Australia's National
Science Agency

GISERA | Gas Industry Social and Environmental Research Alliance

Project Order

Short Project Title

Microbial communities and their ability to degrade prospective chemicals used in coal seam gas activities

Long Project Title	Microbial degradation of chemicals used in coal seam gas activities and chemical baselining in the Narrabri region, NSW.
GISERA Project Number	W27
Start Date	01 July 2022
End Date	31 December 2023
Project Leader	David Midgley

GISERA State/Territory

- [ ] Queensland
- [x] New South Wales
- [ ] Northern Territory
- [ ] South Australia
- [ ] Western Australia
- [ ] Victoria
- [ ] National scale project

Basin(s)

- [ ] Adavale
- [ ] Amadeus
- [ ] Beetaloo
- [ ] Canning
- [ ] Western Australia
- [ ] Carnarvon
- [ ] Clarence-Morton
- [ ] Cooper
- [ ] Eromanga
- [ ] Galilee
- [ ] Gippsland
- [ ] Gloucester
- [x] Gunnedah
- [ ] Maryborough
- [ ] McArthur
- [ ] North Bowen
- [ ] Otway
- [ ] Perth
- [ ] South Nicholson
- [ ] Surat
- [ ] Other (please specify)

GISERA Research Program

- [x] Water Research
- [ ] Health Research
- [ ] Biodiversity Research
- [ ] Social & Economic Research
- [ ] Greenhouse Gas Research
- [ ] Agricultural Land Management Research
- [ ] Other (please specify)

1. Project Summary

The Narrabri Gas Project (NGP) in northeastern NSW operated by Santos Limited received state and federal government's environmental approval in 2020. The harnessing of unconventional resources such as coal seam gas (CSG), however, has elicited intense public debate about potential impacts to water, especially groundwater quality. In particular, the preservation of clean water for the environment, human consumption, and agricultural uses is of major concern in developing a viable CSG industry in Australia. In providing consent for the development of the NGP, the Independent Planning Commission of NSW[1] identified groundwater management concerns, particularly around the potential cross-contamination of aquifers including migration of groundwater from lower aquifers to the Great Artesian Basin (GAB). Key to addressing this concern and those of the local community is understanding the fate of chemical compounds used in CSG activities in the region if these compounds were to come into contact with the environment. CSIRO's GISERA has developed a growing body of knowledge of microbial degradation of CSG-used chemical compounds and this project will focus on generating new information directly relevant to the Narrabri region for activities of the NGP. Specifically, this project aims to achieve two goals: 1) establish microbial community and chemical baselines in agriculturally important surface and ground waters along with the two major soil types of the Narrabri region (agriculturally important vertosols and the sodosolic soils of the Pilliga Forest); and 2) assess and understand the capacity of microbes of these environments to degrade a range of chemicals likely to be used in CSG activities. Through the application of sensitive state-of-the-art microbial genomic biomonitoring and chemical degradation experiments, we aim to reveal major insights into the microbial diversity of the Narrabri region and their capacity to biodegrade chemicals likely to be used in CSG activities. These outcomes will assist in the assessment of environmental impacts from CSG to the local Narrabri region under development in the NGP.

[1] https://www.ipcn.nsw.gov.au/resources/pac/media/files/pac/projects/2020/03/namrrabri-gas-project/determination/ssd-6456-development-consent.pdf

2. Project description

Introduction

The Narrabri region in northeast, NSW, is the site where a coal seam gas (CSG) project operated by the oil and gas producing company Santos has gained state and federal environmental approval in 2020[2]. However, the harnessing of unconventional resources such as CSG has elicited intense public debate on the apprehensions about potential impacts to water, especially groundwater quality. Of particular concern is the use of a range of chemicals used by CSG companies in drilling, workovers and other activities, including surfactants, biocides, corrosion inhibitors, buffers, friction reducers and viscosity control (e.g., Schinteie et al., 2019 and references therein). Unsurprisingly, 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). In regard to the Narrabri Gas Project (NGP), a chemical risk assessment framework to protect the environment and water resources during CSG operations was assigned to the 2020 environmental approval by the federal government[3].

This project is part of GISERA's efforts to reduce uncertainty around the environmental, social, health, cultural and economic risks associated with CSG activities in the Narrabri local region. In particular, this project aims to investigate some of the risks associated with chemicals likely to be used for the NGP through the following objectives:

1. Establishing microbial community and chemical baselines in aquifer waters and soil samples of sites proximal to prospective coal seam gas activities

Water quality and environmental health can be assessed through various chemical, physical, and biological methods providing various levels of detail. The last few decades have seen biological assessment techniques reach a state where it has been rated more heavily than physicochemical measures in determining the environmental health of water resources (Pawlowski et al., 2018). This outcome has largely been achieved through advances in genomic techniques and their application to cost-effectively and comprehensively assess biological "health" indicators in a timely manner (e.g., Pawlowski et al., 2018). DNA-based counts, in particular, rely on developments in next-generation sequencing which enable the comprehensive "counting" of all organisms in a given environment using environmental DNA (eDNA). Consequently, this allows the identification of all members of various microbial groups present in a particular environment using informative marker genes such as 16S (see Fig. 1). In addition to using fluctuations in microbial communities to measure change, these methods allow the identification of organisms that may be sensitive to particular environmental contaminants.

[2] https://www.ipcn.nsw.gov.au/projects/2020/03/narrabri-gas-project

[3] http://epbcnotices.environment.gov.au/_entity/annotation/b4110561-152e-eb11-82c9-00505684324c/a71d58ad-4cba-48b6-8dab-f3091fc31cd5?t=1606195949773

Indeed, these more sensitive organisms may then be a specific focus of monitoring efforts. This field is generally known as either biomonitoring or metabarcoding and increasingly comprises 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 natural features to be maintained during the development of a CSG industry in NSW. Soil health is defined as positive when soils 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). As with water described above, soil 'quality' can be measured using the same biomonitoring tools. One key difference is that 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 Narrabri region 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. Assessing and understanding the capacity of these microbial communities to degrade a range of chemicals likely to be used in coal seam gas activities in both the major soil types of the region and in relevant aquifer environments

Onshore CSG activities, including exploration and production, use a range of chemicals including surfactants, biocides, corrosion inhibitors, buffers, friction reducers and viscosity control.

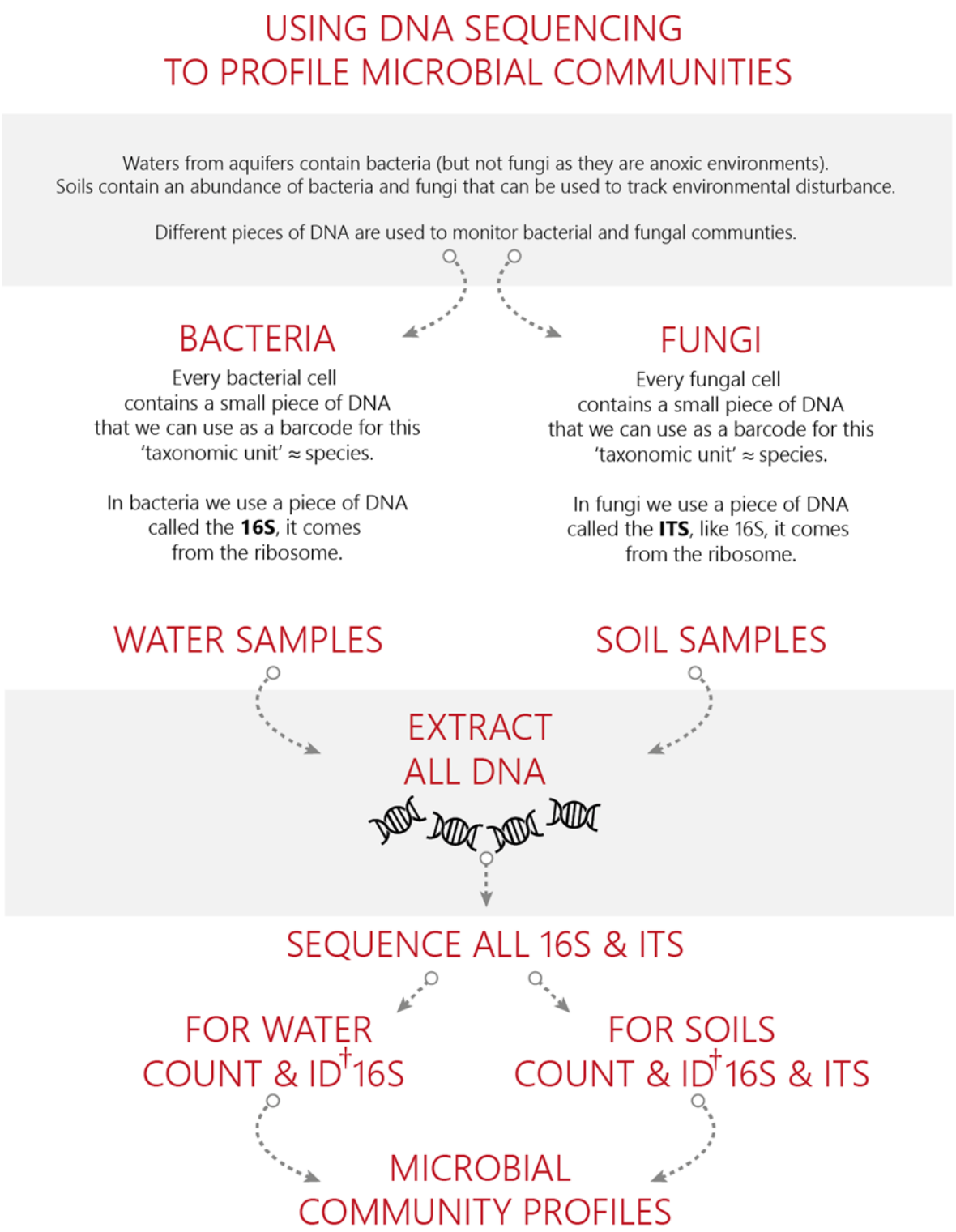

Figure 1: Microbial community profiling using environmental DNA and next-generation DNA sequencing approaches

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

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 Narrabri region. The most abundant soil type in the Narrabri region are clay-rich vertosols in the northern agricultural-dominant area and sodosols in the south, in and around the Pilliga Forest[4] . Since differing soil types have been shown to host markedly different microbial communities (Griffiths et al., 1996; Wieland et al., 2001; Xue et al., 2018), we thus propose to examine each of the two major soil types individually for microbial diversity and conduct experiments to understand their impact on 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. Where possible, chemical biodegradation will be analysed quantitatively with NATA accredited tests.

Prior Research

Currently, to our knowledge, scant microbiological baselining or biomonitoring has been undertaken in the Narrabri region. According to the 'Australian Microbiome' website[5], only limited microbiology studies were conducted at agricultural sites largely to the north of the Pilliga Forest and mostly in intensively cropped areas to the north of Narrabri. Therefore, microbial baselining in the Narrabri region is limited to one small area and is likely not representative of the entire region, particularly of the sodosolic soils to the south of Narrabri.

Several studies exist on groundwater quality and chemistry of the Narrabri area conducted previously by government departments, consultants, and research organisations[6].

A relatively recent hydrogeochemical project (Parsons Brinckerhoff, 2011) indicated that groundwater in the Narrabri Formation is dominated by sodium and chloride, with major ion chemistry in the Gunnedah Formation being spatially variable. This indicates that the aquifer is laterally discontinuous and exhibits zones of differing ion compositions and salinity. The report also observed deteriorating groundwater quality in these formations that make them unsuitable for stock and/or grazing. In addition, a long-term trend of increasing salinity was observed in some Gunnedah Formation bores and attributed to vertical leakage of saline water from the upper aquifer and saline intrusion of pore waters. A similar trend was also observed in one Narrabri Formation and one Cubbaroo Formation bore. Finally, the report pointed to processes influencing the major ion composition of groundwater in this region that include mixing, ion exchange, reverse ion exchange, and dissolution and precipitation of minerals such as carbonates and gypsum (Parsons Brinckerhoff, 2011). Such reports provide useful context, but they generally are not focused around CSG development. As such, the

[4] https://www.bioregionalassessments.gov.au/assessments/11-context-statement-namoi-subregion/1121-physical-geography

[5] https://www.australianmicrobiome.com/

[6] https://www.bioregionalassessments.gov.au/assessments/11-context-statement-namoi-subregion/1142-groundwater-systems

current study seeks to study the waters in agricultural and monitoring bores around the CSG development and north of Narrabri to understand the chemistry of these waters.

Soils in the Narrabri area are typically described as clays, clay loams or sandy loams[7] (e.g., The most abundant soil type in the Narrabri region are clay-rich vertosols in the northern agricultural-dominant area and sodosols in the south where the Pilliga Forest is located. The high clay content vertosol soils are characterised by high water holding capacities when saturated, impeded drainage, hard setting surfaces and restrictive infiltration during heavy rainfall. Among the sodosols of the Pilliga Plateau, by contrast, most of the land is characterised as having severe limitations to agricultural land use. Nevertheless, these lands support a variety of low intensity land uses, such as grazing, forestry and nature conservation[8].

The majority of soil research in microbiomes of the region has focused on the cracking clay vertosols of the region used for agriculture. Comparatively less work has been conducted in the sodosolic soils of the Pilliga area. Data from these previous studies will be collected in Task 2 of this project and will be a benchmark for work done in this study.

Relevant State/Territory Government independent reviews

Independent Review of Coal Seam Gas Activities in New South Wales, NSW Chief Scientist and Engineer (https://www.chiefscientist.nsw.gov.au/independent-reports/coal-seam-gas)

- Outlines findings of a 2013 review that lists numerous technical challenges and risks posed by the CSG industry. It determined that these limitations can generally be managed through a range of implementations including comprehensive environmental monitoring and reporting. This project thus addresses a key goal of this review by providing baseline data for comprehensive environmental monitoring.

Narrabri Gas Project Development Consent, Independent Planning Commission of NSW (https://www.ipcn.nsw.gov.au/resources/pac/media/files/pac/projects/2020/03/narrabri-gas-project/determination/ssd-6456-development-consent.pdf)

- Sets out conditions to prevent, minimise and/or offset adverse environmental impacts, set standards and performance measures of environmental performance that are acceptable, including regular monitoring and reporting requirements and ongoing environmental management. The outcomes of this project contributes data on biodegradation of CSG-related chemicals by microbes indigenous to relevant soils and water from the Narrabri region.

[7] https://www.bioregionalassessments.gov.au/assessments/11-context-statement-namoi-subregion/1121-physical-geography

[8] https://www.bioregionalassessments.gov.au/assessments/11-context-statement-namoi-subregion/1121-physical-geography

Narrabri Gas Project Statement of Reasons, Independent Planning Commission of NSW (https://www.ipcn.nsw.gov.au/resources/pac/media/files/pac/projects/2020/03/narrabri-gas-project/determination/ssd-6456-statement-of-reasons.pdf?la=en&hash=076C39D03F13A74AF7354920214FCC5D)

- Provides a reason from the NSW Government (Independent Planning Commission) for allowing the NGP to be approved. This project thus contributes valuable data on biodiversity and the potential mitigation of environmental impacts resulting from potential spills or contamination events related to CSG activity.

Need & Scope

To help ease public concerns around CSG production and water and soil quality in the Narrabri region, it is important to establish an environmental baseline from which to monitor the environment as the NGP development proceeds.

As described in the Objectives, water quality should be assessed through a variety of measures including the use of biomonitoring. Currently, to our knowledge, only limited microbiological baselining or biomonitoring has been undertaken in the Narrabri region. However, such microbial biomonitoring is key to establishing these indicators prior to the extensive development of the CSG 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 CSG industry in the Narrabri region is unknown. The different soils and aquifers of the region likely host markedly different microbial communities with, potentially, differing capacities for degradation of chemicals prospectively used by industry.

Objective

This project aims to achieve two objectives:

1) Establish microbial community and chemical baselines in aquifer waters and soil samples of sites proximal to prospective coal seam gas activities in Narrabri, NSW, and

2) Assess and understand the capacity of these microbial communities to degrade a range of chemicals likely to be used in coal seam gas activities, in both the major soil types of the region and in relevant aquifer environments. This task is important to provide the community with information about the biodegradation potential of microbes in aquifers and soils in the event of a future spill or leak event.

Methods

During Task 1 project team members will establish the sampling sites for both water and soil samples required through consultation with local stakeholders. Task 1 will review sampling sites to determine the 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. Sampling will take place in October 2022 and March 2023 to provide a more comprehensive understanding of the microbial diversity across time. For these two trips, a total of 96 samples will be collected (24 soil and 24 water samples per trip) for microbial and chemical baselining (Task 4). In addition, four soil and two water samples will be collected in October 2022 for chemical degradation experiments (Task 5).

Task 1 will also determine a representative list of chemicals to be examined in degradation experiments, through consultation with the community, the technical reference group and other relevant stakeholders. This list will be confirmed with the CSG operator of the region (i.e. Santos) to check whether these chemicals are proposed for use in the NGP. In addition, the state regulator NSW Environment Protection Authority (EPA) will be contacted for their input into chemical selection for this study and to confirm concentrations to be examined.

Task 1 will ensure the safe and environmentally sensitive planning, provisioning and logistics for the sampling campaigns.

Task 2 aims to provide a detailed literature review of existing research on the geology, surface water and groundwater chemistry, and microbiology of the Narrabri area. Task 2 will be conducted concurrently with Task 1. Numerous studies of the soil microbiology of the cracking clay vertosols used for cropping in the Narrabri region have been conducted, less, however, is known about the sodosolic soils of the Pilliga area. All data from these studies will be summarized in Task 2. CSIRO Myall Vale will also be contacted to ascertain whether other, as yet unpublished data, are available for the region.

Task 3 will involve staff travelling twice to the Narrabri region (in October 2022 and March 2023) with the purpose of collecting a range of soil and water samples from the sites determined in Task 1. This fieldwork will fulfil the sampling requirements for all downstream activities, including microbial and chemical baselining (Task 4) and chemical biodegradation trials (Task 5). Due to the high diversity and heterogeneity of soil, and to a lesser extent aquifer water, it is important to sample sufficient replicates to strike a balance between statistical rigor and pragmatism.

Task 4 will carry out microbial and chemical baselining activities for the soils and water collected from the Narrabri region. On returning the samples to the laboratory, water and soil samples will be subject to DNA extraction along with 16S rDNA sequencing, and, for the soil samples ITS DNA sequencing.

Task 5 will establish replicated microcosms containing either the soils or aquifer water and will be used to determine the ability of microbes in these environments to degrade a list of CSG-related chemicals (determined in Task 1). Chemical degradation will be determined either through direct measurement of the chemical in the soil or aquifer using analytical chemistry techniques or through microbial growth assays. 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.

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 inform requirements for future research areas and will provide information for a range of stakeholders.

3. Project Inputs

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	>20 years	CSIRO
Richard SCHINTEIE	100 days	Geomicrobiology & geochemistry	>20 years	CSIRO
Tania VERGARA	15 days	Analytical chemistry	>6 years	CSIRO
Nai TRAN-DINH	34 days	Microbial ecology	>20 years	CSIRO
Carla MARIANI	71 days	Microbiology and organic chemistry	>4 years	CSIRO
Kaydy PINETOWN	3 days	GIS and geology	>20 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.	Molecular Research DNA Laboratories, Texas, USA.

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 which may include:

- Santos representative
- NSW Government representatives from:
    - NSW Department of Primary Industry
    - NSW Department of Planning and Environment
    - NSW Environmental Protection Authority
- Expert in hydrogeology

Budget Summary

Source of Cash Contributions	2021/22	2022/23	2023/24	2024/25	2025/26	% of Contribution	Total
GISERA	$0	$383,493	$64,074	$0	$0	80%	$448,567
- Federal Government	$0	$312,400	$52,060	$0	$0	65%	$364,461
- NSW Government	$0	$72,092	$12,014	$0	$0	15%	$84,106
Total Cash Contributions	**$0**	**$384,493**	**$64,074**	**$0**	**$0**	**80.0%**	**$448,567**

Source of In-Kind Contribution	2021/22	2022/23	2023/24	2024/25	2025/26	% of Contribution	Total
CSIRO	$0	$96,123	$16,019	$0	$0	20%	$112,142
Total In-Kind Contribution	**$0**	**$96,123**	**$16,019**	**$0**	**$0**	**20%**	**$112,142**

TOTAL PROJECT BUDGET	2021/22	2022/23	2023/24	2024/25	2025/26	-	TOTAL
All contributions	$0	$480,616	$80,093	$0	$0	-	$560,709
TOTAL PROJECT BUDGET	**$0**	**$480,616**	**$80,093**	**$0**	**$0**	**-**	**$560,709**

4. Communications Plan

Stakeholder	**Objective**	**Channel** (e.g. meetings/media/factsheets)	**Timeframe** (Before, during at completion)
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 and one at the end of the project – these may be updated periodically as project progresses). Project progress reported on GISERA website to ensure transparency for all stakeholders including regional communities. Media release (optional)	From commencement of project and with updates as they come to hand. As required At completion
Government	To report on research being undertaken	Factsheets, newsletters, website or webcast	During
Gas Industry	Industry adopts methods for improving confidence in baseline quality of water and soil through genomic biomonitoring	Presentation of findings at joint Gas Industry/Government Knowledge Transfer Session	At completion
Government	Advice provided to senior bureaucrats / ministers / policy makers	Presentation of findings at joint Gas Industry/Government Knowledge Transfer Session	At completion
Community stakeholders	Presentation of research findings	Presentation of findings through community forums or briefings	At completion
Regional community/wider public, government, scientific community and industry	To report on key findings	Public release of final report	At project completion
Traditional Owner communities	To explore collaboration opportunities for information exchange.	Engagement with representatives of relevant land councils where appropriate to determine interest/availability in making information available to communities	Ongoing
Scientific community	To publish results in international peer-reviewed journals	Manuscript for submission to journals	At completion

5. Project Impact Pathway

Activities	Outputs	Short term Outcomes	Long term outcomes	Impact
Review, logistics, planning and bore and soil selection	Provide a review of existing work being done on the geology, surface water and groundwater chemistry, and microbiology of the Narrabri region. A wide range of literature including publicly accessible reports, scientific journals and web-based resources will be used. Logistics, occupational health and safety, environmental and community concerns, along with detailed sampling procedures. A list of chemicals used by the coal seam gas industry that poses a putative environmental risk in the Narrabri region will be determined through consultation with the Technical Reference Group. A list of available water bores will be compiled. Sites for soil collection will be determined. A briefing document will be prepared for the sampling campaign.	Knowledge on the biodegradation of chemicals that are potentially involved in CSG 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 Narrabri region in which the NGP development is occurring.	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 CSG activities in the Narrabri 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 practices and decision-making to minimise such risks. Increased community awareness of the potential environmental impacts of CSG activities is another long-term outcome of this project.	The impact of this research extends to government, industry and everyday Australians. All Australian communities that are located in CSG 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 CSG activities. The project provides knowledge in the area of both surface and groundwater contamination at several locations in the Narrabri region which will assist those at both the decision-making and policy-making levels of government.
Sampling campaign	Provision of both water and soil samples for the experimental program of this project. Briefing document with details of collection and sample availability will be prepared.			
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.			
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.			

6. Project Plan

Project Schedule

ID	Activities / Task Title	Task Leader	Scheduled Start	Scheduled Finish	Predecessor
Task 1	Logistics, planning, bore and soil selection	Tania VERGARA	July 2022	September 2022	-
Task 2	Literature review	Richard SCHINTEIE	July 2022	August 2022	-
Task 3	Sampling campaign	Richard SCHINTEIE	October 2022	March 2023	Task 1, 2
Task 4	Baselining microbial communities	Richard SCHINTEIE	November 2022	June 2023	Task 1, 2
Task 5	Microbial degradation and sole carbon growth trials	Richard SCHINTEIE	November 2022	June 2023	Task 1, 2
Task 6	Data analysis and project reporting	David MIDGLEY	November 2022	December 2023	Task 1,2,3,4,5
Task 7	Communicate findings to stakeholders	David MIDGLEY	July 2022	December 2023	-

Task description

Task 1: Logistics, planning, bore and soil selection

OVERALL TIMEFRAME: July-September 2022

BACKGROUND: During Task 1 staff will work to establish the sampling sites for soil and water samples required for downstream activities. Chemicals to be tested in subsequent tasks will be determined in Task 1 via consultation with the TRG. Up to 25 chemicals will be tested with priority given to chemicals with the greatest environmental and stakeholder concern. All chemicals will be verified as being planned for use in the Narrabri region by Santos. Task 1 will also include the safe and environmentally sensitive planning, provisioning and logistics for the sampling campaign.

The study proposes to sample up to 48 soil samples and up to 48 surface and ground water samples, taking in agriculturally and ecologically important soils and waters of the region.

TASK OBJECTIVES:

1) Establish water and soil sampling sites within the Narrabri region;
2) Conduct site selection and sampling campaign logistics;
3) Determination of chemicals of relevance for CSG production in the Narrabri region;
4) Preparation of sampling equipment/reagents;
5) Preparation for remote sampling fieldwork; and
6) Identification of any permits, permissions 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: Literature review

OVERALL TIMEFRAME: July 2022-August 2022

BACKGROUND: Task 2 will involve a detailed review of existing work being done on the geology, surface water and groundwater chemistry, and microbiology of the Narrabri region. Furthermore, microbial biodegradation of CSG-related chemicals will be reviewed in light of the relevance to the Narrabri region. A wide range of literature including publicly accessible reports, scientific journals and web-based resources will be used. Task 2 will also help inform Task 1. CSIRO Myall Vale will also be contacted to determine what, if any, unpublished data are available on the microbiology of these soils.

TASK OBJECTIVES:

1) Review previous work as published in reports, journals, and web-based resources; and
2) Use this information to inform Task 1.

TASK OUTPUTS AND SPECIFIC DELIVERABLES: A written document that comprehensively reviews previous work will be prepared.

Task 3: Sampling campaign

OVERALL TIMEFRAME: October 2022 and March 2023

BACKGROUND: Task 3 will involve two staff travelling to the Narrabri region for the purpose of collecting water and soil samples.

TASK OBJECTIVES:

1) Microbially preserved water and soil samples will be collected from sites identified in Task 1 for microbial baselining;
2) Four "live", anoxic water samples will be collected from sites identified in Task 1 for biodegradation experiments;
3) Four large-volume water samples will be collected from sites identified in Task 1 for preparation of media for biodegradation experiments and chemical baselining;
4) Collection of microbially preserved soil samples from each of the two major soil types for microbial baselining; and
5) Large volume soil samples will be collected from sites identified in Task 1 for biodegradation experiments and chemical baselining.

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

Task 4: Baselining chemistry and microbial communities

OVERALL TIMEFRAME: November 2022 – June 2023

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

TASK OBJECTIVES:

1) Complete DNA extractions from all samples;
2) DNA samples prepared and 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 5: Microbial degradation and sole carbon growth trials

OVERALL TIMEFRAME: November 2022 – June 2023

BACKGROUND: This task is designed to provide biodegradation data for chemicals used in onshore gas activities to alleviate community concern in the event of a spill. 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 the CSG industry. Chemicals will be grouped and tested in up to four mock industrial applications (e.g. drilling, workovers, surface facility treatments). These applications, and the chemicals used in them, will be determined through consultation with the TRG. Chemical degradation will be determined via microbial growth assays and community profiling. A small subset of five chemicals will be directly measured using analytical chemistry techniques. These chemicals will be determined through consultation with the TRG to choose those of most concern to the community. In addition, sole carbon growth trials (using all chemicals individually) will be conducted in soil mimicking liquid media and directly in aquifer water samples. Only samples collected in the first sampling collection (October 2022) will be used for setting up biodegradation trials.

TASK OBJECTIVES:

1) Establish replicated microcosms;
2) Spike microcosms with target chemicals at concentrations as consulted with TRG;
3) Incubate at *in situ* 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 four weeks and prepare samples for DNA sequencing and chemical analyses;
5) Harvest all water treatments after twelve weeks and prepare samples for DNA sequencing and chemical analyses;
6) Establish sole carbon source experiments;
7) Incubate at relevant field conditions;
8) Inspect cultures for visual signs of growth and prepare samples for DNA sequencing; 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 6: Data analysis and project reporting

OVERALL TIMEFRAME: July 2022 – December 2023

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 inform requirements for future research 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:

1) Reporting results and analyses from Tasks 3-5;
2) Provide research options for chemicals with high residual risk of environmental impact.

TASK OUTPUTS AND SPECIFIC DELIVERABLES: Final written report encompassing all the tasks outlined above.

Task 7: Communicate project objectives, progress and findings to stakeholders

OVERALL TIMEFRAME: Full duration of project

BACKGROUND: Communications of GISERA research are an important component of outreach and dissemination of findings to diverse audiences.

TASK OBJECTIVES: Communicate project objectives, progress and findings to stakeholders through meetings, knowledge transfer sessions, factsheets and journal articles, in collaboration with GISERA Communications officers.

TASK OUTPUTS AND SPECIFIC DELIVERABLES: Communicate project objectives, progress and results to GISERA stakeholders according to standard GISERA project procedures which may include, but are not limited to:

1) Knowledge Transfer session with Government/Gas Industry

2) Presentation of findings to Community members/groups

3) Preparation of article for GISERA newsletter and other media outlets e.g. The Conversation

4) Revision of project factsheet to include final results (a factsheet is developed at project commencement, and another will be prepared at completion)

5) Peer reviewed scientific manuscript ready for submission to a relevant journal

Project Gantt Chart

		2022/23												2023/24					
Task	**Task Description**	**Jul 22**	**Aug 22**	**Sep 22**	**Oct 22**	**Nov 22**	**Dec 22**	**Jan 23**	**Feb 23**	**Mar 23**	**Apr 23**	**May 23**	**Jun 23**	**Jul 23**	**Aug 23**	**Sep 23**	**Oct 23**	**Nov 23**	**Dec 23**
1	Logistics, planning, bore and soil selection																		
2	Literature review																		
3	Sampling campaign																		
4	Baselining microbial communities																		
5	Microbial degradation and sole carbon growth assays																		
6	Data analysis and project reporting																		
7	Communicate findings to stakeholders																		

7. Budget Summary

Expenditure	2021/22	2022/23	2023/24	2024/25	2025/26	Total
Labour	$0	$281,016	$80,093	$0	$0	$361,109
Operating	$0	$38,600	$0	$0	$0	$38,600
Subcontractors	$0	$161,000	$0	$0	$0	$161,000
Total Expenditure	**$0**	**$480,616**	**$80,093**	**$0**	**$0**	**$560,709**

Expenditure per task	2021/22	2022/23	2023/24	2024/25	2025/26	Total
Task 1	$0	$24,549	$0	$0	$0	$24,549
Task 2	$0	$72,486	$0	$0	$0	$72,486
Task 3	$0	$190,739	$0	$0	$0	$190,739
Task 4	$0	$126,296	$0	$0	$0	$126,296
Task 5	$0	$28,095	$0	$0	$0	$28,095
Task 6	$0	$34,894	$76,435	$0	$0	$111,329
Task 7	$0	$3,557	$3,658	$0	$0	$7,215
Total Expenditure	**$0**	**$480,616**	**$80,093**	**$0**	**$0**	**$560,709**

Source of Cash Contributions	2021/22	2022/23	2023/24	2024/25	2025/26	Total
Federal Govt (65%)	$0	$312,400	$52,060	$0	$0	$364,461
NSW Govt (15%)	$0	$72,092	$12,014	$0	$0	$84,106
Total Cash Contributions	**$0**	**$384,493**	**$64,074**	**$0**	**$0**	**$448,567**

In-Kind Contributions	2021/22	2022/23	2023/24	2024/25	2025/26	Total
CSIRO (20%)	$0	$0	$96,123	$16,019	$0	$112,142
Total In-Kind Contributions	**$0**	**$0**	**$96,123**	**$16,019**	**$0**	**$112,142**

	Total funding over all years	Percentage of Total Budget
Federal Government investment	$364,461	65%
NSW Government investment	$84,106	15 %
CSIRO investment	$112,142	20%
Total Expenditure	**$560,709**	**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	Jul-22	Sep-22	2022/2023	$19,639
Task 2	2.1	Literature review	GISERA	Jul-22	Aug-22	2022/2023	$57,989
Task 3	3.1	Sample collections- soil and water *(1st trip - Oct 2022 / 2nd trip – Mar 2023)*	GISERA	Oct-22	Mar-23	2022/2023	$152,591
Task 4	4.1	Baseline microbial community profiling complete and raw data available	GISERA	Nov-22	Jun-23	2022/2023	$101,037
Task 5	5.1	Chemical degradation and sole carbon growth assays complete and data prepared for final report	GISERA	Nov-22	Jun-23	2022/2023	$22,476
Task 6	6.1	Analysis, integration and interpretation complete and final report delivered to GISERA	GISERA	Nov-22	Dec-23	2023/2024	$89,063
Task 7	7.1	Communicating findings to stakeholder through meetings, seminars, reports/briefings	GISERA	Jul-22	Dec-23	2023/2024	$5,772

8. Intellectual Property and Confidentiality

Background IP (clause 11.1, 11.2)	**Party**	**Description of Background IP**	**Restrictions on use (if any)**	**Value**
				$
				$
Ownership of Non-Derivative IP (clause 12.3)	CSIRO			
Confidentiality of Project Results (clause 15.6)	Project Results are not confidential.			
Additional Commercialisation requirements (clause 13.1)	Not Applicable			
Distribution of Commercialisation Income (clause 13.4)	Not Applicable			
Commercialisation Interest (clause 13.1)	**Party**		**Commercialisation Interest**	
	CSIRO		N/A	

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