Project Order, Variations and Research Progress

Project Title: Microbial degradation of chemicals and fluids in aquifers of the Limestone Coast, South Australia

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

1 Original Project Order

Project Order

Proforma 2020

1. Short Project Title

Microbial degradation of chemicals and fluids in aquifers of the Limestone Coast, South Australia

Long Project Title	Microbial degradation of onshore gas activity chemicals and fluids in aquifers of the Limestone Coast, South Australia
GISERA Project Number	W.22
Proposed Start Date	01/08/2020
Proposed End Date	31/07/2021
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:

1. Establish microbial community baselines in the Tertiary Limestone Aquifer (TLA) across the Limestone Coast region. A particular focus will be placed on sites of agricultural importance proximal to prospective onshore gas activities.
2. Understand the microbial degradation of a range of chemicals likely to be used in onshore gas activities, in waters of the TLA.

1. Establish microbial community baselines in the TLA

Access to clean water is critical for both the environment and anthropogenic activities. The TLA is the primary groundwater supply for the Limestone Coast region and used for many purposes, including town water supplies, industrial uses, stock watering and widespread irrigation use. With the putative development of the gas resource in the region, a key task is to ensure this is done with no impacts to water quality. Water is protected through government policy and law such as: the Australian Water Act, 2007 (C2020C00058) or the Environment Protection (Water Quality) Policy 2015 (South Australian Government, v.1.6.2019), while internationally water is protected through the Clean Water Act (in the USA; CWA 33 U.S.C; USA Environmental Protection Agency) and analogous regulatory frameworks in Europe.

Such laws and policy include various testing regimes to maintain certain standards. These testing regimes are moving to include biological and microbiological measures of environmental health (Pawlowski et al., 2018). Modern methods for counting and identifying microbes use Next Generation Sequencing (NGS) technologies to profile entire microbiomes. While in surface waters, microbes profiled for environmental health tend to include eukaryotes (fungi, invertebrates and other small animals), such organisms are largely absent from anoxic aquifers, and prokaryotic (bacterial and archaeal) communities tend to be preferred. This method is reliant on a process which includes the following steps: (1) extract environmental DNA, (2) amplify, using polymerase chain reaction (PCR), groups using primers that amplify known marker genes (e.g. 16S – a structural RNA, Figure 1), (4) sequencing amplified PCR products and (5) analyse using bioinformatics.

This approach allows for the observation of the microbial baseline prior to the initiation of an onshore gas industry, and the identification of indicator organisms. The process is called 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).

2. Understand the microbial degradation

Onshore gas production and exploration uses a range of chemical products including various biocides, buffers, corrosion inhibitors, friction reducers, surfactants and viscosity control agents. While the risks associated with these chemicals are well-characterised from an environmental and human health perspective, most studies do not characterise their degradation. Moreover, little is known about the particular microbiology of the waters of the TLA. Microbes in this aquifer have the potential to detoxify and catabolise (those parts of metabolism that relate to the breaking down of compounds) these chemicals. As such, microbes offer an additional layer of environmental protection above and beyond the significant regulatory and operating controls placed both on, and by, industry, respectively.

Aquifers are complex discontinuous environments with disparate microbiology influenced by local factors. In the TLA, local microbial communities are likely impacted by surface land use as vertical recharge occurs in some sections of the TLA. Elsewhere, the water in the aquifer is ancient (~20,000 years old), and is either not recharged actively, or is recharged horizontally (Tertiary Limestone Aquifer, Information Sheet 2/4 South Australian Government). This complex discontinuity in the aquifer means that microbial capacity to degrade a range of compounds likely varies spatially across the region.

Experiments in GISERA South Australia Project Microbial degradation of onshore gas-related chemical compounds (W15) demonstrated that most chemicals were either completely degraded or had organisms capable of growth on the chemical as a sole source of carbon in South Australian loam soils in the Penola region. In contrast, results from W15 for degradation of the chemicals in the single aquifer sample indicated that in aquifer microcosms the chemicals were either partially degraded or not degraded at all. It was not, however, clear whether this phenomenon was limited to the single aquifer sample examined, or whether given more time, there would be further degradation.

The aim of the current study is to demonstrate the potential for microbial degradation of chemicals used by industry across the TLA, taking in areas with important stakeholders including viticulture, cattle and sheep farms, grain farms along with fruit, vegetable and tree nut farms. Sampling of the TLA in this project will consider both aquifer heterogeneity (due to stratification and discontinuity) and the agricultural use. Where existing NATA accredited analytical tests are available, chemical degradation will be analysed quantitatively. In situations where chemicals of interest have no analytical testing method, microbial growth on the chemical as a sole source of carbon will be used as a proxy for degradation. Growth assays in anoxic water samples will provide evidence that microbes from the TLA are capable of degrading and growing on the chemical as a sole source of carbon, however, the rates of degradation and the residual compounds of degradation cannot be determined from these growth experiments. In parallel, metagenomic sequencing

will be undertaken, on a single sample per chemical to attempt to elucidate the genetic basis for the degradation of these chemicals.

Description

This project continues a series of linked steps seeking to holistically characterise the migration and degradation of chemicals used in onshore gas activities that have been previously identified to pose an environmental or human health risk [1][2][3].

The work undertaken in W15 demonstrated for a single, widespread soil type (dark sandy loam) from the Limestone Coast region of South Australia, that in broad terms, limited impacts where observed on the soil microbial communities. Additionally, soil communities rapidly degraded most chemicals tested (analytical chemical tests indicated no detectable levels from soils after 34 days of incubation). In contrast, the single sample of microbially rich water from the TLA after 30 days incubation did not degrade many of chemicals tested. It was not clear whether this was due to insufficient incubation times, nutrient limitation or some innate quality of the single sample tested and led to this research proposal to investigate the reasons.

The hydrogeological discontinuities in the TLA, and its varying recharge methods (vertical and horizontal) mean that data from a single sample may not be indicative of the capacity of microbes across the TLA to respond to environmental contamination. To that end, this project will seek to collect water across the Limestone Coast region from relevant industries (viticulture, cattle and sheep farms, grain farms along with fruit, vegetable and tree nut farms) and working to ensure that aquifer heterogeneity (stratification and discontinuity) are also captured. These waters will be spiked with chemicals from Table 1 and incubated for 120 days to determine whether different microbes and microbial communities can degrade these compounds given sufficient time. In parallel, microbially preserved samples will be collected to establish a baseline from

[1] Australian Government Department of the Environment and Energy (2014) Hydraulic fracturing ('fraccing') techniques, including reporting requirements and governance arrangements (http://www.environment.gov.au/system/files/resources/de709bdd-95a0-4459-a8ce-8ed3cb72d44a/files/background-review-hydraulic-fracturing_0.pdf)

[2] Australian Government Department of the Environment and Energy (2017) Risk assessment guidance manual: for chemicals associated with coal seam gas extraction (http://www.environment.gov.au/system/files/consultations/81536a00-45ea-4aba-982c-5c52a100cc15/files/risk-assessment-guidance-manual-chemicals-associated-csg-extraction-australia-exposure-draft.pdf)

[3] Australian Government Department of the Environment and Energy (2017) National assessment of chemicals associated with coal seam gas extraction in Australia – Overview (http://www.environment.gov.au/system/files/resources/03137f85-1bea-46a4-b9e7-67d985b4aeb5/files/national-assessment-chemicals-overview.pdf)

a range of land-use sites across the TLA in the Limestone Coast region of South Australia. This data not only provides a useful tool for monitoring environmental health but will be able to be integrated with information obtained on taxa capable of microbial degradation. For example, if a bacterial species is shown to be involved in the breakdown of a chemical, then the baseline data will provide information on how widespread this particular species is, giving potential insights into environmental resilience across the region.

It is important to note this study will examine the chemicals in a laboratory setting outside of any industry chemical management best-practices or regulatory controls. That is, this study examines the inherent risks of these chemicals before industry management and regulatory controls are used in the field to minimise residual risks to as low as reasonably possible.

Table 1: Chemicals potentially used in onshore gas production in South Australia and their process role.

Chemicals	Additive role in onshore gas activities
2-aminoethanol	Viscosity management/ drilling additive
2-butoxyethanol	Surfactant
2-ethylhexanol	Surfactant
benzisothiazolinone	Biocide
bronopol	Biocide
c12 alcohol ethoxylate	Surfactant
diesel fuel	Fuel
diethylene glycol ethyl ether	Solvent
d-limonene	Surfactant
eicosane	Surfactant
hydrotreated light petroleum distillate	Carrier fluid
ethylene glycol	Viscosity management
glutaraldehyde	Biocide
glyoxal	Viscosity management/ crosslinker
hexahydro-1,3,5-tris(2-hydroxyethyl)-sym-triazine	Biocide
isopropanol	Surfactant
methanol	Surfactant
methylchloroisothiazolinone	Biocide
methylisothiazolinone	Biocide
naphthalene	Corrosion inhibitor
o-cresol	Biocide
polyacrylamide	Friction reducer
polyoxypropylene diamine	Pipework/Epoxy resins/Hardener
pristane	Surfactant
propylene glycol	Viscosity management
triethanolamine	Viscosity management

This project will sample 60 microbially preserved samples from across the Limestone Coast region and provide a systems view of these samples from the perspective of water chemistry and microbiology. All 60 samples will be subject to microbial community profiling which will be correlated with co-collected data on water chemistry across the TLA. Microbial profiles provide a means of rapidly assaying for perturbations in the aquifer and provide data on aquifer mixing. In parallel, the microbial degradation of ~26 previously identified hazardous chemical compounds (Table 1) associated with onshore gas activities will be examined in five waters representative of heterogeneity across the TLA and of land use (from viticulture, animal grazing, grain, vegetable and orchard land use). This list of chemicals will be confirmed with industry to ensure that it is representative of their intended use in the field as part of Task 1. All quantitative chemical degradation will be analysed for those chemicals that have a commercial, NATA accredited test.

All experiments will be conducted in highly replicated microcosm experiments (n=10). Microcosms will be incubated under field-like conditions (anoxia, temperature, and darkness) and with industrially relevant concentrations of the target chemicals.

The resultant microbial and chemical data will be subject to statistical comparisons to confirm chemical degradation or microbial community change. The project will use estimated cell counts and DNA sequencing targeting ribosomal genes in bacteria (16S rDNA sequencing) to describe effects on the microbial community and to identify indicator taxa (see Figure 1). The study will also sequence one metagenome per chemical, to attempt to identify the genes that might underpin chemical degradation in the TLA.

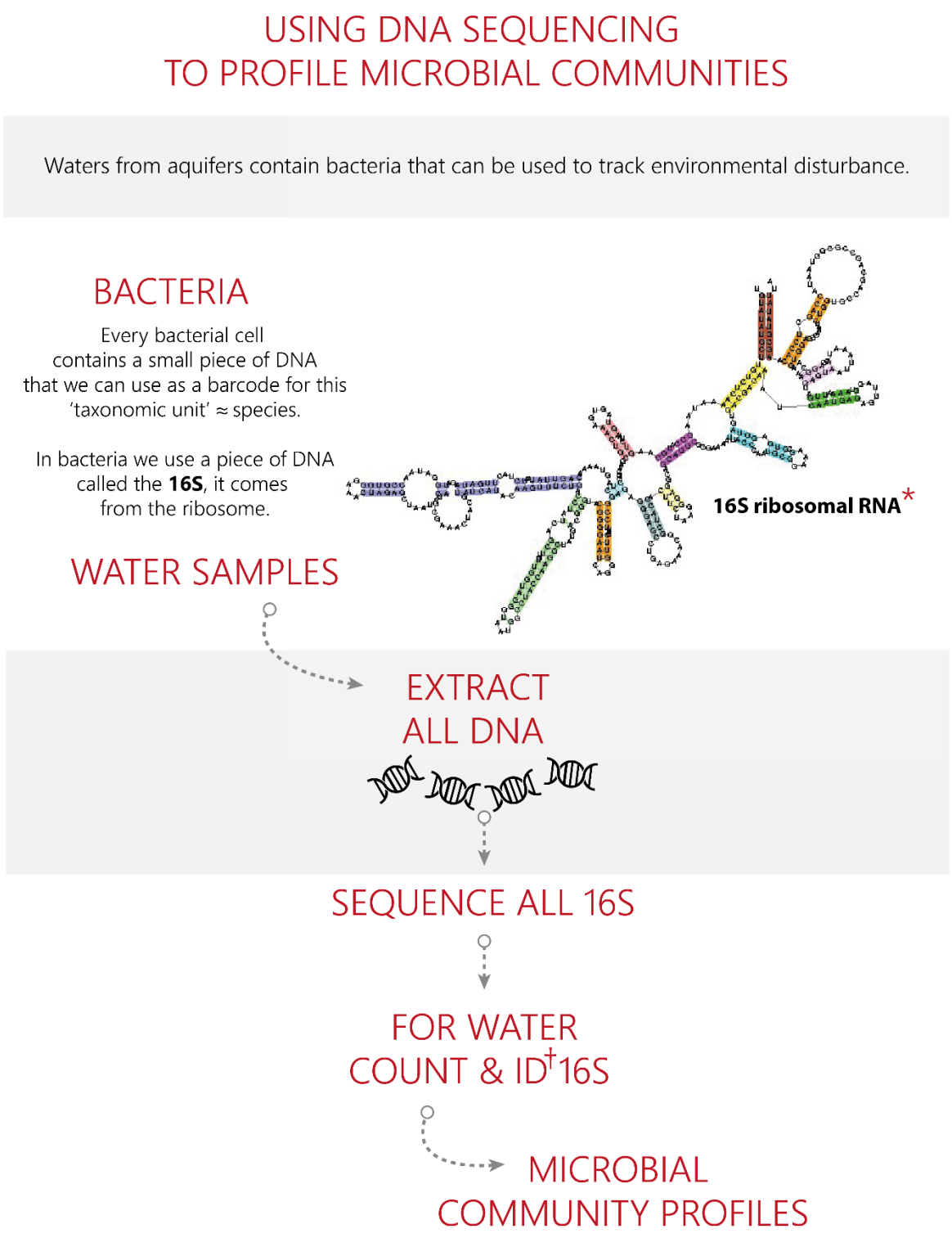

Figure 1: Microbial community profiling

† ID- Identification using Bayesian classifiers and a bacterial 16S database (Cole et al., 2014)

* 16S ribosomal RNA is a structural domain in microbial ribosomes and is ideal for phylogenetic identification as part of these genes are highly conserved from an evolutionary perspective.

It is critically important to establish meaningful baseline data before any extensive development of the industry.

The final report for this project will synthesize all aspects of the work and include:

- Baseline data on microbial communities across the TLA in the Limestone Coast region that can be directly correlated with geochemical data also collected in the present project. These data can be integrated into microbial maps of the TLA which may provide useful insights into resilient and susceptible sites in the region.
- 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 environmental impacts of activities associated with the growth of the onshore gas industry in the Limestone Coast region of South Australia.
- Genomes and genes of those taxa which grow on chemicals as a sole source of carbon and information on the catabolic activity of these pathways.

Need and Scope

There is significant public concern about onshore gas activities and potential environmental and human-health harm that may be caused by an expansion of the industry in the Limestone Coast region. This is despite the industry having almost no incidences of environmental harm recorded in Australia and intensive scrutiny of industry practice through government regulation. Nevertheless, it is vital to properly assess controls to a potential incident to assist in easing public concern, or to provide feedback to industry about chemicals that pose unacceptably high risk.

In order to understand the impact of industrial growth in onshore gas activities on the environment it is critical to understand baseline information on the chemistry and microbiology of the TLA. Such data can be used to monitor the aquifer using microbes as indicators of environmental health. Additionally, it is important to understand whether microbes in the TLA provide an additional level of defence in the unlikely event of a spill or whether, as observed in the single sample examined in W15, some chemicals persist in the waters of the TLA.

Method

Task 1 will involve obtaining geographic and hydrogeological maps of the Limestone Coast region, along with consultation with the South Australian Departments of Energy and Mining (DEM), and Environment and Water (DEW) to guide sample collection. Staff involved in Task 1 will then plot on these maps the locations of land use of interest (e.g. viticulture, animal grazing, grain growing, fruit and tree nut farms, and vegetable

farms) and known hydrogeological and physicochemical heterogeneity across the TLA. To facilitate sample collection, properties in areas where sampling is required will be contacted to ask if they would be willing to share water samples for the project. The project will seek to prioritise agriculturally important sites for collection and will aim to also obtain broad coverage of the region such that aquifer heterogeneity and microbial variation (and variation in their catabolism, if it exists) across the TLA can be captured. The output of this task will be a sampling schedule and map of the region. Task 1 will also include the safe and environmentally-sensitive planning, provisioning and logistics for the sampling campaign.

The list of chemicals examined in this study will be based on those examined in W15, however, additionally diesel and hydrotreated light petroleum distillate will be included (Table 1), and all chemicals will be reconfirmed with industry representatives as still being relevant to their proposed operations in the Limestone Coast region of South Australia.

Task 2 involves two staff travelling to the Limestone Coast region of South Australia to collect triplicate microbially preserved aquifer samples from the 20 sites (total of 60 samples) identified by Task 1. In addition, up to five biological and bulk aquifer samples will be collected and used in Tasks 4 and 5 to measure chemical degradation and microbial indicators of degradation. These five biological and bulk water samples will aim to cover the land use types (viticulture, animal grazing, grain growing, vegetable growing, and orchards) and heterogeneity in the TLA. The biological samples will be collected under an argon, nitrogen or carbon dioxide atmosphere to preserve the anoxic nature of the aquifer and prevent overgrowth of organisms that flourish in oxic environments. The bulk samples will be used for media preparation in Tasks 4 and 5.

On returning samples to the laboratory, Task 3, will commence with the DNA extraction of 60 microbially preserved samples. These will then be subject to 16S PCR using individually-barcoded primers as recommended by the Earth Microbiome Project:

Forward primer :515F (Parada) GTGYCAGCMGCCGCGGTAA;
Reverse primer: 806Rb (Apprill) GGACTACNVGGGTWTCTAAT

The resultant barcoded PCR products (amplicons) will be mixed and sent for sequencing to obtain a baseline of microbial communities across the TLA. In parallel Task 4 will be initiated once samples are returned to the laboratory. This will involve the setup of a replicated experiment (n=10) for each chemical and controls. This experiment will determine which microbes are involved in degradation of the chemicals, and where accredited methods are available, quantify the amount of chemical degraded. For numerous chemicals used by industry, no NATA accredited analysis method exists. Their quantitation is therefore challenging and would require significant R&D to develop a reliable, accurate analytical method. Such experiments are beyond the

focus of this project, and where no method exists, microbial growth and community change will be used as a proxy for degradation. For example, spiking 2-aminoethanol into aquifer water and incubating this water at aquifer temperatures in the dark will result in growth of taxa capable of using 2-aminoethanol as a carbon source. The aquifer water is extremely low in organic carbon, so very little background growth can occur. In this example, an accredited test exists so it will be possible to obtain not only the amount of 2-aminoethanol degraded, but also the main organisms responsible for its degradation and additionally, any potentially sensitive organisms (those that die on exposure to this compound). This latter data can be used to identify useful biological indicators of contamination. These analyses of the outcomes of Task 4, form the majority of Task 5 and is reliant on a range of statistical analyses of bioinformatic data that will come from Task 4. Task 6 will involve sequencing the genome(s) of those taxa whose abundance increases by the greatest margin on each chemical with the hope of identifying genes involved in the degradative pathway.

Task 6 will use DNA produced by Task 4 and will result in genomic DNA sequence data, and where possible genes and pathways responsible for the degradative ability observed in assays. To briefly describe the process: once per chemical, metagenomic sequencing data will be bioinformatically assembled into genomes, subjected to gene calling and annotation, and putative genes for chemical degradation identified. These data will be made available on a public data portal.

Task 7 will synthesize all data from the project, creating a microbial map of the TLA in the Limestone Coast region using data from Task 3 and analysing this in view of the potential for degradation identified through Tasks 4 and 5. This report will also include recommendations for both community, government and industry.

The Tasks from this project are summarised in Figure 2.

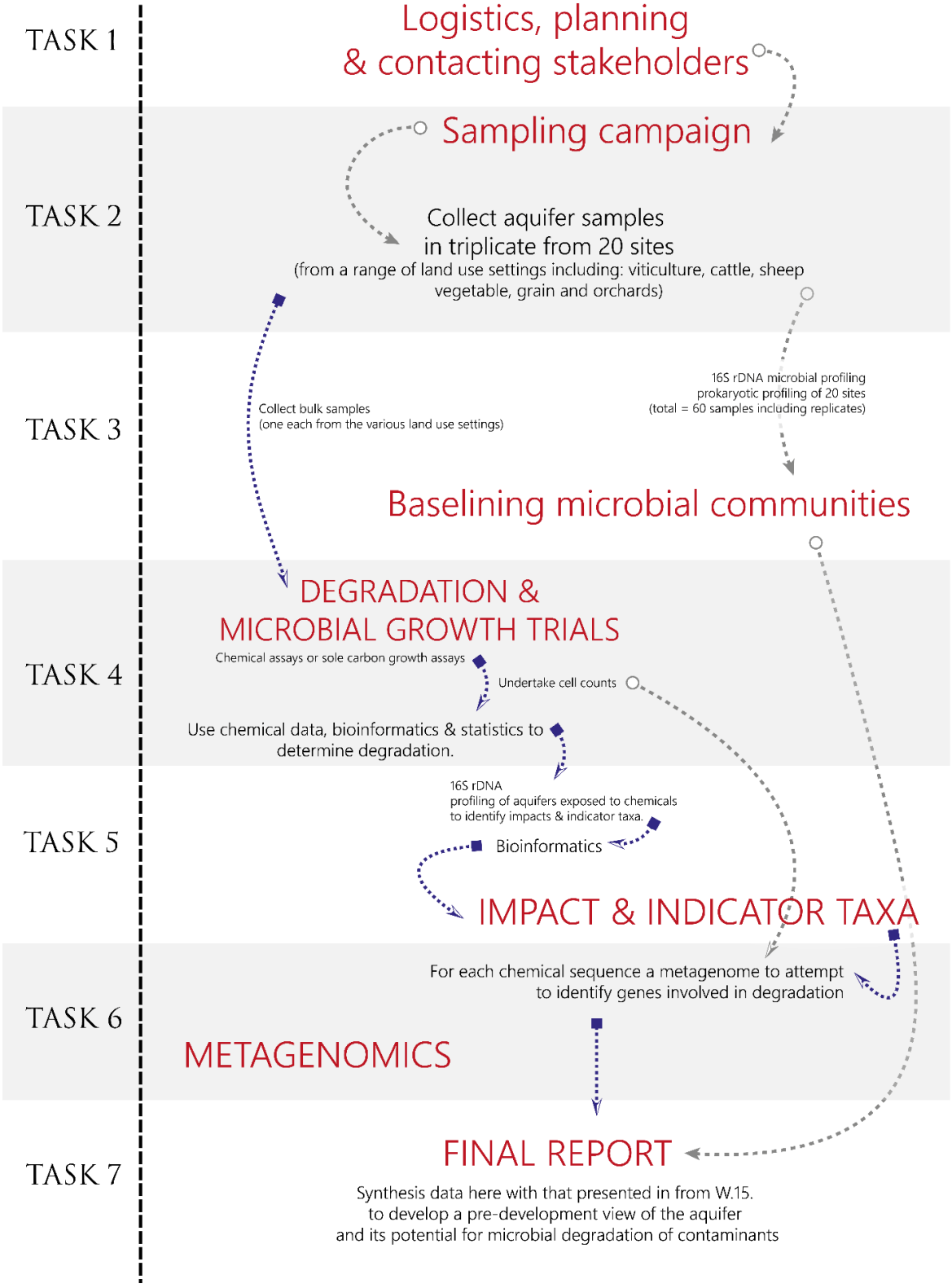

Figure 2: Schedule of tasks and brief description

5. Project Inputs

Research

Chemical degradation investigated in this project will prioritise those chemicals used regularly by industry which have potential as environmental or human health hazards. It is noteworthy, that microbial degradation of those compounds has been largely established in non-aquifer settings (Figure 3) and many of the compounds share common intermediates (see Figure 3).

The majority of what is known about degradation is based on oxic systems (shown in blue arrows in Figure 3). In aquifers and specifically, in the waters of the TLA, nothing is known about their degradation aside from those data presented in W15. This project will seek to establish the capacity (and identity) of microbes that are able to degrade chemicals across the TLA in the Limestone Coast region. Further, this study will synthesize this data with microbial maps of the TLA generated as an output of Task 3 to generalise degradative potential across the region.

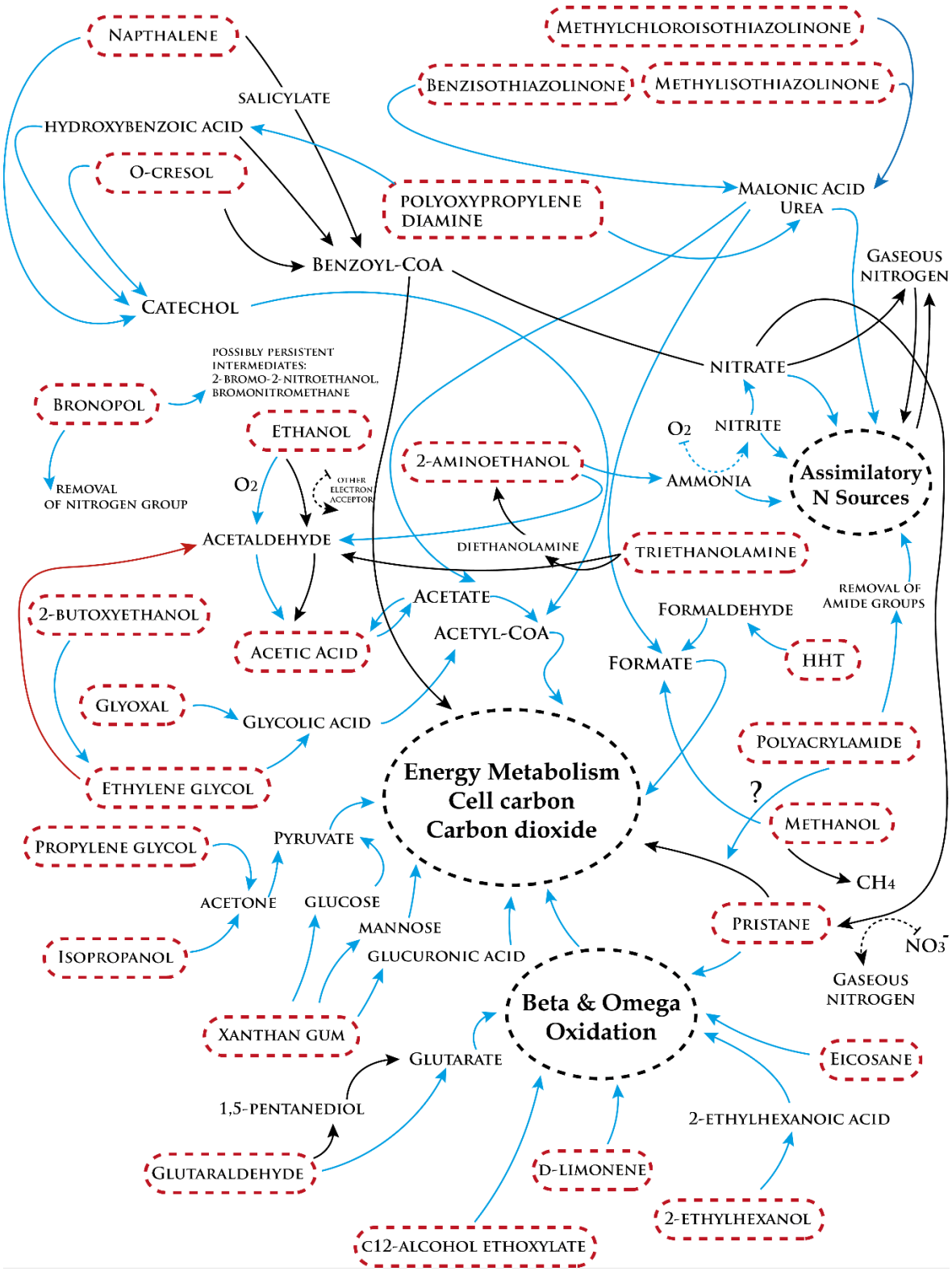

Figure 3:Conceptual model of chemical catabolism in soils and aquifers of south east South Australia

(See GISERA W15 for full report)

Resources and collaborations

Researcher	Time Commitment (project as a whole)	Principle area of expertise	Years of experience	Organisation
David MIDGLEY	38 days	Microbial Ecology & Catabolism	>20years	CSIRO
Kaydy PINETOWN	17 days	Geology	>20 years	CSIRO
Tony ALLEN	10 days	Geologist	>30 years	CSIRO
Nai TRAN-DINH	28 days	Microbial Ecology and molecular biology	>20 years	CSIRO
Carla MARIANI	47 days	Microbiology and geochemistry	>3 years	CSIRO
Richard SCHINTEIE	4 days	Chemistry and microbiology	>15 years	CSIRO

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

GISERA
Gas Industry Social and Environmental Research Alliance

Budget Summary

Source of Cash Contributions	2020/21	2021/22	2022/23	% of Contribution	Total
GISERA	$192,507	$12,620	$0	75%	$205,127
- Federal Government	$142,815	$9,362	$0	55.64%	$152,177
- SA Government	$49,692	$3,258	$0	19.36%	$42,378
Total Cash Contributions	**$192,507**	**$12,620**	**$0**	75%	**$205,127**
Source of In-Kind Contribution	**2020/21**	**2021/22**	**2022/23**	**% of Contribution**	**Total**
CSIRO	$64,169	$4,207	$0	25%	$68,376
Total In-Kind Contribution	**$64,169**	**$4,207**	**$0**	**25%**	**$68,376**

6. Project Impact Pathway

Activities	Outputs	Short term Outcomes	Long term outcomes	Impact
Logistics, planning and water sampling selection	Detailed geographic and hydrogeological maps overlaid with land use (viticulture, cattle and sheep farms, grain farms along with fruit, vegetable and tree nut farms) and known hydrogeological and physicochemical heterogeneity across the TLA. Logistics, occupational health and safety, environmental and community concerns, along with detailed sampling procedures. A list of chemicals to be tested in this project will be determined through consultation with industry and the regulator. A sampling schedule with property contacts and locations of access to the TLA. A total of twenty sites,, for collection of microbially preserved samples (n=60), biological samples (n=15) and bulk samples (n=5). A briefing document will be prepared for the sampling campaign.	Knowledge on the microbial baseline of aquifer waters in the Limestone Coast region used in viticulture, animal grazing, fruit, vegetable and tree nut industries and the microbial biodegradation of chemicals that are potentially involved in onshore gas activities as well as an understanding of microbial community changes as a result of the presence of such chemicals. This project will additionally provide information on potential indicator microbial taxa specific to the Limestone Coast region.	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 onshore gas activities in the Limestone Coast region. 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, leading to improved industry practice and decision-making to minimise such risks.	The impact of this research extends to government, industry and everyday Australians. All Australian communities that are located in onshore 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 contamination at several locations in the Limestone Coast region which will assist those at both the decision-making and policy-making levels of government.
Sampling campaign	Provision of water samples for experimental program of this project. Briefing document with details of collection and sample availability will be prepared.			
Determine the reduced risk of chemicals used in onshore gas activities primarily through microbial degradation.	Technical report containing results from tasks examining the biodegradation of individual chemicals in onshore 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 taxa as potential indicators for environmental health.	Technical report to include the identification of microbial taxa that displays sensitivity towards individual chemicals with the potential to be used as environmental health indicators specific to the region.			
Build upon the results of project W15. Work synergistically with the NT project W16, and a parallel running project investigating stygofauna of the Beetaloo sub-Basin.	Data from project W15 will be used to inform decisions within this project around sample collection, chemical selection and analysis. Demonstrate the value of integration of the two data sets to generate a systems level view of the pre-resource development aquifer networks in the Limestone Coast region. This project's results will extend the knowledge obtained in W15 by examining a greater number of aquifer samples and by providing information regarding microbial biodegradation over a greater time period.			

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.		Increased community awareness of the potential environmental impacts of gas activities is another long-term outcome of this project.	
Prepare and submit scientific manuscripts for publication in peer-reviewed journals	Manuscript submission to peer-reviewed journals.			

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	Briefing document for sampling campaign	Kaydy PINETOWN	August 2020	September 2020	
Task 2	Sample collections- soil and water	David MIDGLEY	September 2020	November 2020	Task 1
Task 3	Baseline microbial community profiling complete and raw data available	Carla MARIANI	November 2020	February 2021	Task 2
Task 4	Chemical degradation and sole carbon growth assays complete and data prepared for final report	Nai TRAN-DINH	December 2020	June 2021	Task 2
Task 5	Impact and indicator taxa identified and data prepared for final report	Nai TRAN-DINH	December 2020	June 2021	Tasks 1, 2, 3 & 4
Task 6	Metagenomics	David MIDGLEY	February 2021	June 2021	Task 4 (partial)
Task 7	Data analysis and reporting	David MIDGLEY	August 2020	July 2021	All other tasks.

Task description

Task 1

TASK NAME: Logistics, planning, contacting stakeholders

TASK LEADER: Kaydy PINETOWN

OVERALL TIMEFRAME: August - September 2020

BACKGROUND: During Task 1 we consult with colleagues in the South Australian Departments of Energy and Mining (DEM), and Environment and Water (DEW) to guide the sampling campaign to ensure that hydrogeological and physicochemical heterogeneity in the TLA is captured. In addition, we will contact relevant landholders who use water from the TLA. This will include replicated samples from viticulture, cattle and sheep farms, grain farms along with fruit, vegetable and tree nut farms, and will ensure that the heterogeneity in the TLA is adequately covered.

TASK OBJECTIVES:

1) Establish contacts with colleagues in the South Australian Departments of Energy and Mining (DEM), and Environment and Water (DEW) to guide the sampling campaign to ensure that hydrogeological and physicochemical heterogeneity in the TLA is captured.
2) Establish contacts with relevant landholders who use water from the TLA and identification of any permits, permission or consultation required for sampling.
3) Confirm the relevance of chemicals being tested in the project i.e. that they are still relevant for onshore gas production in the Limestone Coast region.
4) Identification of sites for aquifer collections. With a view to ensuring a good spread of sampling across the region.
5) Ordering and preparation of sampling equipment/reagents, vehicles and OH&S considerations.

TASK OUTPUTS AND SPECIFIC DELIVERABLES: This task will yield a series of documents describing the contacts, sampling sites, relevant permissions, sampling equipment and OH&S considerations.

Task 2

TASK NAME: Sampling campaign

TASK LEADER: David Midgley

OVERALL TIMEFRAME: September – November 2020

BACKGROUND: Task 2 will involve two staff travelling to the Limestone Coast region of South Australia with the purpose of collecting aquifer samples across the region under a variety of land-use practices.

TASK OBJECTIVES:

1) To collect triplicate preserved aquifer samples from the sites identified by Task 1.

2) To collect triplicate microbiological ('live') aquifer samples (under CO_2 or other gas headspace) from each of the five land use types (viticulture, animal grazing, grain growing, fruit and tree nut farms, and vegetable farms).
3) To collect bulk aquifer samples (4x5L) to match the microbiological ('live') aquifer samples.

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

Task 3

TASK NAME: Baselining microbial communities

TASK LEADER: Carla MARIANI

OVERALL TIMEFRAME: November 2020 to February 2021

BACKGROUND: The microbially preserved aquifer samples will be subject to DNA extraction along with 16S rDNA sequencing.

TASK OBJECTIVES: The task will include the following objectives:

1) Filter microbially preserved samples onto 0.1µM PVDF filters.
2) Complete DNA extractions from all samples.
3) Process DNA for 16S NGS sequencing.

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: Nai TRAN-DINH

OVERALL TIMEFRAME: December 2020 to June 2021

BACKGROUND: Replicated aquifer microcosms containing aquifer water will be established and used to determine the ability of aquifer microbes to degrade chemicals potentially used by industry. Chemical degradation will be determined either through direct measurement of the chemical where available at NATA accredited laboratories or measured indirectly through microbial growth on that compound.

TASK OBJECTIVES: The task will include the following objectives:

1) Establish replicated anoxic microcosms with a CO_2 headspace.
2) Spike microcosms with target compounds at realistic concentrations.
3) Incubate at realistic conditions i.e. aquifer temperature, dark, anoxic.
4) Harvest all water treatments after 120 days and prepare samples for chemical analyses.
5) Establish sole carbon source degradation trials.
6) 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: Bioinformatic analyses and identification of impact and indicator taxa

TASK LEADER: Nai TRAN-DINH

OVERALL TIMEFRAME: December 2020 to June 2021

BACKGROUND: Aquifer microcosms will be subject to microbial community profiling after 120 days 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 aquifer microcosms from Task 4 to determine changes in microbial community profiles.
2) Process DNA for 16S NGS sequencing.
3) Statistical and bioinformatics analyses of the resultant data from Tasks 3 and 5.

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

Task 6

TASK NAME: Metagenomics

TASK LEADER: David MIDGLEY

OVERALL TIMEFRAME: February 2021 to July 2021

BACKGROUND: For each chemical, one aquifer sample will be chosen based on the increase in cell number such that those aquifer samples that respond the most will be selected. DNA will be used from Task 4 and subject to whole genome, shot gun sequencing using 150bp PE reads. From the resultant metagenomic sequences contigs will be assembled and binned into genomes using a tri- or tetra-mer approach. The resultant bins (genomes) will then be subject to gene calling, gene annotation and pathway analyses with a view to identifying putative genes for chemical degradation. These data will be made available via a public data portal.

TASK OBJECTIVES: The task will include the following objectives:

1) Complete DNA sequencing
2) Complete metagenomic analyses (assembly, binning and annotation)
3) Putative genes involved in chemical degradation will be identified and data presented in the final report; and

4) Data will be available via a public portal.

TASK OUTPUTS AND SPECIFIC DELIVERABLES: DNA sequence data, binned metagenomic contiguous sequences and annotations, and data available on a public portal linked to this project.

Task 7

TASK NAME: Data analysis and reporting

TASK LEADER: David MIDGLEY

OVERALL TIMEFRAME: August 2020 to July 2021

BACKGROUND: The final report for this project will bring together microbial baseline data from aquifer samples across the region under differing land use practices. It will identify or describe microbial degradation, microbial community impact and useful indicator taxa for individual chemicals. These data will be combined with results from project W15 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 2-6;
2) Integration of this studies results with those of project W15; 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 W15.

Project Gantt Chart

Task	Task Description	Task Leader	2020-2021											
			Aug-20	Sept-20	Oct-20	Nov-20	Dec-20	Jan-21	Feb-21	Mar-21	Apr-21	May-21	Jun-21	July 2021
1	Logistics, planning, bore and soil selection	Kaydy PINETOWN												
2	Sampling campaign	David MIDGLEY												
3	Baselining microbial communities	Carla MARIANI												
4	Microbial degradation and sole carbon growth assays	Nai TRAN-DINH												
5	Impact and indicator taxa	Nai TRAN-DINH												
6	Metagenomics	David MIDGLEY												
7	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). Project progress reported on GISERA website to ensure transparency for all stakeholders including regional communities. Participation in roadshows, community workshops and meetings and other engagements where appropriate. Maps and visuals - Key findings communicated with the use of maps and visual cues incorporated.	From commencement of project and with updates as they come to hand. Periodically As required Towards completion
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	2020/21	2021/22	2022/23	Total
Labour	$162,576	$16,826	$0	$179,402
Operating	$49,100	$0	$0	$49,100
Subcontractors	$45,000	$0	$0	$45,000
Total Expenditure	**$256,676**	**$16,826**	**$0**	**$273,502**

Expenditure per Task	2020/21	2021/22	2022/23	Total
Task 1	$33,733	$0	$0	$33,733
Task 2	$35,417	$0	$0	$35,417
Task 3	$32,346	$0	$0	$32,346
Task 4	$105,185	$0	$0	$105,185
Task 5	$10,575	$0	$0	$10,575
Task 6	$21,463	$0	$0	$21,463
Task 7	$17,957	$16,826		$34,783
Total Expenditure	**$256,676**	**$16,826**	**$0**	**$273,502**

Source of Cash Contributions	2020/21	2021/22	2022/23	Total
Federal Government (55.64%)	$142,815	$9,362	$0	$152,177
SA Government (19.36%)	$49,692	$3,258	$0	$52,950
Total Cash Contributions	**$192,507**	**$12,620**	**$0**	**$205,127**

In-Kind Contributions	2020/21	2021/22	2022/23	Total
CSIRO (25%)	$64,169	$4,207	$0	$68,376
Total In-Kind Contributions	**$64,169**	**$4,207**	**$0**	**$68,376**

	Total funding over all years	Percentage of Total Budget
Federal Government Investment	$152,177	55.64%
SA Government Investment	$52,950	19.36%
CSIRO Investment	$68,376	25%
Total Other Investment		
TOTAL	**$273,502**	**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	Aug 20	Sep 20	2020/21	$25,300
Task 2	2.1	Sample collections- soil and water	GISERA	Sep 20	Nov 20	2020/21	$26,563
Task 3	3.1	Baseline microbial community profiling complete and raw data available	GISERA	Nov 20	Feb 21	2020/21	$24,260
Task 4	4.1	Chemical degradation and sole carbon growth assays complete and data prepared for final report	GISERA	Dec 20	Jun 21	2020/21	$78,889
Task 5	5.1	Impact and indicator taxa identified and data prepared for final report	GISERA	Dec 20	Jun 21	2020/21	$7,931
Task 6	6.1	Metagenomes available via a public poral.	GISERA	Feb 21	Jul 21	2021/22	$16,097
Task 7	7.1	Data analysis and reporting	GISERA	Aug 20	Jun 21	2021/22	$26,087

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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
28th September 2020	Due to the boarder closures, planning a field campaign has been difficult and therefore delayed.	All milestones have been pushed back by 2 months, the new project delivery date will be September 2021	

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 Regional 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	Briefing document for sampling campaign	Kaydy PINETOWN	August 2020	November 2020	
Task 2	Sample collections- soil and water	David MIDGLEY	September 2020	January 2021	Task 1
Task 3	Baseline microbial community profiling complete and raw data available	Carla MARIANI	November 2020	April 2021	Task 2
Task 4	Chemical degradation and sole carbon growth assays complete and data prepared for final report	Nai TRAN-DINH	December 2020	August 2021	Task 2
Task 5	Impact and indicator taxa identified and data prepared for final report	Nai TRAN-DINH	December 2020	August 2021	Tasks 1, 2, 3 & 4
Task 6	Metagenomics	David MIDGLEY	February 2021	August 2021	Task 4 (partial)
Task 7	Data analysis and reporting	David MIDGLEY	August 2020	September 2021	All other tasks.

Project Schedule Report

THE FIRST TASK IS NOT DUE FOR DELIVERY UNTIL NOVEMBER 2020