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ORIGINAL PAPER

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Biostimulation and bioaugmentation for on-site treatment of weathered diesel fuel in Arctic soil

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Abstract Bioremediation of weathered diesel fuel in Arctic soil at low temperature was studied both on-site in small-scale biopiles and in laboratory microcosms. The field study site was on Ellesmere Island (82°30'N, 62°20'W). Biostimulation was by fertilization with phosphorous and nitrogen. Bioaugmentation was with an enrichment culture originating from the field site. In biopiles, total petroleum hydrocarbons (TPH) were reduced from 2.9 to 0.5 mg/g of dry soil over a period of 65 days. In microcosms at 7 °C, TPH were reduced from 2.4 to 0.5 mg/g of dry soil over a period of 90 days. Inoculation had no effect on hydrocarbon removal in biopiles or in microcosms. Maximum TPH removal rates in the biopiles were approximately 90 µg of TPH g^{-1} of soil day^{-1}, occurring during the first 14 days when ambient temperature ranged from 0 to 10 °C. The fate of three phylotypes present in the inoculum was monitored using most-probable-number PCR, targeting 16S rRNA genes. Populations of all three phylotypes increased more than 100-fold during incubation of both uninoculated and inoculated biopiles. The inoculum increased the initial populations of the phylotypes but did not significantly affect their final populations. Thus, biostimulation on site enriched populations that were also selected in laboratory enrichment cultures.

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Introduction

Numerous Arctic sites have been contaminated with hydrocarbon fuels. With increasing attention towards the preservation of the environment, aboriginal land claim settlements and decommissioning of former military sites, effective and economical means to clean up hydrocarbon-contaminated Arctic sites are increasingly needed.

Biodegradation of hydrocarbons by natural populations of microorganisms represents one of the primary mechanisms by which petroleum and other hydrocarbon pollutants are eliminated or transformed in the environment. It is generally accepted today that organic compounds, especially petroleum hydrocarbons, can be degraded by microorganisms as long as a few factors, such as nutrients (including oxygen), organic compound bioavailability and temperature are controlled and optimized (Alexander 1999; Atlas 1981; Leahy and Colwell 1990; Zhou and Crawford 1995).

Optimum hydrocarbon biodegradation temperatures are usually between 15 °C and 30 °C, but at Arctic sites it may be impractical to achieve temperatures above 10 °C. A small number of published reports have described field experiments on bioremediation of fuel-contaminated soil in polar regions (Aislabie et al. 1998; Braddock et al. 1997; Kerry 1993; Mohn et al. 2001).

Because of the temperature constraints at Arctic sites, it is necessary to optimize other parameters for bioremediation of such sites. One strategy for doing so is inoculation with hydrocarbon-degrading microorganisms. Such microorganisms can be found almost everywhere on Earth, but sometimes bioaugmentation may increase pollutant removal rates (Alexander 1999; Atlas 1981; Colwell and Walker 1977). On the other hand, inocula-

tion of contaminated soil has often failed to enhance hydrocarbon degradation rates or the total amount of hydrocarbons removed (Prince 1998; Stotzky 1997; Vogel 1996). Usually, inoculation has been shown to increase the bacterial concentrations at the start of experiments, without any effect on the final concentrations (Alexander 1999). If the indigenous microbial population of an Arctic soil is low, or for some reason inhibited, inoculation may have a positive effect on the degradation of hydrocarbons. In field studies (Mohn et al. 2001) and laboratory microcosms (Mohn and Stewart 2000), we previously found that inoculation of fuel-contaminated Arctic tundra soils did enhance fuel biodegradation, in addition to enhancement due to nutrient addition. Further studies are necessary to evaluate the general usefulness of inoculation to stimulate petroleum hydrocarbon removal from Arctic tundra soils. In addition, regulatory requirements necessitate investigation of the fate of inocula and evidence that inocula do not harm the environment.

In this study a field experiment and a laboratory microcosm experiment were performed to determine the effect of inoculation on biodegradation of fuel-contaminated Arctic tundra soil. The inoculum used was an enrichment culture originating from the soil that was treated. We previously characterized this culture and detected nine phylotypes in a clone library of 16S rRNA gene fragments (Thomassin-Lacroix et al. 2001). We also developed PCR assays for the three most abundant phylotypes in the library. In this study, the PCR assays were used to monitor populations of those phylotypes. Culture assays were also used to quantify bacterial populations in soil.

Materials and methods

Study site and soil samples

Canadian Forces Station Alert is located on the northeastern tip of Ellesmere Island, Nunavut, Canada (82°30'06" N, 62°19'47" W). Alert is located in an Arctic desert with a mean annual precipitation of 155 mm. The mean daily temperature for January, February and March is –30 °C, with a record low of –50 °C. In July, the mean daily maximum temperature is 6.4 °C with the record high of 20 °C. Alert has only 20–30 frost-free days per year. There is constant daylight for 5 months in the summer and constant darkness for 5 months in the winter.

Soil was collected from the site of a fuel spill near the airstrip at Alert and was used for the field experiments and the microcosm experiments. The fuel spilled appeared to be either Arctic diesel or jet fuel, which are very similar and primarily contain 10- to 18-carbon alkanes. Soil samples were refrigerated or kept on ice during transport and stored in the laboratory at –20 °C. The soil had an initial average contamination level of weathered total petroleum hydrocarbons (TPH) of 3.0 mg/g of dry soil. The soil had a composition of 36.6% sand, 49.3% silt and 14.1% clay. Total organic carbon was 3.77%. Soil pH was 7.2, and the water content was 13%.

On-site biopiles

Small-scale biopiles were built on site at Alert. The experiment consisted of six small-scale biopiles, three inoculated with a hydrocarbon-degrading enrichment culture and three control uninoculated biopiles. The biopile size was approximately 1 m diameter×0.5 m height, with a volume of soil of approximately 0.5 m^3. The biopiles were constructed on top of a polyethylene barrier in a single line, east to west, to minimize differences in the effects of the sun and the wind on each biopile. Nutrients and other soil amendments were added to all biopiles. The fertilizers used were granular urea (46%) and diammonium phosphate (18% N, 46% P_2O_5). Urea and diammonium phosphate were used at concentrations of 1.04 kg and 0.14 kg, respectively, per cubic meter of hydrocarbon-contaminated soil. A surfactant (Biosolve, Westford Chemical) was added according to the manufacturer's recommendation at a final concentration of 1.25 l/m^3 of soil. Gro Brix (Gro Brix Distributors, Mississauga, Ont.), a cocoa-fiber bulking agent, was added to increase airflow and porosity of the soil. Hydrated Gro Brix was used at a ratio of 10% (vol/vol). The original source of the enrichment culture inoculum was soil from Alert, and the culture was grown on jet fuel at 7 °C (Thomassin-Lacroix et al. 2001). Cells were lyophilized for storage and transport and suspended in water prior to use. Ten liters of inoculum (2×10^{13} cells/L) was added to each of the three inoculated biopiles to give a final density of approximately 10^8 cells/g of dry soil. Each biopile had a weight of approximately 750 kg with a water content of 15%. To ensure maximum homogeneity of the amendments in soil, a mobile concrete mixer was used to separately mix the soil for each biopile for 20 min. Each biopile was passively aerated with 5-cm diameter PVC pipes that were perforated with two rows of 1-cm diameter holes spaced at 10 cm. These pipes were placed horizontally at approximately mid-height of each biopile. The biopiles were covered with clear polyethylene to retain moisture and heat, with the aeration pipes protruding from the plastic covers. For all of the above steps in construction of biopiles, as well as for sampling (below), the uninoculated biopiles were manipulated before the inoculated ones in order to prevent transfer of the inoculum into the uninoculated controls.

The biopiles were sampled using a composite sampling method. Three composite soil samples were taken from each biopile at each time point. For each composite sample, eight samples of approximately 100 g each were taken from random locations inside the biopile. The eight samples were thoroughly mixed in a clean beaker, and a 125-ml sterile bottle was filled from the mixture. Sampling was difficult at the last time point (65 days) because the biopiles were completely frozen; therefore only one composite sample was collected from each biopile by taking soil from five different locations inside the pile.

Laboratory microcosms

The soil used in the microcosms was stored at –20 °C for 5 months before the microcosm experiment started. The soil was from the same batch used in the biopiles. The soil was thawed and sieved (2 mm). Microcosms of 80 g of moist soil were prepared in Teflon-septum-sealed 250-ml amber bottles to prevent loss of volatile hydrocarbons. Four treatments in triplicate were: (1) uninoculated control, (2) inoculum of 10^6 cells per g of dry soil, (3) inoculum of 10^9 cells per g of dry soil, and (4) killed uninoculated control with 3 g NaN_3/kg of moist soil. The inoculum was prepared as described above. The bottles were incubated for 92 days in darkness at 7 °C±2 °C. Every week, the soil was well mixed, and samples were removed. For TPH analysis, 3.0-g samples were immediately analyzed. For DNA extraction, 0.5-g soil samples were stored at –20 °C. DNA was extracted from each 0.5-g soil sample using a FastDNA Spin Kit (Bio 101, Quantum Technologies, Calif.).

Most-probable-number PCR assays

Three putatively abundant organisms (phylotypes) in the inoculum were monitored in the biopiles and microcosms using previously described phylotype-specific PCR assays (Thomassin-Lacroix 2001). The PCR assays targeted 16S rRNA genes (rDNA) and

used dilutions of the template to determine a most-probable number (MPN) of the target. The three phylotypes targeted were *Pseudomonas* sp. Ale-1.6, *Sphingomonas* sp. Ale-1.14, and *Rhodococcus* sp. Ale-1.46. Hydrocarbon-degrading *Pseudomonas* and *Rhodococcus* spp. corresponding to the Ale-1.6 and Ale-1.46 phylotypes, respectively, were isolated. Sequence analysis and empirical testing indicated that the assays are generally, but not completely, species-specific. Detection limits, per g of dry soil, were 70 copies of Ale-1.6 rDNA, 500 copies of Ale-1.14 rDNA and 700 copies of Ale-1.46 rDNA.

MPN growth assays

Total culturable heterotrophs and hydrocarbon degraders were enumerated using an MPN method modified from Wrenn and Venosa (1996). Cells were extracted from 1.0 g of soil by vortexing several times in 9.0 ml of saline buffer solution containing 0.80% NaCl plus 0.1% sodium pyrophosphate (pH 7.5). Cell extracts were then diluted. For enumeration of total heterotrophs, 3 g TSB (BBL)/l (10% of normal concentration) was used. For hydrocarbon degraders, Bushnell-Haas medium (Difco) with 5.0 g of filter sterilized (0.2-µm pore size) jet A-1 fuel/l was used. Cultures were prepared in microtiter plates with 180 µl of medium in each well. Each well was inoculated with 20 µl from an appropriate dilution of a cell extract. The microtiter plates were incubated at 7 °C for 29 days. Growth of total viable heterotrophs was determined by visual observation of turbidity. Jet fuel oxidation was detected by adding 50 µl of iodonitrotetrazolium violet (INT) (final conc. 0.6 g/l). In positive wells, INT was reduced to an insoluble formazan that deposits intracellularly as a red precipitate that was visually detected.

Monitoring potential dispersal of the inoculum

Soil samples were taken from the experimental site at Alert before the start of the biopile experiment and after a treatment period after 65 days, in order to determine if the inoculum spread to nearby locations. Samples (125 ml) of surface soil (top 10 cm) were taken along four transects, north, south, east, and west of the location of the biopiles, at distances of 25 m, 50 m, and 100 m (12 samples). All samples were screened with the three phylotype-specific PCR assays to see if any of the three strains could be detected and enumerated at these locations. Remote surface soil samples were taken from areas with no evidence of human disturbance at locations from 5 to 20 km southwest to southeast of Alert (10 samples).

Total petroleum hydrocarbons analysis

TPH were extracted from the soil samples using hexane, after drying the samples with anhydrous sodium sulfate, and quantified using a gas chromatograph-flame ionization detector (GC-FID), as described previously (Mohn and Stewart 2000). Soil water content (%) was measured by weighing known volumes of soil before and after drying overnight in a 110 °C oven.

Statistical analysis

An analysis of variance (ANOVA) was done for the field and the laboratory experiments to test significant differences in TPH concentrations within and between triplicate treatments.

Results

Fuel biodegradation in biopiles and microcosms

The results from the field experiment showed that bioremediation of diesel-fuel-contaminated soil is possible on-site at Alert in biopiles at low ambient temperatures (Fig. 1A). Bioaugmentation with an enrichment culture at a cell density of 10^8 cells per g of soil did not enhance the TPH removal rate or the total amount of TPH removed at the end of the treatment period (65 days). The TPH removal rate in the biopiles was approximately 90 µg TPH g^{-1} of dry soil per day^{-1} during the first 14 days when almost 50% of the TPH were removed. Final TPH concentrations were in the range of 0.50–0.70 mg per g of dry soil. ANOVA (α=0.05) confirmed that there were no significant differences between the TPH concentrations in the control biopiles vs the inoculated biopiles at any time. The ANOVA was also applied to test variability among triplicate treatments and indicated that there were significant differences in TPH concentration between uninoculated control treatments at all times. The average daily temperature for the site ranged from 10 °C to –14 °C during the experiment.

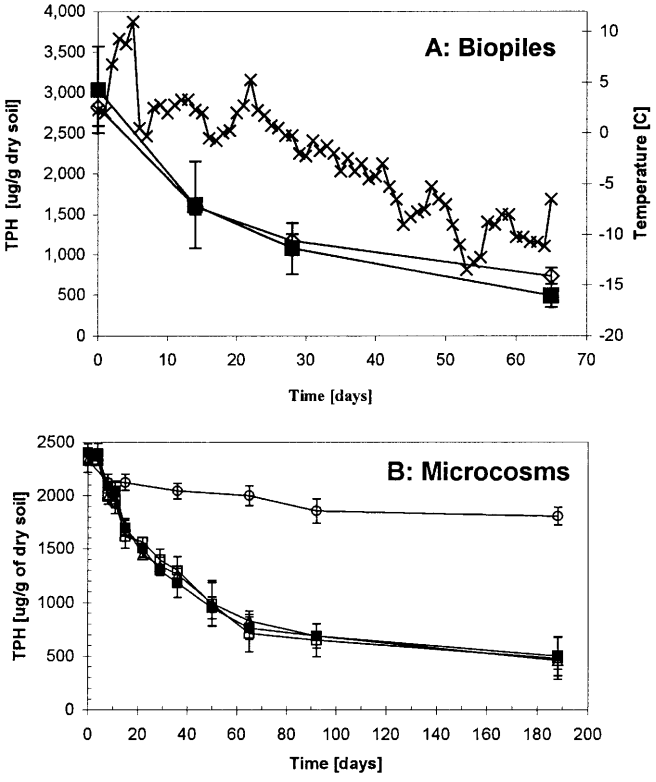

Fig. 1 Removal of total petroleum hydrocarbons (TPH) **A** from soil in biopiles at Alert and **B** from soil in laboratory microcosms at 7 °C. X Daily average outdoor air temperature, ■ uninoculated control, ❑ inoculated 10^6 cells/g of dry soil, ✧ inoculated 10^8 cells/g of dry soil, Δ inoculated 10^9 cells/g of dry soil, ❍ sterile control. *Error bars* Standard deviation (*n*=3)

The treatments in the field experiment were also tested in a laboratory microcosm experiment. The microcosms had two rather than one inoculum density and had more controlled conditions than the biopiles (e.g., more homogeneous and less potential for volatilization of TPH). The results from the microcosm experiment confirmed those of the field experiment (Fig. 1B). There was no significant effect (ANOVA, α=0.05) of inoculation, at either

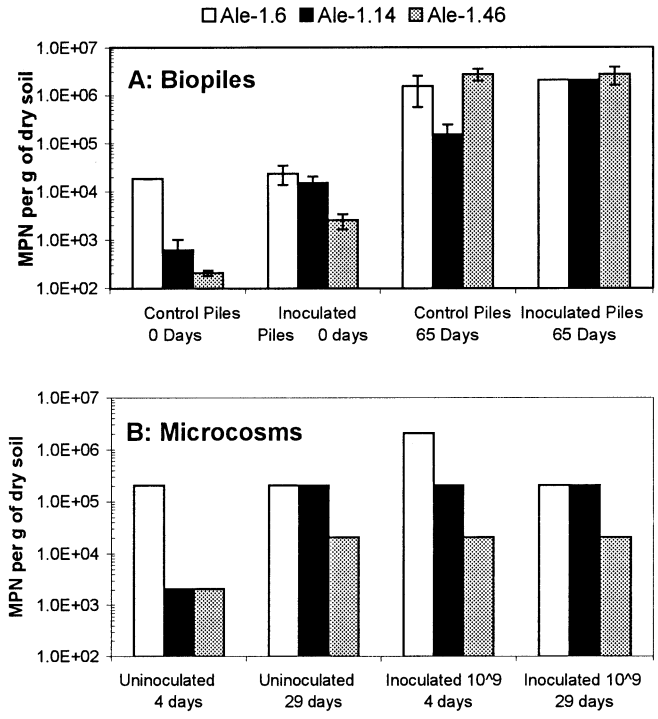

Fig. 2 Most probable numbers of three phylotypes **A** in biopiles at CFS Alert and **B** in laboratory microcosms. *Error bars* standard deviation (n=3)

cell density tested, on TPH removal. The maximum TPH removal rates in the microcosms were lower, but similar to those in the biopiles, at approximately 50 μg g^{-1} of dry soil day^{-1}. The initial TPH concentration in all three microcosm treatments was approximately 2.4 mg/g of dry soil, and the final TPH concentration after 92 days of treatment was approximately 0.65 mg/g of dry soil. There was only slight TPH disappearance in the sterile control, which may have been due to TPH volatilization during sampling periods, indicating that most TPH removal in the microcosms was due to biodegradation. A final set of samples analyzed after 190 days confirmed that degradation had essentially stopped, leaving a TPH concentration of 0.50 mg/g of dry soil. Virtually all hydrocarbons were removed. The six major peaks in the TPH chromatogram were aliphatic non-branched compounds, which were extensively removed after 92 days.

Phylotype populations monitored in biopiles

The three phylotypes monitored, *Sphingomonas* sp. Ale-1.14, *Rhodococcus* sp. Ale-1.46 and *Pseudomonas* sp. Ale-1.6, were all initially detectable in both the inoculated and uninoculated biopiles (Fig. 2A). Ale1.14 and Ale 1.46 were approximately ten times more abundant in the inoculated biopiles than in the uninoculated ones at the start of the experiment. The abundance of Ale 1.6 was not significantly different between the two treatments on day 0, so the inoculation of this phylotype could not be detected. During the treatment period, all three phylotypes increased significantly in both treatments. After 65 days, the abundances of the three phylotypes in each treatment were similar (all approximately 10^6 cells per g of dry soil). The only exception was Ale-1.14, which was ten times less abundant in the uninoculated biopiles than in the inoculated ones.

Phylotype populations monitored in microcosms

Near the start of the microcosm experiment (day 4), all three phylotypes monitored were more abundant in the microcosms inoculated with 10^9 cells per g of soil than in the uninoculated microcosms (Fig. 2B). All phylotypes were initially more abundant in inoculated microcosms than in the above inoculated biopiles (Fig. 2A), in accordance with the more dense inoculum used in the microcosms assayed. From day 4 to day 29, the populations of Ale-1.14 and Ale-1.46 increased in the uninoculated microcosms but were unchanged in the inoculated microcosms (Fig. 2B). The population of Ale 1.6 was unchanged in the uninoculated microcosms but decreased in the inoculated microcosms. The final populations of the three phylotypes were lower in the microcosms than in the above biopiles. However, this may reflect the fact that the final samples were taken from the microcosms on day 29 and from the biopiles on day 65.

Culturable heterotrophs and hydrocarbon degraders

Inoculation did not increase populations of culturable heterotrophs or hydrocarbon degraders in the microcosms. In all treatments, both culturable heterotrophs and hydrocarbon degraders increased from day 4 to day 29. After 4 days of incubation, the number of culturable heterotrophs was approximately 6.0×10^8 propagules per g of dry soil. After 29 days of incubation, the culturable heterotrophs increased in the uninoculated treatment by more than 10^4-fold, while, they increased in both the microcosms inoculated with 10^6 and 10^9 cells per g of dry soil by approximately 10-fold. Culturable hydrocarbon degraders increased by more than 100-fold in all treatments. Culturable hydrocarbon degraders, as a fraction of culturable heterotrophs, increased from approximately 0.22 to 9.0% in the microcosm inoculated with 10^6 cells per g of dry soil, and from approximately 0.13 to 22.9% in the microcosm inoculated with 10^9 cells per g of dry soil during the incubation period. The culturable hydrocarbon degraders, as a fraction of total culturable heterotrophs, did not increase in the control microcosm, because of the large increase in culturable heterotrophs.

Occurrence of phylotypes in the inoculum beyond the biopiles

In soil samples from the Alert region, the Ale-1.14 phylotype appeared to be nearly ubiquitous at densities

bordering on the detection limit, whereas the Ale-1.6 and Ale-1.46 phylotypes were consistently below detectable densities. None of the soil samples from outside of the biopiles had detectable levels of phylotypes Ale-1.6 (<70 rDNA copies per g of dry soil) or Ale-1.46 (<700 copies per g of soil). Only the Ale-1.14 phylotype was detected in any of the soil samples from outside of the biopiles, but it was always close to the detection limit (500–1,000 copies per g of soil). Prior to constructing the biopiles, Ale-1.14 was detected in six of 12 samples from within 100 m of the site for the biopiles. After the biopiles had been in place for 65 days, Ale-1.14 was again detected in six of 12 samples from within 100 m of the site for the biopiles. However, Ale-1.14 was not consistently detected or not detected in samples from the same locations. Ale-1.14 was detected in seven of ten samples from undisturbed, remote locations (5–20 km from the Alert station). These results provide no evidence that organisms from the inoculum spread from the biopiles to the surrounding soil.

Discussion

Degradation of hydrocarbons in soil at low temperature

Both the field experiment and the laboratory microcosm experiment indicated that bioremediation of Arctic soil contaminated with weathered diesel fuel is possible on-site at low ambient temperatures. It is noteworthy that TPH removal rates and the trends shown in the graphs (Fig. 1) were similar in the microcosm experiment and in the biopiles in the field experiment. This indicates that modeling of the removal and degradation of these substances on the basis of laboratory studies can predict on-site bioremediation kinetics. The slight difference in TPH removal between the field experiment and the microcosms was likely due to volatilization of hydrocarbons from the biopiles in the field. These results are consistent with a previous study (Whyte et al. 1999) that showed the potential of the microflora in soil from Alert to mineralize hexadecane at 5 °C. The previous study also showed that fertilization with N plus P stimulated that mineralization activity.

Effect of inoculation on hydrocarbon degradation

Inoculation with a high concentration of hydrocarbon degraders did not enhance either the rate or the final extent of TPH removal. This is consistent with general observations concerning inoculation of contaminated soils (reviewed by Alexander 1999). Inoculation has been shown to be efficient when the contaminants belong to a single type of recalcitrant compound. If few microorganisms exist that are capable of degrading compounds present in soil, inoculation may have the desired effects. However, petroleum hydrocarbons are a diverse mixture of straight and branched aliphatic hydrocarbons, often with additional aromatic compounds, and these compounds have been reported to be degraded by a diverse group of microorganisms (Atlas 1981). Such complex hydrocarbon mixtures presumably select for a diverse hydrocarbon-degrading community in contaminated soil. Often, high numbers of indigenous hydrocarbon degraders exist in contaminated soil, and in this case, a further increase in their number by inoculation is likely to be ineffective. However, as reported previously (Mohn and Stewart 2000; Mohn et al. 2001), hydrocarbon removal from diesel-contaminated Arctic tundra soils was stimulated by inoculation with a cold-adapted enrichment culture. Thus, the soils in the previous studies appeared to have microflora that limited hydrocarbon biodegradation when other factors such as nutrient availability and water were optimized.

Possible explanations for the lack of a stimulatory effect of the inoculum in this study include: (1) low survival of the inoculum in soil, (2) inability of the inoculum to degrade the particular hydrocarbons present in the contaminated soil, and (3) existence of some limiting factor for TPH removal other than the size of the hydrocarbon-degrading population. The first explanation seems unlikely since inoculation increased the populations of three members of the hydrocarbon-degrading consortium (Fig. 2). However, we cannot exclude the possibility that the PCR assays detected DNA from nonviable cells. Since the three populations increased to the same density in both inoculated and uninoculated biopiles, we cannot be sure that cells included in the inoculum were among those that grew. Further, inoculation of the microcosms did not measurably increase initial populations of culturable heterotrophs or hydrocarbon degraders. When tested in liquid laboratory cultures, the proportion of viable cells in the inoculum was greater than 10% (Thomassin-Lacroix 2000). The second explanation is very unlikely, because the extensive growth of the three phylotypes indicates that they could grow on abundant substrates (presumably hydrocarbons) in the soil. The third explanation seems most likely, since with and without inoculation, in biopiles and in microcosms, hydrocarbons were reduced at approximately the same maximum rate to approximately the same final level (Fig. 1). Thus, a common factor appeared to limit TPH removal. The biopile system was designed to optimize factors potentially limiting hydrocarbon biodegradation, such as availability of N, P, O_2 and water as well as conditions of pH and temperature. Surfactant was added to enhance bioavailablity of hydrocarbons to microorganisms; however, there is no direct evidence that the surfactant had this effect.

We suspect that mass transfer of hydrocarbons may have been an important factor limiting TPH biodegradation There is no direct evidence that hydrocarbon bioavailability was a limiting factor, but alternatives appear unlikely in all treatments. Hydrocarbon bioavailability was likely similar in all experimental treatments. The onset of freezing temperature (Fig. 1A) did not appear to stop TPH removal in the biopiles, as a similar level of

residual TPH remained in the microcosms, which were maintained at 7 °C. Furthermore, the remaining TPH included *n*-alkanes that were shown to be degradable (i.e., they were only partly removed). Thus, residual TPH included degradable hydrocarbons that appear to have been unavailable to microorganisms.

Occurrence and fate of organisms in the inoculum

Biostimulation in both biopiles and microcosms selected for some of the same organisms as did a laboratory enrichment culture. The three phylotypes monitored were selected because they appeared to be abundant in the inoculum enrichment culture, on the basis of PCR-amplified 16S rRNA gene fragments (Thomassin-Lacroix et al. 2001). Further, it was also possible to isolate strains representing two of the three phylotypes, Ale-1.6 and Ale-1.46, by conventional means using solid medium and jet fuel as a substrate. The facts that: (1) the three phylotypes were undetectable or barely detectable in uncontaminated soil from the study site, (2) the phylotypes were enriched in hydrocarbon-contaminated soil, and (3) the phylotype's populations increased in the enrichment culture, microcosms, and biopiles all strongly support the conclusion that the MPN-PCR assays detected the intended populations of hydrocarbon degraders. It is possible that one or more phylotypes include multiple strains (e.g., subspecies). The three phylotypes increased greatly in abundance in the soil, in both the biopiles and the microcosms (Fig. 2), whether or not the soil was inoculated with the enrichment culture. Consistent with this finding is that the enrichment culture and soil treatments were exposed to similar conditions, such as high nutrient levels, which would favor fast-growing hydrocarbon degraders. This finding demonstrates that organisms well adapted to laboratory conditions can also compete successfully in a soil environment, albeit, a soil environment disturbed by human activity.

There was no evidence that organisms from the inoculum spread to nearby soil. In uncontaminated soil, two phylotypes from the inoculum that were monitored, *Pseudomonas* sp. Ale-1.6 and *Rhodococcus* sp. Ale-1.46, were never detected. The third phylotype monitored, *Sphingomonas* sp. Ale-1.14, appeared to be ubiquitous in soil in the Alert region at densities near the detection level. There was no evidence that the abundance of any of the three phylotypes increased in soil near the biopiles during the course of the field experiment. In contrast to uncontaminated soils, hydrocarbon-contaminated soil at the site contained all three phylotypes at relatively high densities (Fig. 2A). Thus, these phylotypes appear to be common in soil near Alert at low densities and to be readily enriched in situ by diesel fuel pollution. Use of these phylotypes in an inoculum did not appear to increase their abundance in nearby uncontaminated soil. If these phylotypes are representative of the others in the enrichment culture, such inoculation with indigenous bacteria is unlikely to cause environmental harm by disrupting natural soil communities.

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