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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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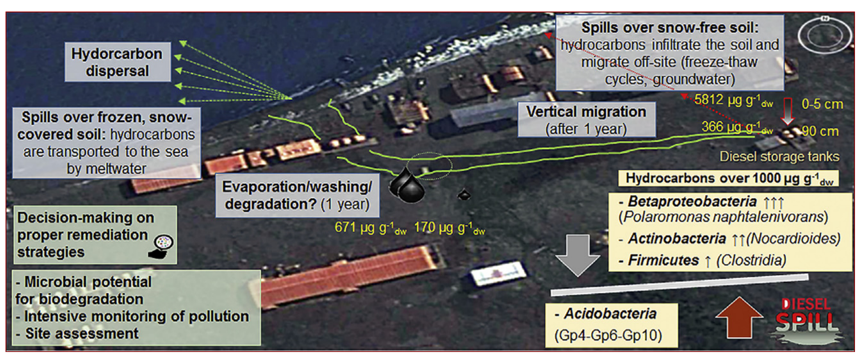
HIGHLIGHTS

- 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,