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Impact of hydraulic fracturing chemicals on soil quality

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Cover Photo

Sampling of soils at Condabri site, Surat Basin (November 2017).

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Abbreviations

Abbreviations	Description
AGRF	Australian Genome Research Facility
amoA	Ammonia oxidation bacterial biomarker
ANOSIM	Analysis of similarities
ANOVA	Analysis of variance
APPEA	Australian Petroleum Production and Exploration Association
ASRIS	Australian Soil Resources Information System
BTEX	Benzene, toluene, ethylbenzene, xylenes
CEC	Cation exchange capacity
CSG	Coal seam gas – naturally occurring gas in coal seams
CMIT	Chloro-methylthioisozolinone
CSIRO	Commonwealth Scientific and Industrial Research Organisation
DNA	Deoxyribonucleic acid
DT_{50}	Time taken for 50% loss of a chemical
DT_{90}	Time taken for 90% loss of a chemical
EC	Electrical conductivity
GA	Glutaraldehyde
GA1	Gibberellic acid
GC-MS	Gas chromatography-mass spectrometry
GUS	Groundwater Ubiquity Score Index
HF	Hydraulic fracturing
ICP-MS	Inductively-coupled mass spectrometry
K_d	Sorption or partition coefficient based on bulk soil
K_{oc}	Sorption or partition coefficient based on organic carbon in soil
K_{ow}	Octanol-water partition coefficient
LC-MS/MS	Liquid chromatography - Mass spectrometry/mass spectrometry
LOD	Limit of detection
LOR	Limit of reporting
MIT	Methylthioisozolinone
MRM	Multiple reaction monitoring
MWHC	Maximum Water Holding Capacity
NATA	National Association of Testing Authorities
NDA	Non-disclosure Agreement
NGS	Next Generation Sequencing

Abbreviations	Description
nifH	Nitrogen fixation bacterial biomarker
nirK	Nitrite reductase bacterial biomarker
nMDS	Nonmetric multidimensional scaling
NMI	National Measurement Institute
OECD	Organisation for Economic Co-operation and Development
OTU	Operational taxonomic unit
PAH	Polycyclic aromatic hydrocarbon
QiiME	Quantitative insights into microbial ecology
qPCR	Quantitative polymerase chain reaction
SIMPER	Similarity percentages
SIMPROF	Similarity Profile Analysis
SIN	Substrate induced nitrification
SIR	Substrate induced respiration
SIM	Selected ion monitoring
TEA	Triethanolamine
TOF MS	Time of flight mass spectrometry
TRH	Total recoverable hydrocarbons

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Executive summary

An assessment by Patterson et al. (2017) of potential exposure pathways for contamination of soils with chemicals established that spills of hydraulic fracturing (HF) fluids and produced water are among the most polluting and plausible pathways affecting unconventional gas operations. Spills are unpredictable and are very site- and event-specific. Therefore, as opposed to a field-based soil contamination assessment, a scenario-based study, mimicking the exposure via spills of HF fluid and produced water under laboratory conditions was considered a more appropriate option.

This study involved exposing soil samples, representative of the five different soil types from the Surat Basin, to HF fluids and produced waters under controlled laboratory conditions. It is noteworthy that while the drilling pads surfaces are artificially created and hence a spill may not make a direct contact with surface soils immediately. However, it is assumed that with time the constituents of any fluid spilled on the drill-pad surface may infiltrate into soils. In such cases, the current assessment represents a worst-case scenario.

A HF fluid for use in the study was prepared by a company that regularly conducts HF activities in Central Queensland. Produced water used in the study was collected from operating gas wells in the Surat Basin. In addition, in a parallel experiment, selected chemicals (methylthioisozolinone (MIT), Chloro-methylthioisozolinone (CMIT), triethanolamine (TEA), phenol and two cresols) were added separately to either produced water or to the ultrapure water and mixed in soils. This allowed a comparison of the degradation behaviour of chemicals in soils in the presence and absence of any additional chemicals present in the HF fluids. The soils treated with ultrapure water served as a control for microbial studies.

Microbial responses to the fluids were measured using indicators such as overall microbial activity (substrate induced respiration - SIR), nitrogen cycling (substrate induced nitrification - SIN) and microbial community structure to provide a comprehensive assessment of the impacts of chemical spills on soil microbiological activities and composition.

In addition, environmental fate of a selected set of potentially hazardous chemicals such as biocides (MIT and CMIT) and TEA (a breaker aid) used in HF fluids, and geogenic chemicals detected in produced water (phenols and two cresols), were also examined. Their rate of breakdown in soils and their binding to soils was studied as predictors of their potential mobility through soils to groundwater.

Chemical breakdown in soils

The half-life of a chemical is often used as an indicator of its persistence. In the present study the times for 50% loss (DT_{50}) in all soils or for 90% loss (DT_{90}) of biocides in all soils were established. The two biocides (MIT and CMIT) that were added to the HF fluid degraded (or transformed) very rapidly in soils. The DT_{90} values for the two biocides in surface soils were less than 1 day and in sub-surface soils less than 2 days. Similarly, the three geogenic compounds detected in produced water (namely phenol, *m*-cresol and *p*-cresol) also showed rapid degradation in soils, with DT_{90}

values <2 days in all soils. The degradation/transformation of TEA was fairly rapid in soils treated with pure water (DT_{90} values <7 days in 4 surface soils), however, it was much slower in soils treated with fracturing fluid with DT_{90} values >30 days in 4 surface and all sub-surface soils.

Effect on soil microbial activities and functions

A significant effect of spills of HF fluid and produced water on overall soil microbial activity (responsible for carbon cycling, as measured by the SIR method) was noted during the experiment. The HF fluid had a greater impact than produced water in any given soil and especially on sub-surface soils, with poorer inherent microbial activities. For example, after nearly two months, the overall microbial activities in surface soils had fully recovered in three out of five cases of spills of produced water. However, in the case of HF fluid spills, the detrimental effect was still noticeable even after this period, such that in four out of five surface and subsurface soils the microbial activity was still significantly lower (by at least 25%) than that in the case of pure water.

The spill of HF fluid had a marked impact on nitrifying microorganisms, such that the exposure to this fluid completely inhibited the nitrification in both surface as well as sub-surface soils during the test period. No recovery was observed after 66 days. In the case of the produced water, the impact was much lower but variable among surface soils, and three out of five surface soils showed complete recovery by the end of the experiment. The response at the molecular level showed that functional genes for nitrogen fixation and carbon cycling were significantly affected with both HF fluid and produced water in some soils. However, the effect was more pronounced in the case of HF fluid. The microbial community composition was also significantly reduced in the HF fluid treated soils. After 28 days, only 2 - 3 taxa were present in soils treated with HF fluid as compared to 4 - 8 in those treated with pure water.

Recommendations

The study shows that the scenario associated with the spill of HF fluid on soils can potentially have a significant adverse impact on soil microbial activity in terms of both the community composition as well as important functions such as nitrification. It is therefore recommended that extra care be taken to minimise and or contain any potential spill of HF fluid.

Since the HF fluid is a mixture of several chemicals, it was not clear which specific chemical/s (or their transformation products, which were not studied here) were responsible for the observed effects. Further studies are desirable to identify the specific HF fluid constituents or their breakdown products that may be responsible for the impact on soil microbial functions. The homogenisation of soils in laboratory conditions can break the microstructure and could eliminate the refugia for microorganisms in soils and hence the recovery may be faster in field conditions. Some recovery of microbial functions was noted and therefore, a longer term field–based study is recommended.

1 Introduction

The recent boom in unconventional natural gas production, which includes shale gas and coal seam gas, has had a significant impact on the global energy economy as it has provided a relatively cheap source of energy. However, concerns persist among the general public and other stakeholders over the potential impacts of these resource extraction operations on water resources and both terrestrial and aquatic ecosystems. The potential impact on air, surface water, groundwater and soil of hydraulic fracturing (HF) operations in coal seam gas production are of general concern to communities living in gas development regions. Community concerns centre around disclosure of the nature and type of chemicals used in the HF operations; potential enhanced mobilization of contaminants from the coal seams; the environmental fate of HF chemicals and geogenic contaminants; and the potential for impacts on human health and the environment. Securing a social licence to operate for the unconventional gas industry has been hampered by the lack of independent scientific studies on the impacts of hydraulic fracturing leading to distrust by the general public.

In order to address these concerns, CSIRO has undertaken a comprehensive investigation of the impacts of hydraulic fracturing (HF) of coal seams on air, soil and water quality at two locations in the Surat Basin, QLD. The air component of the study was conducted by CSIRO Oceans & Atmosphere and the water/soil component by CSIRO Land & Water.

The aims of the water and soil study were to:

(i) Quantify the impacts of HF operations on the concentrations of contaminants in nearby surface waters, groundwater and soils.

(ii) Assess the concentrations of HF chemicals and geogenic contaminants in flowback and produced waters resulting from CSG HF operations.

(iii) Assess contaminant concentrations in the collected water and soil samples with relevant Australian water and soil quality guideline values.

(iv) Conduct a laboratory assessment of various spill scenarios involving spillage of HF fluid and produced waters onto various soils types representative of the Surat Basin.

During the planning phase of the study, two sites operated by Origin Energy were selected in the Surat Basin at Condabri and Combabula. A sampling and monitoring plan for waters and soils was subsequently developed (Apte et al., 2017) and a field sampling program executed from July 2017 to April 2018 during which a range of water and soil samples were collected (Apte et al., 2018a).

A compilation of the laboratory analytical data generated from the samples and details of the analytical methods employed and quality control data are the subject of a separate report (Apte et al., 2018b). The outcomes of the field studies are detailed in two separate reports (Dunne et al.

2019, Apte et al., 2019). The chemical spills laboratory study was conducted as a parallel investigation and is the focus of this report.

Spills of HF fluids and produced water on soils are among the most polluting and plausible pathways of exposure to contaminants (Vengosh et al., 2014; Patterson et al., 2017). Biocides are commonly used in HF fluids and these are designed to be toxic to microorganisms. Therefore, it is important to establish their environmental fate (e.g. mobility and breakdown) in soils. In soils, biocides may undergo a range of processes such as sorption (binding to soil), leaching and degradation, as illustrated in Figure 1. Based on public disclosure by APPEA (www.appea.com.au/wp-content/uploads/2014/08/CSG-fraccing-chemicals.pdf, last accessed 20th September 2018) biocides that are currently used by the CSG industry in Australia as HF additives include CMIT, MIT, sodium hypochlorite, phosphonium sulfate and PigMent Red 5 (also known as Naphthol Carmine). A wider range of biocides including the ones listed above are used in the USA (Kahrilas et al. 2015).

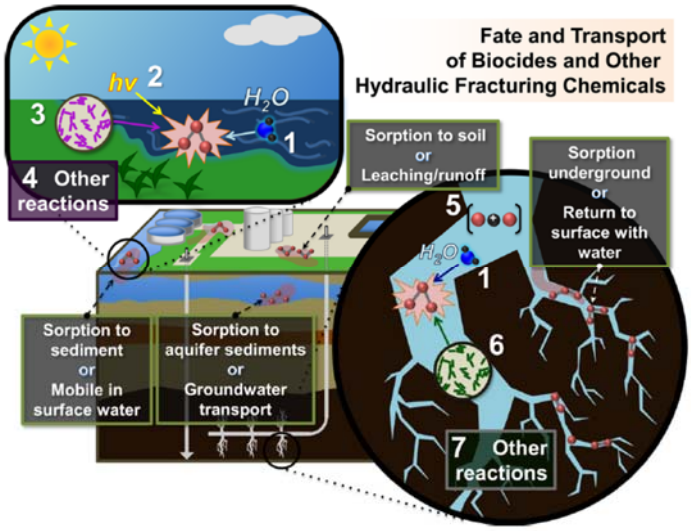

Figure 1. A schematic diagram showing various environment fate and breakdown pathways for biocides in soils. The numbers 1-7 represent different biotic and abiotic pathways of degradation (reproduced from Kahrilas et al., 2015).

A detailed study on some of HF chemicals such as the biocide glutaraldehyde, polyethylene glycol surfactant and polyacrylamide fraction reducer has been reported by McLaughlin et al. (2016). They noted that transformation of certain HF chemicals (polyethylene glycols) was impeded in the presence of the biocide glutaraldehyde (GA) and salts present in HF fluid. They also reported that GA, although undergoing biodegradation (half-life of 10 days), had some inhibitory effect on soil microorganisms. The interactions with other chemicals in HF fluids affected the sorption of GA in soils.

Since the occurrence of spills are unpredictable and are very site- and event-specific, conducting a field-based soil contamination study may not yield meaningful information that can be extrapolated to other locations. Considering the above, a scenario-based assessment mimicking the exposure via spills of HF fluid and produced water (under controlled conditions) was proposed to be a more appropriate approach for our study, as this would generate more broadly applicable information on the potential fate and effects of HF fluid chemicals in soils that could be used to inform future management of spills.

This report describes the studies conducted in relation to fate of chemicals and their impacts on soils under laboratory conditions. The study simulated scenarios involving the spill of HF fluid or produced water onto soils. The impacts on soil microbial functions as well as breakdown of chemicals following such a spill were examined. The binding of biocides (MIT and CMIT) and triethanolamine (TEA) to soils was also assessed to estimate their potential mobility through soils to groundwater.

The overall objectives of the study were to better understand the potential impacts of chemicals present in HF fluid and produced water on soil microbiological activities (through a battery of assays) and estimate their potential mobility through soils to shallow groundwater after a spill.

Although spills of chemicals during transport activities associated with field operations could occur, this scenario was out of the scope of this study. Nevertheless a component of this study that relates to the decay of chemicals in soils is somewhat relevant to such a scenario.

2 Methodology

To make this study relevant to the entire Surat Basin, a range of dominant soil types from the region were collected. The selection of representative soils for this study is discussed below.

2.1 Selection of soils

A wide range of soil types are found in Surat Basin. The map of distribution of soil types in the basin (Figure 2) provides an overview of dominant soil types belonging to different geomorphic units found in the basin (Table 1). Based on their distribution, the five most prominent soil types in the basin were selected for this study. These were clay-rich Vertosols of alluvium/basalt origin, texture contrasting Sodosols (with sodic B horizon), Kandosols and Rudosols (both sandy in nature) of non-quartzose sedimentary origin, and Dermosols of basalt origin (Table 1). Both surface (0-20 cm) and sub-surface (20-40 cm) soils were collected. Contamination of sub-surface soils may occur either because of leaching of chemicals through surface soils due to high volume of spills, and or due to scraping of surface soils during the site operations and reuse of surface soils for rehabilitation of the well lease area, as mentioned earlier. This selection of soils represented a diverse range of soil properties in terms of organic carbon content, clay content, pH, and other physical, chemical and biological properties (Table 2 and Figure 3).

The soils used in the laboratory study were collected on 16-17th April 2018, to ensure their biological integrity, about 3 weeks before the commencement of experiment on 7th May 2018. About 20 kg of each of the surface (0-20) and subsurface soils (20-40 cm) representing the five dominant soil types were collected by digging soil profiles. The surface soils collected from the field were generally dry, despite some rainfall prior to their collection. Sub-surface soils were reasonably moist. The soils were immediately transported to the laboratory by air. All soils had to be air-dried at 40°C for a couple of days before grinding using a mechanical grinder in the laboratory followed by sieving through a 2mm sieve and homogenising by thorough mixing of the sieved-soils prior to their use. Maximum water holding capacities of the soils were determined in

the laboratory. These soils were biologically revived by moistening them and incubating them under optimum temperature (e.g. 20-25°C) for 5 days before the commencement of experiments.

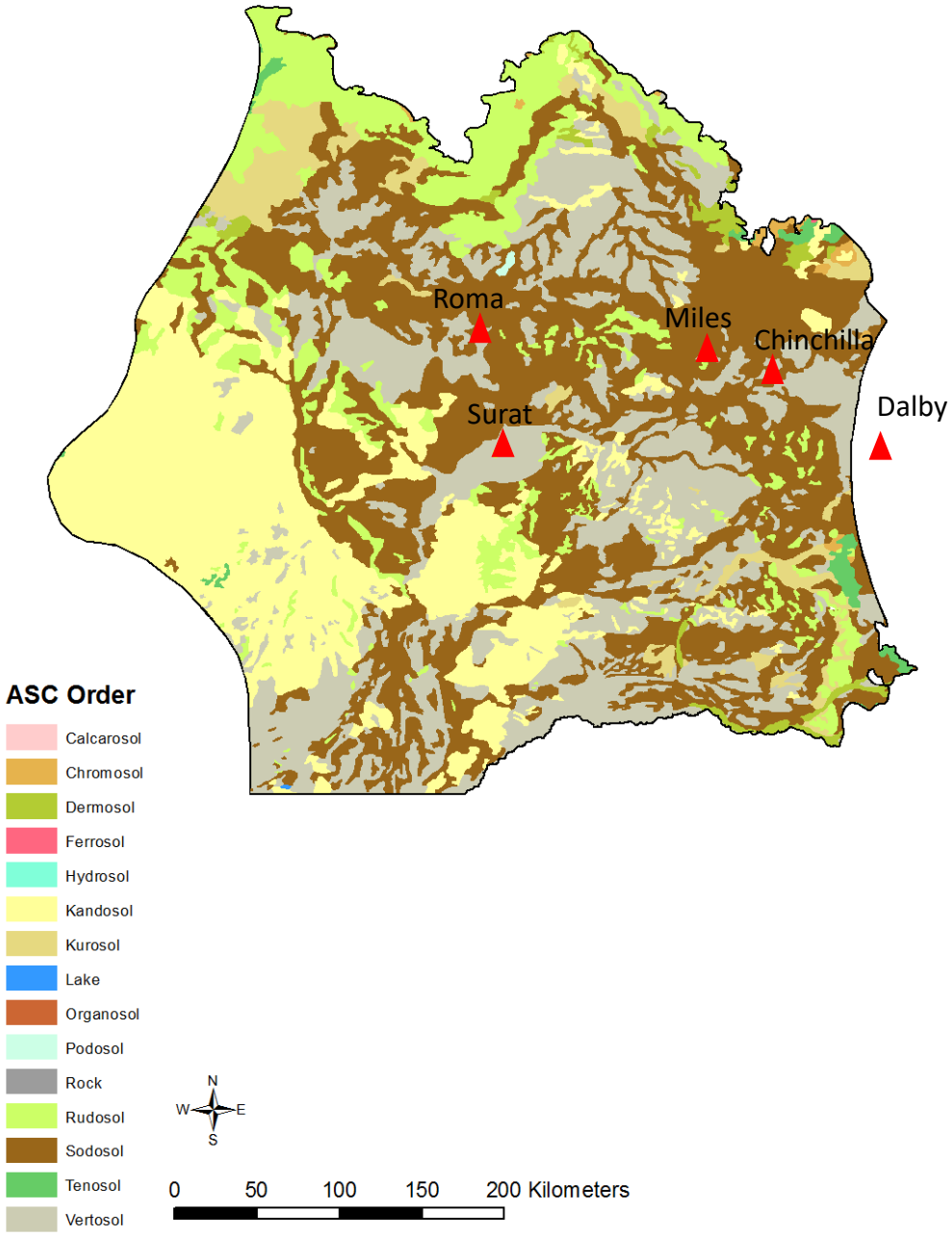

Figure 2 Distribution of soil types in the Surat Basin (Queensland). Soils maps is based on ASRIS Soil Atlas dataset accessed via http://www.asris.csiro.au/themes/Atlas.html February 2018.

Table 1. Geomorphic units and soils types in Surat Basin (after Biggs et al., 2012)

Geomorphic unit	Dominant soil type	Key features
Alluvia	Black and grey Vertosols	Clay rich (> 35%)
Basalt	Vertosols, Ferrosols, Dermosols	Deep clay soils
Quartzose sandstones	Chromosols (rarely found)	Sandy texture-contrast soils
Unweathered to moderately weathered non-quartzose sedimentary rocks	Vertosols, texture contrast Sodosols, Kandosols	Sodosols - Sodic B Horizon
Moderately to strongly weathered non-quartzose sedimentary rocks	Texture contrast Sodosols, Chromosols, Rudosols, Tenosols	Rudosols – Sandy loams Chromosols – Sandy over clayey B horizon

Table 2. Some physico-chemical properties of the soils that were used in this study

Soil type	EC (1:5) dS/m	pH 0.01 M $CaCl_2$	Total Carbon %	Total Nitrogen %	Clay %	Silt %	CEC cmol(+)/kg
				Surface soils			
Dermosol	0.07	5.0	0.89	0.09	17	28	7.9
Kandosol	0.02	4.8	0.61	0.07	10	16	4.2
Vertosol	0.08	5.0	1.30	0.11	37	22	21
Rudosol	0.01	5.1	0.29	0.04	3	5	1.8
Sodosol	0.13	5.2	1.2	0.11	25	15	11
				Sub-surface soils			
Dermosol	0.42	5.7	0.64	0.08	32	21	13.0
Kandosol	0.05	5.6	0.39	0.05	16	16	5.6
Vertosol	0.24	6.4	1.10	0.09	33	24	24
Rudosol	0.01	5.2	0.19	0.04	3	4	1.7
Sodosol	0.26	5.0	0.82	0.09	46	11	21

2.2 Nature of studies conducted on soils

A scenario-based laboratory study involved exposing soil samples to HF fluid and produced water (spill scenarios), to establish the impact of HF fluid and produced water on soil microbial activity as well as to assess the environmental fate of chemicals (i.e. degradation and sorption) that may potentially contaminate soils during a spill. To cover the exposure to chemicals and their potential

impacts as a result of a spill of HF and produced water on soils, three aspects were covered in this study, namely:

(i) degradation rate of selected chemicals present in HF fluid and produced water in soils;
(ii) sorption of selected chemicals in soils to assess their mobility through soils to shallow groundwater, and
(iii) potential impacts of HF fluid and produced water spills on soil microbial health.

A summary of different experiments and associated methodology has been provided in Table 3 and further details have been discussed in specific sections below.

2.2.1 Preparation of hydraulic fracturing and other fluids used in the study

Two types of fluids were seen as the main contributor to the potential contamination of soil via spills during various operations, namely the HF fluid and the produced water. The HF fluid was prepared by the industry using a specific recipe, whereas the produced water reflected the natural properties of the coal seam i.e. groundwater geochemistry. The main difference between the two fluids was that the produced water is naturally highly saline and may contain geogenic chemicals (both organic and inorganic), whereas the composition of HF fluid was known *a priori*. As a reference scenario (control treatment), ultrapure water was included.

The HF fluid was prepared in our laboratory by an engineer of a company involved in hydraulic fracturing in the field, under a Non-disclosure Agreement (NDA) between CSIRO, the company and Origin Energy. The exact recipe and the composition of the HF fluid is proprietary information of the company and cannot be presented in this report (as per the NDA agreement mentioned above). The HF fluid was prepared on the day of commencement of our study (only an hour before its use) using the same products and recipe being used in field. The produced water (60 L) was collected in April 2018 from a well in Combabula region and was kept refrigerated before the use in the experiment. For the ultrapure water, the water from a Milli-Q system (18.2 MΩ.cm resistivity and TOC <5 µg/L) was used. The three fluids used in the study were analysed by NMI or CSIRO for the chemicals of interest only. The data on the composition of the produced water has been presented in Appendix A (Table A2).

2.2.2 Concentrations of chemicals used in degradation and toxicity experiments

As per the NDA, the concentrations of only three compounds used in HF fluid were analysed in this study. These were: CMIT- 3.6 mg/L, MIT- 7.2 mg/L and TEA-1000 mg/L. The initial soil concentrations of these chemicals plus geogenic compounds in the experiments were dependent on soil type because of the variable volumes of fluid that were required to achieve the desired moisture content in different soils. The resultant initial concentrations of chemicals used in the degradation and microbial toxicity experiments for various soils are presented in Appendix A1. The concentrations of organic compounds and inorganic compounds in produced water and ultrapure waters used in the study are presented in Appendices A2 and A3, respectively. The radionuclide concentrations in the produced water used in this study are presented in Appendix A4.

Table 3 **A summary of study design and methodology used in various experiments**

Study Component	Purpose	Study Design	Methodology	Comments
Chemical breakdown	To establish the rate of breakdown of chemicals in surface and subsurface soils	Three replications of 5 surface and 5 subsurface soils were spiked with either HF fluid or produced water spiked with phenol and cresols (Appendix A, Table A2). A pure water treatment served as a control.	Laboratory incubation test in which residues of the chemicals (MIT, CMIT, TEA, Phenol and Cresols) in soils were analysed at different time up to 30 days. Soil samples were collected at 0, 1, 3, 7, 14, 21 and 30 days after treatment. A repeat experiment was also conducted on some of the chemicals (MIT, CMIT, Phenol and Cresols) with samplings on 0, 0.2, 1, 2, 3, 4, and 7 days.	The results provided half-lives of chemicals in soils that were used to assess the groundwater hazard of chemicals.
Chemical mobility	To establish the sorption (binding) of chemicals (MIT, CMIT and TEA) on surface and subsurface soils	A standard OECD protocol was used for sorption studies. Two replicates of 5 surface soils were spiked with 4 different concentrations of the chemicals by serially diluting the HF fluid by 0, 2, 4, 8 times. A blank (pure water) treatment was included as a control.	An aliquot of 2 g soil sample was equilibrated with 10 mL of solution (0.01 M $CaCl_2$ solution + 0.01 M $HgCl_2$) spiked with various chemical concentrations and shaken for 16 hours. The slurry was centrifuged for phase separation (3000 rpm) and solution was decanted for analysis. Due to rapid-degradability of chemicals a soil 0.01 M $HgCl_2$ was used as a microbial inhibitor in the background solution.	The results provided sorption coefficients (Kd) of chemicals in soils that were used to assess the groundwater hazard of chemicals.
Impact on overall microbial activity	To establish the impact of HF fluid and produced water on carbon cycling	Three replications of 5 surface and 5 subsurface soils were treated with either HF fluid or produced water (Appendix A,	Standard OECD protocols for SIR, as optimised by CSIRO for Australian soils (Broos et al., 2007) was used in this study. The SIR is a 6-h incubation after addition of C14 glucose to soil. The soils were subsampled at the beginning (time 0), during (7 days)	The data provided an assessment of impact of HF fluid and produced

		Table A2). The pure water treatment serves as a control.	and end of the experiment (60 days after the addition of fluids).	water on overall microbial activity.
Impact on soil nitrification	To establish the impact of HF fluid and produced water on nitrogen cycling	Three replications of 5 surface and 5 subsurface soils were treated with either HF fluid or produced water (Appendix A, Table A2). The pure water treatment serves as a control.	Standard OECD protocols for SIN as optimised by CSIRO for Australian soils (Broos et al., 2007) was used in this study. The SIN requires a 28-day long incubation of soils after adding ammonium sulfate as a substrate. The soils were subsampled at time 0, 7 days and at 60 days after the addition of fluids.	The data provided an assessment of impact of HF fluid on nitrification, which is considered as a sensitive test.
Impact on microbial functional genes	To establish the impact of HF fluid and produced water on microbial functional genes	Three replications of 5 surface and 5 subsurface soils were treated with either HF fluid or produced water (Appendix A, Table A2). The pure water treatment serves as a control.	The qPCR protocol was used to assess the microbial functional genes. For this assay, the soils were subsampled at 3 and 28 days after the addition of fluids. The functions measured utilised gene probes associated with nitrification (*amo*A), nitrogen fixation (*nif*H), denitrification (*nir*K) and carbon cycling (*GA1*). Total bacterial numbers were estimated using the species conserved 16S gene.	The results provided an assessment of impact of HF fluid and produced water on various genes responsible for microbial functions in soils.
Microbial community structure	To establish the impact of HF fluid and produced water microbial community structures	Three replications of 5 surface and 5 subsurface soils were treated with either HF fluid or produced water (Appendix A, Table A2). The pure water treatment serves as a control.	Next generation sequencing (NGS) was used to assess the microbial community structure (OECD 2015). For this assay, the soils were subsampled at 3 and 28 days after the addition of fluids. Duplicate purified DNA samples from surface soils were sent to the Australian Genome Research Facility (AGRF) for diversity profiling. The NGS diversity used the V3/V4 region of the 16S gene to look at species diversity.	The data provided an assessment on how HF fluid and/or produced water impacted the microbial community composition

2.2.3 Artificial contamination of soils with fluids for spill scenarios

The maximum water holding capacity (MWHC) of all soils was established prior to the commencement of experiments. This allowed assessment of the volumes of different fluids required to saturate the soils. For each surface and sub-surface soil, a sample of 500 g of air-dried soil was weighed into a 1 L amber glass container and spiked with the required volume of fluid to obtain a moisture content of approximately 80% of MWHC of soils. Due to the varying textures of soils (clay-rich Vertosol to very sandy Rudosol), different amounts of water were required for the soils to reach the desired moisture status. The spiked soils were immediately homogenised by mixing well with a spatula, weighed and subsampled for chemical analysis before incubating them in a temperature-controlled chamber (maintained at 25± 2°C). Initial water loss from the containers was allowed, but once the moisture content reached to 50% of MWHC capacity, the soil moisture status was maintained by regular weighing and addition of ultrapure water. The soils were subsampled at pre-determined period for chemical and microbial analysis. The sampling times are given below in Sections 2.1.4 and 2.1.5.

2.2.4 Soil microbiological assessments

Each of the soils that were contaminated with HF fluid or produced water or ultrapure water was subjected to a range of tests (Table 3) for overall microbial activity, specialist functions (e.g. nitrification) and community structure. Further details are given below.

2.2.4.1 Impact on microbial respiration, nitrification and functional genes

Standard OECD protocols for SIR and SIN that had been optimised by CSIRO for Australian soils and published (Broos et al., 2007) were used in this study. The SIR is a 6-h incubation after addition of C14 glucose to soil, whereas SIN requires a 28-day long incubation of soils after adding ammonium sulfate as a substrate. Other details were same as published by Broos et al. (2007). The soils were subsampled at the beginning (time 0), during (7 days) and end of the experiment (60 days after the addition of fluids) for SIN and SIR. In addition, the soil microbial community was evaluated using next generation sequencing (NGS) and quantitative polymerase chain reaction (qPCR) protocols to assess the microbial community structure and functioning, respectively. For this assay, the soils were subsampled at 3 and 28 days after the addition of fluids. The functions measured utilised gene probes associated with nitrification (*amo*A), nitrogen fixation (*nif*H), denitrification (*nir*K) and carbon cycling (*GA1*). Total bacterial numbers were estimated using the species conserved 16S gene. The NGS diversity also used the V3/V4 region of the 16S gene to look at species diversity.

2.2.4.2 Microbial community structure

For the analysis of microbial community structure, duplicate purified DNA samples (using DNeasy PowerSoil Kit; Qiagen) from surface soils were sent to the Australian Genome Research Facility (AGRF) for diversity profiling. 16S PCR amplification and further processing was performed by AGRF using the primer pair 341F-806R and run on a MiSeq Illumina machine. Paired-ends reads were assembled by aligning the forward and reverse reads using PEAR1 (version 0.9.5). Primers were identified and trimmed. Trimmed sequences were processed using Quantitative Insights into

Microbial Ecology (QiiME 1.8)4 USEARCH2,3 (version 8.0.1623) and UPARSE softwares. Using usearch tools sequences were quality filtered, full length duplicate sequences were removed and sorted by abundance. Singletons or unique reads in the data set were discarded. Sequences were clustered followed by chimera filtered using "rdp gold" database as reference. To obtain number of reads in each OTU, reads were mapped back to OTUs with a minimum identity of 97%. Using Qiime, taxonomy was assigned using Greengenes database5 (Version 13_8, August 2013).

A non-metric multidimensional scaling (NMDS) plot was used to visualise the structure among samples, using the taxonomic abundance matrix in PRIMER v6. The plots were generated from 4th root transformed Bray-Curtis similarity index matrixes.

The 30 surface soils DNA were run in duplicate with a negative control to take into account non-specific sequences at low microbial levels. The negative control was an empty tube taken through the extraction to sequencing process. 8,339,566 paired end sequences were generated in total. After assembly and quality assessment a total of 6,974,299 high quality reads were obtained.

2.2.4.3 Assessment of recovery

For the thirty spill scenarios (5 soils x 2 depths x 3 fluid combinations including ultrapure water) tested in the experiment, microbial assessments were carried out at the beginning (immediately after the addition of fluids), during (e.g. 3 or 7 days after addition of fluids), and at the end of the experiment (e.g. 28 or 60 days after the addition). The comparison between the middle and end time points allowed an assessment of any recovery that may have occurred during the experimental period. To eliminate any time-related effect of experimental conditions on the soil's inherent microbial status, the ultrapure water case for each sampling time was used for a direct comparison between the HF fluid and produced water scenarios for each sampling time. The results for microbiological tests were presented as response relative to that observed under chemical-free (ultrapure water) scenario (assumed to be 100%). This eliminated the influence of any confounding factors related to time or experimental conditions (e.g. long incubation times). This is a common practice reported in scientific literature on the effect of chemicals on soil microbial activities (e.g. Dennis et al., 2018).

2.2.5 Soil chemical assessments

From a chemical perspective, two important questions were answered in this study. Firstly, to establish at what rate the chemicals will breakdown in soils and secondly, how mobile these chemicals are likely to be in the soil profile.

The compounds of key concern in HF fluids were two biocides (MIT and CMIT) and one breaker-aid (TEA) that are commonly included in HF formulations. These were used as mixtures. Permission was granted for the analysis of these three chemicals in the HF fluid via the Non-Disclosure Agreement with the company carrying out the HF operations and Origin Energy.

For the produced water case, the choice of chemicals of interest was based on the extensive analysis of produced waters that were collected in the water quality assessment component of the project (Apte et al., 2019). Here, the naturally occurring organic chemicals phenol and two cresols (*m*-cresol and *p*-cresol) were identified as the key chemicals. However, their occurrence was often

at very low concentrations (<0.002 to 0.30 mg/L). Therefore, a model test case for chemical degradation was included where produced water was spiked with phenol and cresols at 200 mg/L to achieve 6-16 mg/kg concentrations in different soils (Appendix A1).

2.2.5.1 Breakdown of toxic chemicals in soils

Soils were incubated in a controlled chamber under constant temperature (25°C) and at a fixed moisture content (60% MWHC). Two sets of experiments were conducted, as we decided to repeat the degradation experiment since, in the first experiment, we observed a faster than expected loss of chemicals.

The first experiment commenced in May 2018. Aliquots (500 g) of air-dried and homogenised soils (all surface and sub-surface soils) were weighed into amber glass jars and the soil was wetted to 30% MWHC (determined prior to commencement) with ultrapure water only. The soils were incubated for 5 days to allow the revival of any loss in microbial activity in dry soils. Then 40-80 mL of the three fluids (HF fluid, produced water and ultrapure water) were added to various soils (depending on their MWHC) to achieve a moisture equivalent to about 80% of MWHC. The soils were homogenised with a stainless spatula, sub-sampled for time zero and the weights were noted. The glass jars with soils plus various fluid additions were transferred to a temperature-controlled chamber set at 25° C. Subsamples (5-10 g) were taken at designated times (0, 3, 7, 14, 28 and 60 days), and were placed in the deep freezer prior to sending these for analysis. The samples were sent (in cool boxes) to National Measurement Institute (NMI) Sydney for residue analysis of phenol, cresols and PAHs. The residues of MIT, CMIT and TEA were analysed by the CSIRO Adelaide laboratory.

In the first experiment, the chemicals were found to degrade much faster than expected, i.e. MIT and CMIT as well as phenol and cresols had completely degraded in 3 days. Hence to verify the findings, the experiment was repeated with finer time resolution and some modifications in the protocol. Instantaneous freezing of subsamples in liquid nitrogen was employed to arrest any losses that may occur during sample handling. The repeat experiment was conducted in August 2018. The soils were revived, as before, by incubation with ultrapure water at 30% MWHC for 5 days before the fluids were added to the soils. In the second experiment, the subsampling was carried out after 0, 0.2 (4 h), 1, 2, 3, 4, 7, 14 and 28 days after treatment. Following snap-freezing by dipping into liquid nitrogen, each sample was stored at -18°C prior to analyses. For the repeat experiment, all analyses were carried out in the CSIRO laboratory to avoid any potential losses of these short-lived chemicals during transit to an external laboratory.

2.2.5.2 Sorption experiment to assess potential mobility of HF chemicals through soils

Ideally, the mobility of chemicals is best studied using intact cores to maintain the structural integrity of soil column during assessment. However, such cores at best only represent the spot they are taken from, due to inherent heterogeneity in soil structure, such as the presence of biopores, cracks or other structural features. It is therefore difficult to represent a large area through column studies. Biopores could also lead to preferential flow of chemicals allowing limited interactions with soil. Besides, the rapid degradation of chemicals can also confound the results of a column transport experiment. Since this study was designed to represent a range of soil types

from the entire Surat Basin, standard batch method was employed for the assessment of mobility via sorption.

Batch sorption experiments were conducted on the two biocides (MIT and CMIT) and TEA in all surface and sub-surface soils. Using a standard OECD protocol, a 2 g soil sample was equilibrated with 10 mL of solution (0.01 M $CaCl_2$ solution + 0.01 M $HgCl_2$ as a microbial inhibitor) spiked with the three chemicals at a range of concentrations. For such readily degradable compounds it was essential to use a microbial inhibitor to arrest losses during sorption experiment. Our previous work established that 0.10 M $HgCl_2$ was most effective for sorption experiments on labile organic compounds (Martin et al. 2018). Four different chemical concentrations were used, by serial dilutions of that present in the fluid. This together with a blank (no chemicals added) allowed four concentrations for the generation of sorption isotherms. All measurements were run in duplicate. The residues analysis after 20-h equilibration was carried out by the method described below.

2.3 Analytical methods

2.3.1 Phenols and cresols

For the first experiment (May 2018) the USEPA Method 8270C was used by NMI for the analysis of phenol and cresols in soils. A 10 g aliquot of soil, treated with sodium sulfate, was extracted in 20 mL of DCM/acetone (50:50) by tumbling for 2 hours. A gas chromatograph (GC - Agilent Model 7890A) coupled to an Agilent Model 5975C Mass Selective Detector operating in the electron impact mode was used for analysis. The GC column was a DB5 MS 30 m X 0.25 mm X 0.25 micron. An Agilent split/splitless capillary injector fitted with silanised glass liner was used as the injection system. Other conditions included: Injection temp – 250°C, helium carrier at 1 mL/minute, GC/MS transfer line 280°C. Oven program: 40°C for 1 minute, ramp 18°C/min to 310°C, hold for 7 min.

For the repeat experiment in August 2018, the analysis was carried out by CSIRO. An aliquot of 5 g soil was extracted with 10 mL of 10% methanol+90% 0.01M $CaCl_2$ by sonication for 15 minutes followed by shaking for 30 minutes. The phenol and two cresols in the extracts were analysed with an AB SCIEX TripleTOF ^{TM} 5600+ system (APCI negative) with a DuoSpray™ Source.

A SCIEX Exion LC AD autosampler with column oven, LC pumps and degasser was used for liquid chromatography (LC) separation. A Varian Pursuit XRs diphenyl 150 x 2.0 mm column was used with a binary mobile phase at a flow rate of 0.4 mL/min. The first 4.0 min of the flow was sent to waste via a 10 port-2-position valve installed post-column in order to prevent the ion source from contamination with matrix components. The optimised separation conditions consisted of a mobile phase of Milli-Q water (A) and methanol (B). The gradient elution was as follows: 0–1.5 min: 10% B, increasing to 95% within 5 min, held at 95% for 5 minute, then was re-equilibrated at 10% B for 3 min with a total run time of 13 min. The column oven and autosampler temperature were set at 30^0C and 10^0C, respectively. The sample volume injected was 10 µL.

The acquisition was scanned over a mass range of 100 – 500 (m/z). Automated calibration was performed using an external calibrant delivery system (CDS) which infuses calibration solution prior to sample introduction. The information dependent acquisition (IDA) methods consisted of a

TOF-MS dependent scan (m/z 100-500) followed by a multiple reaction monitoring (MRM) scan was performed with a collision energy of -20 eV. Data was processed using MultiQuant^{TM} 3.0.2 Software. Recovery tests on soils were conducted to validate the extraction method. The recoveries from soils ranged from 62 to 78 %.

2.3.2 Biocides and triethanolamine

An aliquot of 5 g of soil was extracted in 10 mL of 5% methanol: water with 5% formic acid (10:90) by tumbling for 4 hours. The recovery tests in soils were conducted to validate the extraction method and the recoveries in soils ranged from 65-101 % for MIT and 58-99% for CMIT. The TEA extraction procedure was based on the study by West and Gonsior (1996). Soil samples (5 g) were extracted with 10 mL of 1 N NaOH by tumbling for 4 hr. The recoveries of TEA in soils ranged from 91 – 97%. The residue in aqueous samples from sorption experiments were measured directly after 100 -1000 fold dilutions.

The residue analysis was performed by LC/MS/MS on a Thermo-FINNIGAN Surveyor autosampler Plus and Thermo-FINNIGAN Surveyor MS pump Plus (ThermoFisher Scientific, Waltham, MA, USA). Separation was performed with Waters Atlantis dC18 5µm (2.1 x 150 mm column, Waters, Milford, Massachusetts, USA) and a binary mobile phase at a flow rate of 0.25 mL/min. The first 1.0 min of the flow of the chromatographic run was sent to waste via a 6 port, 2-position valve installed post-column in order to prevent the ion source from contamination with matrix components. The optimized separation conditions were as follows: solvent A: a mixture of 0.1% formic acid and 10mM ammonium formate, solvent B: methanol. The gradient elution was as follows: 0–2.5 min: 95% A, the organic phase B was increased to 85% within 5 min, stay as 85% B for 4 minutes, back to 5% A in 2 minute, the column then was re-equilibrated with 95% A for 5 min. the total run time was 16 minutes. The column oven and the autosampler temperature were set at 35^0C and 10^0C, respectively. The sample volume injected was 10 µL.

Coupled mass spectrometry was performed using Thermo TSQ Quadrupole Mass Spectrometer (ThermoFisher Scientific, Waltham, MA, USA). Electrospray ionisation (ESI) positive was used.

MS source parameters were optimized as: spray voltage: 5 kV (ESI positive mode), sheath gas pressure: 40 arbitrary units, Auxiliary gas pressure: 5 arbitrary units, capillary temperature: 350^0C, collision gas pressure: 1.5 mTorr. High purity nitrogen (>98%) was used for desolvation and as a nebulizer gas. Argon was used as the collision gas. Positive ionisation mode was used for the analysis of three compounds (MIT, CMIT and TEA). Optimization of multiple reaction monitoring (MRM) parameters was performed by direct infusion 200 µg/L of analytical standard at a flow rate 10 µL/min. Collision energy, tube lens voltages were optimised for each MRM transitions, respectively. Data were acquired and processed using the Xcalibur 2.1 software.

2.4 Quality control / quality assurance

All soil samples were collected and analysed using internationally accepted sampling and analysis protocols. Standard test protocols specified by OECD were used for microbiological assays (e.g. SIR and SIN as optimised by CSIRO for Australian soils and published (Broos et al., 2007).

Consistent with the water quality assessment, the chemical contamination of soils at impacted sites (the field study component) were conducted in NATA (National Association of Testing Authorities, Australia) accredited laboratories with rigorous quality control. Similarly, the residue analysis during the first chemical degradation experiment was carried out by NMI. The residue data by NMI and CSIRO for cresols and phenols was found to be consistent with each other.

To avoid any potential impact of soil matrices, the calibration curves were prepared in soil extracts and thus matrices were matched. Suitable blanks and controls were used during the analysis.

Analytical methods were optimised prior to residue analysis. The recoveries of spiked soils and where possible surrogates (isotopically labelled compounds) were used to establish the effectiveness of extractants and the reproducibility of recoveries. For example, for studies on phenol and cresols, each sample was spiked with phenol-D6 surrogate. Blank and spiked control samples were also processed per batch. A matrix spike sample and duplicate was analysed per 20 sample batch where appropriate.

2.5 Statistical analysis

The data on SIR and SIN assays for microbial assessment were presented as relative response ratios. This was necessary to account for soil to soil differences (including between surface and subsoils) as well as any differences in microbial responses related to experimental conditions with time. Response ratios were analysed using an analysis of variance (ANOVA) for each soil depth. Treatment, soil type, time and batch were included as main effects. Two way interactions were also included. Least significant difference (LSD) letters were calculated using the multiple comparisons adjustment method of Benjamini and Hochberg (1995). This was implemented using the LSD.test function in the R agricolae package (de Mendiburu, 2017). A conservative estimate of the LSD considering three variables at once, enabled comparisons across time, treatments and soil types on the same graph. Hadley Wickham's R facet plots were used to display graphical results (Wickham, 2016). Details of the statistical analyses can be found in Appendix B.

Outliers were identified using ANOVA residual plots and excluded from further analysis. Pure water controls were not omitted as they were the basis for the ratios. In all, only 2 (out of 510) nitrate values were omitted and 2 (out of 510) CO_2-C values were omitted. Nitrate production <0.025 mg/kg were considered to be 0.025 (253 out of 510). Nitrate ratios >96th percentile were considered to be 2.06 for 4 triplicate ratios.

To test the significance of fluid on copy numbers of functional genes, qPCR data was analysed by one-way ANOVA (significance set at $p<0.05$ and Dunnett's multiple comparison test) in SigmaPlot 12.5.

ANOSIM was used to test the effects of fluid on the community structure, SIMProf to test the significance of grouping and SIMPER to determine the dominant species contributing to the dissimilarites in community structure.

3 Results and Discussion

Microorganisms in soils (bacteria and fungi) participate in carbon and nitrogen cycling, as well as the breakdown of both naturally occurring and man-made chemicals. While a range of microorganisms are involved in carbon cycling and chemical breakdown (in most cases), nitrogen cycling is mediated by specialist microorganisms and are known to be more sensitive to pollutants (e.g. Radniecki et al. 2008). The impacts of fluids on microbial activities in soils was established using three different modes of biological assessments (Table 3). Overall microbial activity was measured using SIR and qPCR of bacterial and carbon-cycling genes. Nitrifying activity was assessed using SIN and qPCR of key genes involved in nitrogen cycling. The microbial community structure and diversity was also measured with DNA sequencing to determine any community shifts and identify specific taxa driving these shifts. The inherent microbial status of soils was assessed in the absence of any chemicals that may be introduced such as with a spill of HF fluid or produced water.

3.1 Inherent microbial activities in the soils

The data on the inherent microbial activities in soils (Figure 3) show that all surface soils and sub-surface soils were microbiologically active and suitable for the various experiments. The subsoils also had significant activities but much lower (in some cases down to 40-50%) than the surface soils. In terms of nitrifying microorganisms (a specialist group of bacteria), marked differences were noted among surface and sub-surface soils. For example, while the surface layers of Dermosol and Kandosol had very active populations of nitrifying bacteria, their respective sub-surface layers showed very little activity of the same (about 3 to 4% of that in the surface soils). This may be for a number of reasons such as an unfavourable soil environment, previous history and exposure to elemental nitrogen and its compounds. It is noteworthy that all of these soils were from open grasslands with stands of native tree vegetation and not under intensive management and thus free of fertiliser inputs.

3.2 Breakdown of chemicals in soils

Before the impact of spills of HF fluids and produced water on soil microbial activities are discussed, it is important to understand which chemicals were associated with these fluids and how long they persisted in the soils. Such information may help understand the observed impacts on soil microbial processes being discussed later.

3.2.1 Biocides

Based on the information supplied by the operator responsible for HF of wells at the study site, the main biocides used in HF fluids were a mixture of two compounds, MIT and CMIT. These compounds occur in a certain ratio in the product used by the industry. Since little information is available on these compounds in the published literature, we focussed on MIT and CMIT biocides.

The decay of these two chemicals over time in five surface and sub-surface soils is presented in Figures 4 and 5. Both compounds degraded (including transformation) very rapidly in soils. In

surface soils, nearly all (99%) of the applied MIT and CMIT had degraded within a day. Despite the inherent variations in the microbial activities, all soils were able to readily breakdown or transform the two biocides. The transformation products were not identified in this study. In sub-surface soils, the rate of degradation was only slightly slower than that in surface soils. Still, about 90% of the applied chemicals had degraded within a day in most sub-surface soils. By Day 2, essentially all (99%) of the MIT and CMIT had disappeared from or transformed in the sub-surface soils also.

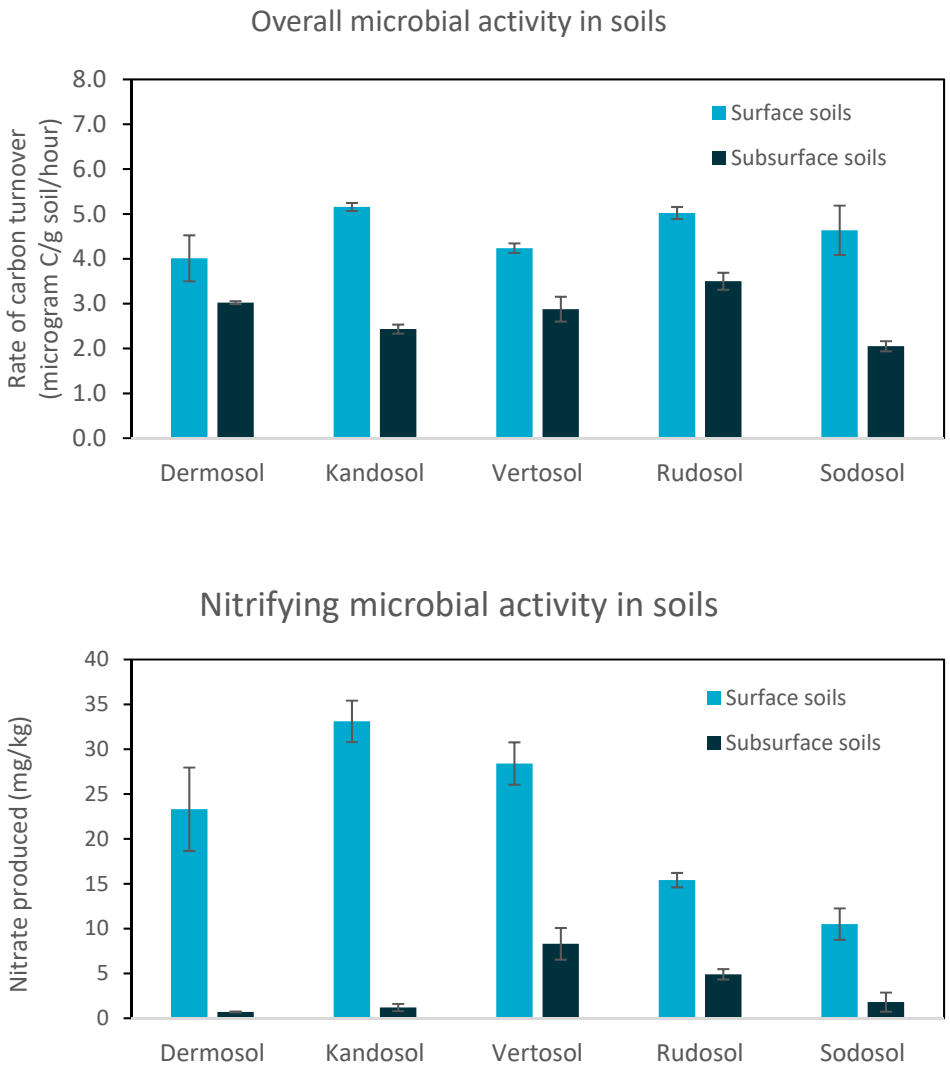

Figure 3. Inherent overall and nitrifying microbial activities in five surface and sub-surface soils, as measured by SIR and SIN assays, respectively. The error bars represent 1 standard deviation around the mean (n=3).

The half-life values (presented as DT_{50}) of the two biocides (which is usually used as an indicator of their rate of degradation/transformation) in surface soils were < 4 h and in sub-surface soils were less than 1 day in most soils. The rates of degradation/transformation of the two chemicals were essentially similar, despite some differences in their chemistry. There are limited published data in literature on the fate of these chemicals in soils, however, the results of this study are consistent

with the only published study that we could find. Krzeminski et al. (1975) observed that these compounds are readily degraded in water and soils through hydrolytic, photochemical and biological actions. They found that, in soils, the degradation was mainly through biodegradation processes. They also noted that, in an inoculated river water, about 90% of the compounds had degraded within 5 days. The European Chemicals Agency (ECHA 2016) committee for risk assessment, in their background document on these two compounds, cites short half-lives (DT_{50}) in aerobic soils ranging from only <0.08 to 0.27 days, which is also consistent with our findings. It was confirmed by repeating the experiment that these compounds are readily degraded in soils of the Surat Basin, under optimum conditions of moisture and temperature. We are not sure if these chemicals were completely mineralised to C, H and water or transformed into breakdown products.

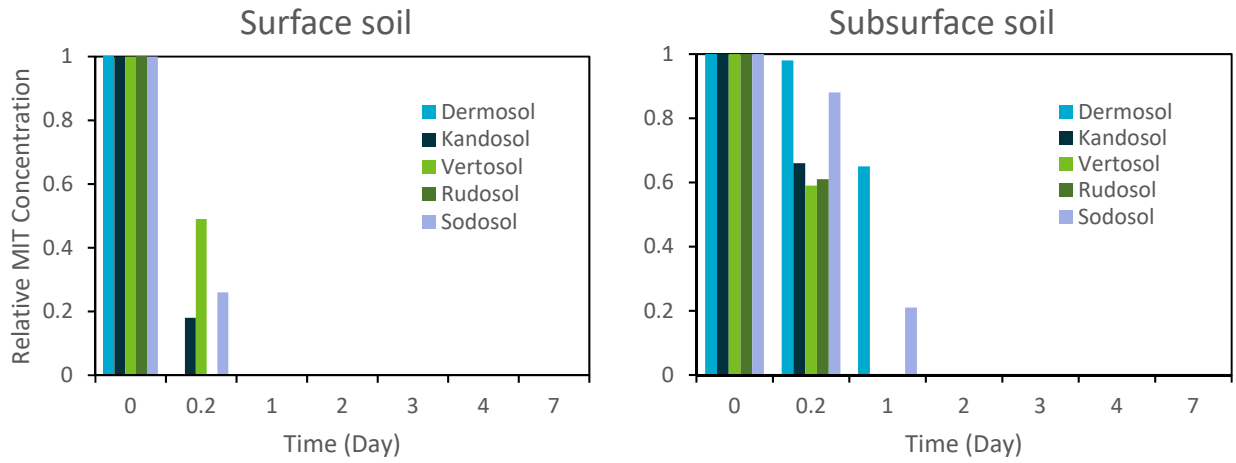

Figure 4. Breakdown of biocide MIT present in hydraulic fracturing fluid in five surface and sub-surface soils

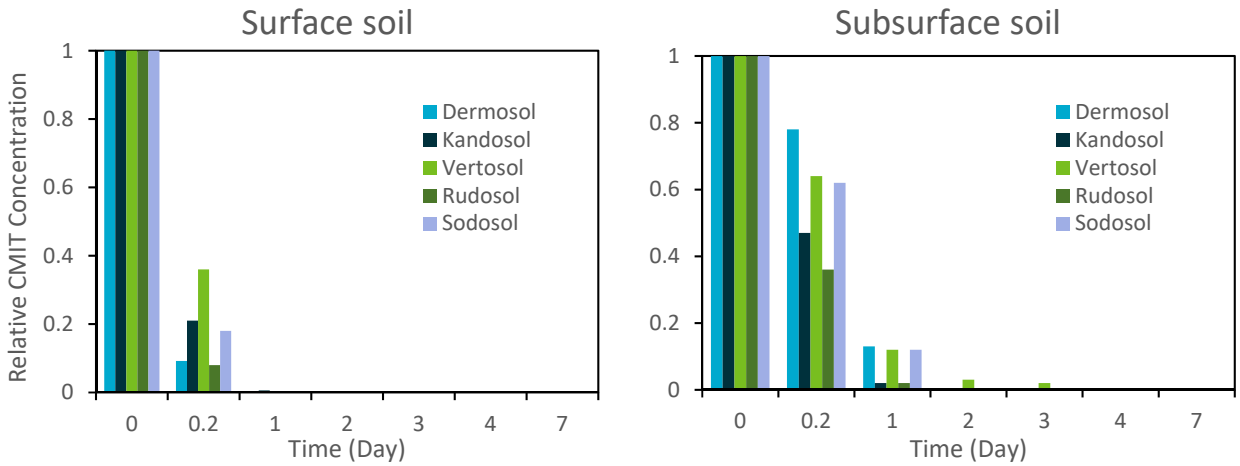

Figure 5. Breakdown of CMIT biocide present in hydraulic fracturing fluid in five surface and sub-surface soils

3.2.2 Triethanolamine

Another chemical of key interest in this study was TEA (triethanolamine or 2,2',2"-nitrilotriethanol), which is used as a low temperature breaker aid in HF fluids. We could not find much published data on the environmental fate of this compound, except one study by West and Gonsior (1996). Hence this chemical was also included in the degradation study.

The data on breakdown of TEA after adding the HF fluid to different soils are presented in Figure 6. Significant degradation of the compound (40-80%) occurred in first three days after adding the fluid containing TEA to soil. However, after this period the rate of degradation (or transformation) slowed down considerably, to the extent that in some soils (e.g. Dermosol, Sodosol) very little further degradation occurred during the next 4 weeks. This may either be due to reduced microbial activity in these soils with time as affected by the HF fluid (see discussion in Section 3.4 of this report). The rate of degradation was much slower in the subsurface soils, such that in 3 out 5 soils approximately 80% or more residue was still detectable 30 days after the addition of HF fluid to soils.

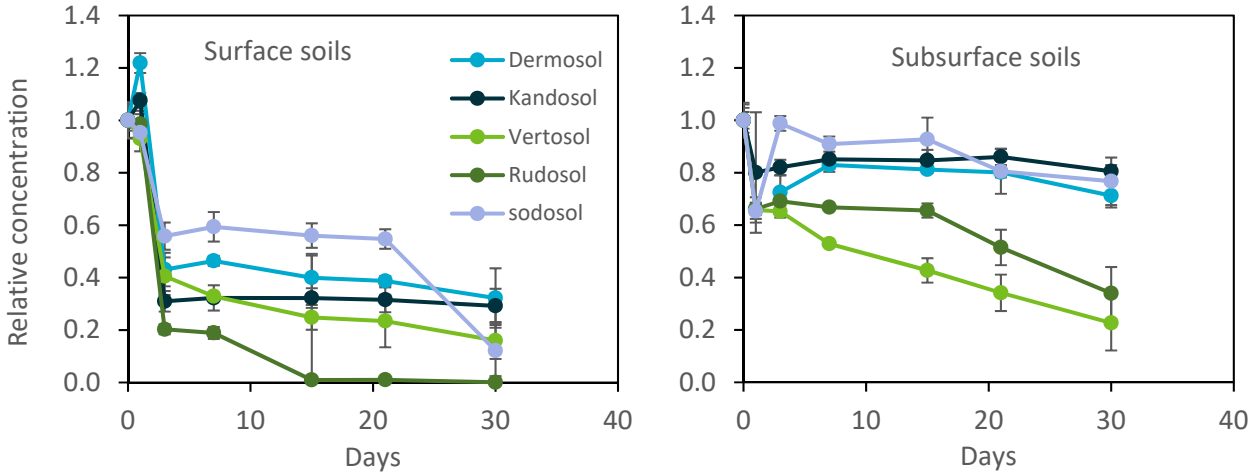

Figure 6. Degradation of triethanolamine in surface and sub-surface soils in presence of HF fluid. The error bars represent the standard error of the mean (n=3).

To understand the role of HF fluid characteristics, degradation of TEA was also measured when applied with pure water in all five surface soils (Figure 7). A comparison of the data on the rate of degradation of TEA between pure water and HF fluid in Figures 6 and 7 clearly show that TEA degraded (or transformed) more rapidly in soils when applied with pure water than with the HF fluid. Unlike in the case of HF fluid, in four out of five soils TEA had degraded (or transformed) within a week, when applied with pure water. Specific transformation products of TEA were not investigated in this study. In both treatments (pure water and HF fluid) the rate of degradation was slowest in Sodosol and fastest in Rudosol. The soil properties had only a minor impact on the rate of breakdown, the major differences observed between degradation in the presence of pure water and HF fluid is related to the fluid characteristics. This may have been caused by the reduced soil microbial activity and altered microbial community structure in the presence of HF fluid (Section 3.4).

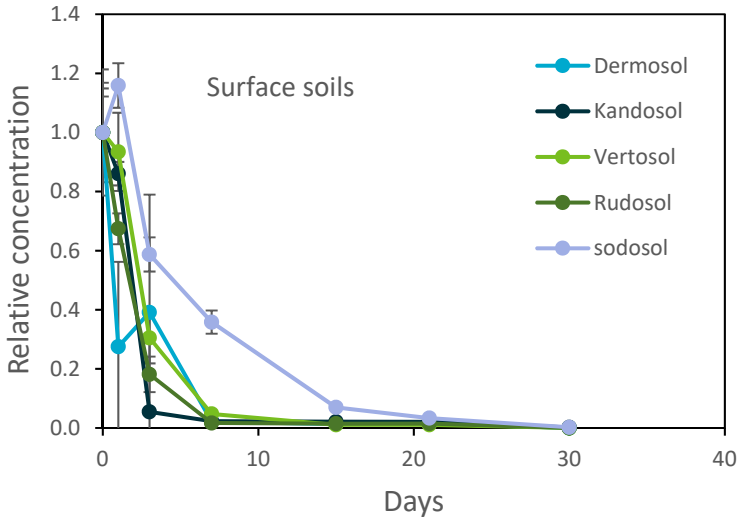

Figure 7. Degradation of triethanolamine in surface soils in the presence of pure water. The error bars represent the standard error of the mean (n = 3).

3.2.3 Phenol and cresols

As part of the comprehensive study on water quality at the study site, produced waters were collected from a number of wells at Condabri and Combabula sites which had been hydraulically stimulated. Their analyses showed low concentrations of phenols (< 1 to 14 µg/L), cresols (< 2 to 300 µg/L) and naphthalene (<0.5 to 1.5 µg/L) in these waters (Apte et al. 2019).

While there is little published data on phenol and cresol degradation in soils, naphthalene is a well-studied compound. Naphthalene is known to be lost from air, water and surface soils through volatilisation, photochemical degradation and biodegradation; with half-lives ranging from 1 to 80 days (or even longer) in soils depending on the conditions such as co-contamination, previous history of exposure, microbial status of soils, soil organic carbon content (e.g. Jury et al. 1984; Cerniglia 1992; Smith et al. 1997; ECB 2007). Due to their susceptibility to volatilisation, the rates of degradation for naphthalene and BTEX compounds in soils are difficult to measure (Smith et al. 1997). However, the European Commission have used 70 days as the half-life for naphthalene in bulk soils in their risk assessment (ECB 2007). Considering the above, we focussed on the degradation study of phenol and two cresols (3-methylphenol also known as *m*-cresol, 4-methylphenol also known as *p*-cresol) that were detected in flowback and produced waters.

The soils spiked with produced water had no measureable background concentrations of these compounds at the time of spike. Therefore, the soils were artificially spiked with phenol and two cresols in produced water in a parallel experiment. The results from this study showed that phenol and cresols had all completely degraded in soils by the first sampling time, i.e. 3 days after spiking (analyses carried out by NMI, Sydney). Therefore the experiment was repeated with much shorter sampling times, i.e. 0, 0.2, 1, 2, 3, 4 and 7 days after the treatment. The repeat experiment (Figure 8) confirmed the findings of the first experiment. Once again, phenol and cresols were found to completely degrade in all surface and sub-surface soils within 2 days, i.e. residues in soils were not detectable after 2 days in soils.

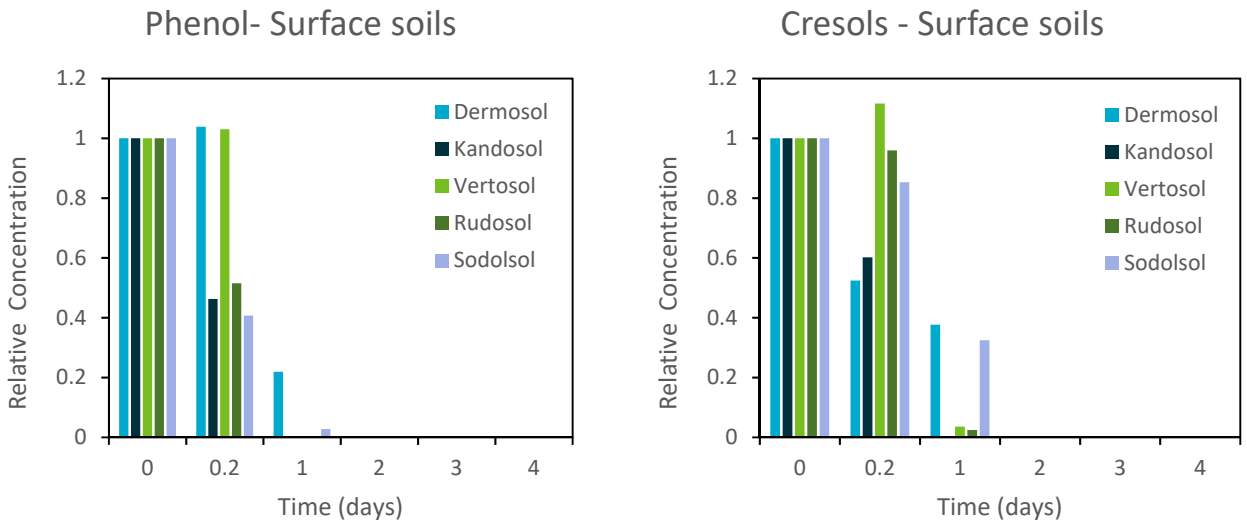

Figure 8. Breakdown of phenol and two cresols (analysed as a mixture) present in produced water in five surface soils

- The two biocides (MIT and CMIT) that are commonly used in HF fluids are readily degraded in soils with complete loss of residue within one day of mixing into the surface soils. The loss rate was only slightly slower in sub-surface soils.
- Triethanolamine degraded relatively rapidly when introduced to the soil with pure water (completely degraded within a week in most soils). However, in the presence of HF fluid, its rate of degradation after 3 days became so slow such that little further breakdown occurred in three out of five soils during the month long experiment. Reduced microbial activity and altered microbial community structure in the presence of HF fluid may have resulted in its longer persistence in soils.
- Phenol and two cresols (*m*-cresol, *p*-cresol) in produced water completely degraded in all surface soils within 2 days.

3.2.4 Sorption (binding) of biocides and triethanolamine onto soils

The potential mobility of chemicals through a soil profile is usually measured through their sorption (binding) to soil particles. Data on sorption of MIT and CMIT are presented in Table 4. Since only a small fraction of the compounds present in water were removed (sorbed) by soils, complete isotherms could not be measured. For example, for MIT the % sorption in different soils ranged from 7.7 to 30.7% and, for CMIT, it was even smaller, ranging from only 1.9 to 5.1%. These values correspond to the maximum concentrations of the two biocides present in the HF fluid (for MIT 144 mg/L and for CMIT 72 mg/L. Similarly for TEA, the % sorption values ranged from 4.1 to 64% at the highest initial concentration (110 mg/L). In only two soils (Sodosol and Vertosol), the sorption was significant enough to establish a sorption isotherm, as shown in Figure 9. The isotherms were nearly linear with K_d values 8.8 and 4.3 L/kg and K_{oc} values of 733 and 331, respectively for the Sodosol and Vertosol. The K_{oc} values of TEA from the single point sorption for the rest of the soils ranged from 71-107. From these data, the two biocides and TEA were expected to be quite mobile in most soils studied here.

Table 4. Sorption of two biocides (MIT, CMIT) and triethanolamine (TEA) in five surface soils

Soil type	MIT	CMIT	TEA
	Sorption, % of initial concentration		
Dermosol	10.2	1.9	16.0
Kandosol	7.7	2.6	10.1
Rudosol	26.6	5.1	4.1
Sodosol	19.1	4.0	64.0
Vertosol	30.7	5.1	45.2

- The two biocides (MIT, CMIT) are weakly sorbed in most soils indicating their ready mobility with water down the soil profile.
- The degree of sorption of TEA varied among soils, being higher in soils richer in organic matter (Sodosol and Vertosol).

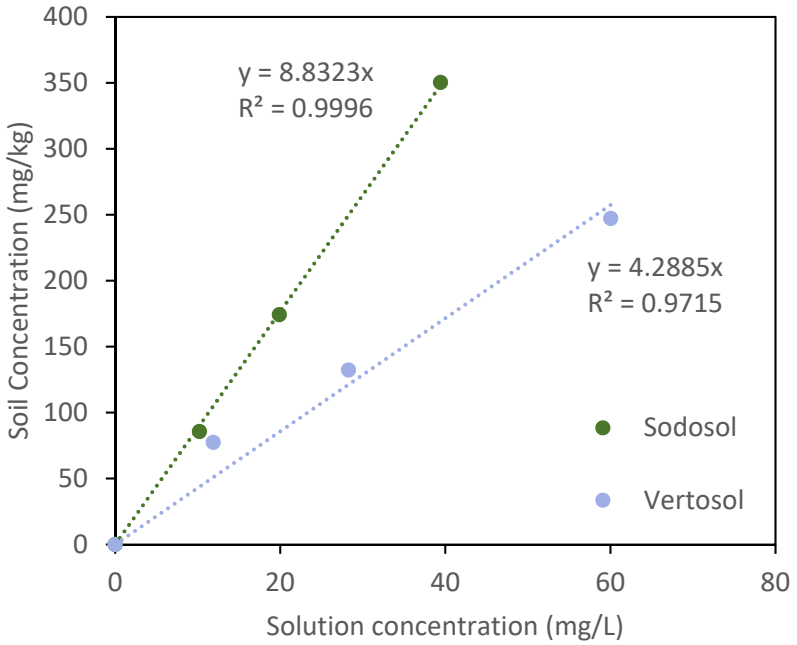

Figure 9. Sorption isotherms of triethanolamine in two of the five surface soils. The amount sorbed was too low to construct isotherms for the other soils.

3.3 Impact of hydraulic fracturing fluid and produced water on soil microbial processes

3.3.1 Microbial processes in soils

The impacts of spills of HF fluid as well as that of produced water on five surface and sub-surface soils are discussed below from the standpoint of (i) overall microbial activity – carbon cycling, (ii)

the specialist microorganisms responsible for nitrogen cycling, (iii) microbial functional gene analysis, and (iv) microbial community structure.

3.3.1.1 Overall microbial activity (carbon cycling)

Overall microbial activity was assessed through the SIR assay, which essentially measures the turnover of a readily available carbon source (glucose in this case) in soils. The SIR data have been presented as a ratio of the respiration rate (microgram C/g soil/h) in a given treatment to that measured in ultrapure water (control treatment). Figure 10 shows the relative responses (ratios) of HF fluid and produced water spills on 5 surface and sub-surface soils during the experiment (3 days after) and at the end of the experiment (60 days).

A significant effect on microbial activity was evident from Figure 10, especially during the experiment. One surface soil (Dermosol) showed some enhancement in overall microbial activity in presence of HF fluid and produced water, even at the beginning of the experiment. The HF fluid had caused a greater reduction in the overall microbial activity than produced water in others, including surface and sub-surface soils. By the end of the experiment (about 2 months after the spill) the microbial activities had fully recovered in 4 out of 5 surface soils spiked with produced water. This also suggests that salts present in produced water did not have a major detrimental impact on soil microbial processes. However, the recovery was negligible in presence of HF fluid. The HF fluid had a stronger impact on the sub-surface soils than on surface soils. However, full recovery was noted in two of the five soils (Dermosol and Rudosol). Such recovery in carbon cycling is commonly observed, as even if a part of microbial population is adversely affected by toxicants, other species are able to take up their role.

> Hydraulic fracturing fluid had a significant effect on soil microbial activity, and showed little recovery with time, especially in surface soils. Addition of produced water had a smaller effect and most soils recovered fully in two months.

3.3.1.2 Specialist microorganisms (nitrogen cycling)

As mentioned before, the nitrogen cycle is mediated by specialist nitrifying microorganisms such as genera of *Nitrosomonas* and *Nitrobacter* bacteria in soils. These bacteria are known to be quite sensitive to contaminants and hence are a good indicator of impact of chemicals on soil microbial health. The impacts of HF fluid and produced water spills on five surface and sub-surface soils from the Surat Basin are presented in Figure 11. The data are again presented as response relative to that observed under the control treatment (in ultrapure water with no chemicals).

The HF fluid had a marked impact on nitrifying microorganisms, such that the exposure to these fluids almost completely inhibited the nitrification in both surface as well as sub-surface soils during the experiment. No recovery was noted even two months after the spill. In the case of produced water, however, the impact was much lower and variable among the surface soils. By the end of the experiment, 3 out of 5 surface soils showed complete recovery of the microbial function. However, the Vertosol and Sodosol did not show any recovery. The impact of produced water on sub-surface soils was greater than that in surface soils. While the reasons for this are unclear, presumably the poor microbial population base (especially of the nitrifying bacteria) may have made subsurface soils more vulnerable. For example, in the Dermosol and Kandosol, the

inherent nitrifying activity in sub-surface soil was nearly 30 times lower than that in the surface soil (Figure 3). In the Sodosol, a 6-fold reduction was noted, whereas in the Vertosol and Rudosol, it was three times lower than in the respective surface soils.

The reasons for such a marked effect on nitrifying bacteria are not clear. As shown earlier (Section 3.2.1), the two biocides (MIT + CMIT) were short-lived in soils, degrading almost fully within 2-3 days after the spill. If they were the primary cause of the effect, then some recovery in soils was expected, unless the bacteria were impacted beyond recovery by the two biocides. It is not clear if the transformation products of these chemicals (not studied here) are toxic or not. Salts and other toxicants present in produced waters may also have contributed to the toxicity to microorganisms. Currently, it is not clear which of the chemicals may have been responsible for the effect on soil-nitrifying bacteria. This aspect is worthy of further investigations.

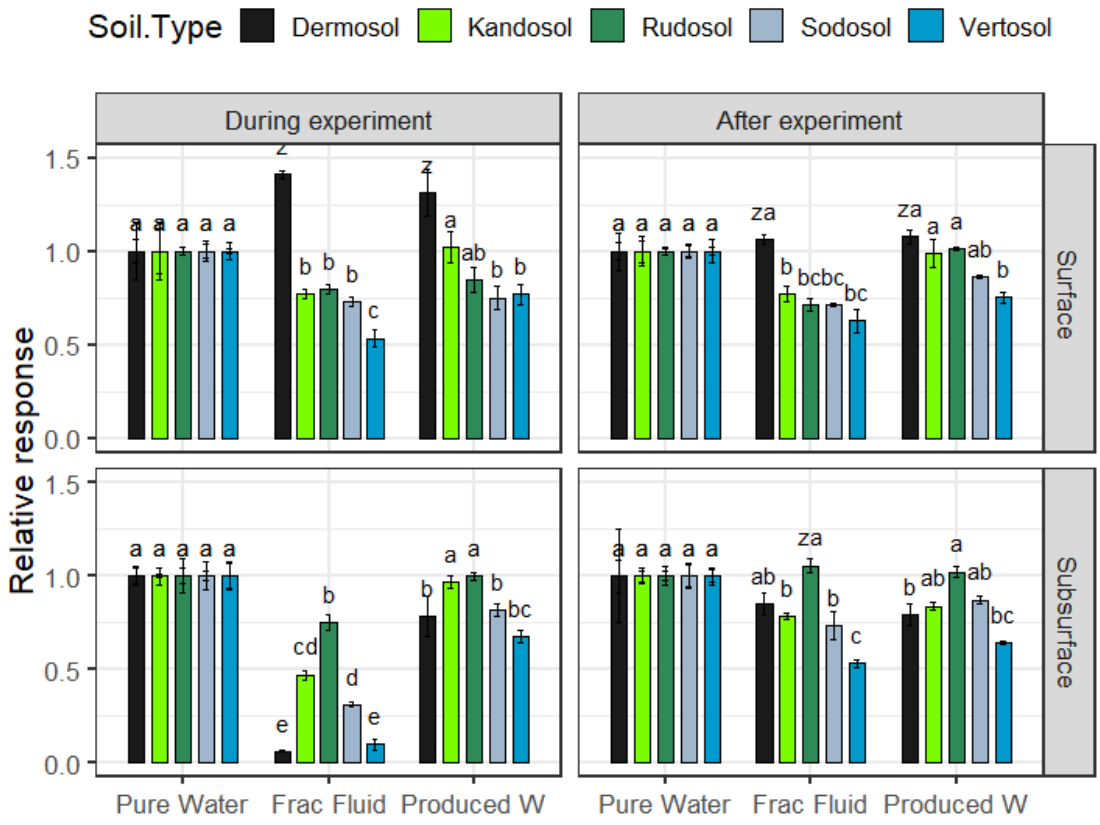

Figure 10. Overall microbial activity in soils as measured through SIR assay. The data on carbon turnover (microgram C/g soil/h) are presented at two times (During experiment after 3 days; after experiment, 60 days after the treatment) in soils treated with HF fluid (Frac Fluid) or produced water. Response is relative to that observed in the control treatment (soils treated with ultrapure water only). The error bars indicate the standard error of the mean (n=3) and the same letter on the bars indicate no statistically significant difference.

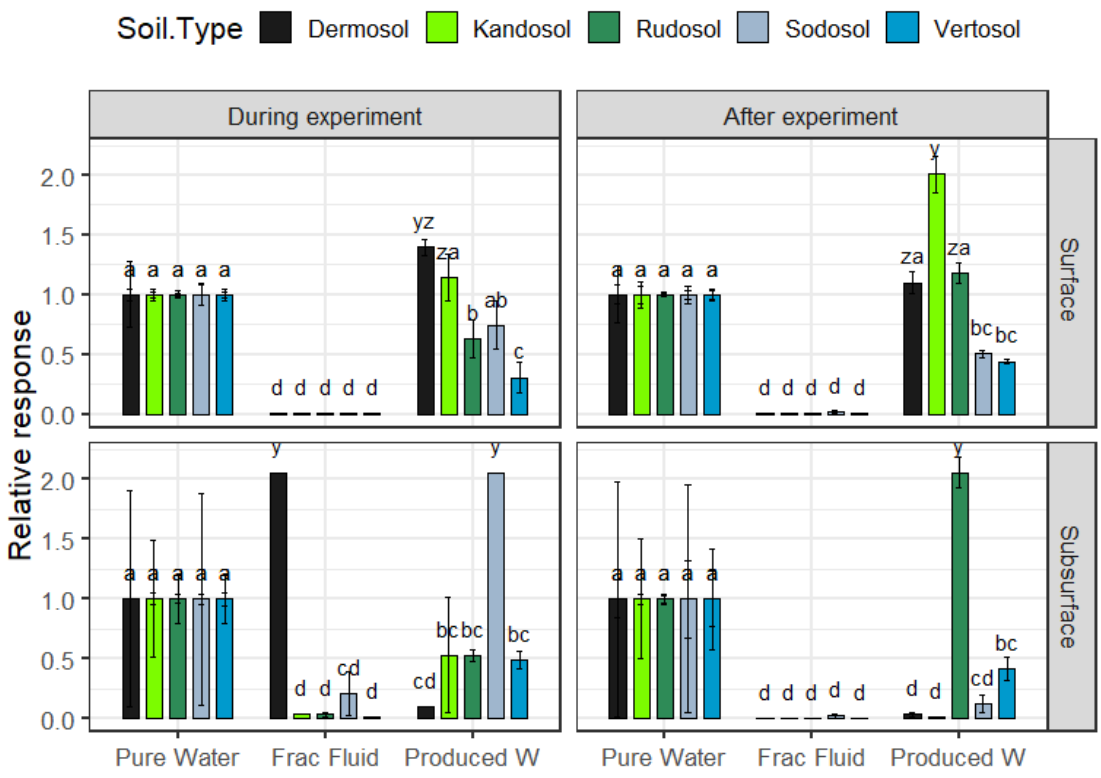

Figure 11. Microbial functioning for the N cycle as measured through nitrate production (mg/kg) using the SIN assay at two times (During experiment after 3 days; After experiment, 60 days after the treatment) in soils treated with HF fluid or produced water. Response is relative to that observed in the control treatment (soils treated with ultrapure water only). The error bars indicate the standard error of the mean (n=3) and the same letter on the bars indicate no statistically significant difference. Note: The nitrification in some soils (e.g. Kandosol and most subsoils) was very small even in the control treatment, leading to large variations in ratios.

- The spill of HF fluid on soils had a marked effect on nitrogen cycling as it completely inhibited the nitrification in soils. The microbial function in soils did not recover even two months after the spill.
- Produced water had a lesser effect on N cycling, with 3 out of 5 soils showing complete recovery within 2 months. Sub-surface soils, inherently deficient in nitrifying bacteria, were more affected than the surface soils.
- It is not clear which constituent of HF fluid or produced water was responsible for the toxic effect. This issue deserves further investigation.

3.3.1.3 Microbial functional gene analysis

qPCR was used to estimate the population size via gene copy number quantification of biomarkers associated with nitrogen fixing bacteria (*nifH*), ammonia oxidising bacteria (*amoA*), denitrifiers (*nirK*) and total bacteria (16S rRNA) in surface soils treated with HF fluid and produced water. The response in terms of gene copy number/ng DNA was compared with those where only ultrapure water was added to the soils. The data are presented in Figure 12.

The abundance of 16S gene decreased in two of the five soils in the HF fluid and three soils with produced water during the experiment. At the end of the experiment this reduction was seen in three and four of the soils for both HF fluid and produced water. The carbon cycling gene (GA1) response was reduced in two soils with HF fluid and three in produced water (Vertosol, Dermosol and Kandosol) and persisted until the end of the experiment. The produced water had a greater recovery by the end of the experiment. In the case of nitrogen-fixing bacteria (*nifH*) a significant effect was observed in the case of two soils (Kandosol and Vertosol) after 3 days of adding HF fluid and produced waters. The Rudosol showed a significant effect with HF fluid only and not with the produced water. Nevertheless, all three soils showed recovery by the end of experiment, as the gene copy numbers were found to be not significantly different to the ultrapure water controls. The gene for ammonia oxidising bacteria (*amoA*) was only detected in one soil (Kandosol) and its gene copy numbers were significantly affected after 3 days of adding HF fluid and produced water. The denitrifier (*nirK*) gene was not detected in any of the soils studied here.

3.3.1.4 Microbial community structure

3.3.1.4.1 Dominant microbial taxa

The majority of sequences among all treatment and incubation times were dominated by bacteria (99.8% on average) followed by archaea (0.2%). Only 0.0008% of sequences were unclassified. The relative abundances of the major bacterial phyla observed are shown in Figure 13. At the beginning of the experiment (Day 3) samples from all soil types were on average dominated by Actinobacteria (55%), Firmicutes (28%), Chloroflexi (10%) and Gemmatimonadetes (3%). After 28 days incubation, the dominant phyla were Proteobacteria (35%), Actinobacteria (29%), Bacteroidetes (8%), Acidobacteria (8%), Firmicutes (6%), TM7 (4%) and Chloroflexi (3%). The replicates exhibited similar data profiles and were pooled for future data analysis. The microbial community structure had changed considerably in the presence of HF fluid and produced water, by the end of the experiment (Table 5). It is notewrothy that some changes in the case of pure water treatment were also noted. Therefore, a comparison of number of taxa remaining in the soils 28 days after the exposure to HF fluid or produced water was seen as a better comparison among different treatments. It is clear from the table (Table 5) that the microbial diversity was affected more markedly in the case of HF fluid than in the presence of produced water, when compared at day 3 and with that in the case of pure water. Following the exposure of soils to HF fluid for 28 days, only 2 to 3 taxa were present in all soils, whereas in the presence of produced water and the pure water, the number of taxa remaining in various soils ranged from 4 to 6 and 4 to 8, respectively. Proteobacteria dominated in most cases, however, Actinobacteria dominated in all treatments in the case of the Vertosol (Figure 13).

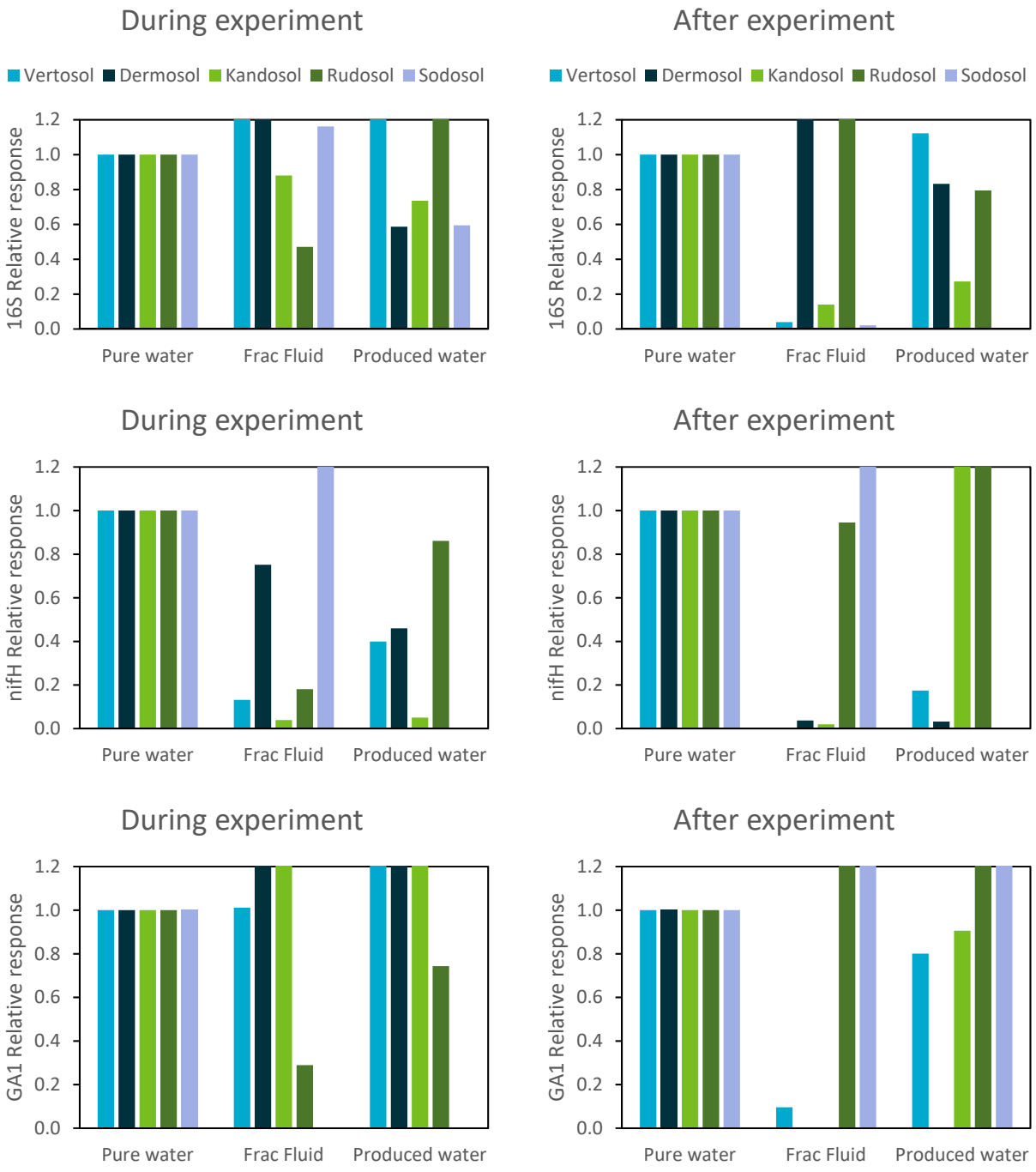

Figure 12. Copy numbers of functional genes involved in nitrogen fixation (*nif*H), carbon cycling (GA1) and total bacteria (16S) as measured via qPCR in five surface soils treated with hydraulic fracturing fluid or produced water. Relative responses >1.2 are not shown.

Table 5. Number of taxa remaining in different soils at 3 and 28 days after exposure to different fluids

Soil types	HF fluid Day 3	HF fluid Day 28	Produced water Day 3	Produced water Day 28	Pure water Day 3	Pure water Day 28
Dermosol	7	2	8	5	8	6
Kandosol	7	3	7	5	7	8
Vertosol	5	3	6	4	6	5
Rudosol	6	3	8	6	8	7
Sodosol	5	3	5	4	6	4

3.3.1.4.2 Community composition

The similarity of the community composition under different treatments was assessed through dendrogram and cluster analysis, as shown in Figures 14 and 15 below. In Figure 14, the red lines show that there is no significant difference between those connecting samples. At the beginning of the experiment (Day 3) the populations between different treatments were found to be at least 90% similar. However, by Day 28 the population formed two main groups, i.e. HF fluid and the rest of the treatments, branching off at the 40% similarity point. This means that the HF group was 60% different to the rest of the sample clusters. This is more clearly illustrated in Figure 15 through the clusters formed by different treatments (based on 40% similarity). At the beginning of the experiment, all treatments clustered as one group, whereas after 28 days the HF fluid stood out as a separate cluster. The produced water and pure water although showed some change with time but still clustered together, with the produced water treatment showing only a small overlap with HF fluid. Overall, the HF fluid had the largest effect on community composition, with an ANOSIM R-value of 0.732 for HF vs. Pure Water and significance level of p=0.008 at Day 28. There were no significant differences at Day 3 or between the others in Day 28 samples.

Hydraulic fracturing fluid when added to soil caused a significant alteration of microbial community composition with time, especially in terms of number of microbial taxa remaining active in soils 28 days after exposure. Produced water had a lower impact on microbial community structure. At the beginning of the experiment the populations between different treatments were found to be at least 90% similar. However, at Day 28 the population in the soils treated with HF was 60% different to that in soils treated with pure water.

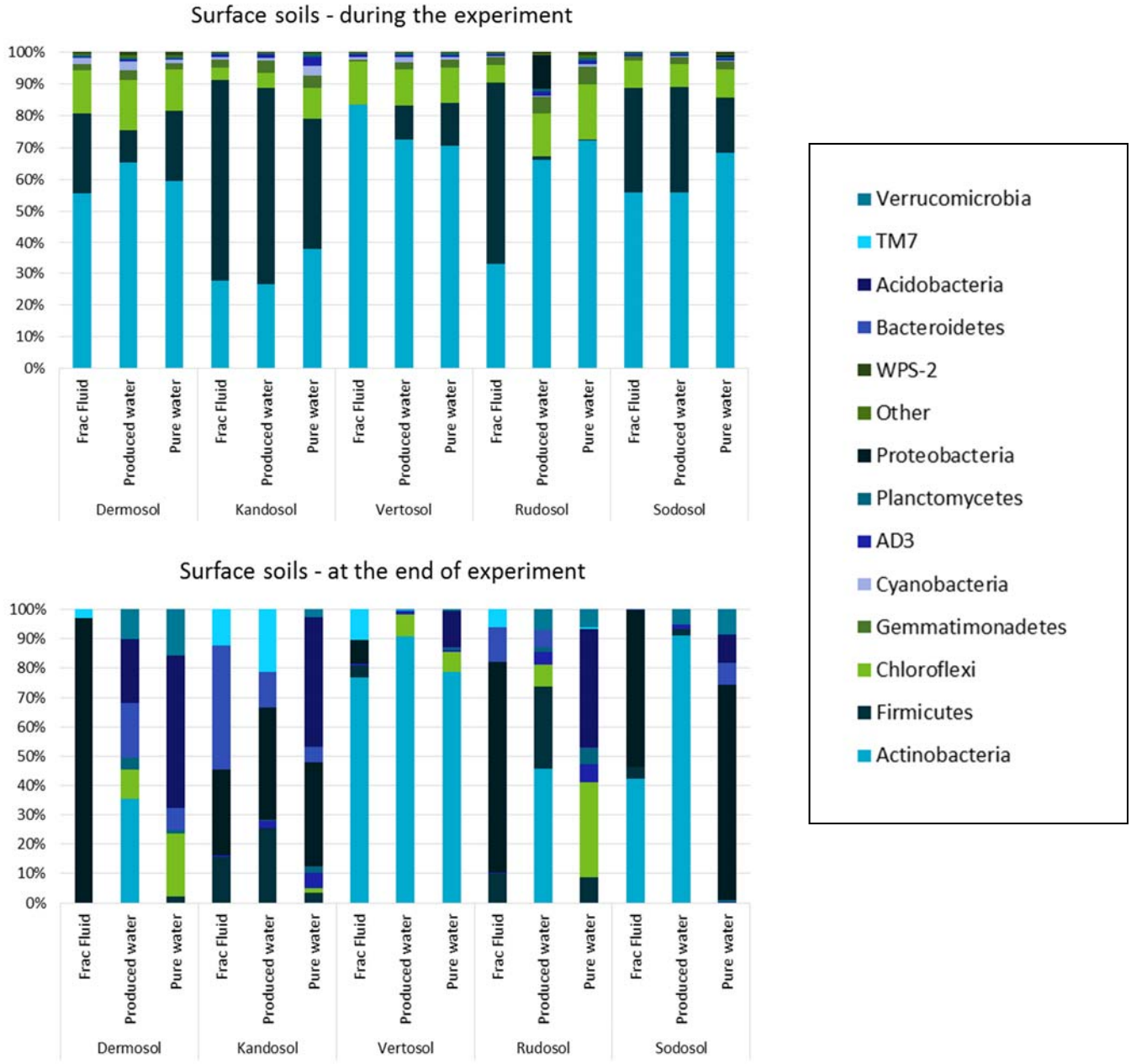

Figure 13. The microbial community diversity in five surface soils as impacted by addition of (HF fluid, produced water or pure water at the beginning (Day 3) and end of the experiment (Day 28).

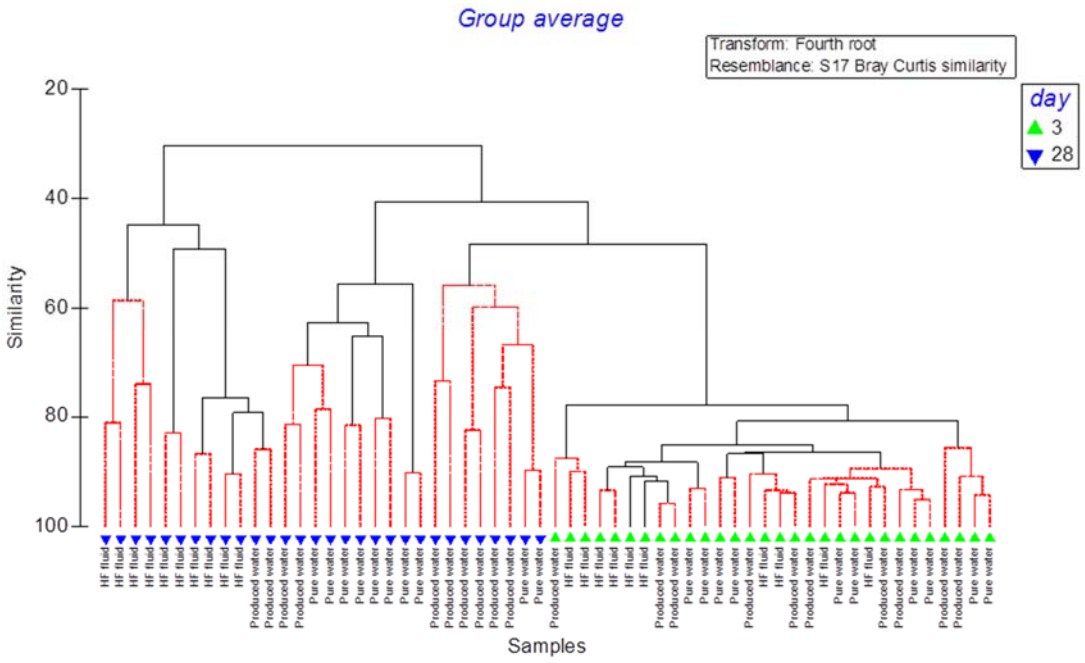

Figure 14. **The dendrogram showing the similarity and diversion points for the microbial populations in various surface soils receiving HF fluid, produced water and pure waters at the beginning (Day 3) and the end of the experiment (Day 28).**

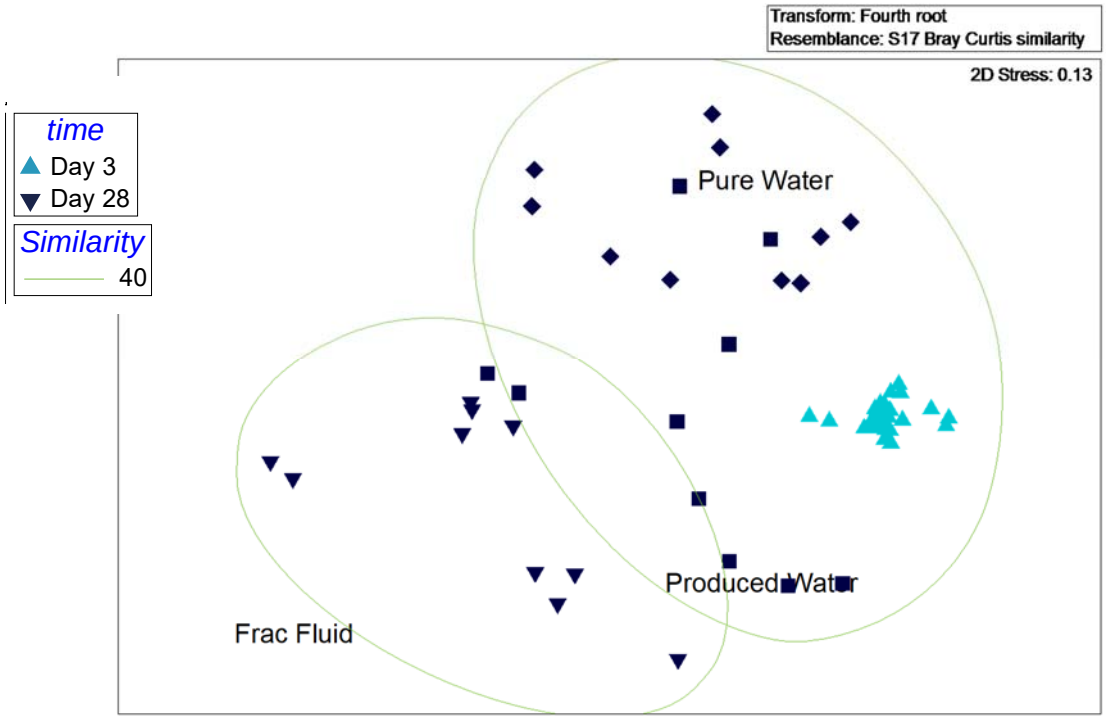

Figure 15. **The clusters of microbial populations (based on 40% similarity) at the end of the experiment (Day 28) in five surface soils treated with HF fluid (▼), produced water (■) and pure water (♦). Note at Day 3 all samples formed a tight cluster (▲).**

3.4 Mobility of chemicals through soils and potential groundwater hazard

The rate of degradation of chemicals in soils (indicated by their half-lives or DT_{50}) and their sorption (K_{oc}) together determine the potential for groundwater contamination. Based on these two parameters, a GUS (Groundwater Ubiquity Score) Index was developed by Gustafson (1989) for assessing groundwater pollution potential of pesticides. GUS is commonly used to categorise chemicals in low, medium or high category from the perspective of groundwater contamination. We used this index for selected chemicals of interest in this study to assess their inherent risk of groundwater contamination as a result of leaching through soils after a spill of HF fluid or produced water. GUS is designed to represent a generic assessment and not a specific spill or leaching event. The large volume of a spill and the presence of preferential flow paths in soils (e.g. cracks, biopores) can facilitate rapid leaching of chemicals and in that situation this assessment approach will not be valid.

The data on degradation and sorption of chemicals were obtained through experiments conducted in this study as well as from the literature (Table 5). While our study did not find a strong impact of soil types on half-lives of biocides, phenol and cresols, the rate of degradation as well as sorption (mobility) of TEA was influenced by soil type. The range of values presented in Table 5 was used as input data for calculating the GUS index of the chemicals.

Table 6. Data on persistence (DT_{50} or half-life) and sorption (K_{oc}) of selected chemicals in soils from Surat Basin from this study along with those previously reported in literature

Chemical	DT_{50} or Half-life (days)	Sorption (K_{oc})	Source
MIT	<2	4-450	This study
CMIT	<2	35-695	This study
TEA	0.5 - >30	71-733	This study; West and Gonsior (1996)
Phenol	1.7-10	7-710	This study; Boyd (1982); Southworth and Keller (1986)
m-Cresol and *p*-Cresol	1.8-13	18-3420	This study; Boyd (1982); Southworth and Keller (1986); Namkoong et al. (1988); Shibata et al. (2006)
Naphthalene	80	200-1470	Lewis et al. (2016)

The mapping of the groundwater hazard data (Table 6) according to the GUS index (Figure 16) showed that in most cases the groundwater hazard was low, mainly due to their rapid degradation (short half-lives). For example, the two biocides were so short lived in all soils (DT_{50} < 2 days) that, despite their high mobility through sandy soils, these were still considered to have low groundwater hazard. However, TEA also fell in the medium to high hazard category, mainly because of its longer DT_{50} (>30 days) when applied with HF fluid. In a small number of cases (phenol, cresols, naphthalene) where sorption of chemicals was very low (e.g. in sandy soils) they were placed in the medium to high hazard category. Naphthalene was ranked as a potentially high risk of groundwater contamination due to its longer average half-life of 80 days reported by Lewis et al. (2016). It is noteworthy that the naphthalene concentration in produced water was very low

(1-2 µg/L) and it is a volatile compound. In warm climates, its loss through volatilisation may further reduce its groundwater contamination potential, unless the spill is large enough to push it deeper into the soil profile.

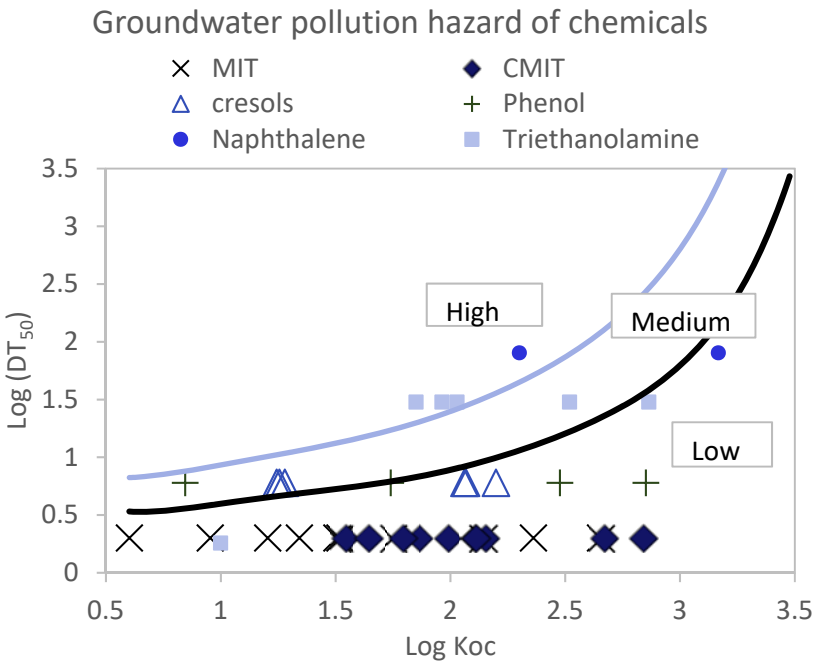

Figure 16 Mapping of key chemicals of interest in terms of their groundwater pollution hazard using the GUS index. The two curves delineate the low (GUS 1.8) and high (GUS 2.8) risk scenarios. The hazard assessment is based on data from this study and those from literature (Table 5) and is generic in nature.

It is noteworthy that the groundwater hazards assessed by the GUS index in Figure 16 are first-tier assessments used for screening purposes. The actual groundwater contamination hazard associated with a chemical will depend on the site conditions and nature of the specific spill event (volume, location, timing, duration etc.). In a scenario of a high volume spill, any of the above chemicals (MIT, CMIT, TEA, phenol, cresols etc.) are likely to move deeper into soil profile due to their high mobility (low sorption) and thus could potentially contaminate groundwaters. Large volume spills could lead to stagnation of water on soil surface and result in changes in redox conditions which may impact the rate of chemical degradation. In such situations, the rapid rate of degradation observed under aerobic conditions in soils in this study may not be applicable. Besides, preferential flow pathways such as through soil cracks or biopores could lead to rapid leaching of chemicals to subsurface soils and even groundwater. Therefore, in case of a specific spill of fluid on soil, site specific investigations are warranted.

While the MIT, CMIT, phenol and cresols were highly mobile (low sorption) they were still rated to have a low inherent groundwater contamination hazard, mainly due to their rapid breakdown in the aerobic soils. Triethanolamine and naphthalene, on the other hand, were classified as a medium to high hazard category in some soils due to their potentially longer persistence in soils.
Large volume spills, however, may result in rapid leaching of MIT, CMIT, phenol and cresols through soils to contaminate groundwater, despite their observed short stability in soils.

3.5 Summary of the key findings

Breakdown of chemicals

- The two biocides (MIT and CMIT) that are commonly used in HF fluids are readily degraded in soils with >90% loss within a day of mixing into the most soils tested. All of the five dominant soils types from the Surat Basin were able to readily degrade the two biocides.
- Triethanolamine (which is used as a gel-breaker aid) degraded relatively rapidly when introduced in the soil with pure water (>90% degraded within a week in four out of five soils). However, in the presence of HF fluid, its rate of degradation after 3 days became so slow that little further loss occurred in three out of five soils during the month long experiment. The reduced microbial activity and altered microbial community structure in the presence of HF fluid appear to have resulted in its longer persistence in soils.
- Three geogenic chemicals that were occasionally detected in produced water (i.e. phenol, *m*-cresol, *p*-cresol) were completely degraded in soils within two days after their mixing with surface soils.

Effect on soil microbial activity

- The hydraulic fracturing fluid had a significant adverse effect on overall microbial activity, especially in sub-surface soils. The microbial activity recovered significantly over time but not fully in 2 months. The produced water had a relatively smaller effect on soil microbial activity in soils and most surface soils recovered fully in two months.
- The spill of HF fluid on soils had a marked effect on nitrogen cycling as it completely inhibited the activity of nitrifying bacteria in soils. The nitrifying function in soils did not recover even two months after the spill. Produced water had a lower effect on the nitrogen cycle, with 3 out of 5 soils showing complete recovery within 2 months.
- The abundance of functional genes for nitrogen fixation and carbon cycling was significantly decreased in some soils with both HF fluid and produced water. However, some recovery was noted with time in soils.
- The bacterial community structure changed over the 28-day test period in all treatments. However, the HF fluid had the largest effect on community composition. After 28 days, only 2 to 3 taxa were present in all soils where HF fluid was applied, in comparison to 4 to 8 in the case of pure water.

3.6 Study limitations and recommendations

While the use of actual HF fluid is a strength of this study, the results presented here are only representative of the fluid used. The fluid composition may change with time and space.

It is noteworthy that while the drilling pads surfaces are artificially created and hence a spill may not make a direct contact with surface soils immediately. However, it is assumed that with time the constituents of any fluid spilled on the drill-pad surface may infiltrate into soils. In such cases, the current assessment represents a worst-case scenario.

The findings presented above in this report indicate that the scenario associated with the spill of HF fluid on soils had the largest impact on soil microbial activity in terms of both the community composition as well as important functions such as nitrification. It is therefore recommended that extra care be taken to minimise and/or to contain any potential spill of HF fluid.

Since the HF fluid is a mixture of several chemicals, it was not possible to clearly identify the specific chemical/s that were responsible for the observed toxicity. Since the biocides were relatively short-lived, it is not clear if the biocides (or their potential transformation products, not studied here) had any detrimental impact or other constituents such as TEA (or its transformation products) which was found to persist longer in these soils. The rate of breakdown of TEA in soils was much slower in the HF fluid soil treatment than in soils treated with pure water. While this was consistent with the impact of HF fluid on microorganisms in soils, it was not clear if the biocides or any other specific constituent of the HF fluid were responsible for this. Further studies are desirable to identify the specific HF fluid constituents (and/or their transformation products) that may be responsible for the impact on soil microbial functions. It is also important to establish the degradation pathways of TEA and potential toxicity of its intermediate products.

In the case of HF fluid, no significant recovery in the soil microbial population and/or functions was noted during the 2 month test period. It is possible that soils may show recovery over a longer time period. Furthermore, the grinding and homogenisation of soils in laboratory conditions can break the microstructure and could eliminate the refugia for microorganisms in soils and hence the recovery may be faster under field conditions. Therefore, it is recommended that a longer-term study be conducted to assess the true impact of HF fluid of soil microbial functions and plant growth, especially under field conditions.

Based on the rate of breakdown and mobility of chemicals, TEA was identified as a potential high groundwater pollution hazard. Since the assessment was indicative in nature, a more targeted study on the leaching and degradation behaviour of TEA, preferably in field or in intact cores is recommended.

The studies recommended above are essential to provide a thorough understanding of HF fluid impact on soils under field conditions, the specific constituents of HF fluid that may have toxic impacts and thus identification of options to minimise any potential detrimental impact on soil health, in the event of a spill of HF fluid.

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Appendices

Appendix A

The concentrations of organic and inorganic chemicals in produced water and ultrapure waters used in this study are presented in various tables below.

Table A1. Concentrations of HF and geogenic chemicals applied to soil (mg/kg) in the degradation and microbial toxicity experiments

	Kandosol	Vertosol	Rudosol	Sodosol	Dermosol
Surface soils					
MIT	5.76	11.52	4.32	5.76	5.76
CMIT	2.88	5.76	2.16	2.88	2.88
TEA	80	160	60	80	80
Phenol	8.0	16.0	6.0	8.0	8.0
m-cresol	8.0	16.0	6.0	8.0	8.0
p-cresol	8.0	16.0	6.0	8.0	8.0
Subsurface soils					
MIT	5.76	11.52	4.32	5.76	5.76
CMIT	2.88	5.76	2.16	2.88	2.88
TEA	80	160	60	80	80
Phenol	8.0	16.0	6.0	8.0	8.0
m-cresol	8.0	16.0	6.0	8.0	8.0
p-cresol	8.0	16.0	6.0	8.0	8.0

Table A2. Concentrations of organic chemicals in produced water and ultrapure water (Milli-Q water) used in the study

Compounds	Unit	Produced water (unspiked)	Produced water (spiked with geogenic chemicals)	Ultrapure water (unspiked)	Ultrapure water (spiked with geogenic chemicals)
MIT	mg/L	<0.005	<0.005	<0.005	<0.005
CMIT	mg/L	<0.005	<0.005	<0.005	<0.005
TEA	mg/L	<0.01	<0.01	<0.01	<0.01
Phenol	µg/L	<1	3100	<1	200,000
3-& 4-Methylphenols (cresols)	µg/L	29	8700	<2	200,000
Other phenols (chlorophenols)	µg/L	<1 - <2	<1 - <2	<1 - <2	<1 - <2
Benzene	µg/L	<1	<1	<1	<1
Toluene	µg/L	<1	<1	<1	<1
Ethylbenzene	µg/L	<1	<1	<1	<1
m,p-Xylene	µg/L	<2	<2	<2	<2
o-Xylene	µg/L	<1	<1	<1	<1
Naphthalene	µg/L	<0.5	<0.5	<0.5	<0.5
Other PAHs	µg/L	<0.5-<1	<0.5-<1	<0.5-<1	<0.5-<1
TRH (C10-C14)	µg/L	<25	<25	<25	<25
TRH (C15-C28)	µg/L	<100	1000	<100	<100
TRH (C29-C36)	µg/L	<100	350	<100	<100

Table A3. Inorganic constituents (and some properties) in produced water and ultrapure water (Milli-Q water) used in the study

Type of water	pH	Conducti-vity (mS/cm)	TOC (mg/L)	DOC (mg/L)	Chrloride (mg/L)	Sulphate (mg/L)	Phosphate (mg P/L)	Nitrate (mg N/L)	Nitrite (mg N/L)	Ammonia (mg N/L)
Produced water (filtered)	-	-	-	2.4	2900	0.3	<0.005	<0.005	<0.005	2.7
Produced water (unfiltered)	8.0	1.62	2.8	-	-	-	-	-	-	-
Milli-Q Water (filtered)	-	-	-	0.1	<0.1	0.1	<0.005	<0.005	<0.005	<0.001
Milli-Q Water (filtered)	-	0.0036	0.2	-	-	-	-	-	-	-

Type of water	Ag (µg/L)	Al (mg/L)	As (µg/L)	B (mg/L)	Ba (mg/L)	Be (µg/L)	Bi (µg/L)	Ca (mg/L)	Cd (µg/L)	Ce (µg/L)	Co (µg/L)	Cr (µg/L)
Produced (filtered)	<0.03	<0.01	0.33	2.1	1.8	<0.01	<1	22	<0.1	<0.01	0.2	<1
Produced (unfiltered)	<0.03	<0.01	0.52	2.2	1.8	<0.01	<1	22	<0.1	<0.01	0.1	2
Milli-Q (filtered)	<0.03	<0.01	0.07	<0.04	<0.001	<0.01	<1	<0.04	<0.1	<0.01	<0.1	<1
Milli-Q (unfiltered)	<0.03	<0.01	0.13	<0.04	<0.001	<0.01	<1	<0.04	<0.1	<0.01	<0.1	<1

Type of water	Cs (µg/L)	Cu (µg/L)	Dy (µg/L)	Er (µg/L)	Eu (µg/L)	Fe (mg/L)	Ga (µg/L)	Ge (µg/L)	Hf (µg/L)	Hg (µg/L)	Ho (µg/L)	In (µg/L)
Produced (filtered)	0.98	2	<0.003	<0.001	0.18	<0.02	<0.04	1.5	0.01	<0.2	<0.001	<0.03
Produced (unfiltered)	0.98	2	<0.003	<0.001	0.18	0.25	<0.04	1.6	0.02	<0.2	<0.001	<0.03
Milli-Q (filtered)	<0.01	<1	<0.003	<0.001	<0.002	<0.02	<0.04	<0.03	<0.01	<0.2	<0.001	<0.03
Milli-Q (unfiltered)	<0.01	<1	<0.003	<0.001	<0.002	<0.02	<0.04	<0.03	<0.01	<0.2	<0.001	<0.03

Type of water	La (µg/L)	Li (µg/L)	Lu (µg/L)	Mg (mg/L)	Mn (mg/L)	Mo (µg/L)	Nb (µg/L)	Nd (µg/L)	Ni (µg/L)	Pb (µg/L)	Pd (µg/L)	Pr (µg/L)
Produced (filtered)	0.021	164	0.002	5.8	0.17	1.4	<0.04	0.005	2.9	<0.4	1.3	0.003
Produced (unfiltered)	0.020	167	<0.001	5.8	0.17	1.9	<0.04	0.005	3.2	<0.4	1.3	0.002
Milli-Q (filtered)	<0.002	<0.01	<0.001	<0.02	<0.002	<0.1	<0.04	<0.002	<0.2	<0.4	<0.03	<0.03
Milli-Q (unfiltered)	0.004	<0.01	<0.001	<0.02	<0.002	<0.1	<0.04	0.005	0.4	<0.4	<0.03	<0.03

Type of water	Rb (µg/L)	Re (µg/L)	Rh (µg/L)	Ru (mg/L)	S (mg/L)	Sb (µg/L)	Sc (µg/L)	Sm (µg/L)	Sn (µg/L)	Se (µg/L)	Sr (mg/L)	Ta (µg/L)	Tb (µg/L)	Te (µg/L)
Produced (filtered)	24	<0.1	<1	<0.1	0.7	0.14	<0.2	<0.1	<1	0.010	4.7	<0.02	<0.001	0.8
Produced (unfiltered)	24	<0.1	<1	<0.1	0.6	0.13	<0.2	<0.1	<1	0.005	4.8	<0.02	<0.001	<0.2
Milli-Q (filtered)	<0.02	<0.1	<1	<0.1	<0.2	<0.04	<0.2	<0.1	<1	<0.001	<0.001	<0.02	<0.001	<0.2
Milli-Q (unfiltered)	<0.02	<0.1	<1	<0.1	<0.2	<0.04	<0.2	<0.1	<1	<0.001	<0.001	<0.02	<0.001	<0.2

Type of water	Te (µg/L)	Th (µg/L)	Tl (µg/L)	Tm (µg/L)	U (µg/L)	V (µg/L)	W (µg/L)	Y (µg/L)	Yb (µg/L)	Zn (µg/L)	Zr (µg/L)
Produced Water (filtered)	0.8	0.005	<0.1	<0.001	0.005	<0.5	10	0.07	<0.004	14	0.12
Produced Water (unfiltered)	<0.2	0.006	<0.1	<0.001	0.012	<0.5	11	0.07	<0.004	14	0.12
Milli-Q (filtered)	<0.2	<0.004	<0.1	<0.001	<0.002	<0.5	<0.01	<0.01	<0.004	<1	<0.02
Milli-Q (unfiltered)	<0.2	<0.004	<0.1	<0.001	<0.002	<0.5	<0.01	<0.01	<0.004	<1	<0.02

Table A4. Radionuclide activities in produced waters

Radionuclide	Unit	Produced water used in this study	Average concentrations in other produced waters analysed	Standard Deviation of other produced waters
^{238}U	mBq/kg	<1	2.35	0.68
^{234}U	mBq/kg	1.5	9.84	5.64
^{226}Ra	mBq/kg	58	58.7	33.05
^{228}Ra	mBq/kg	<0.2	<0.6	-
^{232}Th	mBq/kg	<1	<1	-
^{230}Th	mBq/kg	<5	<5	-
^{228}Th	mBq/kg	<1	5.6	-

Appendix B

Statistical analysis of data on SIR.

CO2_Statistical analysis.pdf

Statistical analysis of data on SIN.

NO3_Statistical aanlysis.pdf

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