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Abstract

A kerosene type hydrocarbon fraction (equivalent to $7\,L\,m^{-2}$) was added to enclosures in the surface layer of high-arctic intertidal beach sediment. The experimental spill was repeated in two consecutive years in the period July-September. The rate and extent of hydrocarbon removal and the accompanying bacterial response were monitored for 79 days (2002) and 78 days (2003). The bulk of added kerosene, i.e. 94 to 98 percent, was lost from the upper 5 cm layer by putatively abiotic processes within 2 days and a residual fraction in the range 0.6 to 1.2 mg per g dry sediment was stably retained. Concomitant addition of oleophilic fertilizer led to higher initial retention, as 24% of the kerosene remained after 2 days in the presence of a modified, cold-climate adapted version of the well-known Inipol EAP 22 bioremediation agent. In these enclosures, which showed an increase in hydrocarbon-degrader counts from $6.5x10^3$ to $4.1x10^7$ per g dry sediment within 8 days, a 17% contribution by biodegradation to subsequent hydrocarbon removal was estimated. Stimulation in hydrocarbon-degrader counts in fertilizer-alone control enclosures was indistinguishable from the stimulation observed with both kerosene and fertilizer present, suggesting that the dynamics in numbers of hydrocarbon-degrading bacteria was primarily impacted by the bioremediation agent.

Keywords

1. Introduction

The risk of accidental oil spills in the polar regions increases with the extent of human activities. The potentially most devastating incidents are obviously related to production and transport of crude oil, but the hazards of contamination from fuel oils are also increased in connection with activities such as tourist cruises, fisheries and research.

In the year-round low temperatures of the Arctic and the Antarctica, evaporation of even relatively light hydrocarbon fractions is low (Fingas, 2004) , while viscosity is increased compared with temperate and warm regions (Jarsjö et al., 1997). Thus, the residence time of e.g. stranded spills must be expected to increase. This effect is further enhanced by the fact that the physicochemical processes of oil removal from the shorelines are largely discontinued for major parts of the year, when the beaches and adjacent waters are frozen and ice-covered.

Hydrocarbon-degrading bacteria have been shown to be ubiquitous members of the indigenous bacterial community in soil and aquatic environments, including permanently cold regions [reviews by Atlas (1981), Leahy and Colwell (1990), Margesin and Schinner (1999)]. Generally, these organisms constitute a small subset of the total community in pristine environments, but their relative numbers tend to increase by orders of magnitude in hydrocarbon-exposed areas. This response has been shown to be rapid, also at low temperatures (Delille et al., 1998; Margesin and Schinner, 1999). In natural soil and aquatic environments, the hydrocarbon-induced bacterial proliferation is generally restricted by too low inherent bioavailability of the macro-nutrients nitrogen and phosphorous. Growth stimulation by N and P supplementation is well documented – frequently accompanied by increases in hydrocarbon biodegradation rates (Atlas and Bartha, 1972; Gibbs, 1975; Braddock et al., 1997; Xia et al., 2006) .

Studies on the use of fertilizers to stimulate bioremediation in intertidal zone systems include laboratory simulations (Swannell et al., 1995; Santas and Santas, 2000; Bachoon et al., 2001; Oh et al., 2001; Röling et al., 2002; Xu and Obbard, 2003), small-scale and pilot-scale on-site experiments (Venosa et al., 1997; Oudot et al., 1998; Swannell et al., 2000; Choi et al., 2002; Delille et al., 2002; Prince et al., 2003; Pelletier et al., 2004) and evaluations of countermeasures after real spill events (Bragg et al., 1994; Swannell et al., 1999; Maki et al., 2003). The intertidal zone poses particular problems of maintaining sufficiently high levels of added macro-nutrients. Specialized slow-release inorganic or oleophilic organic fertilizers, as well as repeated addition of conventional agricultural fertilizers, have been employed as bioremediation strategies. As comprehensively reviewed (Swannell et al., 1996; Lee, 2000; Zhu et al., 2004), nutrient additions seem to accelerate immediate biodegradation rates, but the effect on the overall long-term extent of removal has been more contradictory. The physical nature of the beach substrate, aeration, emulsion formation by wave action (Santas and Santas, 2000) and environmental temperature are among the factors that have been reported to interact with nutrient availability.

A dedicated oil bioremediation agent, like the Inipol EAP 22, which was used extensively during e.g. the Exxon Valdez cleanup (Bragg et al., 1994), combine surfactant properties to counter formation of water-in-oil emulsions, organic N and P sources and a hydrophobic carbon source (oleic acid) to boost bacterial growth (Ladousse and Tramier, 1991). However, the impact of this extra C-source addition on the biodegradation of the oil contamination itself has been questioned. The bacteria, even if carrying hydrocarbon-degrading capacity, complete the consumption of the alternative C sources before they attack the hydrocarbons. This has been demonstrated in pure culture

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of *Marinobacter hydrocarbonoclasticus* (Rivet et al., 1993) and with natural assemblages of intertidal bacteria (Swannell et al., 1995).

The present study addresses the rate and extent of surface layer removal of a relatively light (kerosene type) hydrocarbon contamination contained on a high-arctic intertidal beach location. Particular emphasis is on the swiftness and extent of the growth response by the inherent bacterial community and its capacity to biodegrade the residual contamination with or without amendment with an oleophilic fertilizer / bioremediation agent.

2. Materials and methods

2.1. Field site and sediment characteristics

The field trials were carried out in the periods July-September of 2002 and 2003 at Kapp Wijk in the northern arms of the Isfjorden fjord system on Svalbard (N 78°36.5', E 015°10'). A flat moraine peninsula, with an inlet well protected from high seas, is situated approximately 45 km from the nearest settlement (Longyearbyen) and has generally experienced little impact by human activities, except for occasional boat traffic in the surrounding waters. The grain size distribution in the upper 5 cm of the intertidal beach sediment was determined by serial dry sieving and classification according to the Udden-Wentworth scheme (Table 1). Sediment density was 1.77 g cm^{-3} and water content at low tide was in the range 9.7-17.8 %, depending on the interval between water retraction and sampling.

2.2. Experimental setup

The experimental periods lasted from 9 July to 26 September in 2002 (79 days) and from 7 July to 23 September in 2003 (78 days). Twelve 30 cm (i.d.) x 100 cm PVC tubes were

placed vertically in the intertidal zone and covered and lidded with reflective Al foil to prevent from solar heating. They were dug 40 cm into the sediment and refilled to the same level as the surroundings. The tidal water percolated vertically through the enclosure sediment with just a few minutes delay relative to the surrounding sea surface oscillations. The tubes´ positioning relative to the sea level was such that the orifice was not overflowed at maximum spring tides, while the sediment surface was still submerged at neap tides (Fig. 1). The enclosures were separated by 2 m and organized into 3 groups of 4 treatments each. After being allowed to settle for 3 days, triplicate enclosures were subjected to the following treatments: (1) no additions (control); (2) addition of 400 g (corresponding to 570 mg cm^{-2}) of a 190-250°C bp kerosene (<0.3% aromatics) supplemented with heavier alkanes [3.5% hexadecane (2003 only), 1.7% heptadecane, 2.2% octadecane 1.1% nonadecane, 1.1% eicosane and 1.7% pristane (2,6,10,14-tetramethylpentadecane) by weight]. All hydrocarbons were purum grade Fluka products; (3) Addition of 50 ml of the oleophilic fertilizer/bioremediation agents Inipol EAP 22 (2002) or Inipol$^+$ (2003), produced by CECA SA (France); (4) concomitant addition of 400 g of the kerosene mixture and 50 ml Inipol (10 vol% of hydrocarbons). In the 2003 experiment, addition of 50 ml Inipol$^+$ was repeated after 43 days. To assure an even distribution in the enclosure surfaces, the additions were done at ebb tide, but while the sediments were still submerged. The upper 5 cm layer was assumed to maintain a stable oxic atmosphere due its coarseness combined with the twice-a-day replenishments by fresh air and seawater through the tidal oscillations.

2.3. Sampling

Temperature was measured in the surface sediment inside and immediately outside three randomly chosen enclosures at low tide every sampling date. Duplicate core sediment

samples were collected from each enclosure by pressing 50 ml sterile plastic tubes 5 cm into the surface layer. The samples were subsequently transported on ice to a laboratory facility within 4 hours. The material for microbiological analyses was processed immediately, while the remainder was frozen at -20ºC.

2.4. Hydrocarbon analyses

Two grams of thawed sediment were suspended in 5 ml dichloromethane with 500 µg 1-hexadecene added as an internal standard. The suspension was vortexed twice for 15 s with a 5 min interval. After 30 min rest, 3 ml of the clear supernatant were transferred to glass screw-cap tubes and stored at 5°C. Samples were injected splitlessly by an A200S autosampler (Fisons Instruments, Altrincham, UK) onto a CP-Sil-8 CB column (25 x 0.25 mm i.d., Chrompack International, The Netherlands) installed in a Fisons 8060 Gas Chromotograph connected to a Fisons VG Platform II mass spectrometer. The column temperature was maintained at 50°C for 3 min, then increased by 8°C min^{-1} to 130°C and 1°C min^{-1} to 140°C. Thereafter, the column temperature was ramped to 280°C (15°C min^{-1}) and held there for 5 min. The injector temperature was 230°C, the detector interface temperature 250°C, the ion source temperature 180ºC, and the ionizing voltage 70 eV. Qualitative analyses were performed by scanning from 30 to 400 atomic mass units at a scan rate of 0.9 s/scan. Alternatively, identical GC temperature conditions were programmed on an Agilent 6890N gas chromatograph (Agilent Technologies, Palo Alto, USA) equipped with a similar column (VF-5ms, 30 x 0.25 mm i.d., Varian, Palo Alto, USA) and a flame ionization detector. Individual alkanes were quantitated from MS data by the areas from the total ion current or from flame ionization detector areas.

2.5. Microbiological methods

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Cell suspensions in phosphate-buffered 2% NaCl (PBS) were obtained according to
Anderson et al. (2001) and 10-fold dilution series in PBS were used for viable cell
enumerations. Numbers of viable hydrocarbon degraders (DC) were estimated by a
modified most probable number (MPN) procedure (Brown and Braddock, 1990).
Aliquots of 20 µl were added into 7 separate dilution series in 96-microwell plates
(NUNC A/S, Denmark). The wells were pre-filled with 180 µl 1.5% NaCl-supplemented
Bushnell-Haas mineral medium (Difco) and 5 µl filter-sterilised kerosene mixture. After
incubation in the dark at 6^oC for 3 months (Delille and Delille 2000), each well was
inspected for dispersion of the kerosene ("Sheen Screen") and subsquently added 50 µl
filter-sterilized 3 mg ml^{-1} solution of the respiration indicator INT (2-(4-iodophenyl)-3(4-
nitrophenyl)-5-phenyltetrazolium chloride; Sigma-Aldrich) (Haines et al., 1996). After
over-night incubation in the dark at 6°C, red and pink wells were counted as positive for
growth. A maximum-likelihood estimation of microbial numbers based on 7-fold dilution
series was developed for the Microsoft Excel® for Windows spreadsheet program.
Additionally, DC were estimated from colony counts of the appropriate dilutions on Petri
dishes with solidified Bushnell-Haas medium after incubation at 6°C in a saturated
kerosene atmosphere (Dandie et al., 2001).

For heterotrophic-count estimates (HC), dilution series were plated on Petri
dishes with a half-strength Marine broth/peptone medium (10 g L^{-1} Marine broth 2216
(Difco), 5 g L^{-1} Bacto-peptone (Difco) 15 g L^{-1} NaCl, 15 g L^{-1} agar (Difco). Plates were
incubated for 3 weeks at 6°C.

Suspended cells for epifluorescent microscopy total counts (TC) were subjected
to short-term fixation in 4% paraformaldehyde with subsequent freeze storage in 50%
ethanol according to the procedure of Pernthaler et al. (2001). The fixated cells were
diluted 100-fold in ultrapure water, filtered through Whatman Anodisc 25 filters (0.2 µm

pore size) and stained with SYBR Gold® (Sigma-Aldrich, USA) according to Noble and

Fuhrman (1998), except that stained filters were dried overnight at 40°C prior to

mounting. A total of 500-1000 bacteria-sized cells per filter were counted in a fluorescent

microscope (Leitz DMRBE).

2.6. Nitrogen, phosphorous and carbon content

Sediment samples (25 g) were dried for 24 h at $40^{\circ}C$, passed through a 2 mm mesh sieve

and milled. Ten mg material was wrapped in tin cups and analysed for total C and N

using a Perkin Elmer 2400 CHN Element Analyzer (carrier gases Ar, O_2 and He).

Samples were heated to $640^{\circ}C$ and $926^{\circ}C$.

Inorganic and organic phosphate were extracted (Aspila et al., 1976) and quantified by

the colorimetric method of Murphy and Riley (1962).

3. Results

3.1. Temperature and nutrients

The surface sediment temperatures within the enclosures, as recorded at the sampling

dates (Fig. 2), showed the same basic developments both years, i.e. fluctuations in the

range 6 to 13°C in the period from start to mid-August, thereafter decreasing

temperatures until freezing conditions emerged towards the end of the experiments. The

reflective Al foil cover kept the insides of the enclosures slightly on the cold side of the

naturally exposed beach throughout the summer periods. The differences were in the

range 0.5 to 1 degree on cloudy days and 2 to 3 degrees in sunshine (data not shown).

The 7.4 weight% urea-N in the Inipol bioremediation agent (Swannell et al., 1996) added

5.2 mg cm^{-2} of nitrogen to the fertilized enclosures. This quantity closely equalled the

cumulative amount of total inherent N per cm^2 in the upper 5 cm of the sediment, as

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determined from the un-fertilized enclosures of the 2003 experiment (0.60 ± 0.07 mg per g dry sediment). However, no trend towards increased N levels in fertilized enclosures was detectable at first samplings 2-5 days after fertilizer addition or later throughout the experimental period.

The added 0.5 mg organic P cm^{-2} (0.7% by weight as tri(laureth-4)-phosphate in the Inipol agent; Swannell et al. (1996) was a negligible contribution to total P of this exceptionally phosphorite-rich (Krajewski, 1992) sediment (23 ± 3 mg per g dry sediment). It was equivalent to about one-third of the inherent organic P of the upper 5 cm layer (average 0.16 mg per g dry sediment). Consequently, no significant differences in total or organic phosphate content were observed between treatments.

3.2. Hydrocarbons reduction

The bulk of added hydrocarbons were lost from the surface layer within the first few days. This was most evident in enclosures without accompanying fertilizer. In the 2002 experiment, about 98% of the initial kerosene load of 570 mg per cm^2 was lost from the upper 5 cm of sediment after 2 days, while the corresponding figure for 2003 was 94%. Thereafter, the hydrocarbon reduction levelled out and residual concentrations stabilized at about 0.6 mg per g dry sediment (2002) and 1.2 mg per g (2003) (Fig. 3). With concomitant addition of the Inipol bioremediation agent, a higher fraction of the kerosene was retained in the upper layer. This was particularly evident in the 2003 experiment, when 24% of the initial load (i.e. 15.0 mg per g dry sediment) was retained at day 2. In 2002 the figure was 4.1%. The subsequent removal rates in the fertilized enclosures were, however, higher, resulting in a merging of the retention curves after about a month (2002) or at the time of the termination of the experiment (2003).

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As the present kerosene product, on the whole, contained no biodegradation-recalcitrant components, equal quantities of pristane and heptadecane were included to monitor their ratio change as indicator of biodegradation. None of the kerosene-supplemented enclosures showed reduced ratio in 2002, nor did the un-fertilized enclosures in 2003 (Fig. 4). Rather, the straight-chain alkane tended to be retained in the sediment to a higher degree than its highly branched counterpart. This development seemed caused by physiochemical differences, as comparison of the fate of n-alkanes of various chain lengths confirmed a strict dependence on molecular size (Fig. 5). The C_{17} and heavier n-alkanes disappeared more slowly than pristane, while the C_{16} and shorter molecules showed the opposite pattern. Thus the division point between molecules showing higher and lower retention than pristane did not coincide with heptadecane, but rather was situated in-between C_{16} and C_{17}.

For Inipol-supplemented kerosene enclosures in the 2003 experiment, the n-C_{17} / pristane ratio decreased throughout the experimental period. An abrupt drop by 20% within the first 5 days (which was also observed in the 2002 experiment) was followed by a steady decline to a final ratio of 0.51 (Fig. 4). By disregarding the immediate reduction, which was plausibly abiotic by nature, a biodegradative contribution to overall n-C_{17} loss from the upper layer could be estimated at 17% in the period of day 5 to the termination of the experiment at day 78. This is a minimum estimate, as some accompanying microbial consumption of the pristane may be anticipated.

3.3 Microbial enumerations

The confinement of beach sediment in experimental enclosures seemed, by itself, to have little influence on cell numbers. Total epifluorescent counts (TC), which were in the ranges 3.4 to 6.6 x 10^7 per g dry sediment in 2002 and 1.2 to 2.8 x 10^7 per g in 2003, as

well as viable heterotrophic (HC) and hydrocarbon-degrader counts (DC), all showed
pronounced stability in the control tubes throughout the experimental periods (Fig. 6).
Most-probable -number (MPN) estimates and colony-forming units on solid alkanes-
mineral salts media (data not shown) both gave estimates of DC in the range 0.01-0.03%
of total cells, while the HC figures, on the whole, were 10-fold higher than DC. Based on
colony morphologies and microscopic cell-size estimates, this hydrocarbon-degrading
viable flora was exclusively of bacterial origin.

Addition of the kerosene mixture led to a marked increase in DC in the surface 5
cm layer within the first 2 weeks. For the rest of the experimental periods, the alkane
degraders constituted a major part of viable cells, however still a minor fraction of the
total counts. At most samplings, the HC and DC figures were two orders of magnitude
below TC. The TC development further manifested the rather insignificant growth-
stimulatory effect of hydrocarbons alone, as no increase relative to the controls was
traceable.

In the first-year experiment, the lack of growth stimulation was evident even
when 10 vol% of the Inipol EAP 22 agent was added concomitantly with the kerosene.
However in 2003, the fertilizer-supplemented tubes showed a more notable development.
Both the HC and DC figures exceeded 10^7 per g dry sediment within a week, making
viable cells a substantial fraction of the total microbial load. The growth-stimulatory
effect was also reflected in a 3-fold increase in total counts. On the other hand, the
stimulation was distinctly transient. By the next viable -count sampling 5 weeks later, the
DC was down by two orders of magnitude. Following a second fertilizer addition, the
high load of viable cells recurred. A six-fold increase in TC, peaking at 1.3×10^8 per g
dry sediment within 6 days, seemed exclusively due to the growth of readily cultivable
variants – including a substantial fraction with capacity for alkanes catabolism.

The boost of alkane degraders in the presence of the Inipol agent in 2003 was evidently effected by components of this product. When added without accompanying hydrocarbons, the oleophilic fertilizer caused developments in microbial numbers – alkane degraders included – which basically were indistinguishable from the ones observed in the kerosene + fertilizer enclosures.

4. Discussion

The 94 to 98% loss of kerosene from the upper 5 cm layer within 2 days in unfertilized enclosures was evidently a result of physicochemical processes, i.e. a combination of leaching deeper into the sediment and evaporation. The laboratory studies of Jarsjö et al. (1994) indicate an inherent retention capacity of less than 10 mg per g dry sediment in this rather coarse beach sediment and a shift towards heavier alkanes in the residual fraction (data not shown) indicated a substantial loss also from evaporation. The residual fraction, seemingly recalcitrant to both evaporation and further leaching, was in the range 0.6-1.2 mg per g dry sediment.

Concomitant addition of the Inipol bioremediation agent enhanced retention of the kerosene in the surface layer. The higher hydrocarbon concentration persisted throughout the experimental period in the 2003 experiment, while the residual levels in fertilizer-amended and un-amended enclosures merged after about one month in 2002. This difference suggested an impact of the replacement of the well-established Inipol EAP 22 product in 2002 with Inipol[+] in 2003. Basically, the relative content of the co-surfactant 2-butoxyethanol had been increased relative to the other ingredients to obtain a formulation that worked more efficiently than EAP 22 at low environmental temperatures (F. Périé, personal communication).

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The study confirmed the presence of a significant indigenous population of hydrocarbon degraders, also in remote polar beach sediments supposedly little affected by human activities (Swannell et al., 1995; Delille and Delille, 2000). Addition of the kerosene mixture caused rapid and lasting 1-2 orders of magnitude increases in DC, making hydrocarbon degraders a predominant element of the cultivable bacteria. A highly similar response has previously been observed in sub-Antarctic intertidal sediments (Delille and Delille, 2000). The attained DC levels of 10^5-10^6 per g dry sediment were in the same range as reported for unfertilized plots of comparable field experiments (Delille and Delille, 2000; Delille et al., 2002), however generally lower than what has been observed in laboratory and microcosm simulations of intertidal-zone biodegradation (Swannell et al., 1995; Santas and Santas, 2000; Oh et al., 2001). Even after growth stimulation, hydrocarbon degraders constituted just 1-2% of total epifluorescent counts in the present study. There is ample evidence that substantial fractions of fluorescently labelled bacteria from natural marine environments are inactive or even dead (Lebaron et al., 2001; Luna et al., 2002). Consequently, the contribution by the hydrocarbon degraders to the actively metabolizing subset of microbes may have been substantially higher. On the other hand, the observation that total counts were basically unaffected by kerosene-alone addition both years, pointed to a modest impact on the overall biomass. Thus, the microbial growth seemed severely restricted by the availability of inorganic nutrients. Additionally, loose attachment of the hydrocarbon-degraders to the substrate may have resulted in repeated reductions in numbers by flushing out of the surface layer during tidal cycles. The low impact of the hydrocarbon-degrading flora was also manifested by the striking stability of the residual kerosene fraction after the initial, supposedly abiotic reduction. In 2002, this stability was

complete from two weeks on, while a minor reduction was observed during the last month of the 2003 experimental period.

Addition of Inipol EAP 22 did not lead to stimulation in hydrocarbon-degrader counts beyond that of kerosene alone in the 2002 experiment. This contrasts previous studies, where experimental units amended by the product typically have reached 1-2 orders of magnitude higher viable counts than un-fertilized controls (Swannell et al., 1995; Choi et al., 2002; Delille et al., 2002). Seemingly, insufficient quantities of the bioremediation agent resisted drainage out of the upper layer by the tidal flushings to make a difference with respect to microbial growth. Inipol EAP 22's susceptibility to leaching from irrigated sediments has been documented in other studies (Xu and Obbard, 2003). As experimental conditions – temperature regime included – were virtually identical the two years, the distinctly stronger growth stimulation by the Inipol$^+$ applied in 2003 seemed ascribable to the above-mentioned adjustment in the recipe of the product. Most likely, more of the easily accessible organic nutrients were retained in the surface layer.

Both years the growth stimulation in enclosures added just the fertilizer, was basically identical with what was observed in enclosures added Inipol and kerosene. The 2003 experiment, in particular, suggested that the numerical dynamics of the hydrocarbon-degrading community was distinctly more impacted by the bioremediation agent than by the hydrocarbons *per se*. One claimed effect of the Inipol products is to initiate an early start of bacterial growth, implicitly of strains with the capacity to catabolize hydrocarbons. This characteristic has primarily been attributed to the presence of about 25% oleic acid (Ladousse and Tramier, 1991). As mentioned, the benefit of this growth boost for subsequent biodegradation has been questioned (Rivet et al., 1993; Swannell et al., 1995; Pelletier et al., 2004). From the 2003 results of the present study,

even the ability of the Inipol-induced flora to sustain on the hydrocarbons when more readily degradable C sources are depleted seems limited. The rapid alkane-degrader proliferations observed after each Inipol[+] addition were equally transient both if the product was added as kerosene supplement or if it was added to the enclosures alone.

Decline over time in the quantitative ratios between the isoprenoid hydrocarbons pristane and phytane and the respective n-alkanes heptadecane and octadecane has a long record as indicators of biodgradation in oil (Blumer and Sass, 1972), and the general notion that reductions in these ratios are true reflections of biodegradation still prevails. However, there have also been repeated warnings that quantitative conclusions, at least should be drawn with caution. Both bacterial isolates and consortia have been shown to catabolize the highly branched isoprenoid chains, although mostly at lower rates than straight-chain alkanes (Jobson et al., 1972; Oudot et al., 1998; Garrett et al., 2003; Xu et al., 2003).

When the ratios between presumed biodegradable and recalcitrant molecules are used as indicator of biodegradation, the possibility of abiotically induced ratio change is implicitly disregarded. To our knowledge, the validity of this premise has not been thoroughly re-examined in available literature. From the present study, however, it seems questionable with respect to the heptadecane / pristane pair. Both years the ratio dropped by 20% of its original value within the first 5 days in Inipol-amended enclosures. These periods were too short in time and alkane-degrader loads were too modest to make biodegradation a plausible explanation. As corresponding developments were not observed in the kerosene-only enclosures, this immediate reduction seemed caused by interaction between the hydrocarbons and the bioremediation agent. Furthermore, the removal rates of the n-alkanes relative to pristane in the subsequent weeks were sensitive to carbon chain length in a manner that seemed incompatible with microbial degradation

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and the heptadecane and pristane retentions did not coincide. Rather, the removal of the straight-chain hydrocarbon proceeded somewhat slower.

In the light of the above-mentioned, putatively non-biotic influences on the C_{17}/ pristane ratio, the steady decline in the 2003 kerosene + Inipol enclosures throughout the experimental period suggested some contribution by biodegradation to the overall hydrocarbon removal. The stronger stimulation in alkane-degrader counts, with peak MPN figures exceeding 10^8 per g dry sediment, pointed in the same direction. The minimum estimate of 17% biodegradative contribution to the overall removal of residual hydrocarbons is, however, a modest figure compared with previous observations in intertidal sediments (Swannell et al., 1995; Wright et al., 1997; Delille et al., 2002).

Conclusions

The present study addressed the fate of a light fuel oil (kerosene) spill in high-arctic intertidal-zone sediment and the biodegradative response by the inherent microbial community. The major findings were as follows:

(1) In the upper 5 cm layer, a residual fraction in the range of 0.6-1.2 mg per g dry sediment seemed recalcitrant to abiotic removal processes throughout experimental periods of nearly 80 days.

(2) Concomitant addition of an oleophilic fertilizer / bioremediation agent slowed the immediate abiotic removal, leaving substantially higher concentrations of the kerosene in the upper layer for weeks or months.

(3) In fertilizer-amended experimental enclosures, the dynamics in numbers of hydrocarbon-degrading cells seemed primarily impacted by the fertilizer; the

dynamics in control enclosures with fertilizer only was indistinguishable from what was observed with both kerosene and fertilizer added.

(4) Use of the ratio between heptadecane and pristane as biodegradation indicator was obscured by evidently physicochemical differences in removal rates. However, it may be concluded that biodegradation was negligible in un-fertilized experimental enclosures. Fertilization gave mixed results, as the established commercial variant of the employed bioremediation agent caused no observable biodegradation, while a minimum 17% contribution by microorganisms to overall hydrocarbon removal was estimated with a modified, cold-climate adapted variant of the product.

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Figure legends

Figure 1 Placement of the tube enclosures in the intertidal zone.

Figure 2 Sediment surface temperatures within enclosures in the experimental periods 9 July to 26 September (79 days) in 2002 (?) and 7 July to 23 September (78 days) in 2003 (?).

Figure 3 Total residual extractable hydrocarbons in the enclosures throughout the experimental periods 9 July to 26 September in 2002 (A) and 7 July to 23 September in 2003 (B); (?), kerosene mixture added alone, (¦), concomitant addition of Inipol, with re-addition at day 43 in 2003 (arrow). Error bars represent standard deviation between triplicate enclosures.

Figure 4 Development in the heptadecane / pristane ratios throughout the experimental periods 9 July to 26 September in 2002 (A) and 7 July to 23 September in 2003 (B). The values in the original kerosene mixture (day 0) are scaled to 1.0; (?), kerosene-only enclosures; (¦), kerosene + Inipol enclosures. Error bars represent standard deviations between triplicate enclosures.

Figure 5 Ratios between individual n-alkanes and pristane in kerosene-only enclosures of the 2003 experiment. The values in the original kerosene mixture were scaled to 1.0; white column, 10 days; light grey column, 24 days; dark grey column, 43 days. Error bars represent standard deviations between triplicate enclosures.

Figure 6 Microbial enumerations by epifluorescent microscopy counts (?), viable heterotrophic plate counts (|) and viable alkane-degrader MPN counts (?). Figures A-D give the 2002 data (9 July to 12 September) and figures E-H the 2003 data (7 July to 23 September); A and E, control enclosures (no additions); B and F, enclosures added kerosene alone; C and G, control enclosures (Inipol alone); D and H, enclosures added kerosene + Inipol. Error bars represent standard deviations between triplicate enclosures; if non-detectable, the error bars are shielded by the point markers. Arrows indicate Inipol re-addition.
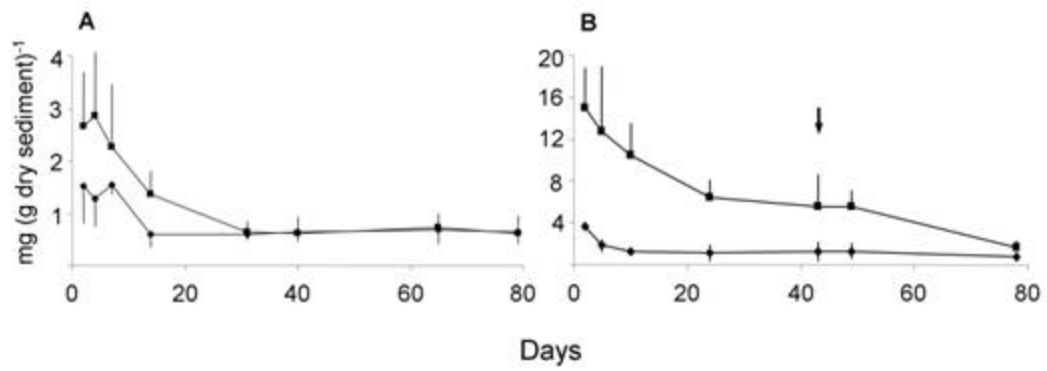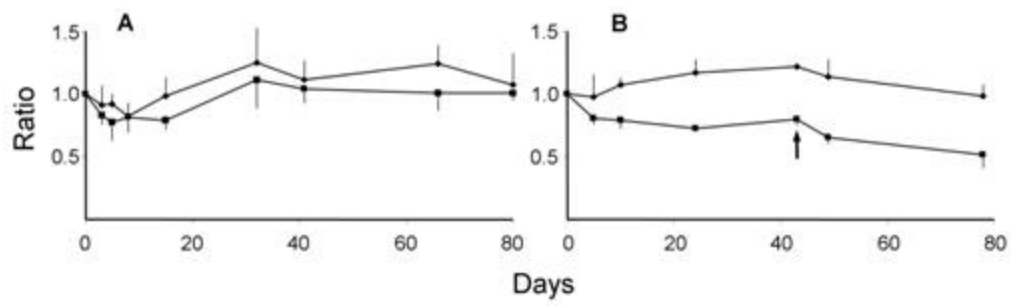
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Fig.1
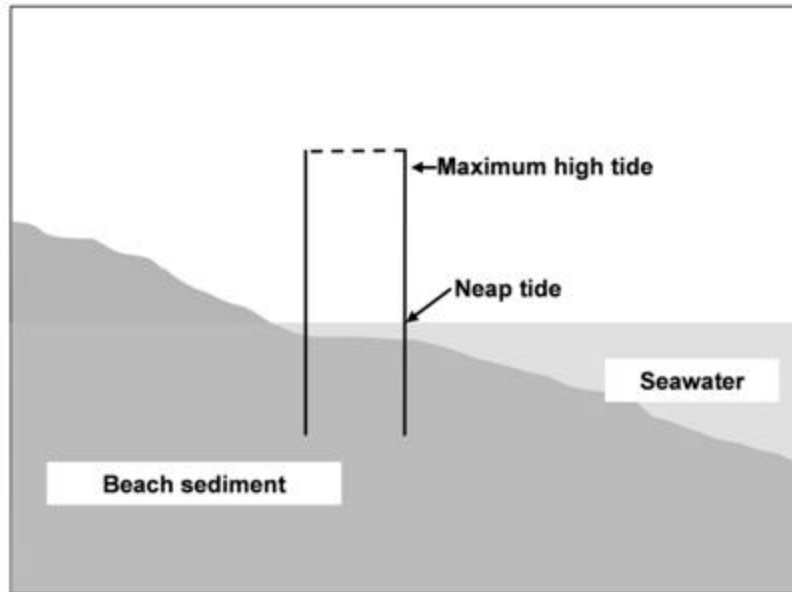
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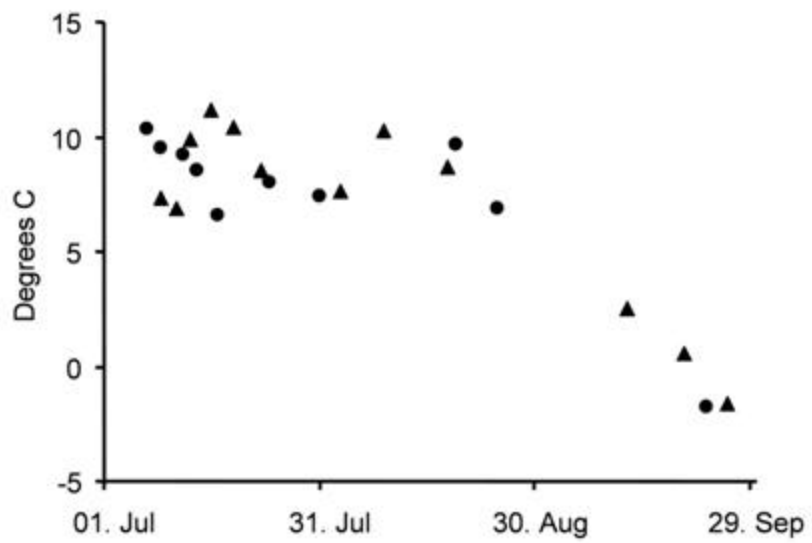
Fig.3

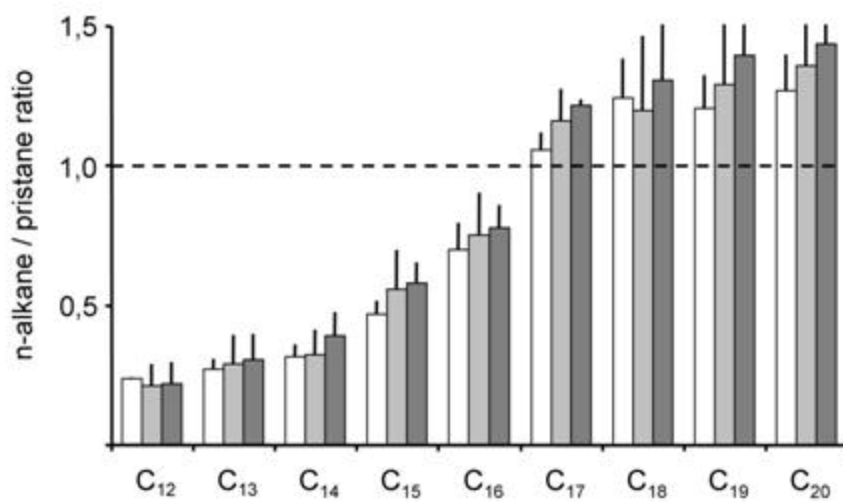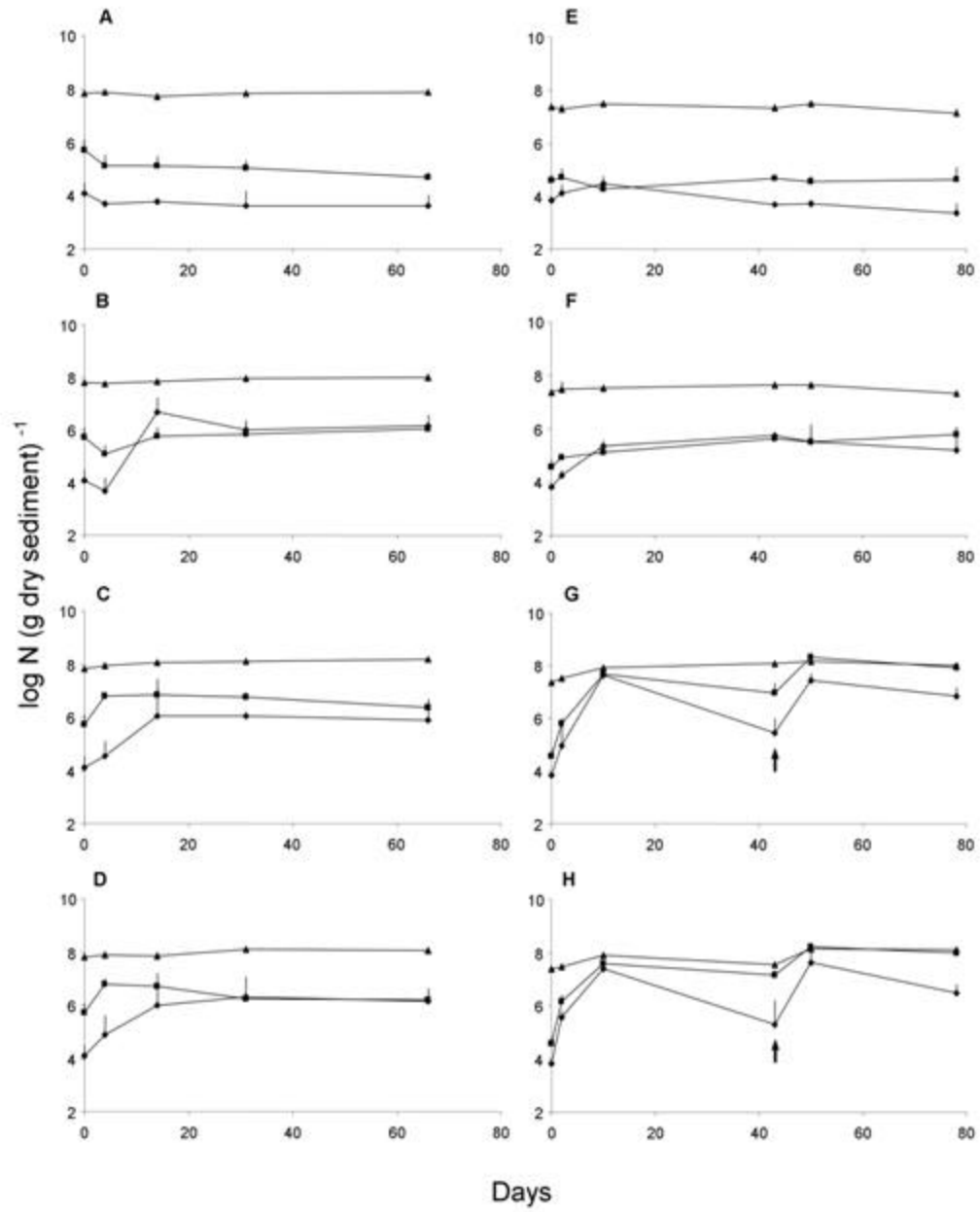Fig.4

Fig.5

Fig.6

Table 1. Sediment grain size distribution and classification (according to the Wentworth scale) of three individual samples distributed throughout the experimental field.

Classification	Particle size (mm)	% of dry sediment (S.D)
Medium gravel	8 - 16	6.3 (4.9)
Fine gravel	4 - 8	31.3 (6.0)
Very fine gravel	2 - 4	17.9 (2.6)
Very coarse sand	1 - 2	11.9 (3.0)
Coarse sand	0.5 – 1.0	14.8 (1.1)
Medium sand	0.25 - 0.50	13.7 (4.0)
Very fine sand	0.063 - 0.125	0.3 (0.0)