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Effluent Organic Nitrogen (EON): Bioavailability and Photochemical and Salinity-Mediated Release

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The goal of this study was to investigate three potential ways that the soluble organic nitrogen (N) fraction of wastewater treatment plant (WWTP) effluents, termed effluent organic N (EON), could contribute to coastal eutrophication - direct biological removal, photochemical release of labile compounds, and salinity-mediated release of ammonium (NH_4^+). Effluents from two WWTPs were used in the experiments. For the bioassays, EON was added to water from four salinities (~0 to 30) collected from the James River (VA) in August 2008, and then concentrations of N and phosphorus compounds were measured periodically over 48 h. Bioassay results, based on changes in DON concentrations, indicate that some fraction of the EON was removed and that the degree of EON removal varied between effluents and with salinity. Further, we caution that bioassay results should be interpreted within a broad context of detailed information on chemical characterization. EON from both WWTPs was also photoreactive, with labile NH_4^+ and dissolved primary amines released during exposure to sunlight. We also present the first data that demonstrate that when EON is exposed to higher salinities, increasing amounts of NH_4^+ are released, further facilitating EON use as effluent transits from freshwater through estuaries to the coast.

Introduction

The Pew Oceans Commission (2003) reports that two-thirds of estuaries and bays in the United States are either moderately or severely degraded due to eutrophication - the increase in the production of phytoplankton due largely to excessive nutrient additions. Eutrophication is a key driver causing a number of pressing environmental problems including reductions in light penetration and resulting seagrass mortality, increases in harmful algal blooms, and hypoxic and anoxic conditions resulting from the decay of biomass. Wastewater treatment plants (WWTPs) are a substantial source of nitrogen (N) to natural waters worldwide and thus contribute to eutrophication (*1*). Discharge limits are enforced at these facilities in order to reduce their impact on the environment. This is a critical issue because to lessen N pollution and its effects, more stringent N discharge limits are being imposed on wastewater treatment utilities in many coastal regions of the world; in the Chesapeake Bay watershed, discharge limits ranging from 3 to 8 mg N L^{-1} will be required by 2011 (Chesapeake Bay Program 2006). A factor that will affect further nutrient reduction from WWTPs in the future is whether the soluble organic N fraction of effluent, which we term effluent organic N (EON), is included in permitted discharge allowances. One hypothesis is that EON is refractory and therefore can be excluded from discharge limits. The opposing hypothesis is that EON is bioavailable to estuarine and coastal microbial communities and therefore should be regulated in permitting decisions. Determining the potential for EON to contribute to eutrophication is the subject of the current study.

Effluent from WWTPs includes both inorganic and organic N. The conventional biological nutrient removal (BNR) systems that incorporate coupled nitrification/denitrification have the potential to remove total N down to about 8−12 mg N L^{-1} and, in selected cases, down to 5 mg N L^{-1} with tertiary filtration, for example. Newer and more expensive technologies can achieve so-called enhanced nutrient removal (ENR) and can reach effluent total N levels of 3 mg N L^{-1} (*2*). The BNR approach is very efficient at removing inorganics and can eliminate most of the dissolved inorganic N (DIN), which is composed of ammonium (NH_4^+), nitrate (NO_3^-), and nitrite (NO_2^-) (*2*). As a result of efficient ENR processing, a substantial fraction of the residual N in effluent is organic. With ENR plants, EON is typically >1 mg N L^{-1} or upward of 30% of the maximum amount of N that these plants release (*3*). Historically, this EON has been assumed to be refractory and therefore biologically unavailable. As a result of this common perception, some dischargers are applying to regulatory agencies to amend their nutrient discharge allowances to exclude EON (*4*). In the Chesapeake Bay region, current permits regulate total N (TN), which includes EON (*5*). Discounting the EON in effluents could substantially reduce construction costs and plant upgrades to improve N removal. The question is whether this cost saving is consistent with the goal of reducing coastal eutrophication in this watershed.

The traditional belief that organic N is refractory has its roots in oceanographic literature. The growth of primary producers in many parts of the world's ocean is limited by the availability of N - specifically DIN. DIN concentrations in oceanic surface waters are generally at the limit of analytical detection (i.e., < 0.42 μg N L^{-1}). In contrast, concentrations of dissolved organic N (DON) are consistently greater than 56 μg N L^{-1} (*6*). The persistence of this large DON pool was the basis for the traditional dogma that DON is refractory and not important to phytoplankton N nutrition - if phytoplankton could use it, they would and it would become depleted. The DON that was removed was believed to be remineralized by bacteria only over long time-scales.

In the 1970s and 1980s, using newly developed isotopic techniques, it was discovered that uptake and production of NH_4^+ and amino acids were tightly coupled in oceanic and estuarine surface waters, and this explained their very low concentrations in the environment (*7*−*9*). Similarly, in the early 1990s, another newly developed ^{15}N tracer technique was employed, which showed that planktonic DON release

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rates were high (*10, 11*) and that rates of DON uptake and release were similar in magnitude, thus explaining the apparent invariant nature of DON concentrations in marine systems (*12, 13*).

Research over the past decade has also made great strides in chemically characterizing the DON pool in aquatic systems (*14*). The greatest challenge in working with DON, however, is that the composition of the pool at any given time is unknown and is expected to change over relatively small spatial and temporal scales (*6*). As a result, the DON pool is generally treated as a "black box". Research confirms that a large fraction of the DON includes truly recalcitrant components that persist in the environment for months to hundreds of years, lending credence to the pool's refractory reputation (*15, 16*). However, mixed in with the refractory pool are the "doughnuts" of the DON world - highly labile compounds, which include urea, dissolved free amino acids (DFAA), and nucleic acids, that turn over on the order of seconds to days (*6, 16*). Unfortunately, the traditional dogma that organic N is refractory still persists in some disciplines.

Similar to the situation for DON in the ocean, the origin and composition of EON is largely unknown and only about 10% of EON is identifiable using current techniques (*17*). Like DON in the ocean (*18*), EON is thought to be largely of amide functionality (*19, 20*). It is also likely that a significant fraction of EON is derived from metabolic products generated by microbes present in the wastewater treatment process itself (*21, 22*). In this respect, it may have a number of similarities in composition to the small labile subpool of DON in the ocean, which is also produced largely by microbial processes. Other compounds identified in EON include chelating agents, pharmaceuticals, and soluble microbial products produced during biological treatment (*17*).

In the current study we analyzed EON from two WWTPs that use different treatment technologies. The objectives of the study were 3-fold. First, to determine the fraction of EON derived from wastewater streams that is potentially bioavailable and can stimulate algal growth along an estuarine gradient during light/dark incubations with natural plankton communities. We hypothesized that not all EON is recalcitrant and that its bioavailability would vary with changes in salinity, both because salinity can alter the chemical structure of organic compounds and because the composition of the microbial community varies with salinity. Second, to determine whether exposing EON to sunlight would result in significant photochemical release of low molecular weight (LMW) labile N, including NH_4^+, dissolved primary amines (DPA), and NO_2^-. Third, to determine whether EON would release NH_4^+ when exposed to elevated salinities. We hypothesized that photochemical release and salinity-mediated release of labile N are two abiotic mechanisms that can make N associated with EON available to the estuarine and coastal plankton communities.

Materials and Methods

Effluent Selection and Pretreatment. EON4 was collected from a WWTP with a very small (<0.05 million gallons per day) membrane bioreactor system and a solids residence time of 20−30 days that discharges within the Chesapeake Bay watershed. It receives highly variable influent composed of both sewage and septage but no known industrial sources. The plant uses biological TN removal with influent equalization, 4-stage Bardenpho with submerged membranes in the reaeration stage, and ultrafiltration with hollow fiber membranes followed by UV disinfection; a grab sample of effluent used in this study was collected prior to UV disinfection. EON5 was isolated from a domestic WWTP (40 million gallons per day) located in the arid western U.S. This facility uses a sophisticated multistage process that includes a suspended culture A/O biological phosphorus removal process with a solids retention time of 3 days, followed by a nitrifying trickling filter and a methanol-fed fluidized bed denitrification process. Grab samples for this work were collected prior to the disinfection (chlorination) stage. At the point of sample collection, the effluent still contained large amounts of microbial biomass from the treatment system itself, and so the samples were filtered through a 1 μm pleated sediment cartridge (Safe Water Technologies Inc., Elgin, IL) prior to packaging and shipping.

These plants were chosen because they are both ENR facilities, and the effluents were expected to have a high percent of DON relative to DIN in the effluent (*3*). In August 2008, the effluents were collected in polycarbonate carboys, transported to Old Dominion University, Norfolk, VA, filtered through a 0.2 μm cartridge filter, and concentrated from 13 L to 270 mL (EON4) and 19 L to 405 mL (EON5) using rotary evaporation. Rotary evaporation is a gentle concentration step that does not result in the loss of EON.

Field Sampling. Water was collected along a transect in the James River in southeastern VA on August 19 and 20, 2008. Four salinities, 0.9 (often referred to as freshwater in this paper), 10, 22, and 30, were chosen to mimic an effluent's path from freshwater to saltwater. Water was collected aboard the *R/V Fay Slover* using Niskin bottles mounted on a rosette, pooled in a polycarbonate carboy to ensure homogeneity, transported to the Virginia Institute of Marine Science (VIMS), and then dispensed into acid washed 500 mL PETG bottles.

Bioassay Protocols. The bottles were placed in an on-deck incubator maintained at ambient light and temperature aboard the *R/V Fay Slover* during the cruise and then in an incubator at VIMS with a 13.5/10.5 h light/dark cycle at a constant temperature of 25 °C. Bottles were divided into three groups. Once in the lab (<12 h from collection), to start the bioassays, the first group received a 427 μg N L^{-1} addition of concentrated EON4 effluent; the effluent was 56% DON, 43% NO_3^-, 0.3% NH_4^+, and 0.3% NO_2^-. The second group received a 369 μg N L^{-1} of concentrated EON5 effluent; the effluent was 97.5% DON, 0.7% NO_3^-, 0.5% NH_4^+, and 1.3% NO_2^- (Table 1). The third group served as a control and received no effluent addition. At 0, 12, 24, and 48 h, duplicate bottles of each treatment were filtered through a precombusted Whatman GF/F filter (2 h at 450 °C); filters were frozen and later analyzed for chlorophyll *a* (Chl *a*). We note that in the marine literature dissolved compounds are generally defined as those that pass through a 0.2 to 0.7 μm filter (*6*). GF/F filters used in these experiments have a nominal pore size of 0.7 μm. The GF/F filtrate was collected and frozen for later analysis of NH_4^+, NO_3^-, NO_2^-, urea, DPA, total dissolved N (TDN), and phosphate (PO_4^{3-}).

Photochemical Release Assays. To measure the rate of photoproduction of labile N compounds, 28 mL of the concentrated EON4 or EON5 effluent was added to 1.9 L Barnstead water (BW; ASTM Type I purified water, > 18MΩ-cm) and filter sterilized through a prerinsed (200 mL BW) 0.2 μm Supor filter. For each effluent, quartz tubes were filled and half were then wrapped in foil to serve as dark controls. The tubes were placed on a white tray and incubated in natural sunlight (*23*). The tray was plumbed with continuously flowing seawater to keep the tubes at ambient water temperature. Triplicate tubes of each treatment were removed at 0, 9, and 33 h, and samples were frozen for later analysis of NH_4^+, NO_2^-, DPA, and PO_4^{3-}.

Salinity-Mediated Release Assays. To determine the effect of salinity on EON, polypropylene centrifuge tubes were filled with 20 mL of BW that had been adjusted to different salinities (0, 20, 44, and 60) through the addition of precombusted seawater salts (NaCl, $MgSO_4$, and $NaHCO_3$). A dilute solution of effluent was made by adding 4 mL of concentrated effluent to 250 mL of BW. For each effluent, 20 mL of the diluted effluent was added to triplicate tubes with water of each of

TABLE 1. Concentrations of Ammonium (NH_4^+), Nitrite (NO_2^-), Nitrate (NO_3^-), Dissolved Organic Nitrogen (DON), Urea, Dissolved Primary Amines (DPA), and Chlorophyll *a* (Chl *a*) at the Start of the Experiment, Prior to the Effluent Addition, for Each of the Four Samples Collected along the Salinity Gradient[a]

salinity	NH_4^+ (μg N L^{-1})	NO_2^- (μg N L^{-1})	NO_3^- (μg N L^{-1})	DON (μg N L^{-1})	urea (μg N L^{-1})	DPA (μg N L^{-1})	Chl *a* (μg Chl *a* L^{-1})
0.9	6.0 ± 0.1	13.5 ± 0.1	85.4 ± 0.3	298.2 ± 1.4	13.9 ± 0.8	2.5 ± 0.1	17.6 ± 3.8
10	4.1 ± 0.3	60.1 ± 0.8	18.5 ± 0.1	298.2 ± 24.2	11.5 ± 1.8	3.9 ± 3.4	9.0 ± 1.2
22	7.4 ± 0.7	0.0 ± 0.0	2.2 ± 0.1	238.0 ± 8.4	1.4 ± 0.8	3.8 ± 0.6	8.6 ± 1.5
30	6.7 ± 1.5	0.0 ± 0.0	1.4 ± 0.4	149.8 ± 4.2	2.9 ± 0.4	3.2 ± 0.3	0.2 ± 0.0
effluent additions	**NH_4^+ (μg N L^{-1})**	**NO_2^- (μg N L^{-1})**	**NO_3^- (μg N L^{-1})**	**DON (μg N L^{-1})**	**urea (μg N L^{-1})**	**DPA (μg N L^{-1})**	**Chl *a* (μg Chl *a* L^{-1})**
EON4	1.4 ± 2.2	1.3 ± 1.5	183.4 ± 12.6	240.8 ± 14.0	2.4 ± 1.0	1.5 ± 1.1	nm[b]
EON5	2.0 ± 1.1	4.8 ± 3.2	2.4 ± 0.12	359.8 ± 11.2	5.3 ± 1.7	11.5 ± 2.8	nm[b]

[a] When effluent was added to the incubations, the concentration of a number of compounds increased; the bottom portion of the table shows the change in concentrations of the different substrates averaged over all four salinities. [b] nm = not measured.

the different salinities resulting in treatments with final salinities of 0, 10, 22, and 30, chosen to correspond to the bioassay experiments. After 30 min, samples were frozen for later analysis of NH_4^+ concentrations.

Laboratory Analysis. Concentrations of NO_3^- and NO_2^- were run in duplicate on a Lachat QuikChem 8500 autoanalyzer (detection limit 1.4 μg N L^{-1} and 0.7 μg N L^{-1}, respectively) along with known standards (*24*). Concentrations of NH_4^+ were measured in triplicate on a Shimadzu UV-1601 spectrophotometer following the manual phenol hypochlorite method (detection limit 0.7 μg N L^{-1}) (*25*). Phosphate concentrations were measured in duplicate on an Astoria Pacific autoanalyzer (detection limit 1.5 μg P L^{-1}).

TDN was measured in triplicate after persulfate oxidation, and the concentration of DON was calculated by difference after subtracting the concentration of NH_4^+, NO_3^-, and NO_2^- (*26*); propagation of error was used to determine the standard deviation of the final DON concentration. Urea concentrations were measured in duplicate on an Astoria Pacific autoanalyzer using the monoxime method (detection limit 0.35 μg N L^{-1}) (*27*). Dissolved primary amine concentrations were measured in triplicate on a Shimadzu RF-1501 spectrofluorometer following the OPA (*o*-phthaldialdehyde) method (detection limit 0.6 μg N L^{-1}) (*24*). The water left in the bioassay after all samples were taken was filtered through a prerinsed (200 mL BW) 0.2 μm Supor filter and frozen for detailed chemical characterization using electrospray ionization (ESI) coupled with Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR-MS); these analyses are described in detail in a separate manuscript (*28*). Chlorophyll *a* (Chl *a*) was measured in duplicate fluorometrically on a Turner Design Model 10-AU fluorometer (*29*). Particulate N (PN) concentrations were measured on a Europa 20/20 isotope ratio mass spectrometer.

Results and Discussion

Effluent Characterization. The effluents from the two plants studied had very different characteristics. The EON4 effluent was 44% DIN, the majority of which was in the form of NO_3^- (Table 1). In contrast, the EON5 effluent was 98% DON. Urea and DPA contributed a minor fraction of the EON in both effluents, representing 1.6% of the DON in EON4 and 4.7% of the DON in EON5 (Table 1).

Bioassays. The initial concentration of NO_3^- in the four estuarine water samples decreased as salinity increased (Table 1). In the control and EON5 incubations, all the NO_3^- was taken up in the first 24 h (Figure S1A-D). In contrast, in the EON4 incubations, all the NO_3^- was removed by 48 h with the exception of the highest salinity, which had 161 μg N L^{-1} NO_3^- remaining after 48 h (Figure S1A-D); all the NO_2^- was consumed within 48 h in all incubations (data not shown). In the case of NH_4^+, the ambient concentrations were relatively uniform at all sampling sites, and the effluent addition of NH_4^+ was small (Table 1). There was a decrease in NH_4^+ concentrations during the first 12 h in all incubations, but the concentration of NH_4^+ never neared the limit of detection in any of the treatments (Figure S1E-H). Ambient concentrations of PO_4^{3-} ranged from 2.5 μg P L^{-1}, at the high salinity site, to 36.2 μg P L^{-1} at the salinity 10 station. Phosphate concentrations in the bioassays neared the limit of detection by 24 h in the EON5 incubation at the lowest and highest salinity stations (Figure S2). Concentrations of Chl *a* increased to the greatest extent in incubations with EON4, likely due to the high concentrations of NO_3^- (Figure S3A-D). In incubations with EON5, Chl *a* generally increased over the initial 24 h and then decreased during the final 24 h. Particulate N concentrations generally showed a similar pattern to Chl *a* with the greatest accumulations observed in incubations with added EON4 followed by incubations with EON5 (Figure S3E-H).

The ambient concentration of DON decreased from the lowest to highest salinity sites (Table 1). In the control there was net production of DON in the freshwater treatment incubation but a net consumption of DON in the three saline treatments (Figure S4A-D); neither trend was significant at the $p < 0.05$ level. In samples with added EON4 effluent, there was a net decrease in DON in samples from salinity 10, but no net change in DON concentrations at the other salinities (Figure S4A-D). In samples that received EON5 effluent there was a decrease in DON concentrations over the incubation period in treatments from all salinities (Figure S4A-D). Dissolved primary amines, which are highly labile N substrates, were only a minor component of the ambient estuarine DON pools (Table 1). Concentrations of DPA either decreased slightly or remained unchanged during the first 24 h in the control and EON4 incubations but then did not change or increased over the next 24 h (Figure S4E-H). The EON5 effluent added a substantial amount of DPA (11.5 μg N L^{-1}) to the incubations, and most of this DPA was removed in the first 24 h of the incubations. Concentrations of urea did not decrease significantly during the course of the bioassays (data not shown), which suggests that there were other more desirable N substrates available. This result is different from our earlier bioassays where urea removal was large (*30*).

To estimate the percentage of EON that was labile during the incubation, the decrease (or increase) in DON concentrations in the incubations with added effluent was subtracted from the decrease (or increase) in DON concentrations in the control run in parallel. In the case of the EON4

TABLE 2. Maximum Decrease in the Concentration of Effluent Organic Nitrogen (EON), Measured As Dissolved Organic Nitrogen (DON), Observed during Bioassays with Added EON4 and EON5[f]

	net EON removal (μg N L^{-1})		EON that is labile (%)	
salinity	EON4	EON5	EON4	EON5
0.9	22.4 ± 14.0	84.0[d] ± 14.0	9.1[c]	22.8[c]
10	12.6 ± 14.0	74.2[d] ± 14.0	4.7[a]	20.2[c]
22	4.2 ± 15.4	49.0 ± 15.4	1.7[b]	14.1[c]
30	−9.8 ± 23.8	42.0[e] ± 15.4	–4.3[a]	11.7[c]

[a] Data from T_{12} hours. [b] Data from T_{24} hours. [c] Data from T_{48} hours. [d] Significantly different from starting concentration at $p < 0.03$ using a Student's t test. [e] Significantly different from starting concentration at $p < 0.01$ using a Student's t test. [f] Negative values indicate production. Net EON removal is the change in DON concentrations in the EON treatment minus the change in the DON concentrations in the control run in parallel. The % EON that is labile is the net EON removal divided by the amount of DON added with the effluent.

incubations, there was net consumption at the lowest three salinities where up to 9.1% of the added EON was removed; at the highest salinity there was net production of DON (Table 2). In the case of the incubations with EON5 effluent, the decrease in DON concentration, as a percentage of the amount of DON added to the sample as effluent (EON), consistently decreased from 23% at the lowest salinity to 12% at the highest salinity (Table 2).

Under the conditions of our bioassays two trends in EON lability were evident. First, a larger percentage of the EON from both plants was removed in the lower salinity waters (Table 2). Second, based on changes in DON concentrations, the effluents differed in relative lability with EON5 appearing to be more labile than EON4 (Table 2). The difference in the EON use is likely affected by the presence of high concentrations of NO_3^-, in the case of the EON4 bioassays, and the relative absence of NO_3^- in the EON5 bioassays. In the present study, EON lability was much lower than that observed in a previous bioassay study done during the spring, where 80 and 85% of the EON was removed in effluents from two BNR plants with different treatment processes (*30*). This finding is not surprising considering that the EON a given plant produces is a function of the influent composition and the biotic and abiotic processes occurring within the plant at any point in time, many of which are particularly sensitive to temperature. Additionally, phosphorus limitation at some of the salinities may have also affected our results. In our previous study (*30*), the effluents also contained PO_4^{3-}, but PO_4^{3-} was never drawn down to the degree it was in the incubations reported here.

An important note regarding the interpretation of bioassay results is that one cannot distinguish between consumption of DON that was present in the ambient water and the DON added with the effluent. Further, from bulk DON measurements, it is impossible to say whether DON was altered abiotically or due to the activity of microbes during the incubations. In the case of the EON4 treatments, however, there was net consumption of DON at the lowest three salinities. The increase in DON at the highest salinity indicates production of DON by the microbial community, which would mask the utilization of components of the EON pool. Conversion of NH_4^+ and NO_3^- to DON by the microbial community is a common observation in planktonic systems (*11*). For EON5, the removal of DON during the incubations at all salinities exceeded that in the control incubation.

Using net changes in DON concentrations may limit our ability to assess the bioavailability and reactivity of organic matter in environmental samples. FT-ICR-MS analyses run on the low salinity bioassay samples at the beginning and end of the bioassay showed that 79 to 100% of the compounds present at the start of the incubation were removed during the incubation with new compounds produced (*28*). This indicates that the EON pools were much more dynamic than the relatively small changes in DON concentrations would indicate. These results reinforce the need for advanced chemical techniques like FT-ICR-MS for accurately interpreting bioassay results.

Photochemical Release. When the EON4 and EON5 effluents were exposed to ambient sunlight, significant photoproduction of NH_4^+ was measured (Figure 1; $p < 0.03$). There was also signification photoproduction of DPA measured in both EON4 and EON5 ($p < 0.04$). Significant production of NO_2^- was observed with EON4 ($p < 0.002$) but not in EON5. Phosphate photoproduction was observed in EON4 ($p < 0.05$) but again not in EON5 (data not shown).

Photochemical reactions are known to affect the lability of organic material along estuarine gradients, and UV exposure can convert "recalcitrant" compounds into reactive material (*31, 32*). Previous work has shown that NH_4^+ and NO_2^- can be photochemically released from dissolved organic matter (DOM) (*23, 33*). Further, biologically recalcitrant DOM that has been converted into bioavailable N via photochemical reactions can stimulate N-limited microbial food webs

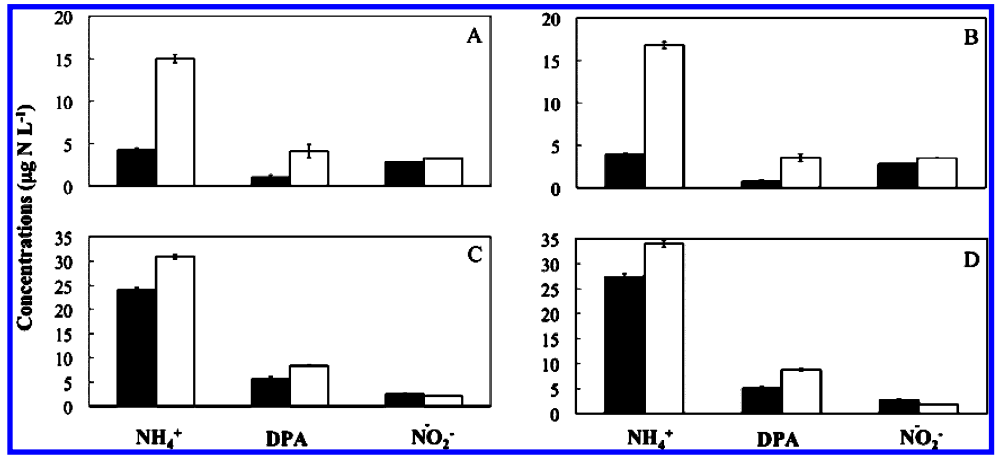

FIGURE 1. Concentrations of ammonium (NH_4^+), dissolved primary amines (DPA), and nitrite (NO_2^-) in effluent exposed to natural sunlight (white bars) or in dark controls (dark bars): (A) EON4 after 9 h and (B) 33 h of exposure and (C) EON5 after 9 h and (D) 33 h of exposure. Differences between light and dark treatments were significant at the $p < 0.04$ level (Student's t test) with the exception of NO_2^- in incubations with EON5.

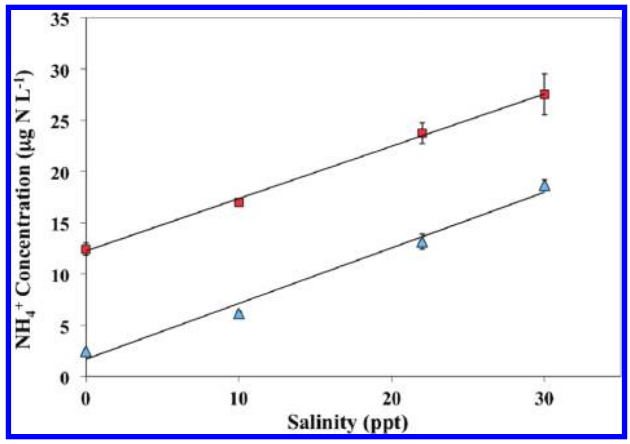

FIGURE 2. The concentration of ammonium (NH_4^+) in effluent from EON4 (red squares) and EON5 (blue triangles) added to waters with increasing salinities. The equation of the lines are EON4 $y = 0.511x + 12.2$ ($r^2 = 0.998$) and EON5 $y = 0.543x + 1.7$ ($r^2 = 0.986$).

(*34*). This release may explain why bacterial growth efficiency, bacterial nutrient demand, and bacterial biomass and respiration rates are influenced by light (*35*). Therefore, light is a critical link in assessing EON bioavailability (*36*).

Salinity-Mediated Release. Salinity can affect the transport of labile N associated with organic compounds. Humic substances in rivers are capable of adsorbing NH_4^+ from the surrounding waters to cation binding sites located on the humic structure (*16, 37*). This adsorption of NH_4^+ makes humic substances a potentially important shuttle, the 'humic shuttle', for transporting N that is produced upriver to the estuary and eventually the coastal ocean. As the humics move downriver and encounter more saline waters, salt ions can displace the loosely bound amino groups on the humic structure, which are then released into the environment. When humics isolated from three different rivers were exposed to increasing salinities, concentrations of free NH_4^+ increased and the release of NH_4^+ was rapid and reproducible (*37*). Similarly, biomass derived organo-amino compounds are believed to comprise a significant fraction of EON (*19, 20*). When these reduced forms of N are released from the plant as EON, loosely associated amino groups may dissociate from the EON as it reaches higher salinities - in effect resulting in an "EON shuttle" as material moves down-estuary. Here we provide evidence that EON operates in a similar fashion to humics. When effluent was added to a series of artificial seawater samples with increasing salinity, there was an increase in the concentration of free NH_4^+ in the solution (Figure 2). Though filtered, EON could also contain a colloidal fraction, which could also be the source of the released NH_4^+. This finding may be significant from a treatment perspective because if NH_4^+ binds to EON within the WWTP, it may not be accessible to the coupled nitrification/denitrification process in BNR facilities that would normally remove it.

Salinity was also a factor in the bioassay results; note that the bioassays were incubated indoors in a light and temperature controlled incubator in plastic bottles so photochemical release would not be occurring during the incubations due to lack of UV exposure. In the EON4 incubations, based on the abiotic release of NH_4^+ measured in the salinity-mediated release assays, 24% and 100% of the observed DON loss in the salinity 10 and 22 bioassays respectively was likely the result of abiotic release of NH_4^+ due to the increase in salinity. Similarly, in EON5 salinity-mediated release assays, 10, 39, and 47% of the decrease in DON concentrations observed in the incubations with the three highest salinities, respectively, could be accounted for by abiotic release of NH_4^+ from EON. These data indicate that release of labile NH_4^+ due to increases in salinity is likely an important mechanism contributing to EON bioavailability in saline environments.

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Supporting Information Available

Graphs showing changes in concentrations of nitrate, ammonium, phosphate, dissolved organic nitrogen, dissolved primary amines, chlorophyll *a*, and particulate nitrogen over time in the bioassays. This material is available free of charge via the Internet at http://pubs.acs.org.

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