



GISERA
Gas Industry Social and
Environmental Research Alliance

Project Order, Variations and Research Progress

Project Title: Environmental monitoring and microbial degradation of onshore shale gas activity chemicals and fluids

This document contains three sections. Click on the relevant section for more information.

- Section 1: [Research Project Order as approved by the GISERA Research Advisory Committee and GISERA Management Committee before project commencement](#)
- Section 2: [Variations to Project Order](#)
- Section 3: [Progress against project milestones](#)



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GISERA
Gas Industry Social and
Environmental Research Alliance

1 Original Project Order

Project Order

Proforma 2019

1. Short Project Title

Environmental monitoring and microbial degradation of onshore shale gas activity chemicals and fluids

Long Project Title

Microbial profiling for environmental monitoring and microbial degradation of chemicals in drilling and hydraulic fracturing fluid in the Northern Territory

GISERA Project Number

W17

Proposed Start Date

20 May 2019

Proposed End Date

21 February 2020

Project Leader

David Midgley

2. GISERA Region

- | | | |
|--|--|---|
| ☐ Queensland | ☐ New South Wales | ☒ Northern Territory |
| ☐ South Australia | ☐ Western Australia | ☐ Victoria |

3. GISERA Research Program

- | | | |
|---|---|---|
| ☒ Water Research | ☐ GHG Research | ☐ Social & Economic Research |
| ☐ Biodiversity Research | ☐ Agricultural Land
Management Research | ☐ Health Research |

4. Project Summary

Objective

This project aims to achieve two objectives:

1. Establish microbial community baselines in aquifer waters and soil samples of sites proximal to prospective unconventional gas activities in the Northern Territory (using wells previously sampled for GISERA water project - [Baseline monitoring of groundwater properties in the Beetaloo sub-Basin](#) (project W.16).
2. Understand the microbial degradation of a range of chemicals likely to be used in unconventional gas activities, in both the five major soil types of the region and in relevant aquifer environments.

1. Establish microbial community baselines in aquifer waters and soil samples

One key aspect to development of a shale gas industry in the Northern Territory is the preservation of clean water for the environment, human consumption and agricultural uses.

Internationally, water quality and environmental health has been protected through regulation. Examples in the northern hemisphere include the Clean Water Act (CWA 33 U.S.C; USA Environmental Protection Agency), and the European Union's Framework Directives (e.g. 2000/60/EC & 2008/56/EC). These regulations seek to protect water resources and maintain high water standards through various testing regimes. In Europe, the last two decades have seen the addition of biological assessments to water testing frameworks. Indeed, in the EU, biological assessments are rated more heavily than physicochemical measures in determining the environmental health of water resources (Pawlowski et al., 2018). Before 2010, most of these biological measures were counts of eukaryotic (and mostly multicellular) organisms such as benthic invertebrates, planktonic organisms or macroalgae. Such counts are obviously of high value, but they are resource and labour intensive to gather and of limited usefulness in groundwater where the vast majority of organisms are microscopic and prokaryotic (bacteria and archaea).

Since ~2010 DNA-based assessments of biological "health" indicators have increasingly replaced these traditional counts (Pawlowski et al., 2018). DNA-based counts rely on developments in next-generation sequencing which enable the "counting" of all organisms in a given environment using environmental DNA (eDNA). This results in the ability to identify all members of various microbial groups present in a given environment through the use of informative marker genes such as 16S (see Fig. 1). In addition to using changes in microbial communities to measure change, these methods allow the identification of organisms that may be sensitive to particular environmental pollutants (these more sensitive organisms may then be a specific focus of monitoring). This field is generally known as biomonitoring, or metabarcoding and

increasingly, is the subject of a body of research which demonstrates its efficacy for monitoring and managing environmental change (e.g. (Bohmann et al., 2014; Cristescu, 2014; Darling et al., 2017; Deiner et al., 2017; Keck et al., 2017; Leese et al., 2018; Valentini et al., 2016).

Along with water, soils represent important resources to be maintained during the development of a shale gas industry in the Northern Territory. Unlike waters, soils are not typically defined along a gradient of pristine to polluted. Instead, soils are defined as healthy when they have the capacity *“to function as a vital living system, within ecosystem and land-use boundaries, to sustain plant and animal productivity, maintain or enhance water and air quality, and promote plant and animal health”* (Doran and Zeiss, 2000). Like water, soil ‘quality’ can be measured using same biomonitoring tools used for water quality monitoring. One key difference is while soils contain diverse prokaryotic communities they also include a range of microscopic eukaryotes. The most important group of these organisms, the fungi, can be profiled using the ITS marker gene (see Fig. 1).

Taken together, data on prokaryotic diversity from waters of the Beetaloo sub-Basin and on the fungal and prokaryotic diversity of the major soil types of the region provides a baseline from which any disturbance can be tracked.

2. Understand the microbial degradation of a range of chemicals likely to be used in onshore shale gas activities in the Northern Territory in major soil types of the region and in relevant aquifer environments.

Onshore shale gas activities, including exploration and production, use a range of chemical products in drilling and stimulation as surfactants, biocides, corrosion inhibitors, buffers, friction reducers and viscosity control.

The risks associated with these chemicals have been the focus of numerous reviews into potential environmental and human health impacts (Australian Government Department of the Environment and Energy Reports 2014, 2017). While the risks of these chemicals have been identified, nothing is known regarding the microbial degradation of these chemicals in edaphic and subsurface environments in the Northern Territory. The region hosts five broad soil types of the region: tenosols, rudosols, kandosols, vertosols and chromosol (Northern Territory Government report, “Soils of the Northern Territory”). The dominant soil type of the region is a kandosol. Differing soil types host markedly different microbial communities (Griffiths et al., 1996; Wieland et al., 2001; Xue et al., 2018), it is thus proposed to examine each of the five soil types individually for microbial diversity and impact of exposure to individual industry chemicals, along with the ability of microbes from each soil type to use the individual industry chemicals as a sole source of carbon (see Fig. 2). Similarly, samples of the Cambrian limestone aquifer will be grouped using data from project W.16, such that representative samples from the region will be examined for microbial chemical degradation, in the same fashion as soil samples (see Fig. 2). Where possible, chemical degradation will be analysed quantitatively with NATA accredited tests.

USING DNA SEQUENCING TO PROFILE MICROBIAL COMMUNITIES

Waters from aquifers contain bacteria (but not fungi as they are anoxic environments).
Soils contain an abundance of bacteria and fungi that can be used to track environmental disturbance.

Different pieces of DNA are used to monitor bacterial and fungal communities.

BACTERIA

Every bacterial cell contains a small piece of DNA that we can use as a barcode for this 'taxonomic unit' $\approx$ species.

In bacteria we use a piece of DNA called the **16S**, it comes from the ribosome.

FUNGI

Every fungal cell contains a small piece of DNA that we can use as a barcode for this 'taxonomic unit' $\approx$ species.

In fungi we use a piece of DNA called the **ITS**, like 16S, it comes from the ribosome.

WATER SAMPLES

SOIL SAMPLES

EXTRACT
ALL DNA



SEQUENCE ALL 16S & ITS

FOR WATER
COUNT & ID[†]16S

FOR SOILS
COUNT & ID[†]16S & ITS

MICROBIAL
COMMUNITY PROFILES

Figure 1: Microbial community profiling

[†] ID- Identification using Bayesian classifiers and a bacterial 16S database (Cole et al., 2014) and a fungal ITS database (Deshpande et al., 2016).

Description

The final report from the Scientific Inquiry into Hydraulic Fracturing in the Northern Territory acknowledged public concerns regarding hydraulic fracturing, but made a number of recommendations that, if adopted and implemented, would reduce the environmental, social, health, cultural and economic risks associated with hydraulic fracturing involved in unconventional gas production in the Northern Territory.

This project is part of an effort to reduce the environmental, social, health, cultural and economic risks associated with unconventional gas production in the Northern Territory. In particular, this project will develop complimentary microbial community profiles for aquifers examined in project W.16 and also the five major soil types of the Beetaloo sub-Basin. The second objective of the project will develop an understanding of the potential for microbes to degrade the common chemicals used by industry in the development of the shale gas resource in major soils of the region and representative aquifer samples.

The baselining component of the project will involve profiling the 55 aquifer/bore water collections examined in project W.16. This will provide 55 aquifer microbial profiles which can be correlated with geochemical data collected in project W.16. These profiles provide a means of rapidly assaying for perturbations in the aquifer and provide data on aquifer mixing. In addition, representative soil sampling will be undertaken for the five soil types of the region. Prokaryotic microbial community profiling will be carried out on all samples. Soil fungal communities will be profiled for the various soil types.

The second objective for this project will examine the microbial degradation of ~30 previously identified hazardous chemical compounds associated with onshore gas activities. For this project, the final list of compounds to be investigated will be determined through consultation with industry and the Northern Territory Government regulator, as part of Task 1. Microbial chemical degradation will be investigated in soil for each of the five soil types (tenosols, rudosols, kandosols, vertosols and chromosols), as well as in five representative anoxic water samples to be determined in Task 1. Quantitative chemical degradation will be analysed for those compounds that have a commercial, NATA accredited test.

All experiments will be conducted in triplicated microcosms. Microcosms will be incubated under field-like conditions and temperatures. Statistical comparisons will be undertaken to confirm chemical degradation by microbial communities. The project will use DNA sequencing targeting ribosomal genes in bacteria (16S rDNA sequencing) and fungi (ITS sequencing) to describe effects on the microbial community and to identify indicator taxa (see Fig. 1).

It is important that this work is undertaken prior to any extensive development of the industry.

The final report for this project will include:

- Baseline data on microbial communities across the Beetaloo sub-Basin that can be directly correlated with geochemical data available on the completion of project W.16.
- Information on microbial degradation of a range of common compounds used by industry.
- Identification of microbial taxa as indicators that could be used to monitor the growth of industry to ensure it does not adversely impact the environment.

Need and Scope

The Northern Territory's 'Scientific Inquiry into Hydraulic Fracturing' acknowledged public concerns regarding hydraulic fracturing in particular with regard to water quality. To help ease public concern, it is important to establish an environmental baseline from which to monitor the environment as the industry expands.

As described in the Objectives, water quality should be assessed through a variety of measures including the use of biomonitoring. Currently, no biological baselining or biomonitoring has been undertaken in the Beetaloo sub-Basin. It is key to establish these indicators prior to the extensive development of the Beetaloo sub-Basin gas resource to ensure that any unintended environmental disturbance can be detected at the earliest time.

Additionally, as well as their role as biomonitors, microbes have the potential to mitigate significant environmental harm through the assimilation, degradation or detoxification of a range of environmental contaminants. Currently, the capacity for microbes to act as mitigants of compounds prospectively used by the shale gas industry in the Beetaloo sub-Basin, Northern Territory is unknown. The different soils and aquifers of the region likely host markedly different microbial communities with, potentially, differing capacity for degradation of chemicals used by industry.

Method

During Task 1, staff will work with team members of project W.16 to establish the sampling sites for large volume water samples required. Task 1 will review sampling sites from W.16 to determine relevance for potential impacts to the environment via industrial activities, emphasizing those locations where impact may be greatest. In particular, fertile agricultural soils will be prioritized. Alluvial aquifers and sediments will be considered in the sampling regime. Additionally, the list of compounds to be examined will be determined through consultation with industry stakeholders and the Northern Territory Government regulator. This task will also identify the sampling sites for soil collection. Task 1 will also include the safe and environmentally-sensitive planning, provisioning and logistics for the sampling campaign (see Fig. 2). During Task 1, team members will interact with researchers conducting investigations of stygofauna found in the Beetaloo sub-Basin groundwater for the purposes of coordination, sampling, data comparison and information exchange.

Task 2 will involve staff travelling to the Beetaloo sub-Basin with the purposes of collecting: triplicate microbially preserved water samples from the 55 sites examined by project W.16 along with 50 soil samples

(ten each of the five major soil types: tenosols, rudosols, kandosols, vertosols and chromosols of the Beetaloo sub-Basin). Due to the heterogeneity of soil, it is important to sample sufficient replicates. Ten samples per soil type strikes a balance between statistical rigor and pragmatism (see Fig. 2). In addition, five large volume water samples will be collected anoxically for microbial degradation assays (identified in Task 1). On returning the samples to the laboratory, the 55 microbially preserved water samples and the 50 soil samples will be subject to DNA extraction along with 16S rDNA sequencing, and, for the soil samples ITS DNA sequencing (Task 3; see Fig. 2). Replicated microcosms containing either the soils or aquifer water will be established and used to determine the ability of microbes in these environments to degrade chemicals potentially used by industry. Chemical degradation will be determined either through direct measurement of the chemical in the soil or aquifer using analytical chemistry techniques or microbial growth assays (Task 4; see Fig. 2).

For most chemicals no NATA-accredited analytical method exists, in these cases a sole carbon source growth trials will be conducted on solid media and in anoxic water samples. Growth assays provide evidence that microbes are able to grow on the chemical compounds as a sole source of carbon, however, the rates of degradation and the residual compounds of degradation cannot be ascertained from the growth assay. The production of biomass by microbes in these assays, however, in the absence of other carbon, demonstrates carbon from the chemical of interest is being used for a range of biological molecules.

In the soils and the water microcosms, microbial community profiling will also be undertaken after exposure to individual chemicals to ascertain impacts on microbial communities and to potentially identify putatively useful indicator taxa for monitoring environmental impacts (Task 5; see Fig. 2).

The final report for this project will collate baseline data with microbial degradation, microbial community impact and useful indicator taxa for individual chemicals (Task 6). These data will be combined with results from project W.16 to inform requirements for future toxicological studies and will provide information for a range of stakeholders (see Fig. 2).

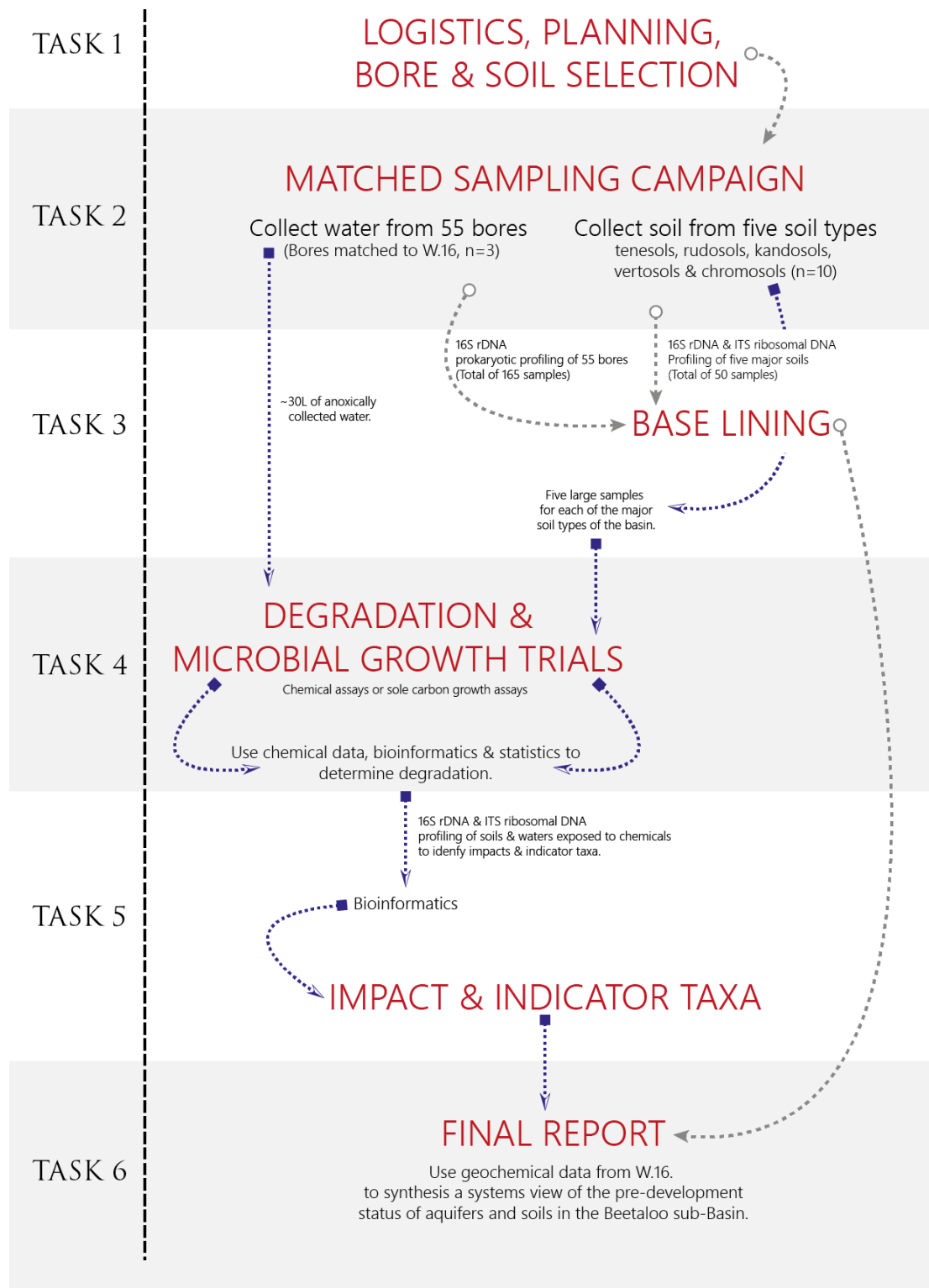


Figure 2: Schedule of tasks and brief description

5. Project Inputs

Research

The list of chemical compounds used in shale gas activities, as determined during Task 1, will prioritise compounds shown to pose a potential risk of accidental release, leaks or spills. Mechanistic aspects of microbial degradation of these chemical compounds have been mostly investigated in other settings. Soils and aquifers of the Northern Territory, Australia, however, are relatively poorly studied and little is known about degradation of these compounds under these conditions. The present study will determine the impact of these compounds on microbial communities and their degradation by microbes in conditions specific to the Northern Territory.

Resources and collaborations

Researcher	Time Commitment (project as a whole)	Principle area of expertise	Years of experience	Organisation
David MIDGLEY	14 days	Microbial Ecology & Catabolism	>20years	CSIRO
Kaydy PINETOWN	19 days	Geology	>20 years	CSIRO
Tania VERGARA	20 days	Analytical chemistry	>5 years	CSIRO
Nai TRAN-DINH	10 days	Microbial Ecology	>20 years	CSIRO
Tony ALLAN	13 days	Geologist	>30 years	CSIRO
Stephen SESTAK	10 days	Analytical chemistry	>20 years	CSIRO
Carla MARIANI	28 days	Microbiology and geochemistry	>2 years	CSIRO



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Subcontractors (clause 9.5(a)(i))	Time Commitment (project as a whole)	Principle area of expertise	Years of experience	Organisation
ALS	1-2 weeks turnaround on receipt of samples.	Testing	Many. Commercial laboratory.	ALS. NATA-accredited laboratory.
Sequencing service provider	6-8 weeks turnaround on receipt of samples.	DNA sequencing, microbiomes.	Many. Commercial DNA sequencing facility.	Ramaciotti Centre for Genomics, UNSW

Project Budget Summary

Source of Cash Contributions	2018/19	2019/20	% of Contribution	Total
GISERA	\$25,530.75	\$160,442.25	75%	\$185,973.00
- Federal Government	\$7,659.23	\$48,132.68	22.5%	\$55,791.90
- NT Government	\$7,659.23	\$48,132.68	22.5%	\$55,791.90
- Origin Energy	\$3,404.10	\$21,392.30	10%	\$24,796.40
- Santos	\$3,404.10	\$21,392.30	10%	\$24,796.40
- Pangaea	\$3,404.10	\$21,392.30	10%	\$24,796.40
Total Cash Contributions	\$25,530.75	\$160,442.25	75%	\$185,973.00
Source of In-Kind Contribution	2018/19	2019/20	% of Contribution	Total
CSIRO	\$8,510.25	\$53,480.75	25%	\$61,991.00
Total In-Kind Contribution	\$8,510.25	\$53,480.75	25%	\$61,991.00

6. Project Impact Pathway

Activities	Outputs	Short term Outcomes	Long term outcomes	Impact
Logistics, planning and bore and soil selection	Logistics, occupational health and safety, environmental and community concerns, along with detailed sampling procedures. A list of chemicals used by the shale gas industry that poses a putative environmental risk in the Northern Territory will be determined through consultation with industry and the regulator. A list of available water bores matched to project W.16 (up to 55 water samples). Fifty sites for soil collection, inclusive of the five soil types found in the Beetaloo sub-Basin. These soil samples will include five large (15kg) soil samples for Task 4. A briefing document will be prepared for the sampling campaign.	Knowledge on the biodegradation of chemicals that are potentially involved in shale gas activities as well as an understanding of microbial community changes as a result of the presence of such chemicals.	Assist in informing governments, regulators as well as policy-makers on the microbial impact of a selected list of chemicals that may be used in future shale gas activities in the Northern Territory. Characterisation of microbial communities and their sensitivity to chemical exposure will lead to information on microbial variability and the identification of potential indicator microbial species. The project will expand on the understanding of surface and groundwater contamination impacts due to leaks and spills of individual chemicals related to gas activities,	The impact of this research extends to government, industry and everyday Australians. All Australian communities that are located in shale gas regions as well as industry will benefit from the outcomes of this research, through increased understanding and awareness of environmental impacts that may result from the use of certain chemicals in future shale gas activities. The project provides knowledge in the area of both surface and groundwater
Sampling campaign	Provision of both water and soil samples for experimental program of this project. Briefing document with details of collection and sample availability will be prepared.	This project will additionally provide information on potential indicator microbial taxa specific to the chosen regions of the Northern Territory.		
Determine the reduced risk of chemicals used in shale gas activities primarily through microbial degradation.	Technical report containing results from tasks examining the biodegradation of individual chemicals in shale gas activities, to include microbial growth and any changes in microbial communities upon exposure to the prescribed chemicals.			
Develop a set of identifiable chemical-specific microbial	Technical report to include the identification of microbial taxa that displays sensitivity towards individual chemicals with the potential to be used			

taxa as potential indicators for environmental health.	as environmental health indicators specific to the region.			
Work synergistically with the “Baseline assessment of groundwater characteristics in the Beetaloo sub-Basin, NT” project (W.16), and a parallel running project investigating stygofauna of the Beetaloo sub-Basin.	Data from project W.16 will be used for selection of five large anoxic collections of water for Tasks 3-5. Coordination and integration of technical report from this study with both project W.16 and the stygofauna project. Demonstrate the value of integration of the three data sets to generate a systems level view of the pre-resource development aquifer networks in the Beetaloo sub-Basin.			
Develop fact sheets with key findings	GISERA Communications will develop a plain English factsheet at project commencement. Completed fact sheet(s) with key findings for distribution via the GISERA website and at community engagement events.			
Prepare and submit scientific manuscripts for publication in peer-reviewed journals	Manuscript submission to peer-reviewed journals.			

lending to improved industry practice and decision-making to minimise such risks. Increased community awareness of the potential environmental impacts of gas activities is another long term outcome of this project.

contamination at several locations in the Northern Territory which will assist those at both the decision-making and policy-making levels of government.

7. Project Plan

Project Schedule

ID	Activities / Task Title (should match activities in impact pathway section)	Task Leader	Scheduled Start	Scheduled Finish	Predecessor
Task 1	Logistics, planning, bore and soil selection	Kaydy PINETOWN	May 2019	July 2019	Project W.16
Task 2	Sampling campaign	Tony ALLAN	July 2019	July 2019	Task 1
Task 3	Baselining microbial communities	Nai TRAN-DINH	August 2019	October 2019	Task 2
Task 4	Microbial degradation and sole carbon growth trials	Tania VERGARA	August 2019	October 2019	Task 2
Task 5	Impact and indicator taxa	Nai TRAN-DINH	November 2019	January 2020	Tasks 2, 3 & 4
Task 6	Final Report	David MIDGLEY	May 2019	February 2020	All other tasks.

Task description

Task 1

TASK NAME: Logistics, planning, bore and soil selection

TASK LEADER: Kaydy PINETOWN

OVERALL TIMEFRAME: May-July 2019

BACKGROUND: During Task 1, staff will work with team members of GISERA project W.16 to establish the sampling sites for large volume water samples required. This task will also identify the sampling sites for soil collection. Chemicals to be tested in subsequent tasks will be determined in Task 1 via consultation with industry and a regulator. Task 1 will interact with the parallel running project investigating stygofauna in the Beetaloo sub-Basin to ensure project compatibility. Task 1 will also include the safe and environmentally-sensitive planning, provisioning and logistics for the sampling campaign.

TASK OBJECTIVES:

- 1) Establish water and sampling sites within the Beetaloo sub-Basin;
- 2) Liaise with project W.16 and stygofauna project teams for site selection and sampling campaign logistics;
- 3) Determination of chemicals of relevance for onshore gas production in the Northern Territory;
- 4) Identification of sites for soil collection for use in Tasks 2-5;
- 5) Preparation of sampling equipment/reagents;
- 6) Preparation for remote sampling fieldwork; and
- 7) Identification of any permits, permission or consultation required for sampling.

TASK OUTPUTS AND SPECIFIC DELIVERABLES: A briefing document will be prepared for the sampling campaign describing the outcomes of task objectives 1-6.

Task 2

TASK NAME: Sampling campaign

TASK LEADER: Tony ALLAN

OVERALL TIMEFRAME: July 2019

BACKGROUND: Task 2 will involve two staff travelling to the Beetaloo sub-Basin with the purposes of collecting: triplicate microbially preserved water samples from the 55 sites examined by project W.16 along with 50 soil samples (ten each of the five major soil types: tenosols, rudosols, kandosols, vertosols and

chromosols of the Beetaloo sub-Basin). In addition five large volume water samples will be collected anoxically for microbial degradation assays (identified in Task 1).

TASK OBJECTIVES: To collect oxic and anoxic samples:

- 1) Microbially preserved water samples will be collected from sites identified in Task 1/project W.16;
- 2) Five large volume anoxic water samples will be collected from sites identified in Task 1/project W.16;
- 3) Collection of 10 replicated soil samples from each of the five soil types completed; and
- 4) Five large volume soil samples will be collected from sites identified in Task 1.

TASK OUTPUTS AND SPECIFIC DELIVERABLES: Collection of preserved samples, oxic and anoxic samples to establish microcosms.

Task 3

TASK NAME: Baseline microbial communities

TASK LEADER: Nai TRAN-DINH

OVERALL TIMEFRAME: August 2019 to October 2019

BACKGROUND: The 55 microbially preserved water samples and the 50 soil samples will be subject to DNA extraction along with 16S rDNA sequencing, and, for the soil samples ITS DNA sequencing.

TASK OBJECTIVES: The task will include the following objectives:

- 1) Complete DNA extractions from all samples;
- 2) DNA samples sent to external sequencing provider; and
- 3) Bioinformatics completed for microbial baselining of all samples.

TASK OUTPUTS AND SPECIFIC DELIVERABLES: Raw sequencing data from microbial community profiling available.

Task 4

TASK NAME: Microbial degradation and sole carbon growth trials

TASK LEADER: Tania VERGARA

OVERALL TIMEFRAME: August 2019 to October 2019

BACKGROUND: Replicated microcosms containing either the soils or aquifer water will be established and used to determine the ability of microbes in these environments to degrade chemicals potentially used by

industry. Chemical degradation will be determined either through direct measurement of the chemical in the soil or aquifer using analytical chemistry techniques or microbial growth assays

TASK OBJECTIVES: The task will include the following objectives:

- 1) Establish replicated microcosms;
- 2) Spike microcosms with target compounds at realistic concentrations;
- 3) Incubate at realistic conditions i.e. for soil microcosms, incubate at field relevant conditions (local temperatures and day/night cycle will be reproduced in the laboratory) for aquifer water microcosms relevant subsurface temperature will be used in the absence of light;
- 4) Harvest all soil treatments after two weeks and prepare samples for chemical analyses;
- 5) Harvest all water treatments after six weeks and prepare samples for chemical analyses;
- 6) Establish sole carbon source experiments;
- 7) Incubate at a relevant field conditions;
- 8) Inspect cultures for visual signs of growth and where possible (in the aquifer cultures) measure biomass; and
- 9) Statistical analyses of the resultant data.

TASK OUTPUTS AND SPECIFIC DELIVERABLES: Replicated Experimental data on the degradation of target compounds. Data prepared for analysis and final reporting.

Task 5

TASK NAME: Impact and indicator taxa

TASK LEADER: Nai TRAN-DINH

OVERALL TIMEFRAME: November 2019 to January 2020

BACKGROUND: In the soils and the water microcosms, microbial community profiling will also be undertaken after exposure to individual chemicals will be carried out to ascertain impacts on microbial communities and to potentially identify putatively useful indicator taxa for monitoring environmental impacts.

TASK OBJECTIVES: The task will include the following objectives:

- 1) Extract DNA from soil and water from Task 4 to determine changes in microbial community profiles;
- 2) Statistical and bioinformatics analyses of the resultant data.

TASK OUTPUTS AND SPECIFIC DELIVERABLES: Experimental data on the microbial community changes in response to exposure to shale gas activity-related chemicals. Analyses complete and prepared for final report.

Task 6

TASK NAME: Project management, data analysis and reporting

TASK LEADER: David MIDGLEY

OVERALL TIMEFRAME: May 2019 to February 2020

BACKGROUND: The final report for this project will collate baseline data with microbial degradation, microbial community impact and useful indicator taxa for individual chemicals. These data will be combined with results from project W.16 to inform requirements for future toxicological studies and will provide information for a range of stakeholders.

Critical evaluation of the results is needed to understand the experimental outcomes of this study.

TASK OBJECTIVES: The task will include the following objectives:

- 1) Reporting results and analyses from Tasks 2-5;
- 2) Integration of this studies results with those of project W.16; and
- 3) Provide recommendations of chemicals with potential high residual risk requiring toxicological studies.

TASK OUTPUTS AND SPECIFIC DELIVERABLES: Final written report encompassing all the tasks outlined above and integration with the related project W.16.

Project Gantt Chart

Task	Task Description	Task Leader	May-19	Jun-19	Jul-19	Aug-19	Sep-19	Oct-19	Nov-19	Dec-19	Jan-20	Feb-20
1	Logistics, planning, bore and soil selection	Kaydy PINETOWN										
2	Sampling campaign	Tony ALLAN										
3	Baselining microbial communities	Nai TRAN-DINH										
4	Microbial degradation and sole carbon growth assays	Tania VERGARA										
5	Impact and indicator taxa	Nai TRAN-DINH										
6	Project management, data analysis and reporting	David MIDGLEY										

8. Technical Reference Group

The project will establish a Technical Reference Group (TRG) aimed at seeking peer-to-peer technical advice on contextual matters and to discuss research needs as well as outputs as the project progresses. The TRG will include the project leader and a group of different stakeholders as appropriate.

9. Communications Plan

Stakeholder	Objective	Channel	Timeframe
Government and industry	To facilitate a deeper understanding of research findings and implications for policy, programs, planning, and other initiatives	Knowledge transfer sessions and through stakeholder workshops and meetings.	From commencement of project and with updates as they come to hand.
Regional Community/Wider public	To communicate project objectives and key messages from the research	Fact sheets (including development of one at commencement of project which will explain in plain English the objective of the project – this will be updated periodically as project progresses).	From commencement of project and with updates as they come to hand.
		Project progress reported on GISERA website to ensure transparency for all stakeholders including regional communities.	As required.
		Participation in roadshows, community workshops and meetings and other engagements where appropriate.	As required
		Maps and visuals - Key findings communicated with the use of maps and visual cues incorporated.	Towards completion
Traditional Owner communities	To pursue relations with Traditional Owner communities (via cultural monitors)	Engagement with TO communities – as a wider context as part of CSIRO communications (considered mutually beneficial)	Ongoing

Regional Community/ Wider public, Government, Scientific community and Industry	To report on key findings	Final Report	At completion
Scientific community	To publish results in international peer-reviewed journals	Manuscript for submission to journals	At completion

10. Budget Summary

Expenditure	2018/19	2019/20	Total
Labour	\$26,041	\$119,403	\$145,444
Operating	\$8,000	\$25,400	\$33,400
Subcontractors	\$0	\$69,120	\$69,120
Total Expenditure	\$34,041	\$213,923	\$247,964

Expenditure per Task	2018/19	2019/20	Total
Task 1	\$24,981	\$5,226	\$30,207
Task 2	\$0	\$48,421	\$48,421
Task 3	\$0	\$30,473	\$30,473
Task 4	\$0	\$92,980	\$92,980
Task 5	\$0	\$24,795	\$24,795
Task 6	\$9,060	\$12,028	\$21,088
Total Expenditure	\$34,041	\$213,923	\$247,964

Source of Cash Contributions	2018/19	2019/20	Total
Federal Government (22.5%)	\$7,659.23	\$48,132.68	\$55,791.90
NT Government (22.5%)	\$7,659.23	\$48,132.68	\$55,791.90
Origin Energy (10%)	\$3,404.10	\$21,392.30	\$24,796.40
Santos (10%)	\$3,404.10	\$21,392.30	\$24,796.40
Pangaea (10%)	\$3,404.10	\$21,392.30	\$24,796.40
Total Cash Contributions	\$25,530.75	\$160,442.25	\$185,973.00

In-Kind Contributions	2018/19	2019/20	Total
CSIRO (25%)	\$8,510.25	\$53,480.75	\$61,991.00
Total In-Kind Contributions	\$8,510.25	\$53,480.75	\$61,991.00

	Total funding overall years	Percentage of Total Budget
Federal Government Investment	\$55,791.90	22.5%
NT Government Investment	\$55,791.90	22.5%
Origin Energy	\$24,796.40	10%
Santos	\$24,796.40	10%
Pangaea Resources	\$24,796.40	10%
CSIRO Investment	\$61,991.00	25%
TOTAL	\$247,964	100%



Task	Milestone Number	Milestone Description	Funded by	Start Date (mm-yy)	Delivery Date (mm-yy)	Fiscal Year Completed	Payment \$ (excluding CSIRO contribution)
Task 1	1.1	Briefing document for sampling campaign	GISERA	May 2019	July 2019	2019-20	\$22,655.25
Task 2	2.1	Sample collections- soil and water	GISERA	July 2019	July 2019	2019-20	\$36,315.75
Task 3	3.1	Baseline microbial community profiling complete and raw data available	GISERA	August 2019	October 2019	2019-20	\$22,854.75
Task 4	4.1	Chemical degradation and sole carbon growth assays complete and data prepared for final report	GISERA	August 2019	October 2019	2019-20	\$69,735.00
Task 5	5.1	Impact and indicator taxa identified and data prepared for final report	GISERA	November 2019	January 2020	2019-20	\$18,596.25
Task 6	6.1	Analysis, integration and interpretation complete and final report delivered to GISERA	GISERA	May 2019	February 2020	2019-20	\$15,816.00

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2 Variations to Project Order

Changes to research Project Orders are approved by the GISERA Director, acting with authority provided by the GISERA National Research Management Committee, in accordance with the [National GISERA Alliance Agreement](#).

The table below details variations to research Project Order.

Register of changes to Research Project Order

Date	Issue	Action	Authorisation
27 September 2019	Due to complexities discovered during logistics and planning, and loss of equipment in transit, an additional \$24,000 (\$18,000 GISERA funding & \$6,000 CSIRO in-kind) is required for task 2 field campaign to sample soil and water collections.	An additional \$24,000 (\$18,000 GISERA funding & \$6,000 CSIRO in-kind) is allocated for task 2 field campaign to sample soil and water collections.	
11 June 2020	Due to several significant, unplanned and unforeseen events, the research proponent has requested that the delivery dates for milestones 3-6 be moved. Additional funding has also been requested to cover the additional OPEX costs incurred.	There have been changes to the delivery dates of milestones 3-6 and an overall project budget increase of \$20,000 (\$15,000 GISERA funding and \$5,000 CSIRO funding). This results in an overall total project budget of \$291,964 and a project delivery date of 30 November 2020.	The NT RRAC approved the project variation including the additional \$20,000 funding. Refer to Minutes.

3 Progress against project milestones

Progress against milestones are approved by the GISERA Director, acting with authority provided by the GISERA National Research Management Committee, in accordance with the [National GISERA Alliance Agreement](#).

Progress against project milestones/tasks is indicated by two methods: Traffic Light Reports and descriptive Project Schedule Reports.

1. Traffic light reports in the Project Schedule Table below show progress using a simple colour code:

- **Green:**
 - Milestone fully met according to schedule.
 - Project is expected to continue to deliver according to plan.
 - Milestone payment is approved.
- **Amber:**
 - Milestone largely met according to schedule.
 - Project has experienced delays or difficulties that will be overcome by next milestone, enabling project to return to delivery according to plan by next milestone.
 - Milestone payment approved for one amber light.
 - Milestone payment withheld for second of two successive amber lights; project review initiated and undertaken by GISERA Director.
- **Red:**
 - Milestone not met according to schedule.
 - Problems in meeting milestone are likely to impact subsequent project delivery, such that revisions to project timing, scope or budget must be considered.
 - Milestone payment is withheld.
 - Project review initiated and undertaken by GISERA Research Advisory Committee.

2. Progress Schedule Reports outline task objectives and outputs and describe, in the 'progress report' section, the means and extent to which progress towards tasks has been made.



Project Schedule Table

ID	Activities / Task Title (should match activities in impact pathway section)	Task Leader	Scheduled Start	Scheduled Finish	Predecessor
Task 1	Logistics, planning, bore and soil selection	Kaydy PINETOWN	May 2019	July 2019	Project W.16
Task 2	Sampling campaign	Tony ALLAN	July 2019	July 2019	Task 1
Task 3	Baselining microbial communities	Nai TRAN-DINH	August 2019	August 2020	Task 2
Task 4	Microbial degradation and sole carbon growth trials	Tania VERGARA	August 2019	August 2020	Task 2
Task 5	Impact and indicator taxa	Nai TRAN-DINH	November 2019	November 2020	Tasks 2, 3 & 4
Task 6	Final Report	David MIDGLEY	May 2019	November 2020	All other tasks

Project Schedule Report



TASK 1

TASK NAME: Logistics, planning, bore and soil selection

TASK LEADER: Kaydy PINETOWN

OVERALL TIMEFRAME: May-July 2019

BACKGROUND: During Task 1, staff will work with team members of GISERA project W.16 to establish the sampling sites for large volume water samples required. This task will also identify the sampling sites for soil collection. Chemicals to be tested in subsequent tasks will be determined in Task 1 via consultation with industry and a regulator. Task 1 will interact with the parallel running project investigating stygofauna in the Beetaloo sub-Basin to ensure project compatibility. Task 1 will also include the safe and environmentally-sensitive planning, provisioning and logistics for the sampling campaign.

TASK OBJECTIVES:

- 1) Establish water and sampling sites within the Beetaloo sub-Basin;
- 2) Liaise with project W.16 and stygofauna project teams for site selection and sampling campaign logistics;
- 3) Determination of chemicals of relevance for onshore gas production in the Northern Territory;
- 4) Identification of sites for soil collection for use in Tasks 2-5;
- 5) Preparation of sampling equipment/reagents;
- 6) Preparation for remote sampling fieldwork; and
- 7) Identification of any permits, permission or consultation required for sampling.

TASK OUTPUTS AND SPECIFIC DELIVERABLES: A briefing document will be prepared for the sampling campaign describing the outcomes of task objectives 1-6.

PROGRESS REPORT:

All seven of the objectives listed for Task 1 were achieved.

1. Project staff consulted team members of GISERA project W.16 to establish the sampling sites and acquire information useful for planning purposes. Electronic geoscience data, such as ESRI files for ArcGIS, other GIS data and borehole location data were purchased for a small fee from the relevant Northern Territory government department, acquired from CSIRO colleagues involved in W16 and provided by the supporting companies (Origin Energy and Santos). Maps were constructed for planning the sampling program and the preferred water sampling sites were selected. A detailed sampling schedule was also produced as a starting point in discussions with company representatives.
2. Following the first meeting project members of the W17 and W18 (the stygofauna project) liaised regularly to coordinate a joint sampling campaign for both projects as requested by company representatives. Coordinating a joint sampling campaign resulted in a slight delay and hence the field campaign was conducted between August 4th and August 20th.
3. A list of the relevant chemicals was confirmed by company representatives, with some additions by Santos.



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4. Electronic data to produce soil maps of the study region were also obtained. These maps were used to select sites for soil collection for use in Tasks 2-5. The soil sampling activities were also included in the detailed sampling schedule.
5. An initial batch of the sampling equipment/reagents was prepared and shipped to Darwin using Toll Priority. The shipment was accidentally sent by road freight by store personnel and would not have reached the destination in time to be collected by the field teams. Attempts were made to recover and divert the shipment but after Toll was unable to establish the location of the shipment while in transit, a new batch of the sampling equipment/reagents was prepared and transported via Qantas freight with the first field team. The initial batch of sampling equipment/reagents was eventually recovered. This incident added a small additional cost to the project, but the alternative arrangements ensured that sampling and other activities could take place as planned for a successful sampling campaign.
6. All internal safety documentation was completed including an Activity Risk Assessment and a Fieldwork Trip Plan. The team was also provided with Spot Trackers, EPIRBS and satellite phones, along with a daily call-in schedule. Spot Trackers allowed the base contacts to check the movements of field team members online. Field team members and base contacts also communicated regularly through a group chat on Facebook Messenger whenever the field teams had mobile phone reception.
7. Permission for sampling was first discussed with company representatives who then appointed their own field representatives to organise borehole access permission with individual landowners. Permission for sampling from NT government owned bores was organised with the relevant government department and keys to locked boreholes were provided to field teams by government representatives. In a few cases CSIRO was required to communicate with landowners directly for access permission and this was done predominantly via email and phone. In some cases planned boreholes were either closed in, unequipped with pumps or permission was not granted, such as in the case of Amungee Mungee. In these instances, company field representatives organised collection from alternative boreholes.

All arrangements for the joint sampling campaign was completed in time for the field trip to commence.

TASK 2

TASK NAME: Sampling campaign

TASK LEADER: Tony ALLAN

OVERALL TIMEFRAME: July 2019

BACKGROUND: Task 2 will involve two staff travelling to the Beetaloo sub-Basin with the purposes of collecting: triplicate microbially preserved water samples from the 55 sites examined by project W.16 along with 50 soil samples (ten each of the five major soil types: tenosols, rudosols, kandosols, vertosols and chromosols of the Beetaloo sub-Basin). In addition five large volume water samples will be collected anoxically for microbial degradation assays (identified in Task 1).

TASK OBJECTIVES: To collect oxic and anoxic samples:

- 1) Microbially preserved water samples will be collected from sites identified in Task 1/project W.16;



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- 2) Five large volume anoxic water samples will be collected from sites identified in Task 1/project W.16;
- 3) Collection of 10 replicated soil samples from each of the five soil types completed; and
- 4) Five large volume soil samples will be collected from sites identified in Task 1.

TASK OUTPUTS AND SPECIFIC DELIVERABLES: Collection of preserved samples, oxic and anoxic samples to establish microcosms.

PROGRESS REPORT:

All four of the objectives listed for Task 2 were achieved.

1. Thirty eight microbially-preserved, replicated water samples from planned sites as per the sampling schedule (Task 1) and from alternative sites organised by company field representatives. Bores collected include (RN008481, RN029027, RN033608, RN033609, RN033670, RN033671, RN037665, RN038817, RN038818, RN040930, RN007658, RN025291, RN030325, RN037654, RN037655, RN037666, RN038179, RN038580, RN038581, RN005942, RN024616, RN028082, RN029012, RN031243, RN031382, RN031397, RN032961, RN033135, RN035130, RN035146, RN036654, RN036775, RN038630, RN038811, Stuart Plains Homestead Bore, Hayfield Shenandoah Homestead Bore , Amungee NW1, Motel Bore)
2. Six large volume anoxic water samples were collected from selected sites as per the sampling schedule (Task 1)
3. Fifty replicate soil samples were collected from 10 locations (n=5), from each of the main five soil types in the region.
4. Five large volume soil sample were collected from five locations, one from each of the main five soil types in the region.

The joint field campaign was conducted successfully and there were no safety concerns. Six CSIRO staff members conducted the field campaign, four from W17 and two from W18, accompanied by some staff from Charles Darwin University. The hire vehicle of the north team had a flat tyre soon after reaching Daly Waters but team members and company field representatives were able to manage the situation efficiently and make adjustments to the sampling schedule as required, still ensuring the success of the campaign. To ensure that all samples arrived at North Ryde laboratories safely and in a timely manner, samples were packaged in Darwin on August 20th and transported by road with TSS Sensitive Freight. Samples and equipment/reagents arrived at North Ryde laboratories on August 28th and work on the following project tasks has commenced.

TASK 3

TASK NAME: Baseline microbial communities

TASK LEADER: Nai TRAN-DINH

OVERALL TIMEFRAME: August 2019 to October 2019

BACKGROUND: The 55 microbially preserved water samples and the 50 soil samples will be subject to DNA extraction along with 16S rDNA sequencing, and, for the soil samples ITS DNA sequencing.

TASK OBJECTIVES: The task will include the following objectives:



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- 1) Complete DNA extractions from all samples;
- 2) DNA samples sent to external sequencing provider; and
- 3) Bioinformatics completed for microbial baselining of all samples.

TASK OUTPUTS AND SPECIFIC DELIVERABLES: Raw sequencing data from microbial community profiling available.

PROGRESS REPORT: This milestone is complete.

Task objectives 1 & 2) All soils and aquifer baseline samples have been DNA extracted, subject to required PCR, and subsequently uniquely barcoded and purified. These purified PCR products were then pooled and sent to Macrogen Korea for sequencing.

Task objectives 3) Sequencing data was received back in Australia and has been subject to bioinformatic analyses. In brief, the amplicon library was demultiplexed (a process whereby DNA reads are assigned to bins based on their barcode), these bins were then subject to quality control (removing short, erroneous or chimeric reads). The bins are then combined to create a single pool of reads from which singletons are discarded and a clustering process is undertaken to create OTUs ($\approx$ species). The original reads from the bins are then back-mapped onto the OTUs to create counts for each OTU in each bin.

TASK 4

TASK NAME: Microbial degradation and sole carbon growth trials

TASK LEADER: Tania VERGARA

OVERALL TIMEFRAME: August 2019 to October 2019

BACKGROUND: Replicated microcosms containing either the soils or aquifer water will be established and used to determine the ability of microbes in these environments to degrade chemicals potentially used by industry. Chemical degradation will be determined either through direct measurement of the chemical in the soil or aquifer using analytical chemistry techniques or microbial growth assays

TASK OBJECTIVES: The task will include the following objectives:

- 1) Establish replicated microcosms;
- 2) Spike microcosms with target compounds at realistic concentrations;
- 3) Incubate at realistic conditions i.e. for soil microcosms, incubate at field relevant conditions (local temperatures and day/night cycle will be reproduced in the laboratory) for aquifer water microcosms relevant subsurface temperature will be used in the absence of light;
- 4) Harvest all soil treatments after two weeks and prepare samples for chemical analyses;
- 5) Harvest all water treatments after six weeks and prepare samples for chemical analyses;
- 6) Establish sole carbon source experiments;
- 7) Incubate at a relevant field conditions;
- 8) Inspect cultures for visual signs of growth and where possible (in the aquifer cultures) measure biomass; and

9) Statistical analyses of the resultant data.

TASK OUTPUTS AND SPECIFIC DELIVERABLES: Replicated Experimental data on the degradation of target compounds. Data prepared for analysis and final reporting.

PROGRESS REPORT: This milestone is complete.

Task objectives 1, 2 & 3) Replicated microcosms were established for soils (oxic conditions) or aquifers (anoxic conditions) with and without chemical spikes.

Task objectives 4, 5, 6 and 7) All replicated microcosms were harvested and subject to chemical testing and DNA analyses (see: Task 5; Impact and indicator taxa). All sole carbon source experiments were established and incubated at relevant field conditions.

Task objective 8) Data on visual growth recorded at multiple time points and biomass estimated using DNA yields from Task 5.

TASK 5

TASK NAME: Impact and indicator taxa

TASK LEADER: Nai TRAN-DINH

OVERALL TIMEFRAME: November 2019 to January 2020

BACKGROUND: In the soils and the water microcosms, microbial community profiling will also be undertaken after exposure to individual chemicals will be carried out to ascertain impacts on microbial communities and to potentially identify putatively useful indicator taxa for monitoring environmental impacts.

TASK OBJECTIVES: The task will include the following objectives:

- 1) Extract DNA from soil and water from Task 4 to determine changes in microbial community profiles;
- 2) Statistical and bioinformatics analyses of the resultant data.

TASK OUTPUTS AND SPECIFIC DELIVERABLES: Experimental data on the microbial community changes in response to exposure to shale gas activity-related chemicals. Analyses complete and prepared for final report.

PROGRESS REPORT: This milestone is complete.

Task objectives 1) All soils and aquifer spiked microcosm samples have been DNA extracted, subject to required PCR, and subsequently uniquely barcoded and purified. These purified PCR products were then pooled and sent to Macrogen Korea for sequencing along with the DNA from Task 3.

Task objective 2) Sequencing data was received back in Australia and has been subject to bioinformatic analyses. In brief, the amplicon library was demultiplexed (a process whereby DNA reads are assigned to bins based on their barcode), these bins were then subject to quality control (removing short, erroneous or chimeric reads). The bins are then combined to create a single pool of reads from which singletons are discarded and a clustering process is undertaken to create OTUs ($\approx$ species). The original reads from the bins are then back-mapped onto the OTUs to create counts for each OTU in each bin. In order to compare chemical data, statistical comparisons were undertaken and p-values determined.



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TASK 6

TASK NAME: Project management, data analysis and reporting

TASK LEADER: David MIDGLEY

OVERALL TIMEFRAME: May 2019 to February 2020

BACKGROUND: The final report for this project will collate baseline data with microbial degradation, microbial community impact and useful indicator taxa for individual chemicals. These data will be combined with results from project W.16 to inform requirements for future toxicological studies and will provide information for a range of stakeholders.

Critical evaluation of the results is needed to understand the experimental outcomes of this study.

TASK OBJECTIVES: The task will include the following objectives:

- 1) Reporting results and analyses from Tasks 2-5;
- 2) Integration of this studies results with those of project W.16; and
- 3) Provide recommendations of chemicals with potential high residual risk requiring toxicological studies.

TASK OUTPUTS AND SPECIFIC DELIVERABLES: Final written report encompassing all the tasks outlined above and integration with the related project W.16.

PROGRESS REPORT: This milestone is underway. Visualisations of data from Task 3, 4 and 5 are on-going and will be complete shortly. The report will follow the completion of these visualisations.