

INFLUENCE OF LIQUID WATER AND SOIL TEMPERATURE ON PETROLEUM HYDROCARBON TOXICITY IN ANTARCTIC SOIL

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Abstract—Fuel spills in Antarctica typically occur in rare ice-free oases along the coast, which are areas of extreme seasonal freezing. Spills often occur at subzero temperatures, but little is known of ecosystem sensitivity to pollutants, in particular the influence that soil liquid water and low temperature have on toxicity of petroleum hydrocarbons (PHC) in Antarctic soil. To evaluate PHC toxicity, 32 locations at an aged diesel spill site in Antarctica were sampled nine times to encompass frozen, thaw, and refreeze periods. Toxicity was assessed using potential activities of substrate-induced respiration, basal respiration, nitrification, denitrification, and metabolic quotient as well as microbial community composition and bacterial biomass. The most sensitive indicator was community composition with a PHC concentration effecting 25% of the population (EC25) of 800 mg/kg, followed by nitrification (2,000 mg/kg), microbial biomass (2,400 mg/kg), and soil respiration (3,500 mg/kg). Despite changes in potential microbial activities and composition over the frozen, thaw, and refreeze period, the sensitivity of these endpoints to PHC did not change with liquid water or temperature. However, the variability associated with ecotoxicity data increased at low liquid water contents. As a consequence of this variability, highly replicated ($n = 50$) experiments are needed to quantify a 25% ecological impairment by PHCs in Antarctic soils at a 95% level of significance. Increases in biomass and respiration associated with changes in community composition suggest that PHC contamination in Antarctic soils may have irrevocable effects on the ecosystem.

Keywords—Soil respiration Nitrification Denitrification Diesel contamination

INTRODUCTION

Petroleum hydrocarbons (PHC) are the most common type of contamination in cold regions [1], but there are no environment specific ecotoxicology data available for this region [2]. Most contamination in Antarctica is attributed to human activity associated with research stations that are located mainly in ice-free coastal oases. These rare habitats make up <0.05% of the land mass of Antarctica [3] and are important areas for birds, seals, vegetation, soil invertebrates, and microorganisms. Yet there is no information on the sensitivity of these areas to PHC contamination, and there are currently no remediation guidelines for Antarctica.

Toxicity guidelines for PHCs might differ in polar environments because of unique environmental characteristics, such as subzero temperatures and low liquid water content. In polar ecosystems, soil organisms remain active at temperatures below 0°C but are limited by liquid water content in frozen soil [4,5]. Liquid water is present in soil at temperatures below 0°C because of freezing point depression, in which salinity, capillary, and adsorptive forces lower the freezing point of water [6].

Liquid water content can be measured by time-domain reflectometry (TDR), a technique that measures the dielectric constant of the medium. Because the dielectric constant of water ($K_{\text{water}} = 80$) is much higher than other soil constituents ($K_{\text{air}} = 1$; $3 < K_{\text{soil}} < 7$; $K_{\text{ice}} = 3.2$), the dielectric constant of the medium is proportional to the amount of liquid water [7]. This technique is capable of measuring small quantities of

liquid water down to 0.05 m³ H₂O/m³ soil [7]. However, TDR probes must be calibrated to account for salinity, temperature, and soil type [8–10].

Toxicity of PHC is attributed mainly to a narcotic mode of action that is characterized by the disruption of the cellular membrane [11]. Petroleum hydrocarbons are widely reported to disrupt key soil ecosystem functions, such as respiration and nitrogen cycling [12–15]. Soil functions, such as microbial activity and community composition, can be used to assess the toxicity of contaminants in a soil ecosystem [13,16,17]. Nitrification and soil respiration are two important enzymatic activities carried out by soil microorganisms that are affected by PHC contamination [17–19]. Phospholipid fatty acid (PLFA) analysis provides information on microbial community composition and has been used by many researchers to show that soil microbial community composition is sensitive to PHC contamination [13,16,20,21]. Phospholipid fatty acid analysis is useful in ecotoxicity evaluations because it offers a technique that reflects the physiological stress, nutritional status, and viable biomass of the microbial population [16].

The purpose of the present study is to estimate PHC toxicity to key biogeochemical cycles for a fuel spill at an Antarctic research station. It is hypothesized that decreases in liquid water content will increase PHC toxicity to soil biogeochemical cycles because the organisms responsible for these processes will be under significant osmotic and thermal stress.

MATERIALS AND METHODS

Site description

Soils were sampled at a six-year-old PHC-contaminated spill site at Casey Station, East Antarctica (66°17'S, 110°32'E)

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during the austral summer of 2005 to 2006. The spill occurred during the 1999 austral winter when approximately 5,000 L of Special Antarctic Blend (SAB) diesel fuel leaked from a storage tank near the main powerhouse (MPH) [22]. Special Antarctic Blend fuel is a diesel fuel that contains a higher portion of the lighter fractions of PHCs [23]. Six years following the initial spill event, the contamination levels still exceeded 25,000 mg/kg.

A sampling grid was established at the MPH site using a Nikon total station (Nikon DTM-332). Thirty-two sampling locations were selected along a transect orthogonal to the diesel plume and identified by easting (m) and northing (m) based on a reference point (northeast corner of the boom surrounding the fuel tank on the north side of the MPH) that was given the coordinates (0, 0). Sampling locations encompassed a range of PHC contamination, from uncontaminated (0 mg/kg) to approximately 25,000 mg/kg. The concentration of fuel at each location was analyzed from a hexane extract (details on analysis in *Supporting Information*; <http://dx.doi.org/10.1897/08-434.S1>) at the beginning, middle, and end of the field season using a gas chromatograph fitted with a flame ionization detector [23] (*Supporting Information*; <http://dx.doi.org/10.1897/08-434.S1>). Soil from each location was also characterized for particle size distribution [24] (Horiba LA-950, laser scattering particle size distribution analyzer, Horiba Instruments), total organic carbon (loss on combustion at 550°C), and gravimetric water content ($[\text{mass of wet soil} - \text{mass of dry soil}]/[\text{mass of dry soil}]$; mass of dry soil was determined by drying at 105°C for 24 h) (*Supporting Information*; <http://dx.doi.org/10.1897/08-434.S1>). At each site, thermocouples and TDR probes were installed vertically in the soil at a depth of 8.5 cm to measure temperature and liquid water content.

Soil was sampled nine times throughout the field season to encompass frozen, thaw, and refreeze periods. Soil was sampled at a depth of 0 to 15 cm and passed through a 4.75-mm sieve to remove large rocks. Soils were subdivided into three portions—one portion was processed immediately for potential microbial activity, a second portion was stored at -20°C for soil characterization, while the remaining sample was stored at -80°C for PLFA analysis. Because regression analysis was the main statistical technique used in the present study, the number of sampling locations within the grid was optimized by taking one replicate per location per sampling time.

Soil temperature and volumetric water content were measured at time of sampling for each location. Temperature was measured using type K thermocouples (Cole-Parmer) and a handheld Digi-Sense Dual JTEK thermocouple thermometer (Cole-Parmer). Liquid water content was measured using TDR probes (constructed by researchers) with a TDR100, SDM50 50-Ω multiplexer, CR10X data logger, and PS100 12.0-V power supply (all from Campbell Scientific). To account for salinity and soil type, probes were calibrated at room temperature using uncontaminated site soil at eight different volumetric water contents (data not shown). Detailed calibration procedure for TDR probes is described by Siciliano et al. [25]. The probes are also sensitive to temperature; however, extensive calibrations would improve accuracy of frozen water only by 2.5% [26]. Since we expected the field variation of liquid water to be much greater than 2.5%, we did not calibrate the probes at frozen temperatures.

Toxicity assays

For each sampling period, soil from each location was analyzed for the following potential microbial activities: nitrification [27], denitrification (production and consumption of nitrous oxide) [28], basal respiration [19], substrate-induced respiration [19], and metabolic quotient [29]. Because regression analysis was performed on the ecotox data, only one replicate per location was collected per sampling period. These assays are standard techniques and are described in detail in the *Supporting Information* (<http://dx.doi.org/10.1897/08-434.S1>). The authors would like to emphasize that these assays are a measure of the potential activity under optimal conditions (nutrients, temperature, and liquid water).

Microbial community composition was assessed spatially (32 locations) and temporally (frozen, thaw, and refreeze periods) using PLFA analysis [16,20]. The fatty acids chosen to represent bacterial PLFA include i15:0, a15:0, 15:0, i16:0, 16:1 ω7c, 17:0, i17:0, cy17:0, 18:1 ω7c, and cy19:0 and were further broken down into Gram-positive (i15:0, a15:0, i16:0, i17:0) and Gram-negative (cy17:0, 18:1 ω7c, cy19:0) groups [16]. Fungal biomarkers include 18:2 ω6,9c as well as 16:1 ω5c and 18:1 ω9c [29]. Details for PLFA analysis and fatty acid nomenclature can be found in the *Supporting Information* (<http://dx.doi.org/10.1897/08-434.S1>).

Statistical analysis

Ecotoxicity data was analyzed for spatial and temporal differences in potential microbial activity and community composition. Regression analysis was performed on dose-response relationships between PHC concentration and potential microbial activities using SigmaPlot (Ver 9.01). Model of best fit was chosen from reparameterized equations for linear, logistic, and exponential models based on the optimal r^2 value [30]. The EC25 values were derived from model of best fit and represent the concentration that causes a 25% difference from the control response. The EC25 value represents a concentration value that will protect 75% of the soil ecosystem function and thus can be considered a site-specific remediation guideline for the particular endpoint the calculation has been based on. Significant differences in EC25 values between sampling periods were tested for using Zajdlik's ad hoc method number 2 [30].

Phospholipid fatty acid data was analyzed using nonmetric multidimensional scaling (NMS), an ordination technique suited to data that are nonnormal or on arbitrary or discontinuous scales [31], using PC-Ord (Ver 5.10). The parameters used for the NMS analysis were Sorensen (Bray-Curtis) distance, random starting configurations, and 250 runs with real and randomized data. The PLFA content was transformed on a logarithmic scale prior to NMS analysis. The correlation between environmental/soil variables (liquid water content, soil temperature, soil organic matter, PHC concentration) and NMS scores were determined. To compare between sampling times (frozen, thaw, and refreeze), a multiresponse permutation procedure was performed on the data. Multiresponse permutation is a nonparametric procedure that allows for testing the hypothesis of no difference between groups [31].

RESULTS

Petroleum hydrocarbon contamination altered PNA and soil respiration activities (Fig. 1), indicating that these activities are good indicators of PHC toxicity. Petroleum hydrocarbon

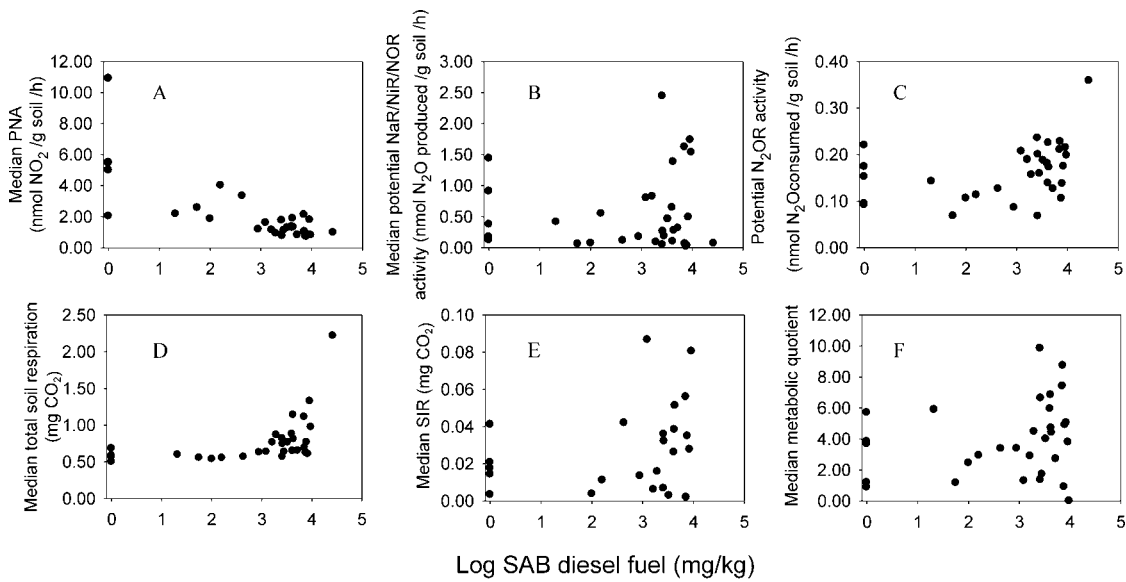


Fig. 1. Dose–response curves for potential microbial activities and diesel fuel contamination at the main powerhouse (MPH) spill site at Casey Station. The median response from nine sampling times at each sampling location is graphed for each ecotoxicological measurement endpoint (potential nitrification activity [PNA]) (A), potential nitrate reductase/nitrite reductase/nitric oxide reductase (NaR/NiR/NOR) activity (B), potential nitrous oxide reductase (N₂OR) activity (C), potential basal respiration (D), potential substrate-induced respiration (SIR) (E), and metabolic quotient (F) and are given on the y axis with Special Antarctic Blend diesel fuel concentration (mg/kg) on a log scale on the x axis.

contamination decreased PNA in an exponential fashion with an EC₂₅ of 2,000 mg/kg observed for the entire season, modeled by the equation $y = 5.731 \cdot 0.25^{x/3.302}$, $r^2 = 0.618$, where x is the log concentration of SAB diesel fuel and y is the potential nitrification activity. In contrast, PHC increased soil respiration in a logistic fashion with an EC₂₅ of 3,500 mg/kg observed for the entire season, modeled by the equation $y = 0.589 / \{1 + [(x/3.55)^{5.912}] \cdot (-0.25/1.25)\}$, $r^2 = 0.780$, where x is the log concentration of SAB diesel fuel and y is soil respiration. The other potential microbial activities, including denitrification (N₂O production and consumption), substrate induced respiration (SIR), and metabolic quotient, were not sensitive to PHC contamination (Fig. 1). The median value for each potential microbial activity were as follows: 1.3 nmol NO₂/g soil dry weight/h for PNA, 0.5 mg CO₂ for basal respiration, 0.01 mg CO₂ for SIR, 0.3 nmol N₂O produced/g soil dry weight/h for NaR/NiR/NOR enzyme activity, 0.2 nmol N₂O consumed/g soil dry weight/h for N₂OR enzyme activity, and 0.2 mg CO₂/μg C/g soil dry weight for metabolic quotient.

Similar to PNA and soil respiration, microbial community composition and microbial biomass were altered by PHC contamination during the frozen, thaw, and refreeze periods (Fig. 2). Approximately 83% of the variation in PLFA data was explained by axis 1 (23%) and axis 2 (60%). Axis 1 was correlated with TPH, $r = 0.408$, and $r = 0.216$ for axis 2. The decrease in axis 1 scores was linearly related to TPH concentration as modeled by the equation $y = 0.153 - [(0.25 \cdot 0.53x) / 800]$, $r^2 = 0.202$, where x is the concentration of SAB diesel fuel and y is the NMS score for axis 1.

The TPH dependence on community composition is seen in all three sampling periods (frozen, thaw, and refreeze). When the community composition between sampling periods are compared by multiresponse permutation analysis, a difference is observed between frozen and thawed soil ($p = 0.07$) as well as between thaw and refreeze samples ($p = 0.02$) but not between frozen and refreeze sampling periods ($p = 0.27$). The multiresponse permutation analysis indicates that a shift

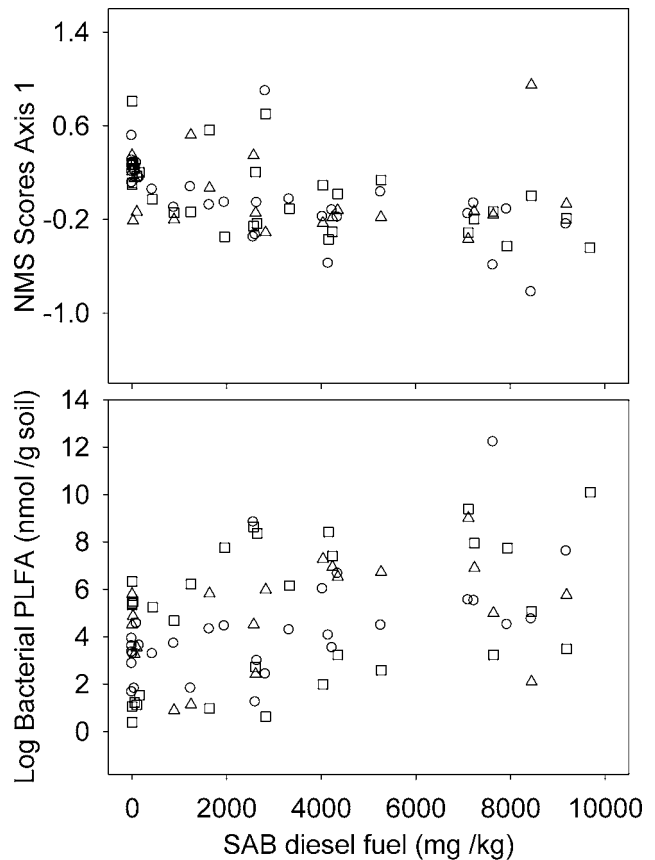


Fig. 2. Microbial community composition changes for frozen, thaw, and refreeze periods. Nonmetric multidimensional scaling (NMS) scores for axis 1 are shown as a function of Special Antarctic Blend (SAB) diesel fuel concentration ($r = 0.408$) (top panel). Log of bacterial phospholipid fatty acid (PLFA) content is shown as a function of SAB diesel fuel concentration (bottom panel). Δ = frozen period; \circ = thawed period; \square = refreeze period.

Table 1. Concentration values effecting 25% of the population (EC25) and regression parameters for select measurement endpoints

	Frozen	Thawed	Refreeze	Combined ^a
Potential nitrification activity				
Model	Exponential	Exponential	Linear/exponential	Exponential
EC25 ^b (mg/kg)	200AB	1,000AC	20B/24,000C	2,000AC
SE ^c	400	1,600	6.70/62,000	2,500
<i>r</i> ²	0.353	0.420	0.420/0.443	0.618
Potential basal respiration				
Model	Logistic	Logistic	Logistic	Logistic
EC25 (mg/kg)	5,400D	2,400D	3,000D	3,500D
SE	9,600	3,100	3,500	1,700
<i>r</i> ²	0.447	0.284	0.276	0.780
Microbial community composition				
Model	Linear	Linear	Linear	Linear
EC25 (mg/kg)	NA ^d	670E	880E	800E
SE	NA	175	220	170
<i>r</i> ²	NA	0.385	0.306	0.202
Bacterial biomass				
Model	Linear	Linear	Linear	Linear
EC25 (mg/kg)	3,800F	1,700F	2,500F	2,400F
SE	2,700	680	1,500	730
<i>r</i> ²	0.145	0.338	0.151	0.202

^a Combined refers to the all nine sampling periods.

^b Different capital letters represent significant differences between sampling periods at the 95% level of significance. Statistical significance in EC25 was tested only between sampling period and not between measurement endpoint.

^c SE = standard error.

^d NA = not available.

in microbial community occurs when the soil goes from a frozen to thawed state but then returns to the same condition when the soil refreezes.

Microbial biomass increased linearly as PHC contents in soil increased (Fig. 2, bottom panel) with an EC25 value of 2,400 mg/kg for the combined season, modeled by the equation $y = 3.5 - [(-0.25 \cdot 0.35x)/2,400]$, $r^2 = 0.202$, where x is the concentration of SAB diesel fuel and y is the log bacterial PLFA content. While general bacterial PLFA biomarkers indicated an increase in bacterial population with an increase in PHC concentration, the biomarkers assigned specifically to Gram-negative and Gram-positive bacteria did not show a change with PHC contamination. Similarly, the fungal population did not show a response to PHC contamination.

Potential nitrification activity, soil respiration, microbial community composition, and bacterial biomass were sensitive indicators of PHC toxicity with EC25 values of 2,000, 3,500, 800, and 2,400 mg/kg soil, respectively, for combined sampling periods (Table 1). The EC25 values were not calculated for the remaining potential microbial activities because of poor dose–response curves generated by these endpoints. Over the frozen, thaw, and refreeze period, EC25s fluctuated but did not significantly change, with the exception of the refreeze period for PNA (Table 1). However, the dose–response relationship obtained for PNA during the refreeze sampling period was difficult to model, as EC25 values vary greatly, depending on the type of regression model applied (20 mg/kg determined by linear model and 24,000 mg/kg determined by exponential model, with similar r^2 values of 0.443 and 0.420, respectively). Therefore, we are reluctant to interpret the significant differences in EC-25 values for PNA as real differences. For the remaining endpoints, confidence limits for EC25 values overlapped between sampling periods, indicating that there is no difference in toxicity between sampling periods.

Despite liquid water content and temperature ranging from

0.04 to 0.56 cm³ water/cm³ soil and –11.2 to 12.1°C, there were no associated changes with potential microbial activities (Fig. 3). However, variability of the potential activities increases at low liquid water content, especially for PNA (Fig. 3). The PNA variability was much higher than that observed for basal respiration with a maximum coefficient of variation

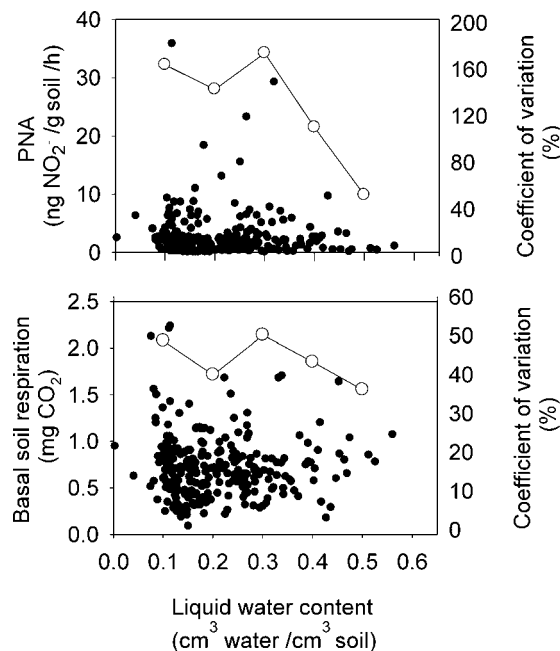


Fig. 3. Potential nitrification activity (PNA) and soil basal respiration as a function of liquid water content. Coefficient of variation (CV) increases at low liquid water content, especially for PNA. To calculate CV, volumetric liquid water content was binned into six equal bins, ranging from 0 to 0.59 cm³ water/cm³ soil, with a minimum bin size of 40.

(CV) of 174% for PNA compared to 56% for basal respiration. For both PNA and basal respiration, CV was linked to liquid water contents with $y = -0.2557x + 2.0542$, $r^2 = 0.677$, for PNA and $y = -0.0219x + 0.5018$, $r^2 = 0.345$, for basal respiration, where x is the liquid water content and y is the potential nitrification activity and soil respiration, respectively. Potential nitrification activity and soil basal respiration are the only microbial activities shown in the graph because of their PHC dependence; however, the variance of all microbial activities increased at low liquid water contents. For example, maximum CV for N_2O consumption and N_2O production were both observed at 0.10 to 0.19 cm^3 water/ cm^3 soil with values of 175 and 82%, respectively.

DISCUSSION

The present study may be the first of its kind to study the effect of liquid water content and soil temperature on the toxicity of PHC. Despite large shifts in liquid water, from 0.04 to 0.56 cm^3 water/ cm^3 soil, and temperature, from -11.2 to $12.1^\circ C$, there was relatively little change in PHC toxicity to potential microbial activity or community composition. It should be noted that this does not imply that in situ activities are insensitive to temperature or liquid water but rather that toxicity assays used to assess soil functioning in response to toxicant impacts are not confounded by large temperature or liquid water changes at the study site. Thus, potential soil functioning tests may be an effective means for toxicologists to reduce the confounding effects of variable climatic and moisture parameters when assessing PHC impact at a contaminated site.

Although the potential activity assays used in the present study were indicative of PHC toxicity, they may be either underestimating or overestimating in situ toxicity. The potential microbial activity assays were performed at $10^\circ C$, and the optimal temperature for cold-adapted soil microorganisms living in Antarctic soil may not be the same. Therefore, the results obtained by these assays could be different if performed at a different temperature. In addition, the nature of the assays (i.e., shaken in a slurry with additional nutrients) changes other variables that affect microbial activity. For example, nitrification activity is affected by temperature, moisture, NH_4^+ , and oxygen, all of which are altered in the PNA assay by the change in temperature, addition of nutrients, and the mechanical shaking of the slurry. While these limitations do not affect the validity of the results, they are important factors to consider to ensure that the data are not overinterpreted.

Although little evidence was found for either liquid water content or soil temperature having an influence on the toxicity of PHC to potential microbial activities, we did observe an increase in variability of microbial activity at low liquid water contents. High variability in soil properties is often associated with soil in polar regions because of a process known as cyroturbation, where soil from the subsurface is mixed with surface soil by frost heave action [32]. In the present study, high variability was observed in biological parameters as a function of liquid water content at subzero temperatures. Other researchers have shown phytotoxicity tests results to be exceptionally variable in Arctic soils compared to temperate soils [33]. High variability leads to large minimum detectable differences between treatments [34].

Experimental designs for ecotoxicological studies in polar soils should consider taking a large number of samples in order to improve the ability to detect toxicological effects in these

systems. Otherwise, there is a significant risk of investigators detecting false negatives during their investigations of polar sites. From our results, we suggest that when liquid water content is low, a minimum of 50 samples be taken from both contaminated and reference sites in order to detect a 25% toxicological impact from contamination. When abundant liquid water is present, the minimum number of samples for soil respiration can be reduced to 20; however, for PNA, the minimum sample number should be kept at 50.

A study conducted on sub-Antarctic soil spiked with diesel fuel shows similar dose-response relationships for potential microbial activity to PHC contamination [19]. In the previous study, PNA decreased, while basal respiration and SIR increased, and denitrification activity remained unchanged by diesel fuel contamination. However, PHC toxicity was an order of magnitude greater in the study with spiked diesel fuel. The EC20 values determined by the laboratory study were approximately 200 mg/kg for PNA and basal soil respiration, whereas the EC25 values for the same measurement endpoints in the present study were approximately 2,000 and 3,500 mg/kg, respectively. This difference in toxicity may be due to the age of the fuel spill or chemical composition of the fuel as it ages. The spiked soil contains a higher portion of light, volatile, short-chained hydrocarbons, which are generally more toxic than the long-chained hydrocarbons that dominate an aged fuel spill. In an aged fuel spill, many of the short-chained hydrocarbons would be lost via volatilization; therefore, soil microbiota may be able to tolerate higher concentrations of PHC in an aged spill. Although we cannot directly compare the effective concentration (ECx) values of these two studies because of physical, chemical, and biological differences between the two soils, the present study is indicative of a decrease in toxicity as a fuel spill ages. Bioremediation studies have also demonstrated a decrease in PHC toxicity over time, especially when biostimulation (addition of nutrients) or land farming (tilling of contaminated soil) techniques are used but also when natural attenuation is used as a remediation technique [35,36].

Potential nitrification activity decreased with an increase in PHC contamination of an aged diesel spill. In contrast, other researchers found that over a prolonged period of time, nitrification activity recovered at a PHC-contaminated site [37]. In Antarctica, nitrification activity was still impacted in areas of high contamination six years after the initial diesel fuel spill. Petroleum hydrocarbons have a specific mode of toxic action on the ammonium monooxygenase enzyme, with alkanes, alkenes, and alkynes having an inhibitory effect on this enzyme via competitive and noncompetitive binding [18,38]. Thus, it appears that PHC contamination prevented nitrifiers from metabolizing, and consequently their populations declined and have not yet recovered. In a study using soil from Macquarie Island, the prevalence of the gene encoding ammonium monooxygenase declined in response to PHC concentration [39]. Using that data, we can calculate an EC25 for ammonium monooxygenase prevalence of 2,400 mg/kg, modeled by

$$y = 2.96 + \{a / (1 + [(x/2400)^{5.37} \cdot \{13.6 / [0.75 \cdot (2.96 + 13.6) - 2.96] - 1])\},$$

$$r^2 = 0.212,$$

where x is PHC concentration and y is ammonium monooxygenase prevalence. The difference in environmental conditions or the nitrifying community itself may be responsible for

the incapability of the Antarctic nitrifying bacteria to recover from the PHC contamination. It remains to be seen if this effect is present only in Antarctic PHC-contaminated sites or if it is also found in Arctic sites.

Community composition was the most sensitive endpoint to PHC contamination, clearly indicating that toxicity is occurring in situ. While community composition changed, microbial biomass and respiration increased. This suggests that these terrestrial microbial ecosystems are severely carbon limited and that PHC contamination stimulates the microbial community. Comparable results have been reported by other researchers [13,16,40]; however, these other studies have also observed an increase in Gram-negative bacteria that was not observed in the present study. These results are troubling because they suggest that once Antarctic soil is contaminated, the microbial ecosystem may be irrevocably altered with little hope of returning Antarctic soil microbial ecosystems to their pristine condition.

In conclusion, the present study demonstrates that potential microbial activities, such as nitrification and soil respiration, can be used as sensitive indicators of PHC contamination in polar soil. Petroleum hydrocarbon contamination is associated with a decrease in PNA and an increase in potential soil respiration. In a polar environment, where N is very limited, a decrease in nitrification, coupled with an increase in soil respiration (which places extra demand on available nutrients), may place additional stress on the soil ecosystem because of N limitation. This nutrient stress, in addition to PHC toxicity, likely explains the shift in the microbial community composition in response to PHC contamination. This interaction between N limitation and PHC toxicity suggests that remediation systems that use extensive fertilization may only temporarily reduce toxicity to the overall ecosystem. Nitrification tests should be used to confirm that reductions in toxicity associated with fertilization remediation schemes in polar systems have resulted in a sustainable ecosystem. It is doubtful if current remediation systems will be able to return microbial ecosystems to their preimpact state.

SUPPORTING INFORMATION

Table S1. Soil characteristics for each sampling location at the Main Powerhouse (MPH) spill site.

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