1}$) | DON ($\mu$mol N $L^{-1}$) | Urea ($\mu$mol N $L^{-1}$) | DPA ($\mu$mol N $L^{-1}$) | $PO_4^{3-}$ ($\mu$mol P $L^{-1}$) | DOC ($\mu$mol C $L^{-1}$) | DIN:$PO_4^{3-}$ | DOC:DON |
|---|---|---|---|---|---|---|---|---|---|---|---|
| May 2016 | 872 ± 7 | 3.22 ± 0.01 | 781.7 ± 2.4 | 2.39 ± 0.04 | 85 ± 2 | 3.1 ± 0.1 | 0.17 ± 0.02 | 78.2 ± 1.7 | 621 ± 17 | 10 ± 0 | 7 ± 0 |
| June 2016 | 787 ± 15 | 2.58 ± 0.03 | 697.3 ± 2.6 | 2.16 ± 0.00 | 85 ± 3 | 2.8 ± 0.0 | 0.39 ± 0.05 | 62.5 ± 0.2 | 626 ± 14 | 11 ± 0 | 7 ± 0 |
| December 2016 | 822 ± 8 | 1.77 ± 0.09 | 664.4 ± 3.7 | 2.82 ± 0.00 | 153 ± 4 | 4.2 ± 0.2 | 0.45 ± 0.16 | 59.4 ± 2.5 | 744 ± 8 | 11 ± 0 | 5 ± 0 |
| January 2017 | 889 ± 5 | 3.25 ± 0.00 | 691.3 ± 2.4 | 2.25 ± 0.21 | 192 ± 3 | 3.9 ± 0.0 | 0.32 ± 0.06 | 76.9 ± 5.7 | 712 ± 8 | 9 ± 1 | 4 ± 0 |
| **AVERAGE** | **843 ± 47** | **2.70 ± 0.70** | **708.7 ± 50.7** | **2.41 ± 0.29** | **129 ± 53** | **3.5 ± 0.7** | **0.33 ± 0.12** | **69.3 ± 9.7** | **676 ± 62** | **10 ± 1** | **6 ± 2** |

| **UV** | TDN ($\mu$mol N $L^{-1}$) | $NH_4^+$ ($\mu$mol N $L^{-1}$) | $NO_3^-$ ($\mu$mol N $L^{-1}$) | $NO_2^-$ ($\mu$mol N $L^{-1}$) | DON ($\mu$mol N $L^{-1}$) | Urea ($\mu$mol N $L^{-1}$) | DPA ($\mu$mol N $L^{-1}$) | $PO_4^{3-}$ ($\mu$mol P $L^{-1}$) | DOC ($\mu$mol C $L^{-1}$) | DIN:$PO_4^{3-}$ | DOC:DON |
|---|---|---|---|---|---|---|---|---|---|---|---|
| May 2016 | 793 ± 8 | 3.03 ± 0.01 | 725.4 ± 2.3 | 2.22 ± 0.04 | 63 ± 2 | 2.9 ± 0.1 | 0.27 ± 0.15 | 78.4 ± 4.1 | 577 ± 2 | 9 ± 1 | 9 ± 0 |
| June 2016 | 739 ± 1 | 2.70 ± 0.03 | 697.7 ± 1.1 | 2.21 ± 0.02 | 36 ± 1 | 2.8 ± 0.0 | 0.33 ± 0.05 | 57.0 ± 0.8 | 605 ± 11 | 12 ± 0 | 17 ± 1 |
| December 2016 | 823 ± 3 | 1.88 ± 0.07 | 661.9 ± 4.8 | 2.88 ± 0.09 | 157 ± 5 | 4.3 ± 0.0 | 0.25 ± 0.03 | 56.5 ± 6.7 | 717 ± 12 | 12 ± 1 | 5 ± 0 |
| January 2017 | 879 ± 4 | 3.31 ± 0.06 | 693.5 ± 2.6 | 2.41 ± 0.01 | 180 ± 2 | 3.9 ± 0.0 | 0.32 ± 0.03 | 75.9 ± 0.8 | 722 ± 11 | 9 ± 0 | 4 ± 0 |
| **AVERAGE** | **809 ± 58** | **2.73 ± 0.62** | **694.6 ± 26.0** | **2.43 ± 0.31** | **109 ± 70** | **3.5 ± 0.7** | **0.29 ± 0.04** | **67.0 ± 11.8** | **655 ± 75** | **11 ± 2** | **9 ± 6** |

| **CL** | TDN ($\mu$mol N $L^{-1}$) | $NH_4^+$ ($\mu$mol N $L^{-1}$) | $NO_3^-$ ($\mu$mol N $L^{-1}$) | $NO_2^-$ ($\mu$mol N $L^{-1}$) | DON ($\mu$mol N $L^{-1}$) | Urea ($\mu$mol N $L^{-1}$) | DPA ($\mu$mol N $L^{-1}$) | $PO_4^{3-}$ ($\mu$mol P $L^{-1}$) | DOC ($\mu$mol C $L^{-1}$) | DIN:$PO_4^{3-}$ | DOC:DON |
|---|---|---|---|---|---|---|---|---|---|---|---|
| May 2016 | 900 ± 3 | 1.71 ± 0.05 | 802.9 ± 0.9 | 0.08 ± 0.01 | 96 ± 1 | 3.3 ± 0.1 | 0.31 ± 0.06 | 91.3 ± 4.7 | 738 ± 20 | 9 ± 0 | 8 ± 0 |
| June 2016 | 751 ± 15 | 1.77 ± 0.06 | 694.7 ± 10.5 | 0.07 ± 0.00 | 55 ± 10 | 3.0 ± 0.0 | 0.39 ± 0.04 | 59.4 ± 0.5 | 640 ± 7 | 12 ± 0 | 12 ± 2 |
| December 2016 | 770 ± 7 | 1.58 ± 0.05 | 668.5 ± 2.2 | 0.08 ± 0.00 | 100 ± 2 | 4.1 ± 0.2 | 0.39 ± 0.02 | 63.0 ± 1.5 | 677 ± 14 | 11 ± 0 | 7 ± 0 |
| January 2017 | 865 ± 3 | 2.09 ± 0.02 | 690.1 ± 5.4 | 0.08 ± 0.00 | 173 ± 5 | 3.9 ± 0.0 | 0.39 ± 0.04 | 82.0 ± 8.1 | 737 ± 8 | 9 ± 1 | 4 ± 0 |
| **AVERAGE** | **822 ± 72** | **1.79 ± 0.22** | **714.0 ± 60.3** | **0.08 ± 0.01** | **106 ± 49** | **3.6 ± 0.5** | **0.37 ± 0.04** | **73.9 ± 15.2** | **698 ± 48** | **10 ± 2** | **8 ± 3** |

Supplemental Table 3. Effluent nutrient concentrations and ratios of biological nitrogen and phosphorus removal (BNPR) disinfection procedures. Concentration of total dissolved nitrogen (TDN), ammonium ($NH_4^+$), nitrate ($NO_3^-$), nitrite ($NO_2^-$), dissolved organic nitrogen (DON), urea, dissolved primary amines (DPA), phosphate ($PO_4^{3-}$), dissolved organic carbon (DOC), DIN: $PO_4^{3-}$ ratio, and DOC:DON ratio in the NO process with three disinfection procedures- no disinfection (ND), ultraviolet radiation (UV), and chlorination (CL). Values are the average over four samplings plus or minus the standard deviation. BD indicates below detection.

| **ND** | TDN ($\mu$mol N $L^{-1}$) | $NH_4^+$ ($\mu$mol N $L^{-1}$) | $NO_3^-$ ($\mu$mol N $L^{-1}$) | $NO_2^-$ ($\mu$mol N $L^{-1}$) | DON ($\mu$mol N $L^{-1}$) | Urea ($\mu$mol N $L^{-1}$) | DPA ($\mu$mol N $L^{-1}$) | $PO_4^{3-}$ ($\mu$mol P $L^{-1}$) | DOC ($\mu$mol C $L^{-1}$) | DIN:$PO_4^{3-}$ | DOC:DON |
|---|---|---|---|---|---|---|---|---|---|---|---|
| May 2016 | 63 ± 3 | 1.72 ± 0.04 | 0.08 ± 0.02 | 0.16 ± 0.02 | 61 ± 0 | 2.9 ± 0.0 | 0.42 ± 0.00 | 3.00 ± 0.00 | 752 ± 8 | 1 ± 0 | 12 ± 0 |
| June 2016 | 59 ± 6 | 0.76 ± 0.06 | BD | 0.09 ± 0.01 | 58 ± 0 | 2.2 ± 0.0 | 0.14 ± 0.02 | 0.93 ± 0.07 | 789 ± 32 | 1 ± 0 | 14 ± 0 |
| December 2016 | 78 ± 2 | 1.08 ± 0.14 | 0.06 ± 0.00 | 0.16 ± 0.01 | 77 ± 0 | 4.3 ± 0.0 | 0.41 ± 0.00 | 1.58 ± 0.04 | 1050 ± 9 | 1 ± 0 | 14 ± 0 |
| January 2017 | 64 ± 2 | 0.97 ± 0.00 | 0.16 ± 0.00 | 0.22 ± 0.00 | 63 ± 0 | 3.9 ± 0.0 | 0.25 ± 0.00 | 1.65 ± 0.04 | 919 ± 15 | 1 ± 0 | 15 ± 0 |
| **AVERAGE** | **66 ± 8** | **1.13 ± 0.41** | **0.08 ± 0.07** | **0.16 ± 0.05** | **65 ± 8** | **3.3 ± 1.0** | **0.31 ± 0.13** | **1.79 ± 0.87** | **877 ± 135** | **1 ± 0** | **14 ± 1** |

| **UV** | TDN ($\mu$mol N $L^{-1}$) | $NH_4^+$ ($\mu$mol N $L^{-1}$) | $NO_3^-$ ($\mu$mol N $L^{-1}$) | $NO_2^-$ ($\mu$mol N $L^{-1}$) | DON ($\mu$mol N $L^{-1}$) | Urea ($\mu$mol N $L^{-1}$) | DPA ($\mu$mol N $L^{-1}$) | $PO_4^{3-}$ ($\mu$mol P $L^{-1}$) | DOC ($\mu$mol C $L^{-1}$) | DIN:$PO_4^{3-}$ | DOC:DON |
|---|---|---|---|---|---|---|---|---|---|---|---|
| May 2016 | 56 ± 1 | 1.62 ± 0.03 | 3.77 ± 0.01 | 0.15 ± 0.00 | 51 ± 0 | 2.9 ± 0.0 | 0.45 ± 0.02 | 3.83 ± 0.03 | 739 ± 13 | 1 ± 0 | 15 ± 0 |
| June 2016 | 45 ± 1 | 0.76 ± 0.01 | 0.04 ± 0.01 | 0.10 ± 0.02 | 44 ± 0 | 2.3 ± 0.0 | 0.10 ± 0.04 | 0.92 ± 0.02 | 809 ± 34 | 1 ± 0 | 18 ± 0 |
| December 2016 | 70 ± 3 | 1.13 ± 0.03 | 0.07 ± 0.00 | 0.17 ± 0.00 | 68 ± 0 | 4.2 ± 0.1 | 0.31 ± 0.03 | 1.34 ± 0.07 | 996 ± 16 | 1 ± 0 | 15 ± 0 |
| January 2017 | 68 ± 3 | 1.04 ± 0.02 | BD | 0.15 ± 0.02 | 67 ± 0 | 3.8 ± 0.1 | 0.35 ± 0.01 | 1.58 ± 0.10 | 918 ± 5 | 1 ± 0 | 14 ± 0 |
| **AVERAGE** | **60 ± 12** | **1.14 ± 0.36** | **0.97 ± 1.87** | **0.14 ± 0.03** | **58 ± 12** | **3.3 ± 0.9** | **0.30 ± 0.15** | **1.92 ± 1.30** | **865 ± 114** | **1 ± 0** | **15 ± 2** |

| **CL** | TDN ($\mu$mol N $L^{-1}$) | $NH_4^+$ ($\mu$mol N $L^{-1}$) | $NO_3^-$ ($\mu$mol N $L^{-1}$) | $NO_2^-$ ($\mu$mol N $L^{-1}$) | DON ($\mu$mol N $L^{-1}$) | Urea ($\mu$mol N $L^{-1}$) | DPA ($\mu$mol N $L^{-1}$) | $PO_4^{3-}$ ($\mu$mol P $L^{-1}$) | DOC ($\mu$mol C $L^{-1}$) | DIN:$PO_4^{3-}$ | DOC:DON |
|---|---|---|---|---|---|---|---|---|---|---|---|
| May 2016 | 74 ± 5 | 1.72 ± 0.05 | 0.37 ± 0.02 | 0.06 ± 0.00 | 72 ± 0 | 3.3 ± 0.0 | 0.50 ± 0.07 | 3.92 ± 0.07 | 830 ± 2 | 1 ± 0 | 12 ± 0 |
| June 2016 | 48 ± 2 | 1.09 ± 0.01 | 0.15 ± 0.00 | 0.05 ± 0.01 | 47 ± 0 | 2.5 ± 0.0 | 0.29 ± 0.01 | 0.89 ± 0.03 | 761 ± 13 | 2 ± 0 | 16 ± 0 |
| December 2016 | 74 ± 1 | 1.57 ± 0.02 | 0.24 ± 0.01 | 0.07 ± 0.00 | 72 ± 0 | 4.3 ± 0.0 | 0.51 ± 0.09 | 1.57 ± 0.14 | 1053 ± 22 | 1 ± 0 | 15 ± 0 |
| January 2017 | 72 ± 2 | 1.34 ± 0.04 | 0.39 ± 0.02 | 0.08 ± 0.01 | 70 ± 0 | 3.9 ± 0.0 | 0.52 ± 0.00 | 1.72 ± 0.05 | 931 ± 7 | 1 ± 0 | 13 ± 0 |
| **AVERAGE** | **67 ± 13** | **1.43 ± 0.28** | **0.29 ± 0.11** | **0.07 ± 0.01** | **65 ± 12** | **3.5 ± 0.8** | **0.46 ± 0.11** | **2.03 ± 1.31** | **894 ± 127** | **1 ± 0** | **14 ± 2** |

Supplemental Table 4. Effluent nutrient concentrations and ratios of biological nitrogen and phosphorus removal and chemical phosphorus removal (BNCPR) disinfection procedures. Concentration of total dissolved nitrogen (TDN), ammonium ($NH_4^+$), nitrate ($NO_3^-$), nitrite ($NO_2^-$), dissolved organic nitrogen (DON), urea, dissolved primary amines (DPA), phosphate ($PO_4^{3-}$), dissolved organic carbon (DOC), DIN: $PO_4^{3-}$ ratio, and DOC:DON ratio in the NO process with three disinfection procedures- no disinfection (ND), ultraviolet radiation (UV), and chlorination (CL). Values are the average over four samplings plus or minus the standard deviation. BD indicates below detection.

| **ND** | TDN ($\mu$mol N $L^{-1}$) | $NH_4^+$ ($\mu$mol N $L^{-1}$) | $NO_3^-$ ($\mu$mol N $L^{-1}$) | $NO_2^-$ ($\mu$mol N $L^{-1}$) | DON ($\mu$mol N $L^{-1}$) | Urea ($\mu$mol N $L^{-1}$) | DPA ($\mu$mol N $L^{-1}$) | $PO_4^{3-}$ ($\mu$mol P $L^{-1}$) | DOC ($\mu$mol C $L^{-1}$) | DIN:$PO_4^{3-}$ | DOC:DON |
|---|---|---|---|---|---|---|---|---|---|---|---|
| May 2016 | 47 ± 3 | 1.66 ± 0.03 | 0.11 ± 0.03 | 0.10 ± 0.00 | 45 ± 0 | 2.1 ± 0.1 | 0.12 ± 0.01 | 0.84 ± 0.03 | 663 ± 12 | 2 ± 0 | 15 ± 0 |
| June 2016 | 41 ± 3 | 0.82 ± 0.04 | 0.16 ± 0.00 | 0.06 ± 0.00 | 40 ± 0 | 1.7 ± 0.0 | 0.11 ± 0.04 | 0.45 ± 0.02 | 561 ± 5 | 2 ± 0 | 14 ± 0 |
| December 2016 | 59 ± 2 | 1.07 ± 0.06 | BD | 0.10 ± 0.00 | 58 ± 0 | 3.6 ± 0.1 | 0.22 ± 0.02 | 0.88 ± 0.04 | 815 ± 12 | 1 ± 0 | 14 ± 0 |
| January 2017 | 62 ± 1 | 1.34 ± 0.05 | 4.87 ± 0.01 | 0.51 ± 0.01 | 55 ± 0 | 3.7 ± 0.0 | 0.08 ± 0.04 | 0.88 ± 0.10 | 929 ± 13 | 8 ± 0 | 17 ± 0 |
| **AVERAGE** | **52 ± 10** | **1.22 ± 0.36** | **1.29 ± 2.39** | **0.19 ± 0.21** | **50 ± 8** | **2.8 ± 1.0** | **0.13 ± 0.06** | **0.77 ± 0.21** | **742 ± 163** | **3 ± 3** | **15 ± 1** |

| **UV** | TDN ($\mu$mol N $L^{-1}$) | $NH_4^+$ ($\mu$mol N $L^{-1}$) | $NO_3^-$ ($\mu$mol N $L^{-1}$) | $NO_2^-$ ($\mu$mol N $L^{-1}$) | DON ($\mu$mol N $L^{-1}$) | Urea ($\mu$mol N $L^{-1}$) | DPA ($\mu$mol N $L^{-1}$) | $PO_4^{3-}$ ($\mu$mol P $L^{-1}$) | DOC ($\mu$mol C $L^{-1}$) | DIN:$PO_4^{3-}$ | DOC:DON |
|---|---|---|---|---|---|---|---|---|---|---|---|
| May 2016 | 50 ± 3 | 1.62 ± 0.01 | 10.9 ± 0.0 | 0.11 ± 0.00 | 37 ± 0 | 2.0 ± 0.0 | 0.09 ± 0.01 | 1.91 ± 0.01 | 626 ± 9 | 7 ± 0 | 17 ± 0 |
| June 2016 | 41 ± 1 | 0.88 ± 0.04 | 0.06 ± 0.00 | 0.06 ± 0.00 | 40 ± 0 | 1.8 ± 0.0 | 0.12 ± 0.01 | 0.47 ± 0.00 | 566 ± 8 | 2 ± 0 | 14 ± 0 |
| December 2016 | 58 ± 1 | 1.15 ± 0.00 | 0.06 ± 0.01 | 0.11 ± 0.00 | 57 ± 0 | 3.7 ± 0.0 | 0.20 ± 0.03 | 0.74 ± 0.04 | 821 ± 2 | 2 ± 0 | 14 ± 0 |
| January 2017 | 64 ± 1 | 1.35 ± 0.02 | 4.97 ± 0.00 | 0.51 ± 0.00 | 57 ± 0 | 3.8 ± 0.0 | 0.09 ± 0.01 | 0.86 ± 0.03 | 914 ± 11 | 8 ± 0 | 16 ± 0 |
| **AVERAGE** | **53 ± 10** | **1.25 ± 0.31** | **4.00 ± 5.15** | **0.20 ± 0.21** | **48 ± 11** | **2.8 ± 1.1** | **0.13 ± 0.05** | **1.00 ± 0.63** | **732 ± 163** | **5 ± 3** | **15 ± 1** |

| **CL** | TDN ($\mu$mol N $L^{-1}$) | $NH_4^+$ ($\mu$mol N $L^{-1}$) | $NO_3^-$ ($\mu$mol N $L^{-1}$) | $NO_2^-$ ($\mu$mol N $L^{-1}$) | DON ($\mu$mol N $L^{-1}$) | Urea ($\mu$mol N $L^{-1}$) | DPA ($\mu$mol N $L^{-1}$) | $PO_4^{3-}$ ($\mu$mol P $L^{-1}$) | DOC ($\mu$mol C $L^{-1}$) | DIN:$PO_4^{3-}$ | DOC:DON |
|---|---|---|---|---|---|---|---|---|---|---|---|
| May 2016 | 45 ± 3 | 1.66 ± 0.01 | 0.30 ± 0.00 | 0.06 ± 0.01 | 43 ± 0 | 2.5 ± 0.0 | 0.20 ± 0.06 | 1.03 ± 0.07 | 709 ± 22 | 2 ± 0 | 16 ± 0 |
| June 2016 | 44 ± 3 | 1.06 ± 0.08 | 0.14 ± 0.01 | 0.05 ± 0.01 | 42 ± 0 | 2.0 ± 0.1 | 0.31 ± 0.04 | 0.48 ± 0.01 | 564 ± 10 | 3 ± 0 | 13 ± 0 |
| December 2016 | 58 ± 1 | 1.20 ± 0.01 | 0.33 ± 0.00 | 0.06 ± 0.00 | 57 ± 0 | 3.7 ± 0.1 | 0.43 ± 0.01 | 0.87 ± 0.01 | 822 ± 12 | 2 ± 0 | 15 ± 0 |
| January 2017 | 61 ± 1 | 1.11 ± 0.02 | 5.64 ± 0.00 | 0.07 ± 0.01 | 54 ± 0 | 3.8 ± 0.1 | 0.27 ± 0.01 | 0.95 ± 0.00 | 886 ± 8 | 7 ± 0 | 16 ± 0 |
| **AVERAGE** | **52 ± 9** | **1.26 ± 0.27** | **1.60 ± 2.69** | **0.06 ± 0.01** | **49 ± 8** | **3.0 ± 0.9** | **0.30 ± 0.10** | **0.83 ± 0.24** | **745 ± 141** | **3 ± 3** | **15 ± 2** |

Supplemental Table 5. Nutrient concentrations from Site 1 – June 2016 bioassays. Starting, final, and consumed concentrations of total dissolved nitrogen (TDN), ammonium ($NH_4^+$), nitrate ($NO_3^-$), nitrite ($NO_2^-$), dissolved organic nitrogen (DON), urea, dissolved primary amines (DPA), phosphate ($PO_4^{3-}$), silica (Si), dissolved organic carbon (DOC), and DOC:DON ratio. Values are the average plus or minus the standard deviation. BD indicates below detection. ND indicates sample not determined. Negative values indicate production.

| Treatment | TDN (μmol N $L^{-1}$) | $NH_4^+$ (μmol N $L^{-1}$) | $NO_3^-$ (μmol N $L^{-1}$) | $NO_2^-$ (μmol N $L^{-1}$) | DON (μmol N $L^{-1}$) | Urea (μmol N $L^{-1}$) | DPA (μmol N $L^{-1}$) | $PO_4^{3-}$ (μmol P $L^{-1}$) | Si (μmol Si $L^{-1}$) | DOC (μmol C $L^{-1}$) | DOC:DON |
|---|---|---|---|---|---|---|---|---|---|---|---|
| CONT T0 | 13 ± 1 | 0.36 ± 0.03 | 0.40 ± 0.03 | BD | 12 ± 1 | 0.2 ± 0.0 | 0.27 ± 0.00 | 0.06 ± 0.02 | 28.3 ± 0.3 | 313 ± 18 | 25 ± 2 |
| CONT TF | 13 ± 1 | 0.15 ± 0.01 | 0.16 ± 0.02 | BD | 13 ± 1 | 0.2 ± 0.0 | 0.22 ± 0.06 | 0.04 ± 0.00 | 25.6 ± 1.3 | 329 ± 17 | 27 ± 2 |
| **CONSUMED** | **0** | **0.21** | **0.24** | **BD** | **0** | **0.0** | **0.05** | **0.02** | **2.7** | **-16** | |
| NO ND T0 | 305 ± 2 | 0.44 ± 0.01 | 262.4 ± 3.9 | 0.39 ± 0.02 | 42 ± 4 | 0.5 ± 0.0 | 0.26 ± 0.03 | 17.4 ± 0.2 | 41.5 ± 1.6 | 358 ± 13 | 9 ± 1 |
| NO ND TF | 258 ± 15 | 1.19 ± 0.94 | 237.7 ± 9.9 | 2.07 ± 0.39 | 17 ± 4 | 0.4 ± 0.2 | 0.30 ± 0.03 | 14.1 ± 1.4 | 4.79 ± 4.76 | 354 ± 15 | 21 ± 4 |
| **CONSUMED** | **47** | **-0.75** | **24.7** | **-1.68** | **25** | **0.1** | **-0.04** | **3.3** | **36.7** | **4** | |
| NO UV T0 | 302 ± 2 | 0.39 ± 0.09 | 270.7 ± 7.6 | BD | 31 ± 9 | 0.4 ± 0.0 | 0.27 ± 0.02 | 16.7 ± 0.6 | 41.1 ± 3.0 | 425 ± 22 | 15 ± 5 |
| NO UV TF | 232 ± 3 | 0.19 ± 0.01 | 217.6 ± 3.6 | 1.14 ± 0.39 | 13 ± 1 | 0.3 ± 0.0 | 0.36 ± 0.03 | 12.2 ± 0.9 | 1.54 ± 0.39 | 439 ± 9 | 35 ± 2 |
| **CONSUMED** | **70** | **0.20** | **53.1** | **-1.14** | **18** | **0.1** | **-0.09** | **4.5** | **39.6** | **-14** | |
| NO CL T0 | 288 ± 1 | 1.15 ± 0.04 | 261.1 ± 3.9 | 0.37 ± 0.03 | 25 ± 4 | 0.4 ± 0.0 | 0.37 ± 0.01 | 16.0 ± 0.3 | 38.0 ± 1.7 | 376 ± 38 | 15 ± 5 |
| NO CL TF | 241 ± 21 | 0.50 ± 0.28 | 222.8 ± 11.9 | 0.96 ± 0.62 | 17 ± 9 | 0.7 ± 0.4 | 0.30 ± 0.03 | 13.4 ± 1.5 | 3.77 ± 3.22 | 393 ± 10 | 27 ± 12 |
| **CONSUMED** | **47** | **0.65** | **38.3** | **-0.59** | **9** | **-0.3** | **0.07** | **2.6** | **34.2** | **-17** | |
| NR ND T0 | 135 ± 2 | 0.42 ± 0.07 | 120.3 ± 1.8 | 0.38 ± 0.00 | 14 ± 3 | 0.6 ± 0.0 | 0.33 ± 0.01 | 15.6 ± 0.2 | 40.7 ± 0.6 | 351 ± 5 | 23 ± 2 |
| NR ND TF | 90 ± 19 | 2.46 ± 2.19 | 71.2 ± 18.8 | 0.91 ± 0.37 | 15 ± 3 | 0.7 ± 0.3 | 0.38 ± 0.15 | 11.5 ± 2.1 | 5.71 ± 6.40 | 383 ± 19 | 26 ± 4 |
| **CONSUMED** | **45** | **-2.04** | **49.1** | **-0.53** | **-2** | **-0.1** | **-0.05** | **4.1** | **35.0** | **-32** | |
| NR UV T0 | 131 ± 1 | 0.26 ± 0.03 | 110.0 ± 8.2 | BD | 21 ± 6 | 0.6 ± 0.0 | 0.37 ± 0.02 | 14.2 ± 0.4 | 39.6 ± 2.2 | 423 ± 12 | 21 ± 6 |
| NR UV TF | 62 ± 8 | 0.08 ± 0.01 | 47.1 ± 9.3 | 0.43 ± 0.39 | 14 ± 2 | 0.4 ± 0.0 | 0.30 ± 0.08 | 7.03 ± 1.03 | 1.27 ± 0.09 | 457 ± 19 | 32 ± 5 |
| **CONSUMED** | **69** | **0.18** | **62.9** | **-0.43** | **6** | **0.2** | **0.07** | **7.2** | **38.3** | **-35** | |
| NR CL T0 | 119 ± 1 | 0.19 ± 0.02 | 106.8 ± 1.1 | 0.35 ± 0.01 | 12 ± 1 | 0.5 ± 0.0 | 0.36 ± 0.02 | 12.4 ± 0.6 | 40.4 ± 1.9 | 367 ± 36 | 31 ± 5 |
| NR CL TF | 73 ± 8 | 0.10 ± 0.06 | 55.2 ± 8.6 | 0.69 ± 0.29 | 17 ± 3 | 0.4 ± 0.1 | 0.33 ± 0.11 | 10.4 ± 0.7 | 1.81 ± 0.70 | 362 ± 11 | 22 ± 4 |
| **CONSUMED** | **46** | **0.09** | **51.6** | **-0.34** | **-5** | **0.1** | **0.03** | **2.0** | **38.6** | **5** | |
| BNPR ND T0 | 24 ± 1 | 0.67 ± 0.05 | 0.37 ± 0.01 | BD | 23 ± 1 | 0.6 ± 0.0 | 0.31 ± 0.01 | 0.63 ± 0.01 | 41.3 ± 0.3 | 395 ± 20 | 18 ± 1 |
| BNPR ND TF | 21 ± 1 | BD | 0.17 ± 0.01 | BD | 21 ± 1 | 0.3 ± 0.0 | 0.23 ± 0.00 | 0.38 ± 0.08 | 38.1 ± 0.5 | 352 ± 9 | 17 ± 1 |
| **CONSUMED** | **3** | **0.67** | **0.20** | **BD** | **2** | **0.3** | **0.08** | **0.25** | **3.2** | **43** | |
| BNPR UV T0 | 22 ± 0 | 0.22 ± 0.01 | 0.20 ± 0.01 | BD | 22 ± 0 | 0.5 ± 0.0 | 0.38 ± 0.01 | 0.55 ± 0.00 | 41.7 ± 0.7 | 454 ± 9 | 21 ± 1 |
| BNPR UV Day 3 | 20 ± 0 | BD | 0.20 ± 0.11 | BD | 20 ± 1 | ND | 0.18 ± 0.02 | 0.19 ± 0.03 | 14.8 ± 1.3 | 430 ± 14 | 21 ± 1 |
| **CONSUMED** | **2** | **0.22** | **0.00** | **BD** | **2** | | **0.20** | **0.36** | **26.9** | **24** | |
| BNPR CL T0 | 22 ± 0 | 2.04 ± 0.10 | 0.75 ± 0.01 | BD | 19 ± 1 | 0.5 ± 0.0 | 0.20 ± 0.01 | 0.59 ± 0.01 | 43.3 ± 0.2 | 329 ± 11 | 17 ± 0 |
| BNPR CL Day 3 | 20 ± 0 | BD | 0.16 ± 0.01 | BD | 20 ± 0 | ND | 0.20 ± 0.03 | 0.19 ± 0.05 | 37.4 ± 0.4 | 287 ± 4 | 17 ± 0 |
| **CONSUMED** | **2** | **2.04** | **0.59** | **BD** | **-1** | | **0.00** | **0.40** | **5.9** | **42** | |
| BNCPR ND T0 | 21 ± 0 | 1.51 ± 0.04 | 0.37 ± 0.03 | BD | 19 ± 1 | 0.4 ± 0.0 | 0.21 ± 0.02 | 0.19 ± 0.01 | 39.2 ± 0.5 | 373 ± 8 | 19 ± 0 |
| BNCPR ND TF | 18 ± 3 | 1.41 ± 0.88 | 0.18 ± 0.00 | BD | 16 ± 2 | 0.3 ± 0.0 | 0.12 ± 0.07 | 0.06 ± 0.02 | 38.9 ± 0.6 | 381 ± 73 | 24 ± 8 |
| **CONSUMED** | **3** | **0.10** | **0.19** | **BD** | **3** | **0.1** | **0.09** | **0.13** | **0.3** | **-8** | |
| BNCPR UV T0 | 20 ± 0 | 1.67 ± 0.09 | 0.22 ± 0.02 | BD | 18 ± 0 | 0.5 ± 0.0 | 0.21 ± 0.02 | 0.16 ± 0.00 | 40.7 ± 0.7 | 439 ± 4 | 24 ± 1 |
| BNCPR UV Day 3 | 18 ± 1 | 0.28 ± 0.10 | 0.14 ± 0.01 | BD | 18 ± 1 | ND | 0.20 ± 0.04 | 0.07 ± 0.01 | 22.4 ± 1.4 | 393 ± 19 | 22 ± 2 |
| **CONSUMED** | **2** | **1.39** | **0.08** | **BD** | **0** | | **0.01** | **0.09** | **18.3** | **45** | |
| BNCPR CL T0 | 20 ± 0 | 1.57 ± 1.08 | 1.87 ± 0.00 | BD | 17 ± 1 | 0.4 ± 0.0 | 0.19 ± 0.02 | 0.27 ± 0.00 | 43.3 ± 0.8 | 298 ± 5 | 18 ± 1 |
| BNCPR CL Day 3 | 17 ± 0 | 0.23 ± 0.05 | 0.16 ± 0.01 | BD | 17 ± 0 | ND | 0.14 ± 0.02 | 0.06 ± 0.01 | 35.4 ± 1.6 | 277 ± 4 | 17 ± 1 |
| **CONSUMED** | **3** | **1.34** | **1.71** | **BD** | **0** | | **0.05** | **0.21** | **7.9** | **21** | |

Supplemental Table 6. Nutrient concentrations from Site 1 – July 2016 bioassays. Starting, final, and consumed concentrations of total dissolved nitrogen (TDN), ammonium ($NH_4^+$), nitrate ($NO_3^-$), nitrite ($NO_2^-$), dissolved organic nitrogen (DON), urea, dissolved primary amines (DPA), phosphate ($PO_4^{3-}$), silica (Si), dissolved organic carbon (DOC), and DOC:DON ratio. Values are the average plus or minus the standard deviation. BD indicates below detection. Negative values indicate production.

| Treatment | TDN (μmol N $L^{-1}$) | $NH_4^+$ (μmol N $L^{-1}$) | $NO_3^-$ (μmol N $L^{-1}$) | $NO_2^-$ (μmol N $L^{-1}$) | DON (μmol N $L^{-1}$) | Urea (μmol N $L^{-1}$) | DPA (μmol N $L^{-1}$) | $PO_4^{3-}$ (μmol P $L^{-1}$) | Si (μmol Si $L^{-1}$) | DOC (μmol C $L^{-1}$) | DOC:DON |
|---|---|---|---|---|---|---|---|---|---|---|---|
| CONT T0 | 14 ± 0 | 1.62 ± 0.08 | 0.18 ± 0.00 | BD | 12 ± 0 | 0.3 ± 0.0 | 0.26 ± 0.00 | 0.24 ± 0.04 | 29.2 ± 0.8 | 189 ± 3 | 16 ± 0 |
| CONT TF | 12 ± 0 | 0.16 ± 0.02 | BD | BD | 12 ± 0 | 0.1 ± 0.0 | 0.16 ± 0.01 | 0.18 ± 0.01 | 26.8 ± 0.3 | 203 ± 7 | 17 ± 0 |
| **CONSUMED** | **2** | **1.46** | **0.18** | **BD** | **0** | **0.2** | **0.10** | **0.06** | **2.4** | **-14** | |
| NO ND T0 | 416 ± 14 | 24.0 ± 0.1 | 318.4 ± 0.0 | 0.15 ± 0.01 | 74 ± 14 | 1.0 ± 0.0 | 0.24 ± 0.13 | 20.2 ± 0.7 | 42.2 ± 0.4 | 347 ± 1 | 4 ± 0 |
| NO ND TF | 399 ± 16 | 9.92 ± 10.30 | 320.6 ± 5.6 | 0.38 ± 0.28 | 68 ± 10 | 1.3 ± 0.9 | 0.30 ± 0.10 | 18.9 ± 1.8 | 24.2 ± 14.9 | 334 ± 3 | 5 ± 1 |
| **CONSUMED** | **17** | **14.1** | **-2.2** | **-0.23** | **5** | **-0.3** | **-0.06** | **1.3** | **18.0** | **13** | |
| NO UV T0 | 389 ± 3 | 24.3 ± 1.1 | 329.2 ± 0.2 | 0.15 ± 0.03 | 35 ± 3 | 1.1 ± 0.0 | 1.02 ± 0.04 | 21.3 ± 0.2 | 42.5 ± 0.3 | 343 ± 14 | 10 ± 1 |
| NO UV TF | 359 ± 8 | 9.11 ± 5.29 | 328.0 ± 4.0 | 0.21 ± 0.03 | 22 ± 3 | 1.9 ± 0.4 | 0.22 ± 0.03 | 19.9 ± 0.5 | 24.6 ± 7.0 | 332 ± 4 | 16 ± 2 |
| **CONSUMED** | **30** | **15.2** | **1.2** | **-0.06** | **14** | **-0.8** | **0.80** | **1.4** | **17.9** | **10** | |
| NO CL T0 | 426 ± 14 | 24.6 ± 0.2 | 348.2 ± 0.5 | 0.06 ± 0.01 | 53 ± 10 | 1.1 ± 0.0 | 0.20 ± 0.04 | 21.1 ± 0.3 | 41.3 ± 1.3 | 305 ± 8 | 6 ± 6 |
| NO CL TF | 409 ± 15 | 8.37 ± 6.80 | 331.5 ± 2.3 | 0.18 ± 0.09 | 69 ± 6 | 1.6 ± 0.8 | 0.17 ± 0.14 | 19.8 ± 0.9 | 25.6 ± 8.7 | 301 ± 10 | 4 ± 0 |
| **CONSUMED** | **17** | **16.2** | **16.7** | **-0.12** | **-16** | **-0.5** | **0.03** | **1.3** | **15.7** | **4** | |
| NR ND T0 | 146 ± 3 | 2.49 ± 0.10 | 115.1 ± 7.2 | 0.36 ± 0.02 | 28 ± 2 | 0.7 ± 0.0 | 0.22 ± 0.02 | 12.8 ± 0.1 | 45.1 ± 0.2 | 324 ± 3 | 12 ± 1 |
| NR ND TF | 100 ± 12 | 0.21 ± 0.09 | 77.7 ± 12.5 | BD | 22 ± 0 | 0.4 ± 0.0 | 0.57 ± 0.46 | 9.72 ± 0.78 | 19.4 ± 10.4 | 332 ± 11 | 15 ± 0 |
| **CONSUMED** | **46** | **2.28** | **37.4** | **0.36** | **6** | **0.3** | **-0.35** | **3.1** | **25.7** | **-8** | |
| NR UV T0 | 129 ± 0 | 1.87 ± 0.01 | 118.6 ± 1.1 | 0.39 ± 0.02 | 8 ± 1 | 0.8 ± 0.0 | 0.27 ± 0.03 | 12.3 ± 0.1 | 44.6 ± 0.6 | 294 ± 6 | 36 ± 5 |
| NR UV TF | 102 ± 18 | 0.10 ± 0.02 | 90.6 ± 17.3 | BD | 11 ± 2 | 0.4 ± 0.0 | 0.41 ± 0.05 | 10.5 ± 1.3 | 28.2 ± 9.7 | 295 ± 7 | 26 ± 3 |
| **CONSUMED** | **27** | **1.78** | **28.0** | **0.39** | **-3** | **0.4** | **-0.14** | **1.8** | **16.4** | **0** | |
| NR CL T0 | 136 ± 0 | 1.69 ± 0.01 | 114.6 ± 2.1 | 0.06 ± 0.00 | 20 ± 2 | 0.9 ± 0.0 | 0.26 ± 0.00 | 12.4 ± 0.1 | 44.7 ± 0.2 | 278 ± 1 | 14 ± 2 |
| NR CL TF | 129 ± 13 | 0.14 ± 0.07 | 96.3 ± 6.1 | BD | 33 ± 17 | 0.4 ± 0.0 | 0.29 ± 0.04 | 10.3 ± 0.7 | 30.1 ± 3.7 | 289 ± 6 | 10 ± 4 |
| **CONSUMED** | **7** | **1.55** | **18.3** | **0.06** | **-13** | **0.5** | **-0.03** | **2.1** | **14.6** | **-11** | |
| BNPR ND T0 | 20 ± 0 | 2.16 ± 0.10 | 0.18 ± 0.00 | BD | 18 ± 0 | 0.7 ± 0.0 | 0.24 ± 0.02 | 0.48 ± 0.01 | 40.8 ± 1.7 | 348 ± 5 | 19 ± 0 |
| BNPR ND TF | 17 ± 0 | 0.15 ± 0.05 | BD | BD | 17 ± 0 | 0.3 ± 0.0 | 0.18 ± 0.05 | 0.24 ± 0.04 | 43.3 ± 0.7 | 299 ± 7 | 17 ± 0 |
| **CONSUMED** | **3** | **2.01** | **0.18** | **BD** | **1** | **0.4** | **0.06** | **0.24** | **-2.5** | **49** | |
| BNPR UV T0 | 21 ± 0 | 1.57 ± 0.06 | 0.15 ± 0.01 | BD | 19 ± 0 | 0.7 ± 0.0 | 0.28 ± 0.10 | 0.44 ± 0.01 | 40.9 ± 1.2 | 324 ± 4 | 17 ± 0 |
| BNPR UV TF | 19 ± 0 | 0.17 ± 0.06 | BD | BD | 19 ± 0 | 0.4 ± 0.0 | 0.14 ± 0.04 | 0.27 ± 0.04 | 40.7 ± 1.3 | 284 ± 1 | 15 ± 0 |
| **CONSUMED** | **2** | **1.40** | **0.15** | **BD** | **0** | **0.3** | **0.14** | **0.17** | **0.2** | **40** | |
| BNPR CL T0 | 21 ± 1 | 1.74 ± 0.07 | 0.18 ± 0.01 | BD | 19 ± 1 | 0.8 ± 0.1 | 0.29 ± 0.01 | 0.43 ± 0.01 | 42.1 ± 1.1 | 312 ± 3 | 16 ± 0 |
| BNPR CL TF | 18 ± 2 | 0.20 ± 0.02 | BD | BD | 18 ± 2 | 0.3 ± 0.0 | 0.13 ± 0.06 | 0.20 ± 0.02 | 41.3 ± 0.3 | 269 ± 12 | 15 ± 0 |
| **CONSUMED** | **3** | **1.54** | **0.18** | **BD** | **1** | **0.5** | **0.16** | **0.23** | **0.8** | **43** | |
| BNCPR ND T0 | 20 ± 0 | 1.97 ± 0.05 | 0.19 ± 0.01 | BD | 18 ± 0 | 0.7 ± 0.0 | 0.18 ± 0.03 | 0.35 ± 0.01 | 41.2 ± 0.3 | 306 ± 8 | 18 ± 0 |
| BNCPR ND TF | 16 ± 0 | 0.12 ± 0.03 | BD | BD | 16 ± 0 | 0.4 ± 0.0 | 0.14 ± 0.00 | 0.18 ± 0.01 | 42.9 ± 0.5 | 289 ± 4 | 18 ± 0 |
| **CONSUMED** | **4** | **1.85** | **0.19** | **BD** | **2** | **0.3** | **0.04** | **0.16** | **-1.7** | **17** | |
| BNCPR UV T0 | 19 ± 0 | 1.65 ± 0.01 | 0.16 ± 0.00 | BD | 17 ± 0 | 0.7 ± 0.1 | 0.23 ± 0.01 | 0.31 ± 0.02 | 42.0 ± 0.3 | 301 ± 4 | 17 ± 0 |
| BNCPR UV TF | 17 ± 0 | 0.21 ± 0.15 | BD | BD | 17 ± 0 | 0.3 ± 0.0 | 0.21 ± 0.04 | 0.15 ± 0.05 | 41.8 ± 0.7 | 293 ± 1 | 18 ± 0 |
| **CONSUMED** | **2** | **1.44** | **0.16** | **BD** | **0** | **0.4** | **0.02** | **0.16** | **0.2** | **8** | |
| BNCPR CL T0 | 20 ± 1 | 1.61 ± 0.01 | 0.18 ± 0.01 | BD | 18 ± 1 | 0.7 ± 0.0 | 0.21 ± 0.00 | 0.31 ± 0.01 | 43.2 ± 0.6 | 266 ± 4 | 14 ± 1 |
| BNCPR CL TF | 18 ± 0 | 0.14 ± 0.04 | BD | BD | 18 ± 0 | 0.3 ± 0.0 | 0.17 ± 0.03 | 0.15 ± 0.03 | 40.3 ± 2.0 | 245 ± 2 | 13 ± 1 |
| **CONSUMED** | **2** | **1.47** | **0.18** | **BD** | **0** | **0.4** | **0.04** | **0.16** | **2.9** | **21** | |

Supplemental Table 7. Nutrient concentrations from Site 1 – January 2017 bioassays. Starting, final, and consumed concentrations of total dissolved nitrogen (TDN), ammonium ($NH_4^+$), nitrate ($NO_3^-$), nitrite ($NO_2^-$), dissolved organic nitrogen (DON), urea, dissolved primary amines (DPA), phosphate ($PO_4^{3-}$), silica (Si), dissolved organic carbon (DOC), and DOC:DON ratio. Values are the average plus or minus the standard deviation. BD indicates below detection. Negative values indicate production.

| Treatment | TDN (μmol N $L^{-1}$) | $NH_4^+$ (μmol N $L^{-1}$) | $NO_3^-$ (μmol N $L^{-1}$) | $NO_2^-$ (μmol N $L^{-1}$) | DON (μmol N $L^{-1}$) | Urea (μmol N $L^{-1}$) | DPA (μmol N $L^{-1}$) | $PO_4^{3-}$ (μmol P $L^{-1}$) | Si (μmol Si $L^{-1}$) | DOC (μmol C $L^{-1}$) | DOC:DON |
|---|---|---|---|---|---|---|---|---|---|---|---|
| CONT T0 | 14 ± 2 | 0.79 ± 0.09 | 0.08 ± 0.01 | BD | 13 ± 2 | 0.2 ± 0.0 | 0.19 ± 0.03 | BD | 13.7 ± 0.0 | 206 ± 2 | 16 ± 2 |
| CONT TF | 12 ± 0 | BD | BD | BD | 12 ± 0 | 0.2 ± 0.1 | 0.14 ± 0.00 | BD | 9.40 ± 0.37 | 200 ± 1 | 16 ± 0 |
| **CONSUMED** | **2** | **0.79** | **0.08** | **BD** | **1** | **0.0** | **0.05** | **BD** | **4.3** | **7** | |
| NO ND T0 | 268 ± 2 | 0.72 ± 0.02 | 237.8 ± 0.8 | 0.37 ± 0.04 | 29 ± 3 | 0.4 ± 0.0 | 0.23 ± 0.04 | 7.75 ± 0.14 | 26.5 ± 0.3 | 294 ± 4 | 10 ± 1 |
| NO ND TF | 167 ± 1 | 0.08 ± 0.00 | 137.8 ± 4.9 | 2.75 ± 0.14 | 26 ± 5 | 0.4 ± 0.0 | 0.76 ± 0.14 | 0.92 ± 0.03 | 0.62 ± 0.02 | 379 ± 10 | 15 ± 2 |
| **CONSUMED** | **101** | **0.64** | **100.0** | **-2.38** | **3** | **0.0** | **-0.53** | **6.83** | **25.9** | **-85** | |
| NO UV T0 | 310 ± 1 | 0.27 ± 0.03 | 274.1 ± 2.1 | 0.47 ± 0.01 | 35 ± 3 | 0.5 ± 0.0 | 0.22 ± 0.01 | 9.12 ± 0.26 | 32.6 ± 0.8 | 311 ± 8 | 9 ± 1 |
| NO UV TF | 209 ± 4 | 0.10 ± 0.01 | 181.1 ± 3.7 | 3.12 ± 0.36 | 25 ± 1 | 0.6 ± 0.1 | 0.75 ± 0.17 | 2.08 ± 0.26 | 0.80 ± 0.09 | 379 ± 25 | 16 ± 2 |
| **CONSUMED** | **101** | **0.17** | **93.0** | **-2.65** | **10** | **-0.1** | **-0.53** | **7.04** | **31.8** | **-68** | |
| NO CL T0 | 313 ± 11 | 0.23 ± 0.02 | 237.4 ± 0.7 | BD | 75 ± 1 | 0.4 ± 0.1 | 0.28 ± 0.02 | 7.77 ± 0.17 | 32.6 ± 0.4 | 328 ± 4 | 4 ± 0 |
| NO CL TF | 187 ± 7 | 0.06 ± 0.01 | 140.8 ± 0.4 | 3.14 ± 0.42 | 43 ± 7 | 0.6 ± 0.0 | 0.58 ± 0.31 | 0.87 ± 0.25 | 0.63 ± 0.02 | 365 ± 24 | 9 ± 1 |
| **CONSUMED** | **126** | **0.17** | **96.6** | **-3.14** | **32** | **-0.2** | **-0.30** | **6.90** | **32.0** | **-37** | |
| NR ND T0 | 105 ± 1 | 0.68 ± 0.02 | 83.7 ± 1.2 | 0.27 ± 0.00 | 20 ± 1 | 0.8 ± 0.0 | 0.24 ± 0.02 | 7.99 ± 0.08 | 24.2 ± 0.3 | 292 ± 3 | 14 ± 0 |
| NR ND TF | 38 ± 3 | 0.11 ± 0.04 | 15.1 ± 2.6 | 0.45 ± 0.07 | 22 ± 1 | 0.7 ± 0.1 | 0.80 ± 0.14 | 2.32 ± 0.29 | 0.74 ± 0.13 | 361 ± 16 | 16 ± 0 |
| **CONSUMED** | **67** | **0.57** | **68.6** | **-0.18** | **-2** | **0.1** | **-0.56** | **5.67** | **23.5** | **-69** | |
| NR UV T0 | 117 ± 1 | 0.36 ± 0.04 | 97.1 ± 0.4 | 0.34 ± 0.00 | 19 ± 1 | 0.8 ± 0.0 | 0.22 ± 0.03 | 9.28 ± 0.09 | 31.9 ± 1.9 | 317 ± 2 | 17 ± 1 |
| NR UV TF | 51 ± 3 | 0.11 ± 0.01 | 28.2 ± 2.6 | 0.81 ± 0.09 | 22 ± 3 | 0.7 ± 0.0 | 0.69 ± 0.09 | 3.00 ± 0.26 | 0.85 ± 0.11 | 372 ± 9 | 17 ± 2 |
| **CONSUMED** | **66** | **0.25** | **68.9** | **-0.47** | **-3** | **0.1** | **-0.47** | **6.28** | **31.1** | **-54** | |
| NR CL T0 | 124 ± 1 | 0.30 ± 0.04 | 96.2 ± 0.3 | BD | 28 ± 0 | 0.8 ± 0.0 | 0.38 ± 0.02 | 9.14 ± 0.03 | 31.4 ± 0.5 | 297 ± 11 | 10 ± 0 |
| NR CL TF | 50 ± 8 | 0.07 ± 0.03 | 29.6 ± 6.5 | 0.99 ± 0.25 | 19 ± 1 | 0.7 ± 0.0 | 0.31 ± 0.02 | 3.04 ± 0.27 | 0.73 ± 0.05 | 309 ± 9 | 16 ± 1 |
| **CONSUMED** | **74** | **0.23** | **66.6** | **-0.99** | **8** | **0.1** | **0.07** | **6.10** | **30.7** | **-12** | |
| BNPR ND T0 | 20 ± 0 | 0.54 ± 0.04 | 0.12 ± 0.00 | BD | 19 ± 0 | 0.7 ± 0.0 | 0.32 ± 0.02 | 0.23 ± 0.00 | 24.6 ± 0.2 | 332 ± 2 | 17 ± 0 |
| BNPR ND TF | 20 ± 1 | 0.09 ± 0.02 | BD | BD | 20 ± 1 | 0.7 ± 0.0 | 0.24 ± 0.03 | BD | 24.9 ± 0.4 | 325 ± 4 | 16 ± 1 |
| **CONSUMED** | **0** | **0.45** | **0.12** | **BD** | **-1** | **0.0** | **0.08** | **0.23** | **-0.3** | **7** | |
| BNPR UV T0 | 22 ± 1 | 0.25 ± 0.00 | 0.05 ± 0.00 | BD | 22 ± 1 | 0.9 ± 0.0 | 0.26 ± 0.06 | 0.27 ± 0.00 | 28.6 ± 0.3 | 358 ± 2 | 17 ± 1 |
| BNPR UV TF | 20 ± 0 | 0.08 ± 0.03 | BD | BD | 20 ± 0 | 0.7 ± 0.0 | 0.26 ± 0.04 | BD | 28.9 ± 0.2 | 363 ± 6 | 18 ± 1 |
| **CONSUMED** | **2** | **0.17** | **0.05** | **BD** | **2** | **0.2** | **0.00** | **0.27** | **-0.3** | **-6** | |
| BNPR CL T0 | 25 ± 0 | 0.36 ± 0.04 | 0.05 ± 0.01 | BD | 25 ± 0 | 0.9 ± 0.0 | 0.31 ± 0.03 | 0.28 ± 0.01 | 34.2 ± 1.3 | 370 ± 24 | 15 ± 1 |
| BNPR CL TF | 20 ± 1 | 0.06 ± 0.01 | BD | BD | 20 ± 1 | 0.8 ± 0.0 | 0.39 ± 0.08 | BD | 31.1 ± 0.4 | 339 ± 11 | 17 ± 1 |
| **CONSUMED** | **5** | **0.30** | **0.05** | **BD** | **5** | **0.1** | **-0.08** | **0.28** | **3.1** | **31** | |
| BNCPR ND T0 | 20 ± 1 | 0.53 ± 0.09 | 0.21 ± 0.02 | BD | 19 ± 1 | 0.7 ± 0.0 | 0.16 ± 0.00 | 0.13 ± 0.00 | 21.5 ± 0.3 | 305 ± 4 | 16 ± 1 |
| BNCPR ND TF | 18 ± 0 | 0.08 ± 0.02 | BD | BD | 18 ± 0 | 0.6 ± 0.0 | 0.12 ± 0.05 | BD | 24.9 ± 0.7 | 298 ± 4 | 17 ± 0 |
| **CONSUMED** | **2** | **0.45** | **0.21** | **BD** | **1** | **0.1** | **0.04** | **0.13** | **-3.4** | **6** | |
| BNCPR UV T0 | 24 ± 0 | 0.30 ± 0.03 | 0.03 ± 0.00 | BD | 24 ± 0 | 0.8 ± 0.0 | 0.17 ± 0.02 | 0.12 ± 0.00 | 33.7 ± 0.9 | 324 ± 4 | 14 ± 0 |
| BNCPR UV TF | 22 ± 2 | 0.05 ± 0.00 | BD | BD | 22 ± 2 | 0.7 ± 0.0 | 0.13 ± 0.01 | BD | 29.9 ± 0.4 | 342 ± 27 | 16 ± 1 |
| **CONSUMED** | **2** | **0.25** | **0.03** | **BD** | **2** | **0.1** | **0.04** | **0.12** | **3.8** | **-18** | |
| BNCPR CL T0 | 21 ± 1 | 0.27 ± 0.01 | 0.06 ± 0.00 | BD | 21 ± 1 | 0.8 ± 0.0 | 0.23 ± 0.02 | 0.14 ± 0.02 | 34.1 ± 0.7 | 319 ± 34 | 16 ± 0 |
| BNCPR CL TF | 19 ± 2 | 0.06 ± 0.01 | BD | BD | 19 ± 2 | 0.8 ± 0.0 | 0.16 ± 0.02 | BD | 31.1 ± 0.6 | 316 ± 30 | 17 ± 0 |
| **CONSUMED** | **2** | **0.21** | **0.06** | **BD** | **2** | **0.0** | **0.07** | **0.14** | **3.0** | **3** | |

Supplemental Table 8. Nutrient concentrations from Site 1 – March 2017 bioassays. Starting, final, and consumed concentrations of total dissolved nitrogen (TDN), ammonium ($NH_4^+$), nitrate ($NO_3^-$), nitrite ($NO_2^-$), dissolved organic nitrogen (DON), urea, dissolved primary amines (DPA), phosphate ($PO_4^{3-}$), silica (Si), dissolved organic carbon (DOC), and DOC:DON ratio. Values are the average plus or minus the standard deviation. BD indicates below detection. Negative values indicate production.

| Treatment | TDN ($\mu$mol N $L^{-1}$) | $NH_4^+$ ($\mu$mol N $L^{-1}$) | $NO_3^-$ ($\mu$mol N $L^{-1}$) | $NO_2^-$ ($\mu$mol N $L^{-1}$) | DON ($\mu$mol N $L^{-1}$) | Urea ($\mu$mol N $L^{-1}$) | DPA ($\mu$mol N $L^{-1}$) | $PO_4^{3-}$ ($\mu$mol P $L^{-1}$) | Si ($\mu$mol Si $L^{-1}$) | DOC ($\mu$mol C $L^{-1}$) | DOC:DON |
|---|---|---|---|---|---|---|---|---|---|---|---|
| CONT T0 | 12 ± 1 | 0.37 ± 0.01 | 0.06 ± 0.01 | BD | 12 ± 1 | 0.3 ± 0.0 | 0.25 ± 0.02 | BD | 5.39 ± 0.14 | 243 ± 6 | 21 ± 1 |
| CONT TF | 13 ± 0 | 0.11 ± 0.10 | BD | BD | 13 ± 0 | 0.3 ± 0.0 | 0.20 ± 0.02 | BD | 2.15 ± 0.10 | 252 ± 11 | 21 ± 1 |
| **CONSUMED** | **-1** | **0.26** | **0.06** | BD | **-1** | **0.0** | **0.05** | **BD** | **3.24** | **-9** | |
| NO ND T0 | 327 ± 5 | 2.10 ± 0.20 | 261.5 ± 0.0 | 0.22 ± 0.01 | 63 ± 5 | 0.6 ± 0.0 | 0.17 ± 0.01 | 13.4 ± 0.2 | 23.8 ± 0.6 | 316 ± 6 | 5 ± 0 |
| NO ND TF | 229 ± 2 | 0.48 ± 0.31 | 170.2 ± 0.3 | 2.61 ± 0.21 | 56 ± 2 | 0.5 ± 0.0 | 0.24 ± 0.10 | 6.91 ± 0.09 | 0.72 ± 0.03 | 350 ± 9 | 6 ± 0 |
| **CONSUMED** | **98** | **1.62** | **91.3** | **-2.39** | **7** | **0.1** | **-0.07** | **6.5** | **23.1** | **-34** | |
| NO UV T0 | 282 ± 4 | 2.55 ± 0.04 | 261.0 ± 1.2 | 0.41 ± 0.01 | 18 ± 5 | 0.6 ± 0.0 | 0.08 ± 0.01 | 13.5 ± 0.1 | 23.2 ± 0.5 | 272 ± 4 | 16 ± 5 |
| NO UV TF | 215 ± 3 | 0.15 ± 0.02 | 195.3 ± 0.6 | 2.49 ± 0.06 | 17 ± 2 | 0.5 ± 0.0 | 0.21 ± 0.02 | 6.13 ± 0.65 | 0.73 ± 0.05 | 292 ± 2 | 17 ± 2 |
| **CONSUMED** | **67** | **2.40** | **65.7** | **-2.08** | **1** | **0.1** | **-0.13** | **7.4** | **22.5** | **-20** | |
| NO CL T0 | 282 ± 1 | 0.17 ± 0.01 | 262.1 ± 1.4 | BD | 20 ± 0 | 0.6 ± 0.0 | 0.21 ± 0.03 | 13.5 ± 0.0 | 22.0 ± 1.5 | 298 ± 11 | 15 ± 1 |
| NO CL TF | 211 ± 3 | 0.14 ± 0.06 | 204.3 ± 2.0 | 2.85 ± 0.19 | 4 ± 1 | 0.6 ± 0.0 | 0.22 ± 0.02 | 6.65 ± 0.69 | 0.76 ± 0.05 | 306 ± 2 | 77 ± 17 |
| **CONSUMED** | **71** | **0.03** | **57.8** | **-2.85** | **16** | **0.0** | **-0.01** | **6.9** | **21.2** | **-8** | |
| NR ND T0 | 143 ± 3 | 1.07 ± 0.02 | 105.4 ± 0.5 | 0.29 ± 0.00 | 36 ± 3 | 0.7 ± 0.0 | 0.26 ± 0.01 | 13.9 ± 0.1 | 27.2 ± 0.3 | 358 ± 9 | 10 ± 1 |
| NR ND TF | 58 ± 1 | 0.14 ± 0.00 | 33.6 ± 5.8 | 0.86 ± 0.16 | 23 ± 5 | 0.6 ± 0.0 | 0.31 ± 0.07 | 6.80 ± 0.31 | 0.75 ± 0.04 | 375 ± 8 | 16 ± 3 |
| **CONSUMED** | **85** | **0.93** | **71.8** | **-0.57** | **13** | **0.1** | **-0.05** | **7.1** | **26.5** | **-17** | |
| NR UV T0 | 116 ± 1 | 0.53 ± 0.04 | 103.8 ± 0.5 | 0.30 ± 0.01 | 11 ± 1 | 0.7 ± 0.0 | 0.22 ± 0.02 | 13.6 ± 0.1 | 26.7 ± 0.2 | 283 ± 3 | 16 ± 14 |
| NR UV TF | 56 ± 1 | 0.12 ± 0.01 | 37.7 ± 1.8 | 0.84 ± 0.14 | 17 ± 0 | 0.6 ± 0.0 | 0.24 ± 0.02 | 6.11 ± 1.02 | 0.78 ± 0.11 | 302 ± 2 | 18 ± 0 |
| **CONSUMED** | **60** | **0.41** | **66.1** | **-0.54** | **-6** | **0.1** | **-0.02** | **7.5** | **25.9** | **-18** | |
| NR CL T0 | 119 ± 1 | 1.98 ± 0.15 | 104.3 ± 0.4 | BD | 13 ± 2 | 0.7 ± 0.0 | 0.31 ± 0.07 | 13.4 ± 0.2 | 25.9 ± 0.1 | 295 ± 3 | 23 ± 3 |
| NR CL TF | 58 ± 2 | 0.30 ± 0.04 | 32.6 ± 9.8 | 0.69 ± 0.26 | 24 ± 8 | 0.7 ± 0.0 | 0.20 ± 0.02 | 6.75 ± 0.17 | 0.82 ± 0.02 | 309 ± 1 | 13 ± 4 |
| **CONSUMED** | **61** | **1.68** | **71.7** | **-0.69** | **-12** | **0.0** | **0.11** | **6.7** | **25.1** | **-14** | |
| BNPR ND T0 | 26 ± 0 | 7.08 ± 1.15 | 0.10 ± 0.01 | BD | 19 ± 1 | 0.8 ± 0.0 | 0.07 ± 0.03 | 0.29 ± 0.01 | 26.6 ± 0.4 | 404 ± 12 | 22 ± 1 |
| BNPR ND TF | 24 ± 0 | 1.57 ± 0.22 | BD | BD | 22 ± 0 | 0.7 ± 0.0 | 0.11 ± 0.02 | BD | 21.0 ± 0.5 | 412 ± 10 | 19 ± 1 |
| **CONSUMED** | **2** | **5.51** | **0.10** | **BD** | **-4** | **0.1** | **-0.04** | **0.29** | **5.6** | **-9** | |
| BNPR UV T0 | 21 ± 1 | 2.07 ± 0.05 | 0.04 ± 0.00 | BD | 19 ± 1 | 0.8 ± 0.0 | 0.17 ± 0.02 | 0.28 ± 0.01 | 25.9 ± 0.6 | 327 ± 1 | 11 ± 10 |
| BNPR UV TF | 20 ± 0 | 2.04 ± 0.52 | BD | BD | 18 ± 0 | 0.6 ± 0.0 | 0.11 ± 0.03 | BD | 20.9 ± 0.4 | 323 ± 1 | 18 ± 0 |
| **CONSUMED** | **1** | **0.03** | **0.04** | **BD** | **1** | **0.2** | **0.06** | **0.28** | **5.0** | **4** | |
| BNPR CL T0 | 20 ± 0 | 2.44 ± 0.23 | BD | BD | 18 ± 1 | 0.8 ± 0.0 | 0.23 ± 0.01 | 0.27 ± 0.01 | 25.7 ± 0.5 | 334 ± 4 | 18 ± 1 |
| BNPR CL TF | 19 ± 1 | 2.06 ± 1.34 | BD | BD | 17 ± 1 | 0.6 ± 0.0 | 0.12 ± 0.03 | BD | 22.9 ± 0.3 | 331 ± 4 | 19 ± 2 |
| **CONSUMED** | **1** | **0.38** | **BD** | **BD** | **1** | **0.2** | **0.11** | **0.27** | **2.8** | **3** | |
| BNCPR ND T0 | 23 ± 0 | 7.11 ± 0.37 | 0.81 ± 0.01 | BD | 15 ± 0 | 0.8 ± 0.0 | 0.00 ± 0.00 | 0.14 ± 0.01 | 25.7 ± 0.1 | 406 ± 2 | 27 ± 1 |
| BNCPR ND TF | 20 ± 0 | 1.63 ± 0.26 | BD | BD | 18 ± 1 | 0.6 ± 0.0 | 0.20 ± 0.14 | BD | 19.5 ± 0.1 | 460 ± 30 | 26 ± 3 |
| **CONSUMED** | **3** | **5.48** | **0.81** | **BD** | **-3** | **0.2** | **-0.20** | **0.14** | **6.2** | **-54** | |
| BNCPR UV T0 | 20 ± 0 | 2.96 ± 0.02 | 0.73 ± 0.01 | 0.03 ± 0.00 | 16 ± 0 | 0.8 ± 0.0 | 0.15 ± 0.01 | 0.13 ± 0.00 | 26.6 ± 0.5 | 329 ± 2 | 20 ± 0 |
| BNCPR UV TF | 19 ± 0 | 1.26 ± 0.23 | BD | BD | 18 ± 0 | 0.6 ± 0.0 | 0.15 ± 0.01 | BD | 18.9 ± 0.3 | 342 ± 5 | 19 ± 0 |
| **CONSUMED** | **1** | **1.70** | **0.73** | **0.03** | **-1** | **0.2** | **0.00** | **0.13** | **7.7** | **-13** | |
| BNCPR CL T0 | 20 ± 1 | 2.46 ± 0.17 | 0.73 ± 0.01 | BD | 17 ± 1 | 0.8 ± 0.1 | 0.17 ± 0.01 | 0.13 ± 0.00 | 26.9 ± 0.6 | 331 ± 6 | 20 ± 1 |
| BNCPR CL TF | 18 ± 0 | 1.32 ± 0.28 | BD | BD | 17 ± 0 | 0.6 ± 0.0 | 0.12 ± 0.01 | BD | 22.3 ± 0.8 | 330 ± 1 | 20 ± 0 |
| **CONSUMED** | **2** | **1.14** | **0.73** | **BD** | **0** | **0.2** | **0.05** | **0.13** | **4.6** | **1** | |

Supplemental Table 9. Nutrient concentrations from Site 2 – June 2016 bioassays. Starting, final, and consumed concentrations of total dissolved nitrogen (TDN), ammonium ($NH_4^+$), nitrate ($NO_3^-$), nitrite ($NO_2^-$), dissolved organic nitrogen (DON), urea, dissolved primary amines (DPA), phosphate ($PO_4^{3-}$), silica (Si), dissolved organic carbon (DOC), and DOC:DON ratio. Values are the average plus or minus the standard deviation. BD indicates below detection. Negative values indicate production.

| Treatment | TDN ($\mu$mol N $L^{-1}$) | $NH_4^+$ ($\mu$mol N $L^{-1}$) | $NO_3^-$ ($\mu$mol N $L^{-1}$) | $NO_2^-$ ($\mu$mol N $L^{-1}$) | DON ($\mu$mol N $L^{-1}$) | Urea ($\mu$mol N $L^{-1}$) | DPA ($\mu$mol N $L^{-1}$) | $PO_4^{3-}$ ($\mu$mol P $L^{-1}$) | Si ($\mu$mol Si $L^{-1}$) | DOC ($\mu$mol C $L^{-1}$) | DOC:DON |
|---|---|---|---|---|---|---|---|---|---|---|---|
| CONT T0 | 33 ± 1 | 1.41 ± 0.19 | 11.5 ± 0.1 | 0.14 ± 0.00 | 20 ± 0 | 0.9 ± 0.1 | 0.19 ± 0.01 | 0.96 ± 0.02 | 66.7 ± 1.1 | 758 ± 40 | 36 ± 2 |
| CONT TF | 17 ± 0 | 0.06 ± 0.03 | BD | 0.07 ± 0.00 | 17 ± 0 | 0.1 ± 0.0 | 0.10 ± 0.01 | 0.27 ± 0.00 | 65.0 ± 2.3 | 758 ± 15 | 44 ± 1 |
| **CONSUMED** | **16** | **1.35** | **11.5** | **0.07** | **3** | **0.8** | **0.10** | **0.69** | **1.7** | **0** | |
| NO ND T0 | 303 ± 4 | 1.70 ± 0.06 | 268.7 ± 9.3 | 0.48 ± 0.04 | 32 ± 7 | 1.0 ± 0.1 | 0.05 ± 0.01 | 16.6 ± 1.1 | 92.9 ± 2.4 | 679 ± 16 | 22 ± 4 |
| NO ND TF | 264 ± 6 | 0.15 ± 0.02 | 251.3 ± 4.6 | 1.05 ± 0.32 | 11 ± 7 | 0.2 ± 0.1 | 0.12 ± 0.08 | 14.0 ± 0.0 | 61.0 ± 3.4 | 677 ± 32 | 45 ± 1 |
| **CONSUMED** | **39** | **1.55** | **17.4** | **-0.57** | **21** | **0.8** | **-0.07** | **2.6** | **31.9** | **2** | |
| NO UV T0 | 274 ± 13 | 1.58 ± 0.03 | 247.2 ± 1.1 | 0.44 ± 0.02 | 25 ± 13 | 1.0 ± 0.0 | 0.05 ± 0.00 | 16.5 ± 0.6 | 93.1 ± 2.4 | 819 ± 12 | 48 ± 13 |
| NO UV TF | 257 ± 10 | 0.22 ± 0.04 | 237.8 ± 6.9 | 1.00 ± 0.33 | 18 ± 5 | 0.1 ± 0.0 | BD | 13.6 ± 0.5 | 64.2 ± 5.6 | 758 ± 17 | 45 ± 13 |
| **CONSUMED** | **17** | **1.36** | **9.4** | **-0.56** | **7** | **0.9** | **0.05** | **2.9** | **28.9** | **61** | |
| NO CL T0 | 317 ± 8 | 1.55 ± 0.02 | 260.4 ± 11.9 | 0.15 ± 0.01 | 55 ± 2 | 1.0 ± 0.0 | 0.03 ± 0.01 | 16.7 ± 1.5 | 83.9 ± 3.5 | 374 ± 40 | 7 ± 1 |
| NO CL TF | 303 ± 9 | 0.16 ± 0.03 | 253.9 ± 3.9 | 0.65 ± 0.13 | 48 ± 12 | 0.2 ± 0.0 | BD | 14.0 ± 0.2 | 61.4 ± 1.8 | 387 ± 59 | 8 ± 1 |
| **CONSUMED** | **14** | **1.39** | **6.5** | **-0.50** | **7** | **0.8** | **0.03** | **2.7** | **22.5** | **-13** | |
| NR ND T0 | 151 ± 1 | 1.58 ± 0.02 | 125.3 ± 6.2 | 0.51 ± 0.03 | 24 ± 6 | 1.4 ± 0.0 | 0.08 ± 0.07 | 16.2 ± 0.2 | 95.0 ± 2.4 | 717 ± 15 | 31 ± 7 |
| NR ND TF | 122 ± 4 | 0.05 ± 0.01 | 98.4 ± 3.3 | 0.75 ± 0.08 | 23 ± 1 | 0.3 ± 0.0 | 0.09 ± 0.03 | 12.2 ± 0.3 | 68.7 ± 1.7 | 731 ± 8 | 33 ± 1 |
| **CONSUMED** | **29** | **1.53** | **26.9** | **-0.24** | **1** | **1.1** | **-0.01** | **4.0** | **26.3** | **-14** | |
| NR UV T0 | 140 ± 8 | 1.57 ± 0.02 | 116.0 ± 1.8 | 0.45 ± 0.02 | 22 ± 6 | 1.3 ± 0.1 | 0.07 ± 0.01 | 13.2 ± 0.4 | 90.1 ± 3.1 | 724 ± 17 | 33 ± 8 |
| NR UV TF | 101 ± 19 | 0.08 ± 0.04 | 85.8 ± 11.6 | 0.83 ± 0.27 | 14 ± 12 | 0.2 ± 0.0 | BD | 10.3 ± 0.6 | 68.0 ± 7.7 | 713 ± 51 | 33 ± 0 |
| **CONSUMED** | **39** | **1.49** | **30.2** | **-0.38** | **8** | **1.1** | **0.07** | **2.9** | **22.1** | **11** | |
| NR CL T0 | 173 ± 3 | 1.25 ± 0.26 | 126.9 ± 1.2 | 0.12 ± 0.01 | 45 ± 3 | 1.1 ± 0.0 | 0.14 ± 0.11 | 14.6 ± 0.9 | 91.9 ± 1.1 | 411 ± 21 | 9 ± 1 |
| NR CL TF | 137 ± 4 | BD | 88.4 ± 9.8 | 0.71 ± 0.16 | 48 ± 7 | 0.1 ± 0.0 | BD | 12.6 ± 0.4 | 64.7 ± 1.6 | 386 ± 52 | 8 ± 1 |
| **CONSUMED** | **36** | **1.25** | **38.5** | **-0.59** | **-3** | **1.0** | **0.14** | **2.0** | **27.2** | **25** | |
| BNPR ND T0 | 39 ± 1 | 1.64 ± 0.02 | 11.5 ± 0.0 | 0.17 ± 0.01 | 26 ± 1 | 1.3 ± 0.1 | BD | 1.57 ± 0.04 | 96.1 ± 2.5 | 762 ± 18 | 29 ± 0 |
| BNPR ND TF | 25 ± 0 | 0.29 ± 0.04 | BD | 0.09 ± 0.01 | 25 ± 0 | 0.2 ± 0.0 | 0.08 ± 0.04 | 0.40 ± 0.03 | 76.6 ± 1.7 | 748 ± 4 | 30 ± 0 |
| **CONSUMED** | **14** | **1.35** | **11.5** | **0.08** | **1** | **1.1** | **-0.08** | **1.17** | **19.5** | **14** | |
| BNPR UV T0 | 41 ± 3 | 1.62 ± 0.03 | 12.1 ± 0.1 | 0.17 ± 0.02 | 27 ± 2 | 1.1 ± 0.1 | BD | 1.60 ± 0.04 | 93.5 ± 4.1 | 816 ± 18 | 30 ± 3 |
| BNPR UV TF | 29 ± 1 | 0.28 ± 0.02 | BD | 0.07 ± 0.01 | 29 ± 1 | 0.2 ± 0.0 | BD | 0.40 ± 0.01 | 74.5 ± 2.6 | 735 ± 41 | 26 ± 2 |
| **CONSUMED** | **12** | **1.34** | **12.1** | **0.10** | **-2** | **0.9** | **BD** | **1.20** | **19.0** | **81** | |
| BNPR CL T0 | 42 ± 1 | 1.53 ± 0.06 | 11.4 ± 0.1 | 0.14 ± 0.01 | 29 ± 0 | 1.3 ± 0.1 | 0.04 ± 0.03 | 1.56 ± 0.04 | 97.6 ± 1.0 | 475 ± 6 | 17 ± 0 |
| BNPR CL TF | 27 ± 4 | 0.18 ± 0.01 | BD | 0.05 ± 0.00 | 27 ± 4 | 0.1 ± 0.0 | BD | 0.39 ± 0.06 | 75.6 ± 0.6 | 511 ± 9 | 19 ± 3 |
| **CONSUMED** | **15** | **1.35** | **11.4** | **0.09** | **2** | **1.2** | **0.04** | **1.17** | **22.0** | **-35** | |
| BNCPR ND T0 | 37 ± 1 | 1.72 ± 0.01 | 11.5 ± 0.0 | 0.16 ± 0.01 | 24 ± 1 | 1.1 ± 0.1 | BD | 1.13 ± 0.01 | 97.7 ± 2.2 | 728 ± 6 | 31 ± 1 |
| BNCPR ND TF | 23 ± 1 | 0.21 ± 0.01 | BD | 0.08 ± 0.01 | 23 ± 1 | 0.1 ± 0.1 | BD | 0.24 ± 0.00 | 75.2 ± 0.4 | 719 ± 7 | 32 ± 1 |
| **CONSUMED** | **14** | **1.51** | **11.5** | **0.08** | **1** | **1.0** | **BD** | **0.89** | **22.5** | **10** | |
| BNCPR UV T0 | 40 ± 0 | 1.64 ± 0.06 | 13.1 ± 0.1 | 0.16 ± 0.01 | 25 ± 0 | 1.1 ± 0.0 | BD | 1.24 ± 0.01 | 96.3 ± 0.8 | 701 ± 18 | 28 ± 1 |
| BNCPR UV TF | 27 ± 0 | 0.29 ± 0.03 | BD | 0.09 ± 0.02 | 27 ± 0 | 0.2 ± 0.1 | BD | 0.28 ± 0.02 | 75.6 ± 1.0 | 676 ± 4 | 25 ± 1 |
| **CONSUMED** | **13** | **1.35** | **13.1** | **0.07** | **-2** | **0.9** | **BD** | **0.96** | **20.7** | **25** | |
| BNCPR CL T0 | 37 ± 3 | 1.57 ± 0.08 | 11.5 ± 0.0 | 0.16 ± 0.00 | 24 ± 1 | 1.2 ± 0.1 | BD | 1.14 ± 0.05 | 96.8 ± 0.8 | 772 ± 55 | 32 ± 3 |
| BNCPR CL TF | 29 ± 1 | 0.17 ± 0.03 | BD | 0.07 ± 0.01 | 29 ± 1 | 0.2 ± 0.0 | BD | 0.24 ± 0.01 | 76.6 ± 0.6 | 686 ± 8 | 24 ± 0 |
| **CONSUMED** | **8** | **1.40** | **11.5** | **0.09** | **-5** | **1.0** | **BD** | **0.90** | **20.2** | **86** | |

Supplemental Table 10. Nutrient concentrations from Site 2 – July 2016 bioassays. Starting, final, and consumed concentrations of total dissolved nitrogen (TDN), ammonium ($NH_4^+$), nitrate ($NO_3^-$), nitrite ($NO_2^-$), dissolved organic nitrogen (DON), urea, dissolved primary amines (DPA), phosphate ($PO_4^{3-}$), silica (Si), dissolved organic carbon (DOC), and DOC:DON ratio. Values are the average plus or minus the standard deviation. BD indicates below detection. Negative values indicate production.

| Treatment | TDN (μmol N $L^{-1}$) | $NH_4^+$ (μmol N $L^{-1}$) | $NO_3^-$ (μmol N $L^{-1}$) | $NO_2^-$ (μmol N $L^{-1}$) | DON (μmol N $L^{-1}$) | Urea (μmol N $L^{-1}$) | DPA (μmol N $L^{-1}$) | $PO_4^{3-}$ (μmol P $L^{-1}$) | Si (μmol Si $L^{-1}$) | DOC (μmol C $L^{-1}$) | DOC:DON |
|---|---|---|---|---|---|---|---|---|---|---|---|
| CONT T0 | 27 ± 0 | 0.61 ± 0.05 | 11.5 ± 0.3 | 0.12 ± 0.01 | 15 ± 0 | 0.3 ± 0.0 | BD | 1.01 ± 0.06 | 79.7 ± 0.3 | 457 ± 7 | 32 ± 1 |
| CONT TF | 15 ± 0 | 0.17 ± 0.03 | BD | BD | 15 ± 0 | 0.1 ± 0.0 | BD | 0.37 ± 0.02 | 71.0 ± 0.3 | 462 ± 3 | 31 ± 1 |
| **CONSUMED** | **12** | **0.44** | **11.5** | **0.12** | **0** | **0.2** | **BD** | **0.64** | **8.7** | **-5** | |
| NO ND T0 | 395 ± 0 | 24.1 ± 0.9 | 334.9 ± 2.5 | BD | 36 ± 3 | 1.1 ± 0.0 | BD | 20.0 ± 0.6 | 86.4 ± 1.8 | 479 ± 28 | 13 ± 2 |
| NO ND TF | 371 ± 2 | 0.25 ± 0.04 | 334.8 ± 2.7 | 0.49 ± 0.07 | 36 ± 2 | 0.3 ± 0.0 | 0.07 ± 0.05 | 17.2 ± 0.5 | 81.4 ± 0.8 | 529 ± 4 | 15 ± 1 |
| **CONSUMED** | **24** | **23.9** | **0.1** | **-0.49** | **1** | **0.8** | **-0.07** | **2.8** | **5.0** | **-50** | |
| NO UV T0 | 434 ± 2 | 24.4 ± 0.4 | 337.4 ± 1.3 | BD | 72 ± 3 | 1.1 ± 0.0 | BD | 19.5 ± 0.1 | 84.5 ± 2.6 | 559 ± 2 | 8 ± 0 |
| NO UV TF | 407 ± 5 | 1.52 ± 1.65 | 306.1 ± 10.6 | 0.42 ± 0.08 | 99 ± 8 | 0.3 ± 0.0 | 0.04 ± 0.01 | 16.8 ± 1.6 | 80.8 ± 4.2 | 555 ± 5 | 6 ± 0 |
| **CONSUMED** | **27** | **22.9** | **31.3** | **-0.42** | **-27** | **0.8** | **-0.04** | **2.7** | **3.7** | **4** | |
| NO CL T0 | 395 ± 7 | 24.5 ± 0.2 | 324.8 ± 0.2 | BD | 46 ± 7 | 1.0 ± 0.1 | BD | 19.6 ± 0.6 | 87.6 ± 2.1 | 588 ± 6 | 13 ± 2 |
| NO CL TF | 364 ± 4 | 1.53 ± 1.79 | 320.1 ± 13.3 | 0.35 ± 0.03 | 42 ± 11 | 0.3 ± 0.0 | 0.15 ± 0.04 | 16.5 ± 1.3 | 82.6 ± 5.5 | 580 ± 5 | 14 ± 4 |
| **CONSUMED** | **31** | **23.0** | **4.7** | **-0.35** | **3** | **0.7** | **-0.15** | **3.1** | **5.0** | **8** | |
| NR ND T0 | 150 ± 1 | 1.21 ± 0.32 | 117.0 ± 0.0 | 0.14 ± 0.08 | 32 ± 1 | 0.7 ± 0.0 | 0.04 ± 0.00 | 12.2 ± 0.0 | 92.2 ± 3.4 | 504 ± 5 | 16 ± 1 |
| NR ND TF | 128 ± 3 | 0.24 ± 0.09 | 100.2 ± 3.9 | 0.37 ± 0.03 | 27 ± 4 | 0.2 ± 0.0 | BD | 10.1 ± 0.5 | 84.5 ± 0.8 | 510 ± 8 | 19 ± 3 |
| **CONSUMED** | **22** | **0.97** | **16.8** | **-0.23** | **4** | **0.5** | **0.04** | **2.1** | **7.7** | **-6** | |
| NR UV T0 | 154 ± 2 | 0.73 ± 0.05 | 117.4 ± 1.9 | 0.20 ± 0.02 | 36 ± 0 | 0.7 ± 0.0 | 0.05 ± 0.01 | 11.9 ± 0.0 | 95.5 ± 3.3 | 516 ± 38 | 14 ± 1 |
| NR UV TF | 135 ± 4 | 0.05 ± 0.02 | 101.8 ± 6.2 | 0.35 ± 0.07 | 33 ± 3 | 0.3 ± 0.0 | 0.04 ± 0.07 | 10.3 ± 0.2 | 87.8 ± 2.6 | 542 ± 1 | 17 ± 1 |
| **CONSUMED** | **19** | **0.68** | **15.6** | **-0.15** | **3** | **0.4** | **0.01** | **1.6** | **7.7** | **-27** | |
| NR CL T0 | 136 ± 1 | 0.62 ± 0.07 | 116.7 ± 2.1 | BD | 19 ± 1 | 0.7 ± 0.0 | BD | 11.5 ± 0.4 | 96.1 ± 3.2 | 568 ± 4 | 30 ± 2 |
| NR CL TF | 112 ± 0 | 0.54 ± 0.64 | 93.2 ± 8.8 | 0.13 ± 0.14 | 18 ± 9 | 0.3 ± 0.0 | BD | 9.04 ± 0.17 | 84.3 ± 4.8 | 564 ± 7 | 35 ± 14 |
| **CONSUMED** | **24** | **0.08** | **23.5** | **-0.13** | **1** | **0.4** | **BD** | **2.5** | **11.8** | **4** | |
| BNPR ND T0 | 37 ± 1 | 0.48 ± 0.06 | 11.4 ± 0.0 | 0.14 ± 0.01 | 25 ± 1 | 0.6 ± 0.2 | BD | 1.24 ± 0.03 | 93.2 ± 1.2 | 551 ± 5 | 22 ± 0 |
| BNPR ND TF | 23 ± 0 | 0.02 ± 0.01 | 0.16 ± 0.01 | BD | 23 ± 0 | 0.2 ± 0.0 | BD | 0.35 ± 0.03 | 87.1 ± 3.9 | 514 ± 12 | 23 ± 1 |
| **CONSUMED** | **14** | **0.46** | **11.2** | **0.14** | **2** | **0.4** | **BD** | **0.89** | **6.1** | **37** | |
| BNPR UV T0 | 35 ± 0 | 0.50 ± 0.12 | 11.5 ± 0.0 | 0.13 ± 0.01 | 23 ± 0 | 0.7 ± 0.1 | BD | 1.22 ± 0.03 | 92.2 ± 2.3 | 573 ± 7 | 25 ± 0 |
| BNPR UV TF | 22 ± 1 | BD | 0.11 ± 0.01 | BD | 22 ± 1 | 0.2 ± 0.1 | BD | 0.35 ± 0.04 | 85.9 ± 1.6 | 534 ± 3 | 25 ± 1 |
| **CONSUMED** | **13** | **0.50** | **11.4** | **0.13** | **1** | **0.5** | **BD** | **0.87** | **6.3** | **39** | |
| BNPR CL T0 | 34 ± 4 | 0.53 ± 0.05 | 11.6 ± 0.0 | 0.12 ± 0.00 | 22 ± 4 | 0.6 ± 0.0 | BD | 1.23 ± 0.01 | 92.3 ± 2.0 | 600 ± 5 | 29 ± 6 |
| BNPR CL TF | 23 ± 0 | BD | 0.12 ± 0.02 | BD | 23 ± 0 | 0.3 ± 0.0 | BD | 0.37 ± 0.02 | 85.6 ± 2.3 | 557 ± 14 | 24 ± 1 |
| **CONSUMED** | **11** | **0.53** | **11.5** | **0.12** | **-1** | **0.3** | **BD** | **0.86** | **6.7** | **43** | |
| BNCPR ND T0 | 32 ± 3 | 0.92 ± 0.12 | 11.5 ± 0.0 | 0.12 ± 0.01 | 19 ± 3 | 0.6 ± 0.0 | BD | 1.12 ± 0.03 | 93.0 ± 2.1 | 530 ± 7 | 25 ± 2 |
| BNCPR ND TF | 22 ± 0 | 0.37 ± 0.05 | 0.08 ± 0.03 | BD | 22 ± 0 | 0.2 ± 0.1 | BD | 0.34 ± 0.08 | 80.8 ± 5.9 | 523 ± 10 | 24 ± 1 |
| **CONSUMED** | **10** | **0.55** | **11.4** | **0.12** | **-2** | **0.4** | **BD** | **0.78** | **12.2** | **8** | |
| BNCPR UV T0 | 33 ± 0 | 0.87 ± 0.02 | 11.4 ± 0.1 | 0.12 ± 0.01 | 21 ± 0 | 0.5 ± 0.0 | BD | 1.10 ± 0.01 | 90.9 ± 2.1 | 536 ± 1 | 26 ± 0 |
| BNCPR UV TF | 21 ± 1 | 0.39 ± 0.02 | BD | BD | 21 ± 1 | 0.2 ± 0.0 | BD | 0.38 ± 0.04 | 88.3 ± 2.2 | 521 ± 18 | 25 ± 0 |
| **CONSUMED** | **12** | **0.48** | **11.4** | **0.12** | **0** | **0.3** | **BD** | **0.72** | **2.6** | **15** | |
| BNCPR CL T0 | 32 ± 0 | 0.71 ± 0.04 | 11.6 ± 0.1 | 0.11 ± 0.01 | 20 ± 0 | 0.5 ± 0.0 | BD | 1.06 ± 0.04 | 93.8 ± 2.3 | 567 ± 2 | 28 ± 0 |
| BNCPR CL TF | 21 ± 1 | BD | 0.04 ± 0.04 | BD | 21 ± 1 | 0.2 ± 0.0 | BD | 0.31 ± 0.03 | 84.3 ± 0.8 | 557 ± 6 | 27 ± 1 |
| **CONSUMED** | **11** | **0.71** | **11.6** | **0.11** | **-1** | **0.3** | **BD** | **0.75** | **9.5** | **11** | |

Supplemental Table 11. Nutrient concentrations from Site 2 – January 2017 bioassays. Starting, final, and consumed concentrations of total dissolved nitrogen (TDN), ammonium ($NH_4^+$), nitrate ($NO_3^-$), nitrite ($NO_2^-$), dissolved organic nitrogen (DON), urea, dissolved primary amines (DPA), phosphate ($PO_4^{3-}$), silica (Si), dissolved organic carbon (DOC), and DOC:DON ratio. Values are the average plus or minus the standard deviation. BD indicates below detection. Negative values indicate production.

| Treatment | TDN (μmol N $L^{-1}$) | $NH_4^+$ (μmol N $L^{-1}$) | $NO_3^-$ (μmol N $L^{-1}$) | $NO_2^-$ (μmol N $L^{-1}$) | DON (μmol N $L^{-1}$) | Urea (μmol N $L^{-1}$) | DPA (μmol N $L^{-1}$) | $PO_4^{3-}$ (μmol P $L^{-1}$) | Si (μmol Si $L^{-1}$) | DOC (μmol C $L^{-1}$) | DOC:DON |
|---|---|---|---|---|---|---|---|---|---|---|---|
| CONT T0 | 38 ± 1 | 1.54 ± 0.03 | 25.9 ± 0.1 | 0.07 ± 0.00 | 11 ± 1 | 0.5 ± 0.0 | BD | 0.36 ± 0.02 | 102.2 ± 2.6 | 261 ± 9 | 26 ± 2 |
| CONT TF | 30 ± 1 | BD | 23.2 ± 0.1 | 0.07 ± 0.01 | 7 ± 0 | 0.2 ± 0.0 | BD | 0.24 ± 0.01 | 105.0 ± 2.1 | 260 ± 4 | 39 ± 3 |
| **CONSUMED** | **8** | **1.54** | **2.7** | **0.00** | **4** | **0.3** | **BD** | **0.12** | **-2.8** | **1** | |
| NO ND T0 | 310 ± 1 | 1.75 ± 0.05 | 255.7 ± 0.9 | 0.57 ± 0.01 | 52 ± 2 | 1.0 ± 0.0 | 0.04 ± 0.01 | 7.30 ± 0.22 | 101.3 ± 5.4 | 341 ± 5 | 7 ± 0 |
| NO ND TF | 310 ± 2 | 0.13 ± 0.03 | 256.4 ± 0.5 | 0.56 ± 0.01 | 53 ± 2 | 0.9 ± 0.0 | BD | 7.36 ± 0.50 | 114.8 ± 3.9 | 342 ± 7 | 7 ± 0 |
| **CONSUMED** | **0** | **1.62** | **-0.7** | **0.01** | **-1** | **0.1** | **0.04** | **-0.06** | **-13.5** | **-1** | |
| NO UV T0 | 307 ± 3 | 2.05 ± 0.03 | 255.5 ± 1.0 | 0.57 ± 0.02 | 49 ± 2 | 1.0 ± 0.0 | 0.04 ± 0.02 | 7.65 ± 0.39 | 110.2 ± 7.6 | 406 ± 5 | 8 ± 0 |
| NO UV TF | 306 ± 3 | 0.42 ± 0.07 | 253.6 ± 1.5 | 0.58 ± 0.01 | 51 ± 2 | 1.0 ± 0.0 | BD | 6.80 ± 0.13 | 109.3 ± 6.1 | 368 ± 9 | 7 ± 0 |
| **CONSUMED** | **1** | **1.63** | **1.9** | **-0.01** | **-3** | **0.0** | **0.04** | **0.85** | **0.9** | **37** | |
| NO CL T0 | 283 ± 3 | 1.79 ± 0.08 | 256.7 ± 0.7 | 0.15 ± 0.01 | 24 ± 3 | 1.0 ± 0.0 | 0.05 ± 0.01 | 8.10 ± 0.24 | 111.9 ± 8.4 | 339 ± 16 | 14 ± 2 |
| NO CL TF | 286 ± 3 | 0.34 ± 0.25 | 258.1 ± 0.5 | 0.16 ± 0.02 | 27 ± 2 | 1.1 ± 0.1 | BD | 7.60 ± 0.16 | 118.8 ± 2.7 | 316 ± 6 | 12 ± 1 |
| **CONSUMED** | **-3** | **1.45** | **-1.4** | **-0.01** | **-3** | **-0.1** | **0.05** | **0.50** | **-6.9** | **23** | |
| NR ND T0 | 154 ± 0 | 1.83 ± 0.00 | 123.8 ± 0.4 | 0.51 ± 0.02 | 28 ± 1 | 1.1 ± 0.0 | 0.10 ± 0.05 | 8.93 ± 0.64 | 119.7 ± 1.0 | 352 ± 4 | 13 ± 0 |
| NR ND TF | 148 ± 1 | 0.11 ± 0.02 | 120.6 ± 0.6 | 0.55 ± 0.01 | 27 ± 1 | 0.7 ± 0.0 | 0.07 ± 0.01 | 8.85 ± 0.05 | 120.5 ± 2.5 | 356 ± 9 | 13 ± 0 |
| **CONSUMED** | **6** | **1.72** | **3.2** | **-0.04** | **1** | **0.4** | **0.03** | **0.08** | **-0.8** | **-3** | |
| NR UV T0 | 147 ± 4 | 1.81 ± 0.02 | 123.4 ± 0.1 | 0.52 ± 0.02 | 21 ± 3 | 1.0 ± 0.0 | 0.09 ± 0.01 | 8.17 ± 0.42 | 120.0 ± 2.9 | 372 ± 1 | 18 ± 3 |
| NR UV TF | 142 ± 1 | 0.13 ± 0.02 | 120.7 ± 1.0 | 0.57 ± 0.04 | 21 ± 0 | 0.6 ± 0.0 | 0.05 ± 0.01 | 8.73 ± 0.10 | 119.8 ± 2.4 | 378 ± 1 | 18 ± 0 |
| **CONSUMED** | **5** | **1.68** | **2.7** | **-0.05** | **1** | **0.4** | **0.04** | **-0.56** | **0.2** | **-6** | |
| NR CL T0 | 135 ± 2 | 1.88 ± 0.06 | 124.2 ± 0.2 | 0.14 ± 0.01 | 9 ± 2 | 0.9 ± 0.0 | 0.09 ± 0.01 | 8.78 ± 0.75 | 122.3 ± 2.6 | 316 ± 3 | 37 ± 7 |
| NR CL TF | 131 ± 2 | 0.11 ± 0.01 | 122.5 ± 1.0 | 0.18 ± 0.02 | 8 ± 2 | 0.6 ± 0.1 | 0.03 ± 0.03 | 8.45 ± 0.52 | 119.8 ± 3.3 | 327 ± 7 | 44 ± 14 |
| **CONSUMED** | **4** | **1.77** | **1.7** | **-0.04** | **1** | **0.3** | **0.06** | **0.33** | **2.5** | **-10** | |
| BNPR ND T0 | 48 ± 1 | 1.65 ± 0.06 | 25.8 ± 0.0 | 0.04 ± 0.01 | 20 ± 1 | 1.0 ± 0.0 | 0.10 ± 0.01 | 0.63 ± 0.02 | 114.9 ± 5.2 | 396 ± 3 | 19 ± 0 |
| BNPR ND TF | 41 ± 2 | 0.25 ± 0.05 | 20.2 ± 0.4 | 1.05 ± 0.00 | 20 ± 1 | 0.8 ± 0.0 | 0.09 ± 0.01 | 0.33 ± 0.01 | 118.5 ± 5.9 | 368 ± 5 | 19 ± 1 |
| **CONSUMED** | **7** | **1.40** | **5.6** | **-1.01** | **1** | **0.2** | **0.01** | **0.30** | **-3.6** | **28** | |
| BNPR UV T0 | 47 ± 2 | 1.68 ± 0.18 | 25.7 ± 0.1 | 0.08 ± 0.01 | 20 ± 1 | 1.0 ± 0.0 | 0.07 ± 0.02 | 0.64 ± 0.01 | 123.1 ± 1.0 | 402 ± 4 | 20 ± 2 |
| BNPR UV TF | 41 ± 0 | 0.35 ± 0.09 | 20.4 ± 0.5 | 1.10 ± 0.05 | 19 ± 0 | 0.9 ± 0.0 | 0.08 ± 0.01 | 0.34 ± 0.01 | 124.7 ± 1.3 | 385 ± 3 | 20 ± 0 |
| **CONSUMED** | **6** | **1.33** | **5.3** | **-1.02** | **0** | **0.1** | **-0.01** | **0.30** | **-1.6** | **17** | |
| BNPR CL T0 | 42 ± 0 | 1.62 ± 0.22 | 25.7 ± 0.1 | 0.05 ± 0.00 | 15 ± 0 | 1.0 ± 0.0 | 0.11 ± 0.02 | 0.67 ± 0.02 | 123.5 ± 1.9 | 370 ± 45 | 25 ± 3 |
| BNPR CL TF | 37 ± 0 | 1.84 ± 0.96 | 20.9 ± 0.4 | 1.06 ± 0.04 | 13 ± 1 | 1.1 ± 0.0 | BD | 0.35 ± 0.01 | 119.0 ± 5.8 | 338 ± 5 | 26 ± 1 |
| **CONSUMED** | **5** | **-0.22** | **4.8** | **-1.01** | **1** | **-0.1** | **0.11** | **0.32** | **4.5** | **31** | |
| BNCPR ND T0 | 47 ± 1 | 1.68 ± 0.02 | 25.9 ± 0.0 | 0.05 ± 0.00 | 19 ± 1 | 1.1 ± 0.0 | 0.10 ± 0.02 | 0.54 ± 0.01 | 119.3 ± 1.9 | 377 ± 8 | 19 ± 1 |
| BNCPR ND TF | 41 ± 1 | 0.33 ± 0.26 | 22.1 ± 0.2 | 0.47 ± 0.01 | 18 ± 1 | 0.9 ± 0.0 | 0.03 ± 0.01 | 0.31 ± 0.01 | 121.3 ± 2.8 | 362 ± 6 | 20 ± 1 |
| **CONSUMED** | **6** | **1.35** | **3.8** | **-0.42** | **1** | **0.2** | **0.07** | **0.23** | **-2.0** | **15** | |
| BNCPR UV T0 | 45 ± 1 | 1.57 ± 0.06 | 25.5 ± 0.1 | 0.06 ± 0.00 | 18 ± 1 | 1.1 ± 0.0 | 0.04 ± 0.01 | 0.52 ± 0.02 | 123.5 ± 3.9 | 382 ± 5 | 21 ± 1 |
| BNCPR UV TF | 42 ± 0 | 0.47 ± 0.39 | 23.0 ± 0.4 | 0.46 ± 0.01 | 18 ± 1 | 0.9 ± 0.1 | 0.04 ± 0.05 | 0.33 ± 0.02 | 125.7 ± 1.8 | 372 ± 11 | 21 ± 1 |
| **CONSUMED** | **3** | **1.10** | **2.5** | **-0.40** | **0** | **0.2** | **0.00** | **0.19** | **-2.2** | **10** | |
| BNCPR CL T0 | 45 ± 2 | 1.48 ± 0.17 | 25.8 ± 0.0 | 0.06 ± 0.00 | 18 ± 1 | 1.1 ± 0.0 | 0.07 ± 0.03 | 0.49 ± 0.00 | 121.4 ± 3.3 | 354 ± 3 | 20 ± 2 |
| BNCPR CL TF | 35 ± 1 | 0.16 ± 0.06 | 22.8 ± 0.3 | 0.51 ± 0.02 | 12 ± 1 | 0.9 ± 0.0 | 0.04 ± 0.01 | 0.31 ± 0.02 | 122.6 ± 2.8 | 325 ± 11 | 29 ± 2 |
| **CONSUMED** | **10** | **1.32** | **3.0** | **-0.45** | **6** | **0.2** | **0.03** | **0.18** | **-1.2** | **29** | |

Supplemental Table 12. Nutrient concentrations from Site 2 – March 2017 bioassays. Starting, final, and consumed concentrations of total dissolved nitrogen (TDN), ammonium ($NH_4^+$), nitrate ($NO_3^-$), nitrite ($NO_2^-$), dissolved organic nitrogen (DON), urea, dissolved primary amines (DPA), phosphate ($PO_4^{3-}$), silica (Si), dissolved organic carbon (DOC), and DOC:DON ratio. Values are the average plus or minus the standard deviation. BD indicates below detection. Negative values indicate production.

| Treatment | TDN (μmol N $L^{-1}$) | $NH_4^+$ (μmol N $L^{-1}$) | $NO_3^-$ (μmol N $L^{-1}$) | $NO_2^-$ (μmol N $L^{-1}$) | DON (μmol N $L^{-1}$) | Urea (μmol N $L^{-1}$) | DPA (μmol N $L^{-1}$) | $PO_4^{3-}$ (μmol P $L^{-1}$) | Si (μmol Si $L^{-1}$) | DOC (μmol C $L^{-1}$) | DOC:DON |
|---|---|---|---|---|---|---|---|---|---|---|---|
| CONT T0 | 38 ± 0 | 1.29 ± 0.02 | 23.1 ± 0.1 | 0.11 ± 0.00 | 14 ± 0 | 0.4 ± 0.0 | BD | 0.30 ± 0.02 | 62.8 ± 1.8 | 344 ± 5 | 26 ± 1 |
| CONT TF | 33 ± 0 | BD | 20.5 ± 0.4 | 0.16 ± 0.01 | 12 ± 0 | 0.1 ± 0.0 | 0.06 ± 0.02 | 0.21 ± 0.01 | 63.8 ± 4.1 | 397 ± 26 | 34 ± 3 |
| **CONSUMED** | **5** | **1.29** | **2.6** | **-0.05** | **1** | **0.3** | **-0.06** | **0.09** | **-1.0** | **-53** | |
| NO ND T0 | 283 ± 4 | 1.54 ± 0.04 | 253.1 ± 4.5 | 0.42 ± 0.00 | 28 ± 8 | 0.8 ± 0.0 | BD | 13.1 ± 0.1 | 81.1 ± 3.1 | 427 ± 24 | 16 ± 6 |
| NO ND TF | 273 ± 3 | 0.05 ± 0.02 | 245.0 ± 3.9 | 0.49 ± 0.02 | 27 ± 2 | 0.4 ± 0.0 | BD | 12.0 ± 0.2 | 78.3 ± 2.5 | 442 ± 31 | 16 ± 3 |
| **CONSUMED** | **10** | **1.49** | **8.0** | **-0.07** | **1** | **0.4** | **BD** | **1.1** | **2.8** | **-15** | |
| NO UV T0 | 305 ± 4 | 1.74 ± 0.24 | 276.5 ± 1.1 | 0.52 ± 0.02 | 26 ± 5 | 0.9 ± 0.0 | BD | 13.3 ± 0.0 | 83.0 ± 1.8 | 428 ± 14 | 17 ± 4 |
| NO UV TF | 295 ± 1 | 0.05 ± 0.02 | 267.0 ± 5.1 | 0.54 ± 0.07 | 27 ± 5 | 0.4 ± 0.0 | 0.04 ± 0.05 | 10.9 ± 0.1 | 80.5 ± 1.3 | 452 ± 26 | 17 ± 2 |
| **CONSUMED** | **10** | **1.69** | **9.5** | **-0.02** | **-1** | **0.5** | **-0.04** | **2.4** | **2.5** | **-24** | |
| NO CL T0 | 330 ± 1 | 1.48 ± 0.04 | 280.8 ± 1.2 | 0.12 ± 0.01 | 48 ± 2 | 0.9 ± 0.1 | BD | 13.6 ± 0.1 | 81.5 ± 3.0 | 369 ± 16 | 8 ± 1 |
| NO CL TF | 324 ± 2 | 0.07 ± 0.03 | 277.9 ± 1.4 | 0.15 ± 0.01 | 46 ± 3 | 0.4 ± 0.0 | 0.04 ± 0.07 | 12.5 ± 0.4 | 80.6 ± 2.2 | 368 ± 6 | 8 ± 1 |
| **CONSUMED** | **6** | **1.41** | **2.9** | **-0.03** | **2** | **0.5** | **-0.04** | **1.1** | **0.9** | **1** | |
| NR ND T0 | 146 ± 1 | 2.12 ± 0.08 | 125.1 ± 0.6 | 0.43 ± 0.01 | 18 ± 1 | 0.9 ± 0.0 | 0.13 ± 0.02 | 13.5 ± 0.2 | 81.6 ± 2.3 | 435 ± 11 | 24 ± 2 |
| NR ND TF | 139 ± 1 | 0.09 ± 0.03 | 123.8 ± 1.8 | 0.49 ± 0.01 | 15 ± 1 | 0.4 ± 0.0 | 0.12 ± 0.05 | 12.7 ± 0.4 | 78.8 ± 3.7 | 450 ± 17 | 30 ± 2 |
| **CONSUMED** | **7** | **2.03** | **1.3** | **-0.06** | **4** | **0.5** | **0.01** | **0.8** | **2.8** | **-15** | |
| NR UV T0 | 148 ± 3 | 2.04 ± 0.02 | 127.2 ± 0.3 | 0.46 ± 0.01 | 18 ± 2 | 0.9 ± 0.0 | 0.12 ± 0.04 | 13.7 ± 0.1 | 86.1 ± 0.9 | 431 ± 5 | 25 ± 3 |
| NR UV TF | 143 ± 3 | BD | 124.0 ± 1.5 | 0.51 ± 0.02 | 18 ± 2 | 0.4 ± 0.0 | 0.23 ± 0.22 | 12.3 ± 0.6 | 84.9 ± 3.4 | 470 ± 18 | 26 ± 3 |
| **CONSUMED** | **5** | **2.04** | **3.2** | **-0.05** | **0** | **0.5** | **-0.11** | **1.4** | **1.2** | **-39** | |
| NR CL T0 | 152 ± 1 | 1.83 ± 0.05 | 127.7 ± 0.5 | 0.15 ± 0.01 | 22 ± 1 | 0.9 ± 0.0 | 0.08 ± 0.01 | 13.6 ± 0.2 | 81.8 ± 2.9 | 385 ± 9 | 17 ± 1 |
| NR CL TF | 145 ± 2 | 0.08 ± 0.05 | 124.9 ± 1.3 | 0.22 ± 0.03 | 20 ± 0 | 0.3 ± 0.0 | 0.11 ± 0.04 | 12.3 ± 0.1 | 84.7 ± 1.6 | 388 ± 7 | 20 ± 1 |
| **CONSUMED** | **7** | **1.75** | **2.8** | **-0.07** | **3** | **0.6** | **-0.03** | **1.3** | **-2.9** | **-4** | |
| BNPR ND T0 | 41 ± 0 | 1.57 ± 0.01 | 22.8 ± 0.3 | 0.13 ± 0.00 | 17 ± 1 | 0.9 ± 0.0 | 0.07 ± 0.02 | 0.60 ± 0.03 | 88.2 ± 0.4 | 495 ± 57 | 31 ± 8 |
| BNPR ND TF | 37 ± 1 | 0.10 ± 0.08 | 17.6 ± 1.2 | 0.58 ± 0.03 | 19 ± 0 | 0.4 ± 0.0 | 0.10 ± 0.01 | 0.27 ± 0.02 | 86.6 ± 0.8 | 490 ± 31 | 26 ± 2 |
| **CONSUMED** | **4** | **1.47** | **5.2** | **-0.45** | **-2** | **0.5** | **-0.03** | **0.33** | **1.6** | **4** | |
| BNPR UV T0 | 41 ± 1 | 1.64 ± 0.08 | 23.2 ± 0.1 | 0.14 ± 0.01 | 16 ± 1 | 0.9 ± 0.0 | 0.09 ± 0.04 | 0.58 ± 0.05 | 83.8 ± 2.8 | 518 ± 11 | 31 ± 2 |
| BNPR UV TF | 35 ± 1 | 0.18 ± 0.03 | 17.9 ± 0.4 | 0.58 ± 0.02 | 16 ± 0 | 0.4 ± 0.0 | 0.12 ± 0.04 | 0.27 ± 0.00 | 87.7 ± 0.4 | 513 ± 11 | 31 ± 1 |
| **CONSUMED** | **6** | **1.46** | **5.3** | **-0.44** | **0** | **0.5** | **-0.03** | **0.31** | **-3.9** | **6** | |
| BNPR CL T0 | 46 ± 3 | 1.73 ± 0.04 | 23.2 ± 0.2 | 0.12 ± 0.00 | 21 ± 3 | 0.9 ± 0.0 | 0.05 ± 0.01 | 0.60 ± 0.02 | 85.4 ± 0.2 | 417 ± 7 | 20 ± 2 |
| BNPR CL TF | 38 ± 1 | 0.26 ± 0.15 | 18.1 ± 0.4 | 0.70 ± 0.01 | 19 ± 2 | 0.4 ± 0.0 | 0.06 ± 0.02 | 0.27 ± 0.01 | 85.2 ± 1.4 | 391 ± 16 | 41 ± 1 |
| **CONSUMED** | **8** | **1.47** | **5.1** | **-0.58** | **2** | **0.5** | **-0.01** | **0.33** | **0.2** | **27** | |
| BNCPR ND T0 | 42 ± 1 | 1.75 ± 0.03 | 24.0 ± 0.2 | 0.17 ± 0.01 | 16 ± 1 | 0.9 ± 0.0 | 0.08 ± 0.02 | 0.43 ± 0.01 | 81.8 ± 2.1 | 442 ± 20 | 28 ± 1 |
| BNCPR ND TF | 37 ± 0 | 0.20 ± 0.04 | 20.2 ± 0.7 | 0.59 ± 0.03 | 16 ± 1 | 0.5 ± 0.0 | 0.27 ± 0.29 | 0.24 ± 0.01 | 86.2 ± 1.2 | 476 ± 17 | 29 ± 1 |
| **CONSUMED** | **5** | **1.55** | **3.8** | **-0.42** | **0** | **0.4** | **-0.19** | **0.19** | **-4.4** | **-35** | |
| BNCPR UV T0 | 41 ± 1 | 1.50 ± 0.06 | 23.9 ± 0.1 | 0.18 ± 0.01 | 15 ± 1 | 0.9 ± 0.0 | 0.05 ± 0.02 | 0.44 ± 0.03 | 85.8 ± 2.2 | 454 ± 3 | 28 ± 2 |
| BNCPR UV TF | 36 ± 0 | 0.16 ± 0.06 | 19.8 ± 0.2 | 0.57 ± 0.02 | 15 ± 0 | 0.5 ± 0.0 | 0.16 ± 0.05 | 0.24 ± 0.01 | 85.0 ± 1.9 | 457 ± 21 | 31 ± 2 |
| **CONSUMED** | **5** | **1.34** | **4.1** | **-0.39** | **0** | **0.4** | **-0.11** | **0.20** | **0.8** | **-4** | |
| BNCPR CL T0 | 44 ± 2 | 1.40 ± 0.02 | 24.3 ± 0.1 | 0.11 ± 0.01 | 18 ± 2 | 1.0 ± 0.0 | 0.06 ± 0.05 | 0.45 ± 0.02 | 87.3 ± 0.0 | 415 ± 12 | 23 ± 2 |
| BNCPR CL TF | 38 ± 2 | 0.26 ± 0.09 | 20.9 ± 0.5 | 0.57 ± 0.00 | 16 ± 1 | 0.5 ± 0.0 | 0.16 ± 0.09 | 0.25 ± 0.01 | 87.3 ± 1.8 | 371 ± 1 | 22 ± 2 |
| **CONSUMED** | **6** | **1.14** | **3.4** | **-0.46** | **2** | **0.5** | **-0.10** | **0.20** | **0.0** | **44** | |

**FIGURE LEGENDS**

Figure 1. Flowchart of experimental design.

Figure 2. Study site map. Map of study site in York River, Virginia. Site 1 is the pier at the Virginia Institute of Marine Science. Site 2 is the public pier in Shanghai. Site 2* is the public pier in Walkerton that was sampled in place of Shanghai in March 2017.

Figure 3. Summer Chlorophyll *a* concentrations of treatments at Site 1. Site 1 – June 2016 (a) and July 2016 (b): Chlorophyll *a* concentrations during the bioassays. Error bars represent standard deviation.

Figure 4. Summer Chlorophyll *a* concentrations of disinfection procedures at Site 1. Site 1 – June 2016 (a) and July 2016 (b): Chlorophyll *a* concentrations for each disinfections procedure within each treatment. Error bars represent standard deviation. ND stands for no disinfection. UV stands for ultraviolet radiation. CL stands for chlorination. Note the difference in scale between graphs.

Figure 5. Winter Chlorophyll *a* concentrations of treatments at Site 1. Site 1 – January 2017 (a) and March 2017 (b): Chlorophyll *a* concentrations during the bioassays. Error bars represent standard deviation.

Figure 6. Winter Chlorophyll *a* concentrations of disinfection procedures at Site 1. Site 1 – January 2017 (a) and March 2017 (b): Chlorophyll *a* concentrations for each disinfections procedure within each treatment. Error bars represent standard deviation. ND stands for no disinfection. UV stands for ultraviolet radiation. CL stands for chlorination. Note the difference in scale between graphs.

Figure 7. Summer Chlorophyll *a* concentrations of treatments at Site 2. Site 2 – June 2016 (a) and July 2016 (b): Chlorophyll *a* concentrations during the bioassays. Error bars represent standard deviation.

Figure 8. Summer Chlorophyll *a* concentrations of disinfection procedures at Site 2. Site 2 – June 2016 (a) and July 2016 (b): Chlorophyll *a* concentrations for each disinfections procedure within each treatment. Error bars represent standard deviation. ND stands for no disinfection. UV stands for ultraviolet radiation. CL stands for chlorination. Note the difference in scale between graphs.

Figure 9. Winter Chlorophyll *a* concentrations of treatments at Site 2. Site 2 – January 2017 (a) and March 2017 (b): Chlorophyll *a* concentrations during the bioassays. Error bars represent standard deviation.

Figure 10. Summer Chlorophyll *a* concentrations of disinfection procedures at Site 2. Site 2 – January 2017 (a) and March 2017 (b): Chlorophyll *a* concentrations for each

disinfections procedure within each treatment. Error bars represent standard deviation. ND stands for no disinfection. UV stands for ultraviolet radiation. CL stands for chlorination. Note the difference in scale between graphs.

Figure 1.

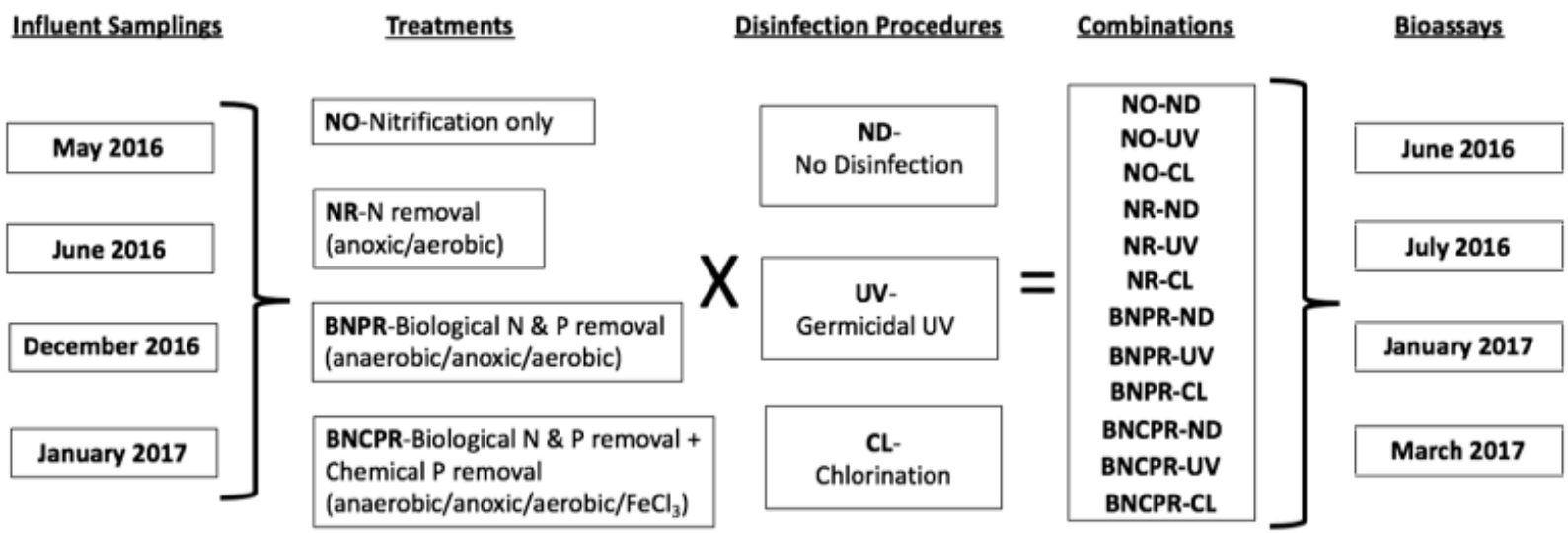

Influent Samplings
Treatments
Disinfection Procedures
Combinations
Bioassays
May 2016
June 2016
December 2016
January 2017
NO-Nitrification only
NR-N removal (anoxic/aerobic)
BNPR-Biological N & P removal (anaerobic/anoxic/aerobic)
BNCPR-Biological N & P removal + Chemical P removal (anaerobic/anoxic/aerobic/FeCl3)
X
ND- No Disinfection
UV- Germicidal UV
CL- Chlorination
=
NO-ND
NO-UV
NO-CL
NR-ND
NR-UV
NR-CL
BNPR-ND
BNPR-UV
BNPR-CL
BNCPR-ND
BNCPR-UV
BNCPR-CL
June 2016
July 2016
January 2017
March 2017

Figure 2.

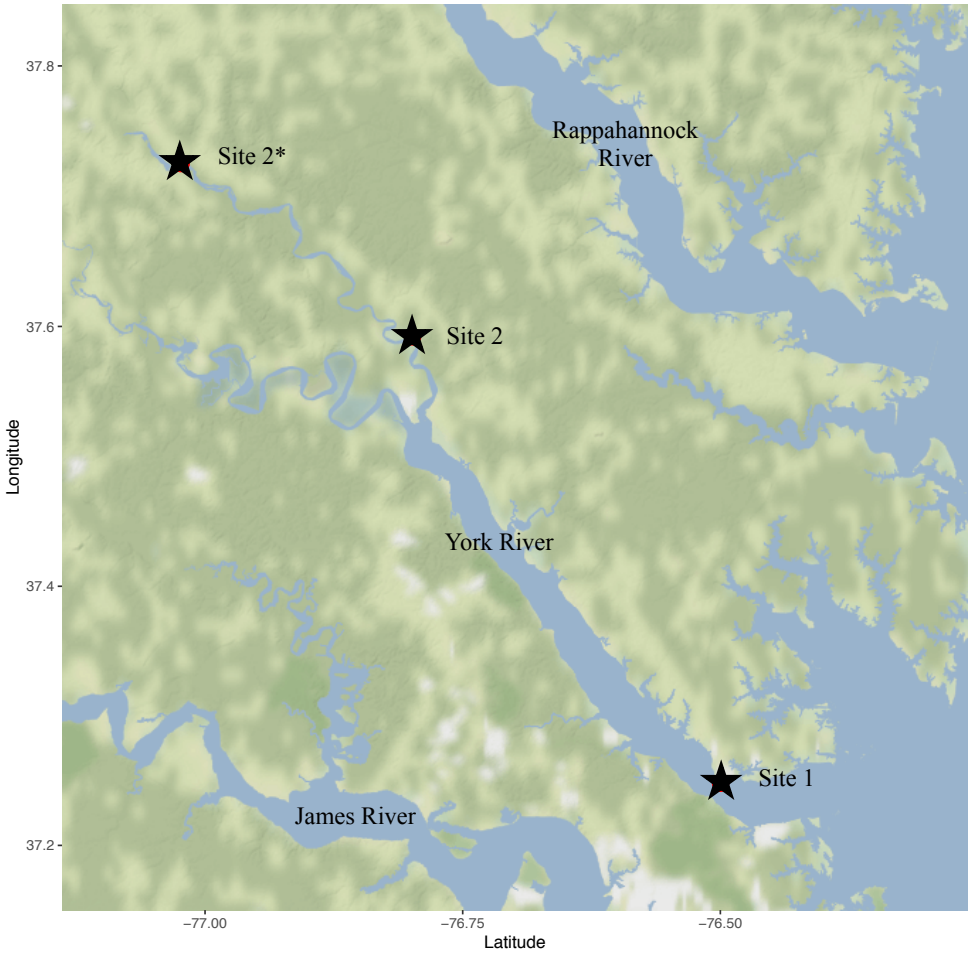

Site 2*
Rappahannock
River
Site 2
York River
Site 1
James River
37.8
37.6
37.4
37.2
Longitude
−77.00
−76.75
−76.50
Latitude

Figure 3.

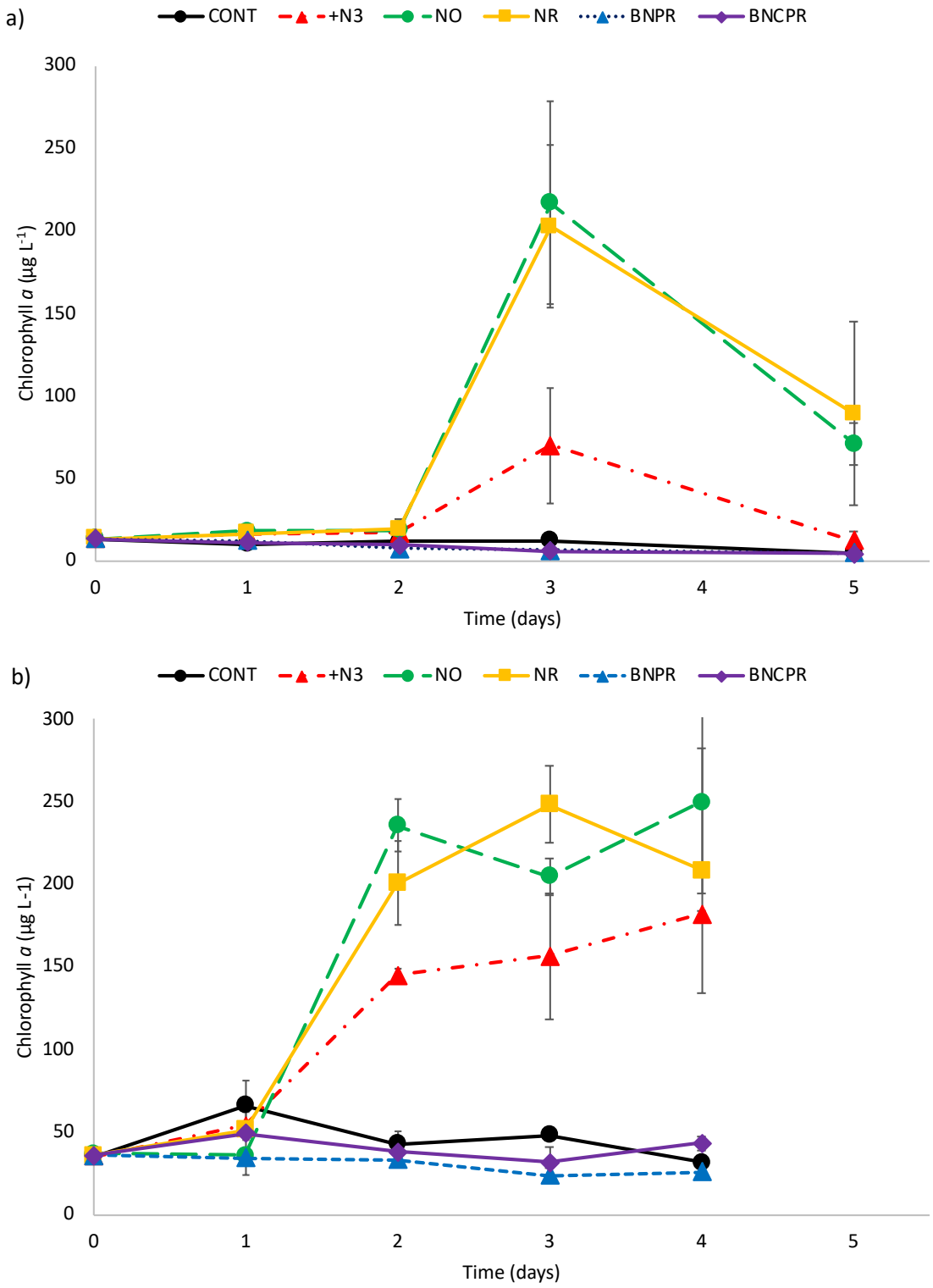

a)
CONT
+N3
NO
NR
BNPR
BNCPR
Chlorophyll a (µg L-1)
Time (days)
b)
CONT
+N3
NO
NR
BNPR
BNCPR
Chlorophyll a (µg L-1)
Time (days)

Figure 4.

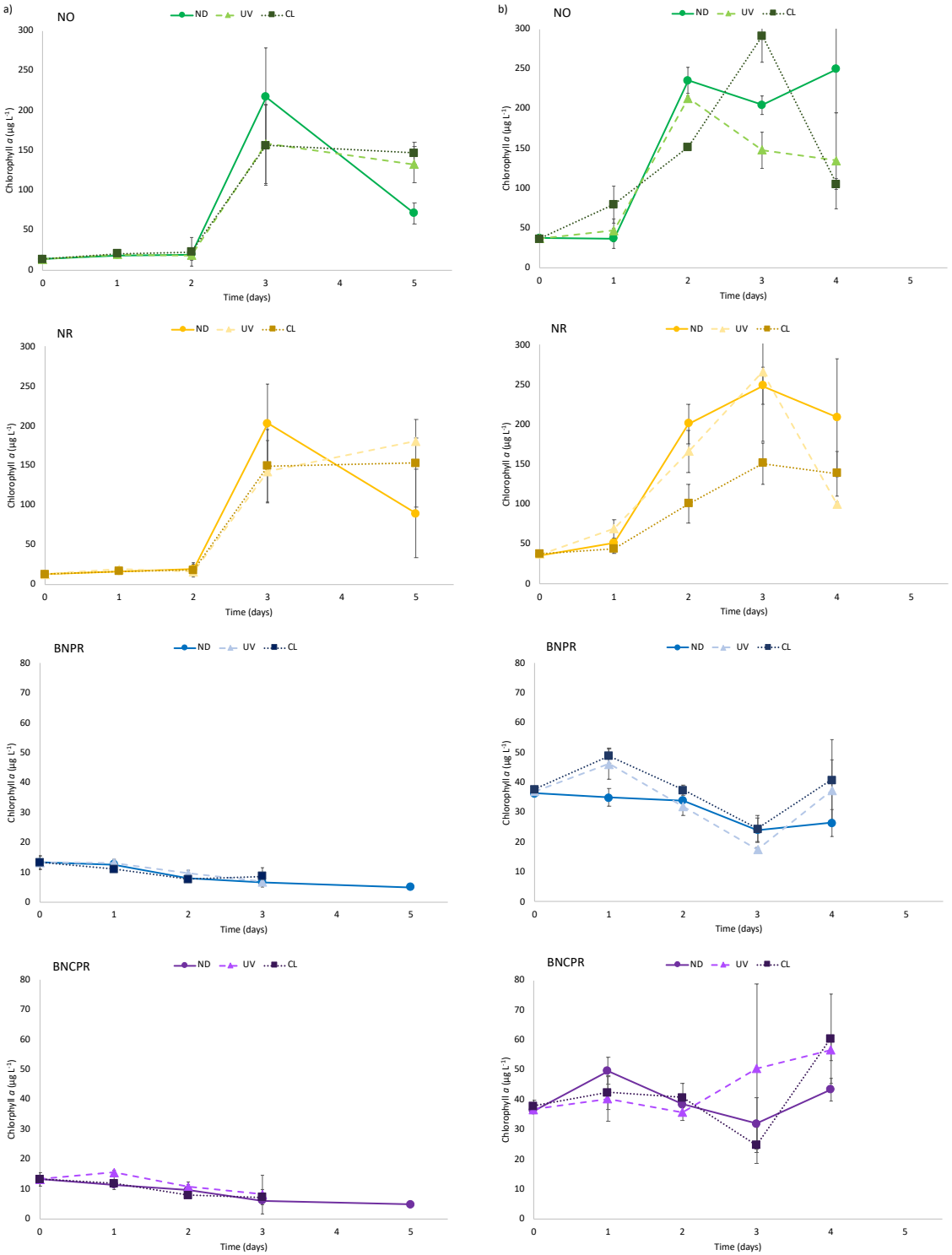


Figure 5.

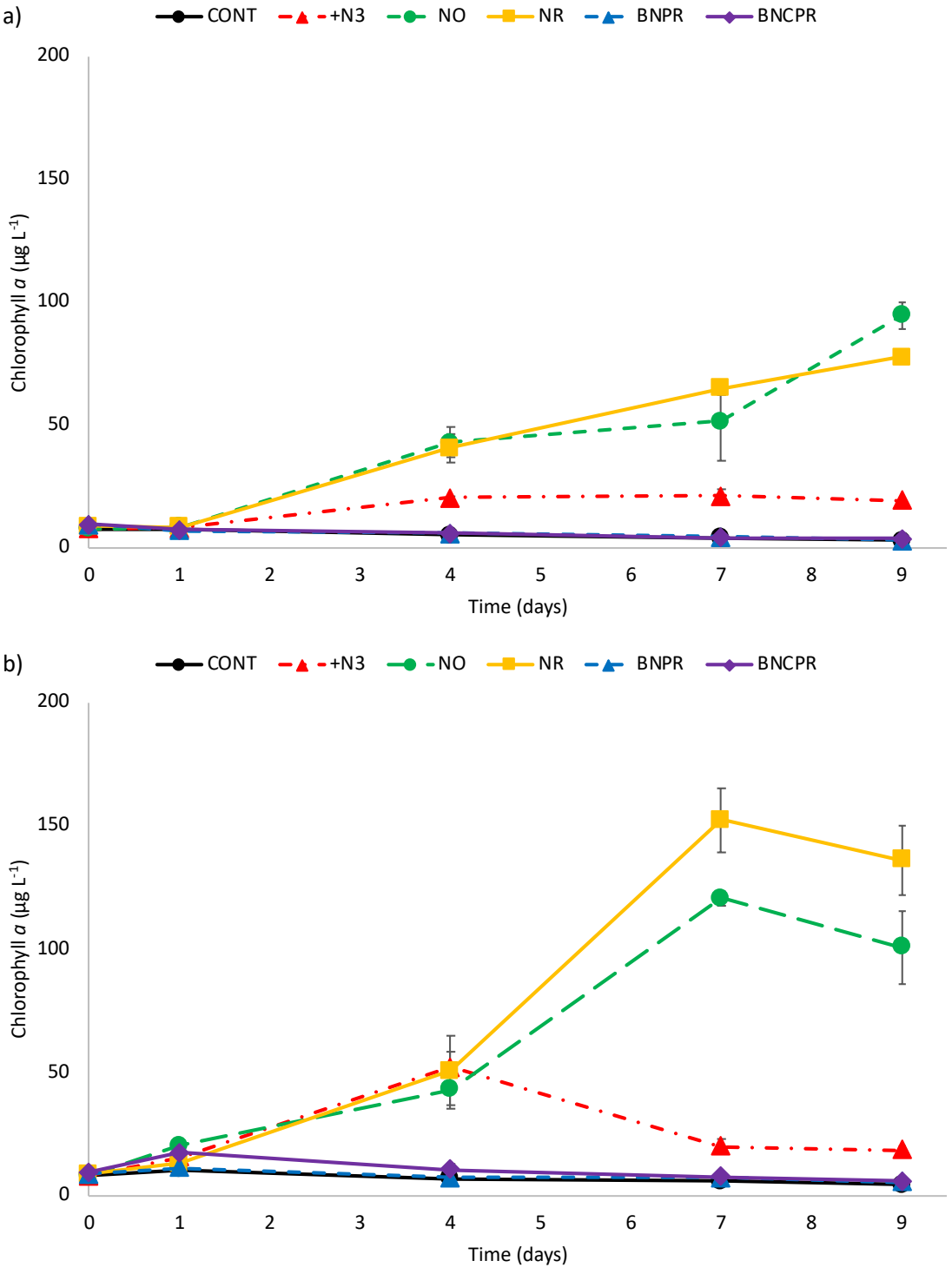

a)
CONT
+N3
NO
NR
BNPR
BNCPR
Chlorophyll a (µg L-1)
Time (days)
b)
CONT
+N3
NO
NR
BNPR
BNCPR
Chlorophyll a (µg L-1)
Time (days)

Figure 6.

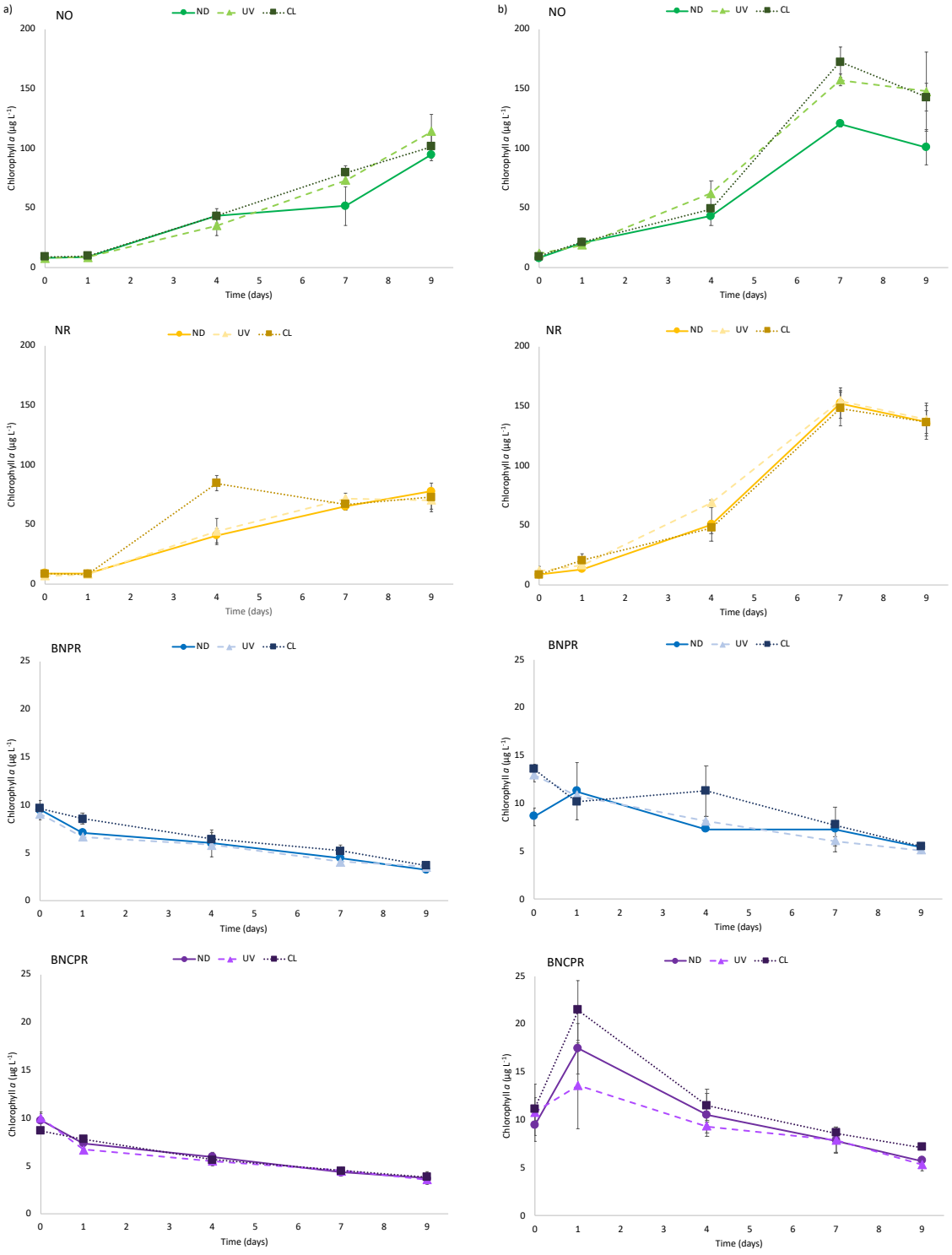


Figure 7.

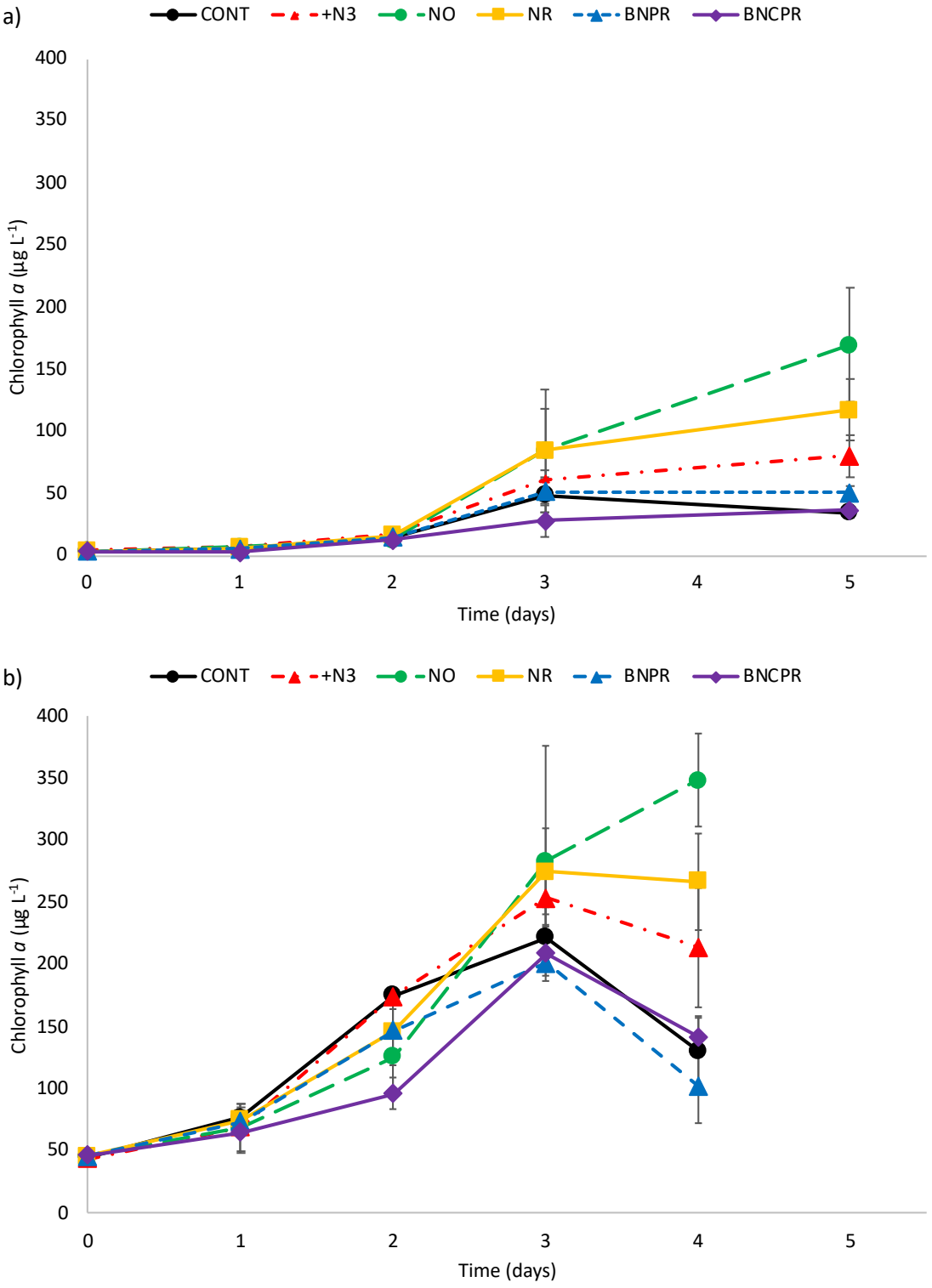

a)
CONT
+N3
NO
NR
BNPR
BNCPR
Chlorophyll a (µg L-1)
Time (days)
b)
CONT
+N3
NO
NR
BNPR
BNCPR
Chlorophyll a (µg L-1)
Time (days)

Figure 8.

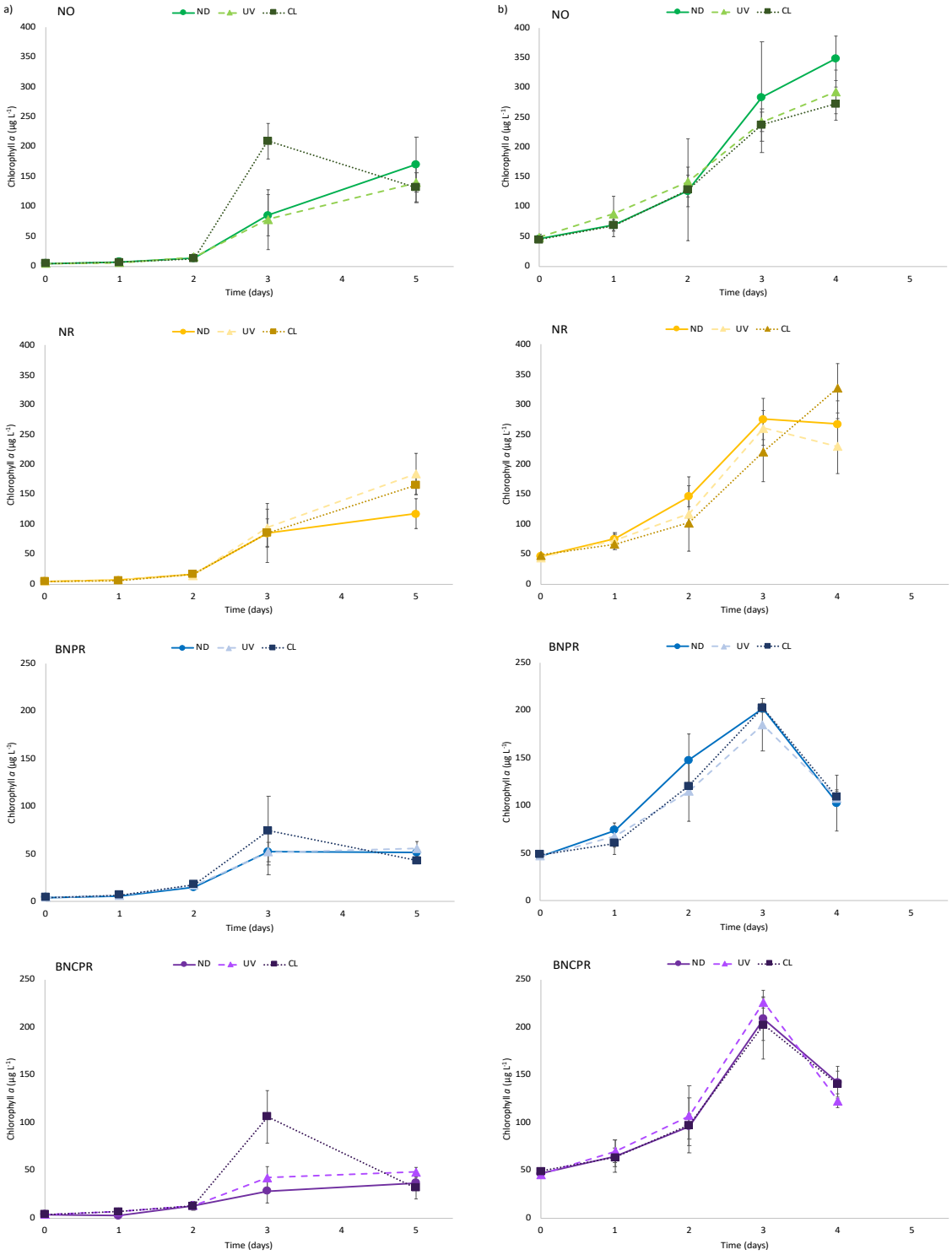

a)
NO
ND
UV
CL
Chlorophyll a (µg L-1)
Time (days)
b)
NO
ND
UV
CL
Chlorophyll a (µg L-1)
Time (days)
NR
ND
UV
CL
Chlorophyll a (µg L-1)
Time (days)
NR
ND
UV
CL
Chlorophyll a (µg L-1)
Time (days)
BNPR
ND
UV
CL
Chlorophyll a (µg L-1)
Time (days)
BNPR
ND
UV
CL
Chlorophyll a (µg L-1)
Time (days)
BNCPR
ND
UV
CL
Chlorophyll a (µg L-1)
Time (days)
BNCPR
ND
UV
CL
Chlorophyll a (µg L-1)
Time (days)

Figure 9.

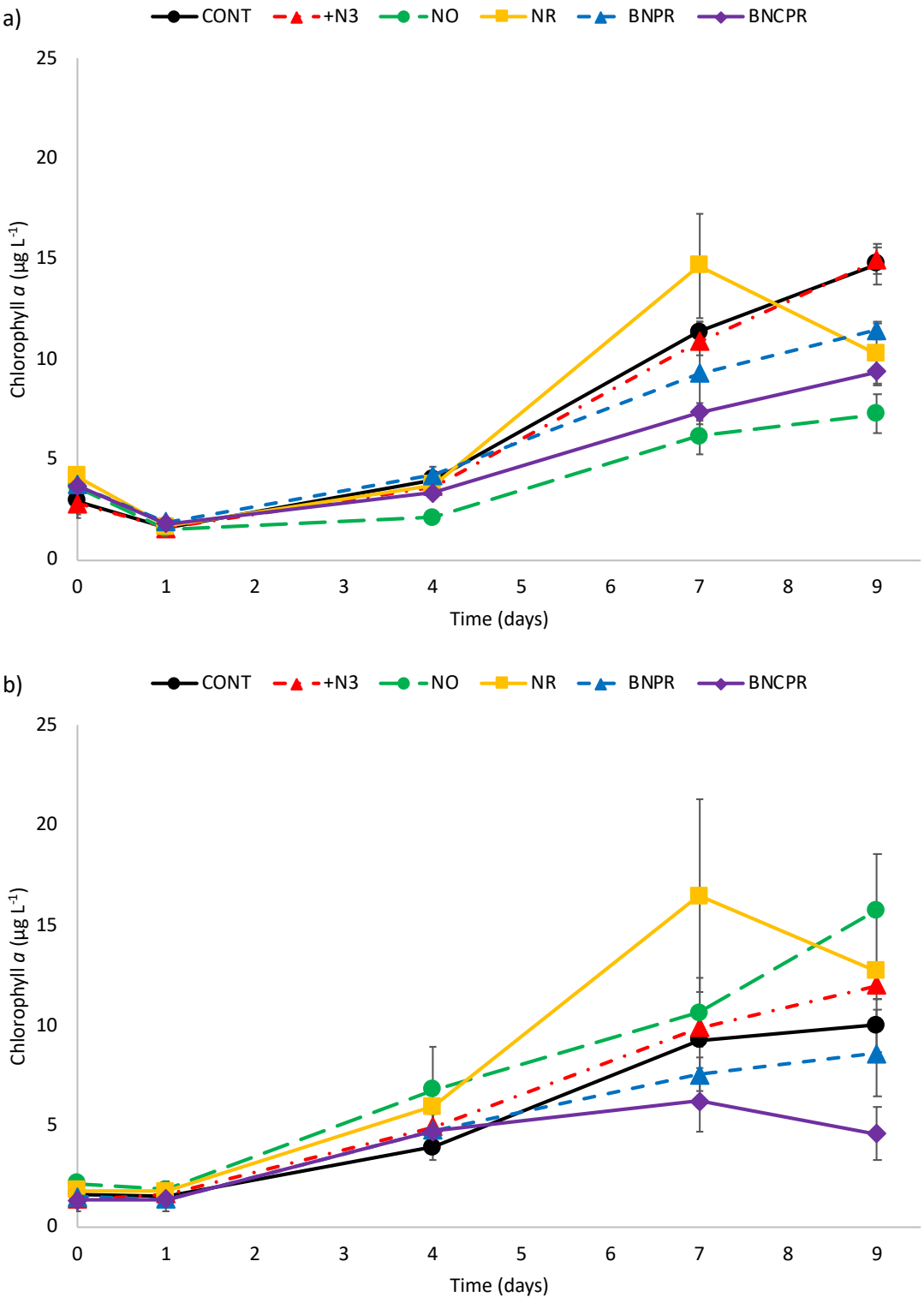
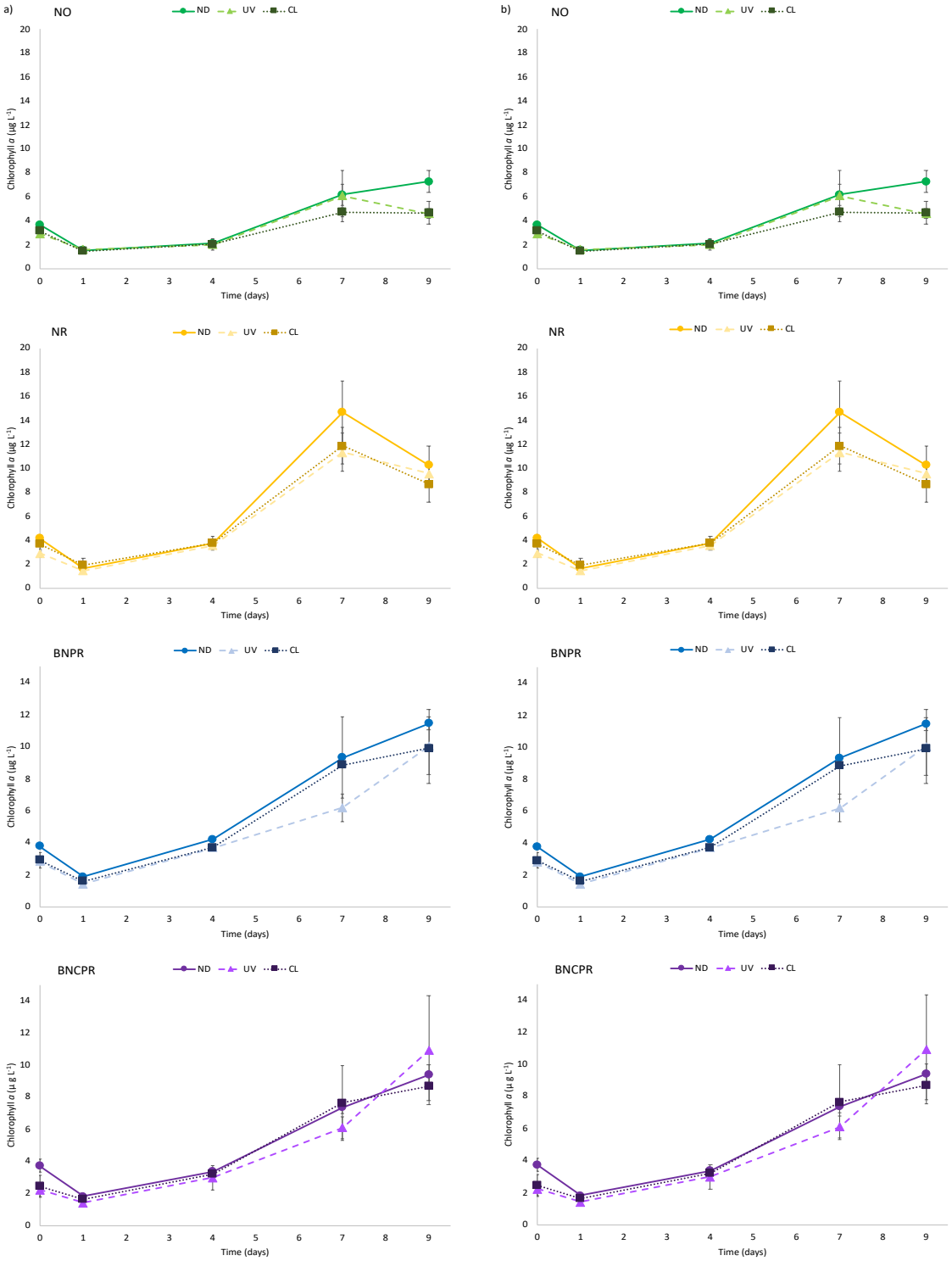

a)
CONT
+N3
NO
NR
BNPR
BNCPR
Chlorophyll a (µg L-1)
Time (days)
b)
CONT
+N3
NO
NR
BNPR
BNCPR
Chlorophyll a (µg L-1)
Time (days)

Figure 10.


a)
NO
ND
UV
CL
Chlorophyll a (µg L-1)
Time (days)
b)
NO
ND
UV
CL
Chlorophyll a (µg L-1)
Time (days)
NR
ND
UV
CL
Chlorophyll a (µg L-1)
Time (days)
NR
ND
UV
CL
Chlorophyll a (µg L-1)
Time (days)
BNPR
ND
UV
CL
Chlorophyll a (µg L-1)
Time (days)
BNPR
ND
UV
CL
Chlorophyll a (µg L-1)
Time (days)
BNCPR
ND
UV
CL
Chlorophyll a (µ g L-1)
Time (days)
BNCPR
ND
UV
CL
Chlorophyll a (µ g L-1)
Time (days)

# CHAPTER 4: CONCLUSIONS

Nitrogen (N) is an essential building block for all life on earth. Anthropogenic activities, however, including the production of human and industrial waste and its release into the environment are causing significant changes in the global N cycle. Nutrient rich effluent from water reclamation facilities (WRFs) is contributing to the declining health of Chesapeake Bay by fueling eutrophication. Though considerable advances in the WRF industry have been made toward the efficient removal of dissolved inorganic nitrogen (DIN), our understanding of and ability to remove effluent dissolved organic nitrogen (EDON) lags far behind. Despite the economic and ecological importance of effluent N, relatively little is known about how different treatment processes impact the biological lability and reactivity of the released EDON.

The first step in dealing with the problem of organics in effluent is to determine what you are working with. It is well known that the concentration and composition of nutrients within the effluent depend upon the characteristics of the influent into the facility, the processes used to treat the wastewater, and the disinfection procedures employed to make the effluent safe prior to discharge. In this study, we investigated how these factors affect the composition of the final effluent.

The experiment included four bench-scale sequencing batch reactors (SBRs) designed to mimic commonly used treatment processes: nitrification only (NO), nitrogen removal (NR), biological nitrogen and phosphorus removal (BNPR), and biological nitrogen and phosphorus removal with additional chemical phosphorus removal (BNCPR). All processes were fed the same influent to remove influent composition as a variable. After treatment, each effluent was then subjected to three disinfection

procedures – no disinfection, ultraviolet radiation, and chlorination. Samples were taken for wet chemical analyses in both the winter and summer because the influent and microbial communities within WRFs can vary greatly with temperature.

Analyses showed that the treatment processes had a wide range of both total dissolved nitrogen (TDN) and phosphate ($PO_4^{3-}$) removal efficiencies, 12 to 98% and 25 to 99%, respectively, and followed the trend NO < NR < BNPR < BNCPR. The least efficient process, NO, had the highest DIN and DON concentrations, but the lowest DON:TDN ratio, while the most efficient process, BNCPR, had the lowest concentrations of DIN and DON, but the highest DON:TDN ratio. Surprisingly, the two low-molecular weight DON species measured, urea and dissolved primary amines (DPAs), accounted for < 2 to 6% in all effluents. Dissolved organic carbon (DOC) removal efficiencies were similar among treatments, ranging from 76 to 81%. Disinfection procedures had very little effect on effluent composition, with the exception of a small decrease in nitrite ($NO_2^-$), < 3.0 μmol N $L^{-1}$ upon chlorination. The results from this study will provide valuable information to the WRF industry on EDON sources and sinks with respect to four common treatment and disinfection procedures, with influent eliminated as a variable.

The second step in dealing with the problem of organics in effluent is to determine if it will be biologically available and therefore contribute to eutrophication. This was assessed through 4 to 9-day bioassays using natural bacterial and phytoplankton communities from two receiving water end members, one fresh water and one brackish water, in the York River, a sub-estuary of Chesapeake Bay. Bioassays were carried out

in both the winter and summer because phytoplankton and bacterial assemblages can vary greatly with temperature.

Effluent from NO and NR, which contained high concentrations of DIN and $PO_4^{3-}$, stimulated phytoplankton growth more than either BNPR or BNCPR, which were primarily composed of DON and had very low concentrations of $PO_4^{3-}$. In some cases, additions of effluent from BNPR and BNCPR inhibited phytoplankton growth. The lability of the EDON varied between 4 to 48%, with NO and NR having higher labilities than BNPR and BNCPR. Overall, disinfection procedures had very little effect on phytoplankton growth. EDON uptake was greater in brackish water than in fresh water where DIN was more abundant. This study provides a bioassay comparison using the same influent for each of the effluent treatments, and the results provide further evidence that regardless of treatment, at least some portion of EDON is biologically available to estuarine microbes. It is likely that the discharge of NO and NR effluents would lead to eutrophication in both N and P-limited receiving waters due to their high inorganic nutrient content and labile EDON. In contrast, the discharge of BNPR and BNCPR effluents, due to their low inorganic nutrient and relatively refractory DON concentrations, is less likely to contribute to eutrophication.

This study was very challenging because it targeted sources with complex matrices. The presence of colloids in the effluent likely caused the high variability observed in some of the wet chemical analyses. Since colloids have a size range from 0.1 μm to 1.2 μm, effluents collected for future work should be filtered through a 0.1 μm filter. This issue may have been further exacerbated by freezing and thawing the effluent. Perhaps effluent should not be frozen, but instead sterile filtered and then stored at 4°C

until use. Another challenge with the analyses was the combination of high DIN:TDN ratios coupled with high dilution factors that occurred as a result of the effluent addition to the site water. Concentrations of DON were calculated as the difference between TDN and the sum of ammonium ($NH_4^+$), nitrate ($NO_3^-$), and nitrite ($NO_2^-$). With high DIN:TDN ratios, variance in DIN measurements can be greater than actual DON concentrations. It is known that high DIN effluents stimulate phytoplankton growth, so to avoid complications in the future, studies should focus on high DON effluents only. Other studies have avoided the high DIN:TDN ratio by isolating DON using anion exchange resins, but these were not used in this study because those methods also have inherent issues including the loss, alteration, and/or the contamination the DON pool, which could all change the potential outcome.

In addition to being analytically challenging, the bioassay portion of the study was really labor intensive and time consuming. For example, each sampling required the set-up of 420 (2 sites x 14 treatments x 3 replicates x 5 timepoints) 250 mL incubation bottles. Filtering at each timepoint involved the coordinated effort of several people and lasted a few hours. In order to minimize errors and produce high quality datasets, bioassays should be scaled down, investigating perhaps a maximum of six treatments. Alternatively, instead of using hundreds of incubation bottles, which can add to variability due to individual bottle effects, bioassays can be conducted in large carboys (20 L) where small volumes can be subsampled at each timepoint to monitor phytoplankton growth.

While the results of this study provided information about how four commonly used treatment processes and two disinfection procedures affect the composition and

bioavailability of effluent N, many questions remain to be answered. As a follow up experiment to this study, I would like to conduct three more bioassays using effluent from BNCPR (or a similar high DON/low DIN effluent) to further assess EDON bioavailability. Low $PO_4^{3-}$ concentrations, as seen in this study, may inhibit N uptake and thus lead to an underestimate of the lability of EDON. So for the first bioassay, I would add $PO_4^{3-}$ to each of the incubation bottles at the beginning of the experiment to ensure P-limitation would not prohibit the uptake of EDON. For the second bioassay, I would investigate whether EDON is more labile in high salinity seawater (e.g. Virginia Beach pier), compared to brackish or fresh water. These waters tend to be N-limited, so bacterial and phytoplankton communities may be more efficient at utilizing EDON. In the third bioassay, I would assess the potential for EDON to stimulate harmful algal blooms (HABs) which occur almost annually in the York River during the summer to early fall when DIN is low. Some HAB species are known to actually prefer DON to DIN, and may therefore have a competitive advantage when supplied with EDON.

In order for managers to successfully mitigate the effect of WRF effluent on eutrophication, research on the bioavailability of EDON needs to continue. No two plants operate under the exact same conditions and receiving waters contain different ambient nutrient concentrations and microbial communities, all of these factors affect bioavailability. Although numerous studies have been conducted on the subject, there is still no established method for assessing the bioavailability of EDON. Pressure on WRFs to reduce N loading to Chesapeake Bay is expected to rise with increasing populations. Not only will WRFs need upgrades to handle larger capacities, but they will also need to implement more efficient strategies for the removal of bioavailable N. Results from

standardized EDON bioavailability studies will help WRF managers find a balance between costly upgrades and minimizing damage to the aquatic environment.