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Bacterial communities and chemical parameters in soils and coastal sediments in response to diesel spills at Carlini Station, Antarctica

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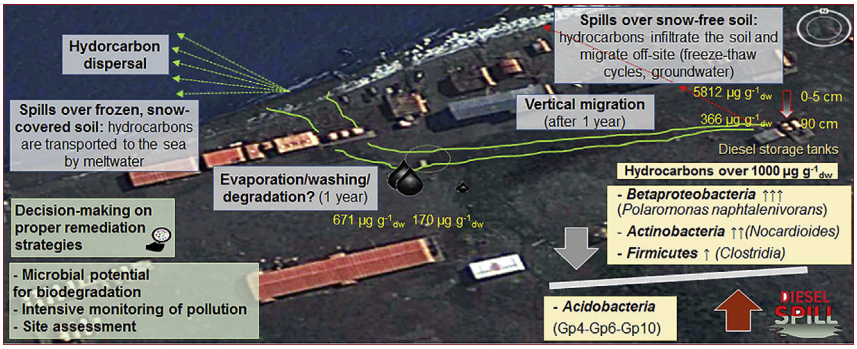
HIGHLIGHTS

- Small to moderate diesel spills are frequent in the Antarctic Peninsula and pose a threat to the biota.
- Diesel spilled over frozen and snow-covered soil or ice-free ground follow different dynamics.
- Bacterial communities in soils around Carlini Station quickly react to hydrocarbons.
- Bacteria related to *Polaromonas naphthalenivorans* and *Nocardioides* dominate contaminated soils.
- Monitoring the fate of hydrocarbons and microbial response are key factors in deciding on remediation strategies.

GRAPHICAL ABSTRACT



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ABSTRACT

A diesel spill occurring at Carlini Station (King George Island (Isla 25 de Mayo), South Shetland Islands) in 2009 started the study of the fate of the hydrocarbons and their effect on the bacterial communities of the Potter Cove ecosystem. Soils and sediments were sampled across the 200-meter long diesel plume towards Potter Cove four and 15 months after the spill. The sampling revealed a second fuel leakage from an underground pipeline at the spill site. The hydrocarbon fraction spilt over frozen and snow-covered ground reached the sea and dispersed with the currents. Contrary, diesel that infiltrated unfrozen soil remained detectable for years, and was seeping with ground water towards coastal marine sediments. Structural changes of the bacterial communities as well as hydrocarbon, carbon and nitrogen contents were investigated in sediments in front of the station, two affected terrestrial sites, and a terrestrial non-contaminated reference site. Bacterial communities (16S rRNA gene clone libraries) changed over time in contaminated soils and sediments. At the underground seepage site of highest contamination (5812 to 366 µg g^{-1}_{dw} hydrocarbons from surface to 90-cm depth), communities were dominated by *Actinobacteria* (18%) and a betaproteobacterium closely related to *Polaromonas naphthalenivorans* (40%). At one of the spill sites, affected exclusively at the surface, contamination disappeared within one year. The same

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bacterial groups were enriched at both contaminated sites. This response at community level suggests that the cold-adapted indigenous microbiota in soils of the West Antarctic Peninsula have a high potential for bioremediation and can support soil cleaning actions in the ecosystem. Intensive monitoring of pollution and site assessment after episodic fuel spills is required for decision-making towards remediation strategies.

1. Introduction

Despite increasing scientific activity in coastal research stations in Antarctica, particularly in the West Antarctic Peninsula (WAP), little is known about the fate and impact of hydrocarbons on Antarctic shallow water ecosystems. Most of terrestrial fuel spills happen locally during refueling of vehicles, refilling of fuel tanks, or transport of fuels to powerhouses (Aislabie et al., 2004). The most commonly used fuels in Antarctica are lubricant oil and gasoline for boat engines, marine gasoil, jet fuel, and light diesel. These fuels contain a high content of aliphatic compounds (≥75%), including resolved alkanes, acyclic-isoprenoids and unresolved complex mixtures (UCM) (Snape et al., 2005). The hydrocarbons most commonly encountered in Antarctic soils are *n*-alkanes from C_9–C_{15}, with a low content of heavier aliphatic fractions and polycyclic aromatic hydrocarbons (PAHs), mainly represented by naphthalene and methylnaphthalenes (Aislabie et al., 2004). The conventional remediation technologies that are used to clean up these contaminants are difficult to implement in Antarctica, as temperatures are low and most areas are covered by ice and snow at least eight months a year. Furthermore, coastal soils are mostly sandy, with poor water holding capacity and limited nutrient availability (Simas et al., 2015).

The Antarctic Treaty and its Protocol on Environmental Protection (ATCP, 1991) requires the signatory parties to comprehensively protect the Antarctic Environment, which should be kept as pristine and unaltered as possible. Environmental protection not only implies avoiding pollution but furthermore requires investigation of the impact coming from coastal research stations. This includes development of action plans for accidental fuel spills to prevent or reduce off-site migration of contaminants. Costs involved in removing contaminated soil and carry out *on-site* treatments are high. Therefore, decisions on whether to treat or not and which treatment to apply in each case of actual contamination differ depending on the spill volume and scenario (superficial soil, aquatic, underground leakage etc.), the type of fuel, and the prospective effects on the local ecosystem (Aislabie et al., 2001). Case studies of contamination scenarios, as well as routine monitoring of areas with potential contamination risk (e.g., coastal research stations) are needed to facilitate decision making, which could result in "doing nothing", allow "natural attenuation" or apply "remediation activities". Hence, a better knowledge of the capacity of a local soil and its microbiota to support remediation is necessary for risk assessment.

Micro- and mesocosms experiments have shown that the bacterial communities in chronically affected Antarctic soils around fuel tanks can grow and support bioremediation, if the water content and nutrient ratios are in balance (Ferguson et al., 2003; Vázquez et al., 2009; Aislabie et al., 2012; Cury et al., 2015 and Martínez Álvarez et al., 2015). Even in soils with no history of pollution, some members of the native microbiota possess the ability to metabolize hydrocarbons (Okere et al., 2012). So far, most research on hydrocarbon degradation in Antarctic soils deals with remediation experiments and was focused on heterotrophic cultivable bacteria and whole communities from chronically contaminated sites (reviewed in Aislabie et al., 2006 and de Jesus et al., 2015). However, bacterial communities in soils recently affected by spills have been reported for East Antarctica and sub-Antarctica (Aislabie et al., 2001; Saul et al., 2005; Snape et al., 2006 and Powell et al., 2010) but, particularly in the coastal ecosystems of the Antarctic Peninsula, most reports on contamination refer to heavy metals and aromatic hydrocarbons (Santos et al., 2005; Curtosi et al., 2007). Very few of these include references to aliphatic hydrocarbon contamination, and most of the existing information is for marine sediments (Kennicutt et al., 1992; Martins et al., 2004; Prendez et al., 2011 and Dauner et al., 2015). In this work, we investigated the response of the bacterial communities of soils and sediments to accidental diesel contamination on ice-free and ice-covered soils at Carlini Antarctic Station, Antarctica and measured the concentrations of hydrocarbons, carbon and nitrogen contents at the contaminated sites to understand the fate of the spilled hydrocarbons and identify the microbial groups indicative of both, the contaminated and uncontaminated state of the soils around the station.

2. Materials and methods

2.1. Site description

The Argentinean Antarctic Station Carlini (formerly known as Jubany Station) is located in an ice-free area on the southern coastline of Potter Cove in the southwestern region of King George (25 de Mayo) Island, South Shetland Islands, WAP (62°14′S, 58°40′W, Fig. 1). With a polar maritime climate, with summer air temperatures generally above 0 °C during the day, repeated freeze-thaw events and higher precipitations than in continental Antarctic areas (Ganzert et al., 2011), Potter Cove and its surroundings are coastal ecosystems of great biological richness and diversity, including an Antarctic Specially Protected Area (ZAEP 132). Carlini Station, located at its borders, has been permanently active since 1953, first as a refuge and then as a research station since 1982. Among its facilities are the main and auxiliary powerhouses, installations for fuel pumping, and two fuel tank arrays. The power generation to support all station activities including the use of vehicles requires transport and handling of fuel, which creates the risk of accidental spills. Antarctic Gasoil (AGO) is the most commonly used fuel in the station, and Jet Propulsion fuel (JP1), gasoline, diesel engine and lubrication oils are used to a lesser extent for aircraft, boats and vehicles. In October 2009, two spills occurred at the location of the station fuel tanks: in one case diesel leaked from a broken pipeline onto frozen and ice-covered ground (Spill 1), and the second occurred from another pipe leakage below the containment basin into non-frozen ground with the consequent infiltration of the spilled hydrocarbons (Spill 2). A closer description of the spills is presented in Supplementary section S1.

2.2. Sample collection

Samples were collected from Carlini Station (soil) and Potter Cove (sediment) during austral summer in February 2010 and January 2011, four and fifteen months after the spills (Fig. 1). All the field studies and samplings performed during this work were previously reported to the environmental protection department of the Argentine Antarctic Institute and received the corresponding permits. A reference to all samples including sample names is given in Table 1.

Ten sites for soil sampling were selected within the area affected by the spills according to visual inspection. Four non-affected sites were also sampled as reference sites representing the background level of contamination within the station area: sites 1 and 3 at the shoreline, site 8 behind the storage tank containment basin, and site 9 behind the tanks. Site 10, which is located further inland behind the storage tanks, was considered a non-contaminated control site. This site is close to a lagoon from which water is drawn for drinking (Fig. 1). Sites 2, 4, 5, 6, and 7 were selected along the superficial hydrocarbon plume. Soils were collected to a depth of 5–10 cm (surface layer,

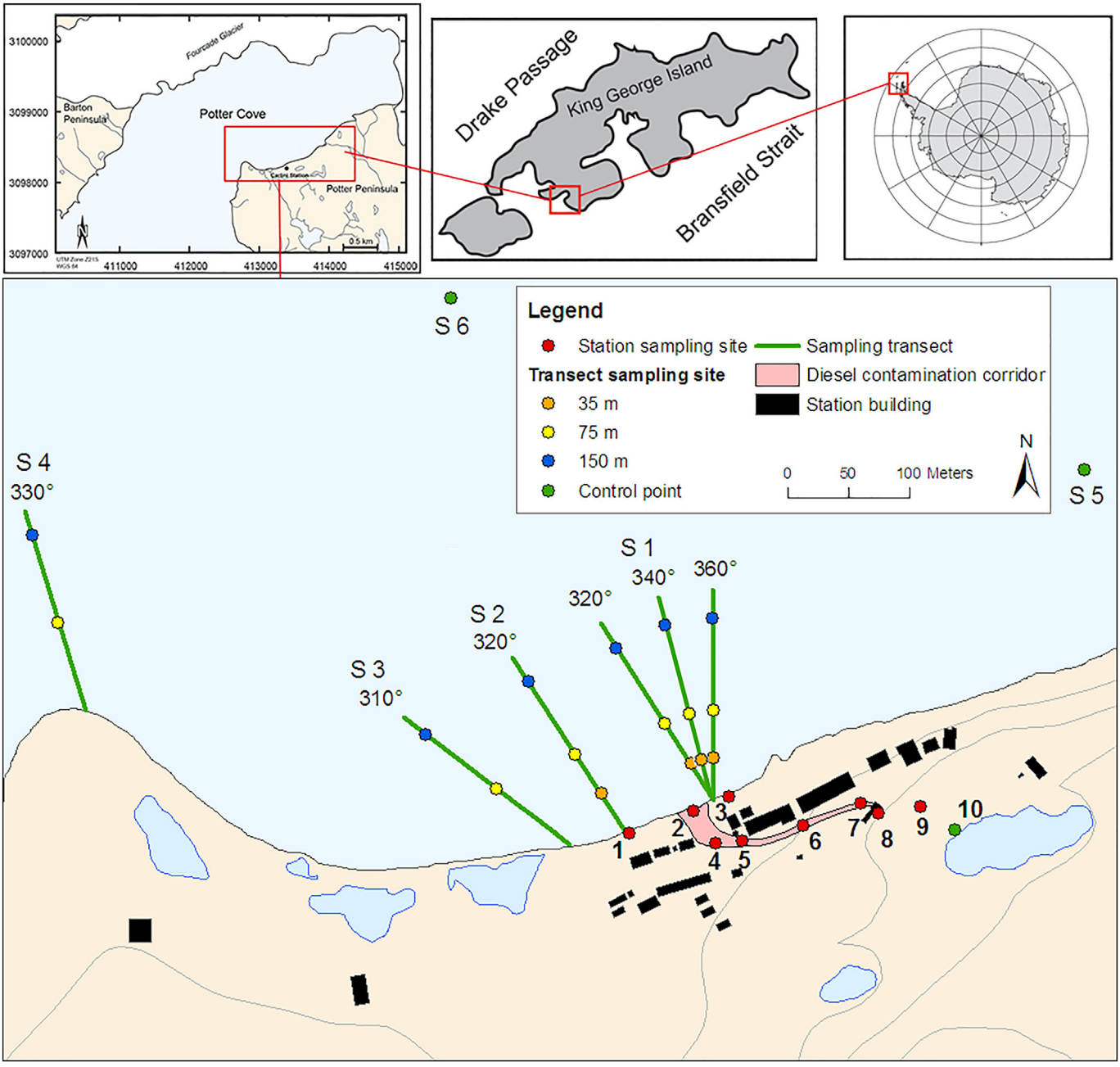

Fig. 1. Location of sampling sites at Carlini Station area and Potter Cove, King George Island (Isla 25 de Mayo), South Shetland Islands, West Antarctic Peninsula (62°14′18″ S; 58°40′05″ O). Soil sampling sites (numbered 1 to 10) and the names and direction of transects for sediment sampling (S1 to S4) are shown. Colored dots (orange, yellow and blue) indicate the starting points in land and the locations sampled along the transects. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

referred to as A), 30–35 cm (subsurface layer, referred to as B) and 90–95 cm (subsurface layer, referred to as C). Four soil samples were collected at each depth from pits dug into the soil at each sampling location using a hexane-rinsed trowel. The four sub-samples (≈200 g each) collected from the walls in each pit for each depth layer were combined into one homogenous composite sample per location and depth layer. Some sites could not be sampled in 2010 because the ground was covered by ice or flooded by meltwater.

Marine sediments were sampled at different distances offshore and along six transects, starting at four points on the shore and covering the area of potential contamination. Two more samples were taken at control sites (S5 and S6) close to the glacier and on the opposite side of the cove (Fig. 1). Samples were collected by scuba-divers with hand-held corers (10-cm diameter). The upper 0–5 cm and 10–15 cm depth sections of the cores were homogenized and used for chemical and microbial analyses. The two samples from each core are hereafter referred to as S (surface) and D (deep) sediment samples. For logistic reasons, not all sites of the six transects could be sampled in 2010, but every location was sampled in 2011. Soil and sediment samples were placed in glass jars washed with 10% hydrochloric acid, rinsed with MilliQ water and then with hexane. The samples were stored at −20 °C and transported to our laboratory at the University of Buenos Aires.

2.3. Chemical analysis

Total carbon (TC) and total nitrogen (TN) contents were measured using a CNS analyzer (Euro EA-CN Elemental Analyzer HEKAtech GmbH, Germany). Soils and sediments were dried (105 °C) and ground to fine powder in an agate ball mill (Retsch GmbH, Haan, Germany, agate ball diameter: 1 cm). The powder was again dried for 12 h at

Table 1
Reference to sample names, indicating the year of sampling (2010 or 2011), the sampling sites and depth in cm below soil surface or sea floor. Location and references to the sampling sites are shown in Fig. 1.

Soil						Sediment							
Year 2010			Year 2011			Year 2010				Year 2011			
Sample	Site	Depth (cmbs)	Sample	Site	Depth (cmbs)	Sample	Site	Distance offshore	Depth (cmbsf)	Sample	Site	Distance offshore	Depth (cmbsf)
5A_10	5	5–10	1A_11	1	5–10	S1A1S_10	S1A.1	75 m	0–5	S1A1S_11	S1A.1	75 m	0–5
7A_10	7	5–10	1B_11	1	30–35	S1A2S_10	S1A.2	150 m	0–5	S1A1D_11	S1A.1	75 m	10–15
7B_10	7	30–35	2A_11	2	5–10	S1A3S_10	S1A.3	35 m	0–5	S1A2S_11	S1A.2	150 m	0–5
8A_10	8	5–10	2B_11	2	30–35	S1B1S_10	S1B.1	75 m	0–5	S1A2D_11	S1A.2	150 m	10–15
8B_10	8	30–35	3A_11	3	5–10	S1B2S_10	S1B.2	150 m	0–5	S1B1S_11	S1B.1	75 m	0–5
9A_10	9	5–10	3B_11	3	30–35	S1B3S_10	S1B.3	35 m	0–5	S1B1D_11	S1B.1	75 m	10–15
9B_10	9	30–35	4A_11	4	5–10	S1C1S_10	S1C.1	75 m	0–5	S1B2S_11	S1B.2	150 m	0–5
10A_10[a]	10	5–10	4B_11	4	30–35	S1C2S_10	S1C.2	150 m	0–5	S1B2D_11	S1B.2	150 m	10–15
10B_10[a]	10	30–35	5A_11	5	5–10	S1C3S_10	S1C.3	35 m	0–5	S21S_11	S2.1	75 m	0–5
			5B_11	5	30–35	S21S_10	S2.1	75 m	0–5	S21D_11	S2.1	75 m	10–15
			6A_11	6	5–10	S22S_10	S2.2	150 m	0–5	S22S_11	S2.2	150 m	0–5
			6B_11	6	30–35	S23S_10	S2.3	35 m	0–5	S22D_11	S2.2	150 m	10–15
			7A_11	7	5–10	S51S_10[a]	S5	75 m	0–5	S31S_11	S3.1	75 m	0–5
			7B_11	7	30–35	S51D_10[a]	S5	75 m	10–15	S31D_11	S3.1	75 m	10–15
			7C_11	7	90–95	S61S_10[a]	S6	75 m	0–5	S32S_11	S3.2	150 m	0–5
			8A_11	8	5–10	S61D_10[a]	S6	75 m	10–15	S32D_11	S3.2	150 m	10–15
			8B_11	8	30–35					S41S_11	S4.1	75 m	0–5
			9A_11	9	5–10					S41D_11	S4.1	75 m	10–15
			9B_11	9	30–35					S42S_11	S4.2	150 m	0–5
			10A_11[a]	10	5–10					S42D_11	S4.2	150 m	10–15
			10B_11[a]	10	30–35					S51S_11[a]	S5	75 m	0–5
										S51D_11[a]	S5	75 m	10–15
										S61S_11[a]	S6	75 m	0–5

[a] Control samples (assumed not to be impacted by hydrocarbons). For soil samples, names refer to site number followed by depth (5–15 cmbs = A; 30–35 cmbs = B; 90 cmbs = C) and year of sampling. For sediment samples, names refer to the transect (with directions of transects S1 called 360° = A, 320° = B, 340° = C), followed by the sampling point in each transect (points sampled 75 m offshore = 1; 150 m = 2; 35 m = 3), depth of sediment layer (surface samples (0–5 cmbsf) = S, deep samples (10–15 cmbsf) = D) and year of sampling. cmbs: centimeters below surface; cmbsf: centimeters below sea floor.

105 °C and stored in a desiccator until analysis. Approximately 50 mg were transferred into clean tin capsules and weighed to the nearest 0.01 mg. The determination was done against acetanilide at 1020 °C, with times of combustion of 25 s and measurement of 420 s. The content of total inorganic carbon (TIC) was determined using a CM 5012 CO_2 coulometer coupled to a CM 5130 acidification module (UIC, USA). Total organic carbon (TOC) was calculated as the difference between TC and TIC (TOC% = TC% − TIC%). To guarantee the precision and accuracy of the methods repeated measurements of samples and in-house standards (Peru-1a, UT-S, Loess 1.5) were used. The precision of the analysis was ≤3.1% (TC), 25% (TN) and 1.2% (TIC) and its accuracy ≤0.4% (TC), −4.1% (TN) and 0.8% (TIC), respectively. Extraction and analysis of PAHs was performed according to Curtosi et al. (2007), with a limit of detection (LOD) between 0.16 and 0.35 ng g^{-1} and a limit of quantification (LOQ) between 0.53 and 1.17 ng g^{-1}, depending on the compound (see Supplementary Fig. S1). Concentration of total petroleum hydrocarbons (TPH) was determined by GC-FID using a HP 5890 Series II gas chromatograph with a DB-5 ms Ultra Inert column (30 m × 0.25 mm × 0.25 μm capillary tube) equipped with a flame ionization detector, with a LOD of 16 μg g^{-1} and a LOQ of 50 μg g^{-1}. Samples (12 g of soil or sediment) were extracted with 10 mL dichloromethane, adding 1 mL of a mixture of 50 μg mL^{-1} *p*-terphenil (CAS: 92-94-4) and 250 μg mL^{-1} cyclooctane (CAS: 292-64-8) in dichloromethane as internal standards. The average recovery using dichloromethane was 93.7% and the relative standard deviation as a measure for the precision of this method was 13.6% (n = 3). Helium was used as carrier gas at a flow rate of 4.43 mL min^{-1}. Samples (5 μL) were introduced in splitless mode at 285 °C injector temperature, cross calibrated with an in-house GOA standard. For calibration, we used 5 concentrations and performed a linear regression analysis with a correlation coefficient of 0.998. Initial oven temperature was 50 °C, held for 3 min followed by a ramp to 300 °C at 15 °C min^{-1}, held for 5 min and then a second ramp to 325 °C at 15 °C min^{-1} and held for 2.33 min. The FID was heated to 325 °C. Total area under resolved peaks and the UCM was integrated relative to internal standards.

Soil dry weight (dw) was determined from the weight loss after heat treatment (105 °C until constant weight) and the calculated water content used to express the TPH concentrations per gram of dry material.

Contour maps of TC/TN ratios in Potter Cove surface and deep sediments (Fig. 2a–c) were generated using Surfer v11 (Golden Software Inc., Colorado) following the Kriging procedure. Classed plots of TPH concentrations in soils (Fig. 2d–f) were constructed using the same software.

2.4. Bacterial community analysis

2.4.1. DGGE

Our first survey on the diversity of the soil and sediment bacterial assemblages was conducted by denaturing gradient gel electrophoresis (DGGE). Total genomic DNA was extracted from 2 g of well-homogenized soil or sediment material as previously described in Vázquez et al. (2009). One to 5 μL of the original or diluted DNA extracts were applied as template in 16S rRNA gene specific PCR reactions with GM5 plus GC-clamp as forward primer and 907RM as reverse primer (Muyzer et al., 1993). PCR conditions were as described in Gerdes et al. (2005). Fifteen μL of a pool of two independent PCR reactions per sample were analyzed by DGGE, based on the protocol of Muyzer et al. (1993) by using a gradient-chamber (DCode system, Bio-Rad Laboratories, USA). The patterns of the DGGE-bands were manually aligned and visually inspected for comparison.

2.4.2. Construction of 16S ribosomal RNA (rRNA) gene clone libraries and sequencing

Based on the DGGE-results six soil and two sediment samples were selected for reconstructing clone libraries to obtain detailed information on the community structure. Nearly full-length 16S rRNA gene

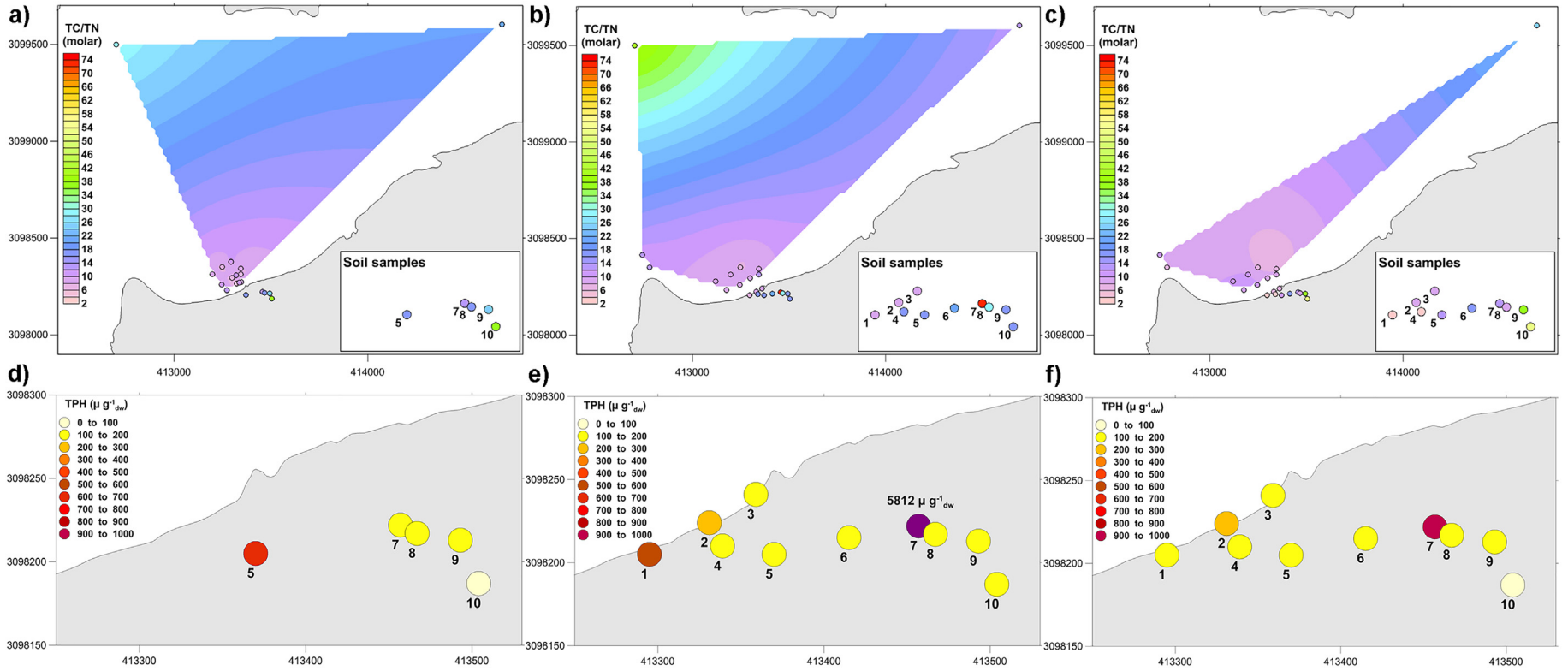

Fig. 2. Spatial distribution of the molar ratios of mean total carbon and total nitrogen (TC/TN) and hydrocarbon concentrations (TPH, in μg g^{-1}) in soils from around Carlini Station and sediments from Potter Cove, sampled in austral summers 2010 and 2011. a) TC/TN, surface sediment and soil samples, 2010; b) TC/TN, surface sediment and soil samples, 2011; c) TC/TN, deep sediment and subsurface soil samples, 2011; d) TPH, surface soil samples, 2010; e) TPH, surface soil samples, 2011; f) TPH, subsurface soil samples, 2011. Values for subsurface soils and deep sediments sampled in 2010 are (TC/TN; TPH): 7B_10 (49.7; 65.0), 8B_10 (45.4; 422), 9B_10 (35.8; 142), 10B_10 (40; 120), S51D_10 (26.7; below the detection limit) and S61D_10 (41.3; below the detection limit).

sequences were amplified by PCR and products were purified as described in Brinkmeyer et al. (2003). 16S rRNA gene clone libraries were constructed with the TOPO® TA Cloning® Kit (Invitrogen, USA). Approximately 120 clones were randomly picked from each sample and amplified by PCR with the flanking vector primers M13F and M13R. Amplified rDNA restriction analysis (ARDRA; Massol-Deya et al., 1999) was used to screen for 16S rRNA gene diversity within the clone libraries, using the restriction enzymes *Rsa*I and *Hae*III (5 U each). The resulting restriction patterns were clustered with the Bionumerics Gelcompare software (Applied Maths, Sint-Martens-Latem, Belgium) using Ward and Pearson correlation method and at least one representative of an ARDRA pattern group shared across all libraries was selected for sequencing, without losing information about the abundances of each pattern per library. Sequencing reactions were prepared using ABI BigDye and run on an ABI Prism 3700 DNA analyzer (Applied Biosystems, USA).

2.4.3. Clone libraries data analysis

All sequences were inspected for appropriate length and quality using the BioEdit software (Tom Hall, Ibis Biosciences). The final sequence dataset from the eight clone libraries (821 sequences in total) were processed using the MOTHUR software package v1.33.3 following the standard procedure for phylotype-based analysis (Schloss et al., 2009; https://www.mothur.org/wiki/454_SOP). Sequences were aligned against the SILVA 16S rRNA reference database file, filtered and screened to a length of 1383–1509 bp. Taxonomic assignment was done against the RDP database and the dataset was normalized to 96 sequences per sample (see Supplementary information containing the taxonomic assignment of the unique sequences and their frequency in each sample). For sample comparison, OTU abundances were standardized as percentage relative abundances. The relationship among bacterial community structures was evaluated using three-dimension non-metric multidimensional scaling (NMDS), with the Jaccard and Bray-Curtis dissimilarity metrics as a measure of community compositional distance between the eight clone libraries. The OTUs that were most important for the observed ordination were identified by Spearman correlation, and the vectors were overlaid on the NMDS.

Representative partial 16S rRNA gene sequences from each OTU were deposited in GenBank (NCBI) under the accession numbers KY190334–KY190953.

3. Results

3.1. Soil analyses

The total data set for TC and TN concentrations in all samples, TIC and TOC in sediments and water content in soils is listed in Supplementary Table S1. The TC/TN ratios are presented additionally in Fig. 2a–c. Total nitrogen in soils was very low (≤0.03%) whereas total carbon was quite variable (0.039–1.79%). Mean TC/TN molar ratios ranged from 5.5–74. Only for the highly-contaminated sample 7A_11 this ratio reflected the TPH content, while for the other samples with lower hydrocarbon concentrations (200–1000 μg g^{-1}_{dw}) the ratios were within the same range as those of the non-contaminated soils (except for 8B_10, with the lowest TN recorded). Sites 9 and 10, not directly affected by the fuel spill, had higher TC/TN ratios in the subsurface than in the surface layer because of very low nitrogen contents in subsurface soils. Molar TOC/TN ratios in Potter Cove sediments ranged from 4.6–10.3 (except for sample S1B3S_10: 2.2), with both control sites S5 and S6 displaying the highest content of inorganic carbon among all sediment samples. The highest contents of TOC were found in surface sediments taken 150 m offshore (transects S1, S2, S3 and S4, samples 2S, see Fig. 1 and Supplementary Table S1) with values between 0.45% and 1.18%. The high TOC values in those samples relate to more fine-grained sediments (Wölfl et al., 2014), with a slightly larger proportion of silt and organic rich material. Interestingly, in 2011 site S1B1 showed evidence of eutrophication with TN and TOC contents of 0.49% and 3.6% in the surface layer and 0.2% and 1.7% in the subsurface layer.

The water content by weight of soil samples was variable, ranging from 7.7% to 19.2% in subsurface samples and from 4.5% to 18.2% in surface soils except for sample 5A_2010 (31.0%). Site 5 lies in a small depression where meltwater running down from the higher surroundings accumulates.

The TPH contents of all sediment samples were below the detection limit. Soil samples from control sites 9 and 10 had low hydrocarbon concentrations, between 50 and 150 μg g^{-1}_{dw} (Fig. 2d–f). Four months after the spills, only the surface soil at site 5 had a slightly elevated TPH concentration of 671 μg g^{-1}_{dw} (Fig. 2d). At that time, a slight accumulation of hydrocarbons was also observed at a depth of 30 cm at site 8, with 422 μg g^{-1}_{dw}. One year later, the TPH concentration of surface soil at site 5 had returned to control level, indicating washout and/or degradation of hydrocarbons over time. In contrast, we found increased hydrocarbon concentrations in surface soils at site 1 (564 μg g^{-1}_{dw}), 2 (205–210 μg g^{-1}_{dw}) and 7 (5812 μg g^{-1}_{dw}) (Fig. 2e), as well as at a depth of 30 cm at sites 2 (209 μg g^{-1}_{dw}) and 7 (969 μg g^{-1}_{dw}) (Fig. 2f). The highest content of TPH in 2011 was detected at site 7 in front of the containment basin of the tanks, where the station crew detected Spill 2 (see Supplementary section S1).

The concentrations of total aromatic hydrocarbons (PAHs) were measured only in selected samples (sites 5 and 7 as representatives of contaminated soil, site 10 as a non-contaminated control, and site S1B1 as potentially affected sediment). The total content of PAHs varied between 37.4 and 1095 ng g^{-1} (Fig. 3). Corresponding with the TPH concentration measured, the highest total PAH content was found in the surface soil at site 7 sampled in 2011. With respect to the concentrations of the individual compounds (Supplementary Fig. S1) the highest values were again found at site 7A_11 where phenantrene, fluorene, pyrene and acenaphthene were the most abundant PAHs, except for benzo(a)anthracene, which was conspicuously higher in samples 10A_10 (close to the water lagoon: 45% of PAH_{total}) and 5A_11 (in the central station area: 80% of PAH_{total}). The concentrations of the low molecular weight (LMW) PAHs (2–3 rings, mainly phenanthrene, fluorene and acenaphthene) ranged between 3.3 and 629 ng g^{-1}, whereas the high molecular weight (HMW) PAHs (4–6 rings, mainly pyrene, benzo(a)anthracene and dibenzo(a,e)pyrene) varied between 30.4 and 467 ng g^{-1} ((Fig. 3).

3.2. Structure of bacterial communities

3.2.1. DGGE profiles

All samples were tested using DGGE fingerprints to assess differences in bacterial community structures between sampling sites and to detect sites that warranted further analysis. The DGGE banding patterns obtained revealed highly diverse communities with multiple taxa occurring in low abundances, resulting in multiple contiguous bands spanning the entire gradient, most of them of low intensity (Supplementary Fig. S2). Concerning soil samples, banding patterns from sites 2 and 6 (2011) showed almost no change with depth. At the other locations, surface and subsurface soil patterns were different. Also, the sediments sampled in 2011 along the transects S2 and S3 shared similar banding patterns regardless of depth, and the same pattern was observed in all sediments sampled along transect S4. However, samples taken closer to the coast (35–75 m, where sediments are mostly coarse-grained sand) and samples taken nearly 150 m offshore (where sediments are more fine-grained sandy mud) had remarkably different patterns. Due to the high number of thin low-intensity bands we had to visually analyzed the main differences between samples and selected eight representative samples for the construction of clone libraries: four soils from the origin site of the spill (samples 7A_10 and 7A_11) the site of accumulation of meltwater containing hydrocarbons (samples 5A_10 and 5A_11), two soils from a site not affected by the spills (samples 10A_10 and 10A_11), and two sediments from the

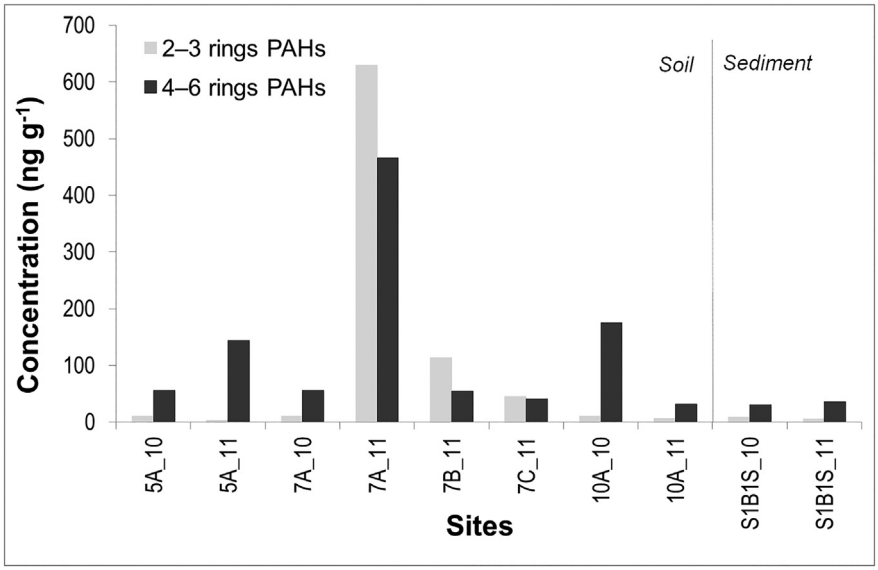

Fig. 3. Concentrations of total aromatic hydrocarbons (2–3 rings PAHs and 4–6 rings PAHs) in four sites from Carlini Station (soils) and one site from Potter Cove (sediments) sampled during austral summers 2010 and 2011.

area where the hydrocarbon plume reached the sea (samples S1B1S_10 and S1B1S_11). A direct comparison of the DGGE profiles of these eight samples is shown in Fig. 4, showing the differences in the bacterial communities of site 7 sampled in 2010 and 2011.

3.2.2. Clone libraries

A summary of the number of sequences, OTUs (phylotypes) defined and alpha diversity analysis of the eight clone libraries is shown in Table 2. Rarefaction curve analysis showed that the clone libraries almost reached full coverage of the bacterial communities (Supplementary Fig. S3). The Good's estimator of coverage was calculated to estimate the percent of the total species that was represented in each clone library, which was in the range of 70.8–84.4% (genus level) and 87.5–99% (phylum-class level). Sample 7A_11 had the lowest number of OTUs (only 30 OTUs). The number of clone sequences appearing only once (singletons) represented 44% of total OTUs for both sediment samples and 56–63% for the soil samples, except for 5A_11 with 40% singletons. Clones appearing only one and two times (singletons and doubletons) made up 67% of total OTUs in both sediment libraries and of 66–78% for the soil libraries.

The diversity indices calculated for the clone libraries indicate an overall loss in diversity in the highly-contaminated sample 7A_11 at both, low and high taxonomic rank, based on a loss of richness and evenness in the bacterial community, as indicated by the Shannon H′, Simpson 1/D, and Equitability J′ indices. The diversity of the bacterial communities from soil samples with low hydrocarbon content was similar to that of the control soils, and the diversity of sediment communities was similar to that of soil communities at genus level, but lower at higher taxonomic ranks.

The relative abundances of the dominant taxa found in soils and sediments are presented in Fig. 5a–b. Both sediment libraries showed a high abundance of *Bacteroidetes*, *Planctomycetes*, *Verrucomicrobia* and α-, γ- and δ-*Proteobacteria* (Fig. 5b). However, the sediment communities sampled in 2010 and 2011 were quite different. The most abundant lineages found in 2011 were α-*Proteobacteria*, *Verrucomicrobia* and *Bacteroidetes* whereas *Planctomycetes*, *Acidobacteria* and *Actinobacteria* were scarce. The most abundant taxa detected in the control and low-impacted soil libraries belong to the groups *Acidobacteria*, *Actinobacteria*, *Bacteroidetes*, *Gemmatimonadetes*, and α-, β-, and γ-*Proteobacteria*. *Actinobacteria* occurred in all soil samples, with different genera found in non-impacted vs. impacted soils. For example, *Nocardioides* were only present in the diesel-contaminated samples 5A_10 and 7A_11. On the contrary, *Acidobacteria* (affiliated mostly to the Gp4 and Gp6 groups) were almost absent in the sample with high content of hydrocarbons. The most striking feature of 7A_11 was the considerable enrichment of the betaproteobacterium *Polaromonas* sp. (related to *P. naphthalenivorans*). We found this bacterium only in

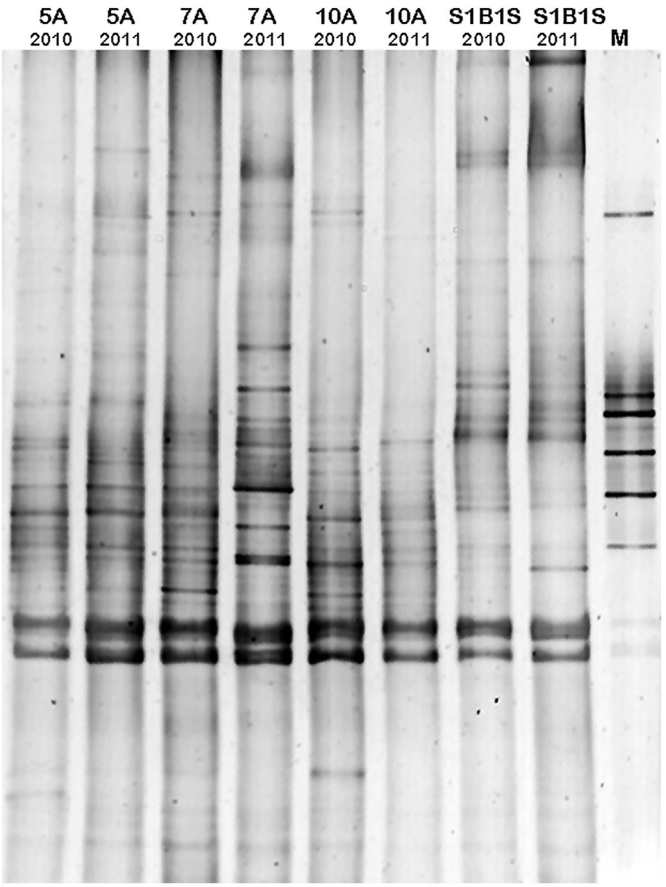

Fig. 4. DGGE profiles of the eight samples selected for construction of 16S rRNA gene clone libraries. Soil samples from sites 5, 7, and 10 were taken from the 5–10 cmbs horizon. Sediment samples from site S1B1 were taken from the surface layer (0–5 cmbsf) 75 m offshore from point S1, 320°.

Table 2
Summary of the alpha diversity analysis of 16S rRNA gene clone libraries obtained from the soil and sediment samples.

	5A_10	7A_10	10A_10	5A_11	7A_11	10A_11	S1B1S_10	S1B1S_11
Total number of clones/sequences: 768 (96 per clone library)								
Total number of OTUs (phylotypes) at low rank (genus, level 1) based on RDP taxonomy: 152								
Total number of OTUs (phylotypes) at high-rank (class-phylum, level 4) based on RDP taxonomy: 40								
OTUs at genus level (S_{obs})	46	39	41	47	30	48	34	36
Number of singletons	27	22	26	19	17	28	15	16
Number of doubletons	9	5	1	16	5	9	8	8
Shannon H′ (diversity)	3.493	3.291	3.378	3.698	2.585	3.617	3.168	3.285
Simpson 1/D (diversity)	28.861	23.147	28.679	54.286	6.230	41.081	21.209	25.909
Equitability J′	0.912	0.898	0.910	0.960	0.760	0.934	0.898	0.917
Good's estimator of coverage	0.7188	0.7708	0.7292	0.8021	0.8229	0.7083	0.8438	0.8333
OTUs at class level (S_{obs})	24	19	20	21	12	29	13	10
Number of singletons	9	7	7	5	5	12	3	1
Number of doubletons	4	2	1	3	1	5	0	2
Shannon H′ (diversity)	2.788	2.478	2.671	2.598	1.814	2.930	2.273	1.981
Simpson 1/D (diversity)	14.295	10.270	13.694	10.224	4.574	14.569	9.030	6.514
Equitability J′	0.877	0.842	0.892	0.853	0.730	0.870	0.886	0.860
Good's estimator of coverage	0.9063	0.9271	0.9271	0.9479	0.9479	0.8750	0.9688	0.9896

Equitability was calculated using the formula H′/lnS_{obs}, in which S_{obs} is the observed number of species (phylotypes). Good's estimator of coverage was calculated using the formula: 1 − (singletons / individuals).

diesel-impacted soils even when hydrocarbons were no longer detectable in high amounts.

The low-abundance community members (singleton and doubleton OTUs) were affiliated mainly with the *Proteobacteria* phylum (47%; α-, β-, γ- and δ-groups) and to the *Bacteroidetes* (18%), *Actinobacteria* (11%), *Acidobacteria* (7%), *Planctomycetes* (7%), *Firmicutes* (5%), and *Chloroflexi* (4%) phyla. The taxonomic position of most of the bacterial taxa found in soil and sediment samples are shown in four phylogenetic trees (Supplementary Fig. S4), indicating a close relationship of several clones to different hydrocarbon-degrading bacteria.

The OTUs identified by Spearman correlation as significantly decisive for the NMDS ordination of the samples are shown in Fig. 6. The presence of OTU7 (unclassified *α-Proteobacteria*) and the absence of OTU8 (unclassified *β-Proteobacteria*), OTU20 (*Rhizobacter*), OTU17 (*Conexibacter*), and OTU24 (*Acidobacteria* Gp7) significantly influence the separation of sediment from soil communities. The abundances of OTU1 (*Polaromonas*) and to lesser extent OTU19 (*Illumatobacter*) are the main factors for the separation of the diesel-impacted sites from the control sites in axes 1 and 3, whereas the absence of OTU3 (*Acidobacteria* Gp4) and OTU11 (unclassified *Chloroflexi*) reinforce the separation of sample 7A_11 from the other soils. The absence of OTU23 (*Nitrospira*) also influences the separation of site 7 from the other sites sampled on land.

4. Discussion

4.1. The fuel spill scenario

Baseline values of hydrocarbons in Carlini Station soils not directly affected by the diesel spills were below the threshold of 200 μg g^{-1} as recommended for environmental risk assessment for terrestrial environments within station limits (Australia, 2012). Four months after Spill 1, only surface soils at site 5 clearly exceeded this threshold value (Fig. 2d) and one year later, the soil samples collected within the area of the diesel plume were all below 200 μg g^{-1}. Directly at the site of the spills (Fig. 1, sample site 7) we detected high hydrocarbon

Fig. 5. a) Stacked bar graph illustrating the percent abundance of the 30 most abundant OTUs (phylotypes) at low rank (genus, level 1) based on RDP taxonomy and the percent abundance in all other remaining OTUs (1–6% abundant each) for the eight samples selected for clone libraries construction. Each of the 30 most abundant OTUs were putatively assigned to the genus level (RDP taxonomy). Assignments were as follows: OTU1, β-*Proteobacteria*: *Polaromonas*; OTU2, *Acidobacteria*: GP6; OTU3, *Acidobacteria*: GP4; OTU4, unclassified bacteria; OTU5, unclassified γ-*Proteobacteria*; OTU6, *Gemmatimonadetes*: *Gemmatimonas*; OTU7 and 28, unclassified α-*Proteobacteria*; OTU8 and 22, unclassified β-*Proteobacteria*; OTU9, α-*Proteobacteria*: *Sphingomonas*; OTU10, unclassified *Actinobacteria*; OTU11, unclassified *Chloroflexi*; OTU12 and 21, unclassified α-*Proteobacteria*; OTU13, unclassified *Planctomycetes*; OTU14, *Bacteroidetes*: *Haliscomenobacter*; OTU15 and 29, unclassified δ-*Proteobacteria*; OTU16, *Verrucomicrobia*: *Rubritalea*; OTU17, *Actinobacteria*: *Conexibacter*; OTU18, unclassified *Flavobacteria*; OTU19, *Actinobacteria*: *Ilumatobacter*; OTU20, γ-*Proteobacteria*: *Rhizobacter*; OTU23, *Nitrospira*: *Nitrospira*; OTU24, *Acidobacteria*, GP7; OTU25, *Bacteroidetes*: *Ferruginibacter*; OTU26, *Actinobacteria*: *Nocardioides*; OTU27, *Planctomycetes*: *Planctomyces*; OTU30, α-*Proteobacteria*: *Loktanella*. b) Stacked bar graph illustrating the percent abundance of the different high-rank taxonomic groups for all samples (class-phylum, based on RDP taxonomy).

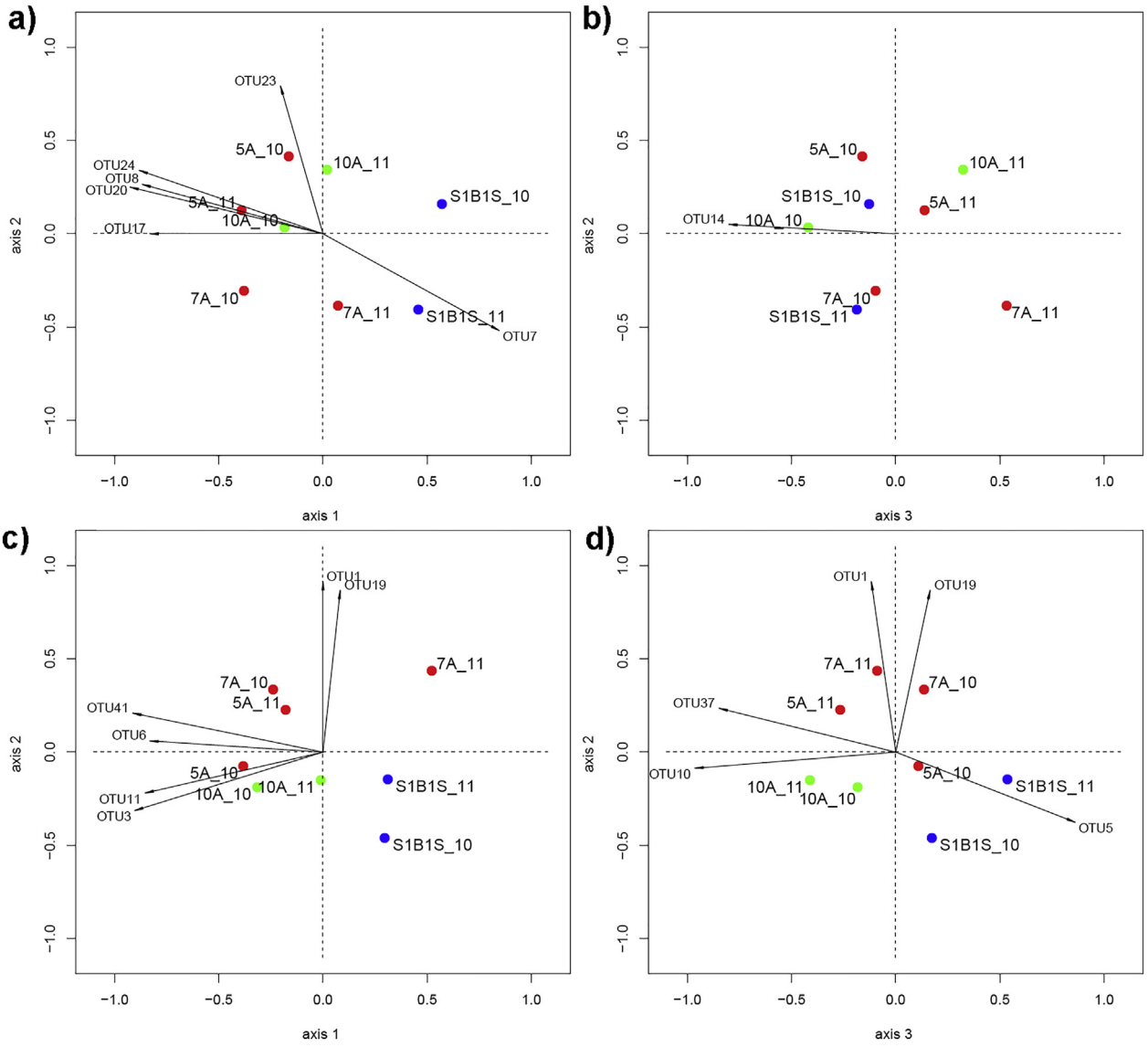

Fig. 6. NMDS plot based on Jaccard (a, b) and Braycurtis (c, d) distances between the eight samples selected for clone libraries construction with biplot of the OTUs significantly correlated (Spearman correlation) with the NMDS axes [stress = 0.16 (Jaccard), 0.13 (Braycurtis)]. NMDS, nonmetric dimensional scaling; OTU, operational taxonomic unit. Taxonomic assignment of OTUs: OTU1, β-*Proteobacteria*: *Polaromonas*; OTU3, *Acidobacteria*: GP4; OTU5, unclassified γ-*Proteobacteria*; OTU6, *Gemmatimonadetes*: *Gemmatimonas*; OTU7, unclassified α-*Proteobacteria*; OTU8, unclassified β-*Proteobacteria*; OTU10, unclassified *Actinobacteria*; OTU11, unclassified *Chloroflexi*; OTU14, *Bacteroidetes*: *Haliscomenobacter*; OTU17, *Actinobacteria*: *Conexibacter*; OTU19, *Actinobacteria*: *Ilumatobacter*; OTU20, γ-*Proteobacteria*: *Rhizobacter*; OTU23, *Nitrospira*: *Nitrospira*; OTU24, *Acidobacteria*, GP7; OTU37, *Acidobacteria*: GP3 and OTU41, unclassified *Proteobacteria*.

concentrations in surface soils (sample 7_2011, Fig. 2e) which diminished with depth. We attribute this to the underground leakage of Spill 2 into unfrozen ground, which is also likely the source of elevated hydrocarbon content in the subsurface soil layers (30 cm) at site 8 in 2010 (Supplementary section S1). We will continue studying Spill 2 to gain knowledge about the fate and migration of the hydrocarbons as they infiltrate soils (spills occurring on ice-free soils) in the coastal areas of Potter Peninsula.

Unlike hydrocarbons from Spill 2 that remained trapped underground at the time of sampling, hydrocarbons from Spill 1 that reached the cove transported by runoff waters were not detectable in Potter Cove sediments. This confirms previous analyses by Dauner et al. (2015) and matches observations of Prendez et al. (2011) for sediments in the vicinities of other coastal research stations in Antarctica. The fact that hydrocarbons do not accumulate in sediments shows that the natural dispersal and remediation capacity of the coastal marine areas is currently protecting the environment. However, Brussaard et al. (2016) proved that even small oil spills rapidly result in dissolved oil compounds with toxic effects for the biota, calling for a thorough monitoring of marine ecosystems for bioavailable toxic substances.

Total PAH concentrations in soil samples were within the range, but also higher, than those previously reported (Curtosi et al., 2007). According to the classification system of Benlahcen et al. (1997), the surface layer at site 7 next to the fuel tanks must be considered as highly contaminated (total PAH > 500 ng g^{-1}), whereas the other sites would be classified as slightly contaminated (total PAH < 250 ng g^{-1}) and do not represent an environmental hazard. Importantly, we measured exceptionally high concentrations of benzo(a)anthracene in samples 10A_10 and 5A_11, which are 5 or more times higher than those reported by Curtosi et al. (2007) for surface soils in Potter Peninsula (site C sampled in 2004), Curtosi et al. (2009) and Dauner et al. (2015) for sediments in Potter Cove (sampled in 2005 and 2011, respectively), as well as from soils with none or low contamination from other locations in Antarctica (Aislabie et al., 2004). As benzo(a)anthracene is formed when gasoline or garbage is burned and deposited as soot or black carbon, in the present case the sources would be the power

generation and the organic waste incineration. Naphthalene and methylnaphthalenes were present only in low amounts or were even absent in our samples although these compounds make up most of the aromatic hydrocarbons in Antarctic diesel. Curtosi et al. (2007) suggested that the low levels of PAHs in soils from Carlini Station are partly due to downward filtration of these substances towards the Potter Cove sediments, which eliminates LMW faster than HMW PAHs. This process of selective transport of LMW PAHs towards the sea and the marine ecosystems represents an increasing challenge to the conservation of the presently "near pristine" state of the marine environment of Potter Cove. The problem will exacerbate with climate change and aerial warming which currently ranges at 2.5 °C over 50 years in this region (Schloss et al., 2012) and accelerates thawing of the frozen soils on Potter Peninsula. Presently, most of the spilled fuels are released over frozen and snow-covered ground and mostly discharge via meltwater streams into Potter Cove where dispersion and transport with the circulating currents theoretically reduces the threat for the local ecosystems.

Elevated total carbon concentrations and unbalanced TC/TN ratios can be indicative of an anthropogenic impact, as shown for Antarctic soils from the Ross Sea Region (Aislabie et al., 2001, 2012 and Saul et al., 2005). Concerning organic carbon, molar TOC/TN ratios in Potter Cove sediments are representative of marine organic matter (Rumolo et al., 2011) and the variable TC and TN concentrations in Carlini Station soils and sediments provided no clear indications of the contamination event. Only the higher TC concentrations and TC/TN ratios of sample 7A_11 (related to Spill 2) with its very high hydrocarbon content could be clearly linked to the diesel contamination, whereas samples with hydrocarbon concentrations under 1000 μg g^{-1} did not show such extreme ratios, and could not be clearly distinguished from the ratios observed in sites with very low nitrogen content or in soils covered by mosses or vegetation. High TC values and TC/TN ratios can therefore give additional information on the general presence of hydrocarbons but cannot be used as indicators to detect oil contamination, especially in soils that contain terrestrial organic matter (mainly plant material in our study area) (Lee et al., 2009).

We know that the native bacterial communities from soils at Carlini Station have the metabolic capacity to promote remediation under proper oxygen and nutrient supplies (Mac Cormack et al., 2011). Yet, how this could affect hydrocarbon contaminations has never been studied. As Antarctic soils are in general very low in carbon, nitrogen, and phosphorous (Fritsen et al., 2000 and Tarnocai and Campbell, 2002), carbon levels will rise when diesel spills happen over the nutrient-poor sandy soils of the coastal areas of the Antarctic islands. This results in a C/N/P imbalance which prevents growth of microorganisms and limits degradation of the contaminants, particularly at low temperatures (Saul et al., 2005 and Aislabie et al., 2006). Thus, hydrocarbons can stay on the ground for years (Bargagli, 2008).

4.2. The response of bacterial communities to the spills

Bacterial communities in Antarctica are highly sensitive to differences in environmental conditions like water content, pH, nutrients, salinity or contaminants, all of which determine their structure (Chong et al., 2012). Our clone libraries constructed from six soils and two sediments had a high proportion of single phylotypes (44 to 63%), suggesting communities with a broad phenotypic diversity and metabolic versatility and thus able to react and adapt to the extremely changeable environmental conditions typical for the maritime Antarctic region. After a spill, the soils turn eutrophic only for carbon, and the toxicity of the fuel becomes a stress factor. Those members of the microbiota with the metabolic capacity for hydrocarbon degradation, N_2 fixation, and enhanced tolerance to stress can overgrow and dominate the community. To this end, we found a reduction in bacterial diversity in soil sample 7A_11 which had high content of hydrocarbons, with its microbiota becoming less rich and more even. This was earlier reported for other mineral soils in Antarctica, where an enrichment in hydrocarbon degraders was observed after major hydrocarbon contamination events (Saul et al., 2005; Chong et al., 2009; Aislabie et al., 2004, 2012 and Cury et al., 2015). In sample 7A_11 we found a strong enrichment of a bacterium closely related to *Polaromonas naphthalenivorans*, which is capable of naphthalene degradation and N_2 fixation (Hanson et al., 2012). Antarctic diesel has a low content of PAHs, which are mainly naphthalene and methylnaphthalenes (Aislabie et al., 2004). The very low concentrations of these PAHs persisting in the soil two years after the contamination event could likely be the result of bacterial degradation. We have no clear evidence yet for diazotrophic metabolism and in situ utilization of hydrocarbons for the local *Polaromonas* variation. Their enrichment in the contaminated soil provides, however, strong support for the assumption that *Polaromonas* might have selective advantages over other bacterial populations when temperatures drop below the freezing point, nitrogen is scant, and toxic compounds are present. In contrast, we found no distinctive pattern between the bacterial communities in soils with hydrocarbon concentrations below 1000 μg g^{-1} and the non-impacted soils. This proves that the autochthonous microbiota can indeed tolerate and even metabolize a certain quantity of organic compounds.

Most reports on bacterial communities from hydrocarbon polluted soils in Antarctica refer to heterotrophs, mainly cultivable bacteria, and were carried out in microcosms with simulated spills and involving different treatments to achieve remediation (a revision of literature on this topic was done in Aislabie et al., 2004, 2012 and de Jesus et al., 2015). Among the few studies done with untreated long-term contaminated soils, Saul et al. (2005) found that the phyla *Acidobacteria*, *Bacteroidetes*, *Firmicutes*, and Candidate TM7 (also known as phylum candidatus *Saccharibacteria*) were present only in control (non-contaminated) mineral soils on Ross Island. We found those phyla in both, control and contaminated soils, and *Firmicutes* were even enriched in the soil with high TPH content. On the contrary, *Acidobacteria*, a bacterial group known as one of the most abundant phyla in the soil habitats playing an important role in biogeochemical processes, dominated in soils with none or low hydrocarbon content but were nearly absent in the highly-contaminated soil. *Proteobacteria* were reported as dominant in oiled soils from King George Island (Foong et al., 2010), mainly represented by the genera *Sphingomonas*, *Sphingobium*, *Pseudomonas*, and *Variovorax*. In a study on King Georg Island at Carlini Station and neighboring areas on Potter Peninsula, Flocco et al. (2009) used the occurrence and diversity of *ndo* genes to trace the genetic potential for PAH biodegradation. Diverse *nah*Ac-like genes closely related to *Pseudomonas* species were found, and their relative abundance increased in response to anthropogenic pollution. In our clone libraries, we found the same dominant phyla (*Proteobacteria*, *Actinobacteria* and *Bacteroidetes*) reported by Cury et al. (2015) from sites with similar hydrocarbon contents at Keller Peninsula, South Shetland Islands. In addition, we also detected *Acidobacteria* and *Gemmatimonadetes* in Carlini soils with TPH content lower than 1000 μg g^{-1}. Moreover, Cury and colleagues postulated the absence of *Nitrospira*, *Verrucomicrobia*, *Chloroflexi*, and *Planctomycetes* in their samples to be a likely consequence of the toxicity of hydrocarbons, whereas we found those groups in soils with and without hydrocarbon contamination. Another difference is their report on candidate division TM7 as the most abundant member of the bacterial communities in soils with 155–426 μg g^{-1} TPH, whereas we found this group to represent only a very low proportion of the bacterial soil community. The samples in both studies underwent similar analytical treatments, and both sets are from the same island although from different locations, but obtained in the same year 2010. The observed differences in community composition can presumably be explained by the different primer sets we used, amplifying longer gene fragments in our case. This emphasizes the need to standardize the methodology to compare across locations and the need for customized bioremediation strategies even for neighbored places in Antarctica.

Our data underline the statements of Aislabie et al. (2012) that contamination changes the structure of Antarctic soil bacterial

communities in a site-specific and dynamic manner, depending on the environmental conditions. Therefore, only well-defined patterns of community change can be taken as indicators of hydrocarbon contamination, and those should be evaluated for each type of soil, environment, and age of contamination. In the case of the region studied here, a strong reduction in the abundance of *Acidobacteria* accompanied by a higher proportion of *Actinobacteria* (*Nocardioides*) and β-*Proteobacteria* appears to be indicative of the presence of hydrocarbons at concentrations over 1000 μg g^{-1}.

The two sediment clone libraries described here are the first recorded data for benthic bacterial communities in Potter Cove. They were constructed with the aim to understand the effect of diesel discharged in coastal waters and sedimentary microbiota. The site sampled to construct the sediment libraries (S1B1) was located within the area of the spill plume (Fig. 1) and showed carbon and nitrogen contents much higher in 2011 than in 2010. Both libraries appear quite similar although in 2011 the diversity at high-rank taxonomy decreased and the amount of chemoheterotrophic and aerobic bacteria increased. Particularly, this situation is indicative of communities living under eutrophic conditions, which agrees with the elevated C and N content found at site S1B1 in 2011 compared to 2010. Our search for typical hydrocarbon-degrading bacteria was, however, unsuccessful.

5. Conclusion

Diesel spills are frequent and nearly unavoidable incidents during handling of fuels at Antarctic stations. In coastal areas of the maritime WAP, soils underlie seasonal freeze-thaw cycles with and without snow cover, and it depends on the timing of a spill whether hydrocarbons can infiltrate the soil and migrate off-site or reach the sea and disperse. We had the opportunity to study both situations at Carlini Station and to learn more about different diesel spill scenarios. Hydrocarbons released over frozen and ice-covered ground quickly disperse with meltwater and precipitation. Once reaching the open water they are transported with surface waters and do not remain in soils and sediments for longer time, causing less damage to terrestrial and coastal marine biota. Although surface transport prevents contaminants from reaching the sediments and affecting the surrounding coastal area, the effect on the pelagic and shallow water communities before dilution and dispersal of the toxic compounds should, however, not be underrated (de Oliveira et al., 2015). Conversely, when spills occur over ice-free non-frozen soils or deeper down in the soil, as in case of the broken pipe under the Carlini fuel tanks, the fuel infiltrates the ground and slowly permeates into subsurface layers and migrates downhill (at the risk of reaching the coast and seafloor) altering the soil microbiota. In Antarctica, acting towards remediation is a major problem because the ground is free of ice only two to three months per year. In addition, the active layer is frozen most of the year favoring the persistence of hydrocarbons underground, while the repeated cycles of freezing and thawing and circulation of groundwater support their underground dispersal.

Our results from Carlini Station indicate that autochthonous microbiota in soils of the western maritime Antarctic have the potential to metabolize toxic compounds generated by human activity, including minor fuel spills. While this sounds like good news concerning the status of contamination by hydrocarbons on polar stations in the area, our findings reinforce the need of dense monitoring of environmental pollution and the development of appropriate contingency plans to quickly counteract accidental spills. A detailed description of the spill, the distribution of the hydrocarbons and the type of bacteria present in the affected soil with potential for biodegradation is mandatory to generate the background knowledge needed to assess feasibility of remediation and decide on the best strategies for removal of contaminants.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.scitotenv.2017.06.129.

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Title: Bacterial communities and chemical parameters in soils and coastal sediments in response to diesel spills at Carlini Station, Antarctica

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Section S1: Description of the spills and chronological presentation of the events.

The main spill (Spill 1, on ice-covered ground) occurred in spring 2009, when a pipe connecting the two auxiliary tanks which carried fuel to the powerhouse leaked an unknown amount of Antarctic diesel into the snow which, at that moment, almost completely covered the containment pool of the fuel tanks (Fig. 1, sample site 7, in the main text). Most of the contaminated snow was removed to recover the fuel, and further dispersion was contained using oil absorbent socks and pillows. The plume formed by the non-recovered diesel migrated downslope at the interface between snow and ice or frozen soil. Later, during early summer 2010 with the onset of snowmelt, diesel moved by surface runoff and reached Potter Cove (Fig. 1 in the main text). Once in the cove, the mixed tidal regime and the wind-driven clockwise circulation (Schloss et al. 2012) surface waters transported the hydrocarbons towards Maxwell Bay with surface waters. Nevertheless, some diesel migrated at the boundaries between snow and ice and infiltrated the soil wherever it was not frozen. The austral summer in 2010 was colder than usual and while the melting period usually starts in early December in this region, the soil was mostly covered by ice and snow until early March, when it rained for several days and the remaining ice melted. It was then that the station crew detected a new diesel spot on the ground that came from a leaking pipe buried below the spill containment basin, which was bypassed immediately (Spill 2, used as a model of spills occurring over ice-free ground). This place could not be sampled until the summer of 2011.

Image showing the two pipes that leaked diesel oil causing the two spills studied in this work

Schloss IR, Abele D, Moreau S, Demers S, Bers AV, Gonzalez O, Ferreyra GA. 2012. Response of phytoplankton dynamics to 19-year. 1991–2009. climate trends in Potter Cove. Antarctica. Journal of Marine Systems 92:53–66. doi: 10.1016/j.jmarsys.2011.10.006

Table S1: Mean total carbon (TC) and total nitrogen (TN) concentrations and TC/TN molar ratios of soils at Carlini Station and sediments from Potter Cove, sampled in austral summers 2010 and 2011 (for sample references see Table S1). For sediment samples also mean total inorganic carbon (TIC), total organic carbon (TOC) and TOC/TN molar ratios are shown.

	Sample	Water content (wt.%)	TN (wt.%)	TC (wt.%)	TC/TN ratio (molar)	
					Mean	Range
2010 (Soil)	**5A_10**	31.0	0.025 ± 0.005	0.309 ± 0.010	14.505	11.687 - 18.740
	7A_10	10.0	0.013 ± 0.003	0.140 ± 0.004	12.868	9.989 - 17.650
	7B_10	9.9	0.006 ± 0.002	0.273 ± 0.008	49.719	38.595 - 68.194
	8A_10	10.9	0.008 ± 0.002	0.106 ± 0.003	15.600	12.570 - 20.155
	8B_10	9.4	0.005 ± 0.001	0.180 ± 0.006	45.357	36.546 - 58.601
	9A_10	8.5	0.010 ± 0.002	0.194 ± 0.006	23.642	19.050 - 30.546
	9B_10	8.9	0.007 ± 0.001	0.226 ± 0.007	35.838	28.876 - 46.302
	10A_10	8.8	0.008 ± 0.002	0.251 ± 0.008	37.044	29.848 - 47.860
	10B_10	8.8	0.007 ± 0.001	0.231 ± 0.007	39.955	32.194 - 51.622
2011 (Soil)	**1A_11**	11.6	0.011 ± 0.002	0.073 ± 0.002	7.579	6.106 - 9.791
	1B_11	16.8	0.008 ± 0.002	0.039 ± 0.001	5.941	4.787 - 7.675
	2A_11	14.7	0.009 ± 0.002	0.076 ± 0.002	9.333	7.520 - 12.059
	2B_11	19.2	0.009 ± 0.002	0.054 ± 0.002	7.206	5.806 - 9.309
	3A_11	11.1	0.012 ± 0.002	0.096 ± 0.003	9.085	7.320 - 11.738
	3B_11	13.4	0.010 ± 0.002	0.063 ± 0.002	7.083	5.707 - 9.151
	4A_11	12.8	0.016 ± 0.003	0.206 ± 0.007	14.911	12.014 - 19.265
	4B_11	16.9	0.008 ± 0.002	0.039 ± 0.001	5.498	4.430 - 7.103
	5A_11	18.2	0.023 ± 0.005	0.314 ± 0.010	15.790	12.723 - 20.400
	5B_11	11.1	0.011 ± 0.002	0.100 ± 0.003	10.362	8.349 - 13.387
	6A_11	7.4	0.021 ± 0.004	0.335 ± 0.011	18.525	14.926 - 23.934
	6B_11	10.2	0.030 ± 0.006	0.451 ± 0.015	17.283	13.926 - 22.330
	7A_11	10.2	0.028 ± 0.007	1.794 ± 0.055	73.721	57.228 - 101.117
	7B_11	8.9	0.016 ± 0.004	0.195 ± 0.006	13.897	10.788 - 19.061
	7C_11	9.5	0.013 ± 0.003	0.202 ± 0.006	17.626	13.683 - 24.176
	8A_11	4.5	0.014 ± 0.003	0.323 ± 0.010	26.401	21.272 - 34.110
	8B_11	7.8	0.007 ± 0.001	0.054 ± 0.002	8.796	7.087 - 11.364
	9A_11	5.1	0.016 ± 0.003	0.251 ± 0.008	17.816	14.355 - 23.018
	9B_11	7.7	0.007 ± 0.001	0.225 ± 0.007	37.339	30.086 - 48.242
	10A_11	9.2	0.014 ± 0.003	0.188 ± 0.006	15.440	12.441 - 19.949
	10B_11	10.6	0.006 ± 0.001	0.290 ± 0.009	53.458	43.074 - 69.068

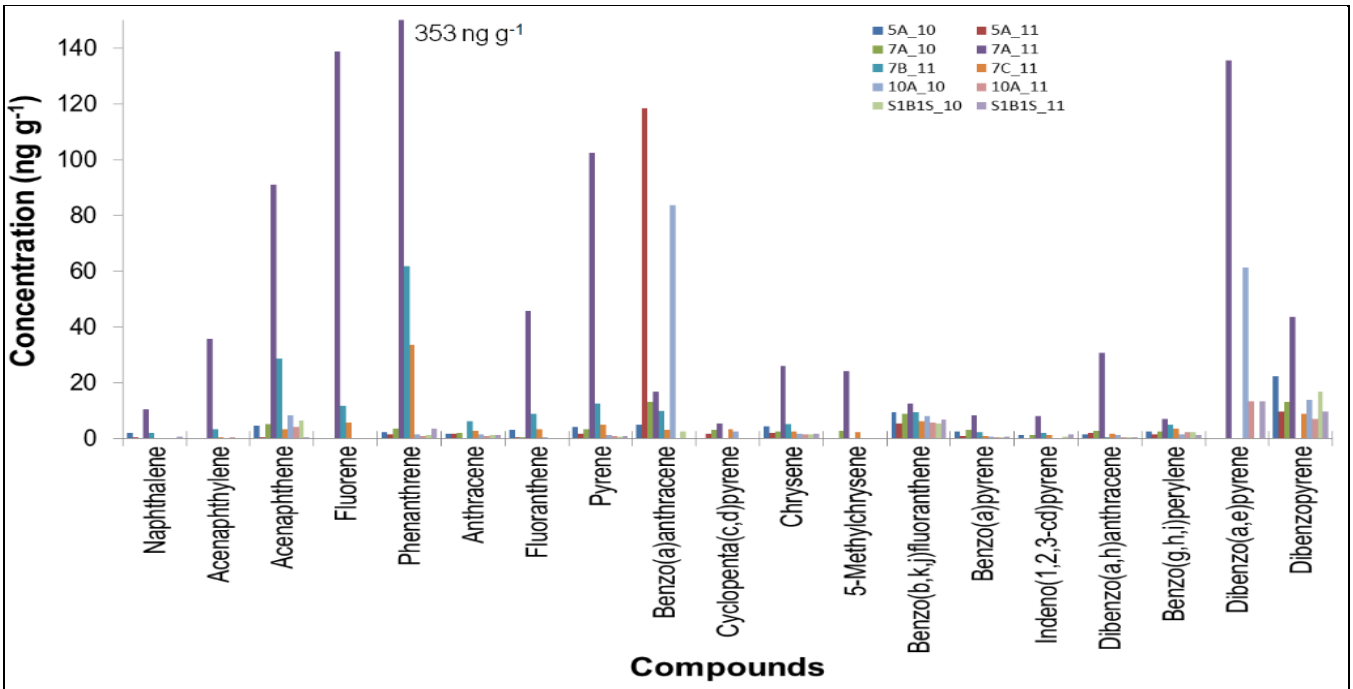
Table S1: continued

	Sample	TN (%)	TC (%)	TIC (%)	TOC (%)	TOC/TN ratio (molar)		TC/TN ratio (molar)	
						Mean	Range	Mean	Range
2010 (Sediment)	**S1A1S_10**	0.024 ± 0.005	0.156 ± 0.005	0.046 ± 0.001	0.109 ± 0.006	5.414	4.278 - 7.121	7.701	6.205 - 9.949
	S1A2S_10	0.118 ± 0.024	0.808 ± 0.026	0.068 ± 0.001	0.740 ± 0.027	7.347	5.895 - 9.529	8.022	6.464 - 10.365
	S1A3S_10	0.014 ± 0.003	0.116 ± 0.004	0.060 ± 0.001	0.056 ± 0.004	4.631	3.547 - 6.261	9.655	7.780 - 12.474
	S1B1S_10	0.026 ± 0.006	0.155 ± 0.005	0.037 ± 0.000	0.117 ± 0.005	5.304	4.064 - 7.363	6.995	5.430 - 9.594
	S1B2S_10	0.133 ± 0.033	0.829 ± 0.026	0.072 ± 0.001	0.757 ± 0.026	6.652	5.144 - 9.156	7.284	5.654 - 9.991
	S1B3S_10	0.028 ± 0.007	0.102 ± 0.003	0.049 ± 0.000	0.052 ± 0.004	2.194	1.639 - 3.116	4.256	3.304 - 5.838
	S1C1S_10	0.026 ± 0.005	0.255 ± 0.008	0.090 ± 0.001	0.165 ± 0.009	7.326	5.756 - 9.686	11.321	9.122 - 14.627
	S1C2S_10	0.067 ± 0.013	0.593 ± 0.019	0.103 ± 0.001	0.490 ± 0.020	8.594	6.858 - 11.203	10.391	8.372 - 13.425
	S1C3S_10	0.018 ± 0.004	0.166 ± 0.005	0.057 ± 0.001	0.109 ± 0.006	7.053	5.549 - 9.314	10.699	8.620 - 13.823
	S21S_10	0.015 ± 0.003	0.137 ± 0.004	0.054 ± 0.001	0.084 ± 0.005	6.516	5.096 - 8.650	10.696	8.618 - 13.819
	S22S_10	0.064 ± 0.013	0.519 ± 0.017	0.074 ± 0.001	0.445 ± 0.018	8.051	6.438 - 10.476	9.380	7.558 - 12.119
	S23S_10	0.014 ± 0.003	0.148 ± 0.005	0.082 ± 0.001	0.066 ± 0.006	5.605	4.262 - 7.624	12.519	10.087 - 16.175
	S51S_10	0.035 ± 0.007	0.604 ± 0.020	0.328 ± 0.004	0.276 ± 0.023	9.221	7.026 - 12.519	20.175	16.256 - 26.065
	S51D_10	0.018 ± 0.004	0.508 ± 0.016	0.357 ± 0.004	0.152 ± 0.021	9.887	7.111 - 14.060	33.135	26.698 - 42.810
	S61S_10	0.031 ± 0.006	0.793 ± 0.026	0.538 ± 0.006	0.255 ± 0.032	9.524	6.935 - 13.416	29.607	23.856 - 38.252
	S61D_10	0.018 ± 0.004	0.780 ± 0.025	0.622 ± 0.007	0.158 ± 0.033	10.354	6.838 - 15.638	51.226	41.275 - 66.183

Table S1: continued

	Sample	TN (%)	TC (%)	TIC (%)	TOC (%)	TOC/TN ratio (molar)		TC/TN ratio (molar)	
						Mean	Range	Mean	Range
2011 (Sediment)	**S1A1S_11**	0.049 ± 0.010	0.467 ± 0.015	0.204 ± 0.002	0.263 ± 0.018	6.301	4.898 - 8.411	11.177	9.005 - 14.440
	S1A1D_11	0.018 ± 0.004	0.144 ± 0.005	0.043 ± 0.001	0.102 ± 0.005	6.719	5.309 - 8.838	9.558	7.701 - 12.348
	S1A2S_11	0.113 ± 0.023	0.845 ± 0.027	0.041 ± 0.000	0.804 ± 0.028	8.274	6.651 - 10.713	8.696	7.006 - 11.235
	S1A2D_11	0.101 ± 0.025	0.568 ± 0.018	0.057 ± 0.000	0.512 ± 0.018	5.936	4.587 - 8.176	6.593	5.118 - 9.044
	S1B1S_11	0.488 ± 0.121	3.769 ± 0.116	0.133 ± 0.001	3.635 ± 0.118	8.696	6.740 - 11.944	9.015	6.998 - 12.365
	S1B1D_11	0.230 ± 0.057	1.819 ± 0.056	0.108 ± 0.001	1.710 ± 0.057	8.676	6.718 - 11.928	9.226	7.162 - 12.654
	S1B2S_11	0.152 ± 0.038	0.988 ± 0.031	0.070 ± 0.001	0.918 ± 0.031	7.029	5.440 - 9.669	7.566	5.874 - 10.378
	S1B2D_11	0.063 ± 0.016	0.408 ± 0.013	0.114 ± 0.001	0.294 ± 0.014	5.478	4.186 - 7.624	7.611	5.908 - 10.439
	S21S_11	0.030 ± 0.006	0.251 ± 0.008	0.075 ± 0.001	0.176 ± 0.009	6.859	5.420 - 9.022	9.755	7.860 - 12.604
	S21D_11	0.012 ± 0.003	0.110 ± 0.004	0.031 ± 0.000	0.080 ± 0.004	7.456	5.902 - 9.791	10.314	8.310 - 13.326
	S22S_11	0.033 ± 0.007	0.255 ± 0.008	0.047 ± 0.001	0.208 ± 0.009	7.435	5.930 - 9.699	9.106	7.337 - 11.765
	S22D_11	0.012 ± 0.002	0.105 ± 0.003	0.044 ± 0.001	0.061 ± 0.004	5.885	4.587 - 7.836	10.095	8.134 - 13.043
	S31S_11	0.041 ± 0.008	0.313 ± 0.010	0.058 ± 0.001	0.256 ± 0.011	7.243	5.775 - 9.448	8.881	7.156 - 11.474
	S31D_11	0.026 ± 0.005	0.269 ± 0.009	0.041 ± 0.000	0.228 ± 0.009	10.108	8.077 - 13.160	11.931	9.613 - 15.415
	S32S_11	0.169 ± 0.034	1.258 ± 0.041	0.077 ± 0.001	1.182 ± 0.042	8.178	6.570 - 10.596	8.709	7.017 - 11.252
	S32D_11	0.065 ± 0.013	0.577 ± 0.019	0.080 ± 0.001	0.497 ± 0.020	8.950	7.159 - 11.643	10.387	8.369 - 13.420
	S41S_11	0.011 ± 0.002	0.113 ± 0.004	0.057 ± 0.001	0.057 ± 0.004	5.743	4.415 - 7.739	11.508	9.273 - 14.869
	S41D_11	0.079 ± 0.016	0.530 ± 0.017	0.067 ± 0.001	0.463 ± 0.018	6.857	5.488 - 8.913	7.847	6.323 - 10.138
	S42S_11	0.122 ± 0.024	1.129 ± 0.036	0.139 ± 0.002	0.990 ± 0.038	9.499	7.605 - 12.347	10.831	8.727 - 13.993
	S42D_11	0.034 ± 0.007	0.319 ± 0.010	0.110 ± 0.001	0.209 ± 0.012	7.153	5.625 - 9.450	10.910	8.791 - 14.096
	S51S_11	0.065 ± 0.013	0.721 ± 0.023	0.230 ± 0.003	0.491 ± 0.026	8.851	6.979 - 11.664	12.995	10.470 - 16.789
	S51D_11	0.020 ± 0.004	0.396 ± 0.013	0.227 ± 0.003	0.169 ± 0.015	10.015	7.574 - 13.684	23.485	18.923 - 30.342
	S61S_11	0.021 ± 0.004	0.730 ± 0.024	0.561 ± 0.007	0.169 ± 0.030	9.503	6.497 - 14.023	41.034	33.063 - 53.015

Fig. S1: Concentrations of PAHs at four sites from Carlini Station (soils) and one site from Potter Cove (sediments) sampled during austral summers 2010 and 2011. bdl: below detection limit. nd*: not determined as the compound was not present in the reference standard (the compounds were detected in the samples but not calibrated)



Site	Naphthalene	Acenaphthylene	Acenaphthene	Fluorene	Phenanthrene	Anthracene	Fluoranthene	Pyrene	Benzo(a)anthracene	Cyclopenta(c,d)pyrene	Chrysene	5-Methylchrysene	Benzo(b,k,j)fluoranthene	Benzo(a)pyrene	Indeno(1,2,3-cd)pyrene	Dibenzo(a,h)anthracene	Benzo(g,h,i)perylene	Dibenzo(a,e)pyrene	Dibenzopyrene
5A_10	1.86	bdl	4.57	bdl	2.24	1.68	3.02	4	4.82	bdl	4.3	bdl	9.44	2.54	1.21	1.33	2.52	bdl	22.3
7A_10	bdl	bdl	5.12	bdl	3.59	2.01	0.07	3.2	12.9	3	2.61	2.65	8.75	2.89	1.18	2.66	2.45	bdl	12.9
10A_10	bdl	bdl	8.17	bdl	1.39	1.32	0.03	1.15	83.6	2.58	1.81	bdl	7.94	0.71	bdl	1.04	1.54	61.3	13.9
5A_11	0.042	bdl	0.12	bdl	1.43	1.75	0.44	1.73	118	1.74	2.05	bdl	5.5	0.79	bdl	1.86	1.37	bdl	9.61
7A_11	10.5	35.74	91	138.76	353	bdl	45.7	102	16.7	5.35	26.1	24	12.5	8.29	8.09	30.6	6.88	135	43.6
7B_11	1.99	3.22	28.5	11.72	61.7	6.08	8.69	12.6	9.76	bdl	5.06	bdl	9.43	2.13	1.89	0.4	4.81	bdl	bdl
7C_11	bdl	0.39	3.29	5.61	33.5	2.83	3.35	4.96	2.97	3.16	2.55	2.23	6.19	0.78	1.25	1.59	3.65	bdl	8.89
10A_11	bdl	0.24	4.17	bdl	0.87	0.99	bdl	0.78	bdl	bdl	1.38	bdl	5.54	0.44	bdl	0.39	2.19	13.4	6.97
S1B1S_10	bdl	bdl	6.5	bdl	1.13	1.22	bdl	0.77	2.53	bdl	1.42	bdl	5.29	0.32	0.68	0.49	2.15	bdl	16.7
S1B1S_11	0.77	bdl	0.32	bdl	3.53	1.14	bdl	0.94	bdl	bdl	1.64	bdl	6.71	0.57	1.55	0.3	1.24	13.2	9.65
LOD (ng g^{-1})	0.16	0.21	0.25	0.22	0.18	0.20	0.25	0.27	0.22	nd*	0.25	nd*	0.23	0.27	0.35	0.32	0.30	nd*	nd*
LOQ (ng g^{-1})	0.53	0.70	0.83	0.73	0.60	0.66	0.84	0.90	0.72	nd*	0.83	nd*	0.97	0.91	1.17	1.10	1.00	nd*	nd*

Fig. S2: DGGE profiles of all soil (A) and sediment (B) samples. PCR was performed with a proper dilution of each genomic DNA, using primers GM5clamp and 907R and DGGE was performed in 6% acrylamide, 30-70% formamide gels (100V for 18 h).The two thick bands at the lower part of each profile should not be considered as representative of any population within the communities, as these are primer-derived structures that did not run out of the gel under the conditions used.

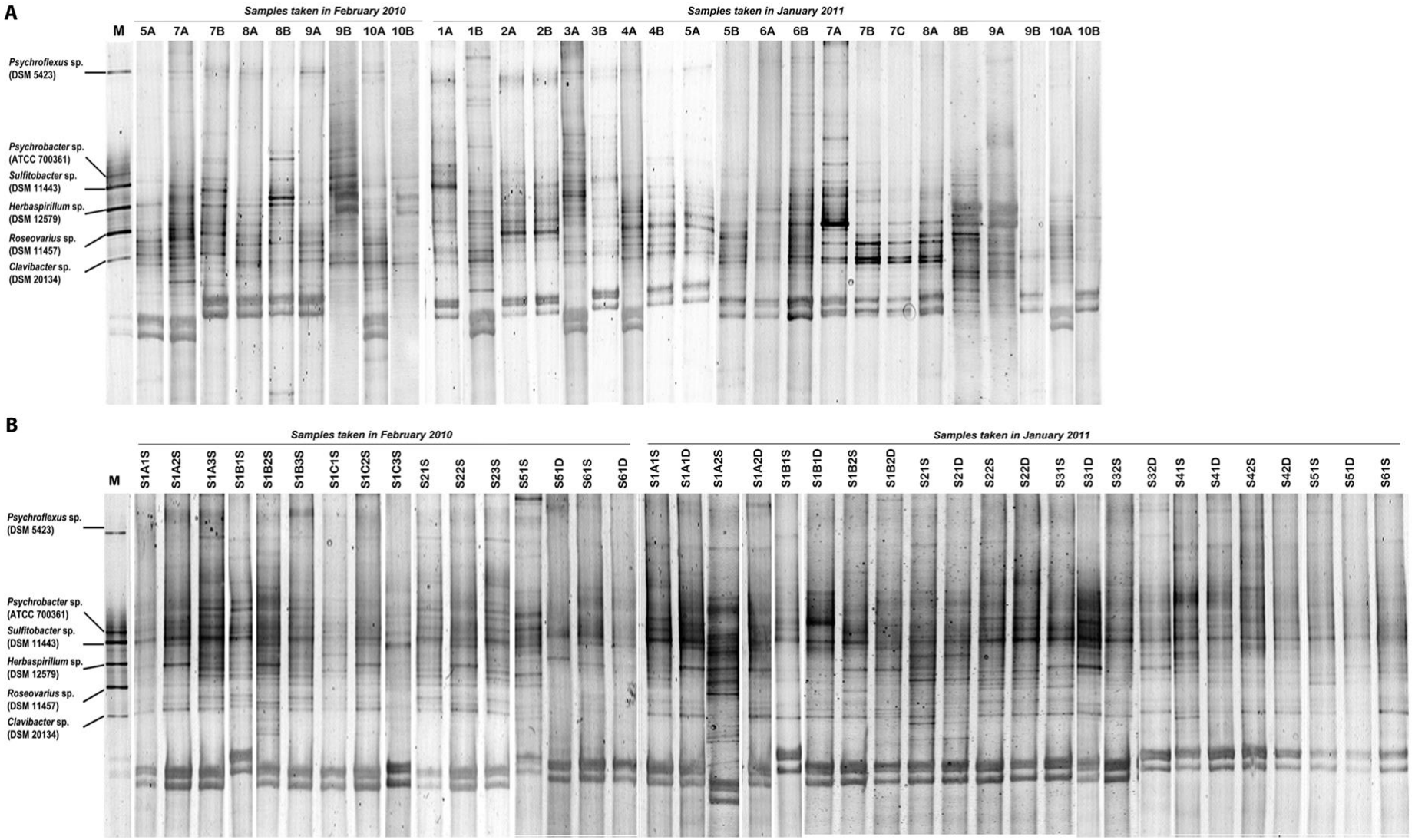


Fig. S3: Rarefaction curves generated for the 16S rRNA genes in the bacterial libraries (96 clones each) from diesel impacted soils (5A_10, 7A_10, 5A_11, 7A_11), non-impacted soils (10A_10, 10A_11) and sediments (S1B1S_10, S1B1S_11). The different clones were assigned to OTUs based on their affiliation at low and high taxonomy rank levels (genus, level=1 and phylum-class, level=4) using the phylotype-based analysis implemented in MOTHUR. The dotted lines indicate the 95% confidence interval.

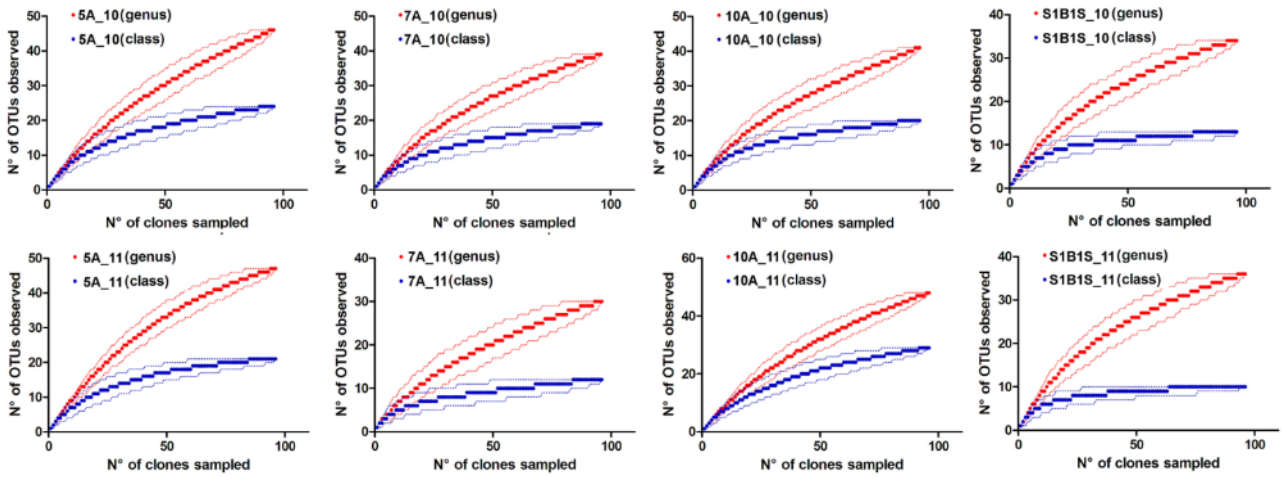
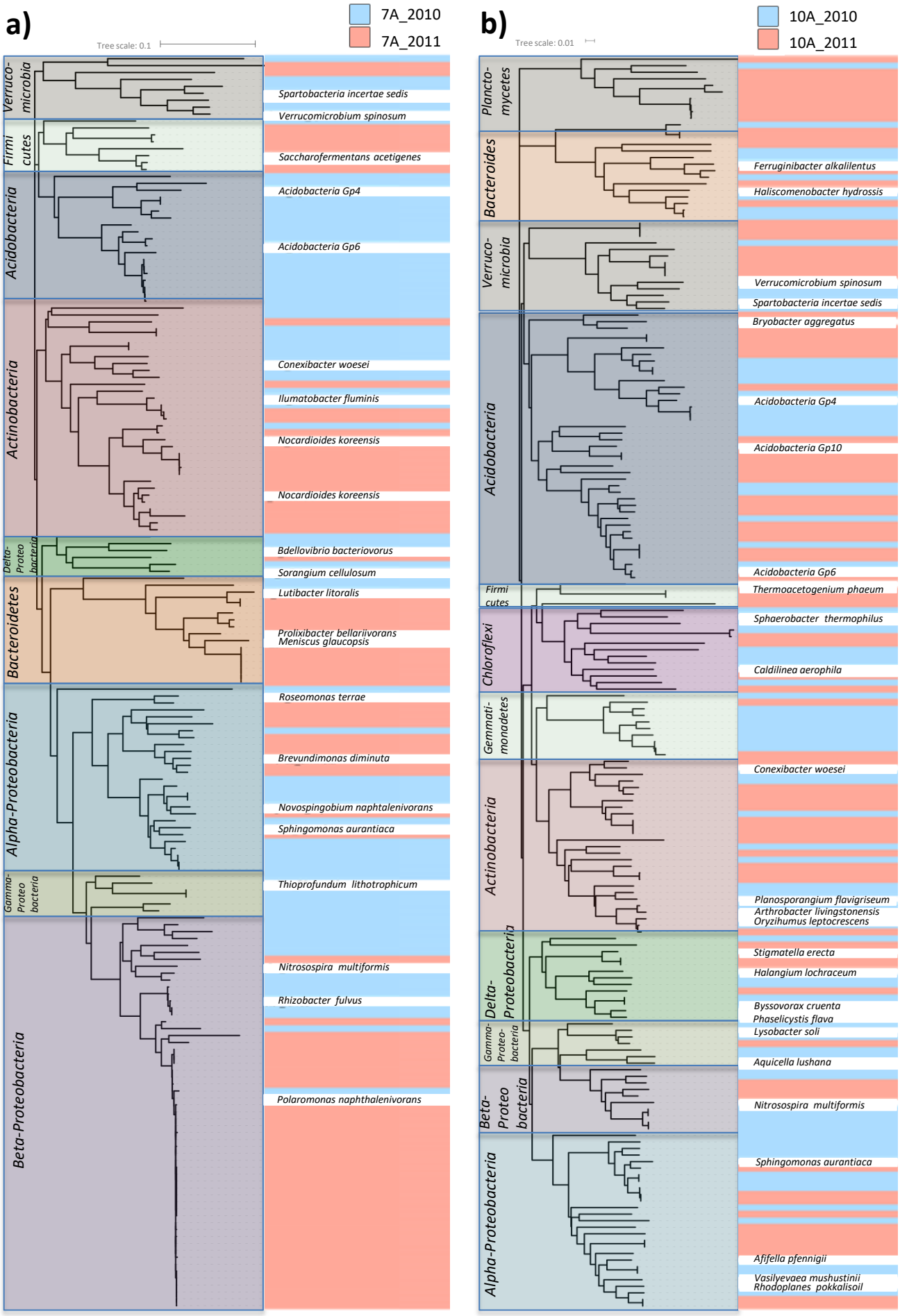
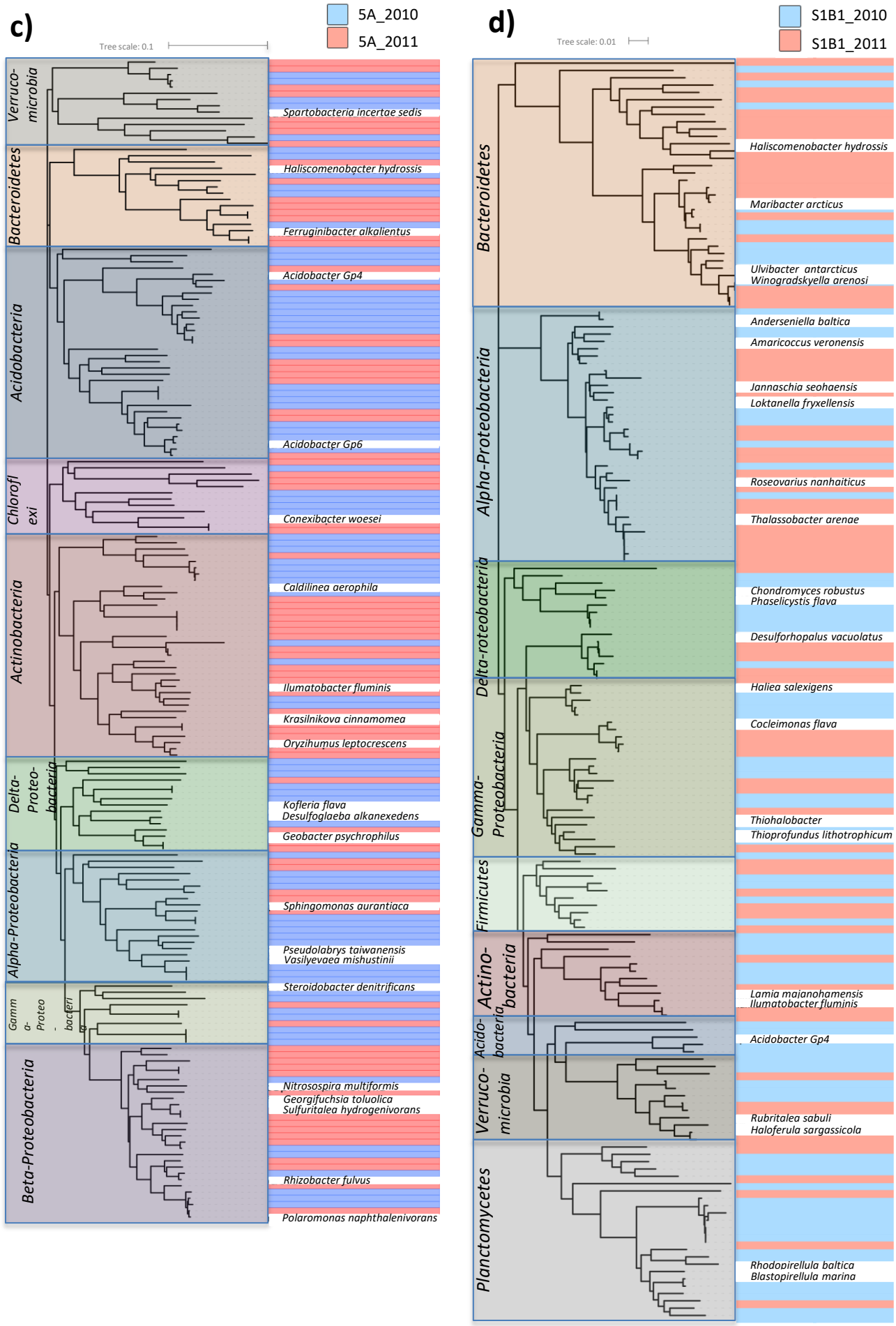


Fig. S4: Phylogenetic trees showing bacterial community structure in selected representative samples taken after two diesel spills at Carlini Station, South Shetland Islands, Antarctica. The trees were reconstructed using maximum-likelihood analysis. The scale bars indicate 1 or 10 % estimated sequence divergence. Sequences were aligned by means of the SINA Alignment Service (http://www.arb-silva.de/aligner/) and analyzed with the ARB software package (http://www.arb-home.de, arb release 5.5). Phylogenetic trees were reconstructed with the maximum-likelihood algorithm using only those sequences containing at least 1200 bases. The frequencies of all 16S rRNA gene ARDRA phylotypes obtained from all the clones analyzed per sample were considered in the tree. Several tree topologies were tested by performing distance matrix and parsimony analysis including different filter sets. The trees were displayed by the web-based tool iTOL (http://www.itol.embl.de).

a, b, c) Phylotypes present in soils at Carlini Station. 16S rRNA gene clones were recovered from contaminated sites 7A and 5A and a control site 10A. All sites were sampled in 2010 (blue) and in 2011 (red). Panel a) shows the sequences obtained from samples 7A_10 and 7A_11, panel b) shows the sequences obtained from samples 10A_10 and 10A_11, and panel c) shows the sequences obtained from samples 5A_10 and 5A_11. d) Phylotypes present in sediments from Potter Cove. 16S rRNA gene clones were recovered from site S1B1 in 2010 (blue) and one year later in 2011(red).


a)
7A_2010
7A_2011
Tree scale: 0.1
Verruco-microbia
Firmi cutes
Acidobacteria
Actinobacteria
Delta-Proteo bacteria
Bacteroidetes
Alpha-Proteobacteria
Gamma-Proteo bacteria
Beta-Proteobacteria
Spartobacteria incertae sedis
Verrucomicrobium spinosum
Saccharofermentans acetigenes
Acidobacteria Gp4
Acidobacteria Gp6
Conexibacter woesei
Ilumatobacter fluminis
Nocardioides koreensis
Nocardioides koreensis
Bdellovibrio bacteriovorus
Sorangium cellulosum
Lutibacter litoralis
Prolixibacter bellariivorans
Meniscus glaucopsis
Roseomonas terrae
Brevundimonas diminuta
Novospingobium naphtalenivorans
Sphingomonas aurantiaca
Thioprofundum lithotrophicum
Nitrosospira multiformis
Rhizobacter fulvus
Polaromonas naphthalenivorans
b)
10A_2010
10A_2011
Tree scale: 0.01
Plancto-mycetes
Bacteroides
Verruco-microbia
Acidobacteria
Firmi cutes
Chloroflexi
Gemmati-monadetes
Actinobacteria
Delta-Proteobacteria
Gamma-Proteo-bacteria
Beta-Proteo bacteria
Alpha-Proteobacteria
Ferruginibacter alkalilentus
Haliscomenobacter hydrossis
Verrucomicrobium spinosum
Spartobacteria incertae sedis
Bryobacter aggregatus
Acidobacteria Gp4
Acidobacteria Gp10
Acidobacteria Gp6
Thermoacetogenium phaeum
Sphaerobacter thermophilus
Caldilinea aerophila
Conexibacter woesei
Planosporangium flavigriseum
Arthrobacter livingstonensis
Oryzihumus leptocrescens
Stigmatella erecta
Halangium lochraceum
Byssovorax cruenta
Phaselicystis flava
Lysobacter soli
Aquicella lushana
Nitrosospira multiformis
Sphingomonas aurantiaca
Afifella pfennigii
Vasilyevaea mushustinii
Rhodoplanes pokkalisoil


c)
5A_2010
5A_2011
Tree scale: 0.1
Verruco-microbia
Bacteroidetes
Acidobacteria
Chlorofl exi
Actinobacteria
Delta-Proteo-bacteria
Alpha-Proteobacteria
Gamm a- Proteo - bacteri a
Beta-Proteobacteria
Spartobacteria incertae sedis
Haliscomenobacter hydrossis
Ferruginibacter alkalientus
Acidobacter Gp4
Acidobacter Gp6
Conexibacter woesei
Caldilinea aerophila
Ilumatobacter fluminis
Krasilnikova cinnamomea
Oryzihumus leptocrescens
Kofleria flava
Desulfoglaeba alkanexedens
Geobacter psychrophilus
Sphingomonas aurantiaca
Pseudolabrys taiwanensis
Vasilyevaea mishustinii
Steroidobacter denitrificans
Nitrosospira multiformis
Georgifuchsia toluolica
Sulfuritalea hydrogenivorans
Rhizobacter fulvus
Polaromonas naphthalenivorans
d)
S1B1_2010
S1B1_2011
Tree scale: 0.01
Bacteroidetes
Alpha-Proteobacteria
Delta-roteobacteria
Gamma-Proteobacteria
Firmicutes
Actino-bacteria
Acido-bacteria
Verruco-microbia
Planctomycetes
Haliscomenobacter hydrossis
Maribacter arcticus
Ulvibacter antarcticus
Winogradskyella arenosi
Anderseniella baltica
Amaricoccus veronensis
Jannaschia seohaensis
Loktanella fryxellensis
Roseovarius nanhaiticus
Thalassobacter arenae
Chondromyces robustus
Phaselicystis flava
Desulforhopalus vacuolatus
Haliea salexigens
Cocleimonas flava
Thiohalobacter
Thioprofundus lithotrophicum
Lamia majanohamensis
Ilumatobacter fluminis
Acidobacter Gp4
Rubritalea sabuli
Haloferula sargassicola
Rhodopirellula baltica
Blastopirellula marina