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Mineralization of 2,4,6-Trinitrotoluene (TNT) in Coastal Waters and Sediments

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14. ABSTRACT

Mineralization rates of 14C-TNT, -DNT, and -DAT were measured in surface sediments, with depth in sediment cores, and in the water column during 12 research cruises over the past 4 years in the San Francisco Bay, Chesapeake Bay, and Hawaii. Mineralization rates were also compared to uptake and incorporation rates of TNT, DNT, and DAT into the natural microbial assemblage. In general, the bacterial mineralizations rates were similar, or an order of magnitude faster, than those for organic hydrocarbons measured at the same time. Using seawater sampled from a historical UXO field, we found that while bacterial mineralization was rapid (>1 ug L-1 d-1, unfiltered water in dark), photodegradation was even faster (16 ug L-1 d-1, filtered water in light) and the combination of light and unfiltered water was greater than the sum of the two rates (103 ug L-1 d-1), suggesting either that the prescnce of active phytoplankton is important or photodegradation enhances bacterial mineralization.

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MINERALIZATION OF 2,4,6-TRINITROTOLUENE (TNT) IN COASTAL WATERS AND SEDIMENTS

INTRODUCTION

The fate and transformation of the 2,4,6-Trinitrotoluene (TNT) has been studied extensively in terrestrial and freshwater systems (Spain et al. 2000, Esteve-Nunez et al. 2001); however, similar investigations in the marine environment have been limited to date (Darrach et al. 1998, Lotofu et al. 2001). These systems are particularly important given historical and current ordnance disposal and training activities that may liberate energetic compounds through detonation or slow release from unexploded ordnance (UXO). Though these compounds persist in soil, freshwater sediments, and groundwater (Spain et al. 2000), preliminary investigations into their fate in marine systems have demonstrated a rapid biological and photochemical degradation. The conflict in these latter findings relative to those of other researchers may directly relate to the ecosystem studied. Heterotrophic bacteria in terrestrial and groundwater environments are widely known to be phosphorus-limited, whereas the growth of marine microbiota is nitrogen-limited (Vitousek and Howarth 1991, Carpenter and Capone 1983). Although TNT is not commonly found in nature, it can supply a valuable, growth-limiting nutrient to estuarine and marine ecosystems. This may be due to the denitration step in TNT transformation that liberates either NO_3^- or NH_4^+ depending on oxygen availability (Spain et al. 2000). Additionally, genetic evidence supports the degradation of these compounds by bacteria that metabolize other types of naturally occurring organic matter (polycyclic aromatic hydrocarbons (PAHs), lignin; Suen et al. 1996). If proven to the extent that these findings are accepted by the Environmental Protection Agency (EPA), they have dramatic implications for lowering Navy UXO recovery and compliance costs and ecological damage liability. Currently, there are no acceptable methods for cleanup of energetics in estuarine sediments, as even dredge and haul practices are complicated by the presence of UXO buried within the sediment. Land farming and composting are the most commonly reported strategies for soil cleanup, while for groundwater cleanup, slurry bioreactors (Steffan and Drew 1995), binding to organics (Fant et al. 2001), and reduction by Zero Valent Iron (ZVI) and related compounds have been proposed or attempted at the pilot scale (for reviews, see Peres and Agathos 2000, Rodgers and Bunce 2001).

Abiotic – Chemical

Once released, either through detonation, casing breakage, or by slow leaks from UXO, TNT may sorb onto particulates, partition to dissolved organic matter (DOM), or remain dissolved in aqueous media. TNT has nitro groups (NO_2^-) on its ring structure that are polarizing and form hydrogen bonds with water. This characteristic increases the affinity of the TNT for aqueous solutions and charged surfaces (Haderlein et al. 2000, van Beelen and Burris 1995, Lotufo et al. 2001). In addition, TNT has an aromatic ring bound to the nitro groups which leads to substantially greater water solubility than other nitrogenous energetic compounds (NECs; e.g., RDX, HMX) (Lynch et al. 2001). Adsorption of nitroaromatics (Haderlein and Schwarzenbach 1993, Haderlein et al. 1996, Weissmahr et al. 1997, Daun et al. 1998, Weissmahr et al. 1999) and nitramines (Leggett 1985, Sheremata et al. 2001, Brannon et al. 2002, Monteil-Rivera et al. 2003) to clays, specifically phyllosilicates, was determined to dominate sorptive processes in aqueous suspensions and groundwater. With smectite clays, the binding of the nitro groups of TNT essentially replace the calcium in the clay matrix, thus integrating the energetic (Li et al.

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2004). Under anoxic conditions in water, the nitro groups of TNT can be reduced to amines (Barrows et al. 1996) which also affects its reactivity (Achtnich et al. 2000). The presence of reduced colloidal or particulate iron (ZVI) can also reduce these nitro groups and enhance both the reactivity and mineralization of TNT in aqueous environments (Agrawal and Tratnyek 1996, Devlin et al. 1998, Brannon et al. 2002) though transformation rates based on laboratory experiments may overestimate the expected rates in the field (Bandstra et al. 2005). In addition to ZVI, zinc was also found to reduce TNT and longer term reactivity was enhanced by the presence of corrosion promoters like chloride (Hernandez et al. 2004).

TNT has a low octanol-water partition coefficient (K_{ow}), indicating a limited affinity for organic matter relative to other organic contaminants which have a higher K_{ow} and commensurate higher affinity for organic matter (e.g., PAHs, PCBs (Karickhoff 1984, Schwarzenbach et al 1993)). Significant differences in TNT sorption behavior were observed between low (0.02%) and high (8%) organic matter soils (Sheremata et al. 1999). Absorption into natural organic matter (NOM) has been identified as a significant process for organic contaminants in environments where NOM is greater than >0.2% (Schwarzenbach et al. 1993), which would be expected in estuarine systems. Many of these terrestrial and groundwater studies identify the association of TNT and particulate organic matter (POM) as an irreversible binding (Selim et al. 1995, Comfort et al. 1995, Pennington et al. 1995, Achtnich et al. 1999, Pennington et al. 1990) but in the presence of live bacterial assemblages, it would be difficult to differentiate radiolabel incorporated into bacterial macromolecules from covalent binding to POM. The presence of colloidal and dissolved organic matter (DOM), may also impact the fate of TNT in the marine water column and sedimentary porewater, as indicated in previous investigations on the enhanced water solubility of several organic pollutants by dissolved humic and fulvic acids (Chiou et al. 1986). Dissolved humic acids decreased the aqueous concentration of TNT and its metabolites in adsorption experiments under anaerobic conditions (Daun et al. 1998); however, an investigation using topsoil-derived DOM produced conflicting results, with no changes in the apparent nitroaromatic solubility (Sheremata et al. 1999). Using soil composting material, Thorn and coworkers (Thorn and Kennedy 2002, Thorn et al. 2002) found that TNT was reduced to nitramines and then covalently bound to humins in soil and this binding was increased by the presence of peroxidases. The majority of these previous investigations have focused predominantly on soil and groundwater environments that have limited available organic material (dissolved and particulate) (Haderlein et al. 1996, Weissmahr et al. 1999). Estuarine sediments have significant differences in organic geochemistries (Hedges and Oades 1997), which may limit the extrapolation of TNT behavior from terrestrial systems to marine systems.

Abiotic -- Photolysis

Photochemical degradation (i.e., photolysis or photooxidation) of TNT in aqueous solutions has been investigated as a treatment strategy for contaminated soils, slurries, and waters—particularly in conjunction with catalysts, such as TiO_2 (Schmelling et al. 1996, Son et al. 2004), or with additions of peroxide and ozone (Rodgers and Bunce 2001), borohydride (Larson et al. 1996), H_2O_2 (Ho 1986), and Fenton's reagent (H_2O_2 and Fe^{2+}). ZVI has also been used to enhance contaminant degradation, which results in the reduction of the contaminant, generating a suite of reaction products (Oh et al. 2003). Collectively known as advanced oxidation processes (AOPs), these techniques employ reactive oxidizing species such as hydroxyl radical (OH·) and superoxide to facilitate the opening of aromatic rings and the ultimate mineralization of organic pollutants to CO_2 and H_2O (Rodgers and Bunce 2001). Because both OH· and superoxide ion are very effective oxidants for organic compounds, they hold much promise for *in situ* chemical oxidations of organic pollutants.

As sunlight (or artificial UV light) decomposes TNT, it is converted into a variety of aromatic photolysis products including the nitroamines and azoxydimers found in biodegradation (Andrews and Osmon 1977). Direct photolysis of ^{14}C-labelled TNT by high energy UV for a period of six days was

reported to partially cleave the ring and degrade 17% of labeled TNT to $^{14}CO_2$ (Andrews and Osmon 1975). Under natural light conditions, TNT half lives are reportedly as short as 10 min for sunlit Holston River water, 20 min for Searsville Pond water, 90 min for Waconda Bay water to 11-22 hours for distilled water (Spanggord et al. 1983, Mabey et al. 1983, Talmage et al. 1999). There are few reports of TNT degradation in seawater though Hoffsommer and Rosen (1973) found it to be stable for 108 days. Nitroaromatics (TNT, DNT) absorb electromagnetic radiation in the ultraviolet (UV) range of 200 nm and 400 nm (Bartolo et al. 1979). The absorption spectra are exponential, thus the absorption in the environmentally-relevant UV (290 to 400 nm) is much less than at wavelengths <270 nm (Meenakshisundaram et al. 1999). This relationship means that sunlight energy is often too weak to cause much direct photolysis deep within an estuarine water column, but research has shown that the presence of oxidizing agents and catalysts may facilitate complete degradation of TNT with sunlight. These agents and catalysts may already be present in the water column under certain environmental conditions.

Laboratory bench top studies and batch reactor applications in the late 1970's confirmed that UV radiation (high energy at 254 nm) and H_2O_2 photochemically degraded TNT, RDX, HMX, 2,4 DNT, 2,6 DNT, and Explosive D (ammonium picrate) when in solution (Andrews 1980, Andrews and Osmon 1977). DiGnazio et al. (1998) hypothesized photodegradation as a partial explanation for nitrate generated from explosives stored in lagoons at the Ammunition Burning Ground at the Crane Naval Surface Warfare Center. This suggests that NEC may naturally degrade in sunlight, even though absorption is relatively weak. The oxidation of an organic pollutant with some combination of UV, H_2O_2, Fenton's reagent, or catalyst generates various degradation products which may or may not be amenable to further photochemical degradation. Recently, Liou et al. (2004) has determined that TNT degradation follows first order kinetics and has deduced the steps of degradation for the photo-Fenton process, though these experiments used distilled water. Characterization of the reaction products via each oxidation pathway in natural estuarine waters is critical to ensure that a mitigation strategy does not produce a more persistent compound of greater toxicity (i.e., azo, azoxy and nitroso compounds), as enhanced toxicity in bioassays has been observed during the photodegradation of TNT (Davenport et al. 1994).

On the laboratory scale, Ho (1986) used a UV/H_2O_2 reaction system to remediate 2,4-DNT and delineated the reaction pathways from the pollutant to low molecular weight carboxylic acids to CO_2. In the field, Fenton's reagent (Fe^{2+}/H_2O_2) has been used in recent years to remove or transform a variety of pollutants, including TNT, found in groundwater or wastewater treatment streams (Zoh and Stenstrom 2002, Oh et al. 2003). Li et al. (1999) found that Fenton's reagent was more effective at pH 6 than pH 3 due to the precipitation of Fe^{3+} at pH >5. Because marine ecosystems have a pH near 8, Fenton chemistry may mediate some component of TNT degradation through active photochemical redox cycling of iron (Miller et al. 1995), an important component of which is H_2O_2 generation.

The efficacy of photochemical degradation and the associated mechanistic pathways, such as those described above, is fundamentally related to the aquatic system in which the degradation is taking place. Freshwater ecosystems at mid-latitudes, such as surface water disposal lagoons (e.g., NSWC Crane, IN), are likely poor environments for substantial degradation of NEC by photochemical degradation. These aquatic ecosystems often have high concentrations of CDOM, effectively limiting the photic zone where photochemical reactions may occur (less than 1 cm to several meters). Atmospheric conditions (clouds, chemistry) and seasonal variations in insolation, the ground level solar radiation reaching surface waters, may also limit the photon efficiency for photolysis. At mid-latitudes, this means that strong seasonal variation in the efficiency of photochemical reactions is expected. In contrast, marine ecosystems at subtropical latitudes (Hawaii, Puerto Rico, and the Bahamas) have very low amounts of particles and CDOM, thus the photic zone may extend much deeper and insolation is much greater than freshwater ecosystems. For example, we have measured very low sunlight attenuation off the coast of Oahu, HI, such that the effective photic zone depth may extend to depths of 40 m (the limit of detection of our radiometer) and perhaps greater depths where there may be substantial photochemical degradation. The

photochemical degradation of TNT produces biologically-important inorganic N (nitrate, ammonium ion) from the denitration of nitro groups on TNT (and its derivatives) and the deamination of amino groups of RDX and HMX. Thus photochemistry may be an important process for fueling increased production in aquatic ecosystems and therefore enhanced TNT degradation.

Biotic – Primary Producers

Microalgae

Although the transformation of TNT via bacterial and photolytic mediation has been examined, little information is available on the transformation of TNT by marine microalgae. This aspect of cycling and fate of TNT is particularly relevant because nitrogen (N), a key component of energetic compounds, frequently controls the rate of primary production in marine, estuarine and some freshwater ecosystems (Sharp 1991). Most work on TNT and microalgae has described the toxic effects of the energetic (Smock et al. 1976, Won et al. 1976, Burton et al. 1994, Tandros et al. 2000) though many of these effects can be reversed if the algae are maintained in flow through systems (Pavlostathis and Jackson 2002). There are some reports on uptake and metabolism of TNT by microalgae (Mondecar et al. 1994, Pavlostathis and Jackson 1999, 2002) as a potential environmental cleanup strategy.

Phytoplankton (planktonic eukaryotic microalgae and cyanobacteria) are the main contributor to aquatic primary production (Paerl et al. 2003, Sellner 1997). In addition to their central roles in carbon and nutrient cycling in aquatic ecosystems, they are also highly sensitive to changes in nutrients and toxic compounds. These microorganisms are thus uniquely suited as potential indicators of regional contamination by TNT, and also mediators for remediation. Initial work has focused on cyanobacteria, specifically *Anabaena* sp., in marine environments found in association with TNT manufacturing effluent (Pavlostathis et al. 1998). Cyanobacteria and other microalgae are known to transform aromatic contaminants such as naphthalene, phenanthrene and other hydrocarbons (Ellis 1977, Cerniglia and Gibson 1979, Cerniglia et al. 1980a,b, Narro et al. 1992, Kuritz and Wolk 1995, Thies et al. 1996, Kuritz et al. 1997, Kirso and Irha 1998).

Under N limited conditions typical of marine systems, a mixed culture of *Anabaena* and heterotrophic bacteria efficiently removed (96% at 58 mg L^{-1} starting concn., ca. 50 mg TNT L^{-1} d^{-1}) and metabolized N from TNT (Pavlostathis and Jackson 2002). This was compared to rates about 30-fold lower for the bacteria only culture media. The difference between the two treatments was attributed to uptake by *Anabaena* alone (Pavlostathis and Jackson 2002) though it should be noted that if there was enhanced removal by bacteria in the presence of *Anabaena*, this removal would have been attributed to *Anabaena* by these researchers. These works highlight the possible use of marine cyanobacteria for TNT remediation. Despite this potential, many cyanobacterial species (e.g., *Anabaena*) are considered nuisance species because they can be toxic and can fix atmospheric N, thus contributing to N overenrichment (i.e., eutrophication) (Paerl 1988, Paerl et al. 2001, 2003, Sellner 1997). They are often a poor food source for higher organisms, and can accumulate and form unsightly blooms. These factors illustrate the importance of determining the underlying mechanisms of energetic degradation by marine microorganisms.

Macrophytes

TNT metabolism and degradation by macrophytes has involved the study of whole plants, plant cell culture, and transgenic plants. The types of whole plants that have been studied include the bush bean (Harvey et al. 1990), hybrid poplar (Thompson et al. 1998), soybean (Adamia et al. 2006), switchgrass (Peterson et al. 1998), ryegrass (Sung et al. 2004), yellow nutsedge (Palazzo and Leggett 1986) and a variety of wetland species (Best et al. 1999 a, b, Bhadra et al. 1999 a, b, Burken et al. 2000, Gorge 1994, Hughes et al. 1997, Nepovim et al. 2005, Paquin et al. 2004, Pavlostathis et al. 1998, Vanderford et al. 1997). Some investigators have used cell cultures (Hughes et al. 1997, Vanek et al. 2002, Vila et al. 2005) or plant extracts (Medina et al. 2004) to differentiate between degradation by the plant cells vs the associated microflora. Commensal bacteria are known to enhance TNT metabolism (Ramos et al. 2005, Van Aken et al. 2004) especially those bacteria found in the rhizosphere (Siciliano et al. 2000). There have also been some recent efforts to model the effect of TNT phytoremediation on natural ecosystems (Schoenmuth and Pestemer 2004, Ouyang et al. 2005).

Plants can sequester TNT as a detoxification mechanism (Bhadra et al. 1999, Sens et al. 1999, Wayment et al. 1999), or incorporate, fully metabolize, or partially degrade it to reduced metabolites (e.g., monoamino derivatives 2- and 4-ADNT) (Best et al. 2005). TNT degradation products found in the plant tend to be localized primarily on membranes of the endoplasmic reticulum, mitochondria, and plastids (Adamia et al. 2006). The degradation products were mainly low molecular weight organics in the roots and higher molecular weight biopolymers in the stems and leaves (Adamia et al. 2006). TNT transformation occurs primarily as two types of enzymes systems are used, oxidative and nitroreductive, with the oxidative enzymes most associated with detoxification and the nitroreductive with metabolism (Best et al. 2005) with many of the degradation products being similar to those found in microbial degradation (Bhadra et al. 1999). The oxidative enzyme system (peroxidase, phenoloxidase) is generally constitutively produced and confers resistance to the presence of the TNT in soil and water (Adamia et al. 2006, Best et al. 2005). The nitroreductive enzymes are inducible in the presence of TNT and the levels are correlated to the TNT removal rate by the plant. It is this latter class of enzymes that appears to be most important in determining the suitability of a plant species for TNT phytoremediation (Adamia et al. 2006).

In addition to the correlations between enzymatic activity and TNT transformation in wild-type plant species, there have been several reports of the creation of transgenic plants that have incorporated and expressed a bacterial nitroreductase gene (French et al. 1995, French et al. 1998, French et al. 1999, Hannink et al. 2001, Kurumata et al. 2005). Kurumata et al. (2005) found that transgenic seedlings increased their uptake of TNT by seven to eight times when exposed to TNT in solution. Tobacco plants were developed that harbored the bacterial *onr* gene that encodes the pentaerythritol tetranitrate reductase from *Enterobacter cloacae* (French et al. 1995, French et al. 1998, French et al. 1999). These plants showed detectable expression of the reductase enzyme in leaf and root tissue and were able to germinate and grow in the presence of TNT concentrations that were inhibitory to the wild-type plants.

Biotic – Secondary Producers

The rate of TNT attenuation below the photic zone in coastal waters and sediments may be largely controlled by metabolism by heterotrophic bacteria. Virtually all of the published information on bacterial NEC metabolism is derived from work in terrestrial soil (e.g., Drzyzga et al. 1999, Steffan and Drew 1995), groundwater (e.g., Krumholz et al. 1997) and freshwater systems (e.g., Spain et al. 2000). These studies provide a substantial amount of information on enzymatic pathways (see review by Peres and Aganthos 2000), production of intermediates or dead end products (Drzyzga et al. 1998), strain identification (Lessner et al. 2002, Smets and Mueller 2001) and some genetic information on the natural assemblage (Fuller and Manning 1998). Typically, some combination of aerobic and anaerobic

metabolism is required for near complete transformation of TNT carbon and this often starts with denitration prior to ring cleavage (Fiorella and Spain 1997). There are also many reports of incomplete metabolism resulting in the production of intermediates or dead end by-products that may be reactive with natural organic matter (e.g., humics) (Knicker et al. 1999, Bruns-Nagel et al. 2000, Thorn et al. 2002). These partial degradation products may be more toxic than the parent compound which may result in increased risk to the ecosystem. There are also some early reports on the use of recombinant bacteria for TNT metabolism (Duque et al. 1993). To date, most work on NEC transformation by bacteria has been performed with environmental isolates rather than natural assemblages.

TNT biodegradation has been reported for a wide variety of bacterial isolates cultured from freshwater or terrestrial environments including *Pseudomonas aeruginosa* (Alvarez et al. 1995, Kalafut et al. 1998, Oh et al. 2003), *P. fluorescens* (Pak et al. 2000), *P. pseudoalcaligenes* (Fiorella and Spain 1997), *Pseudomonas* sp. (Haïdour and Ramos 1996), anaerobic *Desulfovibrio* sp. and anaerobic *Serratia* sp. (Drzyga et al. 1998), *Bacillus* sp. and *Staphylococcus* sp. (Kalafut et al. 1998), actinomycetes (Pasti-Grigsby et al. 1996), *Enterobacter cloacae* (French et al. 1998), *Klebsiella* sp. (Kim et al. 2002), *Clostridium thermoaceticum* (Huang et al. 2000), and *C. acetobutylicum* (Watrous et al. 2003). There are fewer reports of 2,4-DNT biodegradation by cultured *Arthrobacter* sp. (Tope et al. 1999) *Alcaligenes* (Smets and Mueller 2001), and *Burkholderia cepacia* (Johnson et al. 2002), and ADNT by *P. aeruginosa* (Alvarez et al. 1995).

A common finding is that the $-NO_2$ groups of the TNT are reduced to $-NH_2$ or denitrated (*Bacillus* sp., Kalafut et al. 1998) with extracellular release of the various aminotoluene partial degradation products. TNT metabolism in monoculture can be limited by nitroreductase inactivation by these partial degradation products (Riefler and Smets 2002) or by more generally described cytotoxicity (Fuller and Manning 1997). Reduction and cleavage of the aromatic ring of TNT has not been characteristic of most bacterial isolates studied to date (Zaripov et al. 2004). Enzymes involved in TNT metabolism include carbon monoxide dehydrogenase (*C. thermoaceticum*, Huang et al. 2000), NAD(P)H-dependent nitroreductase I (*Klebsiella* sp., Kim and Song 2005), NAD(P)H-dependent flavoprotein oxidoreductase (*P. fluorescens*, Pak et al. 2000), Fe-only hydrogenase (*C. acetobutylicum*, Watrous et al. 2003) and the flavin-dependent oxidoreductases related to the old yellow enzyme of yeast (Williams et al. 2004). These latter oxidoreductases did yield metabolic products that are indicative of reduction of the aromatic ring (Williams et al. 2004). Atypically, *Rhodococcus erythropolis* and a 4-nitrotoluene-utilizing *Mycobacterium* sp. possess reductive enzyme systems which catalyze ring hydrogenation without evidence of reductive TNT denitration (Vorbeck et al. 1998).

Heterotrophic bacteria can use TNT as a sole nitrogen source (*E. cloacae*, French et al. 1998), sole carbon source via nitrobenzene (*P. pseudoalcaligenes*, Fiorella and Spain 1997), with both the carbon and nitrogen incorporated into macromolecules (*Pseudomonas* sp., Esteve-Nunez et al. 2000). Carbon incorporation can be relatively high (42%; Drzyzga et al. 1998) though the amount mineralized is typically very low (6% or less, *Klebsiella* sp.; Kim et al. 2002). Toluene can be produced from the denitration of TNT (Boopathy and Kulpa 1992) and the relatively simple genetic manipulation of transferring the *P. putida* TOL plasmid pWWO-Km to *Pseudomonas* sp. allowed the transconjugant to grow on TNT as a sole source of carbon and nitrogen (Duque et al. 1993). Horizontal gene transfer efficiency of the TOL plasmid is very high, especially in biofilms, and particularly among non-culturable strains (Sorensen et al. 2005). Such plasmids are often unstable and lost upon exposure to the high nutrient culture conditions typically involved in isolating strains from the natural environment (Sorensen et al. 2005).

2,4-DNT can also be used as a sole carbon, nitrogen and energy source (*B. cepacia*, Johnson et al. 2002). The 2,4-DNT dioxygenase of *B. cepacia*, and the naphthalene dioxygenase enzyme system of *Pseudomonas* share a common ancestor (Suen et al. 1996) and appear to have evolved relatively recently

based on the presence of extraneous transposable elements within the gene fragment (Johnson et al. 2002). Leungsakul et al. (2005) were able to use enzyme engineering strategies to enhance degradation of NECs including 2,5-DNT by *Burkholderia* sp. Relative to the amount of information on TNT biodegradation by cultured isolates, there are only scattered reports using mixed or natural assemblages and these include work on sludge (Kroger et al. 2004), groundwater and aquifer sediment (Krumholz et al. 1997), lake surface water (Spanggord et al. 1983, Talmage et al. 1999, Zeng et al. 2004), lake sediment (Boopathy and Kulpa 1994), and soils (Fuller and Manning 1998, Miyares and Jenkins 2000, Siciliano et al. 1999). It seems difficult to imagine that natural assemblages of bacteria and protozoa grazers would be unable to mineralize NEC carbon and nitrogen given the importance of nitrogen as a growth-limiting nutrient in marine systems and the ubiquity of enzyme systems for metabolizing aromatic carbon compounds in nature:

> *"Although most organisms have detoxification abilities (i.e., mineralization, transformation and/or immobilization of pollutants), microorganisms, particularly bacteria, play a crucial role in biogeochemical cycles and in sustainable development of the biosphere. Next to glucosyl residues, the benzene ring is the most widely distributed unit of chemical structure in nature, and many of the aromatic compounds are major environmental pollutants. Bacteria have developed strategies for obtaining energy from virtually every compound under oxic or anoxic conditions (using alternative final electron acceptors such as nitrate, sulfate, and ferric ions). Clusters of genes coding for the catabolism of aromatic compounds are usually found in mobile genetic elements, such as transposons and plasmids, which facilitate their horizontal gene transfer and, therefore, the rapid adaptation of microorganisms to new pollutants. A successful strategy for in situ bioremediation has been the combination, in a single bacterial strain or in a syntrophic bacterial consortium, of different degrading abilities with genetic traits that provide selective advantages in a given environment."* (Diaz 2004)

Reports of TNT half lives in natural environmental samples range from 1.1 days (Miyares and Jenkins 2000) to weeks (Boopathy et al. 1997) to months (Talmage et al. 1999). Popesku et al. (2004) reported high removal rates but only 4% mineralization of TNT carbon by an oil-degrading assemblage during a long term incubation (163 days). Degradation rates were temperature dependant in soil communities (Miyares and Jenkins 2000) and dependent on the availability of other forms of nitrogen and electron acceptors (Boopathy et al. 1997, Krumholz et al. 1997). Spain and coworkers reported use of 2,4-DNT and 2,6-DNT by soil assemblages (slurry reactors) as sole sources of carbon, nitrogen, and energy (Lendenmann et al. 1998, Nishino et al. 2000, Nishino et al. 1999, Zhang et al. 2000). There is little published information on TNT degradation in seawater or marine sediments aside from the work of Carr and Nipper (2003), which found rapid TNT and 2,4-DNT transformation rates using Puget Sound water and sediment and an increase in heterotrophic activity by the natural assemblage, which was attributed to TNT additions.

Exposure of soil assemblages to TNT can lead to a shift in the bacterial community structure (Fuller and Manning 1998, Siciliano et al. 1999) and lowering of metabolic rates (Zeng et al. 2004). There was a positive correlation between NEC concentrations and the presence of the genes responsible for denitrification (*nir*K, *nir*S or *nos*Z) in DNA extracted from the whole community, as well as in culturable strains isolated from the community, though denitrification rates decreased with increasing TNT concentrations (Siciliano et al. 2000). Using freshwater microbial assemblages, Zeng et al. (2004) found short term exposure to TNT (90 min) had cytotoxic effects (lower plate counts and glucose mineralization rates) that could be reversed by exposure to light. The researchers concluded that the photodegradation products (e.g., 2-amino-4,6-DNT, 4-amino-2,6-DNT, and 3,5-dinitroaniline) were being used as growth substrates by the bacterial assemblage.

In addition to NEC catabolism by heterotrophic bacteria, fungi may play a role in degradation as well. Lignin is degraded by fungi using several key enzyme systems: manganese peroxidase (MnP), lignin peroxidase (LiP), and laccase, which may degrade many different types of chemical bonds (Reddy 1995). These enzymes have also been shown to degrade PAHs, such as anthracene and phenanthrene (Pickard et al. 1999). Because aromatic compounds are synthesized by virtually all organisms, it is reasonable that anthropogenic compounds resembling natural aromatic compounds (amino acids, phenols, quinones; Fritsche and Hofrichter 2000) may be metabolized in microbial communities occurring where these compounds accumulate. Studies of fungal degradation of ^{14}C ring labeled TNT showed release of $^{14}CO_2$, evidence of aromatic ring breakage (Fernando et al. 1990). Lignolytic microbial assemblages may have an enhanced ability to degrade other aromatic compounds, such as PAHs and TNT.

In an effort to displace ^{14}C-TNT covalently bound to soil compost, Gunnison et al. (1998) added ammonium to increase the bioavailability to natural microbial assemblages. Only 0.6% of the total ^{14}C-TNT became mineralized in 30 d, however, 23% and 15% were mineralized from the lignocellulose and fulvic fractions. Their interpretation was that the ammonium caused the bound TNT to become disassociated from the OM fractions, however, the nitrogen amendment could have also selected for heterotrophic bacteria or fungi that degrade all aromatic organic carbon without the need to disassociate the two components. Fungal-mediated TNT mineralization occurs only when fungi are lignolytic; i.e., when fungi produce LiP and MnP, the lignin degrading peroxidase enzymes (Hawari et al. 1999). Under these conditions, substantial carbon mineralization can occur (35%, Fernando et al. 1990; 30%, Kim and Song 2003). Partial TNT degradation products like 4-hydroxylamino-2,6-DNT are potent competitive inhibitors of lignin peroxidase H8 of *P. chrysosporium* (Bumpus and Tatarko 1994, Michels and Gottschalk 1994), further evidence of the relationship between microbial degradation of NEC and lignin.

MATERIAL AND METHODS

Sampling

Subtidal stations were sampled from the Chesapeake and Delaware Bays (Fig. 1), San Francisco Bay (Fig. 2) and in Hawaii: the Ala Wai Canal (Fig. 3), Pearl Harbor (Fig. 4), and offshore of Oahu. Surface sediments were collected using Smith MacIntyer (R/V Point Sur), Petite Ponar, Shipek (R/V Cape Henlopen), or Wilco benthic grabs. Gravity corers (Wilco) were used to sample the top 20 cm of sediment at stations in the lower Chesapeake Bay, San Francisco Bay, Pearl Harbor and the Ala Wai Canal. Divers were used to collect surface sediment offshore of Oahu. Surface (1 m below air-sea interface) and bottom water (1 m above sediment) were collected using a Sea Bird CTD with rosette or Go-Flo bottle. Chesapeake and Delaware Bay were sampled using the R/V Cape Henlopen while the R/V Point Sur was used for the San Francisco Bay. Small watercrafts were used for the Hawaii samplings. All data presented in this report were collected during seven sampling events from 2002-2005 (Table 1). Gravity cores were sectioned every 2-3 cm for subsampling for mineralization or bacterial production in the depth profiles. Surface sediments and core slices were subsampled with a cut off 5 cm plastic syringe for the surveys of mineralization and a cut-off 1-cm plastic syringe for bacterial production.

Mineralization

Organic carbon mineralization assays were initiated within three hours of sediment sample collection using a modification of Boyd et al. (1996) and Pohlman et al. (2002). As radiotracers, we used three sentinel PAHs: UL-^{14}C-naphthalene (spec. act. 18.6 mCi mmol^{-1}), 3-^{14}C-fluoranthene (45 mCi mmol^{-1}), and 9-^{14}C-phenanthrene (47 mCi mmol^{-1}; Sigma Chemical), as well as UL-^{14}C-TNT (spec. act. 4 mCi mmol^{-1}), UL-^{14}C-2,4-DNT (55 mCi mmol^{-1}), UL-^{14}C-2,4-DAT (55 mCi mmol^{-1}; American Radiochemicals Inc.), UL-^{14}C-catechol (1.8 mCi mmol^{-1}; Sigma Chemical), and UL-^{14}C-toluene (60 mCi mmol^{-1}; Sigma Chemical). They were added in separate incubations to surface sediment samples (1 mL

wet volume) or 5 mL of seawater in 100×16 mm test tubes to a final concentration of about 0.2 μg g^{-1} (depending on specific activity). For sediment samples, 0.5 mL of bottom water from the same station was filtered (0.22 μm nom. pore dia. Nuclepore polycarbonate) and added to make slurries. Samples were typically incubated for 24 h at *in situ* temperature in the dark and evolved $^{14}CO_2$ was captured on NaOH-soaked filter papers. H_2SO_4 was added to end incubations and to partition any remaining CO_2 into headspace of the tube and to the filter paper trap. The filter paper traps containing metabolized $^{14}CO_2$ were removed, radioassayed and subsequently used to calculate substrate mineralization. Ambient concentration of the specific compound was used to determine an isotope dilution factor.

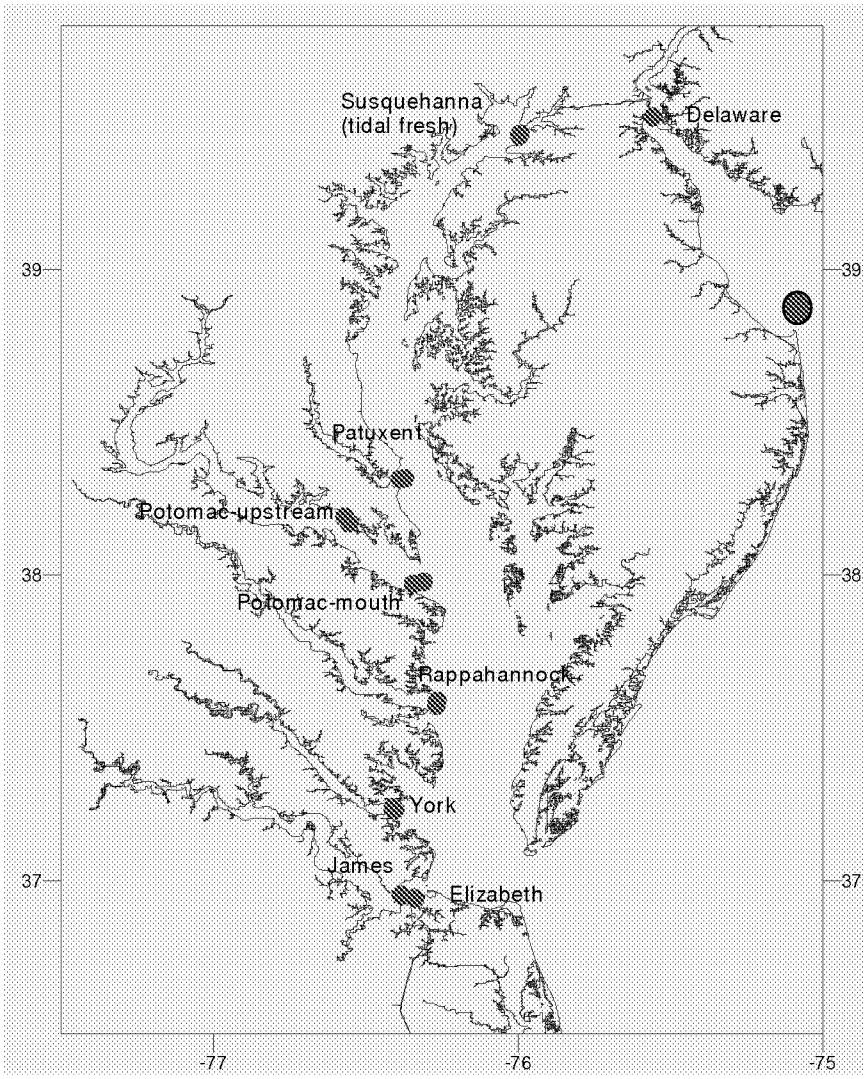

Fig. 1 — Chesapeake Bay station locations

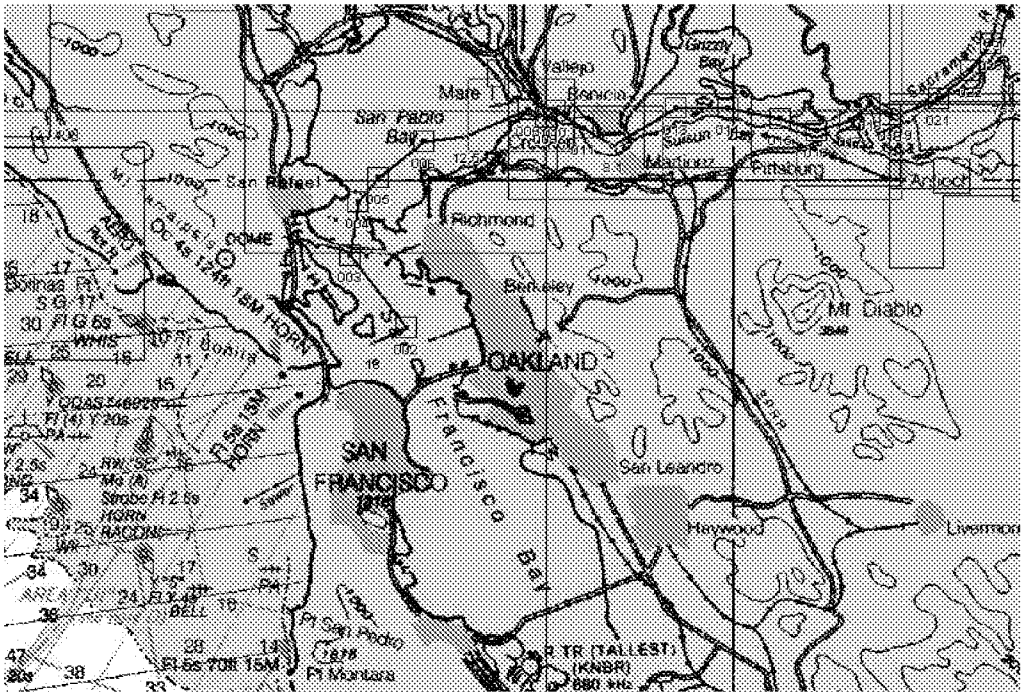

Fig. 2 — Sampling locations (●) for stations in the San Francisco Bay

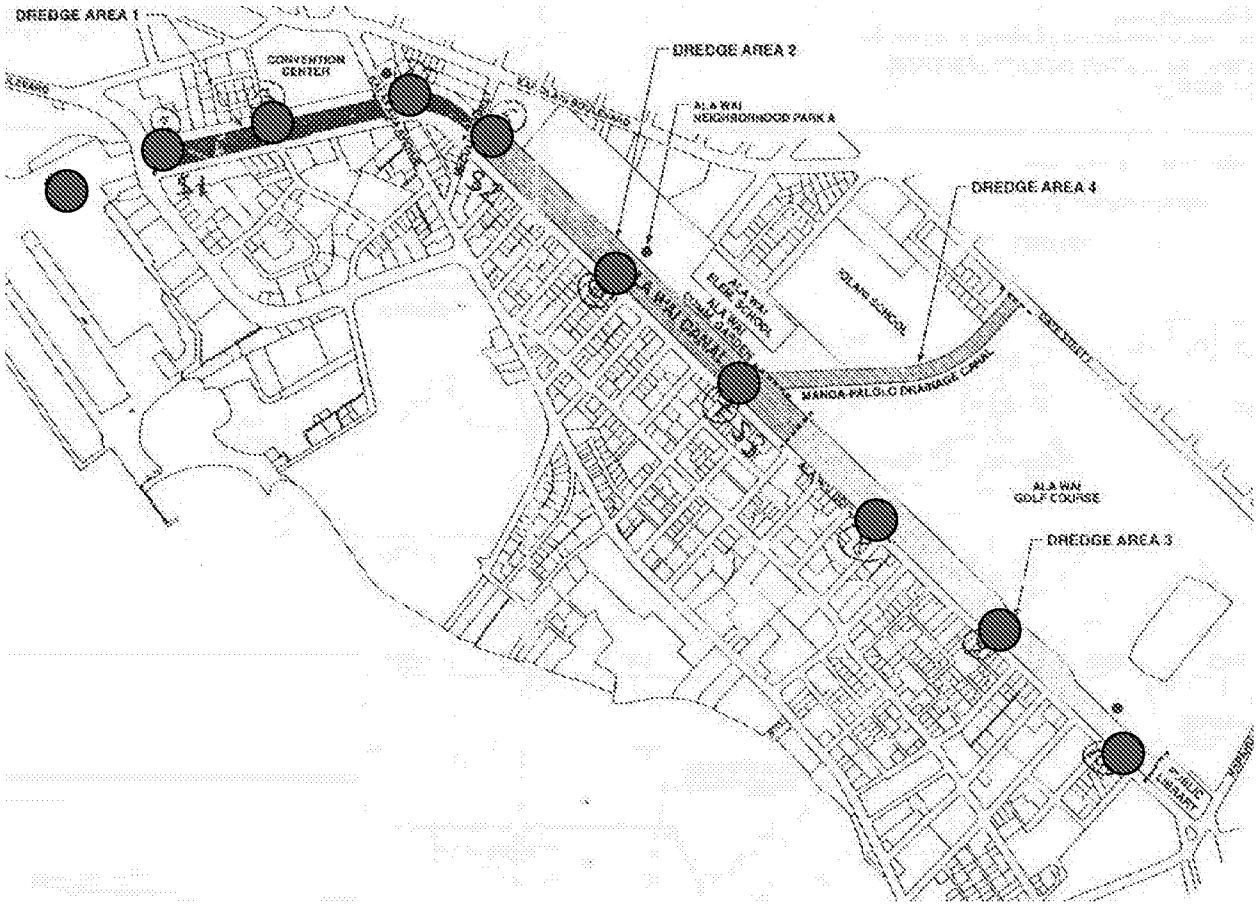

Fig. 3 — Eleven stations (⊗) were sampled in the Ala Wai Canal during July 2003

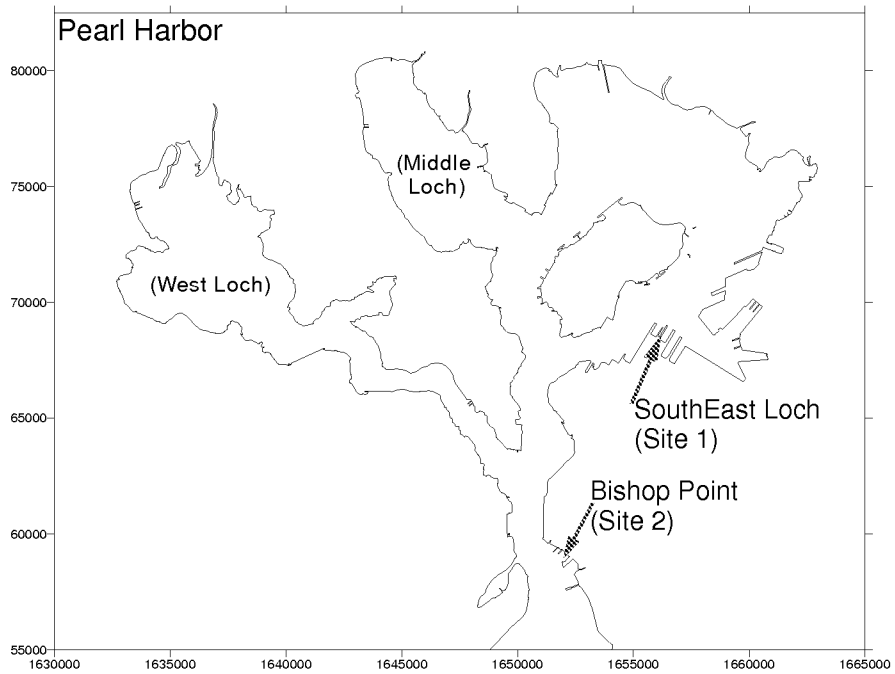

Fig. 4 — Depth profile cores were taken in Bishop's Point and Southeast Loch in Pearl Harbor, HI in December 2002.

Table 1 — Seven Sampling Cruises Were Performed in Three Ecosystems from 2002 to 2005

Ecosystem	Cruise	Stations
Chesapeake Bay	September 2002	17
	March 2004	18
	March 2005	12
Hawaii	December 2002	4
	July 2003	11
	May 2005	2
San Francisco Bay	May 2003	12

Heterotrophic Bacterial Production

The leucine incorporation method (Kirchman et al. 1985, Kirchman 1993, Smith and Azam 1992) was used to measure bacterial production as adapted by Montgomery et al. (1999). A 0.50 µL aliquot of wet surface sediment from each station was added to 2 mL centrifuge tubes (three experimental and one control) which were pre-charged with [^{3}H-4,5]-L-leucine (154 mCi mmol^{-1}). The sediment was extracted from the benthic grab sample and added to the 2 mL tube using a 1 mL plastic syringe with the end cut off. One mL of 0.45 µm nom. pore dia. (Acrodisk, Gelman) filtered bottom water (collected <1 m above bottom) was then added to each tube to form a sediment slurry. Samples were incubated for 1 h at *in situ* temperatures and subsequently processed by the method of Smith and Azam (1992). A constant isotope dilution factor of 1000 was used for all samples. This was estimated from actual measurements of

sediment dissolved free amino acids (Burdige and Martens 1990) and saturation experiment estimates (Tuominen 1995). One mL syringed samples of wet sediment were dried at 50 °C and used to covert production values to dry weight. Leucine incorporation rate was converted to bacterial carbon using factors determined by Simon and Azam (1989).

Microalgal Production

Particulate primary production (PPP) was determined by measuring the uptake of $NaH_2{}^{14}CO_3$ (Parsons et al. 1984). Triplicate 10 mL sub-samples were inoculated with 80 µCi $L^{-1}NaH_2{}^{14}CO_3$ and incubated (1 h) in quartz serum vials. Samples were filtered through Gelman type A/E glass fiber filters (1.0 µm porosity) at ≤125 mm Hg, placed in 20-mL scintillation vials, acidified with 500 µL of 1 N HCL and vented (1 h). Amount of incorporated radioactivity was determined using a Beckman LC 6500. Phytoplankton production of dissolved organic matter (i.e., dissolved primary production (DPP)) was measured using PPP filtrate, which was transferred to glass scintillation vials, acidified as for PPP, purged with air for 20 minutes, and vented (1 h). Measurements were taken at T = 0, 24 and 48 h from separate 2.5 L polycarbonate containers, which were incubated at ambient light and temperature conditions in a 0.5 m deep tank with continuously flowing seawater. Light and dark controls from each detonation area and light and dark plume samples of TNT and RDX were included in the analysis.

Contaminant Concentration

Ambient PAH concentrations of 18 semi-volatile priority pollutants were determined. First, 10 to 15 g of sediment was dried with diatomaceous earth and then extracted in methanol using accelerated solvent extraction. The extracts were concentrated under a N^2 stream (Speedvap) and analyzed by GC/MS (Fisher et al. 1997). *p*-Terphenyl-d_{14} and 2-fluorobiphenyl were used as surrogate standards, following the method described in Pohlman et al. (2002). TNT was measured using standard EPA method 8330.

DGGE of Bacterial Assemblage

Bacterial communities in both the water column and sediment samples were characterized by integrated cultivation and molecular DNA techniques. These approaches analyzed genetic diversity of complex populations. Methods used included DNA polymerase chain reaction (PCR), cloning, denaturing gradient gel electrophoresis (DGGE), fluorescent in situ hybridization (FISH), and numeration by colony counting and the most-probable-number method (Rooney-Varga et al. 1999, Daims et al. 2001, Nogales et al. 2001, Watts et al. 2001, Bouvier and del Giorgio 2003, Ivanov et al. 2003, Sekar et al. 2003, Zwirglmaier et al. 2004). Bacterial community DNA was amplified by PCR using specific primers. A 16S rRNA bacterial specific forward primer (27F) and a universal reverse (1492R) were used for cloning and bacterial specific 16S rRNA primers (b341GC, and b758) for DGGE (Delong 1992, Juck et al. 2000). Archaeal rRNA was similarly amplified with Archaeal specific primers (21F and 958R) (Delong 1992). The DGGE primer set amplifies a region ca. 500 bp that corresponds to positions 341-758 in *Escherichia coli* and resulting PCR products were analyzed with the DCode system (Bio-Rad). Resulting clones and well-separated bands from the DGGE were sequenced in our lab with an Amersham MegaBace 1000 DNA sequencer. Sequences are used for identification and subsequent phylogenetic determination by comparative sequence analysis. DGGE banding patterns among samples are compared to determine community structural differences, which can indicate relative abundances of the main bacterial populations within different samples.

RESULTS AND DISCUSSION

Site Surveys

Seven surveys of coastal ecosystems were conducted to measure NEC mineralization rates using standard radiotracer techniques for interrogation of organic carbon metabolism by natural assemblages (Deming 1992). For these comparisons, the detection limit of our 24 h ^{14}C-radiotracer incubations with natural sediment samples (1 g wet volume) was 1.0×10^{-2} μg C kg^{-1} d^{-1} though a sample was only considered to have detectable NEC mineralization rates if the average of the triplicate live values (minus the kill) was greater than the standard deviation. If the standard deviation was larger than the average mineralization value of the triplicate samples, then that station was listed as a nondetect for TNT mineralization.

In general, TNT mineralization rates by the natural bacterial assemblage in surface sediments were higher than 2,4-DNT and 2,6-DAT mineralization rates measured at the same station. TNT mineralization was detected at 40 out of 56 stations (71%) and ranged from nondetect to 145 (± 16.0) μg C kg^{-1} d^{-1} though the median values of the each survey only ranged from 3.0 (± 0.56) to 17.6 (± 0.49) μg C kg^{-1} d^{-1} (Table 2). These median values may be a more useful for determining the capacity of ecosystem sediments to metabolize TNT. Despite the wide variation in ecosystem types (tropical vs temperate), the median TNT mineralization rates were within an order of magnitude among surveys. In addition, mineralization of 2,4-DNT and 2,6-DAT were also measured in the Chesapeake Bay surveys and the assays were typically conducted at the same stations as those for TNT mineralization. 2,4-DNT and 2,6-DAT mineralization was detected at fewer stations (38% and 58%, respectively) and had a lower median range for stations where mineralization was detected (1.35 ± 0.44 and 9.88 ± 0.48 μg C kg^{-1} d^{-1}, respectively). In San Francisco Bay, only one survey was performed (May 2003) and TNT mineralization was detected at four out of 10 stations with 2,4-DNT mineralization detected at two out of 10 stations. The Hawaii sampling involved surveys of Bishop's Point and South Loch in Pearl Harbor and Ala Wai Canal in Waikiki with 45% of the samples having detectable TNT mineralization rates (median rate = 3.57 ± 1.51 μg C kg^{-1} d^{-1}). When averaged across all data for a sampling cruise, TNT mineralization rates in the Chesapeake Bay were higher than those measured for Hawaii and San Francisco Bay.

Table 2 — Nitrogenous energetic compound (NEC) mineralization rates (μg C kg^{-1} d^{-1}) were surveyed for surface sediments in three coastal waterways from 2002 to 2005. The range and median rate are reported for the collection of samples in each ecosystem where mineralization was detected (AVG: average; SD: one standard deviation).

Ecosystem (# sampling events)	NEC	Total Stations	Detects	Mineralization Range AVG μg C kg^{-1} d^{-1} (± SD)		Median Rate for Detects AVG (± SD)
				Low	High	
Chesapeake Bay (3)	TNT	35	31	0.29 (± 0.13)	145 (± 16.0)	17.6 (± 0.49)
	DNT	26	10	0.14 (± 0.05)	219 (±119)	1.35 (± 0.44)
	DAT	17	10	2.15 (± 0.30)	49.3 (± 22.6)	9.88 (± 0.48)
San Francisco Bay (1)	TNT	10	4	2.96 (± 1.33)	7.42 (± 2.11)	3.08 (± 0.56)
	DNT	12	2	16.0 (± 12.4)	68.2 (± 12.8)	Not Applicable
Hawaii (2)	TNT	11	5	2.38 (± 1.60)	19.9 (± 11.8)	3.47 (± 1.51)

The bacterial assemblage degradation rate of NEC may be related to metabolism of other aromatic compounds like PAHs (^{14}C-naphthalene, -phenanthrene, -fluoranthene), toluene, catechol, or even heterotrophic metabolism in general (bacterial production). Given the wide range of ecosystems and different times of the year of our samplings, we did not find good correlations when comparing TNT mineralization rates with these other parameters on a station by station basis. However, we did find that when averaging rates between stations where TNT mineralization was detected vs not detected, the average rates of bacterial production and mineralization of naphthalene, phenanthrene, fluoranthene, catechol and toluene were all higher at stations where TNT mineralization was detected (Table 3). Also, the range in average PAH and toluene mineralization rates for all the stations (0.80 to 8.04 μg C kg^{-1} d^{-1}) is very similar to that for TNT (3.08 to 17.6 μg C kg^{-1} d^{-1}). This suggests that TNT is mineralized in coastal sediments at rates that are typical for naturally occurring compounds, like phenanthrene and fluoranthene, and even similar to compounds like toluene and naphthalene that are known to be very transient in the estuarine sediments. Average 2,6-DAT mineralization was higher stations where TNT mineralization was detected (9.00 vs 3.84 μg C kg^{-1} d^{-1}). Somewhat surprisingly, average 2,4-DNT mineralization rate for the TNT mineralization non-detect stations was slightly higher (13.8 vs 10.3 μg C kg^{-1} d^{-1}) than for the detect stations suggesting that TNT and 2,4-DNT mineralization may be uncoupled processes amongst the natural assemblage.

Depth Profiles

There is evidence from the literature that both aerobic and anaerobic conditions may be required for complete mineralization of TNT though most of this information is derived from culture work and laboratory conditions. Nonetheless, we examined bacterial TNT mineralization rates with depth in core sections (typically 2-4 cm) down to 20 cm below the water-sediment boundary. Gravity cores were sectioned and then one mL wet volume subsamples of sediment were assayed. These cores were taken

from Pearl Harbor (Bishop's Point, South Loch), the Ala Wai Canal, the lower Chesapeake Bay (including the York and Elizabeth Rivers), and San Francisco Bay (Alameda, Treasure Island, Hunter's Point). Many of these cores were bioturbated to various degrees in the upper sections or throughout.

Table 3 — TNT mineralization was measured on sediment samples (68 total) from the Chesapeake Bay (35), San Francisco (10) and the Ala Wai canal, HI (11). Stations with detectable TNT mineralization rates were compared with those that were below the detection limit. Average (AVG) bacterial production and mineralization rates (µg C kg^{-1} d^{-1}) for other organic compounds were generally higher for samples that had measurable TNT mineralization with the notable exception of DNT.

TNT Mineralization Rate	AVG Production or Mineralization Rate (µg C kg^{-1} d^{-1})							
	Bacterial production	Naphthalene	Phenanthrene	Fluoranthene	Catechol	DNT	DAT	Toluene
Detects (42)	76.3	4.79	8.04	1.06	160	10.3	9.00	4.45
Nondetects (26)	10.1	1.40	4.66	0.80	113	13.8	3.84	3.81

As with the previous survey of surface sediments, the core section data was grouped based on whether or not TNT mineralization was detected or not detected and then compared with other metabolic analyses. In December 2002, four cores were taken at Bishop's Point and South Loch (2 each) in Pearl Harbor, HI. TNT mineralization rates were detected in 6 out of 22 core sections of the 4 cores. Bacterial production averaged among the sections where TNT mineralization was detected was only slightly higher than among those sections where mineralization was not detected (6.50 vs 6.21 µg C kg^{-1} d^{-1}; Table 4). In the comparison of the surface sediments described above, the difference between the TNT mineralization detects and nondetects was much greater with respect to production. Average mineralization rates of the PAHs, naphthalene, phenanthrene, and fluoranthene, were all higher for the core sections where TNT mineralization was detected as was seen in the surveys of the surface sediments from the three ecosystems (Table 4).

Table 4 — Average (AVG) bacterial production and mineralization rates (µg C kg^{-1} d^{-1}) were measured on sections from four sediment cores taken from Pearl Harbor, HI, in December 2002. Although production was similar when averaged among sections where TNT mineralization was not detected relative to sections where it was detected, PAH mineralization rates were higher among the latter sections.

TNT Mineralization Rate	AVG Production or Mineralization Rate (µg C kg^{-1} d^{-1})			
	Bacterial production	Naphthalene	Phenanthrene	Fluoranthene
Detects (6)	6.50	0.20	5.41	1.83
Non-detects (16)	6.21	0.05	2.31	0.02

Bacterial production, as measured by the leucine incorporation method, often decreases dramatically with depth in gravity core sections. We then determined the ratio of TNT mineralization rate to bacterial production for same core sections and compared the ranges in these values between the surface section (0 to 2 cm) and below the surface section (2 to 20 cm). A high ratio indicates that TNT mineralization could potentially comprise a large component of total bacterial production or that a relatively larger fraction of the natural assemblage could metabolize TNT. For the San Francisco Bay cores (which were heavily bioturbated), there was little difference in the range between the ratios in the surface vs deeper in the sediments (Table 5). However there was a higher range of ratios between the surface vs the deep sections

in both the cores from Chesapeake Bay (surface: 0.04-14; deep: 2.87-40.4) and Hawaii cores (surface: 0.29-0.66; deep: 1.18-26.3). This suggests that deeper sediments may harbor a natural bacterial assemblage that may be better adapted for mineralizing TNT or similar types of organic carbon.

Table 5 — TNT mineralization rate (μg C kg^{-1} d^{-1}) as a function of bacterial production was determined as a relative measure of the ability of a bacterial assemblage to metabolize TNT. The range for most surface sediments in the three ecosystems was 0.01 to 1.6, but four surface stations and those in deeper sediments were much higher, suggesting that bacterial assemblages at these stations had a greater capacity to metabolize TNT.

Ecosystem	Sample Type	TNT Mineralization Rate/ Bacterial Production (μg C kg^{-1} d^{-1}, range)	
		Low	High
San Francisco Bay	surface	0.05	1.05
	cores	0.05	0.98
Chesapeake Bay	surface	0.04	14.0
	cores	2.87	40.4
Hawaii	surface	0.29	0.66
	cores	1.18	26.3

Gravity cores were taken from three stations in the Chesapeake Bay system for comparison of bacterial production and mineralization of PAHs and TNT: LY, a relatively bioturbated station in the York River (Fig. 5); M, a relatively unmixed and armored station in the mid bay (Fig. 6); POD, a physically mixed station in the lower bay (Fig. 7) (Arzayus et al. 2001). TNT mineralization with depth is similar to, or higher than, PAH mineralization at the same depth in the Chesapeake Bay cores. The most difference between PAH and TNT mineralization was at the physically mixed station (Fig. 7). The more bioturbated station, LY, had the most sections with detectable PAH and TNT mineralization rates (Fig. 5).

Gravity cores were also taken from three stations in the San Francisco Bay system for comparison of bacterial production and mineralization of PAHs and TNT: Alameda (Fig. 8), Hunter's Point (Fig. 9), and Treasure Island (Fig. 10). Although all cores showed evidence of bioturbation (presence of burrowing worms), the Treasure Island core was characterized by large worms (>20 cm) and light, hydrated sediments. Here the gravity core penetrated 170 cm whereas the other cores were only 20 cm at Alameda and Hunter's Point. Unlike other systems (Fig. 5), bacterial production not as disproportionately elevated in the uppermost sections of the three San Francisco Bay core stations (Figs. 8A, 9A, 10A). At all three stations, TNT mineralization was often higher than that for naphthalene and fluoranthene but lower than phenanthrene mineralization when compared by each depth section (Figs. 8 B, 8C, 9B, 9C, 10B, 10C).

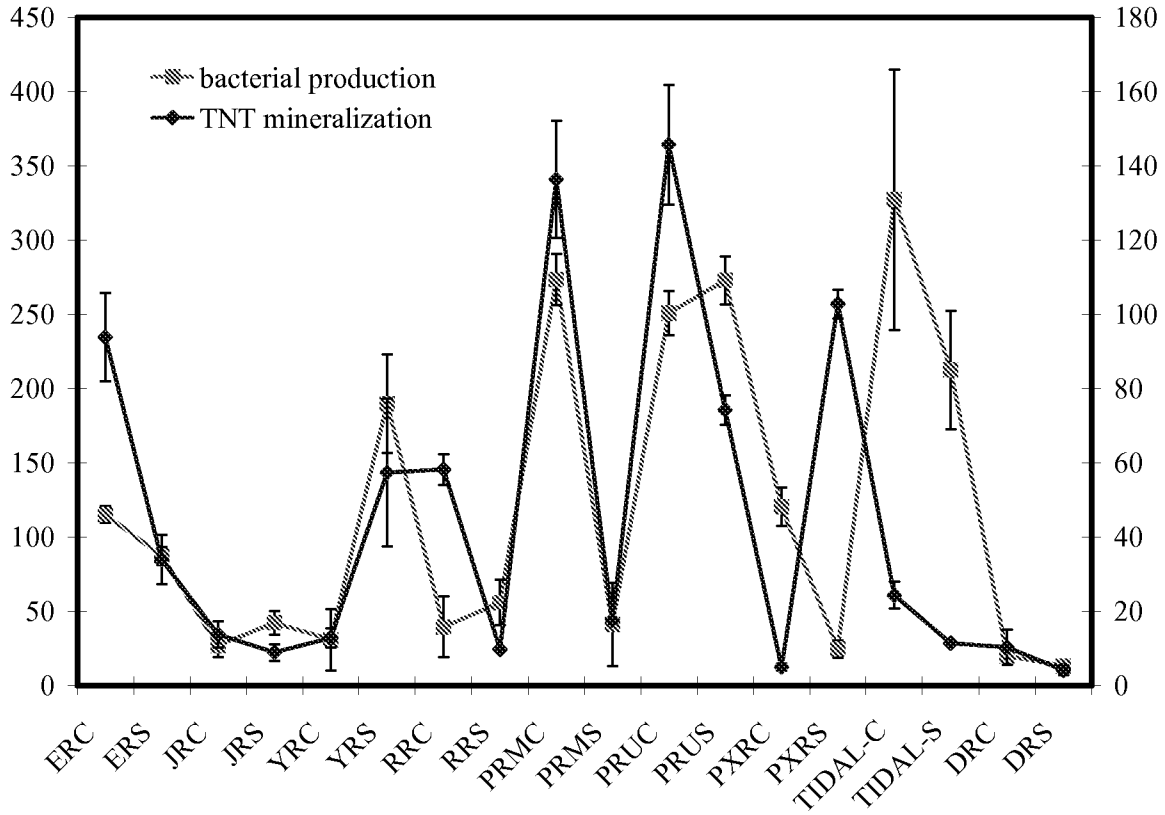

Fig. 5 — Chesapeake Bay production covarying with mineralization except in the tidal fresh and PAX River

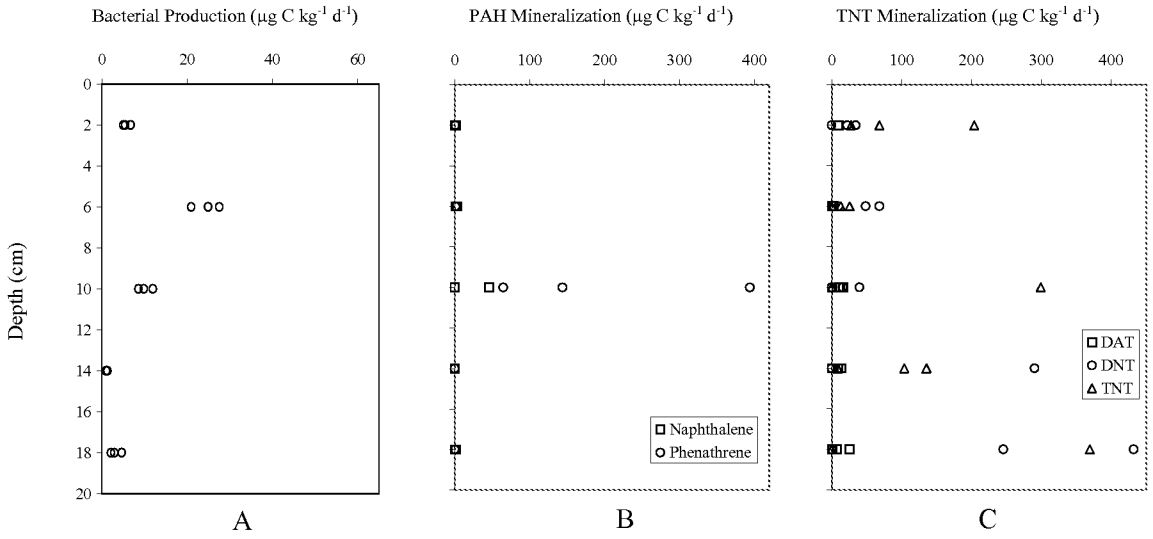

Fig. 6 — Bacterial production (A), PAH mineralization (B) and TNT mineralization (C, µg C kg^{-1} d^{-1}) was measured with depth on a sediment core taken from the armored mid Chesapeake Bay station M3 in September 2002.

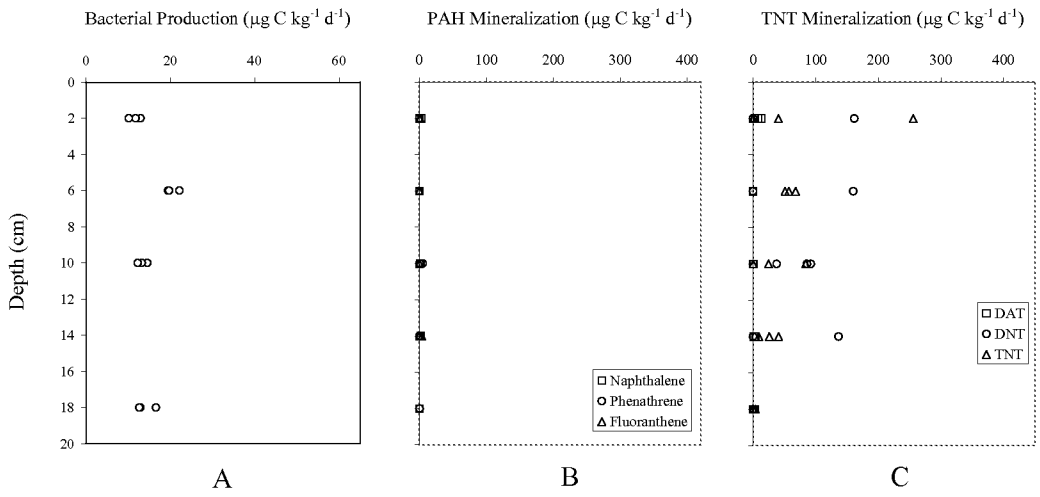

Fig. 7 — Bacterial production (A), PAH mineralization (B) and TNT mineralization (C, µg C kg^{-1} d^{-1}) was measured with depth on a sediment core taken from the physically mixed lower Chesapeake Bay station POD in September 2002.

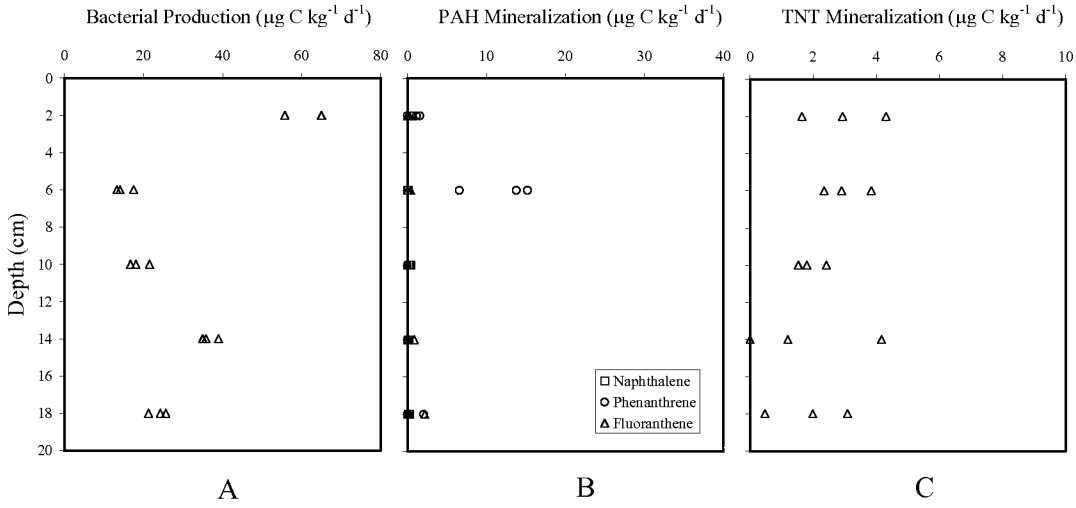

Fig. 8 — Bacterial production (A), PAH mineralization (B) and TNT mineralization (C, µg C kg^{-1} d^{-1}) was measured with depth on a sediment core taken off of Alameda in San Francisco Bay in May 2004.

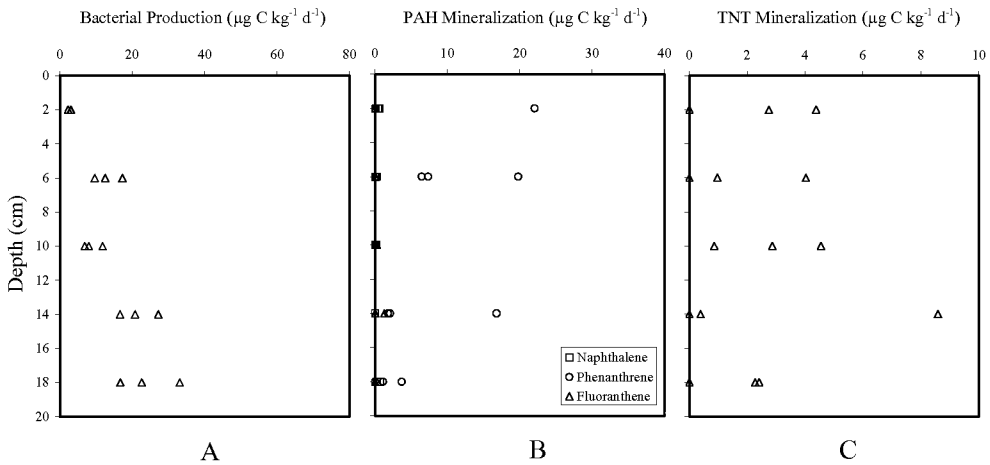

Fig. 9 — Bacterial production (A), PAH mineralization (B) and TNT mineralization (C, µg C kg^{-1} d^{-1}) was measured with depth on a sediment core taken off of Hunter's Point in San Francisco Bay in May 2004.

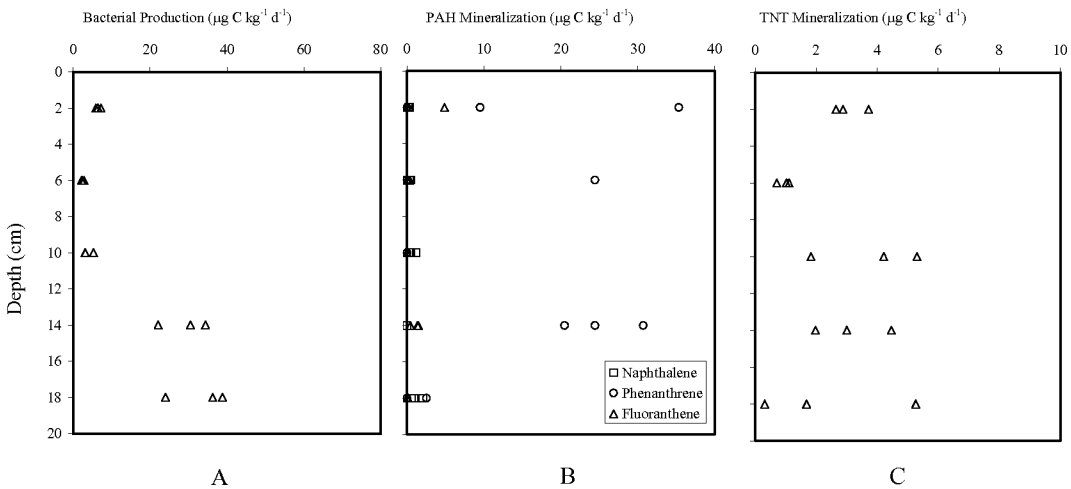

Fig. 10 — Bacterial production (A), PAH mineralization (B) and TNT mineralization (C; µg C kg^{-1} d^{-1}) was measured with depth on a sediment core taken off of Treasure Island in San Francisco Bay in May 2004.

Incorporation and Growth Efficiency

During the estuarine sediment surveys, we found that TNT mineralization was frequently more rapid than 2,4-DNT or 2,6-DAT mineralization at the same station and time point, but had no correlation with toluene mineralization (Fig. 11). This could be due to a greater percentage of the bacterial community metabolizing TNT, more inefficient use of TNT by bacteria (lower % incorporation), or different cellular transport mechanism for TNT vs 2,4-DNT resulting in greater uptake and incorporation of TNT. Estuarine surface water (30‰ salinity) was collected from the mouth of the Delaware Bay in March 2005 and incubated with ^{14}C-TNT, ^{14}C-DNT and ^{14}C-DAT to measure the incorporation and mineralization rates and growth efficiencies of free-living bacteria metabolizing these compounds. Bacteria among the natural assemblage incorporated TNT and 2,6-DAT into macromolecules (TCA precipitate) at a similar

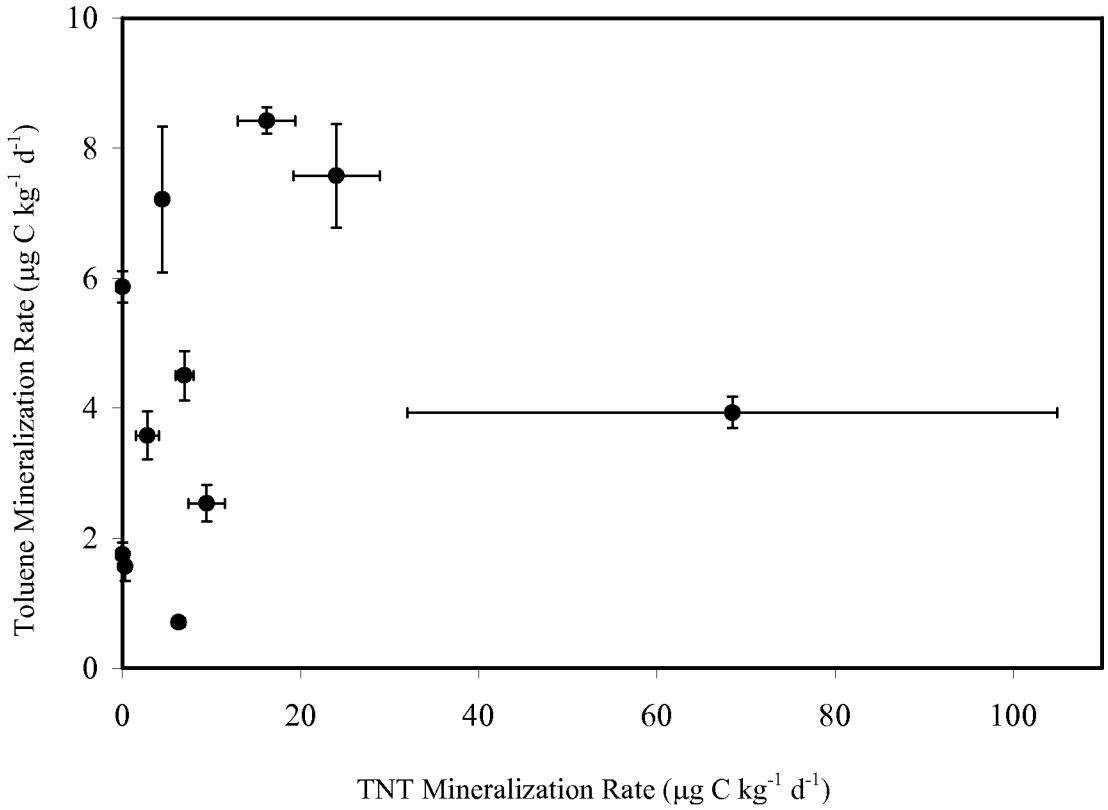

Fig. 11 — Toluene mineralization (µg C kg^{-1} d^{-1}) was uncoupled with TNT mineralization in Chesapeake Bay surface sediments in March 2005.

rate over the course of the 24 h incubation (28.8 ± 12.2 vs 24.5 ± 2.2 µg C L^{-1} d^{-1}, respectively) but had a lower growth efficiency on TNT than 2,6-DAT and the mineralization rate was approximately sixfold less for 2,6-DAT than TNT (3.1 ± 0.9 vs 0.5 ± 0.2 µg C L^{-1} d^{-1}) (Table 6). That is, heterotrophic bacteria processed 2,6-DAT and TNT at about the same rate, but more of the 2,6-DAT carbon was incorporated into macromolecules and more the TNT carbon was respired as CO_2.

Table 6 — Average (AVG) rates of TNT incorporation, mineralization (µg C kg^{-1} d^{-1}) and bacterial growth efficiency (%) using Delaware Bay surface water collected March 2005

Energetic	Rate (µg C L^{-1} d^{-1}, AVG (SD))		% Growth Efficiency
	Incorporation	Mineralization	
TNT	28.8 (12.2)	3.1 (0.9)	90.3
DAT	24.5 (2.2)	0.5 (0.2)	98.0
DNT	3.2 (0.2)	1.1 (0.7)	74.4

At 3.2 (± 0.2) μg C L^{-1} d^{-1}, the incorporation rate of 2,4-DNT was almost an order of magnitude lower than that for both TNT and 2,6-DAT (Table 6). However, at 1.1 (± 0.7) μg C L^{-1} d^{-1}, 2,4-DNT mineralization rate was only about three-fold lower than that for TNT and about twice that for 2,6-DAT due to a much lower growth efficiency for bacterial metabolism of 2,4-DNT (74.4%). Bacteria incorporated a much lower percentage of the 2,4-DNT carbon than they did for 2,6-DAT and TNT. Though there were differences in incorporation efficiencies among these compounds, they are still surprisingly high for organic carbon sources metabolized by a natural assemblage. One hypothesis generated from these results is that these compounds are metabolized efficiently because the N-C subunits of NEC are easily transformed into macromolecules like proteins and nucleic acids. Assimilatory nitrogen metabolism by bacteria may be the preferred transformation process over organic carbon oxidation of the aromatic ring for energy. Although the mineralization rates (μg C L^{-1} d^{-1}) were high relative to other organic carbon substrates, the percentage of assimilated TNT carbon that was mineralized was so low (ca. 10%) that it may actually be due to remineralization of bacterially incorporated ^{14}C by protozoan grazers.

Light vs Dark

The relative importance of photodegradation and marine microbiota was examined using water sampled offshore of Oahu, HI, in July and December of 2003. Both whole surface seawater and filtered seawater (0.22 μm nom. pore dia.) were incubated with 1 ppm of ^{14}C-TNT and exposed to ambient light at *in situ* temperatures (27 °C in July, 25 °C in December) for 48 h. Values for filtered seawater incubated in the dark were used as a control for abiotic/non-photolytic TNT degradation. TNT mineralization in the whole seawater treatment in the dark was attributed to free living heterotrophic marine bacteria. Degradation of the tracer in filtered seawater exposed to ambient laboratory light would be the result of photolytic processes. Mineralization in the whole seawater exposed to light treatment could be both photolysis and heterotrophic bacteria, active marine microalgae, or some more complex interaction between abiotic and biotic processes.

In both the July and December experiments, TNT mineralization was about an order of magnitude higher due to photolysis (filtered, light) than to heterotrophic bacteria (unfiltered, dark), though the absolute value of both treatments was two orders of magnitude lower in the December than in July (Table 7). In the July Light, Unfiltered incubation (LU: 0.93 ± 0.17 μg C L^{-1} d^{-1}), the mineralization rate was somewhat lower than in the Light, Filtered (LF: 1.94 ± 0.54 μg C L^{-1} d^{-1}; Table 7). The LU treatment would be expected to at least be the sum of the LF and DU treatments but the differences between the LF and LU treatments may not be significant. While filtering the sample removed heterotrophic bacteria that would mineralize TNT, it would also remove particles that could absorb or block light thereby reducing photolysis. However, in the December sampling, the LU treatment (0.103 ± 0.014 μg C L^{-1} d^{-1}), had TNT mineralization rate of nearly an order of magnitude higher than the LF treatment (0.016 ± 0.005 μg C L^{-1} d^{-1}; Table 7). Here it may be that active microalgae played some role in TNT mineralization or that the heterotrophic bacterial assemblage was using some photolytic degradation product of the labeled TNT. In either case, the interaction between photolysis and the microbiota appears to be highly variable and complex with respect to degradation of TNT in seawater.

Table 7 — Average (AVG) TNT mineralization rate (μg C kg^{-1} d^{-1}) was higher with the natural microbial assemblage (unfiltered) in light than in the absence of either light or the assemblage during incubations of seawater taken offshore of Oahu, HI, in July 2003 (0.22 ppm TNT added) and December 2003.

Sampling	TNT added (ppm)	^{14}C-TNT Mineralization Rates (μg C L^{-1} d^{-1}, AVG (SD))		
		Dark, Unfiltered	Light, Filtered	Light, Unfiltered
July 2003	0.22	0.21 (0.097)	1.94 (0.54)	0.93 (0.17)
December 2003	0.09	0.0012 (0.016)	0.016 (0.005)	0.103 (0.014)

Effect of Underwater Demolition Detonation

TNT and RDX are used for underwater demolition in construction and to remove hazards such as unexploded ordnance (UXO). In May 2005, we sampled seawater and sediment at two offshore stations where underwater demolition was being performed in separate detonations of 10 lb of TNT and 10 lb of RDX (Fig. 12). We sampled prior to the detonation and then immediately afterwards (within 5 min) in the debris field and then followed the plume sampling every 15 min until it could no longer be seen from the surface (ca. 1 h). We then used the surface water samples collected pre-detonation and immediately post-detonation in shoreside incubations in ambient laboratory light and maintained at *in situ* temperature (27 °C) to determine the degradation rates of the residual TNT and RDX that were expected to result from the incomplete combustion of the detonation charges.

Fig. 12 — Divers collecting sediment samples and setting demolition charge

Residual TNT and RDX were below the detection limit in seawater (LCMS: 0.6 ppm) even in the samples collected in the plume within 5 minutes after the detonation. Therefore, we were unable to determine rates of photolysis and biodegradation of the energetics in the shoreside incubations with the plume samples. However, PUV light profiles were taken at the stations prior to the respective detonations as well as throughout the plume immediately after detonation and every 15 min following the migration of the plume until it dissipated (Fig. 13). They appear to show the dissipation of the effects of the detonation on the water column light penetration as the plume migrates, mixes with seawater outside the plume, and as particles fall out of the water column.

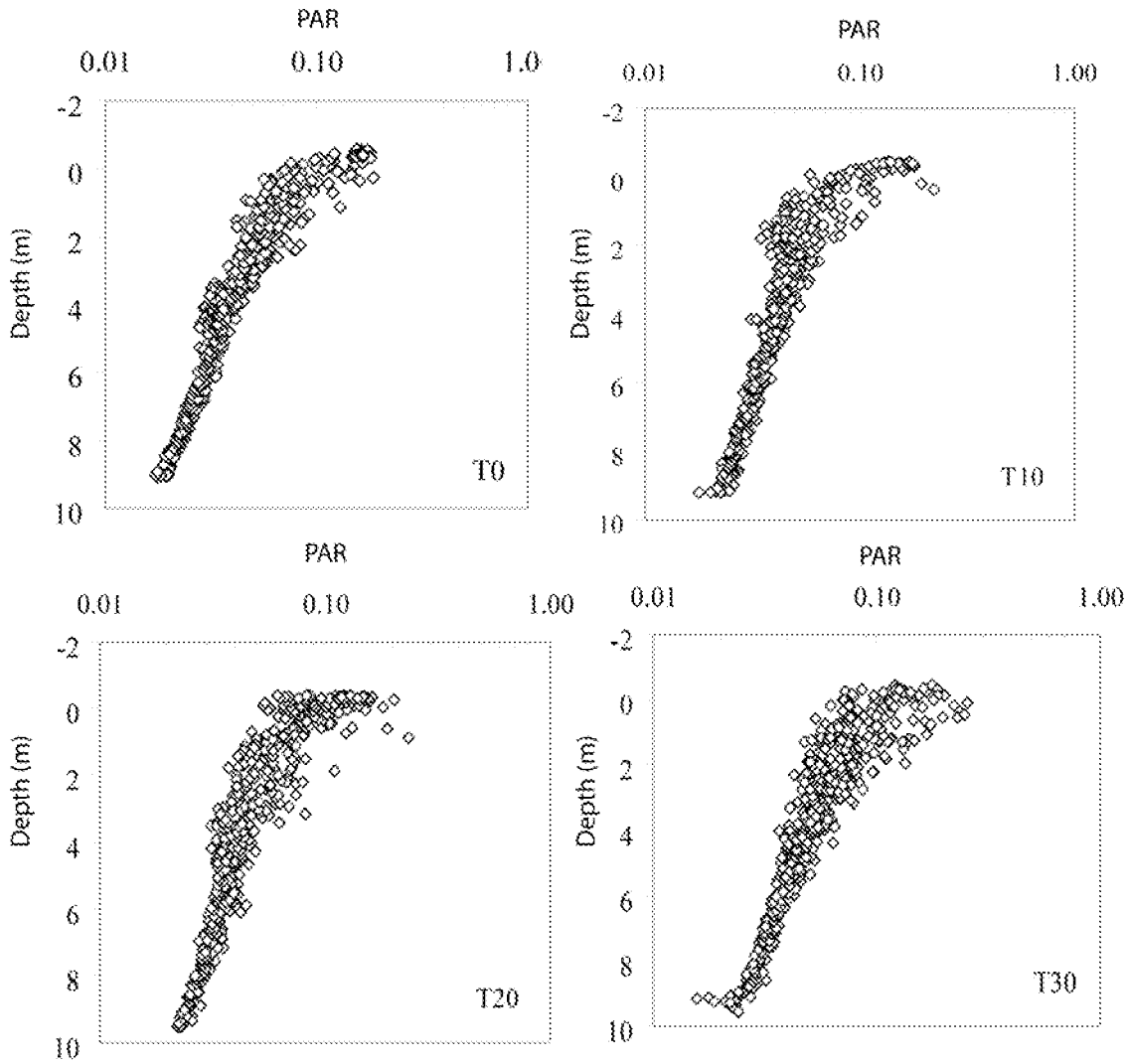

Fig. 13 — Light profiles offshore of Oahu in May 2005

Even though there were no detectable NECs in the plume samples, we were still able to measure some effects of the detonations on the natural microbial assemblage. Bacterial production appeared to be unchanged following the TNT detonation when coming surface seawater taken pre verse post-detonation whereas there was an increase in bacterial production in the plume related to the RDX detonation (Table 8). Even though we were unable to detect NEC present in the plume seawater samples, it is possible that decomposition of the RDX resulting from the detonation could have resulted in a nutrient release (i.e., nitrate) which could stimulate heterotrophic bacterial metabolism. TNT uptake and mineralization was

measured using radiotracer addition to the subsamples of the incubations. Light appeared to increase uptake of TNT in roughly the same relative amount that light increased bacterial production (Table 9). There was no obvious change in bacterial assemblage composition over the 48 h incubations, as determined by DGGE profiles of extracted DNA (data not shown).

Table 8 — Pacific Ocean seawater was sampled pre and post an underwater demolition detonation with 10 lb of TNT or RDX. There was little difference in bacterial production (μg C L^{-1} d^{-1}) as a result of the detonation of TNT, but production roughly doubled in the post RDX detonation sample incubated in either ambient light or dark.

Energetic	Illumination	Detonation	Bacterial Production (μg C L^{-1} d^{-1})	
			AVG	SD
TNT	Light	Pre	3.36	0.03
		Post	3.33	0.37
	Dark	Pre	2.06	0.28
		Post	1.73	0.63
RDX	Light	Pre	1.83	0.30
		Post	4.77	1.42
	Dark	Pre	1.27	0.79
		Post	2.32	0.19

Table 9 — Pacific Ocean seawater was sampled pre- and post- underwater demolition detonation with 10 lb of TNT. There was little difference in bacterial production, TNT mineralization, or uptake rate (μg C L^{-1} d^{-1}) as a result of the detonation (AVG: average; SD, one standard deviation). Incubation under ambient laboratory light increased both bacterial production and TNT uptake (μg C L^{-1} d^{-1}); mineralization was not measured under dark conditions. Added 0.176 ppm of TNT.

Illumination	Detonation	Bacterial Production (μg C L^{-1} d^{-1})		TNT Uptake (μg C L^{-1} d^{-1})	TNT Mineralization (μg C L^{-1} d^{-1})		Primary Production	
		AVG	SD	Range	AVG	SD	AVG	SD
Light	Pre	3.36	0.03	161-170	5.00	2.55		
	Post	3.33	0.37	231-241	4.99	2.39		
Dark	Pre	2.06	0.28	71-123				
	Post	1.73	0.63	88-100				

At T = 0, primary production rates in the light and dark plume samples were not significantly different ($p = 0.05$) from their respective controls (Fig. 14). This was the case for dark samples for the duration of the experiments. T = 24 h, PPP in the light RDX plume sample and the light TNT plume sample were significantly higher than their respective controls. PPP in the light RDX plume sample continued to rise to the end of the experiment, but decreased slightly in the RDX control. Conversely,

PPP in the light TNT plume sample was significantly elevated compared to the control at T = 24 h, but returned to control levels by T = 48 h. It is important to note that PPP in light samples collected from the TNT plume site at T = 0 h was almost double the values from the RDX plume site Also, the absolute difference between the TNT control and plume sample at T = 24 h was greater than the RDX control and plume at the same time. These data indicate that although RDX had a more lasting effect on increasing primary productivity than TNT this may be related to a slower rate of uptake of the RDX daughter products resulting from the detonation by marine algae. It appears that the transformation products (resulting from detonation) of TNT were more labile to phytoplankton and hence were utilized rapidly and exhausted by the end of the experiment. Interestingly, none of the treatments (light vs dark, plume vs control) resulted in a significant difference in the rate of phytoplankton production of dissolved organic matter (also called DPP). DPP production was consistent across treatments and for samples collected from both sites for the duration of the experiment. The introduction of the post-detonation products of RDX and TNT appear only to affect the production of particulate biomass, and not the production of dissolved photosynthate.

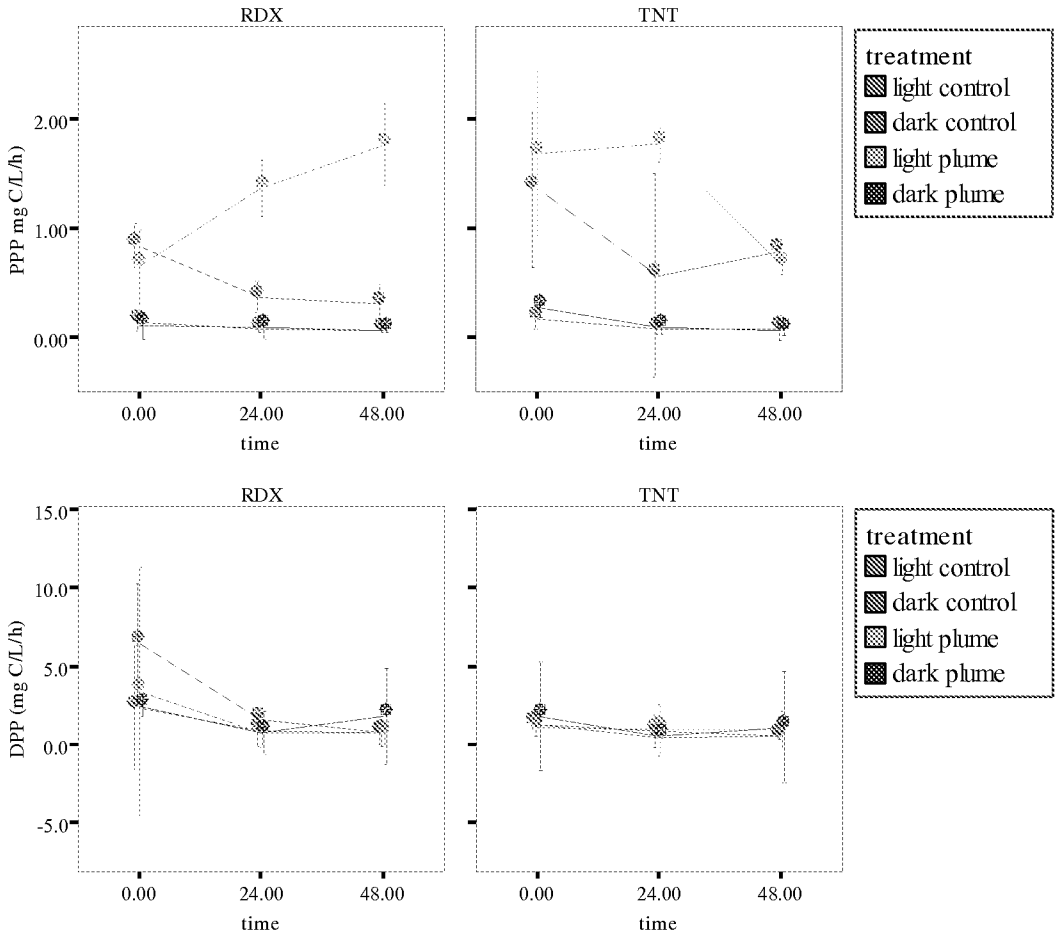

Fig. 14 — Primary production surface water sampled offshore of Oahu in May 2005

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