Anthropogenic petroleum signatures and biodegradation in subantarctic Macquarie Island soils

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HIGHLIGHTS

- Special Antarctic Blend diesel was sometimes spilled at subantarctic Macquarie Island.
- Soils analysed from contaminated areas at “fuel farm” (FF) and main powerhouse (MPH).
- Hydrocarbon signatures of FF and some MPH soils typical of SAB diesel.
- Some MPH sites contaminated with heavier fuel with different biomarker signature.
- Variable levels of biodegradation consistent with slow rate of biodegradation.

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ABSTRACT

Special Antarctic Blend (SAB) diesel is the main fuel used on Macquarie Island and has been identified as the primary contaminant in several past spill events. This study evaluates the environmental impact of petroleum spills at high latitudes, in the soils of subantarctic Macquarie Island. Soil samples were collected from seven locations, including the “fuel farm” and main powerhouse that have been contaminated by petroleum in the past, and five reference locations, away from station infrastructure and from any obvious signs of contamination. Soils were solvent extracted and analysed using gas chromatography-mass spectrometry. The results show that both contaminated and uncontaminated sites contained a suite of different chain-length hydrocarbons. The more contaminated samples from the fuel farm and main powerhouse contained higher concentrations and a greater range of hydrocarbons that typically indicate numerous spills of varying ages. The hydrocarbon signature of samples collected near the fuel farm and at some of the main powerhouse sites was typical of Special Antarctic Blend diesel. However, the hydrocarbon signature at other main powerhouse sites suggest contamination with a heavier fuel with different characteristics, including lower pristane/phytane ratios. Traces of C21–C35 cyclic biomarkers in the spill sites may be derived from additional heavier fuels, and include a signature characteristic of crude oil derived from marine carbonate source rocks. Reference samples had lower concentrations of hydrocarbons, and these were dominated by high molecular weight n-alkanes with an odd-carbon-number predominance, typical of higher-plant derived lipids. Some reference samples also contained geochemical signatures that suggest that they too were contaminated by fuel oil. Variable levels of biodegradation of fuels in soils are consistent with a heterogeneous site and a relatively slow rate of biodegradation. The occurrence of fresh spilled fuel overprinting...
1. Introduction

Most refined petroleum products, such as diesel fuels, are readily biodegraded under aerobic conditions (e.g., Bregnard et al., 1996; McIntyre et al., 2007a). Hydrocarbon-degrading microbes are ubiquitous in polluted and non-polluted environments, but may only compose a small fraction of the microbial community in undisturbed environments (Pudasaini et al., 2017). A petroleum spill can lead to changes in the composition of biological communities, and in particular can lead to a dominance of hydrocarbon degrading bacteria (Powell et al., 2010). The remediation of petroleum-contaminated soils is of particular concern in high latitudes because microbial metabolism is generally reduced at low temperatures and in low nutrient soils (Rayner et al., 2007). Furthermore, in the case of subantarctic Macquarie Island, the pristane/phytane ratio ranging from 5 to 15, dependent on the year and season (Snape et al., 2005, 2006; Brown et al., 2016). Compared to normal diesel, which varies in composition (e.g., Farrell et al., 2007), SAB diesel is produced with a narrower and lower molecular weight compound distribution (Bence et al., 1996), so it has an exceptionally low freezing point making it ideal for use in cold conditions. It contains approximately 70% resolved compounds, with the pristane/phytane ratio ranging from 5 to 15, dependent on the year the SAB diesel was produced (Snape et al., 2006). Although there has been considerable research on the degradation of SAB diesel in Antarctica (Snape et al., 2005, 2006; McIntyre et al., 2007a, 2007b; Powell et al., 2007; Woollenden et al., 2011), it is not known how SAB diesel is degraded in subantarctic biomes. Unlike Antarctic soils which contain little organic matter (Powell et al., 2010), soils of subantarctic islands may contain large amounts of humic material (Snape et al., 2010; Errington et al., 2018a), due to the vegetation cover. Furthermore, hydrocarbons derived from natural organic material are known to occur in the carbon-rich Macquarie Island soils, including high molecular weight n-alkanes with an odd carbon number predominance that are derived from plant lipids (Bray and Evans, 1961; Eglinton and Hamilton, 1967). These have a different chemical signature from the hydrocarbons derived from fresh or biodegraded SAB diesel.

An active remediation program was carried out by the Australian Antarctic Division at Macquarie Island spill sites from 2008 to 2016, using a range of in-situ techniques (Rayner et al., 2007; Wasley et al., 2016; King et al., 2021). The present study investigates soils from areas known to be contaminated with petroleum products (Wasley et al., 2016) that have undergone remediation. In parallel with the remediation program, several ecotoxicological studies have been used to determine the toxicity of fresh and aged hydrocarbons in soils to native Macquarie Island terrestrial biota, in order to predict ecological risks of fuel contaminated soils to soil communities (Schafer et al., 2007; Mooney et al., 2013, 2019, Bramley-Alves et al., 2014, van Dorst et al., 2014, 2020; Macoustra et al., 2015; Wasley et al., 2016; Errington et al., 2018a). Results from these studies with microbes, plants and invertebrates are variable, with different components of biological communities showing different responses, and a range of sensitivities to hydrocarbons. These ecotoxicological studies have utilised both spiked soils (to mimic fresh spills) as well as site-specific soils which contain variably degraded SAB diesel, and most studies have reported only limited information and details of measured hydrocarbon compositions at the different sites tested (van Dorst et al., 2020).

In this paper, we present the first detailed compositional analysis of the hydrocarbons present in the soil at Macquarie Island. The first aim of the study was to clarify which contaminants occur at a range of locations around the Macquarie Island research station. Second, we attempt to elucidate the nature of biodegradation processes in cold, water-logged soils, and what this implies for contamination spill history.

2. Methods

2.1. Soil sample collection

Soil samples were collected from seven locations around the Macquarie Island research station during the 2012–2013 and 2013–2014 field seasons (Fig. 1). These locations include those known to be contaminated in areas to the east and west of the fuel farm, vegetated and unvegetated areas to both the north and south of the main power generation facility (main powerhouse), and generally uncontaminated reference sites, away from station infrastructure and from any obvious signs of contamination to the southwest of the station. Soil samples are provided in Supplementary Table S1, and in Hose et al. (2019).

2.2. Solvent extraction

Samples were mixed to ensure homogeneity and a ~45 g aliquot was removed from each sample. The aliquot was then mixed with sand (previously extracted with an Accelerated Solvent Extractor [ASE300]) using dichloromethane and methanol [9:1, v/v], then combusted at 600 °C for 4 h) and placed in ASE300 cylinders containing pre-extracted glass fibre filters at each end. Samples were solvent extracted with n-hexane using the ASE300 (preheat 5 min, heat 5 min to 70 °C, static 5 min, flush 70% volume, purge 5 min, 1500 psi, 3 cycles). After the first extraction cycle, each sample was removed from the cylinder, mixed, and extracted for a second time using the same ASE300 method. Once the second extraction was complete, the extractable organic matter (EOM) from both extraction cycles was combined and evaporated to ~20 mL using a rotary evaporator. The EOM was treated with HCl-activated copper metal to remove any elemental sulphur present, and then evaporated to ~1 mL using the rotary evaporator and a nitrogen evaporator system. All samples were spiked with an injection standard containing cyclooctane, 1,4-dichlorobenzene, p-terphenyl-d_{14}, and...
either 1-bromoeicosane or n-tetracosane-d_{30}. These standards were chosen due to their differences in retention time and because they are not typically found in petroleum products or indigenously in the environment. For this work, only the p-terphenyl-d_{14} standard was used for semi-quantitation of the n-alkanes, using an experimentally-measured response factor.

2.3. Analysis of hydrocarbons

Samples were analysed using gas chromatography-mass spectrometry (GC-MS). The instrument was a two-dimensional gas chromatograph (Agilent 7890 A) operating in one dimension, coupled to a Leco Pegasus time-of-flight mass spectrometer (GC × GC-ToFMS). Aliquots of the raw EOMs (1 μL) were injected through a split/splitless injector operating at 310 °C in splitless mode onto a J&W DB5MS column (60 m × 0.25 mm i. d., 0.25 mm film thickness) coated with modified 5% phenyl 95% methyl silicone, with He as the carrier gas. The temperature programme was 40 °C (2 min) to 310 °C (held 45 min) at 4 °C/min, and the detector voltage of the MS was between 1600 v and 1850 v. Aliphatic and aromatic hydrocarbon fractions of an international external standard (North Sea Oil-1) (Weiss et al., 2000) were run using the same programme to aid component identification. The standards were integrated using LECO® Chromat® and quantified relative to the peak area of the p-terphenyl-d_{14} internal standard and the recorded wet weight for each sample. Definitions of parameters and a description of how they were calculated is provided in Supplementary Table S1.

3. Results and discussion

3.1. General characteristics of samples and extent of biodegradation

The total organic carbon (TOC) content and total petroleum hydrocarbons (TPH) have previously been reported for soils in the analysed sites around the Macquarie Island research station (Errington et al., 2018a). In this previous study, TPH was measured over the C_{12}-C_{18} molecular weight range, as that is the typical range for fresh SAB diesel, and excludes most of the hydrocarbons derived from plant lipids (Errington et al., 2018a). The reference sites have a TOC of 1.8-4.9% (average 2.9%) and a TPH of 5-23 mg kg^{-1} (average 16 mg kg^{-1}). The fuel farm sites have a TOC of 1.1-3.0% (average 1.9%) and a TPH of 8-166 mg kg^{-1} (average 37 mg kg^{-1}). The main powerhouse samples have a TOC of 0.8-27.4% (average 3.0%) and a TPH of 2-748 mg kg^{-1} (average 56 mg kg^{-1}).

The reference samples contain low concentrations of petroleum hydrocarbons (C_{10}-C_{37} n-alkanes = 0.14-5.1 μg g^{-1}, average 2.2 μg g^{-1}), most of which is in the C_{29}-C_{37} molecular weight fraction (Supplementary Table S1; Fig. 2a and b). The reference samples are generally dominated by high molecular weight n-alkanes with an odd-carbon-number predominance, although other lipids typical of recent organic matter such as alkanals and alkanols were also detected in total ion chromatograms (TICs) of some of the samples (Fig. 3a and b). The reference samples have a carbon preference index of the high molecular weight n-alkanes (CPI_{24-32}) that is mostly >2 (range 0.87-4.2). The terrestrial-aquatic ratio (TAR) is highly variable (1.7-118) with a high average (43), and most samples have n-alkane maxima between C_{26} and C_{29}. This pattern is typically indicative of higher plant-derived lipids (Bray and Evans, 1961). This is consistent with the vegetated nature of the isthmus on Macquarie Island, although it is also likely that some of these soils contain lipids derived from the excreta of wildlife including elephant seals and penguins (e.g., Huang et al., 2011). It is likely that some of the hydrocarbons in some of the reference samples could have an unanticipated anthropogenic origin. For example, one sample (sample 9) was collected close to the accommodation buildings and has one of the largest amounts of n-alkanes for a reference sample (C_{15}-C_{37} n-alkanes = 4.3 μg g^{-1}), the majority in the C_{10}-C_{22} fraction, the lowest TAR of the reference samples, a primary n-alkane maxima at n-C_{16}, a secondary maxima at n-C_{26}, and a CPI_{24-32} of 2.5 (Supplementary Table S1). This sample is interpreted to represent a mix of higher plant-derived lipids and a petroleum contaminant. A similar conclusion was reached based on significant TPH values for some of the reference samples in the study by Errington et al. (2018a).

Seventeen of the 31 samples from the fuel farm have a different n-alkane distribution compared to the reference samples, with maxima in the C_{12}-C_{18} range (Fig. 3c and d). Abundant low molecular weight isoprenoids are a characteristic of these samples, with the C_{13}-C_{18}, C_{19} (norpristane), C_{19} (pristine; Pr) and C_{20} (phytane; Ph) isomers readily detected. Some of the samples exhibit a hump in the baseline of the TIC in the C_{19-29} region (e.g., Fig. 3d), which mass spectra shows is due to elemental sulphur (m/z 64). This did not interfere with analysis of most of the analytes. Some of the fuel farm samples contain virtually no high molecular weight n-alkanes that are characteristic of higher plant lipids (e.g., sample 370; Fig. 3c), and thus have very low (<0.2) TARs (Fig. 2b). However, other samples from the fuel farm have higher TARs (>3), n-alkane maxima at C_{27} or C_{29}, and CPI_{24-32} of 3.0-6.6, characteristic of a...
A major contribution from higher plant lipids. The fuel farm samples contain a broad range of concentrations of petroleum hydrocarbons (C_{10}-C_{37} n-alkanes = 0.04–15.4 μg g^{-1}, average 3.0 μg g^{-1}), and are dominated by the low molecular weight fraction (C_{10}-C_{22}) in the higher concentration samples (Fig. 2a) that have low TAR values (Fig. 2b). The main powerhouse samples also have widely variable TARs (0.31–82; average 8.3; Supplementary Table S1 and Fig. 2b). A typical main powerhouse sample with a low TAR (sample 308; 1.08) contains C_{10}-C_{36} n-alkanes, with a maxima at C_{17} and significant amounts of high molecular weight n-alkanes (Fig. 3e and f). The CPI_{24-32} of main powerhouse samples varies from 0.64 to 3.02, consistent with varying inputs of higher plant lipids. The main powerhouse samples contain greater amounts of C_{10}-C_{22} n-alkanes (average 1.15 μg g^{-1}) than most of the reference samples, but not such a high proportion of this fraction as for some of the fuel farm samples (Fig. 2a).

Most of the samples show little or no increase in the TIC baseline that can be attributed to the presence of an unresolved complex mixture (UCM; Figs. 3 and 4c), so UCM/n-alkane ratios are <0.25 (Supplementary Table S1). However, three of the fuel farm samples have very prominent UCMs (Fig. 4). The TIC of sample 26 contains virtually no resolvable peaks (Fig. 4a), although the presence of a few n-alkanes was established by monitoring the m/z 57 mass chromatogram. This sample has a UCM/n-alkane ratio of 2.9, the highest of the dataset (Fig. 2c), and is interpreted to contain anthropogenic hydrocarbons that were very strongly biodegraded (Connan, 1984). Biodegradation preferentially removes n-alkanes, which are the most susceptible hydrocarbons to bacterial alteration (Volkman et al., 1983), leading to the large UCM. The low concentration of residual n-alkanes and isoprenoids still in this

\[ \text{Fig. 2. Cross-plots of hydrocarbon parameters for the fuel farm, main powerhouse, and reference samples from Macquarie Island. (a) Total C}_{10}-C_{22} n-alkanes versus total C_{23}-C_{37} n-alkanes, expressed as μg g^{-1} soil, (b) total C_{10}-C_{37} n-alkanes (μg g^{-1} soil) versus terrestrial-aquatic ratio (TAR), (c) the pristane/phytane ratio versus the unresolved complex mixture (UCM)/n-alkane ratio, (d) the pristane/n-C_{17}, versus the phytane/n-C_{18} ratio, (e) the methylnaphthalene ratio (MNR) versus a dimethylnaphthalene ratio (DNR-1), and (f) a trimethylnaphthalene ratio (TNR-2) versus the naphthalene/sum of methylnaphthalenes (N/ΣMN) ratio. For definition of the parameters see Supplementary Table S1. The direction of increasing biodegradation is shown on (c) and (d). The direction of increasing thermal maturity is shown on (e). The linear correlation in (e) is for the 15 fuel farm samples for which both parameters could be measured, and has an R-squared value of 0.61.} \]
sample is consistent with a later phase of minor fuel contamination that overprinted the residue from the biodegraded fuel. Sample 30 has a UCM/n-alkane ratio of 1.4, with a prominent UCM centred at about C_{11}-C_{14}, and n-alkanes with a maxima at C_{16}, and virtually no higher plant derived lipids (Fig. 4b). This geochemical composition is consistent with strong biodegradation of a low molecular weight fuel, followed by re-migration and contamination from a freshly spilled fuel. A few samples from the main powerhouse also show evidence of biodegradation and overprinting by freshly spilled fuel (Fig. 2c). This suggests that contamination at both locations is a result of multiple petroleum spills over time.

n-Alkane/isoprenoid ratios provides an additional indication of biodegradation, because n-alkanes are more susceptible to biodegradation than isoprenoids (Volkman et al., 1983). Samples with Pr/n-C_{17} ratios >0.8 and Ph/n-C_{18} ratios >1.6 are likely to have been at least partially biodegraded (Fig. 2d), irrespective of whether this has resulted in a marked UCM. Based on these parameters about half of the fuel farm and main powerhouse samples have experienced at least light
3.2. Types of spilled fuels

3.2.1. Evidence from n-alkanes and isoprenoids

Hydrocarbons, including biomarkers, can be used to establish the types of fuels that have contaminated the soils at the sample sites, because different fuels exhibit different source and thermal maturity parameters, and these can also be used to differentiate the natural background lipids from plants and animals from those derived from anthropogenic fuels. Whereas about half of the fuel farm samples have n-alkane maxima in the C11–C18, only three of the 19 main powerhouse samples have n-alkane maxima in this range, with most samples being dominated by higher plant lipids (C27+), or by medium molecular weight n-alkanes (Supplementary Table S1). This is consistent with the dominant spilled fuel in the soils at the sites around the fuel farm (Fig. 1) being SAB diesel, which has a chemical composition that is dominantly around the C11–C18 molecular weight (Snape et al., 2006; Brown et al., 2016). SAB diesel is the main fuel that is currently used for power generation on the station, and is stored on the island, so this conclusion is unsurprising. The slightly higher molecular weight n-alkane composition in some of the main powerhouse samples (Fig. 3e) is consistent with a spill of a fuel containing more higher molecular weight hydrocarbons.

The Pr/Ph ratio in samples from the fuel farm that have low UCM/n-alkane ratios (<0.3) ranges from 1.9 to 8.1 (Fig. 2c). This range is significantly higher than for most of the main powerhouse and reference samples. SAB diesel has variable Pr/Ph ratios, dependent on the year the SAB diesel was produced, and these are mostly in the range 5–10 (Snape et al., 2006; McIntyre et al., 2007b). Therefore, a correlative relationship between the fuel farm samples and SAB diesel is clear for the samples with Pr/Ph ratios >5 (Fig. 2c). The fuel farm samples with large UCMs have lower Pr/Ph ratios than the other fuel farm samples, consistent with preferential biodegradation of Pr compared to Ph (Kang and Oulman, 1996; Powell et al., 2006; McIntyre et al., 2007b). As is known from the Pr/n-C17 ratios (Fig. 2d), many of the fuel farm samples are partly biodegraded, and this process is likely to have contributed to the alteration of some of the SAB diesel-inherited high Pr/Ph ratios to somewhat lower values (Fig. 2c). The Pr/Ph ratios of the samples from the main powerhouse (0.11–3.4; average 1.6) are generally lower than the ratios from the fuel farm, even in samples with low UCM/n-alkane ratios (Fig. 2c). The main powerhouse samples plot in a different space on the Pr/n-C17 versus Ph/n-C18 graph compared to the fuel farm samples (Fig. 2d), also consistent with the hydrocarbons in the main powerhouse samples having a different origin compared to the fuel farm samples. Together with the n-alkane differences, these data suggest that most of the samples at the main powerhouse were not contaminated by spilled SAB diesel, but by a different, heavier, petroleum product such as a lubricating oil.

3.2.2. Evidence from aromatic hydrocarbons

Aromatic hydrocarbons are present in most of the fuel farm samples, some of the main powerhouse samples, and are absent from most of the reference samples. The aromatic hydrocarbons that were detected include C1–C3 alkylbenzenes, naphthalene, C1–C3 alkylphenanthrenes, phenanthrene, C1–C3 alkylphenanthrenes, and several other 2–5 ring parent polycyclic aromatic hydrocarbons including biphenyl, dibenzofuran, dibenzo[ghi]fluoranthene, pyrene, benz[a]anthracene, triphenylene, chrysene, benzo[a]pyrene, and benzopyrenes. The 3–5 ring polycyclic aromatic hydrocarbons are not present in sufficient abundance in the sample-set to provide useful information, so were not analysed further. Therefore, in this study, only naphthalene, methyl-naphthalenes (MN), dimethylnaphthalenes (DMN), and trimethylnaphthalenes (TMN) were analysed in detail, as these are generally the most abundant aromatic hydrocarbons in the sample set.

Some samples contain signatures dominated by naphthalene, with progressively diminishing alkylphenanthrene contents with increasing alkylation (e.g. sample 37; Fig. 5a), whilst others contain relatively more...
of the more alkylated isomers (e.g. sample 15; Fig. 5b). The N/ΣMN ratio is generally higher for the fuel farm samples (0.29–3.45; average 1.32) compared to the main powerhouse samples (0.06–1.62; average 0.98; Supplementary Table S1; Fig. 2f). Naphthalene and alkynaphthalenes are some of the most vulnerable aromatic hydrocarbons for evaporation loss and/or biodegradation in the environment (e.g., Bence et al., 1996; Scarlett et al., 2011), oilfields (e.g., Forsythe et al., 2019) and laboratories (Ahmed and George, 2004). The lack of naphthalene and alkynaphthalenes in five of the fuel farm samples and 11 of the main powerhouse samples (Supplementary Table S1), and the variable amounts of alkylated isomers and N/ΣMN ratios in the other samples, are therefore consistent with variable degrees of evaporation, water washing and other weathering processes in the soils of Macquarie Island.

The alkynaphthalene isomers in the samples have a thermally mature distribution that is typical of most crude oils and many fuel oils (e.g., Radke et al., 1982; Alexander et al., 1985; Radke et al., 1986), with high relative abundances of the more stable β-substituted isomers such as 2-MN, 2,6-DMN, 2,7-DMN, and 2,3,6-TMN (Fig. 5). Notably, little to no 1,8-DMN was detected in any of the samples, which is an unstable αα-substituted isomer that is characteristic of immature sediments (Alexander et al., 1985). Three isomer ratios that are thermal-maturity controlled were calculated: the methylnaphthalene ratio (MNR), the dimethylnaphthalene ratio 1 (DNR-1), and the trimethylnaphthalene ratio 2 (TNR-2) (Supplementary Table S1). The cross-plot of MNR versus DNR-1 shows a weak positive linear correlation (Fig. 2e; R² = 0.61), but there is no correlation between these ratios and the TNR-2. There was also no consistent co-variation between TNR-2 and the N/ΣMN ratio (Fig. 2f). The measured ranges of MNR, DNR-1 and TNR-2 are similar to those typically found in the middle to upper part of the oil window (e.g., George and Ahmed, 2002). This is consistent with these compounds being derived from contaminant fuel oils.

Three reference samples (samples 9, 22 and 200) also contain alkynaphthalenes (Supplementary Table S1). Two of these samples have a low (<3) TAR, and all three contain significant amounts of low molecular weight n-alkanes. The MNR, DNR-1 and TNR-2 of these samples are consistent with a thermally mature source for the alkynaphthalenes. Although most of the reference sample sites were selected with the anticipation that they would reflect a natural, biogenic background, these geochemical signatures suggests that they too were contaminated in some way by leaking fuel oil. The possibility that this mature signal is from reworking of thermally mature hydrocarbons from surrounding rocks by the weathering process to form the soils is unlikely, given the dominantly igneous geology of Macquarie Island (Williamson, 1988).

3.2.3. Evidence from cyclic biomarkers

Cyclic biomarkers such as steranes and hopanes are present in most sedimentary rocks and oils, and provide evidence of organic matter inputs, depositional environments, and thermal maturity (Peters et al., 2005). The main hydrocarbon biomarkers are relatively high molecular
weight (C_{21}-C_{35}) and are consequently often only minor constituents of thermally mature crude oil and derived refined fuels. In environmental studies such as this, biomarkers can be used to distinguish between indigenous organic matter in the soil or sediment, and anthropogenic spilled fuel oil contamination.

The main compounds detected in the fuel farm and main powerhouse locations using m/z 191 mass chromatograms were C_{27}-C_{35} hopanes, with generally minor amounts of C_{21}-C_{29} tricyclic terpanes and C_{24} tetracyclic terpane (Fig. 6b and c). Some reference samples lacked any identifiable hopane peaks, but some contained a trace of hopanes, together with other unidentified peaks (Fig. 6a). Some of the unidentified peaks detected in the reference samples were also present in some of the fuel farm and main powerhouse samples, for example, the doublet of peaks eluting shortly before the C_{31} 17α(H),21β(H)- (αβ) hopane 22S isomer, and the prominent peak eluting just before C_{31} 17β(H),21α(H)- (βα) hopane (Fig. 6). Some of the fuel farm and main powerhouse samples also contained an anomalously large amount of the C_{34} αβ hopane 22R isomer, relative to other hopanes, or contain an extra peak in that area of the chromatogram (Fig. 6). Care was taken to avoid interpreting data compromised by these unknown sources. Importantly, however, there is no evidence in any of the samples for “immature” hopane isomer distributions, such as isomers with 17β(H),21β(H)-steareochemistry, a large relative proportion of the moretanes (βα) relative to the more stable αβ isomers, or an excess of the C_{34} αβ hopane 22R isomer which is commonly very abundant in immature sediments (e.g., Philp and Gilbert, 1982; Li et al., 2021). Furthermore, no hopanes were detected in any of the samples, including the reference samples. This was a somewhat surprising outcome, as soils and recent sediments are known to contain hopanes (e.g., Mejanelle et al., 2017; Morgunova et al., 2021). The explanation for this discrepancy may lie in the analytical methods used in this study, which focused on obtaining the hydrocarbons associated with anthropogenic pollution, including the choice of hexane as the extraction solvent. There may well be functionalised cyclic biomarkers in the soils at Macquarie Island, such as bacteriohopanepolys and sterols, but these were not in the analytical window, and did not interfere with analysis of the anthropogenic-derived hydrocarbons.

Small amounts of C_{27}-C_{30} steranes and diasteranes were detected in
some of the fuel farm and main powerhouse samples using m/z 217 mass chromatograms (Fig. 6b and c). The isomers present included 13β(H),17β(H)-diasteranes, and 5α(H),14β(H),17β(H) -αββ steranes (both with 20S and 20R isomers). In most of the samples the αββ steranes were more abundant than the ααα steranes, which is consistent with derivation from a fuel that was originally refined from a thermally-mature crude oil. There was no evidence for large amounts of ααα 20R sterane isomers, which are the “biological isomers” that are thermally less stable than the ααα 20S sterane isomers, nor for the presence of the very thermally unstable 5β(H),14α(H),17α(H)-sterane isomers, which under the analytical conditions used would co-elute with the second (20S) isomer of the C27-C29 αββ doublets (Li et al., 2021). Furthermore, no steranes were detected in any of the samples, including the reference samples, although there were some unidentified peaks in the reference samples, some of which were also detected in some samples from the fuel farm and main powerhouse.

The relative amount of steranes and diasteranes was considerably less than the proportion of hopanes in the sample set, so no further analysis of these biomarkers was carried out.

The overall distribution of the hopanes in most of the fuel farm and main powerhouse samples is typical of a thermally-mature crude oil, and thus are consistent with the presence of anthropogenic spilled fuel. This includes the presence of significant amounts of Ts (C17α(H),18α(H),22,29,30-trisnorneohopane), C29s (18α(H)-30-norneohopane), and the lack of all but a tiny amount of the C27 17β(H)-hopane, which would elute just after the unidentified peak that elutes after Tm (C27 17α(H),22,29,30-trisnorhopane; Fig. 6), Ts/(Ts + Tm) and C29Ts/(C29Ts + C29 αβ β hopane) ratios are mainly dependent on thermal maturity, although can be influenced by source rocks facies (Moldowan et al., 1986). The cross-plot of these two ratios showed a very slight positive correlation, with all values consistent with a thermal maturity window for oil generation (Fig. 7a). The C31 and C32 αβ 22S/(22S + 22R) hopane ratios of the fuel farm and main powerhouse samples ranged from 0.50 to 0.63 (average 0.57) and 0.55–0.74 (average 0.64), respectively (Supplementary Table S1). These values are consistent with samples that have reached or exceeded the start of the oil window (Seifert and Moldowan, 1980). Interestingly, reference samples that contained measurable hopanes (samples 2, 9, 21, 22, 200) also had a similar range for the C31 and C32 αβ 22S/(22S + 22R) hopane ratios (0.52–0.62, average 0.56; 0.50–0.63, average 0.59, respectively) (Supplementary Table S1). This is consistent with these reference samples unexpectedly containing mature hydrocarbons, a clear signal for anthropogenic contamination at some stage. Two other hopane maturity parameters that were measured on the sample set (C29αβ/C29 αββ hopane) cluster mostly around the equilibrium values of 0.90–0.95 (Seifert and Moldowan, 1980), including five reference samples (Fig. 7b). These equilibrium values are typically reached in the main part of the oil window, again consistent with derivation of the hopanes from spilled fuels.

The distribution of the terpanes and hopanes also provides information about the source provenance of the high molecular weight fraction of the spilled fuel. Many of the samples, including all the fuel farm samples, had a hopane profile that contained more of the C29 αβ hopane than the C29 αββ hopane (Fig. 6b). The C29 αβ hopane/C29 αβα hopane ratio ranged from 1.07 to 1.62 (average 1.33) for the fuel farm samples (Fig. 7c). In addition to the high abundance of C29 αβ hopane (17α(H)-30-norhopane), a prominent peak in the fuel farm samples that eluted just after C29 αβ hopane was identified as C30 17α(H)-30-norhopane (Fig. 6b). The presence of these 30-norhopanes is characteristic of marine carbonate or marl source rocks (Subroto et al., 1991; Peters et al., 2005). Further evidence for this source provenance for the fuel

\[ \text{FIG. 7. Cross-plots of biomarker parameters for the fuel farm, main powerhouse, and reference samples from Macquarie Island. (a) The Ts/(Ts + Tm) hopane ratio versus the C29Ts/(C29Ts + C29 αβ β hopane) ratio, (b) the C29 αβ/(αββ + βαα) hopane ratio versus the C30 αβ/(αββ + βαα) hopane ratio, (c) the C29 αβ/C30 αβ β hopane ratio versus the C30 αβ 22R hopane/C30 αβ β hopane ratio, and (d) the C29 22S/C29 22S αβ β homohopane ratio versus the C29 tricyclic terpane/C29 tri-cyclic terpane ratio. For definition of the parameters see Supplementary Table S1. The direction of increasing thermal maturity is shown on (a) and (b).} \]
farm samples is the ratio of C_{21} \alpha\beta 22R hopane relative to C_{29} \alpha\beta hopane (0.30–0.59; average 0.41; Fig. 7c). Ratios >0.35 are strongly characteristic of marine carbonate or marl source rocks (Peters et al., 2005). The C_{35} \alpha\beta 225/C_{34} \alpha\beta 22S hopane ratios for the fuel farm samples were mostly >0.8 (Fig. 7d), consistent with marine carbonate or marl source rocks (Peters et al., 2005), but inconsistent with the high Pr/Ph ratios for these samples (Fig. 2c), suggesting that the isoprenoids have a different origin compared to the high molecular weight biomarkers. Oils derived from carbonate source rocks commonly have tricyclic terpane distributions that have low (<0.6) C_{24} tricyclic terpane/C_{23} tricyclic terpane ratios, and high C_{23} tricyclic terpane/C_{21} tricyclic terpane ratios, compared to oils derived from paralic or lacustrine settings (Peters et al., 2005). The fuel farm samples had C_{23} tricyclic terpane/C_{21} tricyclic terpane ratios ranging from 0.25 to 0.71 (average 0.44; Fig. 7d), and C_{23} tricyclic terpane/C_{21} tricyclic terpane ratios that were variable and average 8.8 (Supplementary Table S1), consistent with a marine carbonate or marl source rock for these biomarkers. This is supported by the low C_{29} tricyclic terpane/C_{25} tricyclic terpane ratios (0.11–0.86; average 0.53).

Some of the main powerhouse samples had hopane and terpane distributions similar to the fuel farm samples (Fig. 7; Supplementary Table S1). However, seven of the main powerhouse samples had a somewhat different distribution, as exemplified by sample 99 (Fig. 6c). In these samples the C_{29} \alpha\beta hopane/C_{29} \alpha\beta hopane ratios were lower (<1.1; Fig. 7c), and there was little C_{28} 17\alpha(H)-30-norhopane present. These characteristics are not so strongly indicative of a marine carbonate source, and suggest that the source of some of the high molecular weight contamination in samples from the main powerhouse was different to that in samples from the fuel farm. The reference samples that contained hopanes had overlapping characteristics with samples from both the fuel farm and main powerhouse (Fig. 7).

### 3.3. Synthesis of contamination history at Macquarie island, based on hydrocarbon distributions

The history of petroleum contamination events at Macquarie Island includes the possible past-practice of burning waste oils in a pit near the old powerhouse pre 1975, a spill from a settling tank of presumably SAB diesel in 1975, a possible spill of SAB diesel near the fuel farm in 1988, and a spill of 1000 L of SAB diesel near the main powerhouse in 2002 (Deprez et al., 1994; Errington et al., 2018b). The current study shows that SAB diesel is the most common but not the only petroleum contaminant. The dominant molecular weight range of hydrocarbons, high Pr/Ph ratios (Fig. 2c), and the dominance of low molecular weight aromatic hydrocarbons (Figs. 2f and 5a) in samples from the fuel farm is consistent with contamination by SAB diesel. Some of the samples from the main powerhouse have hydrocarbons with a similar molecular weight range as the samples from the fuel farm, and high Pr/Ph ratios, so were also likely contaminated by SAB diesel. Several of the samples from the main powerhouse have a somewhat higher molecular weight distribution of n-alkanes (Fig. 3e) and aromatic hydrocarbons (Fig. 5b), and also tend to have lower Pr/Ph ratios (Fig. 2c) and plot in different space on the Pr/n-C_{17} versus Ph/n-C_{18} graph (Fig. 2d), consistent with a genetic difference compared to samples from the fuel farm.

Samples from both the main powerhouse and fuel farm contain small amounts of relatively high molecular weight (C_{21–23}) cyclic biomarkers that are not derived from the extant biosphere in the soils, as they include thermally-stable isomers typical of crude oils and anthropogenic oil spills. SAB diesel has a chemical composition that is predominantly around the C_{17–18} molecular weight (Snape et al., 2006; Brown et al., 2016), so it would be unlikely (but not impossible) for it to contain these cyclic biomarkers, especially the C_{29}–C_{35} hopanes and steranes. The composition of the biomarkers in samples from the fuel farm is consistent with the fuel having been refined from a crude oil that was derived from marine carbonate or marl source rocks (Fig. 7). This conclusion is inconsistent with the high Pr/Ph ratios at the fuel farm (Fig. 2c), so it is highly likely that the low-medium molecular weight fraction of the contamination at the fuel farm is derived from a different source compared to the high molecular weight fraction. A possible explanation is that the SAB in the tanks at the fuel farm contained a minor high molecular weight fraction, and this combined diesel plus heavier oil has been spilled, possibly on several occasions. However, more likely is that fuels other than SAB have been stored and spilled at that location. The chemical composition of this heavy fraction is unlike most Australian-derived crude oils, which are classically formed in terrigenously-dominated fluvial-deltaic depositional settings (e.g., Edwards et al., 2004; Jiang and George, 2020). Australia imports much of its crude oil from the Middle East (BP Statistical Review of World Energy, 2021), where marine-carbonate derived crude oils are common, and this could be the ultimate source of the contaminant. However, the type of refined product(s) that has been spilled on Macquarie Island remains uncertain. Biomarker source parameters (Fig. 7c and d) show that some of the high molecular weight fraction at the main powerhouse was not ultimately derived from a marine carbonate crude oil, thus implying that more than one heavier fuel was stored and spilled here. The area of the main powerhouse was used as a northern fuel farm from 1962 to 1976, and likely contained different fuels compared to SAB (Jeremy Richardson, personal communication, November 2022).

Lastly, the biodegradation history of the hydrocarbons in the spill sites provides evidence of multiple spills, separated by time intervals. Only three of the samples have been strongly biodegraded, resulting in a marked UCM hump (Figs. 4 and 2c), but these also contain n-alkanes. Because n-alkanes are the most susceptible hydrocarbons to bacterial alteration (Volkman et al., 1983), this implies that heavy biodegradation to form the UCM was followed by a later phase of minor fuel contamination that overprinted the residue with fresher spilled hydrocarbons that contained n-alkanes. The pristane/n-C_{17} and phytane/n-C_{18} ratios are strongly controlled by moderate biodegradation, which removes n-alkanes preferentially to the isoprenoids, so the wide variety of these ratios (Fig. 2d) is suggestive of mild to moderate biodegradation of about half of the samples. Notably, this means that about half of the sample set have not been appreciably biodegraded, based on their hydrocarbon signatures, and are essentially pristine spilled oils. As the last recorded oil spill event was in 2002, this implies that little biodegradation occurred over the 10 year or greater time interval between spillage and sampling for this study in the 2012–2013 and 2013–2014 field seasons. Therefore, it can be inferred that natural biodegradation sometimes proceeds slowly in this environment, perhaps due to the cold, anoxic, and water-logged soils in some parts, or possibly due to low levels of nutrients such as nitrogen and phosphorus. The rate of weathering of spilled fuel is dependent on environmental conditions and soil parameters, which can be highly variable, even over small spatial scales (van Dorst et al., 2020), thus leading to heterogenous degrees of biodegradation across the site.

### 4. Conclusions

1. The samples analysed from the reference sites contained high molecular weight n-alkanes with an odd-carbon-number predominance, typical of higher-plant derived lipids, but no immature biomarkers that could have interfered with analysis of hopanes and steranes from fuel spills.
2. The main composition of spilled hydrocarbons near the fuel farm is typical of SAB diesel, and this contaminant was also recognised in some of the main powerhouse sites.
3. Some samples from the main powerhouse contained a heavier spilled fuel with characteristics different to SAB diesel, including lower pristane/phytane ratios.
4. Traces of C_{21–23} cyclic biomarkers in the spill sites may be derived from spills of heavier fuels, and include a signal characteristic of crude oil derived from marine carbonate source rocks.
5. Thermal maturity markers based on alkylphenanthrene and hopane ratios indicate mature hydrocarbons in the oil window, consistent with the presence of anthropogenic spilled fuel.

6. Some reference samples contain geochemical signatures that suggest fuel contamination.

7. Levels of biodegradation of the spilled fuels in the soil profiles varies from none through moderate to heavy. The lack of biodegradation in about half the samples, despite at least 10 years elapsing between spillage and sampling, suggests that natural biodegradation may proceed slowly in this environment, perhaps due to the cold, anoxic, and water-logged soils in some parts.

8. Overprinting of fresh spilled fuel on top of earlier biodegraded fuel is compelling evidence of multiple spills and complex mixing in the environment.

Credit author statement

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

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References

