

# Ecosystem Capacity for Microbial Biodegradation of Munitions Compounds and Phenanthrene in Three Coastal Waterways in North Carolina, United States

Michael T. Montgomery,\* Thomas J. Boyd, Nathan S. Hall, Hans W. Paerl, and Christopher L. Osburn



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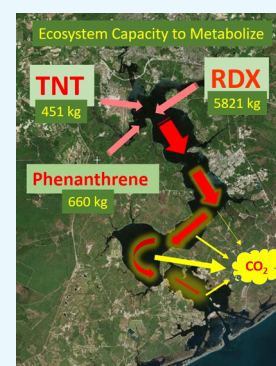


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Supporting Information

**ABSTRACT:** Munitions compounds (i.e., 2,4,6-trinitrotoluene (TNT), octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocin (HMX), and hexahydro-1,3,5-trinitro-1,3,5-triazin (RDX), also called energetics) were originally believed to be recalcitrant to microbial biodegradation based on historical groundwater chemical attenuation data and laboratory culture work. More recently, it has been established that natural bacterial assemblages in coastal waters and sediment can rapidly metabolize these organic nitrogen sources and even incorporate their carbon and nitrogen into bacterial biomass. Here, we report on the capacity of natural microbial assemblages in three coastal North Carolina (United States) estuaries to metabolize energetics and phenanthrene (PHE), a proxy for terrestrial aromatic compounds. Microbial assemblages generally had the highest ecosystem capacity (mass of the compound mineralized per average estuarine residence time) for HMX (21–5463 kg) > RDX (1.4–5821 kg) >> PHE (0.29–660 kg) > TNT (0.25–451 kg). Increasing antecedent precipitation tended to decrease the ecosystem capacity to mineralize TNT in the Newport River Estuary, and PHE and TNT mineralization were often highest with increasing salinity. There was some evidence from the New River Estuary that increased N-demand (due to a phytoplankton bloom) is associated with increased energetic mineralization rates. Using this type of analysis to determine the ecosystem capacity to metabolize energetics can explain why these compounds are rarely detected in seawater and marine sediment, despite the known presence of unexploded ordnance or recent use in military training exercises. Overall, measuring the ecosystem capacity may help predict the effects of climate change (warming and altered precipitation patterns) and other perturbations on exotic compound fate and transport within ecosystems and provide critical information for managers and decision-makers to develop management strategies based on these changes.



## INTRODUCTION

Nitrogen-based munitions compounds (also called energetics), such as 2,4,6-trinitrotoluene (TNT), hexahydro-1,3,5-trinitro-1,3,5-triazin (RDX), and octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocin (HMX), have long been purported to be resistant to microbial biodegradation, though the support for this is largely based on laboratory culture work and groundwater analytical chemistry.<sup>1,2</sup> The premise has been that with little exposure to these anthropogenic compounds that have no known natural source, microbial assemblages would have been unlikely to evolve enzyme systems to metabolize them. The aromatic structure of molecules, like TNT, would also provide intrinsic stability, making it resistant to attack by bacterial enzymes.<sup>3,4</sup>

More recently, using <sup>13</sup>C- and <sup>15</sup>N-labeled TNT, Gallagher et al.<sup>5</sup> demonstrated that both the nitrogen in the side groups and carbon in the aromatic ring are incorporated into nucleic acids of natural bacterial assemblages in estuarine sediment. In addition, relatively rapid mineralization rates of <sup>14</sup>C-labeled energetics to <sup>14</sup>CO<sub>2</sub> have been reported for natural microbial assemblages in water and sediment for numerous coastal ecosystems.<sup>6–8</sup> This may explain why energetic compounds are rarely detected in coastal water and sediment, even in those

areas that are heavily impacted by unexploded and breached ordnance.<sup>9,10</sup>

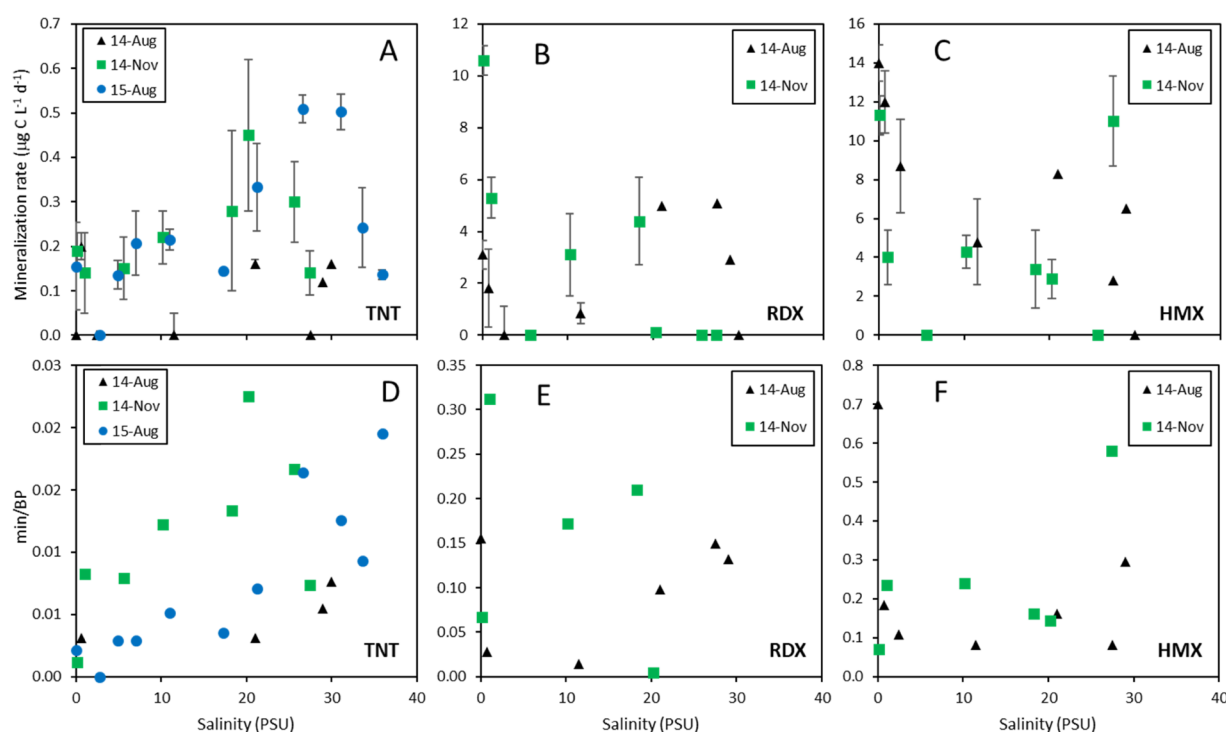
The idea that the degradation of organic compounds is primarily dictated by their chemical structure has become a paradigm that has shaped our understanding of organic matter (OM) processing for decades.<sup>11</sup> However, recent studies in soil and marine biogeochemistry<sup>11</sup> are providing evidence that other factors related to the microenvironment surrounding the molecule including its concentration,<sup>12,13</sup> nutrient availability,<sup>14</sup> microbial ecology, enzyme function, environmental conditions, steric hindrance (i.e., matrix protection),<sup>15</sup> and encounter rate with bacteria<sup>16,17</sup> can be more important than the chemical structure. If universally true for all organic compounds, this new idea represents a paradigm shift within our current understanding of environmental biodegradation processes and bioremediation of anthropogenic contaminants.

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**Figure 1.** Newport River Estuary, North Carolina, United States, values for detectable mineralization rates (AVG  $\mu\text{g C L}^{-1} \text{day}^{-1}$ ) for Tmin (A), RDX mineralization (Rmin) (B), Hmin (C), and ratios of mineralization to BP (min/BP) for TNT (D), RDX (E), and HMX (F) vs salinity for samplings in 14 Aug (black triangles), 14 Nov (green squares), and 15 Aug (blue circles). Error bars are one standard deviation.

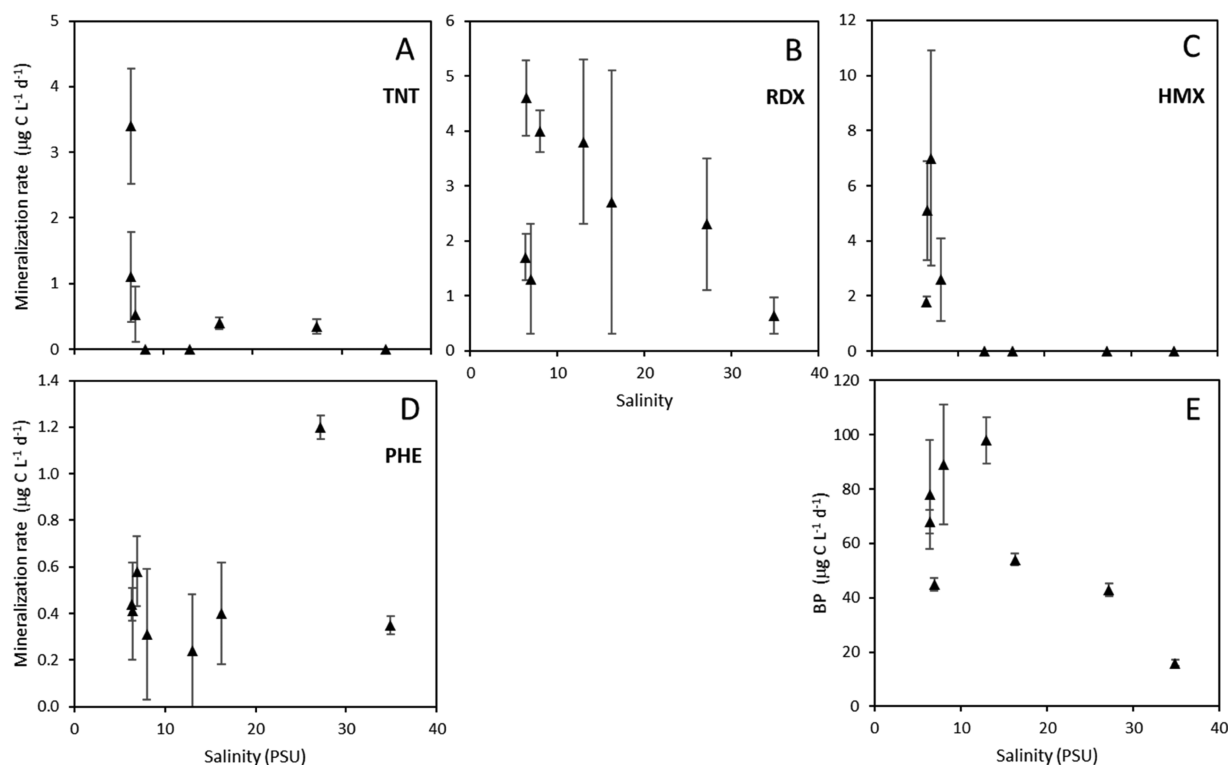
Estuarine ecosystems are “complex adaptive systems” that provide ecosystem services to the adjacent community (see review by Hagstrom and Levin<sup>18</sup>). These services include contaminant removal from waterways prior to the water mass being exported to the open ocean. In a companion paper, Osburn et al.<sup>19</sup> found that antecedent precipitation (AP) events may stimulate aromatic organic carbon metabolism in the lower Newport River Estuary (NPRE), North Carolina, United States. Phenanthrene (PHE) mineralization was used as a proxy for terrestrial aromatic compound biodegradation, but PHE is also an anthropogenic contaminant common in urbanized coastal ecosystems (the most common polycyclic aromatic hydrocarbon (PAH) in marine environments)<sup>20</sup> though there are some terrestrial biogenic sources.<sup>21,22</sup> Estuaries may serve to remove these potential toxins during transport from stream headwaters to the open ocean and thus reduce their negative impacts on biota analogous to the role wetlands can play in ameliorating contaminants in surface runoff (e.g., Truu et al.<sup>23</sup>).

Here, we report on the metabolism of much less commonly encountered contaminants, but ones that may serve as an organic nitrogen source for estuarine bacterial assemblages, namely, TNT, RDX, and HMX. Though this capability may be a relatively common feature of natural bacterial assemblages, what is less clear is how their degradation rates are controlled and what is the ecosystem capacity to modulate episodic fluxes of these contaminants through estuarine systems. If the same environmental parameters controlling natural organic matter (OM) biodegradation also control energetic biodegradation by the same assemblages, then our understanding of their fate and transport can be informed by the large body of literature on coastal OM cycling (e.g., Bauer et al.<sup>24</sup>). Within this context, the ecosystem capacity to metabolize energetics and PHE prior to their potential export to the Atlantic Ocean was determined

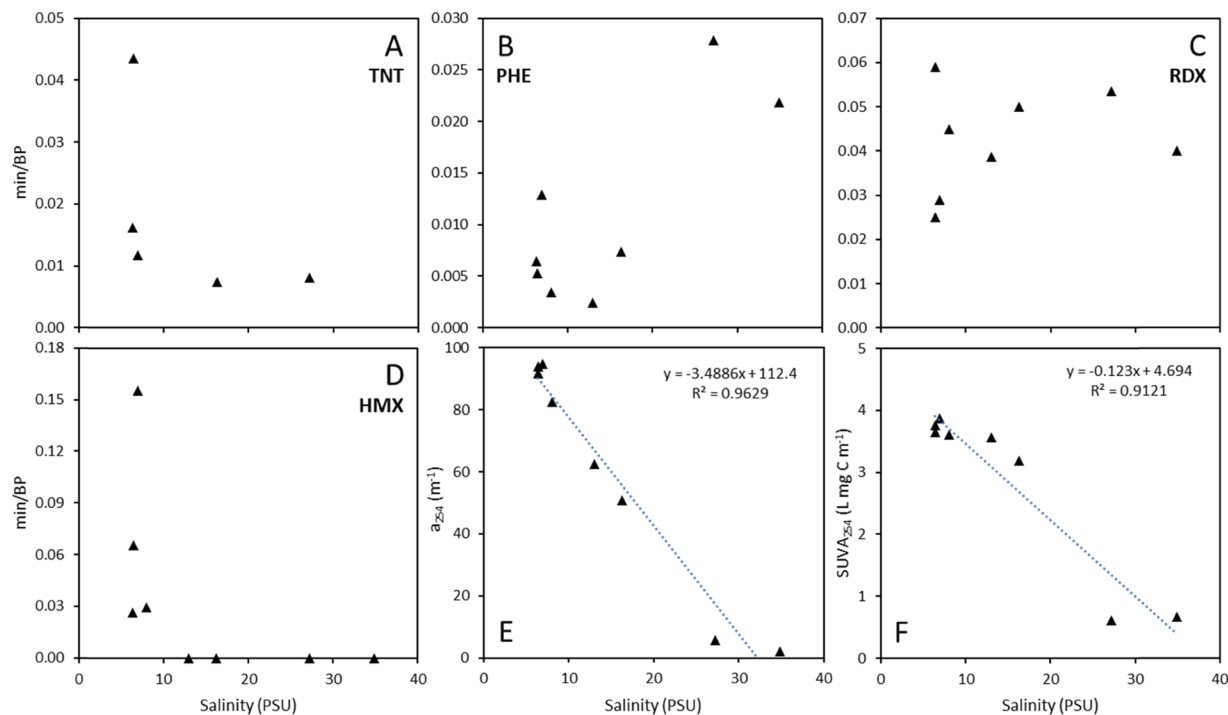
for the NPRE, New River Estuary (NWRE) and Bogue Sound, North Carolina, United States. Here, this capacity is a function of the mineralization rate, media volume, and residence time within the estuary.

## RESULTS

**Energetics Mineralization along the Newport River Estuary Transect.** Substrate mineralization rates were measured by incubation of estuarine samples with <sup>14</sup>C-labeled substrates to evolve <sup>14</sup>CO<sub>2</sub>. Detectable TNT mineralization (Tmin) rates ( $>0.01 \mu\text{g C L}^{-1} \text{day}^{-1}$ ) in water column samples for the NPRE ranged from 0.14 ( $\pm 0.05$ ) to 0.45 ( $\pm 0.17$ )  $\mu\text{g C L}^{-1} \text{day}^{-1}$  for the 14 Nov sampling (low antecedent precipitation (AP): 34 mm over previous 7 day), 0.14 ( $\pm 0.03$ ) to 0.51 ( $\pm 0.03$ )  $\mu\text{g C L}^{-1} \text{day}^{-1}$  for the 15 Aug sampling (moderate AP: 102 mm), and 0.12 ( $\pm 0.05$ ) to 0.20 ( $\pm 0.04$ )  $\mu\text{g C L}^{-1} \text{day}^{-1}$  for the 14 Aug sampling (high AP: 200 mm; Figure 1 and Table S1). These ranges for the low and moderate AP sampling were very similar to each other and slightly lower for the highest AP sampling. In general, Tmin rates were more rapid in the lower estuary (salinity  $>21$ ; Figure 1A), as they were for phenanthrene mineralization (Pmin), as previously reported.<sup>19</sup> Heterotrophic bacterial production (BP) ranged from 7.0 ( $\pm 1.0$ ) at the highest salinity (15 Aug) to 159 ( $\pm 13$ )  $\mu\text{g C L}^{-1} \text{day}^{-1}$  at the Cypress bog headwaters (14 Nov; Table S1 and Osburn et al.<sup>19</sup>) for the three samplings of NPRE. Comparing min/BP can give an indication of the relative importance of carbon substrate metabolism to overall carbon demand by the assemblage. Tmin/BP generally increased with salinity and decreased with higher AP (Figure 1D). If dissolved organic carbon (DOC) associated with precipitation was preferentially metabolized by the estuarine assemblage, one would expect there to be lower contaminant min/BP ratios with higher AP.



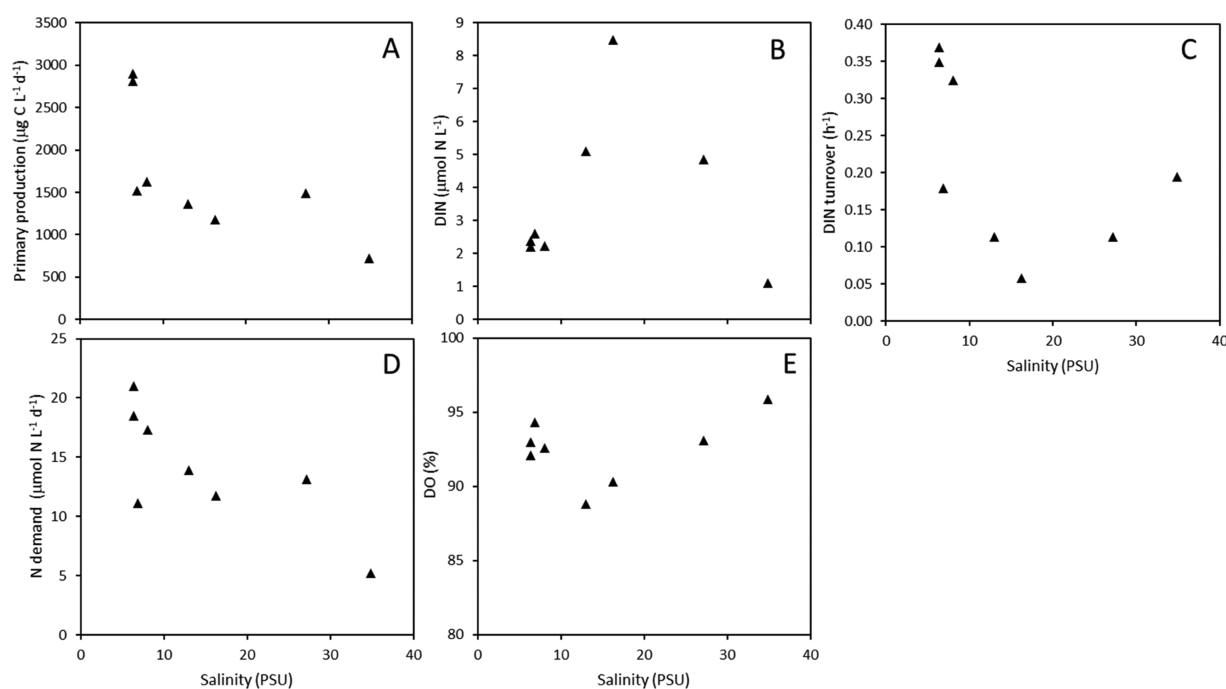
**Figure 2.** New River Estuary, North Carolina, United States, values for detectable mineralization rates (AVG  $\mu\text{g C L}^{-1} \text{day}^{-1}$ ) for TNT (A), RDX (B), HMX (C), PHE (D), and rates of BP (E) vs salinity for 14 Apr sampling. Error bars are 1 standard deviation (SD).



**Figure 3.** New River Estuary, North Carolina, United States, ratios of min/BP for TNT (A), PHE (B), RDX (C), and HMX (D), as well as, values for  $a_{254}$  (E), and SUVA<sub>254</sub> (F) vs salinity for 14 Apr sampling. The linear trend line and the formula are presented for (E) and (F).

RDX and HMX represent nonaromatic carbon and nitrogen compounds that are also unlikely to have any current or historic source of input to the NPRES though they would likely be metabolized as carbon or nitrogen sources in coastal waters.<sup>8</sup> Detectable RDX mineralization (Rmin) rates ( $>0.01 \mu\text{g C L}^{-1} \text{day}^{-1}$ ) ranged from  $0.1 (\pm 0.01)$  to  $10.6 (0.56) \mu\text{g C}$

$\text{L}^{-1} \text{day}^{-1}$  for 14 Nov (AP = 30 mm) and  $1.8 (\pm 0.91)$  to  $5.1 (\pm 1.1) \mu\text{g C L}^{-1} \text{day}^{-1}$  for 14 Aug (AP = 200; Figure 1B). Detectable HMX mineralization (Hmin) rates were somewhat higher ranging from  $2.9 (\pm 1.0)$  to  $11 (\pm 1.0) \mu\text{g C L}^{-1} \text{day}^{-1}$  and  $2.8 (\pm 2.4)$  to  $14 (\pm 0.90) \mu\text{g C L}^{-1} \text{day}^{-1}$  for the respective samplings (Figure 1C). Mean Hmin was higher than



**Figure 4.** New River Estuary, North Carolina, United States, values (AVG) for rates of primary production ( $\mu\text{g C L}^{-1} \text{ day}^{-1}$ ; A), DIN ( $\mu\text{mol N L}^{-1}$ ; B), DIN turnover ( $\text{h}^{-1}$ ; C), N demand ( $\mu\text{mol N L}^{-1} \text{ day}^{-1}$ ; D), and DO (%; E) vs salinity for 14 Apr sampling.

Rmin for both 14 Nov ( $4.6$  vs  $2.9 \mu\text{g C L}^{-1} \text{ day}^{-1}$ ) and 14 Aug ( $7.1$  vs  $2.4 \mu\text{g C L}^{-1} \text{ day}^{-1}$ ); likewise, the mean substrate mineralization to heterotrophic bacterial production ratio (min/BP, unitless) was higher for HMX (14 Nov, 0.24; 14 Aug, 0.23) than for RDX (14 Nov, 0.15; 14 Aug, 0.10) with little effect of AP (Figure 1E,F). Though there was little pattern with estuarine salinity, the highest mineralization rates were often closer to the freshwater bog end member.

**Energetics Mineralization along the New River Estuary Transect.** One sampling was performed along a salinity transect in the NWRE in 14 Apr (AP = 30 mm). Detectable T<sub>min</sub> rates ( $>0.01 \mu\text{g C L}^{-1} \text{ day}^{-1}$ ) in water column samples for the NWRE ranged from  $0.35 (\pm 0.11)$  to  $3.4 (\pm 0.88) \mu\text{g C L}^{-1} \text{ day}^{-1}$  (Figure 2 and Table S2), which are almost an order of magnitude higher than measured in the NPRES for the 14 Nov sampling with similar AP ( $0.14 (\pm 0.05)$  to  $0.45 (\pm 0.17) \mu\text{g C L}^{-1} \text{ day}^{-1}$ ; Table S1). Also, unlike the pattern seen in the NPRES, T<sub>min</sub> was highest closer to the brackish headwaters, which is more similar to that seen with R<sub>min</sub> and H<sub>min</sub> in both estuaries (Figure 2A). The range of detectable R<sub>min</sub> rates ( $0.64 \pm 0.33$  to  $4.6 \pm 0.69 \mu\text{g C L}^{-1} \text{ day}^{-1}$ ) was lower than that for NPRES as were H<sub>min</sub> rates ( $1.8 \pm 0.17$  to  $7 \pm 3.9 \mu\text{g C L}^{-1} \text{ day}^{-1}$ ; Figure 2B,C). The P<sub>min</sub> rate range for the NWRE ( $0.24 \pm 0.24$  to  $1.2 \pm 0.05 \mu\text{g C L}^{-1} \text{ day}^{-1}$ ; Figure 1D) was higher than that reported by Osburn et al.<sup>19</sup> for the NPRES, though most stations fell into the range of the NPRES on the sampling date with the highest AP (14 Aug).

BP generally increased from the headwaters near Jacksonville, North Carolina, United States ( $45 \pm 2.4 \mu\text{g C L}^{-1} \text{ day}^{-1}$ , salinity = 6.9) to  $98 (\pm 8.5) \mu\text{g C L}^{-1} \text{ day}^{-1}$  at mid-estuary (salinity = 13) before decreasing to  $16 (\pm 1.1) \mu\text{g C L}^{-1} \text{ day}^{-1}$  toward the estuary mouth (salinity = 34.9; Figure 2E and Table S2). T<sub>min</sub>/BP ranged from 0.007 to 0.044 (Figure 3A) with most ratios higher than the highest ratio (0.023) from the transects along the NPRES. The P<sub>min</sub>/BP range of 0.002–0.28 (Figure 3B) was very similar to that for the NPRES (the highest

ratio was 0.33), whereas those for R<sub>min</sub>/BP (0.025–0.059; Figure 3C) and H<sub>min</sub>/BP (0.026–0.156; Figure 3D) were generally lower than those for the NPRES. The absorption coefficient at 254 nm ( $a_{254}$ ) was used as a measure of natural aromatic dissolved organic carbon (DOC) and was generally conservative across the salinity transect until the lower estuary (Figure 3E). When normalized for DOC concentration (SUVA<sub>254</sub>), however, aromatic DOC was lower than expected along the transect at a salinity of 27 in Courthouse Bay (Figure 3F).

Because this sampling was performed in collaboration with another project (SERDP's DCERP), additional data on primary production, dissolved nitrogen concentrations, and nitrogen demand were available. Rates of primary production ranged from  $719$  to  $2899 \mu\text{g C L}^{-1} \text{ day}^{-1}$  (Figure 4A) with the highest rates associated with a dinoflagellate bloom (*Prorocentrum minimum*) at the New River headwaters stations, JAX and SW Creek (Table S3). Dissolved inorganic nitrogen (DIN; primarily ammonium) was low ( $2.37 \mu\text{mol}$  to below detect of  $0.1 \mu\text{mol}$ ; Figure 4B), and calculated DIN turnover ( $0.35$ – $0.37 \text{ h}^{-1}$ ; Figure 4C) and N demand based on primary production rates and measured C/N of the particulate organic matter (POM) ( $0.77$ – $0.88 \mu\text{mol N L}^{-1} \text{ day}^{-1}$ ; Figure 4D) were highest at these same stations. Dissolved oxygen (DO) was also elevated (Figure 4E), and the highest min/BP ratios for TNT, RDX and the second highest for HMX were at the JAX station (Figure 3 and Table S2).

**Water and Sediment in Bogue Sound.** Across three stations over four water samplings in Bogue Sound ( $n = 11$ ), BP ranged from  $7.8 (\pm 1.2)$  to  $74 (\pm 4.1) \mu\text{g C L}^{-1} \text{ day}^{-1}$  with both extremes being at the Cat Island station (NC01) following AP extremes, 14 Nov (AP = 30 mm) and 14 Aug (AP = 200 mm), respectively (Table 1). DOC concentration ( $n = 5$ ) ranged from  $2.3$  to  $3.75 \text{ mg L}^{-1}$  with the exception of  $18.4 \text{ mg L}^{-1}$  coinciding with the highest BP values at the Cat Island station, which may have been associated with discharge

**Table 1. Bogue Sound, North Carolina, United States Values for Detectable Rates (AVG (SD)  $\mu\text{g C L}^{-1} \text{day}^{-1}$ ) for BP, Tmin, Rmin, Hmin, Pmin, and Ratios of Mineralization to BP for Water and Surface Sediment<sup>a</sup>**

station (media)	sampling year-month	water temp (°C)	salinity	DOC (mg L <sup>-1</sup> )	$a_{254}$ (m <sup>-1</sup> )	SUVA <sub>254</sub> (L mg C <sup>-1</sup> m <sup>-1</sup> )	rates ( $\mu\text{g C L}^{-1} \text{day}^{-1}$ )														
							BP			Tmin			Rmin			Hmin			Pmin		
							AVG	SD	AVG	SD	AVG	SD	AVG	SD	AVG	SD	AVG	SD	AVG	SD	AVG
NC01 Cat Is. (water)	14 Apr	32	3.75	29	3.355	61	3.1	0.21	0.10	1.9	1.2	4.9	2.3	0.17	0.003	0.031	0.080	0.003			
	14 Aug	17.1	18.4	172	4.060	74	4.1	0	0	6.2	1.5	11	3.0	0.33	0	0.084	0.15	0.004			
	14 Nov	31.9	17	17		7.8	1.2	0	0	0	0	0	0	0.17	0.05	0	0	0.022			
NC02 Emerald Is. (water)	15 Aug					24	1.4	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND			
	14 Apr		3.66	27	3.191	62	7.8	0	0	1.2	0.30	0	0	0.41	0	0.019		0.007			
	14 Aug	25.9	3.33	32	4.183	35	1.7	0	0	3.7	2.4	8.2	2.1	0.83	0	0.11	0.23	0.024			
NC03 Beaufort Inlet (water)	14 Nov	9.7	17.42	18		8.8	0.91	0.18	0.07	6.7	2.0	14	2.8	0.10	0.020	0.76	1.6	0.011			
	15 Aug					39	1.5	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND			
	14 Apr		2.30	15	2.762	42	1.1	0	0	0.79	0.32	1.1	0.50	0.34	0	0.019	0.026	0.008			
NC01 Cat Is. (sediment)	14 Aug	26	3.13	32	4.412	31	0.60	0.11	0.10	0	0	3.5	1.3	1.3	0.004	0	0.11	0.042			
	14 Nov	11.3	32.32	12		8.2	0.74	0	0	7.5	1.6	3.7	2.7	0.013	0	0.91	0.45	0.002			
	14 Apr					551	101	15	1.8	884	305	68	23	0	0.027	1.6	0.12	0			
NC02 Emerald Is. (sediment)	14 Nov					58	28	5.1	0.55	1099	590	104	11	0.18	0.088	19	1.8	0.003			
	15 Aug					126	10	2.9	0.06	ND	ND	ND	ND	0	0.023			0			
	14 Apr					281	126	2.5	16	699	278	75	16	0	0.089	2.5	0.27	0			
NC03 Beaufort Inlet (sediment)	14 Nov					157	13	5.8	0.5	785	288	105	24	0	0.037	5.0	0.67	0			
	15 Aug					211	33	0	0	ND	ND	ND	ND	0.61	0	0.003		0.003			
	14 Apr					362	28	9.2	0.05	306	59	32	9.6	0.38	0.025	0.85	0.088	0.001			
14 Nov					213	16	6.3	2.1	371	193	56	11	1.3	0.030	1.7	0.26	0.006				

<sup>a</sup>Parameters measured in the water column include temperature (°C), salinity, DOC (mg L<sup>-1</sup>),  $a_{254}$  (m<sup>-1</sup>), and SUVA<sub>254</sub> (L mg C m<sup>-1</sup>).

from a combined sewer. With this station located at the west end of Bogue Sound, it was also likely influenced by the outflow from the White Oak River during the high AP event.

Rates of  $T_{min}$  were detectable in three of nine water samples and ranged from 0.11 ( $\pm 0.10$ ) to 0.21 ( $\pm 0.10$ )  $\mu\text{g C L}^{-1} \text{ day}^{-1}$ , which is similar to that measured nearest the mouth of the nearby Newport River (0.14–0.16  $\mu\text{g C L}^{-1} \text{ day}^{-1}$ ; Table 1). Rates of  $P_{min}$  were detectable in all nine water samples and ranged from 0.013 ( $\pm 0.006$ ) to 1.3 ( $\pm 0.07$ )  $\mu\text{g C L}^{-1} \text{ day}^{-1}$  with both extreme rates coming from the NC03 station, which was closest to the mouth of the Newport. Interestingly, same station  $P_{min}$  rates for all three Bogue sites were most rapid for the highest AP sampling (14 Apr, 200 mm) and least rapid for the lowest AP sampling (14 Nov, 30 mm), as was generally observed for NPRES transects (Table 1).

Rates of  $R_{min}$  were detectable in eight of nine water samples and ranged from 0.79 ( $\pm 0.32$ ) to 7.5 (1.6)  $\mu\text{g C L}^{-1} \text{ day}^{-1}$  with both extremes also coming from the NC03; however, the pattern was reversed from  $P_{min}$ , with the most rapid rates corresponding to the lowest AP sampling (14 Nov; Table 1). This range was similar to that observed in the mid to lower estuary (salinity >18) of the NPRES (0.1–5.1  $\mu\text{g C L}^{-1} \text{ day}^{-1}$ ) for all three samplings (Table S1).

Rates of  $H_{min}$  were detectable in seven of nine water samples ranging from 1.1 ( $\pm 0.50$ ) to 14 ( $\pm 2.8$ )  $\mu\text{g C L}^{-1} \text{ day}^{-1}$  and showed a similar pattern to  $R_{min}$  in that the most rapid rate was at the lowest AP sampling (14 Nov) and least rapid at the highest AP sampling (14 Apr; Table 1). This range was similar to that from the NPRES of 2.8–14  $\mu\text{g C L}^{-1} \text{ day}^{-1}$  (Table S1). The relative magnitude of mineralization rates for Bogue Sound water was similar to NPRES and NWRE in that generally  $H_{min} > R_{min} > P_{min} > T_{min}$ .

Surface sediment was also collected for BP at eight stations in Bogue Sound and was generally about an order of magnitude higher (roughly equating a kilogram of sediment to a liter of water by wet volume) than the same station water samples ranging from 58 ( $\pm 28$ ) to 551 ( $\pm 101$ )  $\mu\text{g C kg}^{-1} \text{ day}^{-1}$  (Table 1).  $T_{min}$  was detectable in seven of eight sediment samples and ranged from 2.9 ( $\pm 0.06$ ) to 25 ( $\pm 10$ )  $\mu\text{g C kg}^{-1} \text{ day}^{-1}$ .  $R_{min}$  and  $H_{min}$  were measured in six of six samples with ranges of 306 ( $\pm 59$ ) to 1099 ( $\pm 590$ ) and 32 ( $\pm 9.6$ ) to 105 ( $\pm 24$ )  $\mu\text{g C kg}^{-1} \text{ day}^{-1}$ , respectively. Slowest mineralization rates were for  $P_{min}$ , which was detectable in only four of eight sediment samples and ranged from 0.18 ( $\pm 0.15$ ) to 1.3 ( $\pm 0.79$ )  $\mu\text{g C kg}^{-1} \text{ day}^{-1}$  (Table 1). Relative magnitude of mineralization rates for Bogue Sound sediment was different than observed in the water column surveys with  $R_{min} > H_{min} > T_{min} > P_{min}$ .

$P_{min}/BP$  was generally higher in the water column (0.002–0.042) than in the sediment (0.001–0.006), in fact, only one station (Beaufort Inlet, NC03, 14 Nov) showed a higher ratio in the sediment than water from the corresponding station (Table 1). However,  $T_{min}/BP$  was higher in sediment (0.023–0.089) than water (0.003–0.020), with the same station ratios 2–8-fold higher in sediment.  $R_{min}/BP$  was substantially higher in sediment (0.84–19) than water (0.019–0.76) with same station ratios 2–6-fold higher in 14 Nov and 1–2 orders of magnitude higher in 14 Apr.  $H_{min}/BP$  was only slightly higher in sediment (0.088–1.8) than water (0.026–1.6).

**Ecosystem Capacity for Contaminant Catabolism. New River Estuary.** For each section of NWRE, volume and average residence times<sup>25</sup> were coupled with rates of BP and

mineralization of RDX, HMX, TNT, and PHE to estimate the capacity of NWRE to metabolize a theoretical contaminant addition to the headwater prior to estuarine transport to the Atlantic Ocean. Based on the data from the 14 APR sampling (AP = 30 mm),  $1.4 \times 10^5$  kg of bacterial carbon would be produced within the water column during the average 70 days residence time (Table 2). Calculated microbially mineralized contaminant would be approximately 5800 kg of RDX, 1500 kg of HMX, 660 kg of PHE and 450 kg of TNT, which would represent a combined 6% of the total net bacterially produced carbon (Table 2). Based on this analysis, much of the removal of these compounds would take place mid-estuary (salinity = 13–16.3) in the Lejeune and Stones Bay segments. These two sections represent 53% of the estuary water volume but account for 71% of total BP, 72% of RDX capacity, 65% of that for TNT, and 66% for PHE.  $H_{min}$  was below detect in these segments and at higher salinity so 100% of the HMX biodegrading capacity was calculated to be at salinity <13 in the upper reaches of NWRE (Table 2).

**Newport River Estuary.** Estimated volume, depth, and residence time<sup>26</sup> for the NPRES were available from the literature, but there was no published sectioning of the NPRES system as was performed by Ensign et al.<sup>25</sup> for the NWRE. Sectioning was performed by manually dividing the river estuary map into seven sections that likely include at least one measurement in each section.

The calculated capacity for BP increased with AP from 65 kg (34 mm) to 94 kg (102 mm) to 226 kg (200 mm; Table 3), likely due to the input of nutrients and OM into the estuary from the surface runoff<sup>27</sup> or atmospherically from precipitation.<sup>28</sup> Using  $P_{min}$  rates reported by Osburn et al.,<sup>19</sup> the  $P_{min}$  capacity was somewhat similar for 14 Nov (0.59 kg) and 15 Aug (0.29 kg) but increased to 1.7 kg with the highest AP (14 Aug; Table 3). The segment closest to the river mouth accounted for between 35 and 77% of this PHE-degrading capacity, despite only accounting for 25–32% of total BP in the estuary.

The capacity for  $T_{min}$  was lower for the highest AP sampling (200 mm, 14 Aug) at 0.25 kg than for the other two samplings; 0.80 kg (14 Nov) and 1.2 kg (15 Aug), though 68% of that 0.25 kg was associated with the segment closest to the river mouth (Table 3). Rates of  $R_{min}$  and  $H_{min}$  were only measured for the highest and lowest AP sampling, with the NPRES showing a much higher capacity for both at the high AP sampling, 1.4 vs 6.6 kg for RDX and 21 vs 28 kg for RDX and HMX, respectively.

These analyses assumed that increased AP did not decrease residence time in the NPRES, which is not plausible. Shortening the estimated residence time from 6 to 2 days for the high AP sampling (14 Apr) and comparing that capacity to that of the lowest AP sampling (14 Nov; 6 days residence time) but keeping that 6 days residence time for the latter would produce a similar capacity for both BP, 7.5 vs 6.5 kg, and for  $P_{min}$ , 0.57 vs 0.59 kg (Table 3). The  $R_{min}$  capacity would be higher in the high AP sampling, 2.2 vs 1.4 kg, whereas  $H_{min}$  would be lower, 9.5 vs 21 kg, and  $T_{min}$  would be an order of magnitude lower, 0.08 vs 0.8 kg (Table 3).

**Bogue Sound.** With only three stations in Bogue Sound and no salinity transect, station values were averaged and then multiplied by published water volume and residence time to determine the metabolic capacity.<sup>29,30</sup> For two of the samplings, sediment was processed in addition to water. To determine the capacity of the mixed layer of sediment, BP and

Table 2. New River Estuary, North Carolina, United States, Values (AVG (SD)) for Rates ( $\mu\text{g C L}^{-1} \text{day}^{-1}$ ) of BP, Pmin, Tmin, Rmin, and Hmin for 14 Apr Sampling<sup>a</sup>

stations	section <sup>b</sup>	volume (L)	residence time (days)	rates ( $\mu\text{g C L}^{-1} \text{day}^{-1}$ )												ecosystem capacity mineralized (kg C)			
				BP		Pmin		Tmin		Rmin		Hmin		BP (kg C)	PHE	TNT	RDX	HMX	
				AVG	SD	AVG	SD	AVG	SD	AVG	SD	AVG	SD						
JAX	JAX	$4.06 \times 10^9$	2	78	20	0.41	0.21	3.4	0.88	4.6	0.69	5.1	1.8	633	3.3	28	37	41	
NE Creek	M52			45	2.4	0.58	0.15	0.53	0.42	1.3	1	7	3.9						
SW Creek	M52			68	4.4	0.44	0.07	1.1	0.68	1.7	0.42	1.8	0.17						
NE & SW AVG <sup>c</sup>	M52	$1.63 \times 10^{10}$	9	57		0.51		0.82		1.5		4.4		8289	75	120	220	645	
Morgan/Farnell Bays	M47	$2.44 \times 10^{10}$	13	89	22	0.31	0.28	0	0	4	0.38	2.6	1.5	28 231	98	0	1269	825	
Lejeune	M39	$3.25 \times 10^{10}$	18	98	8.5	0.24	0.24	0	0	3.8	1.5	0		57 330	140	0	2223	0	
Stones Bay	M32 (+172)	$3.66 \times 10^{10}$	20	54	2.2	0.40	0.22	0.4	0.09	2.7	2.4	0		39 528	293	293	1976	0	
Courthouse Bay	M18	$8.13 \times 10^9$	4	43	2.3	1.2	0.05	0.35	0.11	2.3	1.2	0		1398	39	11	75	0	
New River Inlet	M15	$8.13 \times 10^9$	4	16	1.1	0.35	0.04	0.000		0.64	0.33	0		520	11	0	21	0	
total		$1.3 \times 10^{11}$	70											135 929	660	451	5821	1512	

<sup>a</sup>Stations are listed according to sections designated by Ensign et al.<sup>25</sup> along with the corresponding ecosystem capacity for BP (kg C) and total contaminant mineralized (kg C) in each section based on the residence time in each section and the measured mineralization rate. <sup>b</sup>Ensign et al.<sup>25</sup> designations. <sup>c</sup>NE & SW Creek values averaged as both were in MS2.

mineralization averages were multiplied by the published surface area of the sound to a 1 cm deep sediment surface layer for a sediment volume. This assumption was made because the measurement was taken using the top 1 cm of sediment and because water column particles are most likely to mix in the top 1 cm of sediment. Bioturbation can increase PHE and energetic mineralization much deeper than the top 1 cm,<sup>31</sup> but bioturbation depth was not measured here.

Under the constraints of these parameters and assumptions, comparing 14 Nov (AP: 34 mm) water column capacity with sediment capacity, the water column was ca. 8-fold greater for BP ( $0.78$  vs  $5.9 \times 10^3$  kg) and HMX ( $0.48$  vs  $4.3 \times 10^3$  kg), ca. 20-fold greater for PHE ( $3.3$  vs  $68$  kg) but slightly lower for RDX ( $3.4$  vs  $4.1 \times 10^3$  kg; Table 4). For the 14 Apr sampling with similar AP (30 mm), water column capacity was ca. 18-fold higher than sediment for BP ( $2.2$  vs  $40 \times 10^3$  kg), almost 7-fold higher for HMX ( $0.32$  vs  $2.2 \times 10^3$  kg), 2 orders of magnitude higher for PHE ( $0.29$  vs  $221$  kg), but again lower for RDX ( $3.5$  vs  $0.94 \times 10^3$  kg; Table 4). Even normalizing for BP, PHE biodegradation occurred preferentially in the water column and RDX biodegradation occurred preferentially in the sediment.

The high AP (200 mm) Bogue Sound water column sampling event on 14 Aug did not include a sediment sampling. The ecosystem capacity was compared to a low AP (30 mm) water column sampling of Bogue Sound for 14 Apr to try to determine whether the amount of AP had an influence on metabolic capacity differences among associated bacterial assemblages. In addition to the amount of AP, salinity was lower in 14 Aug (17.1–27.5) than 14 Apr (32; Table 4) (clearly as a result of the AP), and though temperature was not measured at the 1 APR Bogue Sound samplings, it was 14.3–17–9 °C in the lower New River the day before and was ca. 26 °C in 14 Aug (Table S2). Despite these differences, the BP capacity in Bogue Sound was very similar between the high AP and low AP sampling ( $33.7$  vs  $39.7 \times 10^3$  kg). TNT biodegradation capacity was about 50% lower during the high AP sampling than during low AP (26 vs 51 kg). In contrast, substantial increases in biodegradation capacity were calculated with higher AP for PHE (592 vs 221 kg), RDX ( $2.4$  vs  $0.94 \times 10^3$  kg), and HMX ( $5.5$  vs  $2.2 \times 10^3$  kg; Table 4). In general, with higher AP in the Bogue Sound, there was a greater capacity for biodegradation of PHE, RDX, and HMX.

## DISCUSSION

**Role of AP on Energetics Biodegradation.** Precipitation within a watershed has been a recent research focus as it can dramatically alter biogeochemical cycles and processing of OM prior to export to coastal oceans. Furthermore, local precipitation patterns are now increasingly altered with climate change.<sup>32</sup> Runoff from shoreside industrial sites is often mentioned as the primary pathway linking anthropogenic contaminants and ecological risk to estuarine biota (e.g., Johnston et al.<sup>33</sup> and references therein). Even relatively moderate amounts of AP ( $13$ – $25$  mm day<sup>-1</sup>) have been shown to impact water quality in NPRES with respect to fecal coliform abundance,<sup>34,35</sup> phytoplankton blooms,<sup>36</sup> DOC,<sup>37</sup> and DIN (nitrate and ammonium<sup>28</sup>). AP can alter the composition of an estuarine bacteria assemblage by changing the competitive balance within the community via chemical flux (terrestrial nutrients and OM) or more directly by mixing terrestrially sourced microbial assemblages with the native estuarine assemblage.

**Table 3. Newport River Estuary, North Carolina, United States, Values for the Ecosystem Capacity for BP (kg C) and Total Contaminant Mineralized for PHE, TNT, RDX, and HMX in Each Section Based on the Residence Time in Each Section and the Measured Mineralization Rate (kg)<sup>a</sup>**

sampling (year- month)	segment	volume (L)	amount mineralized (kg)							amount mineralized (kg)										
			residence time (days)	BP (kg C)	PHE	TNT	RDX	HMX	residence time (days)	BP (kg C)	PHE	TNT	RDX	HMX	residence time (days)	BP (kg C)	PHE	TNT	RDX	HMX
14 Aug	1	$1.3 \times 10^7$	0.03	$6.5 \times 10^{-4}$	$7.0 \times 10^{-5}$	0	0.001	0.005	0.02	$4.3 \times 10^{-4}$	$5.0 \times 10^{-5}$	0	$7.0 \times 10^{-4}$	0.003	0.01	$2.2 \times 10^{-4}$	$2.4 \times 10^{-6}$	0	$3.0 \times 10^{-4}$	0.0015
	2	$3.7 \times 10^8$	0.71	17	0.12	0.052	0.47	3.1	0.47	11	0.08	0.03	0.31	2.1	0.24	5.6	0.04	0.02	0.16	1.0
	3	$2.6 \times 10^8$	0.51	8.6	0.06	0.003	0.24	1.6	0.34	5.7	0.04	0.02	0.16	1.1	0.17	2.9	0.02	0.01	0.08	0.53
	4	$4.6 \times 10^8$	0.89	33	0.16	0	0	3.5	0.59	22	0.11	0	0	2.4	0.30	11	0.05	0	0	1.2
	5	$7.1 \times 10^8$	1.37	79	0.39	0	0	8.5	0.91	52	0.26	0	0	5.6	0.46	2.5	0.13	0	0	2.8
	6	$5.4 \times 10^8$	1.05	34	0.38	0	0.49	2.8	0.70	23	0.25	0	0.33	1.8	0.35	12	0.13	0	0.16	0.92
	7	$7.5 \times 10^8$	1.45	55	0.60	0.17	5.4	9.0	0.97	37	0.40	0.12	3.6	6.0	0.48	18	0.20	0.06	1.8	3.0
total	$3.10 \times 10^9$	6.0	226	1.7	0.25	6.6	28	4.0	151	1.1	0.17	4.4	19	2.0	75	0.57	0.08	2.2	9.5	
14 Nov	1	$1.3 \times 10^7$	0.025	0.02	$4.0 \times 10^{-5}$	$1.0 \times 10^{-4}$	$1.7 \times 10^{-4}$	0.002	0.017	0.014	$3.0 \times 10^{-3}$	0	0.001	0.001	0.008	0.007	$1.0 \times 10^{-4}$	0	$6.0 \times 10^{-4}$	$6.0 \times 10^{-4}$
	2	$3.7 \times 10^8$	0.71	4.7	0.02	0.06	0.08	1.1	0.47	3.1	0.01	0.038	0.54	0.74	0.24	1.6	0.01	0.019	0.27	0.37
	3	$2.6 \times 10^8$	0.51	2.8	0.02	0.037	0.58	0.45	0.34	1.9	0.013	0.025	0.39	0.30	0.17	0.93	0.007	0.012	0.19	0.15
	4	$4.6 \times 10^8$	0.89	8.1	0.04	0.18	0.04	1.2	0.59	5.4	0.03	0.12	0.03	0.78	0.30	2.7	0.01	0.06	0.01	0.39
	5	$7.1 \times 10^8$	1.37	17	0.17	0.29	0	0	0.91	12	0.11	0.19	0	0	0.46	5.8	0.06	0.10	0	0
	6	$5.4 \times 10^8$	1.05	11	0.12	0.08	0	6.3	0.70	7.3	0.08	0.05	0	4.2	0.35	3.6	0.04	0.03	0	2.1
	7	$7.5 \times 10^8$	1.45	21	0.23	0.15	0	12	0.97	14	0.15	0.10	0	8.0	0.48	6.9	0.08	0.05	0	4.0
total	$3.10 \times 10^9$	6.0	65	0.59	0.80	1.4	21	4.0	43	0.39	0.53	0.95	14	2.0	22	0.20	0.27	0.48	7.0	
15 Aug	1	$1.3 \times 10^7$	0.025	0.02	$6.0 \times 10^{-5}$	$4.0 \times 10^{-5}$	$4.0 \times 10^{-5}$	0.002	0.017	0.013	$4.0 \times 10^{-5}$	$3.0 \times 10^{-5}$	0	0.008	0.008	$6.5 \times 10^{-3}$	$2.0 \times 10^{-5}$	$1.0 \times 10^{-5}$	$1.0 \times 10^{-5}$	$1.0 \times 10^{-5}$
	2	$3.7 \times 10^8$	0.71	11	0.04	0.056	0.056	0.47	7.3	0.02	0.037	0.037	0.74	0.24	3.6	0.01	0.019	0.019	0.27	0.37
	3	$2.6 \times 10^8$	0.51	5.4	0.013	0.019	0.019	0.34	3.6	0.009	0.013	0.013	0.30	0.17	1.8	0.004	0.006	0.006	0.19	0.15
	4	$4.6 \times 10^8$	0.89	19	0.06	0.13	0.13	0.59	13	0.04	0.09	0.09	0.78	0.30	6.3	0.02	0.04	0.04	0.19	0.15
	5	$7.1 \times 10^8$	1.37	30	0	0.49	0	8.5	0.91	20	0	0.33	0.78	0.30	11	0.05	0	0	0.16	1.0
	6	$5.4 \times 10^8$	1.05	18	0	0.29	0	6.3	0.70	12	0	0.19	0	4.2	0.35	5.9	0	0.10	0	4.0
	7	$7.5 \times 10^8$	1.45	26	0.22	0.32	0.32	9.0	0.97	18	0.15	0.21	0.21	8.0	0.48	8.8	0.07	0.11	0.11	7.0
total	$3.10 \times 10^9$	6.0	94	0.29	1.2	1.2	4.0	4.0	62	0.19	0.83	0.83	14	2.0	31	0.10	0.41	0.41	7.0	

<sup>a</sup>Three different estimates are given based on the volume (L) and average total residence time (days) within the estuary for 6, 4, and 2 days for each segment of the three sampling events.



**Table 4. Ecological Capacity in Water (L) and Sediment (kg) of Bogue Sound, North Carolina, United States, To Produce Bacterial Carbon via BP (kg 2 day<sup>-1</sup>) and Metabolize TNT, RDX, HMX, and Phenanthrene (PHE) over a 2 days Residence Time**

sampling year-month	media	volume (kg or L)	BP (kg 2 day <sup>-1</sup> )	mineralized (kg 2 day <sup>-1</sup> )			
				TNT	RDX	HMX	PHE
14 Apr	water	3.61 × 10 <sup>11</sup>	39 710	51	936	2166	221
14 Apr	sediment	2.74 × 10 <sup>9</sup>	2181	90	3451	320	0.69
14 Nov	water	3.61 × 10 <sup>11</sup>	5969	43	3417	4260	68
14 Nov	sediment	2.74 × 10 <sup>9</sup>	782	31	4119	484	2.7
14 Aug	water	3.61 × 10 <sup>11</sup>	33 693	26	2383	5463	592

Osburn et al.<sup>19</sup> found enhanced rates of PHE biodegradation in NPRES surface waters positively correlated with increased AP and that Pmin was higher in the lower estuary (across all three samplings) than near the headwaters. In this study, the relationship between AP and Pmin was also seen in Bogue Sound water albeit in a much more limited sampling (Table 1). Tmin was also generally higher in the lower NPRES, but the relationship with AP was generally the opposite with the mean across the transect decreasing with increasing AP although the highest Tmin rates were within the lower estuary with moderate AP (Table S1). The highest biodegradation in the lower estuary for both PHE and TNT may be because both are aromatic, unlike HMX and RDX. Evidence that aromatic CDOM is nonconservatively removed in this area supports this assertion, and hence, mineralization rates of both PHE and TNT could serve as key proxies for aromatic compound biodegradation in estuaries.<sup>19</sup> Whereas PHE may primarily support organic carbon catabolism, TNT is also a source of nitrogen and can be disproportionately incorporated into bacterial biomass relative to that mineralized.<sup>8</sup> Moderate levels of AP and concomitant increase in allochthonous DOM can reduce the phytoplankton to the bacterial biomass ratio and increase zooplankton grazing on bacterial biomass,<sup>38</sup> which could, in turn, increase remineralization rates of bacterially incorporated radiolabeled substrate measured as Tmin. Alternatively, moderate levels of AP may stimulate phytoplankton production in the lower NPRES but higher AP can flush phytoplankton responses out of the estuary.<sup>39,40</sup> Another difference in the NPRES is that PHE flux from surface runoff in urbanized areas would be expected to affect microbial assemblage composition and thus affect measured Pmin rates, whereas there is little likelihood of TNT flux into the NPRES from any source (no unexploded ordnance or the former military use). Unlike with TNT and PHE, biodegradation of RDX and HMX in the water column of NPRES showed little relation to AP though both were often highest nearer the headwaters. If some component of RDX and HMX biodegradation is enhanced by the presence of anaerobic microenvironments in suspended aggregates,<sup>41</sup> then precipitation events could affect rates in different ways. Wind-driven surface sediment resuspension into the water column can co-occur with precipitation events throughout these shallow estuaries,<sup>42</sup> especially nearer to the shallow headwaters where higher Rmin and Hmin were observed. Future studies will focus on the role of estuarine aggregates in RDX and HMX biodegradation in these ecosystems.

**Nitrogen Demand.** Most energetic compounds are nitrogen-containing organics and nitrogen is typically scavenged by natural microbial assemblages in N-limited coastal ecosystems.<sup>43,44</sup> High incorporation rates for <sup>14</sup>C-TNT, -RDX (79–100%), and -HMX (36–100%) into estuarine and marine

bacterial biomass<sup>5,8</sup> suggest that their role as a source of organic nitrogen needs to be taken into account when examining fate and transport through estuaries. With mineralization of the carbon backbone to CO<sub>2</sub>, the measured process is likely to involve assimilatory metabolism and subsequent catabolism. One might assume that only dissimilatory removal of the nitrogen groups takes place when using methods that only measure mineralization of energetic nitrogen to atmospheric N<sub>2</sub>. The latter process is often the more studied pathway (e.g., Smets et al.<sup>45</sup>).

Using marine mesocosms, Tobias, Vlahos, and co-workers performed a series of experiments tracking the fate of <sup>15</sup>N-TNT and <sup>15</sup>N-RDX into the various nitrogen pools, macro and microbiota.<sup>46–50</sup> Although it is difficult to directly compare among different systems and different mineralization methods, with roughly 400 μg of RDX L<sup>-1</sup> added at day 1 and 6% mineralized by day 21, the mineralization rate estimate of ca. 1 μg of RDX L<sup>-1</sup> day<sup>-1</sup> to N<sub>2</sub><sup>44</sup> is comparable to the Rmin rates to CO<sub>2</sub> in coastal waters measured in this study. Smith et al.<sup>47</sup> found that 4.9% of TNT-N was present in the DIN pool after 4 days (same as our incubation time), which roughly converts to 0.4 μg of TNT-C L<sup>-1</sup> day<sup>-1</sup> a rate well within the range of our rates for the NPRES. It was concluded that both aerobic and anaerobic processes were likely accounting for the transformation processes measured in the mesocosms, and that the presence of sediment (and its anoxic microenvironments) was especially important for elevated rates of RDX biodegradation.<sup>48</sup> Though the water columns sampled in this study were aerobic when measured at the macroscale, this does not obviate the possibility that suspended aggregates with hypoxic or anoxic microenvironments<sup>51</sup> impacted measured mineralization rates in the estuary. Especially in shallow estuaries, such as those in this study, biodegradation of organic nitrogen sources is most likely a balance between assimilatory and dissimilatory nitrogen utilization (e.g., ref 52 also see discussion in Montgomery et al.<sup>6</sup>).

Another relationship between the estuarine nitrogen cycle and energetics biodegradation could involve phytoplankton blooms. There are a few examples of microalgae involvement in TNT bioremediation (review by Chekroun et al.<sup>53</sup>), but a more likely role in estuaries might be to limit DIN availability and thus increase DON demand by heterotrophic bacterial assemblages (e.g., Kirchman and Wheeler,<sup>54</sup> Kirchman<sup>55</sup>). Low DIN concentration, rapid DIN turnover, and elevated nitrogen demand (based on primary production rates and measured C/N of POM) all co-occurred with a dinoflagellate bloom and high min/BP ratios for TNT, RDX, and HMX in the New River headwaters. It is possible that high nitrogen demand created by the phytoplankton bloom could have stimulated increased DON scavenging by the heterotrophic assemblage

leading to the higher energetic carbon min/BP ratios in this area of the estuary.

**Ecosystem Capacity.** The capacity for energetics and PHE biodegradation was estimated for three coastal North Carolina water bodies that have significantly different features in terms of hydrology, trophic state, and potential contaminant sources. The NWRE is marine-dominated, relatively wide at mid-estuary with a constrained opening to the Atlantic Ocean and strong urban influence (Jacksonville, North Carolina, United States) at the headwaters. Much of the estuary is surrounded by military property (U.S. Marine Corps Base Camp Lejeune) where training activities occur, so there exists the possibility that energetics could enter the estuary largely via surface runoff from shoreside training ranges. The 70 days mean residence time is the longest of the three systems by an order of magnitude. Much of the calculated biodegradation capacity for these compounds is calculated to occur mid-estuary (salinity = 13–16.3) in two segments that represent half of the estuarine water volume and account for 65–71% of capacity for BP, RDX, TNT, and PHE biodegradation (Table 2).

Similar to the NPRE,<sup>19</sup> Pmin along the NWRE transect was highest ( $1.2 \pm 0.05 \mu\text{g C L}^{-1} \text{ day}^{-1}$ ) in the lower estuary (salinity = 27.2; Figure 2D). Of note is that the lower estuary also exhibited a decrease in aromatic OM ( $a_{254}$  and  $\text{SUVA}_{254}$ ) below the trend line for conservative mixing (Figure 3E,F). This is more evidence that Pmin may be a proxy for aromatic OM biodegradation in estuaries.<sup>19</sup> HMX biodegradation was limited to the upper reaches of the estuary (salinity <13). Though the capacity was elevated mid-estuary, there was no evidence from either mineralization rates or mineralization rates normalized by bacterial production (min/BP) that the microbial assemblage in this area was adapted for energetics catabolism as one might expect if there was a substantial chronic flux of energetics to the surface water.

In contrast to the NWRE, the NPRE has no plausible source of energetic compounds to the watershed. The highest AP in the NPRE watershed coincided with the lowest capacity for TNT biodegradation but the highest capacity for biodegradation of PHE, RDX, and HMX (Table S1). The higher RDX biodegradation capacity in the water column related to high AP is curious given that low flow periods in the NPRE are associated with the highest denitrification flux.<sup>56</sup> Either dissimilatory nitrogen processes are not that important for Rmin in the water column or storm event-related resuspension of aggregates from the surface sediment into the water column-enhanced Rmin rates.

Water from lower NPRE mixes with Onslow Bay through Beaufort Inlet, and this region is an area with high ship traffic and industrial activity. The segment closest to the inlet comprised 24% of the water volume in the NPRE but BP decreased from 32 to 28 to 24% of the total BP for the estuary with increasing AP (Table 3). PHE biodegradation capacity varied from 35 to 75% of that for the NPRE with the highest percentage at moderate AP. Ship traffic and industrial activity (ship terminal offloading) could potentially increase PHE flux to the lower Newport, which could increase selective pressure on the estuarine assemblage toward PHE degraders.<sup>57</sup> After high AP, the highest capacity for degradation of energetics was in the lower estuary segment, as well, with TNT at 67% of the total for the estuary, RDX at 82%, and HMX at 32% (Table 3). Osburn et al.<sup>19</sup> found evidence that this area has enhanced biodegradation of aromatic OM, which supported higher Pmin

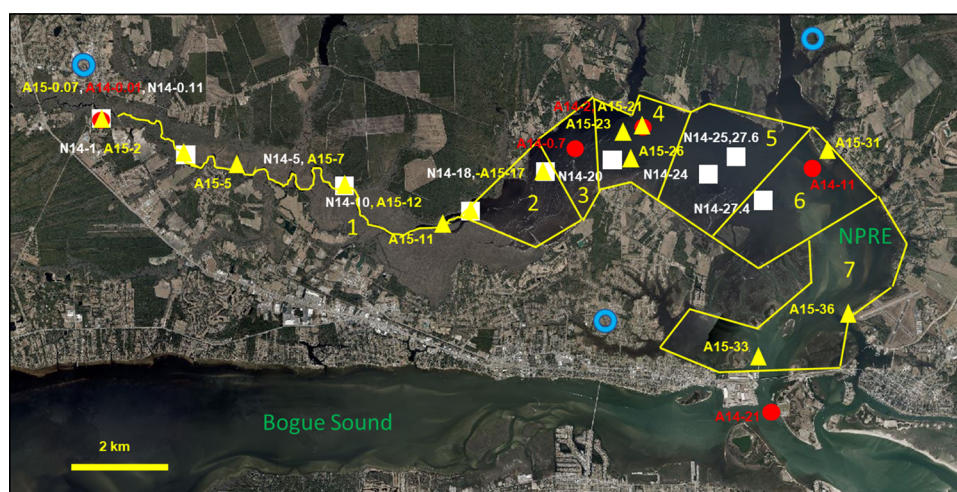
and therefore may have a higher capacity for TNT biodegradation as seen here.

In addition to changing the biogeochemical conditions in the estuary, AP may decrease residence time along a transect. Changing residence time from the mean of 6 days to the low of 2 days<sup>25</sup> for the high AP sampling (14 Aug) resulted in a calculated BP capacity of 75 kg, which is still higher than the 64 kg for the low AP sampling at 6 days residence time (14 Nov; Table 3). This suggests that high AP events may stimulate heterotrophic metabolism capacity even if the higher flow decreases residence time within the estuary by 3-fold. The capacity for PHE degradation was almost unchanged (0.57 vs 0.59 kg) as that for RDX was higher by about 50% in the high AP (2 days residence time) sampling. With the high AP sampling (2 days residence time), the capacity for HMX biodegradation decreases by over 50% and that for TNT decreased by an order of magnitude. This suggests a differential response to AP related events that is not necessarily a simple function of change in overall heterotrophic metabolism by the assemblage.

Tester et al.<sup>36</sup> found that phytoplankton abundance lags precipitation and wind-driven resuspension by 3–4 days at mid-estuary in the NPRE but is modulated by seasonal changes near the lower estuary. Although TNT is aromatic and may have higher rates of biodegradation where PHE is removed, as a source of nitrogen, TNT (along with RDX and HMX) degradation may also be coupled to variability in nitrogen demand, such as that accompanying phytoplankton blooms in the NPRE. In addition, though increased AP can decrease residence time, and thus reduce the amount of contaminant biodegraded in the estuary (resulting in overestimates of capacity), the increased velocity of the river flow may increase respiration rates in situ, which may counter this to some extent.<sup>58,59</sup>

In contrast to the NPRE and NWRE, Bogue Sound is a back-barrier lagoon that receives minimal freshwater input. Bogue Sound also includes a potential source of energetics from a historic bombing target at Cat Island, which is currently a restricted area due to the possibility of unexploded ordnance. In Bogue Sound, TNT biodegradation capacity in the water column was about 50% lower during the high AP sampling, though there was a greater capacity for biodegradation of PHE, RDX, and HMX. These trends are consistent with those seen for NPRE. Despite Cat Island's history of potential energetics exposure, the energetics mineralization rates are similar to the other stations in Bogue Sound and in the NPRE, with no known energetics exposure history.

Though the primary focus of this study was understanding energetics degradation by water column assemblages, there were measurements also made in surface sediment in Bogue Sound. Interestingly, the same station measurements of sediment and overlying waters showed much higher rates in the sediments. Also, min/BP ratios were 2–8-fold higher for TNT and 1–2 orders of magnitude higher for RDX suggesting that the metabolism of these two compounds could potentially be more important to supporting the metabolism of sedimentary assemblages relative to those in the water column. One reason could be that a greater proportion of sedimentary bacteria can metabolize these compounds. Another could be that the anaerobic microenvironments in the lagoon sediment were important for dissimilatory use of these organic nitrogen sources<sup>48</sup> more so than for assimilatory use by free-living bacteria in the water column. Mineralization of organic



**Figure 5.** Newport River Estuary, North Carolina, United States (NPRE) sampling locations for 14 Aug (red circles, A14-salinity), 14 Nov (white squares, N14-salinity), and 15 Aug (yellow triangles, A15-salinity) associated with the seven areas (yellow lines) of the ecosystem capacity determinations. Rainfall collection stations are designated by hollow blue circles (map from 2016 North Carolina Digital Orthoimagery, NC OneMap Geospatial Portal).

nitrogen can be disproportionately occurring in the shallow lagoon sediment of oyster reefs,<sup>60</sup> such as those proximal to the Cat Island station in Bogue Sound, which were the site of the highest sediment Rmin rates. Suspended aggregates in Bogue Sound have been shown to be important in metabolizing polysaccharides<sup>61</sup> and as a source of microbial hydrolytic activity.<sup>62</sup> Resuspension of sediment aggregates may help drive energetic biodegradation in the overlying water column as intense metabolic activity in aggregates can produce anoxic microzones<sup>51,63</sup> that may enhance RDX biodegradation even in oxic surface waters. Given that RDX seemed to be preferentially biodegraded in the sediment, it is possible that storm-related disturbance, such as wind-driven resuspension of sediment assemblages into the water column, could contribute to this higher capacity.

There are important differences in patterns and the location of contaminant degradation among these different coastal ecosystems that may dictate the level of ecological risk to estuarine fauna and whether or not contaminants are exported from an estuary. A key difference between energetics and PHE is the presence of nitrogen, which may supply nutrients to metabolize these compounds in otherwise organic-rich waters and sediment. Despite these differences, the three ecosystems demonstrated relatively high and consistent capacity for the metabolism of contaminants (relative to the total heterotrophic metabolism) with very different chemical structures. These biodegradation rates are likely conservative given that they are based on mineralization, rather than the incorporation rate into bacterial macromolecules, the latter of which can increase the calculated removal rate by an order of magnitude.<sup>8</sup>

## CONCLUSIONS

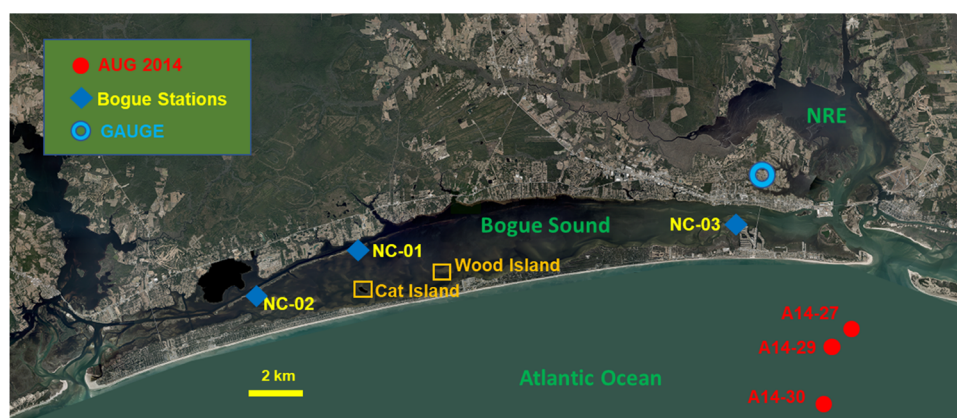
This work represents a unique application of anthropogenic biodegradation rates to estimate the capacity of a coastal ecosystem to assimilate these contaminants and thus reducing their ecological risk prior to export to the open ocean. By comparing biodegradation rates of energetic compounds with those of PHE and rates of heterotrophic bacterial metabolism, one can clarify the relative lability of these compounds in coastal ecosystems. Biodegradation rates can be difficult for ecological risk managers to place within the context of

protecting the environment. These measurements could help managers determine if remedial alternatives protect ecosystems or human health. Energetic compound mineralization rates that are often within a consistent range relative to BP in the environment suggest that the capacity to degrade exotic compounds is not reserved for microbial assemblages from unusual or extreme environments. These findings are consistent with the emerging understanding of OM biodegradation that recalcitrance is not an intrinsic feature of the chemical structure. Further reinforcement of this point is that degradation rates (despite not knowing fluxes) are similar to or higher than PHE, which is the most abundant PAH and has a relatively high flux due to both natural and anthropogenic sources. There is also some evidence that energetic biodegradation may be enhanced in areas of high nitrogen demand (due to phytoplankton blooms) in the NWRE.

The capacity of estuarine ecosystems to metabolize energetics can explain the common lack of detection for these compounds in seawater and marine sediment despite the known presence of unexploded ordnance or recent use in military training exercises. The ecosystem capacity may be the start of analyses to understand the assimilative capacity of an ecosystem to help municipalities and military bases manage land use to be more protective of the adjacent waterways. It should be noted that these types of estimates do not necessarily mean that a given amount of contaminants could be added to an estuarine system without adverse exposure effects on the local biota prior to amelioration. This approach will help elucidate environmental controls on contaminant degradation by placing rates in the context of other OM degradation models of compounds. Overall, measuring ecosystem capacity may help predict the effects of climate change and other perturbations on fate and transport of exotic compounds and provide critical information for decision-makers to create adaptation strategies for these changes.

## MATERIALS AND METHODS

**Site Description.** All three estuaries sampled were located near the Lower Outer Banks in Eastern North Carolina, United States, with the New River farthest South and West, Bogue Sound in the middle and the mouth of the Newport River most

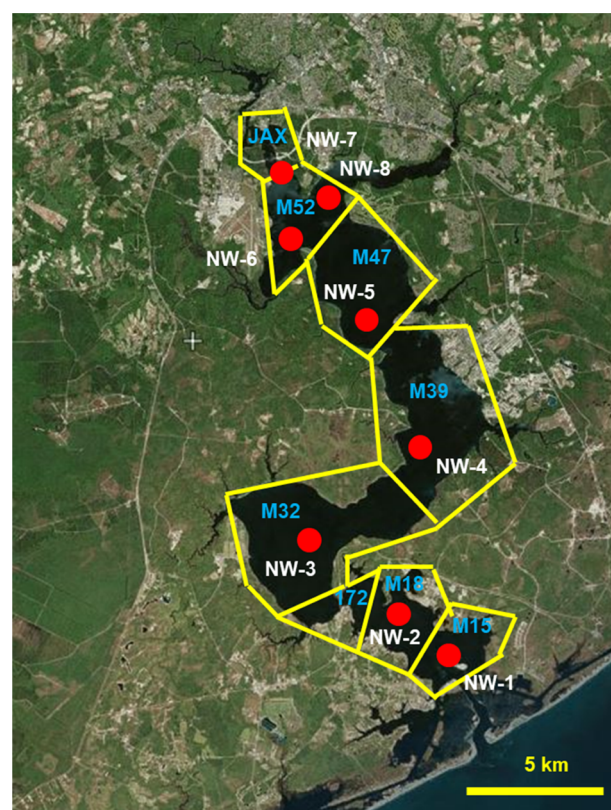


**Figure 6.** Bogue Sound, North Carolina, United States, sampling locations (blue diamonds) including Cat and Wood Islands (orange box) and adjacent Newport River Estuary and its 14 AUG offshore stations (red circles). The rainfall collection station is designated by the hollow blue circle. Offshore Atlantic Ocean seawater was not resolved so it was replaced with a solid fill (map from 2016 North Carolina Digital Orthoimagery, NC OneMap Geospatial Portal).

eastward. NPRE (31 km<sup>2</sup> surface area; mean depth, 1 m; mean residence time, 6 days) is a blackwater river surrounded by wetlands at the headwaters, which includes Cypress swamps and pocosins<sup>25</sup> (Figure 5). Down estuary, the adjacent land is primarily forest and wetlands with some agriculture and urbanized development<sup>25</sup> and no reported input of energetic compounds from ordnance. Bogue Sound (274 km<sup>2</sup> surface area; mean depth, 1.3 m; mean residence time, 2 days) is a shallow, euhaline lagoon estuary bounded by a barrier island (Bogue Banks) and the mainland, both of which are highly developed with single-family residences and vacation homes<sup>29,30</sup> (Figure 6). Inland from Bogue Sound, the watershed is primarily forest and wetland. Two sites in Bogue Sound, Cat and Wood Islands served as bombing targets from 1944 to 1956, and Cat Island may still have live ordnance.<sup>30,64</sup> Note that the Cat Island station (NC01) is in the U.S. Intracoastal Waterway channel ~1.7 km to the north of the restricted area around Cat Island. NWRE (88 km<sup>2</sup> surface area; mean depth, 3 m; mean residence time, 70 days) has the City of Jacksonville at the head of the estuary but is primarily surrounded by Camp Lejeune United States Marine Corps (USMC) Base, which hosts live-fire ordnance exercises on shoreside training ranges<sup>25,30</sup> (Figure 7).

For the Newport River and Bogue Sound, AP was characterized as the cumulative rainfall (inches, converted to millimeters) in the 7 days prior to each sampling recorded at Morehead City, North Carolina (<https://www.usclimatedata.com/climate/morehead-city/north-carolina/united-states/usnc0464>), as reported by Osburn et al.<sup>19</sup> For the New River, precipitation data from the head of the estuary at Jacksonville, North Carolina, United States was used (<https://www.usclimatedata.com/climate/jacksonville/north-carolina/united-states/usnc1305>).

**Sample Collection and Processing.** The 2 days samplings of the NPRE and Bogue Sound took place in August 2014, November 2014, and August 2015, via a small boat, as described in Osburn et al.<sup>19</sup> Sampling for the NWRE took place over one day in April 2014 via the small boat in conjunction with monthly sampling for the SERDP DCERP program (POC: Hans Paerl). At each sampling station, salinity, temperature, and DO were measured using a YSI 6600 (Yellow Springs Inc., Yellow Springs, OH) multiparameter water quality instrument.



**Figure 7.** New River Estuary, North Carolina, United States, 14 Apr sampling locations (red circles) within the segments (blue labels, yellow boxes) described by Ensign et al.<sup>25</sup> (map from 2016 North Carolina Digital Orthoimagery, NC OneMap Geospatial Portal).

**Dissolved Organic Carbon.** DOC concentration measurements were performed as described by Osburn et al.<sup>19</sup> using an OI analytical 1030 TOC analyzer operating in wet chemical oxidation mode and calibrated using standards of caffeine (range: 0–40 mg L<sup>-1</sup>). Error on DOC concentrations was <3%. DOC absorbance was measured on filtered samples from 200 to 800 nm in 1 cm quartz cells. Ultrapure lab water was used as a blank; resulting decadic absorption coefficients were computed and absorptions at 254 nm ( $a_{254}$ ) were divided by

corresponding DOC concentrations to produce specific-UV absorbance ( $SUVA_{254}$ ) measurements.

**Estuarine Hydrology.** There were few published reports on the hydrodynamics of NPRE save for the total area (31 km<sup>2</sup>) and average depth (1 m) from which the total volume could be calculated ( $3.1 \times 10^{11}$  L) and average residence time of 6 days<sup>26,30</sup> (Figure 5). Samplings were largely based on salinity rather than static geolocation. Measurement tools on the Carteret County GIS website (<https://arcgisweb.carteretcountync.gov/maps/>) allowed for sectioning the basin into segments containing at least one station for each of the three samplings. Relative segment areas were summed, and volume and residence time for each segment were calculated as the published volume and published mean residence time multiplied by the relative area. When more than one station was collocated in the same segment, the values for each given parameter were averaged. These estimates could be improved by better resolution of the mean depth for each segment and more accurate areal segmental surface area calculation. This calculation assumes that water entering the estuary originates at the headwater as opposed to the lateral input (e.g., Harlowe Creek, Core Creek).

NWRE sectioning, water volumes, and residence times, reported by Ensign et al.,<sup>25</sup> were correlated with the sample locations in this study. Water volumes of adjacent sections were added together in the cases where our sampling stations did not correlate with the sectioning of Ensign et al.<sup>25</sup> (e.g., M32 + 172). With only three stations and no salinity gradient, Bogue Sound stations BP and mineralization rates were averaged and then multiplied with published total water volume and residence time to determine ecosystem capacities.<sup>29,30</sup>

**Ecosystem Capacity for Energetic Compound Degradation.** Estuary-wide mineralization rates were calculated based on the sum of the measured mineralization rates within each estuarine segment multiplied by segment volume. The ecosystem capacity was then calculated by multiplying the estuary-wide mineralization rate by the average residence time of the estuary. Ecosystem capacity, therefore, defines the mass of a compound that will be degraded in the estuary in the amount of time it takes for the estuary water to be replenished by tides and freshwater inputs.

**Heterotrophic Bacterial Production.** Heterotrophic bacterial production (BP) rate was determined by the Leucine method<sup>65,66</sup> as described in Montgomery et al.<sup>8</sup> using L-[4,5-<sup>3</sup>H]-leucine (specific activity: 154 mCi mmol<sup>-1</sup>; American Radiochemical Corporation (ARC), St. Louis, MO) into bacterial protein.

**Carbon Substrate Mineralization.** Carbon substrates' mineralization to <sup>14</sup>CO<sub>2</sub> was determined for water and sediment by the method described in Montgomery et al.<sup>8</sup> using the following substrates in separate incubations at room temperature (RT): 2,4,6-TNT [ring-<sup>14</sup>C(U)] (4 mCi mmol<sup>-1</sup>, ARC), 9-<sup>14</sup>C-phenanthrene (PHE; 55.7 mCi mmol<sup>-1</sup>, ARC), UL-<sup>14</sup>C-RDX (1.13 mCi mmol<sup>-1</sup>, Defence R&D Canada), and UL-<sup>14</sup>C-HMX (1.97 mCi mmol<sup>-1</sup>, Defence R&D Canada).

**Primary Production and Nitrogen Demand.** For samples collected on April 16, 2014, in the New River Estuary, primary production was measured using the <sup>14</sup>CO<sub>2</sub> method described by Paerl<sup>67</sup> with <sup>14</sup>C-NaHCO<sub>3</sub> (specific activity: 58 mCi mmol<sup>-1</sup>, ICN Radiochemicals). Particulate organic carbon and nitrogen retained on precombusted Whatman GF/F filters were measured on a Costech ECS 4010 elemental

analyzer. Nitrogen demand ( $\mu\text{mol N L}^{-1} \text{h}^{-1}$ ) was estimated as the rate of primary production ( $\mu\text{mol C L}^{-1} \text{h}^{-1}$ ) divided by the C/N molar ratio, and DIN turnover rate ( $\text{h}^{-1}$ ) was estimated as the DIN demand divided by the ambient DIN concentration calculated as the sum of nitrate and ammonium. Nitrate and ammonium concentrations were determined colorimetrically from GF/F-filtered samples using a Lachat Quickchem 8000 autoanalyzer.<sup>38</sup>

## ■ ASSOCIATED CONTENT

### Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acsomega.9b04188>.

Newport River Estuary, North Carolina, United States, rates for BP, Pmin, Tmin, Rmin, and Hmin and respective ratios of min/BP for three samplings. New River Estuary, North Carolina, United States, rates for BP, Pmin, Tmin, Rmin, and Hmin and respective ratios of min/BP, water temperature, salinity, DOC,  $a_{254}$ ,  $SUVA_{254}$ , DO, primary production, N demand, DIN, NH<sub>4</sub>, and DIN turnover for the 14 Apr sampling (PDF)

## ■ AUTHOR INFORMATION

### Corresponding Author

Michael T. Montgomery – Chemistry Division, Naval Research Laboratory, Washington, District of Columbia 20375, United States; [orcid.org/0000-0003-1692-1843](https://orcid.org/0000-0003-1692-1843); Phone: +1-202-404-6419; Email: [michael.montgomery@nrl.navy.mil](mailto:michael.montgomery@nrl.navy.mil)

### Authors

Thomas J. Boyd – Chemistry Division, Naval Research Laboratory, Washington, District of Columbia 20375, United States

Nathan S. Hall – Institute of Marine Sciences, University of North Carolina, Morehead City, North Carolina 28557, United States

Hans W. Paerl – Institute of Marine Sciences, University of North Carolina, Morehead City, North Carolina 28557, United States

Christopher L. Osburn – Department of Marine, Earth, and Atmospheric Sciences, North Carolina State University, Raleigh, North Carolina 27695, United States

Complete contact information is available at: <https://pubs.acs.org/doi/10.1021/acsomega.9b04188>

### Notes

The authors declare no competing financial interest.

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