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Petroleum Hydrocarbon Biodegradation under Seasonal Freeze−Thaw Soil Temperature Regimes in Contaminated Soils from a Sub-Arctic Site

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Several studies have shown that biostimulation in ex situ systems such as landfarms and biopiles can facilitate remediation of petroleum hydrocarbon contaminated soils at sub-Arctic sites during summers when temperatures are above freezing. In this study, we examine the biodegradation of semivolatile (F2: C10−C16) and nonvolatile (F3: C16−C34) petroleum hydrocarbons and microbial respiration and population dynamics at post- and presummer temperatures ranging from −5 to 14 °C. The studies were conducted in pilot-scale tanks with soils obtained from a historically contaminated sub-Arctic site in Resolution Island (RI), Canada. In aerobic, nutrient-amended, unsaturated soils, the F2 hydrocarbons decreased by 32% during the seasonal freeze−thaw phase where soils were cooled from 2 to −5 °C at a freezing rate of −0.12 °C d^{-1} and then thawed from −5 to 4 °C at a thawing rate of +0.16 °C d^{-1}. In the unamended (control) tank, the F2 fraction only decreased by 14% during the same period. Biodegradation of individual hydrocarbon compounds in the nutrient-amended soils was also confirmed by comparing their abundance over time to that of the conserved diesel biomarker, bicyclic sesquiterpanes (BS). During this period, microbial respiration was observed, even at subzero temperatures when unfrozen liquid water was detected during the freeze−thaw period. An increase in culturable heterotrophs and 16S rDNA copy numbers was noted during the freezing phase, and the ^{14}C-hexadecane mineralization in soil samples obtained from the nutrient-amended tank steadily increased. Hydrocarbon degrading bacterial populations identified as *Corynebacterineae*- and *Alkanindiges*-related strains emerged during the freezing and thawing phases, respectively, indicating there were temperature-based microbial community shifts.

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Introduction

A significant number of petroleum-contaminated sites have been identified in polar regions as a result of fuel storage, transportation, military, and industrial activities (*1*). Cold temperatures and the remote locations of these sites pose challenges to site remediation. On-site ex situ bioremediation has been demonstrated in field studies at sub-Arctic sites (*2*). It is generally believed that the potential for bioremediation exists only during the 2 to 3 month summer season when daily mean air temperatures at northern sites are generally in the range of 5−15 °C. The short summer season over which bioremediation is implemented and monitored is often insufficient time for meeting remediation targets, especially where contamination is extensive. Treatment systems are usually left dormant after summer when freezing temperatures set in and revived in the following summer (*3*), and there is little knowledge on the extent of petroleum hydrocarbon degradation occurring in this time interval and on how petroleum hydrocarbon-degrading bacteria respond to these temperature regimes.

Metabolic activity of cold-adapted bacteria has been detected near and below subzero temperatures in permafrost and other pristine Arctic soils, and these microorganisms likely remain active in the unfrozen, liquid water that may be present in the soil matrix over a range of subzero temperatures (*4*). The activity and viability of soil bacteria at near zero and subzero temperatures suggests the possibility of hydrocarbon biodegradation potential under such temperature regimes, but only a few studies have evaluated microbial growth, activity, and hydrocarbon degradation under such conditions.

Eriksson et al. (*5*) reported that C11−C15 n-alkanes in petroleum-contaminated soils collected from a northern site were biodegraded by approximately 60% in nutrient-amended microcosms subjected to the diurnal freeze−thaw cycles between −5 and 7 °C over 48 days. Børresen et al. (*6*) examined the effect of repeated freeze−thaw cycles between −5 and 10 °C on ^{14}C-hexadecane mineralization in microcosms containing petroleum-contaminated soils collected from a Norwegian site. Microcosms subjected to fewer and longer cycles showed higher mineralization. These studies showed that biodegradation was very limited at −5 °C, but it was unclear if the biodegradation observed during freeze−thaw occurred exclusively under thawing conditions or under higher subzero temperatures as well.

In those previous studies (*5, 6*), relatively rapid and uniform freeze−thaw cycles were employed, but in subsurface soil layers (even at depths of up to 30 cm typical of landfarms), gradual, steady cooling and freezing occur in the 2 to 3 months after the summer, and gradual thawing occurs in spring (*7*−*9*). After the active treatment period in summer, landfarm soils are not tilled, and in such situations, diurnal or short-term temperature fluctuations in landfarms may be significantly attenuated compared to those atmospheric temperature changes (*9*). The rate of soil freeze−thaw plays a key role in determining the amount of pore ice, liquid pore water, and the salt concentrations in liquid pore water (*10*), as well as on the associated viability of soil microorganisms (*11*), and thus, may influence hydrocarbon biodegradation rates (*6*).

In this study, we evaluate the extents of biodegradation of semi- and nonvolatile petroleum hydrocarbons and the microbial activity and population dynamics in aged, contaminated soils in pilot-scale systems subjected to the historical daily mean temperatures of a sub-Arctic site for

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the seasonal transition periods preceding and following summer, during which temperatures changed from 2 to −5 °C and thereafter to 4 °C and eventually to 14 °C. Pilot-scale biodegradation experiments were performed in a cold room which was programmed to operate at temperatures that were representative of the daily mean air temperatures at the site for September and October (postsummer freezing phase), mid-May and June (presummer thawing phase), and end of June through early July (presummer period where constant temperature conditions alternate with periods of rapid warming).

The specific objectives of this study were (i) to assess the extent of biodegradation of semivolatile and nonvolatile petroleum hydrocarbons in historically diesel-contaminated soils which were amended with nutrients (N, P) and maintained under aerobic conditions, under representative seasonal freezing and thawing temperature regimes mentioned above and (ii) to examine the changes in microbial respiration activity and population size and composition in these soils during the same temperature regimes.

Materials and Methods

Petroleum-Contaminated Soils. The contaminated soils were obtained from a former military radar site in RI, (61°30′ N, 65°00′ W) where petroleum hydrocarbon contamination occurred between 1954 and 1974 (*3, 12*). Petroleum-contaminated soils were excavated at the RI site and shipped frozen in sealed containers. The total petroleum hydrocarbon (TPH) concentrations in the soils were in the range of 1300 mg kg^{-1} and were comprised of F2 (C10−C16) and F3 (C16−C34) hydrocarbons, with lighter F1 (C6−C10) and heavier F4 (>C34) hydrocarbons present in negligible amounts (*13*). The site soils were nutrient deficient, naturally acidic (pH 4.5), and coarse-grained sands. The detailed results of soil characterization are described elsewhere (*13*).

Pilot-Scale Biodegradation Experiment. Biodegradation studies were conducted in stainless steel tanks (1.0 m L × 0.65 m W × 0.35 m H; soil depth 0.22 m) representing model landfarming operations at the pilot-scale. Soil gas was sampled through perforated horizontal gas collection tubes placed at soil depths of 17.5, 12.5, 7.5, and 2.5 cm and connected to gas sampling ports fitted with valves. The O_2−CO_2 concentrations were measured by an infrared gas monitor that was able to reliably measure gas concentrations at subzero temperatures (ATX 620, Industrial Scientific Co.). Compressed air was continuously supplied into the headspace of the pilot-scale tanks at the slow air-flow rate of 30 L d^{-1}. The supplied air was exhausted through an activated carbon trap.

The soil was amended with a commercial fertilizer (20% N/20% P_2O_5/20% K_2O, Plant-Prod) and 2000 mg $CaCO_3$ kg^{-1} which was presterilized and applied at a dose that yielded a molar-based C_{TPH}/N/P ratio of 100:9:1. The control tank was not amended with nutrients to assess petroleum hydrocarbon losses in the absence of nutrients. The gravimetric soil−water content (w/w) of 12% after addition of various amendments was within 45−60% of the maximum water-holding field capacity. The water content of the unamended soil was 11%.

The temperature profile in the cold room was derived from daily mean air temperature data at the RI site between 1962 and 2007 acquired from National Climate Data and Information Archive of Environment Canada (Figure 1). Soil temperatures and volumetric water contents were monitored using thermocouples and frequency domain reflectometry sensors (Decagon Devices) at three locations in the tank, and the sensors were validated for measurements in frozen soils down to −15 °C (*14*).

^{14}C-Hexadecane Mineralization. To determine the hydrocarbon degradation potential of the soil microbial community during the seasonal freezing and thawing phases, ^{14}C-hexadecane mineralization assays were conducted. Each microcosm was prepared with 10 g of wet soil in triplicate with soils retrieved from the surface, middle, and bottom depths of the pilot-scale tank at different time points. The retrieved samples were spiked with ^{14}C-hexadecane corresponding to 50 000 dpm dissolved in nonradiolabeled hexadecane to provide a total hexadecane concentration of 100 mg kg^{-1} soil and was placed in sealed microcosms. All microcosms, including sterile (control) microcosms, were incubated at 5 °C. Evolved $^{14}CO_2$ was captured in the recovery trap (1 M KOH) which was periodically sampled for 10 weeks and analyzed using a scintillation counter (LS6500, Beckman Coulter).

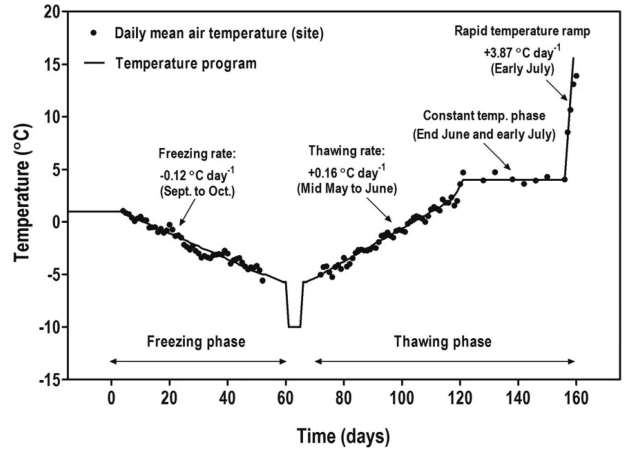

FIGURE 1. Temperature program for the seasonal freeze−thaw pilot-scale experiment conducted in a cold room. The temperature program (solid line) approximated the trend for the daily mean air temperature (data points) of the site.

Petroleum Hydrocarbons Analyses. An analytical method for extractable petroleum hydrocarbons was based on the Canada-Wide Standard for Petroleum Hydrocarbons in Soil (CWS PHC). In the method, TPH is classified into four carbon-number based fractions (F1, F2, F3, and F4) where fraction F1 represents compounds with boiling points lower than C10 (decane), F2 between C10 and C16 alkanes, F3 between C16 and C34 alkanes, and F4 compounds heavier than C34 (*15*).

The soil samples were collected using hand augers and chisels. For each sampling event, five soil samples were randomly collected from each of the surface, middle, and bottom 7 cm layers of the soil tanks. The 15 soil samples for each time point were analyzed for petroleum hydrocarbons. The collected soil samples were preserved at −20 °C before analyses. Petroleum hydrocarbon extractions were conducted in an automatic Soxhlet extractor (Gerhard Soxtherm) and analyzed using a GC-FID (Agilent 6890). In addition, GC-MS analyses were performed to evaluate biodegradation performance by comparing biomarker diagnostic ratios from the nutrient-amended and unamended soils. Individual saturated hydrocarbons (pristane and phytane) and conserved biomarkers (BS) were identified and used for calculating the diagnostic ratios. Extraction, post processing, GC-FID, and GC-MS methods are described in the Supporting Information.

Microbial Analyses. To determine culturable heterotroph population sizes, 1.0 g of soil was vortexed for 30 s in a screw-top test tube containing 2.5 g of sterile 3 mm glass beads and 9.0 mL of minimal salts medium (MSM). Serial dilutions of the MSM were made with 0.1% Na_2PO_7, and 100 μL aliquots were spread onto triplicate R2A agar plates (Becton, Dickinson and Company), incubated at 5 °C for 4 weeks; colony forming units (CFUs) were enumerated. Bacterial (16S rRNA gene)

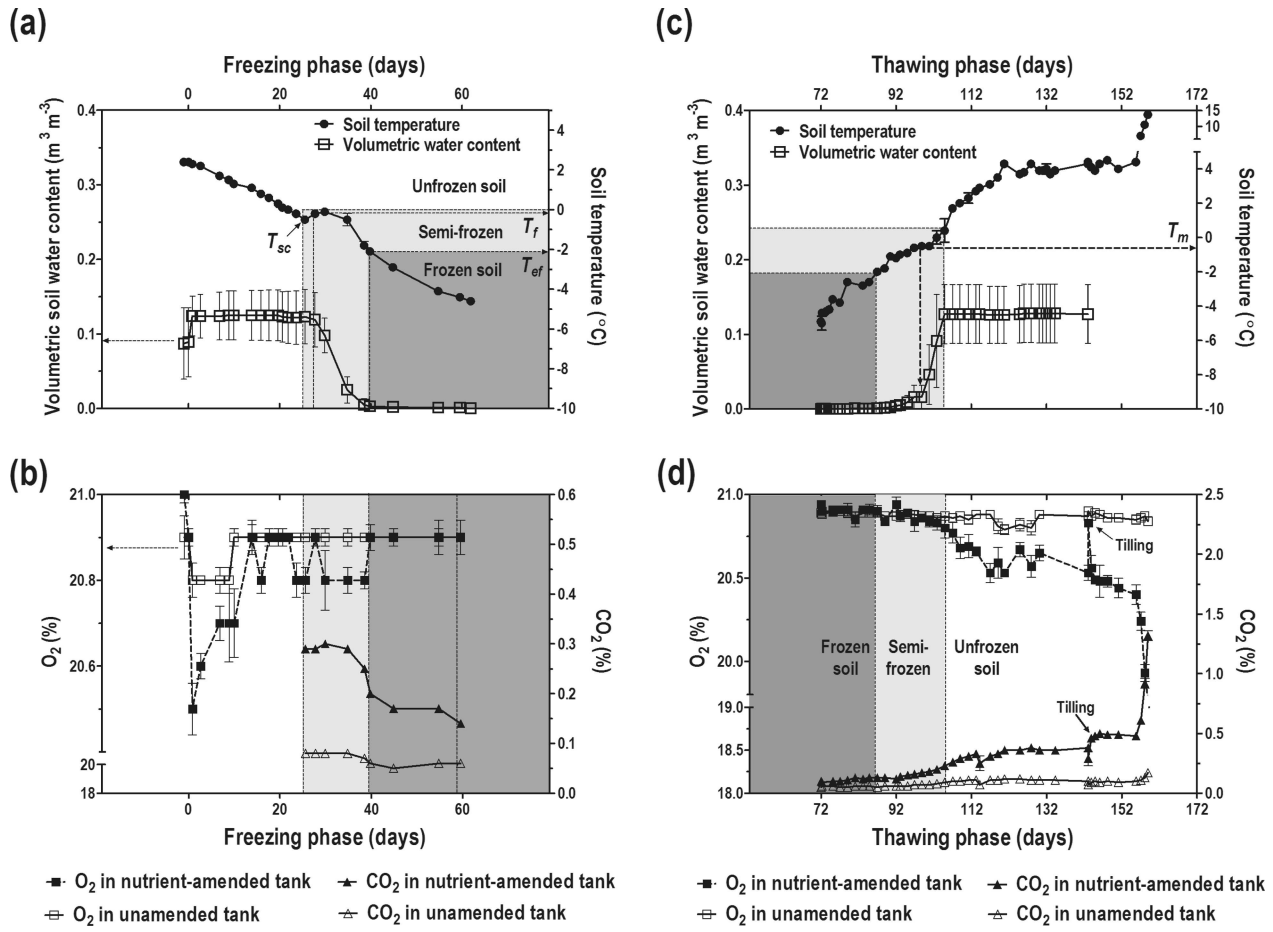

FIGURE 2. Soil temperatures and soil−water content profiles in the freezing phase (a) and thawing phase (c). Corresponding O_2−CO_2 soil gas concentrations in the freezing phase (b) and thawing phase (d). T_{sc}: supercooling temperature = −0.5 °C. T_f: freezing-point depression = −0.2 °C. T_{ef}: effective end point of unbound water = −2.1 °C. T_m: melting temperature = −0.5 °C. The data points for soil temperatures, water contents, and O_2−CO_2 concentrations represent the mean (±SD) of the data obtained from the surface, middle, and bottom depths of the pilot-scale tanks. There were no significant trends in these parameters with depth.

and n-alkane degrader (*alkB* genes) population sizes were determined using the iQ Real-Time PCR Detection System (Bio-Rad Laboratories). Denaturing gradient gel electrophoresis (DGGE) fingerprinting was performed to detect the shifts in total bacterial communities during the seasonal freeze−thaw condition. Details of microbial analyses are presented in the Supporting Information.

Results and Discussion

Changes in Soil Temperatures, Unfrozen Water, and Microbial Respiration during the Post- and Presummer Temperatures. The temperature changes and the unfrozen water content profile for the nutrient-amended soils during the freezing period are shown in Figure 2a. The soil temperature profile shows a characteristic, small temperature increase to the soil freezing temperature, T_f, of −0.2 °C, after reaching T_{sc}, the supercooled temperature of −0.5 °C, which is associated with the release of latent heat during formation of ice (*16*). The water content was unchanged at 0.13 ± 0.033 m^3 m^{-3} until T_{sc} was reached but decreased steadily until reaching a water content less than 0.003 m^3 m^{-3} at −2.1 °C (T_{ef} or effective end point of unbound water) at approximately Day 40 of the freezing phase. Between Day 21 and 40, significant and measurable amounts of liquid pore water coexisted with ice, and thus, the soil was designated to be in a "semifrozen" state. After Day 40, unfrozen pore water contents were below 0.003 m^3 m^{-3}, and soils were, thus, designated to be effectively "frozen" during this period (Figure 2a).

Significant microbial respiration activity, indicated by changes in O_2 and CO_2 concentrations, was detected in the nutrient-amended tank in the unfrozen and semifrozen soils during the freezing phase (Figure 2b). In comparison, the respiration activity measured in the control tank was negligible. In this study, the microbial respiration activity in the nutrient-amended tank continued at the subzero temperatures at which unbound unfrozen water was available. The respiration activity was significantly decreased at around Day 40, and this coincides with the lack of availability of unbound unfrozen water at temperatures below −2.1 °C (T_{ef}). There appears to be a strong relationship between changes in unbound unfrozen water during seasonal freezing and thawing and microbial respiration activity in petroleum-contaminated soils.

The soil thawing temperature and unfrozen water content profiles are presented in Figure 2c,d. The temperature and water content profiles mirrored the patterns observed during the freezing phase, although the frozen and semifrozen states were of shorter duration due to the applied thawing rate of +0.16 °C d^{-1} being slightly higher than the freezing rate of −0.12 °C d^{-1}. The onset of microbial respiration during the semifrozen period in the thawing phase (Figure 2d) was synchronized with the increase in unfrozen water availability and soil temperatures (Figure 2c). During the constant-temperature phase of 4 °C, respiration activity increased moderately. The constant-temperature period was maintained to determine whether accelerated respiration rates, often described as a "CO_2 burst" during soil thawing would

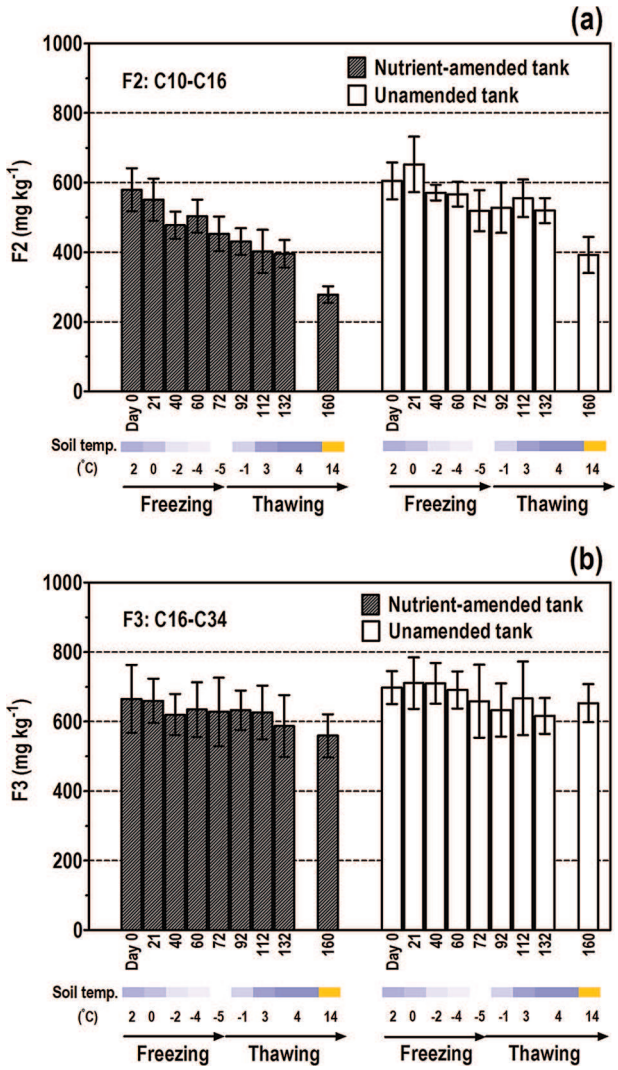

FIGURE 3. Changes in F2 (a) and F3 (b) hydrocarbon concentrations during the experiment in the nutrient-amended and unamended tanks. The data points of the F2 and F3 concentrations represent the mean concentrations (±SD) obtained from the three depths of the pilot-scale soil tanks. There were no significant trends in hydrocarbon concentrations with depth.

occur at these temperatures (*17*). However, respiration activity was very significantly increased only when the soil temperature changed rapidly from 4 to 14 °C between Day 155 and 160. Tilling in both the nutrient-amended and control tanks at the constant 4 °C phase resulted in only a modest increase in respiration activity, indicating O_2 availability to the microorganisms was not limited, and microbial respiration was primarily sensitive to temperature. The CO_2 burst during the thaw season has been attributed to the availability of a new pool nutrients and substrates from microbes killed during the freezing phase (*17*). In our study, the rapid rise in temperature and the presence of amended nutrients were key factors in enhanced respiration and biodegradation activity.

Hydrocarbon Biodegradation in the Pre- and Postsummer Temperatures. Figure 3a shows a significantly larger decrease in F2 hydrocarbons in the nutrient-amended tank compared to that in the unamended tank (32% and 14%, respectively) over the freeze−thaw period (Day 0−132). The F2 concentration decreased by 13%, from 579 ± 62 mg kg^{-1} to 503 ± 48 mg kg^{-1} in the nutrient-amended tank during the freezing phase (Day 0−60). This decrease is statistically significant (two-way ANOVA, $p < 0.05$) and occurred over a period where soil temperatures ranged from 2 to −5 °C and volumetric water contents ranged between 0.13 and 0.003 $m^3\ m^{-3}$. A similar pattern of F2 losses in the nutrient-amended and unamended tanks were noted in the thawing period between Day 72 and 132. Between Day 21 and 92, the subzero temperature period, F2 concentrations decreased by 22% (Bonferroni post-test, $p < 0.001$), which indicates substantial biodegradation at subzero temperatures. The lack of F2 hydrocarbon losses during the freezing phase in the unamended tank which was maintained at the same temperature regime and the significantly higher O_2 consumption and CO_2 production in the nutrient-amended tank compared to the control suggests that these hydrocarbon losses in the nutrient-amended tank are due to biodegradation.

Diagnostic ratios of pristane ($C_{19}H_{40}$) and phytane ($C_{20}H_{42}$) based on the conserved diesel biomarker, BS (*m*/*z* 123), decreased over time between Day 0 and 132, as shown in Figure S1 (Supporting Information), indicating that biodegradation of petroleum hydrocarbons in the nutrient-amended soils occurred under the subzero temperature regimes during the freeze−thaw phases. Significant biodegradation of pristane and phytane in petroleum hydrocarbon-contaminated soils has been reported (*18*), but BS is considered to be a conserved biomarker (*19*). Between Day 0 and 132, the diagnostic ratios were stable in the unamended soils.

The loss of the higher molecular weight hydrocarbon fraction (F3) was limited during the entire experiment (Figure 3b). In the nutrient-amended tank, the initial F3 concentration of 665 ± 97.9 mg kg^{-1} decreased to 559 ± 61.9 mg kg^{-1} by Day 160, which was lower than the F3 of 652 ± 54.5 mg kg^{-1} in the unamended tank on Day 160 (Bonferroni test, $p < 0.01$). The cumulative removal of F3 hydrocarbons was 16% and 6% in the nutrient-amended and unamended tank, respectively.

The literature on biodegradation of field-aged hydrocarbons at or below 0 °C is limited. In microcosm experiments with contaminated soils from an Arctic site, Eriksson et al. (*5*) observed biodegradation rates of 0.95 mg $kg^{-1}\ d^{-1}$ of *n*C11 to *n*C15 at 0 °C but biodegradation was not observed at −5 °C. Rike et al. (*7*) estimated a biodegradation rate of 3 mg $kg^{-1}\ d^{-1}$ on the basis of in situ O_2 consumption and CO_2 production in petroleum-contaminated Arctic soils over a period where the soil temperatures decreased from near zero to −2 °C during 2 months. In our study, we observed an approximate F2 hydrocarbon biodegradation rate of 1.6 mg $kg^{-1}\ d^{-1}$, between Day 21 and 92, the subzero temperature period. This biodegradation rate is comparable to the degradation rates reported in the studies mentioned above (*5, 7*).

The significant biostimulation observed in the nutrient-amended tank during the freezing and thawing phases was further confirmed by the ^{14}C-hexadecane mineralization assay. The hydrocarbon degradation potential of the soil microorganisms in the nutrient-amended tank increased with time over the freezing and thawing phases, as seen from the ^{14}C-hexadecane mineralization activity in soil samples obtained at different time points from the pilot-scale tanks (Figure 4). It should be noted that the mineralization activity was assessed by incubation at 5 °C and, thus, the different mineralization activity exhibited by the soil samples obtained at different days was not influenced by temperature during the mineralization assays. The data in Figure 4 indicate that the hydrocarbon-degrading microbial community was not eliminated under subzero temperatures in this study, where soils were either semifrozen or frozen.

Characterization of Microbial Populations during the Freezing and Thawing Phases. Plate counts of culturable heterotrophs extracted from the nutrient-amended soils indicated a significant increase in microbial populations from

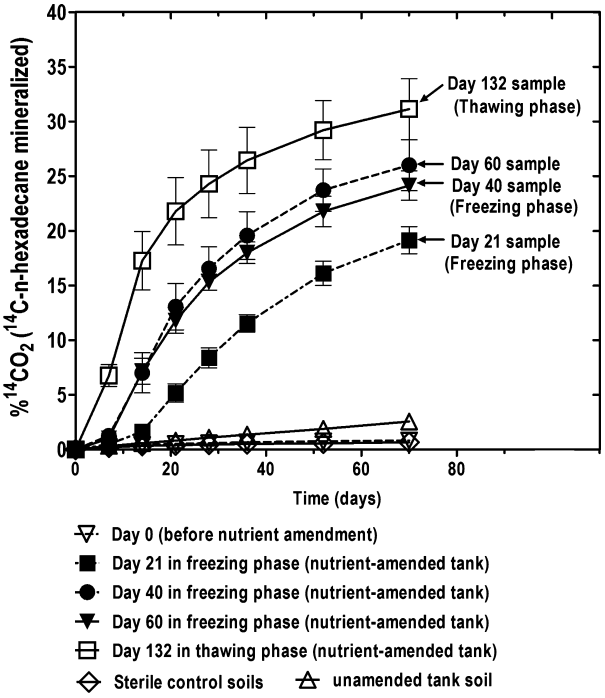

FIGURE 4. Results of ^{14}C-hexadecane mineralization assays of soil samples collected from the soil tanks during the freezing and thawing phase.

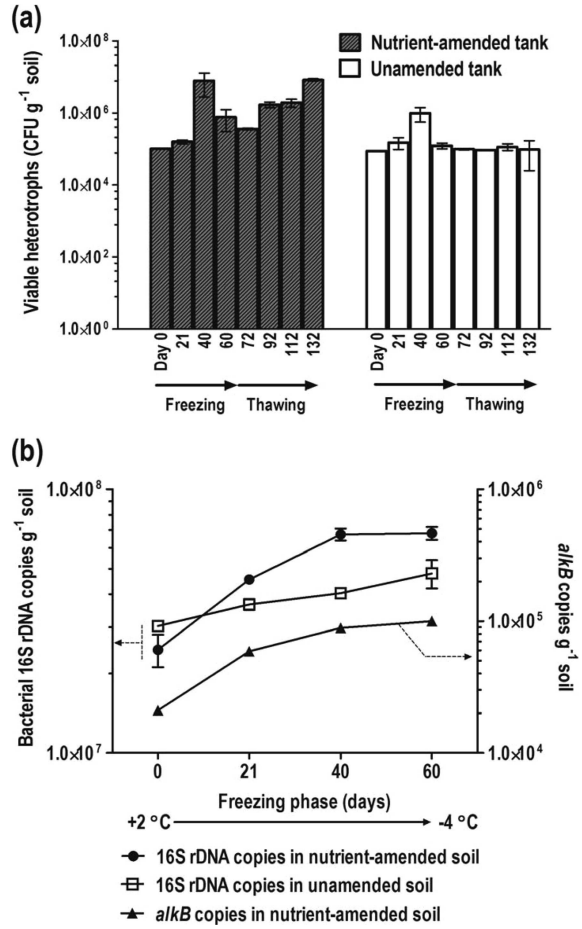

FIGURE 5. (a) Culturable heterotrophs in soils samples collected from the freezing and thawing phase in the nutrient-amended and unamended tanks. (b) 16S rDNA and *alkB* copies in the soils during the freezing phase. The data points (a, b) represent the mean (±SD) of the data obtained from three different depths of the soil tanks.

approximately 1.0×10^5 to 7.9×10^6 CFU g^{-1} soil between Day 21 and 40 (two-way ANOVA, $p < 0.05$, Figure 5a) when the soils were in a semifrozen state. The enhanced heterotrophic populations at Day 40 generally reflected the same trend of enhanced mineralization activity observed in Figure 4. A smaller increase in the heterotroph population was also observed in the control tank during this semifrozen period. There was a 10-fold decline in the heterotroph populations during the frozen state between Day 40 and 60. Subsequently, heterotrophic bacterial populations increased during the thawing phase. The overall trend indicates an increase in microbial numbers as the soils warmed up during the thawing phase, especially in the nutrient-amended soils relative to the control, where culturable aerobic heterotrophs were ~10−100 fold greater in the nutrient-amended soils by Day 132 in the thawing phase.

Culture-independent analyses of 16S rDNA copy numbers from qPCR studies confirmed that maximum population sizes occurred at the end of the semifrozen period and there was no further increase in the frozen state (Figure 5b). Bacterial populations in the nutrient-amended soil increased from 2.5×10^7 to 6.7×10^7 copies 16S rDNA g^{-1} soil by Day 40 in the freezing phase and remained steady up to Day 60. The *alkB* copy numbers showed identical trends. Heterotrophic and bacterial 16S rDNA populations remained approximately near initial levels in the unamended soils during the freezing phase.

Analyses of DGGE banding patterns indicated a shift in the bacterial community in soil from the nutrient-amended tank during the freezing phase (Figure S3a, Supporting Information). While the soil was in a semifrozen state (by Day 40), two DGGE bands appeared and these were classified as *Corynebacterineae* (91%) and *Rhodanobacter* (91−96%), respectively (Ribosomal Database Project-Release 10 (*20*)). The band strongly related to a *Corynebacterineae*-related strain was previously identified in a petroleum-contaminated site in the Antarctica and shown to be capable of hydrocarbon degradation (*21*). In contrast, DGGE banding patterns in soil from the unamended tank revealed a stable microbial community structure, suggesting that the emerging hydrocarbon degrading populations were primarily due to biostimulation from nutrients. Furthermore, the PCR-DGGE analyses showed that the *Corynebacterineae*-related strain that appeared in the freezing period subsequently disappeared after Day 92 in the thawing phase (Figure S3a, Supporting Information). The *Alkanindiges*-related strain (89%) showed increasing band intensity as soil temperature increased in the thawing phase (Figure S3b, Supporting Information). *Alkanindiges*-related microbial populations have been identified in the petroleum-contaminated soils shipped from the RI site (*22*) and are associated with biodegradation of high-molecular-weight hydrocarbons (*23*). Thus, it appears that the hydrocarbon degradation patterns in the freezing and thawing phases are linked to notable community shifts detected with PCR-DGGE analyses.

Implications. This study is the first to demonstrate petroleum hydrocarbon biodegradation under controlled conditions, in field-contaminated soils from a sub-Arctic site during a representative, post- and presummer temperature regime of the site, which included periods of subzero temperatures. The increases in abundance and respiration activity of cold-adapted heterotrophic populations at subzero temperatures were observed when unbound unfrozen water was available in the soils. Microbial activity at subzero temperatures contributed to biodegradation of the F2 hydrocarbons but not the F3 hydrocarbons. The microbial activity was maintained likely due to the slow rate of soil freezing that limits intercellular ice crystallization and cell

damage (*11, 24*). The range of subzero temperatures, for which unbound liquid water can be present, increases with the amount of hydrocarbon contamination and clay content at a given freezing or thawing rate (*25, 26*). Significant unbound liquid water can exist for periods as long as 4 months following summer at some northern sites (*9*) and, thus, may provide more extended periods for slow hydrocarbon biodegradation than that observed in this study.

During the post- and presummer periods over which biodegradation experiments were carried out, the total removals of F2 and F3 hydrocarbon fractions were 52% and 16%, respectively. Of this, a 32% removal of the F2 hydrocarbons was achieved in the temperature range of 2 to −5 to 4 °C. This represents a significant decline in petroleum hydrocarbon concentrations, and maintaining sufficient nutrient levels in landfarm soils at the end of the summer season may be beneficial in lowering hydrocarbon concentrations. It should be noted, however, that the temperature regimes employed in this study have been approximated on the basis of historical atmospheric temperature data and soil temperature trends in other studies. If actual temperature trends are substantially different during the seasonal transition periods in any year or if soil moisture contents are altered substantially for extended time periods by rainfall or snowmelt events, biodegradation and microbial activity patterns may be altered from that observed in this study.

Acknowledgments

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Supporting Information Available

Additional details on methods for hydrocarbon analyses by GC-FID and GC/MS and for microbial population characterization by qPCR and PCR-DGGE; results on changes in abundance of specific hydrocarbons relative to conserved biomarkers (diagnostic ratios) as determined by GC-MS and changes in concentration of specific hydrocarbons as determined by GC-FID, during the freeze-thaw biodegradation experiments; and images of gels from PCR-DGGE analyses showing shifts in microbial communities in those experiments. This material is available free of charge via the Internet at http://pubs.acs.org.

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