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Incorporation and mineralization of TNT and other anthropogenic organics by natural microbial assemblages from a small, tropical estuary

Michael T. Montgomery [a,*], Richard B. Coffin [a], Thomas J. Boyd [a], Christopher L. Osburn [b]

[a] Naval Research Laboratory, Marine Biogeochemistry Section, Code 6114, 4555 Overlook Avenue, Washington, DC 20375, USA
[b] Marine, Earth and Atmospheric Sciences, North Carolina State University, Raleigh, NC 27695, USA

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ABSTRACT

2,4,6-Trinitrotoluene (TNT) metabolism was compared across salinity transects in Kahana Bay, a small tropical estuary on Oahu, HI. In surface water, TNT incorporation rates (range: 3–121 μg C L^{-1} d^{-1}) were often 1–2 orders of magnitude higher than mineralization rates suggesting that it may serve as organic nitrogen for coastal microbial assemblages. These rates were often an order of magnitude more rapid than those for RDX and two orders more than HMX. During average or high stream flow, TNT incorporation was most rapid at the riverine end member and generally decreased with increasing salinity. This pattern was not seen during low flow periods. Although TNT metabolism was not correlated with heterotrophic growth rate, it may be related to metabolism of other aromatic compounds. With most TNT ring-carbon incorporation efficiencies at greater than 97%, production of new biomass appears to be a more significant product of microbial TNT metabolism than mineralization.

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1. Introduction

Chemical constituents of munitions, such as 2,4,6-Trinitrotoluene (TNT), hexadydro-1,3,5-trinitro-1,3,5-triazine (RDX) and octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX) can be found as anthropogenic contaminants in coastal environments due to military exercises and range activities, as well as in production facility effluent. Because many coastal ranges and military sites have to be decontaminated prior to return to the public sector, there has been recent interest in biodegradation and environmental fate and transport of these nitroenergetic compounds (see review by Bernstien and Ronen, 2012).

Early studies on energetic compound residence time and transformations focused on differentiating enzymatic pathways and identification of chemical intermediates (see review by Spain et al., 2000). Lignolytic fungi mineralize significant proportions of TNT to CO_2 (20–100%) but bacterial isolates tend to transform TNT to reduced compounds (e.g. DAT; Hawari et al., 2000) which could then sorb to soil and sediment organic matter (see review by Esteve-Nunez et al., 2001). These findings led to the concept that bacteria would primarily transform TNT to intermediates which would then become abiotically 'humified' (Hawari et al., 2000; Knicker, 2003). However, much of this work used monocultures from terrestrial and groundwater environments where microbial growth is often phosphorus limited. Bacterial assemblage growth in coastal environments is typically nitrogen limited and thus it is likely that an organic nitrogen source, such as TNT, would be scavenged (Montgomery et al., 2011a; Pomeroy, 1970).

Recent surveys of coastal estuarine and marine sediment have demonstrated that natural assemblages can mineralize TNT at rates similar to those for other natural organic matter and on the same scale as heterotrophic bacterial production (Montgomery et al., 2011b). Using natural assemblages from coastal estuarine sediment, Gallagher et al. (2010) recently reported that labeled TNT ring carbon and nitrogen becomes incorporated into bacterial DNA. While this and other findings (Ahmad and Hughes, 2002; and references therein) set precedence for TNT assimilation into bacterial biomass, they do not provide for an incorporation rate by natural assemblages. Work presented here examines the rate of TNT incorporation into bacterial macromolecules and biogeochemical factors that influence TNT residence time in a tropical estuarine ecosystem.

* Corresponding author.
E-mail addresses: michael.montgomery@nrl.navy.mil (M.T. Montgomery), richard.coffin@nrl.navy.mil (R.B. Coffin), thomas.boyd@nrl.navy.mil (T.J. Boyd), closburn@ncsu.edu (C.L. Osburn).

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2. Methods

2.1. Sites and sampling

Subtidal stations were sampled during five events from 2006 to 2011 (Table 1) along a salinity transect from a cove along Kahana Stream, Oahu (freshwater end member), out of the mouth into Kahana Bay and to the Pacific Ocean (marine end member; Maciolek and Timbol, 1981). Kahana Stream outflow is oligohaline (5 Practical Salinity Units (PSU) at the mouth) and typically mixes with more estuarine water over a shallow (0.5–1 m deep) shoal extending from the mouth into the bay. Dramatic differences in stream flow affect the freshwater end member salinity and mixing area location. Average daily stream flow (in $ft^3\ s^{-1}$) for the week prior to each sampling event was obtained from the USGS National Water Information System database (http://waterdata.usgs.gov/hi/nwis/current/?type=flow). Surface water and sediment were collected by wading from shore while salinity was measured using a handheld refractometer (Fisher Scientific).

2.2. Carbon substrate incorporation and mineralization

For aqueous samples, incorporation of radiolabeled substrate carbon into bacterial macromolecules was measured at in situ temperature in the dark by TCA precipitation (Kirchman et al., 1985) as described in Montgomery et al. (2008). Carbon substrates 2,4,6-TNT [ring-^{14}C(U)] (4 mCi $mmol^{-1}$, American Radiochemical Corporation, 99% purity), UL-^{14}C-naphthalene (NAH; 18.6 mCi $mmol^{-1}$), 9-^{14}C-phenanthrene (PHE; 55.7 mCi $mmol^{-1}$), UL-^{14}C-RDX (1.13 mCi $mmol^{-1}$, Defence R&D Canada), and UL-^{14}C-HMX (1.97 mCi $mmol^{-1}$), were added in separate incubations to 100 × 16 mm polycarbonate test tubes (ca. 0.2 μg substrate per tube). Assays were initiated within 2 h of sample collection by adding surface water (5 mL) to each set of four tubes per station and incubated at in situ temperature in the dark. Ice-cold TCA (final concentration = 5% v/v) was added to one tube of each set as a killed control and to end live incubations after 48 h. Samples were then filtered (Nuclepore polycarbonate filters, 0.22 μm nom. pore dia.), rinsed twice with 5% TCA and once with 80% ethanol (5 mL each rinse) prior to radioassay (Beckman LS6500 Liquid Scintillation Counter) to determine amount of energetic carbon that had become incorporated into the bacterial macromolecule precipitate on the filter. Mineralized $^{14}CO_2$ was captured on NaOH-soaked filter papers suspended in the headspace of a separate set of replicate tubes. TCA added to end incubations also partitioned any remaining CO_2 from the aqueous sample into the headspace after incubating killed samples O/N prior to radioassay of filter traps. For sediment samples, mineralization rates were measured similarly to aqueous samples except that one mL wet volume of sediment was added to each tube along with filtered station water (0.45 μm nom. pore dia.; Acrodisk, Gelman; 0.5 mL) to form a slurry (Montgomery et al., 2010). Also, instead of TCA, 2.0 mL of 2.0 M H_2SO_4 was added to end incubations and partition remaining CO_2 into tube headspace and filter paper trap. For both incorporation and mineralization, radioassay values for killed incubations were subtracted from that of live incubations. Assay detection limit was 0.01 μg C kg^{-1} d^{-1} for sediment and 0.01 μg C L^{-1} d^{-1} for aqueous samples and average rates minus one standard deviation lower than this were considered below detection (BD).

2.3. Bacterial production and organotolerance

Bacterial production was measured using leucine incorporation (Kirchman et al., 1985; Smith and Azam, 1992) as adapted by Montgomery et al. (2010). Briefly, an aliquot of wet sediment (50 μL) or surface water (1 mL) from each station was added to 2 mL microcentrifuge tubes (three live and one killed control) that were pre-charged with leucine, L-[4,5-^{3}H] (specific activity: 154 mCi $mmol^{-1}$; final concentration = 20 nM). For sediment, one mL of filtered bottom water (0.45 μm nom. pore dia.), collected at <1 m above the sediment–water interface, was added to each tube and vortexed into a slurry. Samples were incubated (0.5 h for water, 2 h for sediment) at in situ temperature and subsequently processed by the method above. Values for killed controls were subtracted from those of live samples. Using a syringe, 1 cm^3 of wet sediment was placed in cleaned tin dishes and dried (O/N, 50 °C) and the dry sediment mass was then used to convert production to dry weight. Finally, leucine incorporation rate into cellular macromolecules was converted to bacterial carbon production using the theoretical minimum 2-fold isotopic dilution factor as determined by Simon and Azam (1989). Assay detection limit was 0.01 μg C kg^{-1} sediment d^{-1} and average rates minus one standard deviation below this were considered BD. Organotolerance was measured by addition of naphthalene to this bacterial production assay according to the method of Montgomery et al. (2010).

Table 1
Five samplings of surface water and/or sediment performed at Kahana Bay from 2006 to 2011 along with daily mean discharge ($ft^3\ s^{-1}$) for the week prior to the sampling and historical (53 year average) USGS data for the Kahana Stream for those same dates; range of daily mean flow of the Kahana Stream, as well as, the flow of the week prior to the sampling relative to the historic data for those dates (relative percentage increase or decrease from 53 year average for the week).

Date	Total samples		Stream flow ($ft^3\ s^{-1}$, daily mean)		
	Water	Sediment	Relative	Week AVG (historic AVG)	Range
11 May 2006	5	0	+25%	30 (24)	27–42
17 August 2006	5	0	+5%	22 (21)	20–22
13 August 2007	5	5	−23%	17 (22)	16–20
20 July 2010	6	6	−18%	18 (22)	16–21
1 August 2011	7	7	+65%	38 (23)	21–78

2.4. Dissolved organic carbon

DOC was quantified by wet chemical oxidation on 2 mL sample volumes, using concentrated and cleaned sodium persulfate (Osburn and St-Jean, 2007). Assay detection limit via this method was 12 μmol C L^{-1} (0.14 mg C L^{-1}) and reproducibility was <5%. Potassium hydrogen phthalate was used as a calibration standard for DOC concentrations over a range of 83–1666 μM.

2.5. HIX and BIX

Relative aromatic character of DOC was measured by its absorptive and fluorescent properties. Spectral absorption (200–800 nm) was measured using a Varian 300UV spectrophotometer and excitation-emission matrix (EEM) fluorescence using a Varian Eclipse spectrofluorometer on 0.2 μm (nom. pore dia.) filtrates from water samples. Excitation (Ex) wavelengths were sampled from 240 to 450 nm at 5 nm intervals and emission (Em) wavelengths were sampled from 300 to 600 nm at 2 nm intervals. Biological index (BIX) was calculated using the ratio of emission intensities at 380 nm and 430 nm at an excitation of 310 nm (Huguet et al., 2009). BIX values >1.0 correspond to freshly produced DOM of algal or bacterial origin, whereas values <0.6 indicate little freshly produced material. Humification generally increases the aromaticity of DOM, and this was estimated with a humification index, HIX, calculated from the ratio of two integrated emission wavebands: 435–480 nm to 300–345 nm, at 255 nm (Zsolnay et al., 1999). HIX values <10 correspond to relatively non-humified DOM (Birdwell and Engel, 2009) and generally increase with degradation. HIX also shows a direct correlation with aromaticity and is inversely correlated with carbohydrate content (Kalbitz et al., 2003). Optical data processing was performed using in-house codes written in the Matlab® computing environment (Mathworks, Inc.).

2.6. Mixing experiment

A mixing experiment was performed by combining 10 L of each end member (2, 35 PSU) in 20 L carboys, as well as, maintaining two 20 L control treatments of each end member at ambient lab temperature. All three incubations were subsampled for bacterial production, mineralization (TNT, naphthalene and phenanthrene), as well as fluorescence. Subsamples were drawn at initiation of the experiment (T_0) and after 48 h (T_{48}) though mineralization rates were only determined for T_0 mix and end members because assay incubation time was 48 h.

3. Results and discussion

3.1. TNT incorporation

One fate of aromatic organic carbon metabolized by bacteria is incorporation into bacterial macromolecules (e.g. proteins, DNA, lipids, complex carbohydrates). In surface water, incorporation rates of TNT ring carbon (range: 3–121 μg C L^{-1} d^{-1}) were often 1–2 orders of magnitude higher than mineralization rates (0.07–0.95 μg C L^{-1} d^{-1}), suggesting that TNT may provide organic nitrogen to coastal microbial assemblages (Table 2). TNT incorporation rates were often an order of magnitude more rapid than those for RDX (1.2–4 μg C L^{-1} d^{-1}) and two orders more than HMX (0.33–0.89 μg C L^{-1} d^{-1}; Table 3). During average or high stream flow, TNT incorporation by surface water assemblages was most rapid at the stream end member and generally decreased with increasing salinity though little pattern was seen during low flow (Table 2). Highest rate of TNT incorporation was measured during average stream flow conditions (August-06; Table 2) at what appeared to be a frontal boundary between stream and seawater (10 PSU station). The possible role and underlying mechanisms by which a frontal boundary may provide the conditions to enhance