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ORIGINAL PAPER

Bioremediation of hydrocarbon-contaminated soil from Carlini Station, Antarctica: effectiveness of different nutrient sources as biostimulation agents

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Abstract

Logistics and scientific activities carried out in Antarctic stations entail the risk of contamination by fuels. Among remediation strategies, biostimulation significantly improves the efficiency of hydrocarbons (HCs) removal. A 1-year-long field trial was performed in mesocosms filled with soil chronically contaminated with HCs. Three nutrient sources were evaluated as biostimulation agents: inorganic salts (with and without aeration by mixing), a slow-release granular fertilizer (Nitrofoska®) and a commercial bioremediation product (OSEII®). Their performance was assessed considering the number of culturable bacteria, the changes induced in the structure of bacterial communities, the HCs removal efficiencies and the estimation of the abiotic and biodegradative losses of HCs. The soil indigenous microbiota reduced the concentration of hydrocarbons by up to 50% in 50 days and 87% in 365 days depending on the biostimulation agent used. OSEII® (a mixture of surfactants, nutrients, and enzymes) performed better in the medium term, promoting bacterial growth and rapidly inducing changes in the structure of bacterial community, and Nitrofoska® proved to be more efficient for long-term processes, less affecting the size and structure of the microbiota. A mixed strategy combining the fastest action of commercial products acting during summer with slow-release fertilizers acting throughout the year is proposed as a long-term bioremediation treatment for Antarctic areas where the temperature rises above the freezing point and the ground is free of snow shortly during summer. This study highlights the importance of conducting research to develop remediation processes compatible with the Antarctic Treaty, exploiting the metabolic potential of the indigenous microbiota.

Keywords Antarctica · Bioremediation · DGGE · Bacterial communities · Pollution · Hydrocarbons

Introduction

Due to its low cost, effectiveness, and versatility compared to other available options, fuels derived from crude oil are, still today, the most used energy source. These fuels represent an important fraction of the international market (Zhong et al. 2016) and their use at global scale increases the risk of spills or results in severe environmental catastrophes, such the one affecting 2000 km of Brazilian coast in 2019 (Wikipedia contributors 2019). The incidence of hydrocarbons in natural environments denotes an environmental problem throughout the world, including regions such as the Arctic (Kachinskii et al. 2014; Bennett et al. 2015) and Antarctica (Delille and Pelletier 2002; Szopińska et al. 2016; Vázquez et al. 2017; Sutilli et al. 2019), where hydrocarbons are contaminating soils, sediments, and water, mostly around human settlements (de Jesus et al. 2015). Most of the nearly 50 Antarctic stations using fuels derived from crude oil to generate

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energy and heat are settled on the few ice-free regions (less than 0.3% of the continent's total area), where most of the terrestrial biota is also established (Aislabie et al. 2004). To protect these sensitive ecosystems, regulations related to the preservation of the environment were established in the Antarctic Treaty Protocol, signed in 1991 (https://www.ats.aq/e/ats.htm). For this reason, the development of site-specific methods to clean Antarctic soils contaminated with hydrocarbons is required. These methods should be environmentally friendly, simple, inexpensive, and effective at low temperatures. In addition, to meet environmental policies suggested by the Antarctic Treaty and its Protocol on Environmental Protection, they must involve exclusively the use of autochthonous organisms.

Natural attenuation involves physical, chemical, and biological processes and engages autochthonous microorganisms acting without human intervention (EPA 1999). Even though this strategy is considered an effective procedure for restoration of some soils and groundwaters (Mulligan and Yong 2004), in extremely cold areas, the low temperatures, low evaporation rates, long-chain hydrocarbons viscosity, and lower water solubility of the contaminants turn natural attenuation into a poorly efficient process for removal of hydrocarbons from soils (Camenzuli and Freidman 2015). Moreover, in Antarctica, most human settlements are located in the coastal areas of the West Antarctic Peninsula (WAP), where soil temperatures reach values above the freezing point only for about 3 months and the soils remain frozen and covered with ice and snow for the other 9 months of the year. These conditions limit natural attenuation, making it an ineffective strategy to reduce soil contaminants to acceptable levels (Delille and Pelletier 2002; Vázquez et al. 2017; Chaudhary and Kim 2019). For this reason, hydrocarbon removal from WAP soils mostly requires designing and application of active bioremediation strategies (McWatters et al. 2016).

In situ (or, alternatively, on site) bioremediation, involving the activity of a wide array of autochthonous microorganisms, is considered the best approach to reach the maximum removal of hydrocarbons from soil (Goswami et al. 2018). Our previous studies proved that biostimulation with N and P significantly improves removal of aliphatic hydrocarbons from contaminated Antarctic soils (Ruberto et al. 2009; Dias et al. 2015; Martínez Álvarez et al. 2017). On the contrary, bioaugmentation does not seem to provide additional advantages when the soil has long-term exposure to fuels and the indigenous microbiota is well adapted to their presence in the environment (Vázquez et al. 2009; Ruberto et al. 2010). Similar results obtained with different soils and under different climates were reported elsewhere (Zawierucha and Malina 2011; Abed et al. 2014; Wu et al. 2016). The type and quantity of N and P sources are crucial for the success of biostimulation, and several studies have focused on optimizing the amount of nutrients to add to balance the high C level determined by the presence of hydrocarbons. In this sense, low hydrocarbon biodegradation rates have been attributed to deficient amounts of nutrients added as fertilizers (Huesemann et al. 2004), whereas an excess of nutrients could impair hydrocarbon degradation (Ruberto et al. 2003; Liu et al. 2011). Therefore, keeping a right balance of nutrients throughout a bioremediation process is essential for a successful treatment. However, the optimal C:N:P ratio reported for hydrocarbon degradation in soils is widely variable (Lee et al. 2007), so that optimization of nutrient levels represents a site-specific problem. A study by Emami et al. (2014) evidenced that the nature of the fertilizer also plays an important role in the biodegradation of hydrocarbons. Accordingly, many different N and P sources have been tested for bioremediation. Inorganic salts (i.e., ammonia, nitrate, and phosphate) were reported as simple and efficient nutrient sources to sustain hydrocarbon biodegradation (Liu et al. 2011; Martínez Álvarez et al. 2017). However, due to their high solubility in water, they quickly wash off by run-off, making it difficult to maintain adequate concentrations to support microbial growth and minimize the risk of toxicity shortly after the addition. Therefore, alternative sources of nutrients with a better cost/benefit ratio have been tested. Among them, we can mention commercial bioremediation agents, urea, fish meal, slow-release inorganic fertilizers, and various biowastes like soy cake, poultry droppings, oil palm empty fruit bunch and sugarcane bagasse (Adams et al. 2015). Selection of the best source of nutrients seems to depend on the characteristics of the soil under treatment, because the microbiota thriving in each soil is different and can exhibit dissimilar metabolic requirements. Therefore, getting information about the structure and dynamics of microbial communities throughout bioremediation helps to evaluate the performance of the applied strategy. To this purpose, Denaturing Gradient Gel Electrophoresis (DGGE) is a simple and inexpensive tool to process many samples and allows detecting qualitative changes in bacterial populations during a bioremediation process. Concerning oxygen availability, the degradation of hydrocarbons in soils under aerobic conditions proved to be effective, and frequently leads to a high-rate removal. However, in certain remote locations like the Antarctic stations, aeration of huge amounts of soil is expensive and requires a complicated logistics. In addition, precipitation and melting of the snow cover can cause soil saturation that limits oxygen diffusion, resulting in anaerobic conditions (Ruan and Robertson 2017), and even if the soil is aerated, transient anaerobic pockets are formed in the soil matrix due to physical, chemical, and biological factors. Under these situations, microbes able to utilize alternative electron acceptors like nitrate, sulfate, or fumarate could help remediation by undergoing anaerobic degradation of hydrocarbons at contaminated sites (Sampaio et al. 2017).

During in situ remediation studies, hydrocarbons that evaporate and/or migrate with underground runoff are difficult to measure and frequently ignored (Snape et al. 2008), mainly when it comes to field trials, where many conditions cannot be fully controlled. Considering the design of bioremediation strategies to be applied on site in cold regions, it is crucial to distinguish between abiotic and biodegradation losses, which can be inferred from chemical indices sensitive to evaporation or biodegradation. In this regard, Snape et al. (2005) stated that ratios between compounds with different volatilities but equally resistant to biodegradation are adequate to detect hydrocarbon losses due to evaporation, whereas ratios between compounds with similar volatilities but that are degraded differently are more suitable for detecting biodegradation. Also, as resolved compounds are considered to be easily biodegraded than the unresolved complex mixture (UCM), an increase in the relative amount of UCM over time is another potential indicator of biodegradation.

Beyond biological efficiency, financial and logistic issues must be considered when a bioremediation process is planned. This becomes highly relevant when the contaminated soil to be treated is in Antarctica, where isolation and adverse weather conditions impose additional limitations. For this reason, deciding on the optimal source of N, a key ingredient for bioremediation, is particularly relevant for extreme environments. This crucial choice implies an adequate trade-off between effectiveness, cost, environmental adequacy, and adaptation to the conditions inherent to the soil to treat and the only way to find this balance is through experimentation.

Considering all the above stated, the aim of this study was to evaluate the performance of different biostimulation agents and the practice of aeration by mixing on the removal of diesel hydrocarbons from Antarctic soils using mesocosms as experimental systems. Abiotic and degradative hydrocarbon losses have been inferred from gas chromatography profiles of the residual hydrocarbons, and heterotrophic and hydrocarbon-degrading viable bacterial counts and DGGE fingerprints were used to infer bacterial community dynamics throughout a field bioremediation experiment.

Materials and methods

Study area and experimental design

The experiment was carried out at Carlini Station (62° 14′ S, 58° 40′ W), located on Potter Peninsula, Isla 25 de Mayo (King George Island), South Shetland Islands, Antarctica (Fig. 1a). The soil was collected from a chronically contaminated area near the diesel fuel (Antarctic gasoil: AGO) storage tanks. After collection, large stones (> 1 cm diameter) were manually removed and the soil was sieved (10-mm mesh), vigorously mixed to homogeneity and finally disposed in plastic containers simulating small biopiles with 15 kg of soil each (Fig. 1b). Total petroleum hydrocarbons (TPH) in the soil, measured as the average of TPH content in all mesocosms ($n = 15$) at the start of the experiment, was 7620 ± 680 mg per kg of dry weight soil (mg kg^{-1} dw), representing a highly contaminated soil (Cury et al. 2015; Sampaio et al. 2017).

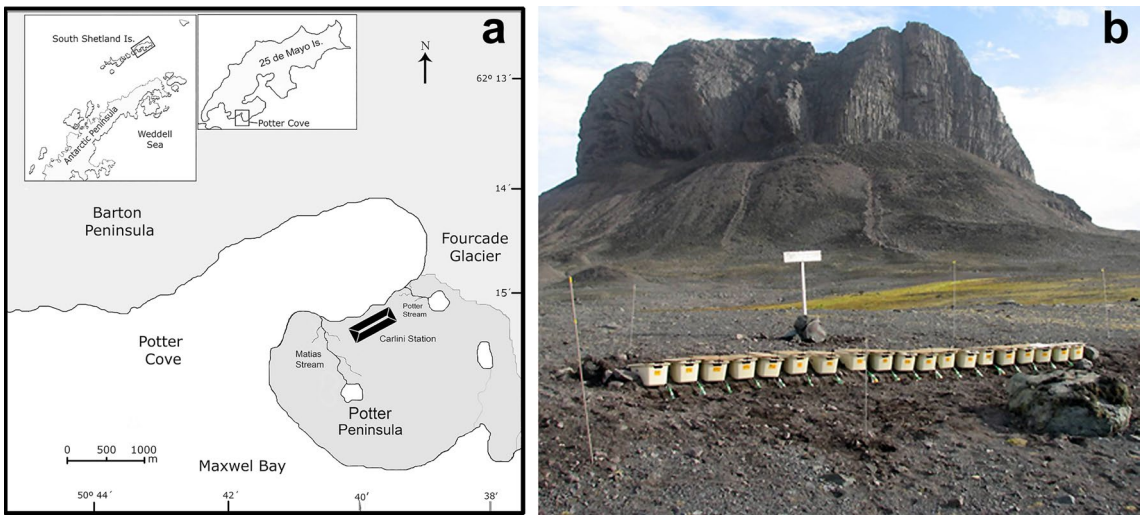

Fig. 1 **a** Geographic location of Potter Cove and Potter Peninsula showing Carlini Station (black rectangle), where a field bioremediation experiment was carried out. **b** Mesocosms containing hydrocarbon-contaminated Antarctic soil plus nutrient amendments were kept outdoors in an area with no traffic and exposed to environmental conditions

Biostimulation treatments using different nutrient sources were performed in triplicate (Fig. 1b, Online Resource 1, 2). Based on the total organic C estimated from the TPH content, necessary amounts of nitrogen (N) and phosphorous (P) were calculated to reach a C:N:P ratio of 100:10:1. To reach this nutrient's ratio, two experimental systems were biostimulated with inorganic salts (NH_4NO_3 0.17% w w^{-1}, Na_2HPO_4 0.027% w w^{-1}, final concentrations) added as sterile water solutions (autoclaved at 121 °C for 15 min). Additional amounts of salts solutions (corresponding to 20% of that added at the time of preparation of the mesocosms) were added after each sampling to compensate for washing out. To assess the effect of periodic soil mixing (a practice performed by manually turning the soil to accomplish aeration in small biopiles) on the extent of hydrocarbons degradation, IN (inorganic salts, intended to be aerobic) was weekly mixed to aerate the soil, whereas ANA (inorganic salts, intended to be anaerobic) was not mixed at all, promoting an anoxic environment. NPK mesocosms were supplied with the commercial slow-release granular fertilizer Nitrofoska® (0.5% w w^{-1}, final concentration) containing nitrogen (N), phosphorous (P), and potassium (K), to favor a more constant availability of nutrients. CP mesocosms were supplemented with the multi-enzymatic bioremediation agent OSEII® (Oil Spill Eater International Corp.), a commercial product listed in the US Environmental Protection Agency's National Contingency Plan for Oil Spills and reported as a biological enzyme additive containing nutrients, surfactants, and vitamins to promote microbial growth. According to the manufacturer's instructions, 15 mL kg^{-1} dw of OSEII® were added. Two control mesocosms were also included: AC, an abiotic control, poisoned with 0.3% w w^{-1} $HgCl_2$, to evaluate non-biological loss of hydrocarbons, and CC, a non-biostimulated control to evaluate the activity of the indigenous microbial community. All mesocosms except ANA were aerated weekly by mixing. All experimental units were kept outdoors for 1 year, under natural environmental conditions but covered with a plastic lid as protection against wind, rain, and snowfall, preventing excessive washing of nutrients and hydrocarbons. Sampling was performed during the austral summer, at days 0 (T0), 10 (T1), 20 (T2), 30 (T3), 40 (T4), 50 (T5) and a final sample was taken at day 365 (T6), 1 year later. Because sudden adverse weather conditions prevented sampling immediately after placing the mesocosms in the field, the initial sample was taken about 12 h after the addition of the nutrients, considered the start of the experiment (day-0). Samples were composed of three core subsamples randomly taken from each mesocosm with 1.5-cm-diameter PVC tubes (Online Resource 3) and were frozen at − 20 °C until processed, except for bacterial counts and pH measurements which were carried out immediately after sampling in the laboratory at Carlini Station.

Soil pH and bacterial counts

pH was measured with a pH meter in the supernatant of a soil suspension (1 g of soil in 10 mL of 0.1% NaCl solution) after settlement of soil particles. Culturable heterotrophic aerobic bacteria (HAB) and hydrocarbon-degrading bacteria (HDB) counts were performed according to Martínez Alvarez et al. (2017). Plates were incubated at 10 °C for 1 week (HAB) or 3 weeks (HDB), to avoid underestimation in counts as some bacteria may grow at slower rates when hydrocarbons are the sole carbon and energy source. Results were expressed as colony-forming units per gram of dry weight soil (CFU g^{-1} dw) and the significance of the differences between mean values from the composite samples taken from the three replicate mesocosms representing each treatment was calculated using one-way ANOVA and post-hoc Tukey's test. A value of $p < 0.05$ was considered statistically significant.

Analysis of hydrocarbons

The qualitative patterns of hydrocarbons were detected by gas chromatography using a flame-ionization detector (GC-FID) using dichloromethane (HPLC grade, Sintorgan) as solvent, and the quantification by infrared spectrophotometry (IR) was performed as described in Martínez Álvarez et al. (2017). To improve precision in quantification by IR, three subsamples (5 g) from each composite sample were extracted and measured independently, and TPH content in the corresponding mesocosm was expressed in mg AGO kg^{-1} dw, as the average of the triplicate subsamples. Hydrocarbon's removal efficiencies were calculated from the differences in TPH content at day-50 or day-365 and the initial time day-0 and the values corresponding to triplicate mesocosms were averaged to obtain the removal efficiencies of each treatment or the control. The significance of the differences between treatments was calculated using one-way ANOVA and post-hoc Dunnett test. A value of $p < 0.05$ was considered statistically significant.

Fingerprinting of bacterial communities

For bacterial communities fingerprinting, total DNA was extracted from 0.5 to 1 g of well-homogenized soil using the PowerLyzer Power Soil DNA Isolation Kit™ (Mobio), following the manufacturer's instructions with some modifications: after addition of Bead Solution, 20-µL lysozyme (300 mg mL^{-1}) was added and the tubes were incubated for 30 min at 37 °C. After that, samples were subjected to three cycles of freezing (− 80 °C) and thawing (65 °C). Fragments

of the V3 hypervariable region of the bacterial 16S rRNA gene were amplified using the GC-341F and 534R primers (Muyzer et al. 1993). PCR reactions contained 40 ng of total DNA, 0.5 µmol L^{-1} of each primer, 0.2 mmol L^{-1} of each deoxyribonucleotide triphosphate, 0.25 mg mL^{-1} of bovine serum albumin, 1.5 U GoTaq™ (Promega), and 1× GoTaq™ buffer, in a final volume of 50 µL. PCR amplification consisted of an initial denaturation step for 5 min at 94 °C; 35 cycles consisting of 94 °C for 45 s; 55 °C for 45 s; 72 °C for 45 s, followed by a final extension at 72 °C for 5 min. The quality and quantity of extracted DNA and PCR products were analyzed by agarose gels electrophoresis (1.5% w v^{-1}) stained with GelRed™ (Biotium, Inc.).

Denaturing gradient gel electrophoresis

Differences in the structure of the bacterial communities present in all samples were inferred by Denaturing Gradient Gel Electrophoresis (DGGE) fingerprinting. Three independent PCR amplifications were done from each sample, which were then pooled, concentrated to 35 µL using a Vacuum Concentrator (Hetovac VR-1), and loaded (500 µg DNA) into gels containing 8% (w v^{-1}) polyacrylamide (acrylamide: *N*,*N*′-methylene bisacrylamide, 37.5:1) in 1× TAE buffer, with a denaturing gradient ranging from 45 to 60% (with 100% denaturant containing 7 M urea and 40% v v^{-1} formamide). DGGE was performed in a TV400-DGGE system (Scie-Plas Ltd.). Gels were run at 65 °C for 16 h at 60 V, stained for 1 h with SyberGold™ (Invitrogen) in 1X TAE buffer and documented under UV light. DGGE profiles were compared based on the presence or absence of bands using the Jaccard similarity coefficient and dendrograms were constructed according to Unweighted Pair Group Method with Arithmetic mean (UPGMA) using the Gel Compar II™ software package (Applied Maths).

Results

pH and bacterial counts

Soil pH was neutral to slightly alkaline, ranging from 7 to 8. Only in the CC1 mesocosm did the pH drop to 5.2 after 1 year. In the CP mesocosms, the pH decreased to 6.6–6.7 after the addition of OSEII® but rose back to neutrality by day-10 (Online Resource 4).

Biological activity was inferred from the changes observed in the cultivable fractions of HAB and HDB (Fig. 2). No colonies grew from the AC mesocosms, confirming an efficient poisoning of the soil microbiota by the $HgCl_2$. As a general trend, average ($n = 3$) HAB counts in all mesocosms increased in one (CC) to two (biostimulation treatments) orders of magnitude from the start (day-0) to the end (day-365) of the experiment (Fig. 2a). In the non-fertilized CC mesocosms HAB counts raised from 2.11×10^6 CFU g^{-1} dw at day-0 to 4.63×10^7 CFU g^{-1} dw at day-30, evidencing the positive effect of mixing and aeration on bacterial growth. However, counts decreased after day-30, making it the system with the lowest HAB counts at day-50 (2.06×10^7 CFU g^{-1} dw) and day-365 (2.99×10^7 CFU g^{-1} dw). CP mesocosms had an early increase in HAB counts (from 2.69×10^6 CFU g^{-1} dw at day-0 to 2.72×10^7 CFU g^{-1} dw at day-10), reaching values that remained higher than those recorded for any other treatment until the end of the experiment (from 1.02×10^8 CFU g^{-1} dw at day-30 to 2.65×10^8 CFU g^{-1} dw at day-365). On the other hand, HAB evolved differently in NPK mesocosms, with no

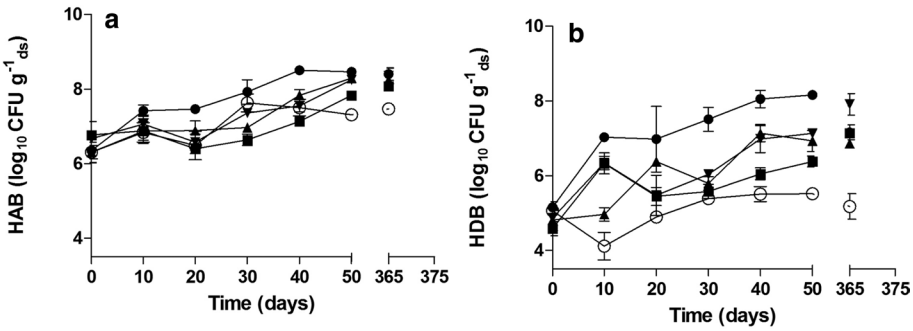

Fig. 2 **a** Total cultivable heterotrophic aerobic bacteria (HAB) and **b** hydrocarbon-degrading bacteria (HDB) expressed as colony-forming units per gram of dry soil (CFU g^{-1} ds) recorded during a field bioremediation experiment for all mesocosms with hydrocarbon-contaminated Antarctic soil amended with different nutrient sources. CC (open circle): non-fertilized control, IN (filled inverted triangle): biostimulation with inorganics salts, ANA (filled triangle): biostimulation with inorganic salts without mixing, NPK (filled square): biostimulation with slow-release granular fertilizer, CP (filled circle): biostimulation with a commercial bioremediation agent. Symbols represent the mean of three independent replicate mesocosms for each treatment. Values are expressed as the mean of three replicate mesocosms. Error bars indicate standard deviation (SD)

remarkable change until day-30 (from 5.95×10^6 CFU g^{-1} dw to 4.48×10^7 CFU g^{-1} dw), followed by a slight but continuous increase until day-50 (6.82×10^7 CFU g^{-1} dw). After 1 year, the HAB counts in the biostimulated mesocosms (1.22×10^8–2.65×10^8 CFU g^{-1} dw) were similar to those recorded on day-50 and significantly higher compared to the control ($p < 0.05$; except NPK).

HDB counts at day-0 (average of triplicate mesocosms, $n = 3$) ranged between 3.97×10^4 and 1.46×10^5 CFU g^{-1} dw (Fig. 2b). In CC mesocosms, HDB counts dropped from 1.17×10^5 CFU g^{-1} dw to 1.59×10^4 CFU g^{-1} dw by day-10 and then steadily increased but remained below the counts of the other treatments at any sampling time, ending with significantly lower counts by day-50 (3.4×10^5 CFU g^{-1} dw) and day-365 (1.81×10^5 CFU g^{-1} dw) ($p < 0.05$). Biostimulation favored the cultivable fraction of HDB, evidenced by the significantly higher ($p < 0.05$) counts recorded at day-50 for all fertilized mesocosms (2.47×10^6–1.45×10^8 CFU g^{-1} dw), compared to the control, confirming that the addition of nutrients is essential to promote the growth of the HDB in this soil. Like with HAB counts, HDB in CP promptly increased from 1.46×10^5 to 1.07×10^7 CFU g^{-1} dw by day-10 to reach 1.45×10^8 CFU g^{-1} dw at day-50, one to two orders of magnitude higher than in the other fertilized mesocosms. HDB in NPK mesocosms showed a similar trend to HAB counts, with values lower than those of the other treatments, likely due to the slow release of N and P. Finally, after 1 year, all the fertilized mesocosms reached HDB counts between 7.40×10^6 and 9.14×10^7 CFU g^{-1} dw, all significantly higher ($p < 0.05$) compared to the control (1.18×10^5 CFU g^{-1} dw).

Efficiency of the biostimulation agents on removal of hydrocarbons from soil

The hydrocarbon removal efficiency at day-50 and day-365 is shown in Fig. 3. After 50 days, no significant differences ($p > 0.05$) were detected between the controls, AC ($12 \pm 3\%$) and CC ($18 \pm 3\%$) ($n = 3$). On the other hand, all the biostimulated treatments except ANA showed significantly higher hydrocarbon removal efficiencies ($p < 0.05$) compared to AC. The mesocosms added with the slow-release granular fertilizer (NPK) and the commercial agent (CP) were considerably more efficient ($32 \pm 6\%$ and $50 \pm 9\%$, $n = 3$, respectively) than the non-fertilized control CC ($p < 0.05$) after 50 days of treatment (Fig. 3a). The higher removal efficiency in CP was in accordance with the early increase observed in bacterial counts.

After 1 year, CP, NPK, IN, and ANA systems reached hydrocarbon removal efficiencies of $79 \pm 14\%$; $87 \pm 13\%$; $66 \pm 12\%$ and $71 \pm 13\%$ ($n = 3$), respectively, all significantly higher ($p < 0.05$) than the $31 \pm 9\%$ ($n = 3$) recorded for the abiotic control AC, which indicates that biodegradation contributed to removal of hydrocarbons from the soil, in addition to abiotic loss (Fig. 3b). However, at this time only NPK treatment showed values significantly higher ($p < 0.05$) than the non-fertilized control CC ($55 \pm 11\%$, $n = 3$).

Online Resources 5 and 6 show the composition of the diesel used by Argentina in Antarctica (AGO) and the GC-FID chromatograms obtained from soil samples at days 0, 50, and 365. Identification and quantification of *n*-alkanes (n-$C_{10–16}$) and isoprenoids (i-$C_{13–16}$) were conducted and the ratios between specific compounds were calculated to distinguish between abiotic and biodegradation losses of hydrocarbons occurred in the controls (AC and CC), and the two fertilized systems showing the higher HCs removal efficiencies, NPK and CP (Fig. 4, Online Resource 7). During the first 50 days of the experiment, n-C_{10-13}/i-C_{16}, i-C_{13}/i-C_{16} and, to a lesser extent, n-C_{14-15}/i-C_{16} ratios decreased in AC, suggesting loss by evaporation of the lighter compounds (up to n-C_{13}). The evaporated fraction in AC was greater after 1 year and more intense for the shorter chain *n*-alkanes (Online Resource 6a–c; Fig. 4a–g). Considering the same ratios, no major changes were observed on day-0 between

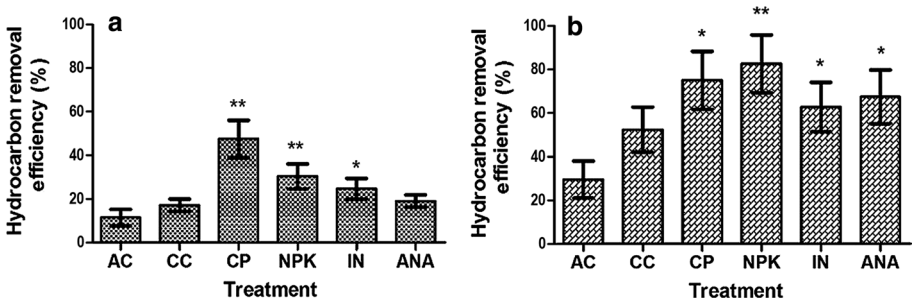

Fig. 3 Hydrocarbon removal efficiency (%) at **a** T5, day-50 and **b** T6, day-365 achieved in the experimental treatments applied using different biostimulation strategies for the bioremediation of an Antarctic soil chronically exposed to diesel. *AC* abiotic control, *CC* non-fertilized control, *IN* biostimulation with inorganics salts, *ANA* biostimulation with inorganic salts without mixing, *NPK* biostimulation with slow-release granular fertilizer, *CP* biostimulation with a commercial bioremediation agent. (*) Indicates significant differences compared to AC and (**) indicates significant differences compared to AC and CC ($p < 0.05$)

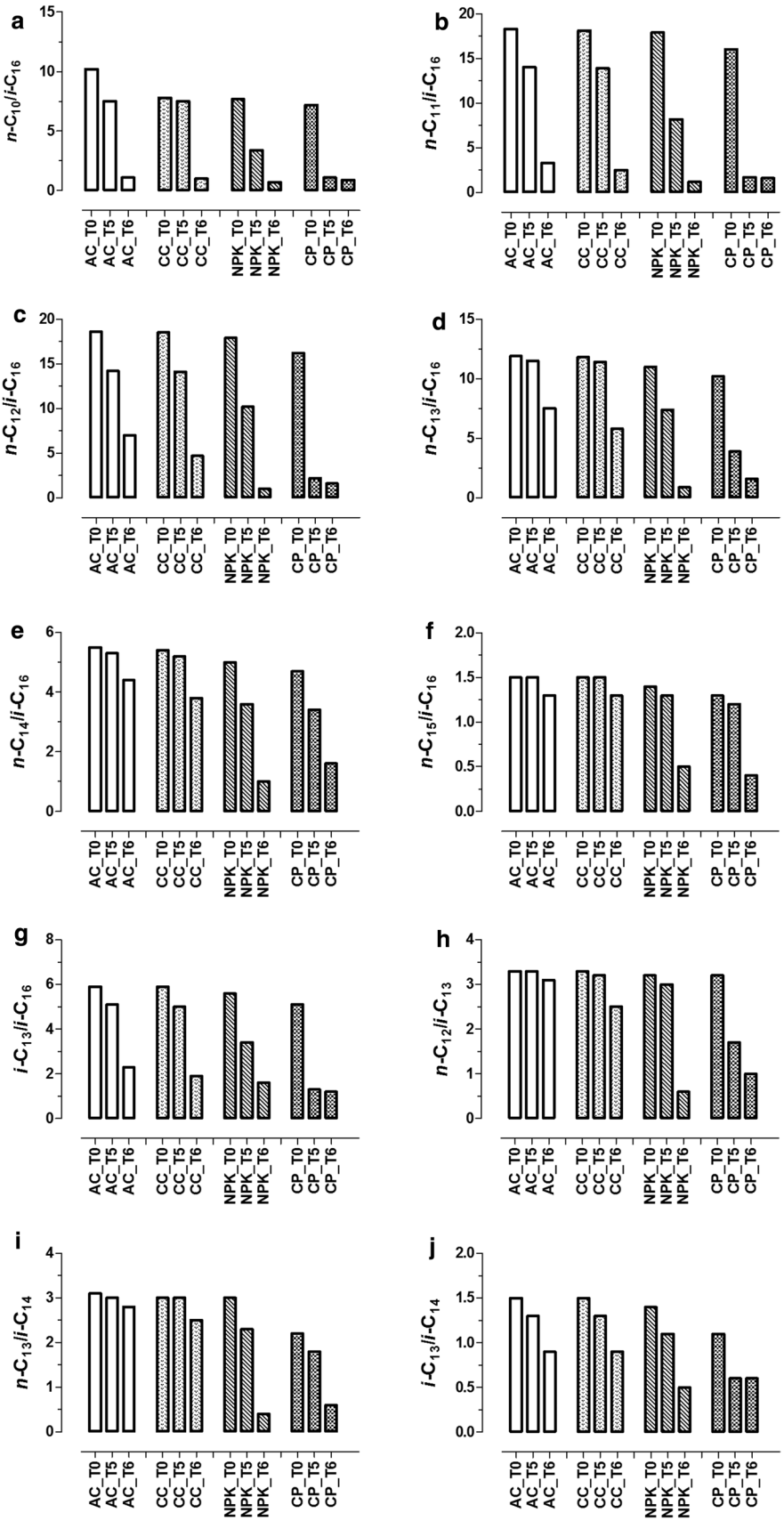

Fig. 4 Concentration ratios of individual hydrocarbons in soil samples from the most efficient treatments (*CP* commercial product OSEII® and *NPK* slow-release fertilizer Nitrofoska®), the non-biostimulated control (CC) and the abiotic control (AC) at T0, day-0 and at T5, day-50 and T6, day-365 of a field bioremediation experiment in mesocosms with hydrocarbon-contaminated Antarctic soil

CC and the most efficient treatments (NPK and CP) compared to AC, except for n-C_{10}/i-C_{16}, suggesting that n-C_{10} could have been degraded to some extent by the microbiota stimulated by the aeration produced by mixing during and after the preparation of the mesocosms, until sampling. In CP, the same was observed to a lesser extent also for n-$C_{11\text{-}13}$, probably due to the stimulation of the microbiota by the rapid action of OSEII® that improved the bioavailability of hydrocarbons and the supply of nutrients. At day-50, no substantial changes in the chromatographic pattern of residual hydrocarbons was observed in CC, compared to day-0 and all ratios in CC and AC were rather similar (Online Resource 6d, e). At this sampling time, NPK showed n-$C_{10\text{-}15}$/i-C_{16} values lower than AC and CC and these ratios were even lower in CP, where the i-C_{13}/i-C_{16} ratio, involving less biodegradable compounds with different rate of evaporation, reached the lowest value (Online Resource 6g, h, j, k, Fig. 4a–g). After 1 year, n-$C_{10\text{-}13}$/i-C_{16} ratios were lower in the non-biostimulated control CC than in AC, and even much lower in NPK and CP, here also including n-$C_{14\text{-}15}$/i-C_{16} ratios. Differences observed at day-50, which were more pronounced at day-365, suggest that the lighter hydrocarbons (up to i-C_{13}) in all mesocosms and even compounds up to n-C15 in NPK and CP were not only removed by evaporation but also by biodegradation. Contrary to the fuel used in the Australian Antarctic Stations (SAB), in the AGO used by Argentina i-C_{13} is more abundant than i-C_{14} (Online Resource 5). Therefore, and since i-C_{13} has shorter chain length and is more volatile than i-C_{14}, when the ratio i-C_{13}/i-C_{14} becomes less than 1, it is a sign of evaporation alone or accompanied by advanced degradation. This change in i-C_{13}/i-C_{14} ratio slightly occurred in AC and CC controls and only after 1 year (Online Resource 6 b, c, e, f, Fig. 4j), due to the progress of evaporative losses. Nevertheless, the inversion in the relative concentration of these two isoprenoids was more pronounced in NPK (Online Resource 6 h, i; Fig. 4j) and even faster (already at day-50) in CP mesocosms (Online Resource 6 k, l, Fig. 4j), indicating advanced biological degradation promoted by the biostimulation in those mesocosms. In addition, it is important to note that both CP and NPK showed higher proportions of UCM at day-50 and day-365 than at day-0, another potential indicator of biodegradation. This increase in UCM was not observed in AC or CC (Online Resource 6).

Effect of the biostimulation agents on the structure of bacterial communities

DGGE banding patterns from soils fertilized with different nutrient sources were clearly different, as seen in the photographs of the gels (Online Resource 8) and in the dendrograms constructed after the presence/absence analysis of DGGE bands (Fig. 5). Although the soil used was thoroughly homogenized, the addition of different nutrients produced fast and differential changes in the structure of the indigenous bacterial communities, as depicted by the differences observed in the DGGE profiles between CC and the other treatments, even shortly after the preparation of the mesocosms (Online Resource 8).

Bacterial community structure in CC changed by day-50 towards an enrichment in populations characterized by medium/high GC% content in the amplified region of the 16S rRNA gene sequences. These changes could be related to a positive effect of the soil aeration on the growth of some aerobic members of the indigenous microbiota, able to utilize or at least tolerate the hydrocarbons. Although different from CC, the bacterial communities in the IN and ANA mesocosms shared similar DGGE banding patterns until day-50 (T5) suggesting that, under biostimulation with inorganic salts, aeration by mixing did not produce substantial changes in the number and size of the populations detected by DGGE (as inferred qualitatively by the band intensities observed visually), at least under the assayed conditions (Online Resource 8). Nevertheless, except for one replicate mesocosm at day-50, IN had a rather constant DGGE profile, with slight changes occurred in the first 10 days (T1), whereas ANA had more variable profiles with few bands of medium and high GC% with higher intensity or even extra bands at day-50 compared to IN. However, after 365 days (T6), the IN and ANA communities diverged (28% similarity). The structures of bacterial communities present in NPK mesocosms at the beginning of the experiment resembled those observed in IN and ANA, clustering together at 65% similarity. Towards day-30 (T3), the DGGE profiles were dominated by bands of low-GC% DNA sequences already present since day-0 (T0), a trend that continued at least until day-50 (T5) (Fig. 5b, Online Resource 8). The most notorious and fast changes in bacterial community structure were observed in CP mesocosms, where OSEII® induced the enrichment of populations with low-GC% 16S rRNA gene. These bands were not detected in the CC control, and determined the differential clustering of CP, even at T0. Over time, the intensity of the few bands in CP already shared with CC at the beginning decreased, with a simultaneous increase in the intensity of other bands almost not detected in any other treatment, probably corresponding to bacteria favored only when nutrients and hydrocarbons are more readily assimilable due to the surfactants and enzymes supplied by OSEII®. Finally, and as revealed by their similar DGGE banding patterns, the structures of the bacterial communities present in the independent replicate mesocosms of each experimental treatment on day-0 (T0) and day-50 (T5) were very similar to each other (Fig. 5a, b, Online Resource 8). However, on day-365 (T6), the DGGE profiles from replicates were no longer as similar to each other and showed multiple low intensity bands spanning the entire gradient, denoting a greater richness and

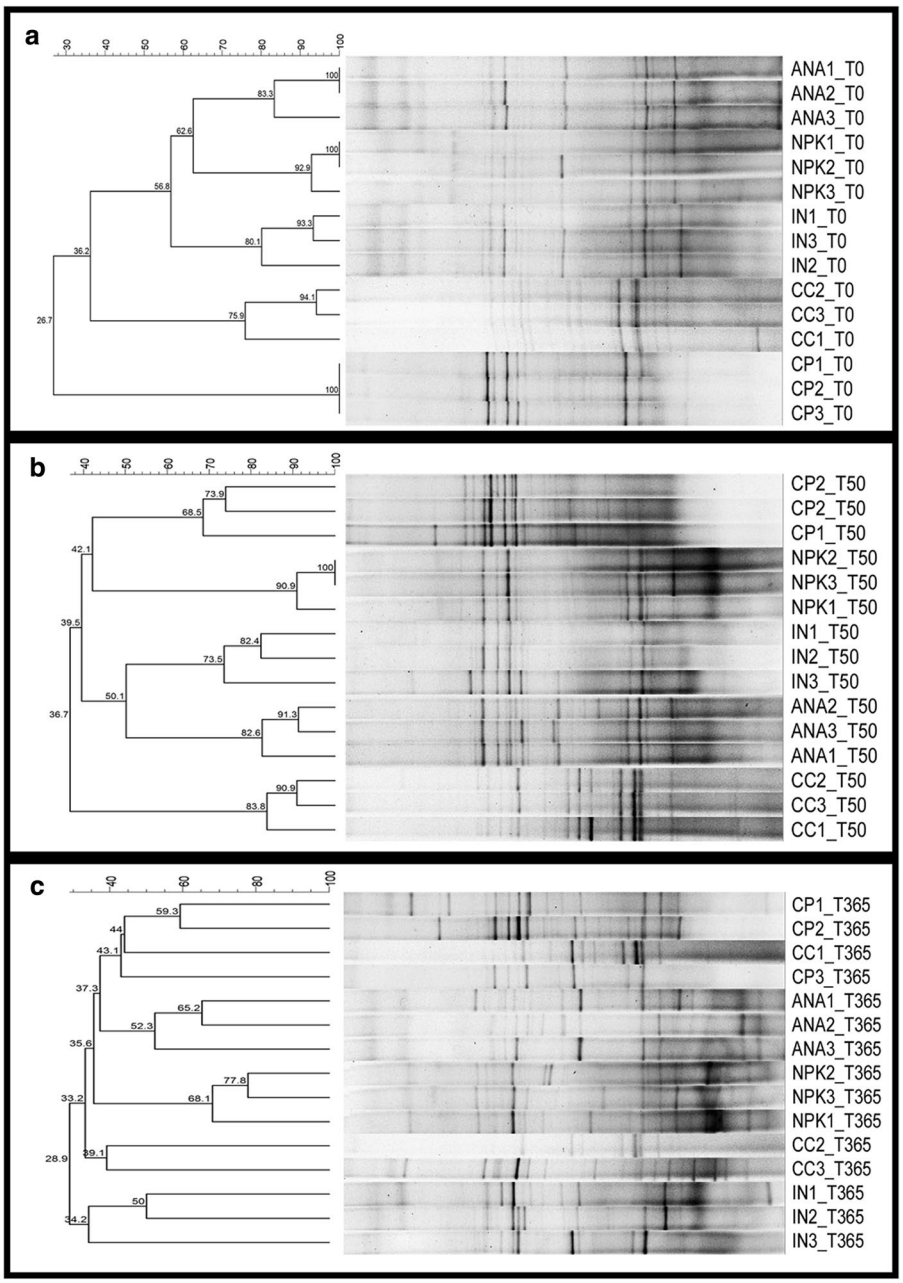

Fig. 5 UPGMA (unweighted pair group method with arithmetic mean) dendrogram resulting from cluster analysis of bacterial communities represented by the presence/absence of 16S rDNA DGGE (denaturing gradient gel electrophoresis) bands at **a** T0, day-0, **b** T5, day-50 and **c** T6, day 365 of a field bioremediation experiment in mesocosms with Antarctic soil chronically exposed to diesel fuel, using different biostimulation strategies with **a**, **b**, **c** denoting pannels in bold-type. *CC* non-fertilized control, *IN* biostimulation with inorganics salts, *ANA* biostimulation with inorganic salts without mixing, *NPK* biostimulation with slow-release fertilizer Nitrofoska®, *CP* biostimulation with a commercial bioremediation agent OSEII®

diversity than on day-50 (T5). Nevertheless, even at day-365 (T6), the communities evolved under the same treatments were more similar to each other than to those from the other treatments, except for the community in CC1 mesocosm that diverged from its replicas, probably caused by the decrease in pH recorded in this mesocosm (Fig. 5c, Online Resource 6).

Discussion

On-site remediation has the advantage of avoiding soil transport and the associated costs and logistics, which is crucial when deciding on the appropriate strategy to be applied in Antarctica. Moreover, considering the Antarctic Treaty statement that prohibits the introduction of exogenous microorganisms, the study of remediation approaches should be based on indigenous microorganisms. Our previous studies at Carlini station have shown that autochthonous bacteria isolated from contaminated soils are adapted to the presence of hydrocarbons, having the ability to metabolize these compounds. (Ruberto et al. 2005; Vázquez et al. 2013). We

also found that bacterial growth and degradation efficiency were closely related to the concentration of N and P in the treated soil (Dias et al. 2012; Martínez Álvarez et al. 2017). This work confirmed these observations, since biostimulation favored the biodegradation of hydrocarbons by the indigenous microbiota of the chronically contaminated soil.

The relationship between high bacterial counts (total and hydrocarbon degraders) and high biodegradation rates has been previously reported and, although this data alone is not sufficient indicator of degrading activity, it is considered a good estimator of the capacity of response that a bacterial community has to the environmental change caused by pollution (Delille and Pelletier 2002; Diplock et al. 2009; Abdulsalam et al. 2011). In a study on the degradation of PAHs, whose concentrations in soils are not sufficient to be used as a carbon source capable of promoting significant increases in bacterial biomass, Lors et al. (2010) did not find such a correlation but observed a significant increase in counts when only the specific fraction capable of degrading contaminants was evaluated. Accordingly, in this work (where the amounts of hydrocarbons were high enough to sustain an increase in bacterial biomass), degradation activity was accompanied by a significant rise in both HAB and HDB counts. In contrast, the low removal efficiency of the non-fertilized CC control was accompanied by low HAB and HDB counts, proving that the lack of nutrients in Antarctic soils makes fertilization with N and P essential, as was also highlighted by others who worked with Antarctic and Subantarctic soils, as those from Scott Base (Aislabie et al 2012) and Port aux Français Station in Kerguelen Islands (Delille and Coulon 2008). Results also showed that aeration by mixing favored an initial growth of the aerobic microbiota, but that this effect was ephemeral, probably because the previously mentioned scarce nutrients prevented bacterial counts to further increase also leading to a decrease in total aerobic counts from day-30 until the end of the study, while the number of degraders remain constant.

In relation to nutrients, and even though under certain conditions, inorganic salts have been one of the best options for biostimulation (Vázquez et al. 2009; Abed et al. 2015); in this study it was not the case, since the IN system performed better than the control but did not reach the hydrocarbon removal efficiencies of NPK and CP systems, coupled with a large fluctuation in HDB counts. Since the mesocosms were carried out outdoors, exposed directly to the weather, the fluctuation observed in HDB counts in the IN mesocosm and also in ANA could be reflecting a temporary lack of nutrients due to the washing of the water-dissolved salts, caused by repeated freezing and thawing of the soil and rainfall, which hampers maintaining adequate concentrations of nutrients to sustain a constant activity of hydrocarbon degradation. Moreover, the fluctuations found in bacterial counts (Fig. 2) and bacterial community structures (Online Resource 8) in these and other mesocosms could be caused in part by the sudden changes in temperature and water content, as seen around sampling times T2 (day-20) and T3 (day-30) and before T5 (day-50) (Online Resource 9), which affected differently the mesocosms, sometimes even the replicates from the same treatment or control. It is also possible that, although the amount of N and P needed to balance the C:N:P ratio in the soil has been added, the fractional supply of nutrients could have led to fluctuating C:N:P ratios, which often differed from the optimal. In a previous study, Dias et al. (2012) observed no significant differences in degradation efficiency of the biostimulated in comparison with the non-biostimulated microbiota in a 45-day long study performed in land plots, where the inorganic salts were added at once at the beginning of the experiment. However, we found biostimulation with inorganic salts successful in other trials (Vázquez et al. 2009; Ruberto et al. 2010; Martínez Álvarez et al. 2017), which was also reported by other authors working with soils in cold environments (Margesin and Schinner 2001; Powell et al. 2006). Keeping nutrient concentrations close to the optimal by fertilizing with inorganic salts and, at the same time, avoiding osmotic stress and washing is difficult in practice, especially when working in open systems with Antarctic mineral soils that have low water holding capacity and experience periodic cycles of freezing and thawing and snowmelt during summer (Delille and Coulon 2008). In this context, the design of closed or full covered experimental units that prevent washing by rainfall and snowmelt becomes a good alternative when fertilization with inorganic salts is the option of choice (McWatters et al. 2016; Martínez Álvarez et al. 2017).

The most relevant finding in this work was represented by the NPK and CP systems, which promoted the higher increases in bacterial numbers and the fastest and greatest degradation activity in 50 days (T5), along with a fast increase in HDB. This suggests that OSEII®, recommended by EPA for bioremediation in cold areas, is adequately designed to promote a rapid response of the bacterial communities from Carlini Station soils to the presence of hydrocarbons, and supports what was reported by Dias et al. (2012) who found that OSEII® was the most efficient fertilizer to promote hydrocarbon removal from an aged, contaminated Antarctic soil, after 45 days of treatment. In comparison, the system fertilized with the slow-release NPK, though less efficient to promote hydrocarbons removal in the short term (T5,day-50), showed the best performance after 1 year of treatment. It was reported that the major challenge for the application of slow-release fertilizers is how to control the releasing rates to maintain optimal nutrient concentrations over long periods of time (Becker et al. 2016). In this study, the use of Nitrofoska®, based on its low cost and good efficiency as nutrients source (Zhu et al. 2004), led to the highest elimination of hydrocarbons after 1 year, evidence that

this product maintained its long-term fertilization capacity. This finding makes slow-release fertilizers specially promising for their use during the Antarctic winter, a long period where human intervention is not possible. The lower short- to medium-term efficiency of Nitrofoska® could have been due, to some extent, to the low soil temperatures that reduce the permeability of the slow-release particle coating (Lee et al. 1993), decreasing nutrient release rates, which could then not meet the high demand of the actively growing bacteria in the early stages of the process.

It is known that diesel fuels components differ in their susceptibility to microbial attack (Chikere et al. 2011) and that the lightest fractions are biodegradable but also more susceptible to abiotic loss. Although their degradation under natural conditions was proved and the corresponding catabolic pathways are known, other compounds, like the isoprenoids, are more recalcitrant to biological degradation due to their branched chemical structure (Varjani 2017) and are frequently used to estimate rates of hydrocarbon removal mediated by the biota (Wang et al. 2018). Biodegradation produces enrichment in the UCM components by removal of resolved compounds and accumulation of new ones, together with lower n-alkane to isoprenoid ratios, both fuel signatures consistent with a weathered or degraded fuel. Gas chromatography profiles in this experiment revealed a significant loss by volatilization and washing, which was the main cause of hydrocarbon removal in the non-biostimulated mesocosms. However, considering that all the mesocosms had the same geometry and experimental design, the higher hydrocarbon removal reached by the fertilized mesocosms can be attributed to the activity of the microbiota. Chromatograms obtained from soil samples at the beginning and after 50 and 365 days of treatment with NPK and CP showed higher biodegradation than AC and CC. This fact was evidenced not only by the relative differences in the concentration of resolved compounds but also by the enhanced UCM proportions. Degradation of the resolved compounds was lower the longer their carbon chain was, and linear alkanes were degraded more readily than the corresponding isoprenoids of similar carbon chain length., in accordance with those reported for bioremediation of other contaminated Antarctic soils (Aislabie et al. 2006; Coulon and Delille 2006). In complex mixtures of hydrocarbons, relative amounts of certain compounds could be indicative of the extent of biodegradation or the abiotic loss. In a biodegradation experiment without loss by evaporation, Snape et al. (2005) found that ratios such as n-C_{12}/i-C_{13} and n-C_{13}/i-C_{14} decreased in comparison with abiotic controls and the isoprenoids did not change throughout the experiment. They also found that the n-alkane/isoprenoid ratios of similar volatilities changed as biodegradation proceeded. By comparing these ratios in the CC and AC controls at each time interval (T0, day-0–T5,day-50; T5, day-50–T6, day-365) it seems that the light hydrocarbons in CC were mainly lost by lixiviation and evaporation, with some biodegradation at the end of the experiment. Conversely, in NPK after 1 year and in CP at day-50, n-C_{12}/i-C_{13} and n-C_{13}/i-C_{14} decreased considerably to 50% or less of the values in AC, indicating that the changes observed were also of biological origin. This supports our observation about the effectiveness of CP to induce the necessary changes in the microbial community to promote biodegradation in a short time, resulting in an efficient bioremediation treatment.

Microbial communities from contaminated ecosystems had been described as less diverse than those inhabiting unstressed environments (Cury et al. 2015). Diversity is influenced by the complexity of chemical contaminants and by the time such populations have been exposed to the pollutants. In this regard, and as has been mentioned above, it should be kept in mind that the soil used in this study was chronically contaminated, but also suffered fresh contamination by frequent diesel leaks before being collected, turning it into a complex and heterogeneous environment with many microhabitats suitable for different bacterial populations. In this scenario, not only well adapted oligotrophic microbes are established, but also opportunistic fast-growing copiotrophs can quickly react and play a relevant role in biodegradation (Martínez-Alonso et al. 2010). In this work, this heterogeneity was reflected by the differences in bacterial community structure evidenced along the process between systems, also suggesting that the indigenous microbiota was able to rapidly react, playing an active role in the removal of hydrocarbons. In this sense, the fast rise of the bacterial counts able to degrade hydrocarbons in the presence of OSEII® could be evidenced by the DGGE bands that increased its relative abundance in the soil from CP mesocosms at day-50. Using the same commercial product with chronically contaminated soil at Carlini Station, Dias et al. (2012) also found a significantly higher hydrocarbon removal in the presence of OSEII® and, although reported minor differences in the DGGE patterns relative to the non-biostimulated microbiota when working with a highly aged soil, they also observed marked changes in community structure when studying a soil that had a combination of old and recent hydrocarbon contamination (Dias et al. 2015), as was the case in the present study.

Although DGGE banding patterns are not always considered accurate enough to calculate diversity indices due to artifacts associated with the presence of multiple bands produced by a unique strain (Neilson et al. 2013), it is still useful for describing differences and similarities between communities (Kuc et al. 2019). In our work, we found similar DGGE banding patterns associated with independent replicates from the same treatment at day-0 and day-50 whereas the patterns differed between treatments. This suggests that, during the first 50 days, and beyond the differences between

replicates in terms of water content or presence of frozen soil or snow cover, the dynamics followed by the soil bacterial microbiota would be mainly driven by the biostimulation strategy applied, more than by the environmental factors. The high reproducibility obtained with our replicates soil samples allowed us to use the presence/absence of shared bands to build a dendrogram and report on the main differences between treatments regarding the evolution of bacterial communities, as was also done elsewhere (Schauer et al. 2000; Gafan et al. 2005; Chong et al. 2009; Bevivino et al. 2014; Festa et al. 2016).

At T5 (day-50) all fertilized mesocosms evolved to a high abundance of bacteria with medium to low-GC% 16S rRNA gene sequence according to the position of the DGGE bands in the gel, compatible with fast hydrocarbon degraders like *Pseudomonas* and other Proteobacteria, though not providing taxonomic identification of the detected bacteria is a limitation of fingerprinting methods and this could only be confirmed by amplicon sequencing. Conversely, non-fertilized mesocosms (CC) showed DGGE patterns dominated by high-GC% 16S rRNA gene bands, compatible with actinobacteria and other more oligotrophic and slow-growing bacteria, adapted to resource-limited conditions. At T6 (day-365), DGGE banding patterns obtained from all the biostimulated treatments showed notorious and distinct changes in their community structures, with higher richness and evenness compared to the original community. These changes were accompanied by ~ 50% reduction of the initial concentration of hydrocarbons in all mesocosms (except for the poisoned abiotic control), suggesting that degrading microorganism remained active and continued their catabolic activity during winter, despite the very low temperature of the frozen soil, probably inhabiting microenvironments where the water remained liquid. In this sense, Yan et al. (2016) postulated that a successional variation in the composition of the bacterial community represents a sensitive ecological indicator of in situ remediation, and White et al. (1998) stated that pollution disappearance accompanied by consequent changes in the structure of microbial communities is indicative of a reduced stress in the microniche environment influencing the local microbiota, even if there has not been a return to the baseline community. It is possible that the different dynamics observed in the bacterial communities from the different biostimulation strategies were related to the gradual reduction of the environmental stress exerted by the hydrocarbons on the indigenous microbiota, which occurred at different rates and extent according to the applied strategy. It is relevant to mention that, once the biostimulation agents were added, the balance of nutrients under such stress could have triggered the development of the fast-growing hydrocarbon-tolerant members of the microbiota, capable of catabolizing the easily degradable fraction of the contaminants at the early stages of the process. This could have precluded the detection by DGGE of other more sensitive or slow-growing hydrocarbon degraders that would be present in the soil but at very low proportions.

Finally, in our study and after 1 year, the biological degradation of the organic contaminants that lead to bacterial growth and nutrient depletion in biostimulated mesocosms may have promoted the establishment of bacterial communities dominated by K-strategists, capable of coping with reduced availability of resources, and also foster the divergence observed in the structure of the community, even in the replicated mesocosms. In this sense, Johansen et al. (2019) found that the structure of the bacterial and fungal communities in replicates became more divergent with longer incubation periods, and that this could be due to interactions between organisms that drive population dynamics, differently changing mesocosm resources.

Conclusion

This study confirmed that in the first stages of a remediation treatment, the microbiota from these contaminated Antarctic soils is able to remove up to 50% of hydrocarbons in 50 days and 87% in 365 days, depending on the biostimulation agent used and that, therefore, bioaugmentation would not be necessary. Beyond that, the results contributed with novel and relevant knowledge about the long-term treatment strategy to be applied to these soils. In this sense, the use of a biostimulation agent like that applied to the CP mesocosms (OSEII; a mixture of surfactants, nutrients, and enzymes) generated a better mid-term biodegradation performance, whereas biostimulation with slow-release fertilizers, such as the Nitrofoska® used in the NPK mesocosms, is more efficient for long-term processes. These findings suggested that a mixed strategy, combining the fastest action of commercial products like OSEII® during the Antarctic summer, with a slow-release fertilizer like Nitrofoska®, more active throughout the year, could be an efficient long-term bioremediation treatment for soils at Carlini Station and similar coastal areas of Antarctica. Lastly, understanding how biological interactions, nutrients, and differences in quantity and bioavailability of hydrocarbons affect the structure and biodegradation capability of a microbial community represents a key factor to improve bioremediation strategies for Antarctic soils, which can also be extrapolated to other cold regions, highlighting the relevance of conducting research on this topic.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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Title: Bioremediation of hydrocarbon-contaminated soil from Carlini Station, Antarctica: effectiveness of different nutrient sources as biostimulation agents

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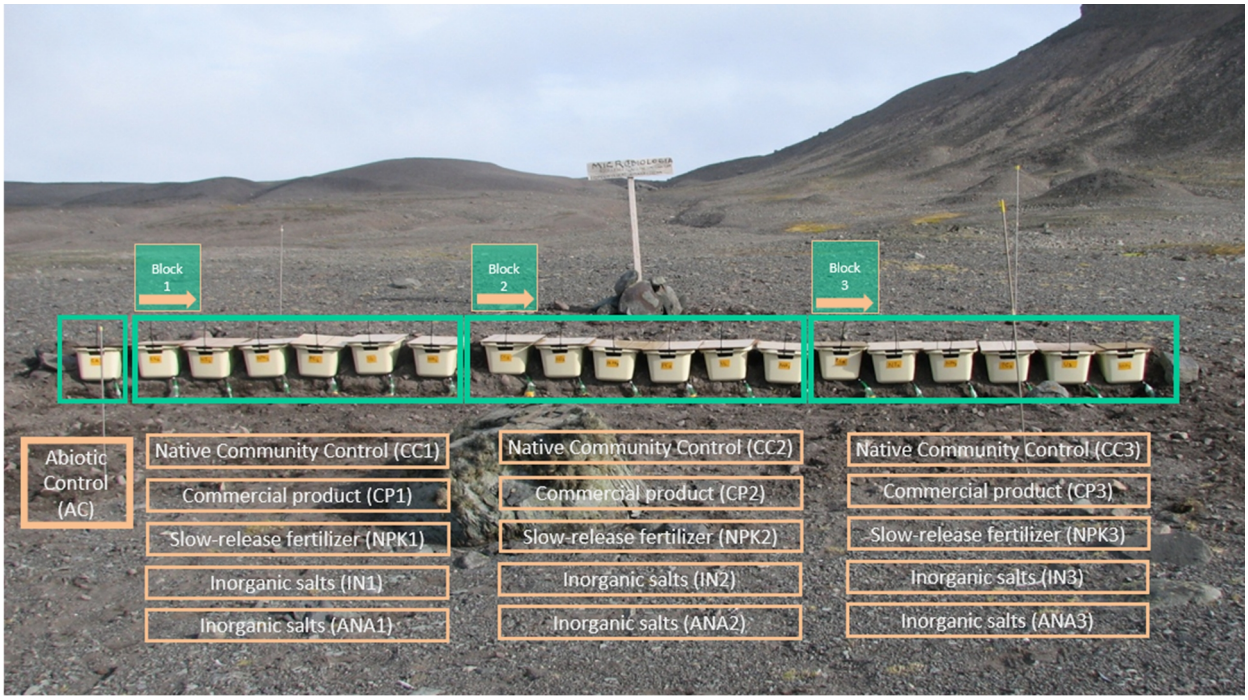
Online Resource 1: Experimental design



The mesocosms were distributed in the soil in three blocks, each block containing one of the three replicates of the biostimulated treatments (IN, ANA, NPK, CP) and the non-fertilized control (CC), so that the influence of the impact of the wind, rain, snowfall and temperature fluctuations were more homogeneous between treatments.

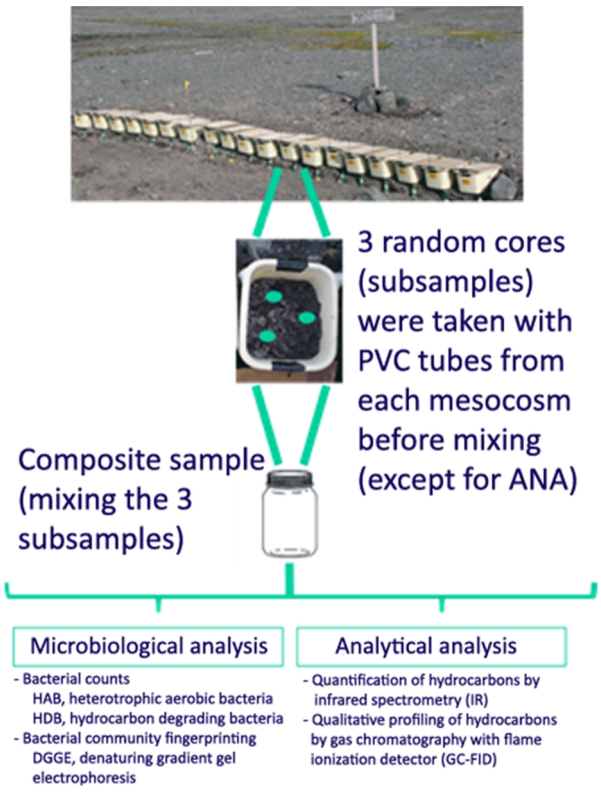
Online Resource 2: Mesocosms detail

Mesocosm	Treatment	Nutrient source*	Weakly mixing	Hg Cl_2
AC	Abiotic control$^{\delta}$	-	+	+
CC	Non-biostimulated indigenous microbial community control	-	+	-
IN	Bioestimulation	Inorganics salts: NH_4NO_3 (0.17% w w^{-1}) and Na_2HPO_4 (0.027% w w^{-1}), added to reach a C:N:P ratio of 100:10:1 based on total organic carbon	+	-
ANA	Bioestimulation	Inorganics salts: NH_4NO_3 (0.17% w w^{-1}) and Na_2HPO_4 (0.027% w w^{-1}), added to reach a C:N:P ratio of 100:10:1 based on total organic carbon	-	-
NPK	Bioestimulation	Commercial slow-release granular fertilizer: Nitrofoska® (0.5% w w^{-1})	+	-
CP	Bioestimulation	Commercial product: OSE II® according to manufacturer's instructions (15 mL kg^{-1}dw)	+	-

* Concentrations given are final concentrations in the experimental units

$^{\delta}$ Poisoned with $HgCl_2$ (0.3% w w^{-1} final concentration)

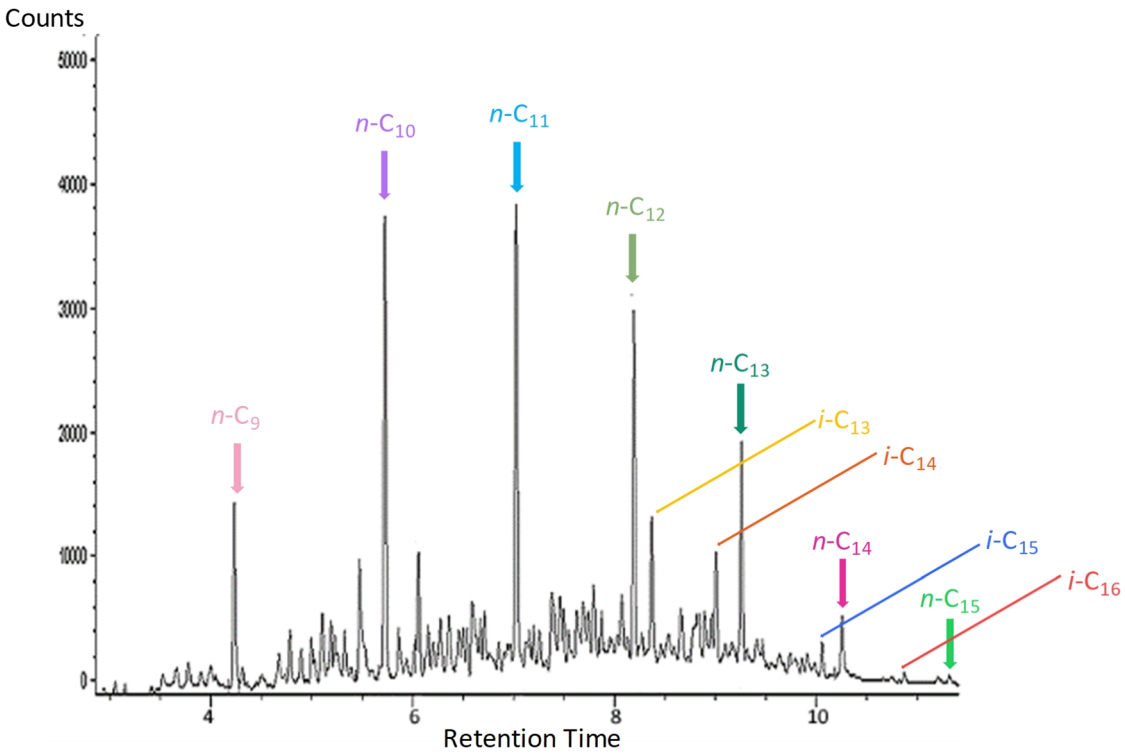
Online Resource 3: Sampling strategy



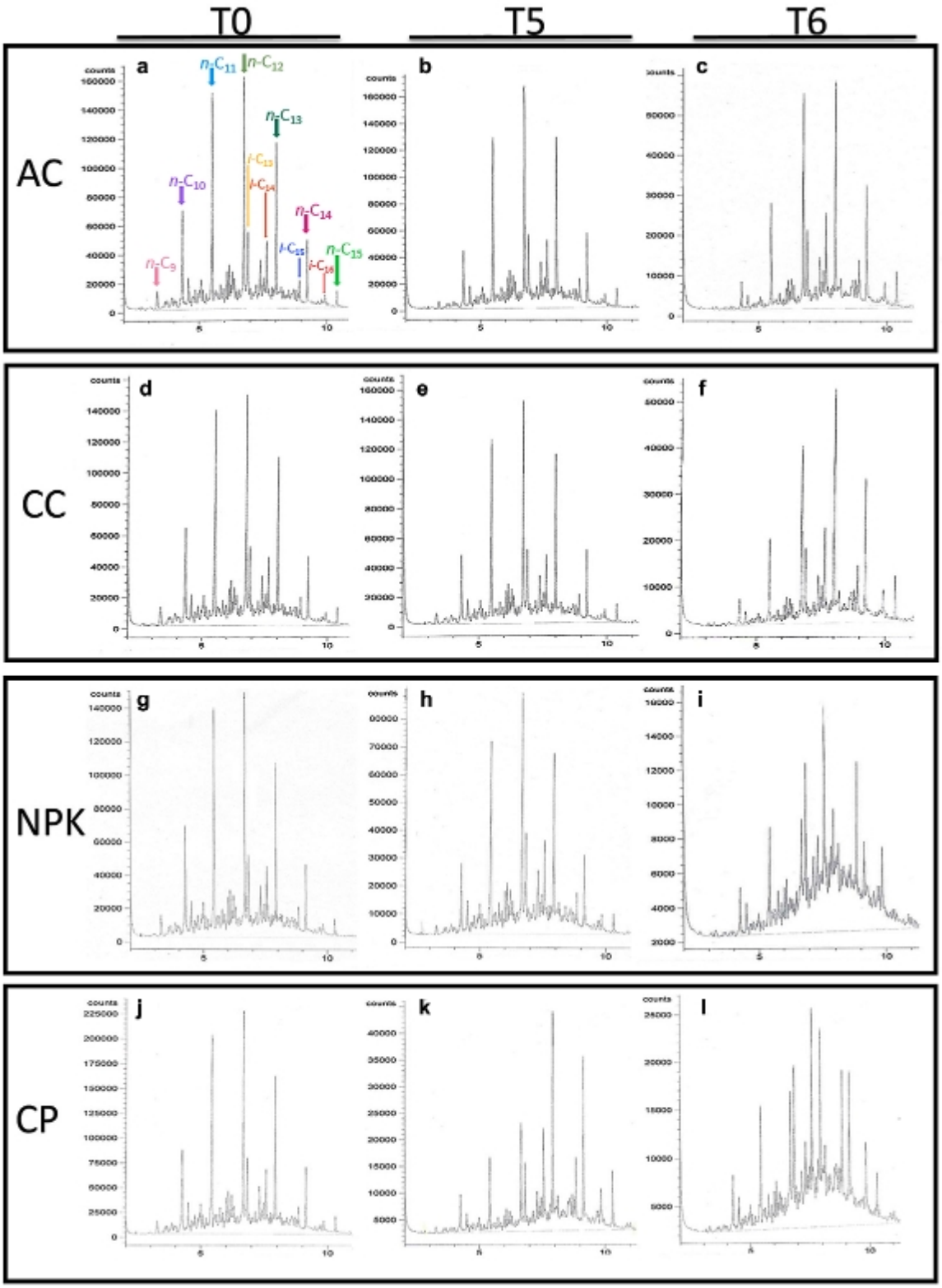
Online Resource 4: pH measured in soil samples from each mesocosm at the beginning of the experiment (T0) and after 10 (T1), 20 (T2), 30 (T3), 40 (T4), 50 (T5) and 365 (T6) days of treatment.

	pH						
Mesocosm	T0	T1	T2	T3	T4	T5	T6
CA	7.2	8.1	8.07	7.51	7.95	7.9	7.7
CC1	7.1	7.61	7.57	7.72	7.91	7.82	5.2
CC2	7	7.81	7.64	7.68	7.9	7.81	7.2
CC3	7.1	7.71	7.68	7.71	8.04	7.76	7.5
NI1	7.2	7.03	7.45	7.36	7.72	7.54	7.5
NI2	7.2	7.4	7.3	7.37	7.56	7.44	7.1
NI3	7.1	7.37	7.43	7.09	7.36	7.23	7.1
ANA1	7.2	7.79	7.6	7.87	7.61	7.67	7.4
ANA2	7.2	7.55	7.8	7.75	7.8	7.68	7.5
ANA3	7	7.43	7.65	7.73	7.64	7.5	7.9
NPK1	7.1	7.04	7.07	7.22	7.24	7.32	7.3
NPK2	7	7.09	7.08	7.2	7.23	7.35	7
NPK3	7.2	6.92	7.08	7.13	7.15	7.03	7
CP1	6.7	7.69	7.61	7.22	7.35	7.54	7
CP2	6.7	7.89	7.53	7.22	7.58	7.46	7.3
CP3	6.6	7.86	7.43	7.23	7.61	7.47	7.4

Online Resource 5: GC-FID chromatogram profile of the diesel fuel (Antarctic gasoil: AGO) used at Carlini Station, showing the main lineal alkanes and isoprenoids.



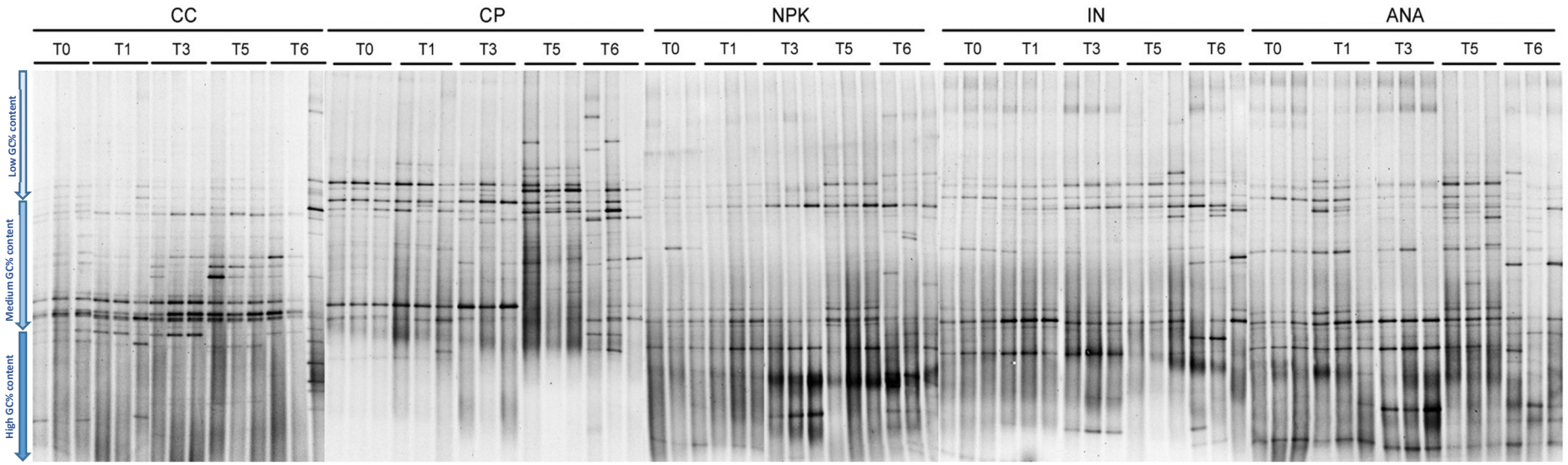
Online Resource 6: Gas chromatography (GC-FID) of the hydrocarbons extracted from soil samples taken at day-0 (T0), day-50 (T5) and day-365 (T6) of a field bioremediation experiment in mesocosms with hydrocarbon-contaminated Antarctic soil amended with different nutrient sources. a-b-c abiotic control (AC); d-e-f non-fertilized control (CC); g-h-i slow-release fertilizer Nitrofoska® (NPK) and j-k-l commercial product OSEII® (CP) treatments. Arrows indicate individual compounds



Online Resource 7: Ratios between concentrations of individual hydrocarbons in soil samples from the most efficient treatments (CP and NPK), the non-biostimulated control (CC) and the abiotic control (AC) at the beginning of the experiment (T0) and after 50 (T5) and 365 (T6) days of treatment.

		AC			CC			NPK			CP		
		T0	T5	T6	T0	T5	T6	T0	T5	T6	T0	T5	T6
Evaporation	*n*-C_{10}/*i*-C_{16}	10.2	7.5	1.1	7.8	7.5	1	7.7	3.4	0.7	7.2	1.1	0.9
	n-C_{11}/*i*-C_{16}	18.3	14	3.3	18.1	13.9	2.5	17.9	8.2	1.2	16	1.7	1.6
	n-C_{12}/*i*-C_{16}	18.6	14.2	7	18.5	14.1	4.7	17.9	10.2	1	16.2	2.2	1.6
	n-C_{13}/*i*-C_{16}	11.9	11.5	7.5	11.8	11.4	5.8	11	7.4	0.9	10.2	3.9	1.6
	n-C_{14}/*i*-C_{16}	5.5	5.3	4.4	5.4	5.2	3.8	5	3.6	1	4.7	3.4	1.6
	n-C_{15}/*i*-C_{16}	1.5	1.5	1.3	1.5	1.5	1.3	1.4	1.3	0.5	1.3	1.2	0.4
	i-C_{13}/*i*-C_{16}	5.9	5.1	2.3	5.9	5	1.9	5.6	3.4	1.6	5.1	1.3	1.2
Degradation	*n*-C_{12}/*i*-C_{13}	3.3	3.3	3.1	3.3	3.2	2.5	3.2	3	0.6	3.2	1.7	1
	n-C_{13}/*i*-C_{14}	3.1	3	2.8	3	3	2.5	3	2.3	0.4	2.2	1.8	0.6
	i-C_{13}/*i*-C_{14}	1.5	1.3	0.9	1.5	1.3	0.9	1.4	1.1	0.5	1.1	0.6	0.6

Online Resource 8: DGGE fingerprinting of bacterial communities present in all the soil samples taken throughout the bioremediation experiment.



Online Resource 9: Daily **a** average air temperature and **b** precipitation recorded in Carlini Station, Antarctica, during the austral summer days in which the bioremediation experiment was carried out