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Enhanced bioremediation of nutrient-amended, petroleum hydrocarbon-contaminated soils over a cold-climate winter: The rate and extent of hydrocarbon biodegradation and microbial response in a pilot-scale biopile subjected to natural seasonal freeze-thaw temperatures

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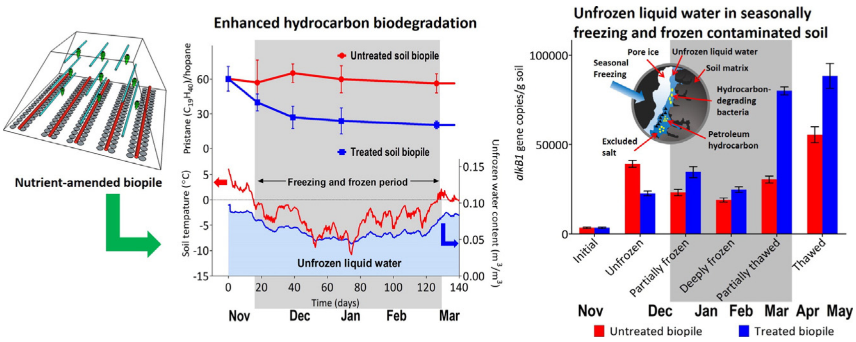
HIGHLIGHTS

- Pilot-scale soil biopiles operated from winter to summer at a cold-climate site.
- Nutrient amendment enhanced hydrocarbon biodegradation during seasonal freeze-thaw.
- Unfrozen water remained detectable in the partially and deeply frozen biopiles.
- The *alkB1* gene copy numbers increased in the partially thawed soil below 0 °C.
- The microbial community compositions shifted during seasonal soil freeze-thaw.

GRAPHICAL ABSTRACT



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ABSTRACT

A pilot-scale biopile field experiment for nutrient-amended petroleum-contaminated fine-grained soils was performed over the winter at a cold-climate site. The rate and extent of hydrocarbon biodegradation and microbial responses were determined and corresponded to the on-site soil phase changes (from unfrozen to partially frozen, deeply frozen, and thawed) associated with natural seasonal freeze-thaw conditions. Treated and untreated biopiles were constructed (~3500 kg each) on an open outdoor surface at a remediation facility in Saskatoon, Canada. The treated biopile received N-P-K-based nutrient and humate amendments before seasonal freezing. Real-time field monitoring indicated significant unfrozen water content in the treated and untreated biopiles throughout the freezing period, from the middle of November to early March. Unfrozen water was slightly more available in the treated biopile due to the aqueous nutrient supply. Soil CO_2 production and O_2 consumption in the treated biopile were generally greater than in the untreated biopile. Total removal percentages for F2 (>C10–C16), F3 (>C16–C34), and total petroleum hydrocarbons (TPH) in the treated biopile were 57, 58, and 58%, respectively, of which 26, 39, and 33% were removed during seasonal freezing and early thawing between November to early March. F3 degradation largely occurred during freezing while F2 hydrocarbons were primarily removed during thawing. Biomarker-based hydrocarbon analyses confirmed enhanced biodegradation in the treated biopile during freezing. The soil treatment increased the first-order rate constants for F2, F3, and TPH degradation by a factor of 2 to 7 compared to the untreated biopile. Shifts in bacterial community appeared in both biopiles as the biopile soils seasonally froze and thawed. Increased *alkB1* gene copy numbers in the treated

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biopile, especially in the partially thawed phase during early thawing, suggest extended hydrocarbon biodegradation to the seasonal freeze-thaw season, due to the nutrients supplied prior to seasonal freezing.

1. Introduction

Cold-climate soil environments are vast, unique, and environmentally sensitive. Large numbers of cold-climate sites, including those in sub-polar and polar regions, have been impacted by petroleum hydrocarbons mainly associated with intensified industrial and human activities (Aislabie et al., 2004; Filler et al., 2015; Siciliano et al., 2008; Snape et al., 2008). Bioremediation has been frequently considered a less destructive remediation technology for petroleum hydrocarbon-contaminated soils in cold climates, including sites in the high Arctic and Antarctica (Aislabie et al., 2006; Camenzuli and Freidman, 2015; Chang et al., 2010; Mair et al., 2013; Margesin, 2004; McWatters et al., 2016; Paudyn et al., 2008; Whyte et al., 2001). Stimulating indigenous cold-adapted hydrocarbon-degrading microbial populations by supplying nutrients (typically nitrogen and/or phosphorous) to cold site soils is effective for active summer remediation at many cold-region sites (e.g., >0 to ~20 °C) (Braddock et al., 1997; Delille et al., 2004; Ferguson et al., 2003; Martínez Álvarez et al., 2017; McCarthy et al., 2004; Thomassin-Lacroix et al., 2002; Walworth et al., 2001; Walworth et al., 2007; Zytner et al., 2001) and for extended treatment periods spanning several annual seasonal cycles (Leewis et al., 2013; McWatters et al., 2016; Paudyn et al., 2008; Sanscartier et al., 2009a).

Field observations of in situ soil CO_2 production and O_2 consumption during seasonal transition periods at northern petroleum-contaminated sites (Norway), and the associated modeling studies, suggest hydrocarbon biodegradation occurs in frozen soils during winter (Rike et al., 2003; Rike et al., 2005). A large-scale biopile remediation project at Antarctic sites demonstrated the successful biostimulation of petroleum hydrocarbon-contaminated soils exposed to an extreme cold climate over a 5-year period (McWatters et al., 2016). Laboratory-controlled soil microcosm experiments conducted at fixed sub-zero temperatures (e.g., −5 °C) and under repeated, cyclic soil freeze-thaw conditions have consistently indicated significant petroleum hydrocarbon biodegradation in nutrient-amended, petroleum-contaminated cold-climate soils (Børresen et al., 2007; Eriksson et al., 2001; Freidman et al., 2016; Karppinen et al., 2017). Chang et al. (2011)'s pilot-scale laboratory bioremediation experiment indicated hydrocarbon biodegradation in nutrient-amended petroleum-contaminated sub-Arctic soils subjected to site-representative seasonal freeze-thaw temperatures, which *slowly* decreased from 2 to −5 °C and then increased to 4 °C at estimated site-specific seasonal rates using a temperature-programmable cold room.

Fixed sub-zero temperatures, rapid soil freeze-thaw cycles, and natural seasonal freeze-thaw cycles might all create different soil microenvironments, especially in terms of unfrozen water availability in the soil matrices (Henry, 2007; Olsson et al., 2003). The rates of soil freezing and thawing (slow or rapid), along with the soil type, composition, surface area, and salinity, regulate the retention of unfrozen liquid water, pore ice formation, and solute partitioning into unfrozen liquid water (Andersland and Ladanyi, 2004; Konrad and McCammon, 1990; Marion, 1995). Natural seasonal soil freeze-thaw processes foster a progressive transition in the thermal phases of cold-region soils, with the exception of surface soils in the topmost few centimeters of cold ground that are most affected by daily temperature fluctuations and other factors such as sunlight and wind speed. In deeper soils subjected to slow seasonal freeze-thaw cycles, solutes (i.e., nutrients) might be excluded from pore ice and potentially remain available in the unfrozen water (Marion, 1995). The average rate of in situ seasonal freezing and thawing in on-site soils is generally slow (Chang et al., 2011; Henry, 2007; Konrad and McCammon, 1990; Olsson et al., 2003). Unfrozen water in freezing and frozen soils is regarded as a prerequisite for microbial survival at sub-zero temperatures (Clein and Schimel, 1995; Panikov et al., 2006; Rivkina et al., 2000). Decreasing unfrozen water content (or unfrozen water film thickness) during soil freezing is correlated with decreasing microbial metabolic activity (Rivkina et al., 2000).

To date, hydrocarbon biodegradation potential has not been studied in detail in contaminated cold-climate soils subjected to seasonal freeze-thaw conditions characterized by gradual thermal phase changes from unfrozen to partially frozen, deeply frozen, partially thawed, and completely thawed using field data. Field bioremediation studies for remote sites in extreme cold climates (e.g., sub-Arctic, high Arctic, and Antarctica) rely largely on short summers to access the sites for soil treatment, sampling and monitoring campaigns, typically once per year (McWatters et al., 2016; Sanscartier et al., 2009a). Few detailed time-dependent datasets exist for hydrocarbon degradation and microbial responses corresponding to sequential changes in soil thermal phases, despite the fact that such field data could reveal an important link between hydrocarbon-degrading microbial survivors and the potential to extend biodegradation activity in contaminated soils during their natural seasonal soil freeze-thaw processes. Such detailed datasets for various cold-climate seasonalities, from moderate to extreme, are lacking.

This field study aimed to demonstrate the rate and extent of hydrocarbon biodegradation, as well as microbial community responses, corresponding to on-site soil thermal phase changes in outdoor pilot-scale biopiles subjected to natural seasonal freeze-thaw temperatures. The treated and untreated (control) biopiles were installed using field-aged, petroleum hydrocarbon-contaminated soils on a large open-surface area of a soil remediation facility in Saskatoon (52°08′N 106°41′ W; Saskatchewan, Canada). Sampling soil at each soil thermal phase, defined based on ambient air and soil temperatures and corresponding unfrozen water content, is very critical in soil freeze-thaw experiments for addressing biodegradation potential throughout a winter season (Henry, 2007). Here, real-time field monitoring of ambient air and soil temperatures and volumetric unfrozen water content in both biopiles was conducted, allowing responsive soil sampling for hydrocarbon and microbial community analyses when the biopile soils were unfrozen, partially frozen, deeply frozen, partially thawed, and completely thawed in the field. To the best of the authors' knowledge, such hydrocarbon and microbial field data in association with detailed observations of on-site seasonal soil thermal phase changes are currently lacking.

2. Materials and methods

2.1. Contaminated soil

The physicochemical and microbial properties of the site soils are presented in Table 1. Briefly, the clayey fine-grained soil (46% w/w coarse-grained and 54% w/w fine-grained particles, based on the Unified Soil Classification System) had a gravimetric water content and soil pH of 15.3% (w/w) and 7.6, respectively. Soluble inorganic nitrogen species from soil extracts, including nitrite, nitrate, and ammonia, were deficient whereas total phosphorus was not limited (432.5 ± 12.6 mg/kg). Viable hydrocarbon-degrading bacteria were enumerated at approximately 2.3×10^4 CFU (colony forming units) per gram of soil using Bushnell Haas nutrient-media plates spiked with 1% (v/v) diesel as the sole carbon source.

Based on the Canada-Wide Standard for Petroleum Hydrocarbons in Soil (CWS PHC) Tier 1 Method, hydrocarbon concentrations in soils are