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Response of the native springtail *Parisotoma insularis* to diesel fuel contaminated soils under field-realistic exposure conditions at subantarctic Macquarie Island.

RUNNING HEAD: Springtail response to fuel contaminated subantarctic soils

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ABSTRACT

A number of sites contaminated by petroleum hydrocarbons from past fuel spills are currently undergoing remediation on subantarctic Macquarie Island. To assess the environmental risks these pose, and to establish remediation targets and guideline values, toxicity data for a range of native biota are required. The availability of data for local biota is limited, especially for soil invertebrates, which are critical to soil health. To examine the response of naturally occurring soil invertebrate communities to fuel contamination, intact soil cores from a range of soil types were collected along an organic carbons gradient. Organic carbon was factored into the toxicity assessment due to its toxicity modifying potential. Soil cores were spiked with Special Antarctic Blend diesel, to mimic a fresh fuel spill at the soil surface. Springtails were the most abundant taxa, with the community heavily dominated by the native species *Parisotoma insularis*. This species was sensitive to fuel contamination (EC20 48 mg/kg, CI 5–188), irrespective of soil organic content. This study is the first to derive

critical effect concentrations (CECs) for a subantarctic springtail species, and

provides important data that will be incorporated into future derivation of site-specific

soil quality guideline values for fuels for Macquarie Island soils and for the

subantarctic region more broadly.

KEYWORDS

petroleum hydrocarbon, toxicity, sensitivity, invertebrate, environmental guidelines

INTRODUCTION

Petroleum hydrocarbon contamination is a significant global issue, particularly

in the Antarctic and subantarctic where low temperatures reduce natural attenuation

and degradation rates of petroleum hydrocarbons, thereby increasing their longevity

and persistence in the natural environment (Snape et al. 2006). Therefore, fuel spills

in these environments may be more damaging and ecosystem recovery slower than in

temperate regions (Raymond et al. 2016). World Heritage listed subantarctic

Macquarie Island has a number of petroleum hydrocarbon contaminated sites that the

Australian Government is remediating (Errington et al. 2018a; Rayner et al. 2007).

The majority of the contaminated sites are located around fuel storage and power

generation structures with the primary contaminant being Special Antarctic Blend

(SAB) diesel, a light diesel comprised of alkanes in the range n-$C_{9\text{-}14}$ peaking at

around n-C_{12}, with trace amounts of n-$C_{15\text{-}23}$.

Previous site-specific physicochemical, ecotoxicological and ecological

studies have been conducted to determine the impacts and toxicity of SAB fuel in

Macquarie Island soils (e.g. Wasley et al. 2016, Rayner et al. 2007, Mooney et al.

2013). Several of these studies have derived critical effect concentrations (CEC) that

can be used for soil quality guideline value derivation as per National Environment

Protection (Assessment of Site Contamination) Measure (NEPM) Guidelines for soils (NEPC 1999). Such guideline values are required to assess sites for environmental risk and to set remediation targets. To date, no soil quality guideline values have been developed specifically for the unique Antarctic or subantarctic environments. A defensible approach to guideline value derivation is to use multiple lines of evidence encompassing chemical analysis, CECs based on ecotoxicological data for native biota, and ecological assessments that assess the impact of contamination on the ecosystem (Dagnino et al. 2008; Snape et al. 2008).

Ecotoxicological studies assessing the toxicity of SAB at Macquarie Island have used diverse methodologies, including soil microbial functionality (Schafer et al. 2007), gene abundance (van Dorst et al. 2014) and single-species responses for an earthworm (Mooney et al. 2013) and eight plant species (Bramley-Alves et al. 2014; Macoustra et al. 2015). With the exception of the tests with plants, most studies indicate that native biota are sensitive to fuel, with IC/EC20 values ranging from 155 to 200 mg SAB/kg soil (Mooney et al. 2013, van Dorst et al. 2014). In these previous studies, sensitivity to fuel was dependent on soil properties, most notably organic carbon (OC) content of the soils which range from sandy to peaty on the island (e.g. Mooney et al. 2013, van Dorst et al. 2014, Macoustra et al. 2015).

Soil OC is known to interact with the soil matrix and alter natural soil chemistry, which in turn influences bioavailability and subsequent toxicity of fuel contaminants (Alexander & Alexander 2000, Chung & Alexander 1998). The greater the percentage of OC in the soil, the greater the amount of contaminant that can be sequestered into the soil matrix, making it less bioavailable (Chung & Alexander 1998, White et al. 1997). In addition, greater proportions of OC are known to decrease

the rate of release (both vapour and/or aqueous phases) of organic contaminants from the soil, as well as to decrease their mobility through the soil (Bouchard et al. 1998).

Toxicity estimates are typically derived from laboratory-based, single-species toxicity tests in which a species is tested in isolation of other species under controlled and standardised conditions. These tests are not representative of complex environmental systems, eliminate natural interactions between species, and are limited in their ability to predict impacts of contaminants at an ecosystem level (Cairns Jr et al. 1996). In contrast, community-based toxicity assessments or toxicity assessments of single species under field realistic conditions, using naturally occurring environmental samples attempt to quantify the effects of contaminants on whole communities and effects on individual species within diverse natural communities. Such assessments can be used to incorporate ecological complexity in order to make more accurate ecological predictions (Baker & King 2010; Arbel et al.. al 2015).

On Macquarie Island, soil invertebrates constitute a large proportion of the island's biodiversity, with the dominant and most ecologically important taxa being Collembola (springtails; Greenslade 2006). These taxa have been used previously in a preliminary assessment of fuel contamination on Macquarie Island, primarily due to their dominance within the soil community at fuel spill sites (Wasley et al. 2016). Springtails play an important role in soil processing, health and function, and are major contributors to nutrient and energy transfer in subantarctic ecosystems by the mineralisation of organic matter (Smith & Steenkamp, 1992; Smith et al., 1993). They are known to be influenced by a range of soil properties, including OC (Gabriel et al., 2001; Ulrich & Fiera, 2009). They are commonly used in single-species toxicity tests (e.g. Fountain and Hopkin 2004; Greenslade and Vaughan 2003) and are also ideal for

demonstrating community changes in response to contamination in an ecosystem (Gillet and Ponge 2003).

The aim of this study was to determine the response of springtail communities and individual species to SAB fuel contamination. We used intact surface soil cores from a wide range of soil types, from sandy to peaty, covering the OC range found in soils on the island. This method provided naturally occurring invertebrate communities for testing to maximise ecological relevance. CEC estimates for SAB fuel-contaminated soils at Macquarie Island were determined for the dominant springtail species *Parisotoma insularis* (Deharveng, 1981), and will be used for future soil quality guideline derivation.

Materials and Methods

Study site and sampling

Macquarie Island is a subantarctic island located in the Southern Ocean, approximately 1500 km south-east of Tasmania, Australian (Figure 1). The island is under Tasmanian state jurisdiction and is protected as a Nature Reserve. A permanently occupied research station is located on the northernmost isthmus (Figure 1). Average day length is variable throughout the year with extremes from 17 h in summer to 7 h in winter. Temperatures are relatively stable throughout the year, with maximum daily temperatures ranging on average between 1.6 and 4.9°C in July (winter) and 5.3 to 8.8°C in January (summer). Annual precipitation approaches 1000 mm, with approximately 70 to 100 mm rainfall/month. Wind speeds are on average more than 30 km/h each month throughout the year (Australian Government, Bureau of Meteorology; climate data for 1948-2018).

Fieldwork was conducted during the 2008/09 and 2009/10 summer seasons, when ecological processes and inverebrates communities are most active. This study

was undertaken early in the Macquarie Island contaminated site remediation program (conducted by the Australian Antarctic Division), and prior to commencement of the Macquarie Island pest eradication program in 2011 (Parks and Wildlife Service, 2014). Three uncontaminated sites near the Macquarie Island research station (Figure 1), which were representative of the isthmus environment were sampled: Site 1, north of the station (-54.4974ºS, 158.9395ºE) was highly vegetated, with a south-west aspect; Site 2, at southern end of the isthmus (-54.5090ºS, 158.9312ºE), with an easterly aspect; and Site 3 in an undisturbed area within the vicinity of the station (-54.4987ºS, 158.9399ºE), with a northerly aspect. Each site is positioned at the base of a slope or gully, with vegetation predominantly small herbs and grasses. Sites 1 and 3 had similar vegetation cover and were not noticeably affected by rabbit grazing. These sites were dominated by the large tussock grass *Poa foliosa*, the mega herb *Stilbocarpa polaris*, and the small-stature *Poa annua* and *Callitriche antarctica.* In contrast, Site 2 showed some evidence of grazing pressure and was dominated by low lying grasses and herbs (*Epilobium pedunculare*, *E. brunescens*, *Acaena magellanica*, and *P. annua*).

Each site consisted of a 50 m transect which encompassed the wide range of soil types found on the island, from coarse sandy soils, low in organic matter, through to peaty soils that were high in organic matter. Collecting over a range of soil types enabled soil OC, which is known to modify fuel toxicity, to be factored into the SAB toxicity assessment. Ten plots (1 x 1 m) were sampled along each transect. Within each plot, seven pairs of soil cores (70 mm diameter x 70 mm deep) were collected using a soil corer, contained within a PVC sleeve to minimise disturbance (Wasley et al. 2016; Terauds et al. 2011). Cores were collected adjacent to each other to ensure individual cores within each pair were as similar as possible. Core pairs were spiked

with SAB fuel at a range of concentrations as described below. One core in each pair was subsequently used for invertebrate extraction (rendering it ineffective for some physicochemical analysis) and the second core used for soil physicochemical analyses. Percentage cover of plant species and bare ground was surveyed for each plot.

SAB addition to soil cores

Core pairs from each plot for Site 1 were spiked with to achieve nominal SAB fuel concentrations of 0, 500, 1000, 5000, 10000, 200000, 400000 mg SAB/kg soil. All concentrations are reported as mg/kg (on a dry mass basis). The two highest concentrations were included to be representative of the maximum concentrations likely to be observed during a fuel spill. However, these concentrations caused total springtail mortality. Therefore, for subsequent sites (2 and 3) these concentrations were replaced with two lower nominal concentrations of 125 and 250 mg SAB/kg soil. A representative soil core was taken from each plot the day before sampling to determine soil moisture content to estimate the required SAB spike volume to achieve the desired nominal concentration.

Soil cores were spiked by injection with a hypodermic syringe and needle into the soil surface layer to achieve environmentally realistic test conditions. This novel method was used to minimise disturbance to soil cores, to maintain the integrity of the soil invertebrate communities, and to deliver fuel in a manner that would best mimic a spill at the soil surface. To ensure even delivery of fuel to soils, spiking was standardised by placing a mesh grid over the surface of soil cores and delivering an even injection distribution pattern standardised for each treatment. Injections in the soil cores were made at a depth of 20 mm, as the greatest percentage of the soil invertebrates occur in the surface layer.

Soil extracts were analysed for Total Petroleum Hydrocarbons (TPH) by gas chromatography using flame ionisation detection (GC-FID; Agilent 6890N with a split/splitless injector) and an auto-sampler (Agilent 7683 ALS) at the Australian Antarctic Division, Hobart, Australia. However, as the spiking method created contaminant "hotspots" throughout the soil cores and did not produce a homogenous mix of contamination, these measured concentrations were not representative of the actual exposure concentrations. Therefore, exposure concentrations (fuel mg/kg, dry mass basis) were calculated for each soil core, based on the volume of fuel added to each soil core and its corresponding dry mass of surface soil to 20 mm (using an average fuel density constant of 0.81 kg/L). The mean calculated soil fuel concentrations for the eight treatments were 350, 800, 1760, 3050, 15890, 32960, 101680 and 417880 mg/kg. Values for the two highest concentrations are high, as peaty cores with high organic carbon content, had a very low dry mass. The discrepancies between calculated concentrations and nominal concentrations were due to difficulties in accurately estimating OC and moisture contents on-site in a remote location with no access to suitable analytical equipment (e.g. muffle furnace).

Test conditions

Spiked cores were incubated for 15 days under field simulated conditions at a temperature of 8±2°C and a diurnal cycle of 16 h light/8 h dark. These conditions mimicked the natural summer field conditions at Macquarie Island, where soil temperatures typically range from 4 to 10°C (Hince et al. 2013). Soil cores from the same treatment concentration were held in sealed containers to prevent fuel volatilization, cross-contamination, and water loss.

Invertebrate extraction and identification

Surviving invertebrates from the 70 cores from each of the three sites were extracted into propylene glycol over 10 days using a temperature gradient extraction unit as per Gabriel et al. (2001). Tops of the cores were covered with coarse mesh and inverted over a collection container. Heating started at 25°C then increased to 30°C after five days. Water at 10°C was circulated around the base of the containers for the full duration. Extracted invertebrates were transferred from the propylene glycol and preserved in 80% ethanol. Taxa were ordinal sorted and springtails separated and identified to species where taxonomic certainty allowed, following Greenslade (2006). The exception to this was *Tullbergia bisetosa* and *Tullbergia templei,* which were pooled as *Tullbergia* spp. due to difficulty differentiating between the two species.

Soil physicochemical analysis

Soil samples collected from each of the three sites were characterised for a range of physicochemical properties. The properties measured were: OC content, moisture content, nutrient concentrations (ammonia, nitrate and phosphate), conductivity, pH, particle size (gravel, sand, mud). Laboratory analysis methods used were based on standard procedures (Clesceri et al. 1998; Rayment and Lyons 2011) and are summarised in Supplemental Information. Plant species % cover (vegetation cover) was also recorded for each plot (Supplemental Tables S1-S3). Regression analysis, on log-transformed and normalised data, was used to determine correlations between OC and other physicochemical properties (Supplemental Tables S4).

Relationships between the springtail assemblage (in control cores) and corresponding environmental data (physicochemical variables and vegetation cover)

were determined using the PRIMER 6 software (Clarke & Gorley, 2006). Biotic data (plot vegetation cover and core springtail assemblage) were square-root transformed prior to analysis to reduce the influence of dominant species. The springtail assemblage was analysed using a Bray-Curtis resemblance matrix. Relationships with associated physicochemical variables and vegetation cover were determined using the Biota and/or Environmental matching (BEST) analysis function, using the Spearman rank correlation, to determine which variables best explain patterns of species abundance in the springtail assemblage.

Modelling species response to SAB fuel

The response of a given species to SAB fuel concentration was modelled as a smooth function of core fuel concentration and soil OC content, using negative-binomial generalized additive models (GAM) with log-link function. Modelled abundances were constrained to be monotone decreasing with respect to fuel concentration, and were fitted using the "scam" package (Pya & Wood, 2015) in R 3.4.1 (R Core Team, 2017). Abundances were highly variable between plots, and so each model also included a factor term for plot. Furthermore, for each species the regression model only used data from those plots where the species occurred in at least three of the seven cores.

The fuel term was log-transformed in each model. This transformation does not force the model term to be strictly log-dependent on fuel, but rather means that the smoothness of the model response will tend to vary on a log-fuel scale (whereas a linear fuel term would yield responses with smoothness variations on a linear fuel scale). Model selection was guided by Akaike's Information Criterion (AIC; Burnham & Anderson 2001).

A Monte Carlo procedure was used to estimate single-species CECs and their associated uncertainty from the fitted models. For a given species, 5000 samples were drawn from the posterior distribution of the fitted model. In effect, this sample is a collection of curves that characterize the response of that species to fuel and soil OC, with more curves being drawn from around the mean of the model fit, and fewer from the extremes. The variability in shape from curve to curve is therefore a reflection of the uncertainty in the model fit. Curves will tend to be similar to one another in regions of predictor space where the uncertainty is low, with higher curve-to-curve variability in regions where uncertainty is high. Single-species CECs were estimated for each curve in the sample, by determining the contaminant concentration at which the modelled abundance of a species dropped by 20% or 50% of its control value. This yielded a distribution of EC20 and EC50 values from which appropriate summaries (mean and uncertainty) were determined.

Results

Fuel contamination impacted springtail communities, with average abundance and species richness declining with increasing fuel concentration (Figure 2). Average abundance of springtails was reduced by nearly half of that in the control at 800 mg/kg, and very few live individuals were found in cores ≥15890 mg/kg (Figure 2A).

The springtail assemblage extracted from soil cores was comprised of 17 taxa, of which seven species were relatively common, occurring in controls at a mean of greater than 5 individuals per core (Figure 2; Table 1). Species abundance was highly variable between cores, with some species in extremely high numbers in comparison to the majority of cores (Table 1; Supplemental Figure S1).

The assemblage was heavily dominated by *Parisotoma insularis* which was present in all plots (Table 1), comprising 77.5% of the springtail assemblage in

controls, and dominating treatments up to and including 3050 mg/kg (Table 1, Figure 2B). All other species occurred at less than 6% abundance in controls, with the next most abundant species being *Folsomotoma punctata*, *Tullbergia bisetosa* and *Ceratophysella denticulata*, representing 6, 4 and 3% of the assemblage, respectively (Figure 2B, Table 1). *Cryptopygus caecus* was widespread, occurring in 2/3 of control cores (Table 1). Most species were native to Macquarie Island, but three were introduced (*Certophysella denticulata, Hypogastura viatica, Lepidocyrtus* sp.). Of these introduced taxa, two were amongst the seven most abundant species (*C. denticulata* and *H. viatica)*. Their representation in the community increased proportionally at the highest treatment concentrations, although this trend should be interpreted with caution as their overall abundance was very low (Figure 2B).

Site vegetation cover and physicochemical soil properties were highly variable both between individual samples and between sites (Supplemental Tables S1-S3). Only weak correlations were found between springtail community composition and any of the properties measured, with site best explaining springtail community patterns (Supplemental Table S5).

There was no obvious relationship between species abundance and soil OC content (Supplemental Table S5; Figure S1). Several species occurred across the entire soil OC range sampled, including the highly abundant *Parisotoma insularis*, along with species such as *Tullbergia* sp, *F. punctata, Cryptopygus antarcticus* and *C. caecus* (Supplemental Figure S1). Some of these species appeared to be more abundant in low to medium OC soils, but patterns were often heavily influenced by single high abundance outliers (Supplemental Figure S1). In contrast, other species had a more restricted range, occurring only in soils with < 15% OC. These included *Hypogastura* sp., *C. lawrencei, C. dubius and C. tricuspis* (Supplemental Figure S1).

Soil OC was correlated with several other environmental parameters (notably, phosphate, soil moisture, and conductivity; $r^2 > 70\%$), hence factors other than OC may also explain springtail community patterns (Supplemental Table S4).

Modelling of individual species response to exposure to fuel was only possible for one species, the highly abundant *P. insularis*. The low abundances and highly patchy nature of other species yielded poorly fitting models that failed diagnostic checking and/or gave estimated CEC values that were inconsistent across models and sensitive to changes in model structure (e.g. the terms included in the model, or their mathematical form). Estimates for other species were, therefore, unable to be determined with any reasonable degree of confidence. Hence we present CECs for *P. insularis* only (Table 2).

The model of *P.insularis* abundance as a function of both fuel concentration and soil OC content provided a statistically better fit than a model with no carbon term (Akaike Information Criterion 1584.8 vs 1590.5). However, the practical effect of carbon on the resulting ECx estimates was minimal (< 5% effect on mean EC20, and around 10% increase in mean EC50 at 50% OC content compared to 10% carbon, Table 2) and those differences were not significant (p > 0.05, Wilcoxon rank sum test). The model with no carbon term provided similar ECx estimates for changes in abundance of *P. insularis*, with EC20 48 (CI 5-195) mg/kg, and EC50 426 (CI 128-859) mg/kg.

Discussion

Springtail communities were negatively impacted by SAB fuel. *Parisotoma insularis* was highly sensitive to fuel with EC20 values <50 mg/kg across all soil types. As there was no significant difference in ECx estimates based on soil organic

carbon content, the average EC20 value of 48 (CI 5–195) mg/kg is representative of the response of *P. insularis* across the full range of soil types and properties that occur on Macquarie Island and is recommended for use in guideline derivation.

This EC20 is comparable to toxicity estimates for SAB fuel from previous studies with native biota from Macquarie Island. In single-species tests with the native earthworm, *Microscolex macquariensis*, an EC20 values of 127 mg/kg was estimated for juvenile production (Mooney et al. 2013). In microbial community toxicity assessments, an IC20 of 190 mg/kg based on nitrification activity as a measure of soil health and function (Schafer et al. 2007), and an EC20 of 155 mg/kg based on changes in abundance of the bacterial *amoA* gene (van Dorst et al. 2014) were determined. In contrast, plants native to Macquarie Island have been found to be relatively tolerant to fuel contamination with EC25 values in some species up to 5000 mg/kg in seed germination tests (Macoustra et al. 2015). Other species, especially *Poa foliosa* were even more tolerant, both in seed germination and in plant growth and physiology tests (Bramley-Alves et al. 2014).

Springtails are widely used in traditional single-species tests worldwide. However no species for which standard methods have been established occur on Macquarie Island (Fountain and Hopkin 2004; Greenslade and Vaughan 2003; Greenslade 2006), and few studies have published estimates for the sensitivity of these taxa to fuel contaminants. While comparisons with other studies are difficult due to the differences in types of fuels tested, methods used, and endpoints assessed, it appears that *P. insularis* is more sensitive to fuel contaminants than other springtails, including the widely tested species *Folsomia candida*. Acute bioassays on oil polluted soils found no effects on the mobility of *F. candida* at concentrations up

to 3300 mg/kg total oil (van Gestel et al. 2001). Similarly, in tests with this species on a petroleum hydrocarbon product mixture, EC25 and EC50 values of 2370 and 5750 mg/kg TPH were reported for an avoidance response (Gainer et al. 2019).

Community testing in ecotoxicology

Our initial aim was to determine toxicity estimates using community structure endpoints to provide CECs with higher ecological relevance than would be obtained from more traditional single species testing. Traditional soil toxicity tests typically use artificially constructed and homogenized soil matrices, with known numbers of a single species introduced to the test system. In contrast, community tests include multiple species in proportion to their natural occurrence in the environment, and therefore allow for complex species interactions (e.g. predation) and provide a more realistic representation of ecosystem function and the dynamics within the entire community.

Working with natural community test systems, however, is complex and poses a number of challenges, primarily associated with sources of heterogeneity. In this study we used intact experimental cores, containing soil invertebrate communities at natural compositions and abundance, and a spiking method representative of a fresh fuel spill at the surface in an attempt to determine the impact of fuel on whole communities under field realistic conditions. Both of these design elements had known trade-offs.

Our use of intact cores obtained from the field without otherwise disturbing biota meant that we had no control nor knowledge of the composition and abundance of communities at the start of tests. As it turns out, natural patterns of abundance of species within invertebrate communities at Macquarie Island were spatially

heterogeneous across even small spatial scales (i.e. soil cores within plots). The prevalence of patchy abundance patterns whereby some cores had 1000s of individuals, while others had none (Figure S1), made the assessment of changes in communities within cores in response to fuel, and subsequent community modelling approaches unsuitable. As variability between replicate cores was so high, the effect of hydrocarbon contamination on springtail communities as a whole could not easily be characterised. While we investigated the entire springtail community using a multispecies dissimilarity matrix to model changes in diversity and abundance to estimate community guideline values, the point estimates from such models were highly variable and sensitive to the model used and the choice of model parameters. As such, community estimates were not consistent nor robust, and were deemed unreliable for inclusion in future guideline derivation. Only one species, *P. insularis*, occurred in high enough numbers consistently across cores to perform concentration-response modelling to determine a CEC value. In order to achieve robust CECs for additional species, and indeed for the whole community as was originally intended, an impractically high number of samples would be required to overcome heterogeneity. The experimental effort required to achieve this would be prohibitive, particularly in remote regions like subantarctic islands where access is limited and operating costs are very high.

Extreme heterogeneity in the springtail community between cores also prevented detection of the influence of other environmental parameters that may have modified the toxicity of fuels. We were especially interested in determining the effect of OC on observed toxicity, and therefore collected cores along a wide OC gradient, from sandy to peaty. Previous studies have shown that soils with high OC are associated with reduced toxicity, due to enhanced sequestration of contaminants

which makes them less bioavailable (White et al. 1997; Chung & Alexander 1998; Alexander & Alexander 2000; Reinecke et al. 2016). Unfortunately, our data were insufficient to differentiate the effect of soil carbon on fuel toxicity to *P. insularis*.

Soil core spiking method

The other main challenge of working with intact experimental soil cores is the application of a homogeneous fuel spike to the test system to facilitate an equal chance of exposure to all test biota. Attempts were made to deliver a homogeneous spike across each soil core, using a grid guide to deliver an even injection pattern across the surface. However, as cores were deliberately not mixed so as not to disturb biota, this method inevitably led to hot spots of contamination.

These hot spots created difficulty for the accurate measurement of fuel concentrations within cores. Fuel concentration (as TPH) was measured in subsamples from all cores using GC-FID, but results were inconsistent with spike volume. As well as the patchy distribution of the spike, this may also have been due to the small fuel volumes delivered (~13.5 µL per core in the lowest treatment of 350 mg/kg), and to the small soil subsample on which measurements were made. Obtaining realistic measured exposure concentrations for TPH was therefore not possible. This necessitated the use of the calculated values for soil fuel exposure concentrations which were based on the volume of SAB injected and the soil dry-mass, on a core by core basis.

A possible further consequence of the heterogeneous nature of the spike was that exposure of soil biota to fuel might not have been uniform across a core. Indeed it is likely that some biota avoided exposure by chance (i.e. were located away from injection points), and that more mobile species may have avoided exposure by

moving to other less contaminated areas within cores. This possible effect was minimized by the standardised gridded approach to spiking. Despite the possibility of some biota avoiding exposure, we still observed a strong decrease in abundance of springtails with increasing soil fuel concentration (Figure 2).

An alternative method of introducing fuel to the soil cores is to individually deconstruct the soil cores and gently mix fuel into the soil to provide a homogenous spike. A pilot trial using this method was undertaken on Macquarie Island during the summer of 2008. However, recovery of soil invertebrates was prohibitively low, so this method was deemed unsuitable. Therefore, it was decided that the best approach was to maintain the soil core integrity at the expense of providing a homogenous spike.

Modelling approach

In this study, we applied a wide range of contamination levels to cores to cover the range of fuel concentrations reported at contaminated sites at Macquarie Island (Wasley et al 2016; Errington et al. 2018b). With any field based ecotoxicological study, limitations on experimental resources mean that it is rarely possible to densely sample the full range of contaminant concentrations. Without prior knowledge of the sensitivity of subantarctic soil invertebrate communities to fuels (at the time this study was initiated), this approach was necessary. In hindsight, a greater number of treatment concentrations and replicates at the lower end of the concentration gradient would have provided greater coverage at the concentration range at which effects on communities were observed. We did modify the experimental design when the two highest concentrations at Site 1 caused 100% mortality, replacing these concentrations with two lower concentrations for Sites 2

and 3. These concentrations were at the limit of what was practically possible to achieve given the very small spike volumes required (as described above).

Despite these attempts to better cover the lower concentration range, our data was still sparse in the concentration range at which tolerance thresholds occurred, making robust modelling of CECs difficult. Parametric models (e.g. log-logistic) are traditionally used to analyse such concentration-response data, and can be less affected by noisy, sparse data because the model form is limited in the range of shapes of response curves that it can produce. However, the point estimates from such models may be sensitive to the choice of model structure. This potentially makes the selection of an appropriate model difficult, and can result in artificially high confidence around point estimates unless the uncertainty to do with model structure is appropriately incorporated into the final estimates.

As an alternative, GAMs provide a flexible modelling approach that estimates the response curve as a combination of smooth functions of one or more predictor variables. The shapes of the smooth functions are estimated from the data using smoothing splines or similar approaches, and can be constrained if appropriate (e.g. monotonically decreasing with respect to contaminant level, as was done here). This reduces the requirement to assume particular model structures, allowing the data to inform the shape of the response curve. Where experimental data are sparse, the model fit will be less precise, naturally reflecting a reduced level of certainty in these regions. Accurate estimates of the confidence intervals of CECs are particularly important when these values are used to support management decision making. The reliable measures of uncertainty around CECs presented here allow us to apply a conservative approach for the management of fuel contaminated soils on the island.

The CECs reported in this study have been derived with careful consideration to model structure and constraints (e.g. smoothness of GAM). This is particularly relevant as the estimated CECs are lower than the minimum spike concentration (350 mg/kg) and are therefore particularly susceptible to the mechanics of the model structure. The high uncertainty on the estimated CECs reflects these factors, and indicates that a precautionary approach should, therefore, be applied to their use.

Conclusions

The present study has provided valuable information on the sensitivity of springtails to fuel contamination in subantarctic soils. Springtails represent one of the most important taxa in terms of soil health and processing on Macquarie Island. Despite the challenges introduced by the use of intact soil cores and the injection spiking method, this approach was necessary to maintain community integrity and environmental relevance of toxicity estimates. We utilised a statistical model to determine CECs, with confidence intervals, based on changes in abundance relative to controls. A 20% decline (EC20) in the dominant springtail *Parisotoma insularis* was estimated at 48 (CI 5–195) mg/kg. Unlike values derived from traditional toxicity tests in which a single-species is tested in isolation, this value represents the response of *P. insularis* within a naturally occurring community and is therefore more ecologically relevant. This CEC for a key springtail species is the most sensitive estimate obtained to date for Macquarie Island biota, and will be used along with sensitivity data for other native taxa, to derive site-specific environmental quality guidelines for Macquarie Island soils and for the subantarctic region as a whole. These guidelines will be used to inform remediation activities and to establish remediation targets to enable contaminated sites to be signed off as no longer posing an environmental risk.

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FIGURES

Figure 1: Map of sampling sites for springtail assemblages on Macquarie Island.
A) The location of Macquarie Island in relation to Australia and New Zealand; B) Macquarie Island and the location of the research station and study sites on the northern isthmus; C) The location of study sites 1, 2, and 3 on the northern isthmus of the island.
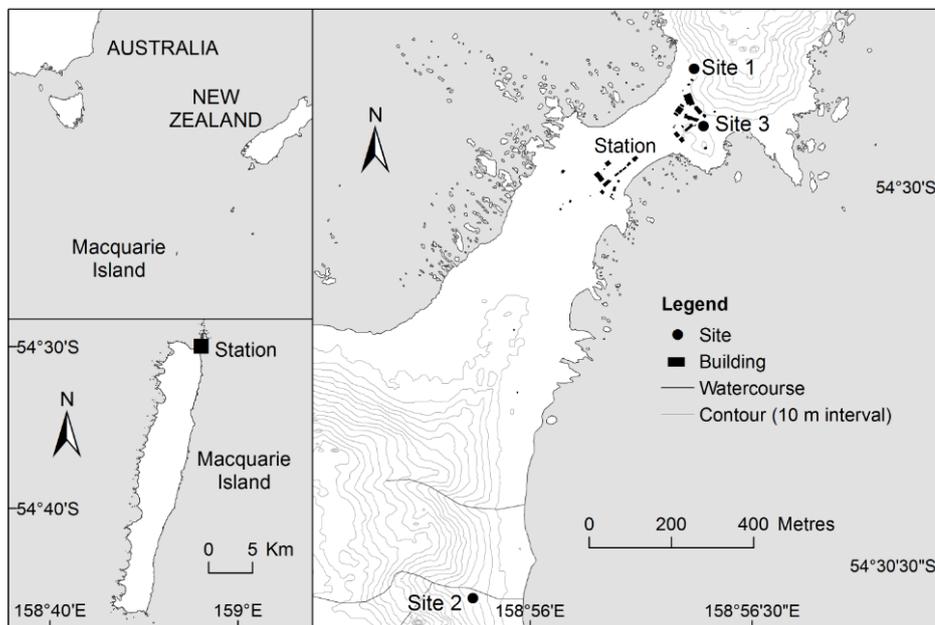
Figure 2: Abundance of Macquarie Island springtail species in response to increasing concentration of Special Antarctic Blend (SAB) fuel contamination in soil. A) Average abundance; B) Percent Abundance. Transects and plots pooled; n = 20 (350 and 800 mg/kg; sites 2 and 3) or 30 (>1000 mg/kg; all sites). Other species (mean <5 in controls, in order of descending abundance): *Megalothorax* sp., *Lepidocyrtus* sp.[†], *Cryptopygus tricuspis*, *Polykatianna davidi*, *Friesea bispinosa*, *Crytopygus lawrencei*, *Cryptopygus dubius*, *Lepidobrya mawsoni*, *Pseudosorensia* sp. and *Katianna banzarei*. Introduced taxa: [†]. *Tullbergia* spp. is *T. bisetosa* and *T. templei*. Two higher treatment concentrations (average: 101680 and 417880 mg/kg) were tested in site 1 but caused total mortality and are therefore are not shown.
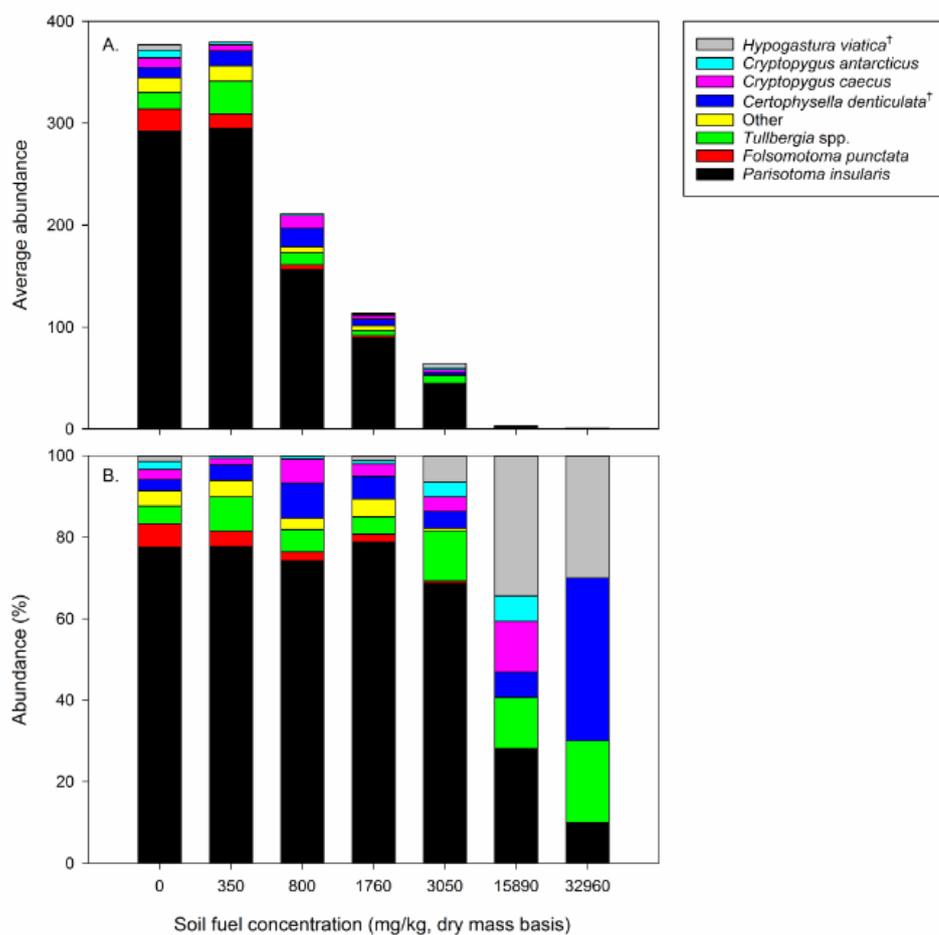
Figure 3: Modelled response of the relative abundance (normalized to unity at zero fuel) of the springtail *Parisotoma insularis* following exposure to Special Antarctic Blend (SAB) fuel contamination in soil. Fuel concentration calculated for individual samples on a soil dry mass basis. Carbon is not included as a term in the model. 95% confidence interval shown in grey. Points on the curve are the estimated EC20 and EC50 values with 95% confidence intervals shown as horizontal error bars.
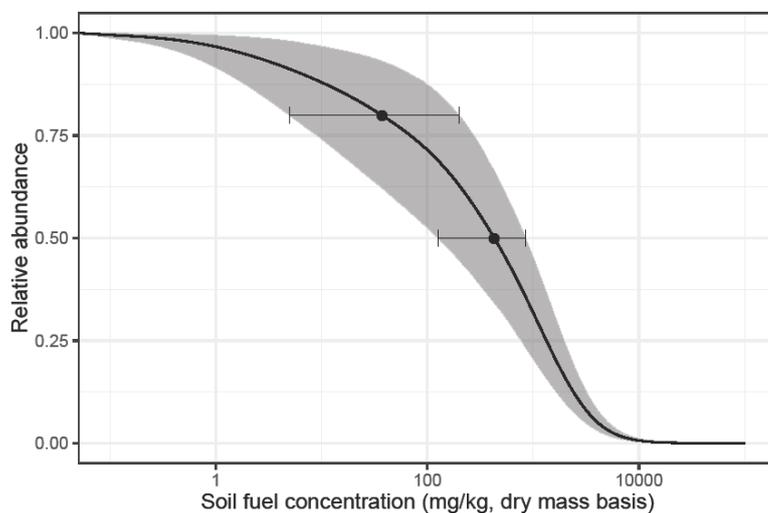
Table 1: Abundance of springtail species in control cores. Taxa are listed in order of occurrence (N; number of cores taxa were present, out of a total of 30 control cores). Other data provided are percent abundance, mean, standard deviation and range (min - max count per core). Two additional species: *Pseudosorensia* sp. and *Katianna banzarei* were found in low abundance in treatment cores but not controls.

Species	N	Abundance (%)	Mean	SD	Min	Max
Total Springtails	30		377	505	20	2386
Parisotoma insularis	30	77.5	292	458	10	2115
Cryptopygus caecus	20	2.4	9	12	0	44
Folsomotoma punctata	18	5.8	22	44	0	202
Polykatianna davidi	14	0.4	1	3	0	13
Tullbergia spp. *	11	4.3	16	49	0	222
Certophysella denticulata†	11	2.8	10	35	0	190
Megalothorax sp.	11	1.2	4	10	0	44
Cryptopygus tricuspis	10	0.5	2	3	0	14
Cryptopygus antarcticus	9	2.0	7	20	0	85

Lepidocyrtus sp.†	8	0.8	3	6	0	23
Hypogastura viatica†	3	1.5	6	23	0	124
Crytopygus lawrencei	3	0.3	1	5	0	24
Cryptopygus dubius	3	0.3	1	4	0	17
Friesea bispinosa	1	0.3	1	7	0	39
Lepidobrya mawsoni	1	0.0	0	0	0	1

† Introduced taxa; *Tullbergia* spp. is *T. bisetosa* and *T. templei*.

Table 2: Critical Effect Concentrations (CECs) for SAB fuel for the springtail *Parisotoma insularis*. Values are modelled with a term for soil organic carbon content, and also modelled without considering soil carbon. EC20 and EC50 estimates represent the change in abundance relative to controls. Estimates for total petroleum hydrocarbon concentrations and associated 95% confidence limits (LL=lower limit, UL=upper limit) are expressed as mg/kg on a soil dry mass basis.

Soil organic content (%)	EC20	LL	UL	EC50	LL	UL
10	**47**	4	202	**394**	100	838
20	**47**	4	202	**414**	98	882
50	**48**	4	217	**440**	94	1053
Model without carbon	**48**	5	195	**426**	128	859