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Energetic contaminants inhibit plant litter decomposition in soil

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ABSTRACT

Individual effects of nitrogen-based energetic materials (EMs) 2,4-dinitrotoluene (2,4-DNT), 2-amino-4,6-dinitrotoluene (2-ADNT), 4-amino-2,6-dinitrotoluene (4-ADNT), nitroglycerin (NG), and 2,4,6,8,10,12-hexanitrohexaazaisowurtzitane (CL-20) on litter decomposition, an essential biologically-mediated soil process, were assessed using Orchard grass (*Dactylis glomerata*) straw in Sassafras sandy loam (SSL) soil, which has physicochemical characteristics that support "very high" qualitative relative bioavailability for organic chemicals. Batches of SSL soil were separately amended with individual EMs or acetone carrier control. To quantify the decomposition rates, one straw cluster was harvested from a set of randomly selected replicate containers from within each treatment, after 1, 2, 3, 4, 6, and 8 months of exposure. Results showed that soil amended with 2,4-DNT or NG inhibited litter decomposition rates based on the median effective concentration (EC50) values of 1122 mg/kg and 860 mg/kg, respectively. Exposure to 2-ADNT, 4-ADNT or CL-20 amended soil did not significantly affect litter decomposition in SSL soil at ≥ 10,000 mg/kg. These ecotoxicological data will be helpful in identifying concentrations of EMs in soil that present an acceptable ecological risk for biologically-mediated soil processes.

1. Introduction

Increased demand for military training and testing resources in recent years has resulted in increased levels of energetic materials (EMs) in soils and has raised concern regarding their environmental impacts at testing and training ranges (Kuperman et al., 2009b). Preservation of soil quality is essential for protecting and sustaining ecological integrity of terrestrial ecosystems at these ranges. Improving our understanding of the potential effects of contaminant EMs on the sustainability of terrestrial ecosystems at defense installations is necessary for achieving this goal. Plant litter decomposition is an essential soil process, and is among the most integrating processes within the soil ecosystem because it involves intricate interactions of microbial and faunal activities with the soil chemical environment (Kuperman et al., 2002; Wentsel et al., 2003). A disturbance that inhibits litter decomposition can result in nutrient losses and declining soil fertility, which can negatively impact ecosystem sustainability. Therefore, an assessment of how rates of organic matter decomposition can be altered by contamination of soil with EMs is critical to understanding the potential impacts of EMs on the overall functioning of the soil ecosystem at military testing and training ranges (Kuperman et al., 2017, 2009b).

Among the common residues present in soil contaminated with EMs are 2,4-dinitrotoluene (2,4-DNT) and nitroglycerin (NG), which are found in propellant residues produced from military live-fire training (Walsh et al., 2011). The physical release of NG and 2,4-DNT encapsulated in nitrocellulose (NC) is dependent upon the physical weathering and subsequent dissolution of the NC (Clausen et al., 2011). Following the release of NG and 2,4-DNT from the NC and the dissolution in water, adsorption onto soil and abiotic and biotic transformation processes can play major roles in determining their overall fate and environmental impact (Bordeleau et al., 2012; Clausen et al., 2011).

When exposed to the environment, 2,4-DNT, like 2,4,6-trinitrotoluene (TNT), does not mineralize either aerobically or anaerobically but can be transformed to mono or diamino derivatives. 2,4-DNT and its amino derivatives frequently co-occur with the relatively stable transformation products of the reductive (bio)transformation pathway of TNT, such as 2-amino-4,6-dinitrotoluene (2-ADNT), and 4-amino-2,6-dinitrotoluene (4-ADNT) in soil contaminated with nitroaromatic EMs (Jenkins, 2004; Monteil-Rivera et al., 2009; Travis et al., 2008). This has precluded investigators from partitioning the effects of the parent materials and their transformation products on the soil microbial

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community and biologically-mediated endpoints, such as organic carbon mineralization (Fuller and Manning, 1998; Gong et al., 2000; Kuperman et al., 2017) and necessitated the present investigation of the ecotoxicological effects of selected individual EMs on biologically-mediated organic matter decomposition using environmentally realistic exposure levels. Concentrations of 117, 19, and 20 mg/kg were reported for 2,4-DNT, 2-ADNT, and 4-ADNT, respectively, in soil at Joliet Army Ammunition Plant (Wilmington, IL) by Simini et al. (1995).

Mineralization of NG in soil has not been reported (Husserl et al., 2010), and lack of NG mineralization in soil contributes to its persistence at contaminated sites. NG concentrations of up to 242 mg/kg have been reported at the Massachusetts Military Reservation (Barnstable County, MA) (Clausen et al., 2011), nearly 2000 mg/kg in surface soils at Canadian Forces Base Valcartier (Quebec) Arnhem Antitank Rocket Range (Jenkins et al., 2004; Reifler and Medina, 2006), and 11,300 mg/kg on an antitank rocket range at Canadian Forces Base Gagetown (New Brunswick) (Jenkins et al., 2004).

An emerging polynitramine EM, 2,4,6,8,10,12-hexanitrohexaazaisowurtzitane (China Lake compound 20, CL-20), can be used as a replacement for the cyclic nitramine explosives hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) and octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX). CL-20 contains multiple electron-withdrawing N-NO_2 functional groups, causing the EM to resist electrophilic attack by oxygenases under aerobic soil conditions resulting in slow and incomplete mineralization and persistence in contaminated soil (Hawari et al., 2004; Hawari, 1999; Monteil-Rivera et al., 2009). Previous studies investigated CL-20 effects on bioluminescence of marine bacteria *Vibrio fischeri*, the cell density of freshwater green algae *Selenastrum capricornutum*, the activities of soil enzymes (Gong et al., 2004), and effects on terrestrial plants (Rocheleau et al., 2008), and on soil invertebrates (Dodard et al., 2005; Kuperman et al., 2006; Robidoux et al., 2004). An improved understanding of ecological impacts of CL-20 release into the environment, including its potential effects on soil organic matter decomposition, is recommended prior to larger-scale production of CL-20.

Notwithstanding the persistence of these EMs in soil, their effects on key soil processes that are important to the regulation, flow, and internal cycling of carbon and nutrients in ecosystems have not been sufficiently investigated (Kuperman et al., 2017, 2009b). Litter decomposition is critical to terrestrial biogeochemical cycles and is key to sustaining the functioning of terrestrial ecosystems (Knacker et al., 2003; Kuperman et al., 2014a; Kuperman, 1999). Understanding the potential impacts of EM contamination on soil ecosystems is important for effective preservation of soil fertility at military installations and for development of environmental quality criteria that can be consistently applied to gauge the potential ecotoxicological impacts of military operations. However, ecotoxicological data acceptable for developing scientifically defensible screening values for biologically-mediated processes in soil are insufficient for 2,4-DNT, 2-ADNT, 4-ADNT, NG, and CL-20. Assessment of EM effects on litter decomposition can generate data that can be used in conjunction with ecological soil screening levels (Eco-SSLs) (USEPA, 2005) within the framework of screening level ecological risk assessments at EM-contaminated sites. To fill the existing data gaps, we conducted definitive studies to test the hypothesis that soil contaminated with selected nitrogen-based EMs can affect plant litter decomposition rates in soil. The development of new ecotoxicological data can aid contaminated site managers in the knowledge-based decision-making process of securing the sustainable use of military testing and training installations.

2. Materials and methods

2.1. Chemicals and reagents

Nitrogen-based EMs used in the present studies included 2,4-DNT (Chemical Abstracts Service [CAS] no. 121-14-2; purity, 97%), 2-ADNT (CAS no. 35572–78-2; purity, 99%), 4-ADNT (CAS no. 19406–51-0; purity, 99%), NG (CAS no. 55–63-0; purity, 99%), and HMX (CAS no. 2691-41-0; purity 99%; used as an internal standard solution). These EMs were obtained from the Defense Research Establishment Valcartier of the Canadian Ministry of National Defense (Val Bélair, QC, Canada). CL-20 (CAS no. 135285-90-4; ε-isomer, purity 99.3%) was obtained from ATK Thiokol Propulsion (Ogden, UT, USA). 1,3-Dinitrobenzene (1,3-DNB; used as an internal standard solution) was obtained from Fluka Chemical (Milwaukee, Wisconsin). High-performance liquid chromatography (HPLC)-grade acetone (CAS no. 67-64-1) was used to prepare individual EM solutions prior to soil amendments. Acetonitrile (CAS no. 75-05-8; HPLC grade), methanol (CAS no. 67-56-1; chromatography grade; purity, 99.9%), and calcium chloride ($CaCl_2$; CAS no. 10043-52-4; reagent grade) were used for the soil extractions and in analytical determinations by HPLC. Certified standards of EMs (AccuStandard, Inc.; New Haven, CT; and Cerilliant; Round Rock, TX) were used in HPLC determinations. ASTM Type I reagent water (18 MΩ cm at 25 °C) (ASTM, 2004) was used throughout the toxicity studies. It was obtained using a Milli-RO 10 Plus system followed by a Milli-Q PF Plus system (Millipore; Bedford, MA). The same grade of reagent water was used throughout the analytical determinations. Glassware was washed with phosphate-free detergent and sequentially rinsed with tap water, ASTM Type II water (> 5 MΩ cm at 25 °C), analytical reagent-grade nitric acid 1% (v/v), and ASTM Type I water.

2.2. Soil collection, characterization, and treatment preparation

Several collections of a natural soil, Sassafras sandy loam [Fine-loamy, siliceous, mesic Typic Hapludults] (SSL), containing indigenous microbial and invertebrate communities, were used in the present studies. This soil was selected for developing ecotoxicological values protective of biologically-mediated processes in soil because (1) it was previously used to establish ecotoxicological benchmarks for the EM effects on soil respiration endpoints (Kuperman et al., 2017) and in standardized plant and soil invertebrate toxicity tests (Dodard et al., 2005; Kuperman et al., 2014b, 2006, 2005; Robidoux et al., 2004); and (2) it has physical and chemical characteristics, including low organic matter and clay contents, that support “very high” qualitative relative bioavailability for organic chemicals in natural soils (USEPA, 2005). Soil was designated with the year of collection as an identifying suffix. SSL2003 was used for studies with CL-20 in 2003. Soil designated SSL2007 was used for studies with 2,4-DNT, 2-ADNT, 4-ADNT, and NG in 2007. Triplicate subsamples of SSL2003 and SSL2007 soil batches were sent to the Pennsylvania State University Agricultural Analytical Services Laboratory to determine soil texture, pH, organic matter content, and cation exchange capacity using methods listed in [http://agsci.psu.edu/aasl/soil-testing/soil-methods]. An average field soil moisture level of 14% dry soil mass was maintained for the duration of the studies by weekly additions of ASTM Type I water.

Freshly collected SSL2003 soil (58% sand, 28% silt, 14% clay, 2.6% organic matter, 9.8 cmol/kg cation-exchange capacity, pH 5.1) was obtained from an open grassland field in the coastal plain on the property of the U.S. Army Aberdeen Proving Ground (APG), Harford County, Maryland, in May 2003. Freshly collected SSL2007 soil (62% sand, 25% silt, 13% clay, 2.2% organic matter, 7.8 cmol/kg cation-exchange capacity, pH 5.0) was obtained from the same location in November 2007. During collection of fresh SSL2003 and SSL2007 soil batches, the O horizon and root zone of the upper soil layer were retained within the collected soil to ensure sufficient abundance of the indigenous soil organisms. Soil was gently passed through a 5 mm sieve to remove large debris and regularize distribution of soil organisms, then stored in covered plastic containers overnight to preserve the initial field moisture level.

For each of the respective studies with 2,4-DNT, 2-ADNT, 4-ADNT, NG, and CL-20, additional SSL soil batches were collected several days before each study commenced for preparation of EM soil concentrates.