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AusME 2019

Australian Microbial Ecology Conference

11 - 13 February 2019

University of Western Australia

The Australian Society for Microbiology
bringing Microbiologists together

THE UNIVERSITY OF WESTERN AUSTRALIA

#AUSME2019

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Welcome

On behalf of the local organising committee and the Australian Society for Microbiology (ASM), it is my pleasure to welcome you to the Australian Microbial Ecology Conference (AusME2019) on the beautiful campus of the University of Western Australia in Perth. AusME is a new, two and a half day, single stream microbial ecology and environmental microbiology conference held for the first time in Melbourne in 2017. Such was the success of AusME in 2017 that it will be a regular fixture on the ASM calendar, running every two years across Australia.

We are proud to present a fantastic scientific program with a range of exciting scientific speakers that includes Students, Early/Mid-Career and Senior Researchers. The conference will present research from across five key thematic areas in environmental microbiology with a range of invited speakers, proffered orals and poster presentations.

I hope you enjoy the conference as much as we have enjoyed putting it together. Take the opportunity to catch up with old friends and new, and while you are here enjoy some great West Australian hospitality.

I would like to thank our sponsors who have greatly enhanced the quality of this meeting with their support - I encourage you to visit their trade exhibits during the conference. And finally, this meeting would not have been possible without the dedication of my colleagues on the local organising committee and I would like to thank them all for their hard work. A special thank you to Linda Blackall as the instigator of the first AusME meeting in Melbourne – her advice has been invaluable and I am sure this conference would not be in such a good her place without her continued support.

I would like to thank the ASM Executive (Dena Lyras, Cheryl Power and Roy Robbins-Brown in particular) and the local WA branch of the ASM (Charlene Kahler in particular) who have provided invaluable support. I would also like to thank ASN Events (especially Jennah Henry) for their work in organising this conference.

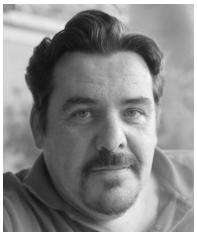
I hope that you enjoy AusME2019 - if you are interested in hosting AusME2021 please come and see me – I promise a rewarding and scientifically enriching experience.

Deirdre B. Gleeson
Local Organising Committee Chair, AusME2019

Speakers

Dr Vanessa Bailey, *Pacific Northwest National Laboratories (US)*
Dr. Bailey's research addresses the role of soil physical structure on microbially-mediated soil C cycling. This research includes a focus on the role played by water as a solvent and transport agent in soils, and uses a suite of molecular chemical and multi-omic approaches to answer questions about the stability and vulnerability of soil carbon. Her research is conducted both in the field, where landscape processes are observed and soils sampled, as well as in the lab where studies occur in controlled microcosms. Research Interests: Microbial community composition and functional potential, Soil microbiology, Carbon cycling, Metagenomics, metabolomics, Terrestrial-aquatic ecosystems

Prof Linda Blackall, *The University of Melbourne*
Linda is an environmental microbial ecologist, who has studied many different complex microbial communities ranging from host associated through to free living in numerous environments. Her research has covered mammalian microbiomes of marsupials, humans, ruminants and horses; and the microbiota of non-mammals including corals and sponges. Environmental microbiomes explored in Linda's research span wastewater treatment (aerobic and anaerobic), solid waste digestion (landfill and composting), bioelectric systems and microbiologically influenced corrosion. The numerous methods she develops and employs in her research allow elucidation of microbial complexity and function in these diverse biomes



Prof Andy Whiteley, *The University of Western Australia*
Winthrop Professor Andy Whiteley is the 10th Australian Premier's Fellow for Western Australia (inaugurated 2012) and Winthrop Professor of Microbial Ecology at UWA. His work has focussed on understanding the hidden microbial diversity beneath our feet. The author of over 100 publications, he has provided groundbreaking research on the function and distribution of microbes in our environment, how they input into food production and are sources and sinks for climate active gases. His research group generated the World's first microbial map of a country, some of the first isotope-molecular ecology direct link analyses and generated the world's first soil sampling Citizen Science program: MicroBlitz. He holds visiting positions at the Chinese Academy of Sciences as a High End Foreign Expert and the Marshall Centre for Infectious Disease Research. His current research works at the interface between large scale survey of microbes and their functions and deploying nationwide Citizen Science projects to empower citizens to determine links between themselves, their environment and their health.

A/Prof Tracy Ainsworth, *UNSW Sydney*
Tracy Ainsworth is an Associate Professor in the School of Biological, Earth and Environmental Sciences (BEES) at The University of New South Wales, Sydney, where she leads research into the impact of environmental change on marine animals. She currently hold a Scientia Fellowship at the University of New South Wales. She has held also held an Australian Research Council Super Science Research Fellowship (2011-2014) and an Australian Research Council Postdoctoral Fellowship (2008-2011). Currently she is focused on the stress biology of corals and this research aims to understand how corals and their microbial symbionts are impacted by a changing environment. Some of her current research projects include: bacterial symbioses of deep water corals, cell death regulation of coral and tissue

regeneration, response of coral to increased sea surface temperatures, conserved bacterial symbioses of coral, bacterial communities of reef fish and microbial ecology in benthic marine communities

A/Prof Treena Burgess, *Murdoch University*
My research field is the biology, ecology and genetics of beneficial and detrimental microorganisms in natural ecosystems, plantation forestry and horticulture, with a focus on biodiversity and bioinvasions. My studies have ranged from use of mycorrhizal fungi to promote tree growth, the global movement of eucalyptus pathogens, role of canker pathogens in stressed ecosystems, the systematics biology and ecology of Phytophthora and the complex biotic interaction in declining ecosystems.

Prof Philip Hugenholtz, *The University of Queensland*
From a PhD in 1994 at the University of Queensland, Phil Hugenholtz developed a career in microbiology and genomics in the USA and in Australia. His last position in the USA was as Staff Scientist (2004-2010) at the DOE Joint Genome Institute. In late 2010 he returned home to establish the Australian Centre for Ecogenomics, now comprising over 50 research and support staff. The Centre conducts culture-independent sequence-based research across a wide range of environmental, engineered and clinical ecosystems underpinned by a genome-based evolutionary framework.

Dr John Moreau, *The University of Melbourne*
As a geomicrobiologist, Dr. Moreau conducts cross-disciplinary research in geochemistry, mineralogy and environmental microbiology on questions that address the impact of microbes on geological materials and processes. His work includes the study of microbial interactions with heavy metals, the evolution of sulfate-reducing bacteria, and the activity of the deep subsurface microbial biosphere. Dr. Moreau employs a range of research approaches involving electron microscopy, advanced chromatography and spectroscopy and genomics. He obtained his Ph.D from the Department of Earth and Planetary Science at the University of California, Berkeley, in 2006, and served as a U.S. National Research Council Postdoctoral Fellow with the U.S. Geological Survey from 2006-2008, prior to taking up his current appointment.

Dr Matthew Stott, *University of Canterbury (NZ)*
Matthew Stott is a lecturer at the School of Biological Sciences, University of Canterbury, in Christchurch New Zealand. His primary research interests focus on the geobiological and ecological interactions of microorganisms and their ecosystems. This work aims to underpin conservation efforts, provide for industrial applications, and to understand the ecosystem services provided by these microbial communities. Much of his research has utilised the extreme geothermal ecosystems in the central north island of New Zealand (Taupō Volcanic Zone), but also includes the subsurface, polluted and mining environments, marine ecosystems and industrial settings. Matthew investigates the taxonomic, genomic and functional diversity of extremophile communities using cross-disciplinary approaches. These include the cultivation and characterisation of novel microbial strains (His research group described the first representatives of a new class, and an order, as well several genera and species), molecular community and geochemical surveys (e.g. the 1000 Springs Project: 1000Springs.org.nz), and the genomic and physiological analyses of isolates and consortium (particularly methanotrophs and atmospheric trace gas scavengers) to understand growth and survival mechanisms within these challenging environments.

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Curtin University

Delegate Information

REGISTRATION DESK – ASN EVENTS

The registration desk is located in the foyer of the Wesfarmers Lecture Theatre. Any enquiries regarding your participation in the AusME 2019 Conference can be directed to the ASN staff onsite.
The registration desk opening hours are:
Monday 11 February: 7.30AM – 5:15PM
Tuesday 12 June: 7:30AM – 5:15PM
Wednesday 13 February: 7:30AM – 1:30PM

SOCIAL PROGRAM

AusME Poster session
Location: Foyer Wesfarmers Lecture Theatre
Date: Monday 11 February 2019
Time: 5:15PM - 6:45PM
Cost: Included in registration
Includes: Grazing table and 1 drink token

Cocktail Event
Location: Bayside Kitchen, 5 Hackett Drv, Crawley
Date: Tuesday 12 February
Time: 7:00PM – 10:00PM
Cost: $20
Includes: Roving canapé dinner & 1 drink token

SPEAKER PREPERATION DETAILS

Presenters are requested to bring their presentation, prepared at 16:9 resolution, on a USB to load directly into the Westfarmers Lecture Theatre in the morning of your session (or the break prior). If you are planning to present using your own computer, please bring your own adapter and also bring your presentation on a USB just in case. There will be a mouse pointer available at the lectern if required. The rooms are fitted with a lectern, microphone and there will be a laser pointer available at the lectern if required.

DISPLAYING YOUR POSTER

Please put your poster up when you arrive and take it down before you leave. Your abstract number will be available onsite at the conference and in the Poster Listing on page 13. Poster boards will be numbered and Velcro will be provided for mounting your poster.

Poster Session
Date: Monday 11 February 2019
Time: 5:15PM - 6:45PM
Location: Foyer

INTERNET ACCESS

Please connect to the internet via EduRoam using the standard username and password credentials you use at your home institution for wireless network access. EduRoam is a secure global roaming wireless network for the research and education sector. For specific instructions on how to connect, please visit the website https://www.eduroam.edu.au

WEB BASED APP

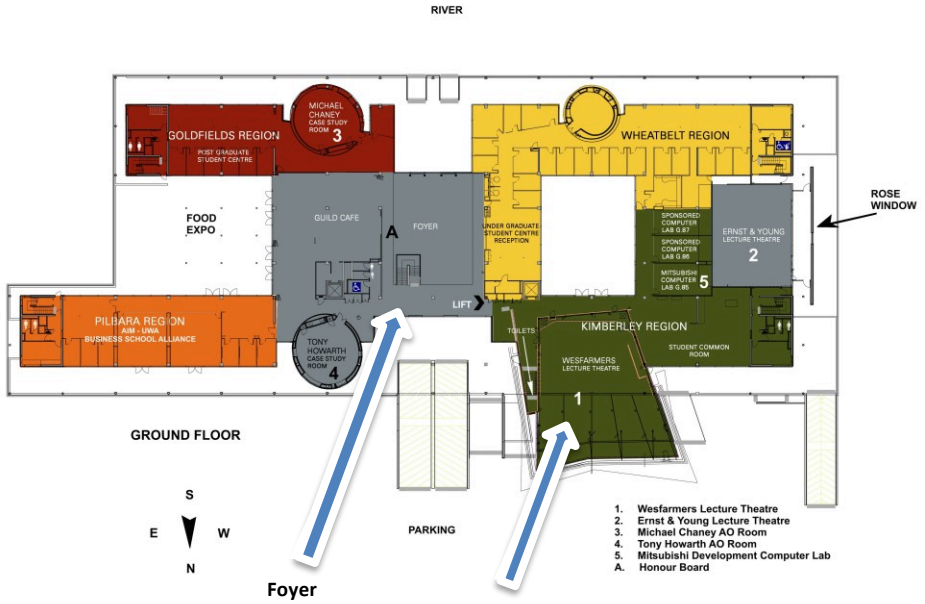
The web based App will keep you organised during the meeting and allow you to view speaker abstracts & bios (if supplied), an up-to-date daily program, save your favourite sessions, conference sponsors and take & save notes on your profile. You can update your profile information too!

How to download the App

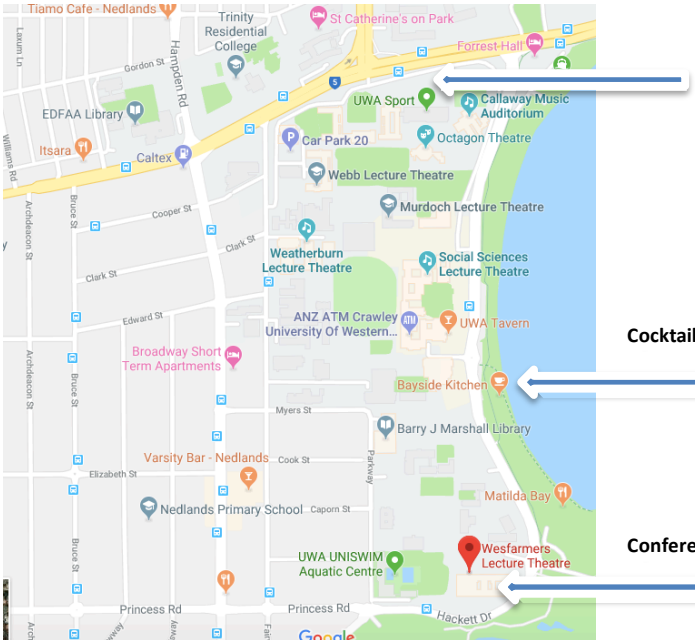
Step 1 Copy http://ausme-2019.m.asnevents.com.au to your browser

Step 2 To install: **Tap the 'share' button in the menu bar**  **Tap the 'Add to home screen' icon.**
This web app icon will now appear on your device homescreen for ease of reference. For further benefits and instructions for Android devices please see please see the ASN staff at the registration desk.

VENUE MAP



UWA CAMPUS MAP



**Please note that it is approx 20 min walk from the main bus stop on Stirling Highway down to the Business School*

Cocktail Event Venue

Conference Venue

Program

Monday 11 February 2019

Registration
7:30AM - 5:15PM Foyer

Acknowledgement of Country and Welcome
9:00AM - 9:30AM Wesfarmers Lecture Theatre
Housekeeping, Dr Deirdre Gleeson
Conference Opening, Prof Tony O'Donnell

Plenary 1 - Through the eye of the needle: Microbes, carbon, water, and their connection through soil pores
9:30AM - 10:15AM Wesfarmers Lecture Theatre
Chair: Deirdre Gleeson

9:30 AM **Vanessa Bailey**
Through the eye of the needle: Microbes, carbon, water, and their connection through soil pores *abs# 1*

Morning Tea
10:15AM - 10:45AM Foyer

Exploring the microbial ecology and diversity of geothermally-heated ecosystems of New Zealand
10:45AM - 11:15AM Wesfarmers Lecture Theatre
Chair: Talitha Santini

10:45AM **Matthew Stott**
Exploring the microbial ecology and diversity of geothermally-heated ecosystems of New Zealand *abs#2*

Terrestrial Systems
11:15AM - 1:15PM Wesfarmers Lecture Theatre
Chairs: Matthew Stott & Vanessa Bailey

11:15 AM **Jen A Middleton**
Spatial zoning of riparian microbial communities in contrasting land uses *abs# 3*
11:30 AM **Angela M Chilton**
Australian biocrusts in arid land rehabilitation *abs# 4*
11:45 AM **Eleonora Chiri**
Termite mounds are biofilters for termite-produced methane and hydrogen *abs# 5*
12:00 PM **Melissa A. Danks**
Microbial assemblages of post-mining soils on Christmas Island: beneficial microbes for agricultural production *abs# 6*
12:15 PM **Gupta Vadakattu**
Biogeography of actinobacteria in Australian and North Antarctica soils *abs# 7*
12:30 PM **Eleonora Egidi**
Identity, distribution and ecology of dominant fungi in global soils *abs# 8*
12:45 PM **Zahra F Islam**
Two Chloroflexi classes independently evolved the ability to persist on atmospheric carbon monoxide and hydrogen *abs# 9*

1:00 PM **Erinne Stirling**
Soil microbial influences on acid sulfate soils of the River Murray: a review and field study inform wetland management *abs# 10*

Lunch
1:15PM - 2:15PM Foyer

Genome-resolved metagenomics of an autotrophic thiocyanate-degrading microbiome enriched from mine tailings
2:15PM - 2:45PM Wesfarmers Lecture Theatre
Chair: Elizabeth Watkin

2:15 PM **John W Moreau**
Genome-resolved metagenomics of an autotrophic thiocyanate-degrading microbiome enriched from mine tailings *abs# 11*

Engineered Systems
2:45PM - 3:30PM Wesfarmers Lecture Theatre
Chairs: John Moreau & Elizabeth Watkin

2:45 PM **Katelyn Boase**
Acid Saline Lakes as a Source of New Biomining Organisms *abs# 12*
3:00 PM **Farhad Shafiei**
Environmental and microbiome-driven factors influencing the efficiency of thiocyanate bioremediation *abs# 13*
3:15 PM **Emma J Gagen**
Microbial iron cycling in the field and harnessed for a pilot-scale attempt at iron duricrust re-formation *abs# 14*

Afternoon Tea
3:30PM - 4:00PM Foyer

Engineered Systems cont.
4:00PM - 5:15PM Wesfarmers Lecture Theatre
Chairs: John Moreau & Elizabeth Watkin

4:00 PM **Karen Gibb**
Whole-community sequencing to identify new indicator bacteria for a tropical waste stabilisation pond *abs# 15*
4:15 PM **Robert Speight**
A snapshot of diversity and function of microbes from a sugarcane bagasse pile *abs# 16*
4:30 PM **Wajid Javed**
Archaea community composition of an acidic mine Pit lake in the semi-arid tropics. *abs# 17*
4:45 PM **Casey K Huang**
Biofilm Ecology in the Premise Plumbing of Drinking Water Distribution Systems *abs# 18*
5:00 PM **Xabier Vázquez-Campos**
Unusual DPANN Archaea from a radioactive legacy site *abs# 19*

Poster Session
5:15PM - 6:45PM Foyer

Tuesday 12 February 2019

Registration
7:30AM - 5:15PM Foyer

Housekeeping
9:15AM - 9:30AM Wesfarmers Lecture Theatre

Plenary 2 - Linda Blackall
9:30AM - 10:15AM Wesfarmers Lecture Theatre
Chair: Andrea Paparini

9:30 AM **Linda Blackall**
The Evolution of Molecular Methods in Microbial Ecology *abs# 20*

Morning Tea
10:15AM - 10:45AM Foyer

What can the microbiome tell us about the future for marine ecosystems?
10:45AM - 11:15AM Wesfarmers Lecture Theatre
Chair: Linda Blackall

10:45 AM **Tracy Ainsworth**
What can the microbiome tell us about the future for marine ecosystems? *abs# 21*

Aquatic Systems
11:15AM - 1:15PM Wesfarmers Lecture Theatre
Chairs: Tracy Ainsworth & Linda Blackall

1:15 AM **Matthew Campbell**
Diversity and function of microorganisms in microbial mat communities from Shark Bay, Western Australia *abs# 22*

11:30 AM **YaJou Chen**
Fermentative and respiratory bacteria are uncoupled in anoxic permeable sediments *abs# 23*

11:45 AM **Marco JL Coolen**
Holocene paleodepositional changes reflected in the sedimentary microbiome of the Black Sea *abs# 24*

12:00 PM **Juliana Mendes Monteiro**
The microbiome of lithifying and non-lithifying microbial mats from hypersaline lakes at Rottnest Island (Western Australia) *abs# 25*

12:15 PM **Matthew W Fraser**
Assessing the contribution of sediment microbes in mediating sulfide intrusion in seagrass ecosystems *abs# 26*

12:30 PM **Lisa R. Moore**
Unicellular cyanobacteria and eukaryotic phytoplankton communities around Australia's oceanic regions *abs# 27*

12:45 PM **Douglas Brumley**
Microbial navigation in marine systems *abs# 28*

1:00 PM **Melanie C Bruckberger**
Investigation of microbial communities in the 1st Gulf war affected, crude oil-contaminated groundwater in Kuwait *abs# 29*

Lunch
1:15PM - 2:15PM Foyer

Interactions among anthropogenic disturbance, an endemic disease and communities of mycorrhizal fungi

2:15PM - 2:45PM Wesfarmers Lecture Theatre

Chair: Anna Hopkins

2:15 PM **Treena Burgess**
Interactions among anthropogenic disturbance, an endemic disease and communities of mycorrhizal fungi *abs# 30*

Symbiotic and Microbial Interactions

2:45PM - 3:30PM Wesfarmers Lecture Theatre

Chairs: Treena Burgess & Anna Hopkins

2:45 PM **Chathurika Daulagala**
Inter-strain interactions of *Rhizobium leguminosarum* influence field pea N nutrition *abs# 31*

3:00 PM **Marnie L Freckelton**
Understanding settlement: the role of common bacterial biofilm elements in the induction and metamorphosis of the polychaete *Hydroides elegans abs# 32*

3:15 PM **Belinda C Martin**
Seagrass rhizosphere; understanding the bottom to manage the top *abs# 33*

Afternoon Tea

3:30PM - 4:00PM Foyer

Symbiotic and Microbial Interactions cont.

4:00PM - 5:15PM Wesfarmers Lecture Theatre

Chairs: Treena Burgess & Anna Hopkins

4:00 PM **Jeff Powell**
Drivers of mycorrhizal fungi and root pathogens in threatened woodlands of the Cumberland Plain *abs# 34*

4:15 PM **Ghislaine Platell**
Intraspecific variation in the gut communities of termites highlights their plasticity and potential for manipulation *abs# 35*

4:30 PM **Rachel J Standish**
Mycorrhizal fungi benefit ecosystem restoration, but how much depends on plant functional type, restoration context and time *abs# 36*

4:45 PM **Sasha Tetu**
Genome-wide screening for genetic factors important in microbe-plant and microbe-microbe ecological interactions *abs# 37*

5:00 PM **Jed Calvert**
Effects of seasonal drought and host identity on endophytic fungal communities in forests of Cape York, northern Australia *abs# 38*

Cocktail Event

7:00PM - 10:00PM Bayside Kitchen

Wednesday 13 February 2019

Registration
7:30AM - 1:30PM Foyer

Housekeeping
9:15AM - 9:30AM Wesfarmers Lecture Theatre

Plenary 3 - Large scale microbial ecology using citizen scientists: Lessons from MicroBlitz
9:30AM - 10:15AM Wesfarmers Lecture Theatre
Chair: Naomi Boxall

9:30 AM **Andrew Whiteley**
Large Scale Microbial Ecology Using Citizen Scientists: Lessons from MicroBlitz *abs# 39*

Morning Tea
10:15AM - 10:45AM Foyer

Single cell viral tagging
10:45AM - 11:15AM Wesfarmers Lecture Theatre
Chair: Andrew Whiteley

10:45 AM **Philip Hugenholtz**
Single cell viral tagging *abs# 40*

Microbial Toolbox
11:15AM - 1:15PM Wesfarmers Lecture Theatre
Chairs: Andrew Whiteley & Philip Hugenholtz

11:15 AM **Sean K Bay**
Soil microbial communities exhibit strong biogeographical patterns at fine taxonomic resolution *abs# 41*
11:30 AM **Jeremy Bougoure**
NanoSIMS for the microbial ecologist *abs# 42*
11:45 AM **Thomas C Jeffries**
Finding keystone taxa in microbial networks *abs# 43*
12:00 PM **Deepa Ruth Varkey**
Dynamic ocean provinces: An outlook for machine learning approaches in microbial ecology *abs# 44*
12:15 PM **Angus Keillar**
Resolving proteomic responses to supercritical carbon dioxide exposure in *Geobacter sulfurreducens abs# 45*
12:30 PM **Kim-Anh Le Cao**
Multivariate microbiome data analysis *abs# 46*
12:45 PM **Christian Rinke**
A proposal for a standardized archaeal taxonomy *abs# 47*
1:00 PM **Ben J Woodcroft**
Community profiling in the age of genomes *abs# 48*

Conference Closing
1:15PM - 1:30PM Wesfarmers Lecture Theatre

Lunch and depart
1:30PM - 2:30PM Foyer

Poster Listing

Microbial toolbox

Symbiotic & Microbial Intera

Aquatic

Engineered

Terrestrial

Abstracts

ORAL ABSTRACTS

1

Through the eye of the needle: Microbes, carbon, water, and their connection through soil pores

Vanessa Bailey[1]

1. Pacific Northwest National Laboratory, Washington, United States

Microbes are critical agents of an enormous number of soil processes, perhaps most importantly, those associated with carbon cycling, greenhouse gas emissions, and global climate feedbacks. Yet the soil system is a physically and chemically complex system within which microbial communities function. Soil structure, soil water, and soil microbiology interlink to regulate the soil carbon cycle. Though it is challenging, these features must be studied together to fully understand how soils function. The physical structure of soil – aggregation and the soil pore network – comprise both the flow paths for resource transport in the aqueous phase and the habitat for soil microbes. The soil water is both the connecting liquid for solute transport and the reagent within which biogeochemical transformations occur. The microbial community both responds to the local environment, decomposing soil organic carbon (SOC) and helps shape the local environment by promoting and sustaining aggregation. To study any of these elements alone is difficult, yet to truly understand soils, we must understand them together.

Our lab builds on a rich legacy of research from scientists past and present to develop an integrated understanding microbial cycling of soil carbon. We combine advanced techniques for molecular characterization of SOC with tomography and sequencing to reveal where in the soil matrix SOC persists, and in what forms. We have imposed treatments to differentiate between physically protected SOC and chemically recalcitrant SOC, and found little evidence for chemical recalcitrance as a C protection mechanisms. We have imposed extreme water cycles, from drought through flood, and found that moisture history matters a great deal to the forms of C in soil, where they are located, and how they contribute to CO2 emitted through heterotrophic respiration. Yet, we find these patterns are expressed differently in different soils and we hypothesize that water and soil structure may explain some of these differences. The complex soil system is difficult to study, but the feedbacks between SOC biogeochemistry and climate require that we meet this challenge.

2

Exploring the microbial ecology and diversity of geothermally-heated ecosystems of New Zealand

Matthew Stott[1, 2]

1. Wairakei Research Centre, GNS Science, Taupō, NZ

2. School of Biological Sciences, University of Canterbury, Christchurch, NZ

Geothermal-influenced ecosystems such as those found in the Taupō Volcanic Zone (TVZ) in New Zealand's central North Island offer challenging physical and chemical conditions for microbial life to grow and/or to persist. The TVZ includes more than 18 volcanic systems across an area of 8000 km^2. These geothermally-influenced soils and aqueous features range from 14°C through to 100 °C and have pH's from less than 0 up to in excess of pH 9. These ecosystems also include substantial concentrations of heavy metals and toxic gases.

In this talk I will describe two unique extremophilic strains isolated and characterised from geothermal soils in the TVZ. The first strain, *Chthonomonas calidirosea*, is a thermophilic bacterial heterotroph that appears to have an oligotrophic scavenger phenotype. When first isolated, *C. calidirosea* was the first cultivated representative of the then candidate phylum OP10; now known as phylum *Armatimonadete*s. The second strain, *Methylacidiphilum infernorum* (phylum *Verrucomicrobia*), is an extremely acidophilic and thermophilic methanotroph and was one of the initial cohort of non-proteobacterial methanotrophs to be described. It was also the first non-neutrophilic methanotroph to be characterised and is currently the most thermophilic methanotroph in culture. In both cases, I will describe our strategy for enriching and isolating these

two bacterial strains along with the results from our phenotypic and genomic investigations that provide insight into the ecological roles these strains play within their host ecosystems. Finally, I will also briefly describe a new project we have just completed (The 1000 Springs Project; 1000Springs.org.nz) which aims to determine the ecological and physicochemical parameters that drive microbial community assembly within geothermal ecosystems.

3

Spatial zoning of riparian microbial communities in contrasting land uses

Jen A Middleton[1], Laura L de Sosa[2], Belinda C Martin[1], Davey L Jones[3], Deidre Gleeson[3]

1. School of Biological Sciences, The University of Western Australia, Perth, WA, Australia

2. School of Environment, Natural Resources & Geography, Bangor University, Bangor, United Kingdom

3. School of Agriculture and Environment, The University of Western Australia, Perth, WA, Australia

Anthropogenic activities have significantly altered global biogeochemical nitrogen (N) cycling leading to major environmental problems including freshwater eutrophication. Soils in the riparian zone, the interface between terrestrial and aquatic ecosystems, may decrease N loads to streams through plant uptake and microbial transformations. However, the ecological functioning of riparian zones are often compromised due to degraded physical and biological conditions (e.g. vegetation clearing, invasive species, and nutrient pollution). Restoration to maximise N retention in riparian zones requires a mechanistic understanding of the processes which underpin the microbial N cycle, particularly the spatial distribution of N and the structure and activity of key N-cycling microbes. Our aim was to assess the spatial organisation of soil microbial communities in riparian zones of contrasting land use (agricultural versus native vegetation ecosystems) using 16S sequencing accompanied with qPCR of archaeal and bacterial nitrogen cycling functional genes (AOA, AOB, nirK, nirS, nosZ). Riparian soil was sampled at 0m (parafluvial zone), 1m, 2m, 5m and 10m distance from one stream within each land use with four latitudinal transects each. Riparian vegetation cover was characterised to measure localised disturbance. Soil physiochemistry (TOC, NH_4+, $N0_3$-), N functional genes and microbial community composition differed between land uses and by distance from the stream. All N functional genes were more abundant at the native site, particularly in the parafluvial zone, likely owing to greater diversity of vegetation habitats and soil physiochemistry compared to the agricultural site. The abundance of nirS and nosZ, key enzymes in the soil N denitrification pathyway, were highest in the native ecosystem parafluvial zone. AOA, nirS and nosZ all increased towards the stream at both sites; indicative of an increase in microbial activity in areas with greater organic deposition and fluctuating anoxic/oxic conditions (due to intermittent inundation). There was no latitudinal pattern for AOB or nirK. These findings highlight the importance of parafluvial soils as catalysts for N processing, especially in stream reaches dominated by native vegetation. Spatial partitioning of N fractions and microbial communities in riparian zones should be accounted for when planning restoration activities.

4

Australian biocrusts in arid land rehabilitation

Angela M Chilton[1], Miriam Munoz-Rojas[3, 4, 2], Brett A Neilan[5, 1]

1. School of Biotechnology and Biomolecular Sciences, University of New South Wales, Sydney, NSW, Australia

2. School of Biological Sciences, The University of Western Australia, Crawley, WA, Australia

3. Kings Park and Botanic Garden, Kings Park, WA, Australia

4. School of Biological, Earth and Environmental Sciences, University of New South Wales, Randwick, NSW, Australia

5. School of Environmental and Life Sciences, University of Newcastle, Callaghan, NSW, Australia

Arid lands encompass over 70% of Australia's mainland and are set to increase and change under predicted climate models. In addition to being biodiversity hotspots, they sustain a range of key industries including agriculture, mining, and tourism. As demand for the resources of arid lands grows, there is inevitably a clash between maintaining ecological productivity and land degradation through continued economic use. Within this context, we seek to understand the natural histories of biocrusts - topsoil assemblages of microorganisms, mosses and lichens – and their potential as agents for arid land rehabilitation.

Using next-generation sequencing, we have profiled biocrust microbiomes at local and intra-continental scales across Australia. Our datasets illustrate the natural status of biocrusts and help establish informed targets to assess and monitor topsoil recovery. The role of cyanobacteria in the formation and maintenance of biocrusts was highlighted. Seasonality of precipitation was identified as a key factor affecting biocrust assembly on an intra-continental scale, indicating biocrust restoration will rely on employing locally-adapted, endemic strains. In addition, we have isolated key biocrust cyanobacteria species and conducted novel microcosm experiments examining the effect of Microcoleus sp. and Nostoc sp. on seedling establishment. We performed bio-priming of seeds with the indigenous cyanobacteria and showed this had significant positive effects on the germination rates of Acacia hilliana and Senna notabilis, two native species used in restoration. Our work highlights the importance of biocrusts in drylands and is developing practical approaches for their integration with current rehabilitation strategies to enhance ecological outcomes.

5

Termite mounds are biofilters for termite-produced methane and hydrogen

Eleonora Chiri[1], Philipp A Nauer[1, 2], Chris Greening[1], Stefan K Arndt[2]

1. School of Biological Sciences, Monash University, Clayton, VIC, Australia

2. School of Ecosystem and Forest Sciences, The University of Melbourne, Burnley, VIC, Australia

It is well-established that hydrogen (H_2) and methane (CH_4) are produced within the anoxic environment of termite hindguts as a result of microbial lignocellulose digestion and methanogenesis. Termites have been found to emit these gases at vastly different rates, depending on feeding groups and species. In contrast, surprisingly little is known about H_2 and CH_4 turnover from the prospective of the oxic environment of mounds and nests that termites live in. Here we present initial results of a comprehensive study on H_2 and CH_4 turnover in termite mounds of Northern Australia. We employed a suite of field- and laboratory-based techniques to quantify H_2 and CH_4 oxidation and identify the responsible microbial communities in mounds of two termite species with different mound architectures and representing the dominant feeding habits. Mounds appeared to be a sink for atmospheric H_2 and a source for CH_4. However, CH_4 emissions were mitigated by microbial CH_4 oxidation. Remarkably, both methanotrophic and hydrogenotrophic communities were able to utilize a vast range of substrate concentration, spanning from the percent range to sub-atmospheric (part per million). While bacterial communities appeared to be evenly distributed among different mound locations (core and periphery), the methanotrophic community was concentrated in the core and differed according to the mound-dwelling termite species. The hydrogenotrophic communities appeared to be highly active, with varying activity according to mound locations. In conclusion, our results suggest that mound-associated microbial communities mitigate emissions of the greenhouse gas CH_4 and influence atmospheric H_2 cycling.

6

Microbial assemblages of post-mining soils on Christmas Island: beneficial microbes for agricultural production

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Agricultural production in post-mining environments is becoming increasingly important globally as many regions are challenged with food security and post-mining land use legacies. Although there are

many advantages in pursuing agriculture at post-mining sites, these substrates have abiotic and biotic challenges for plant growth, including poor fertility, heavy metals, and a lack of beneficial soil microbes. To address the paucity of knowledge about endemic microbes post-phosphate mining on Christmas Island, we investigated the microbial assemblages of various post-mining substrates. Soil samples were collected from seven sites across the island, ranging from kiln-treated mine waste to post-mine soil already under agricultural production. Based on 16S and ITS gene sequence analysis, we identified microbial taxa, such as mycorrhizal fungi and rhizobial bacteria, that are likely to benefit agricultural production in these soils. These results will inform existing agricultural trials and provide insight into the most suitable post-mining environments for successful agriculture on the island. Our work also has important implications for other sites transitioning from mining to agriculture.

7

Biogeography of actinobacteria in Australian and North Antarctica soils

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Actinobacteria, Gram+ bacteria with DNA containing a high G+C percentage, represent one of the most abundant taxon identified in the soils across a diverse range of ecological regions worldwide. Actinobcteria area sought after due to their ability to produce a variety of bioactive metabolites and antibiotics, which has been extensively used to improve human and plant health. The abundance and distribution of soil actinobacteria is considered to be distinct from other microflora due to their size, ability to withstand extreme environments and dispersion abilities. Using the 'Biomes of Australian Soil Environments' (BASE) project generated database for bacterial (16S) diversity across environmental gradients at Australian continental scale, we developed a continental portrait of Australian Actinobacterial communities. A total of 20,931,120 high-quality reads were analysed and 197 different taxonomical groups (assigned at genus level) were classified as Actinobacteria. At the OTU level, 6,887 OTUs were found in the studied samples; 24 OTUs (0.003%) showed more than 100,000 reads each (frequent OTUs), while 4,624 OTUs (67%) showed less than 1,000 reads each (rare OTUs). The most common Actinobacteria families were Actinosynnemataceae, AK1AB1_02E, Frankiaceae, Gaiellaceae and Rubrobacteraceae. Extreme environments (cold - North Antarctica; warm – warm deserts) showed higher numbers of 'endemic' OTUs belonging to the taxa *Euzebya, Nocardioides*, *Gaiella, Mycobacterium, Nocardioides, Pseudonocardia, Robrobacter* and *Streptomyces*. The most pronounced effects on the composition of Actinobacteria at family and genus levels (either in presence or absence of Antarctica data) were associated with pH, climate (humidity and annual temperatures) and Ca availability. Additionally, at the OTU level, a second level of association was found for region, Koppen climate classification, land use, vegetation and soil classification. Although Land use effect was seen at regional and local level, the effects were not observed at continental scale. The high similarity between a group of OTUs found in Antarctica and King Island could be attributed to geogenic factors and the ability of Actinobacteria to persist in the soils for long periods.

8

Identity, distribution and ecology of dominant fungi in global soils

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Soil fungi are among the most diverse and dominant organisms on planet Earth. They play essential roles in key ecosystem functions, and influence diversity and distribution of plants and animals via symbioses and pathogenicity. Yet, unlike their above-ground counterparts, we know little about the identity, distribution and ecological drivers of dominant soil fungi worldwide. In this work, we surveyed 235 sites collected across 18 countries, covering nine biomes (temperate, tropical and dry forests, cold, temperate, tropical and arid grasslands, shrubland, boreal) of the globe to investigate identity, occurrence and ecology of dominant fungal phylotypes in soil. We used a suite of statistical approaches to characterise the distribution and habitat preferences of dominant fungi in relation to edaphic and climatic variables, and mapped their expected geographical distribution and abundance. Additionally, we used whole-genome comparisons to assess the importance of functional attributes in explaining the observed patterns of fungal dominance. Our results provide novel evidence that less than one hundred (< 1% of the retrieved fungi) fungal taxa from a single phylum, Ascomycota, are dominant in soils across the globe, and display a range of environmental preferences, mainly ascribed to differences in climatic conditions. These dominant fungal taxa are characterised by remarkable dispersal abilities, generalist life- styles, and increased genomic potential for resource utilisation, competition and stress tolerance. Our findings suggest that these traits, combined, help explain the success of abundant and ubiquitous fungi in colonising a vast array of ecosystems. This study represents a step-change advancement in our understanding of the patterns and mechanisms driving dominant soil fungal communities in natural ecosystems worldwide. By building a novel, fundamental understanding of distribution, ecological attributes, and patterns of dominance of soil fungi on a global scale, this work opens new leads to develop approaches and strategies aiming at preserving soil fungal diversity and ecosystem functions fungi carry out.

9

Two Chloroflexi classes independently evolved the ability to persist on atmospheric carbon monoxide and hydrogen

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Most bacteria within aerated environments exist within a variety of dormant forms. In these states, bacteria adapt to adverse environmental conditions such as organic carbon starvation by reducing metabolic expenditure and potentially using alternative energy sources. In this study, we investigated the energy sources that could sustain persistence of the environmentally widespread bacterial phylum Chloroflexi. A transcriptome study revealed that *Thermomicrobium roseum* (class Chloroflexia) extensively remodels its respiratory chain after entering stationary phase due to organic carbon limitation. Whereas primary dehydrogenases associated with heterotrophic respiration were downregulated, putative operons encoding enzymes involved in molecular hydrogen (H_2), carbon monoxide (CO), and sulfur compound oxidation were highly expressed. We validated by gas chromatography and microsensor experiments that *T. roseum* oxidizes H_2 and CO at a range of concentrations, including to sub-atmospheric levels, through an aerobic respiratory chain-dependent manner. Phylogenetic analyses suggests that the enzymes mediating these processes, namely group 1h [NiFe]-hydrogenases and type I carbon monoxide dehydrogenases, are widely distributed in Chloroflexi and were acquired on at least two separate occasions through horizontal gene transfer events. In support of this, we confirmed that the sporulating isolate *Thermogemmatispora sp. T81* (class Ktedonobacteria) also scavenges H_2 and CO during persistence. This study provides the first pure culture evidence that atmospheric carbon monoxide supports persistence of microorganisms. In addition, it reports the third phylum capable of mediating the biogeochemically and ecologically important process of atmospheric H_2 oxidation. This adds to growing evidence that trace gases serve as dependable energy sources for dormant microorganisms.

10

Soil microbial influences on acid sulfate soils of the River Murray: a review and field study inform wetland management

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Riverine ecosystems are increasingly threatened by global changes that include drought, extreme weather events, and anthropogenic alterations to natural flows. The health of Australia's longest and most important river system, the Murray-Darling, is dependent on the soils, microorganisms, and plant communities that underpin it. In submerged soils where iron (from soil minerals), organic material and sulfate (from groundwater salts) are available, sulfate reducing microorganisms produce sulfidic materials. These sulfidic materials are typically pyrite (FeS_2); the accumulation of these materials leads to the formation of acid sulfate soils. If these materials are subsequently oxidised (e.g. due to draining), iron oxidising microorganisms enhance the production of sulfuric acid, which causes soil pH to decrease to <4. These are termed acid sulfate soils with sulfuric materials. The development of strongly acidic soil in altered landscapes reduces wetland productivity and water quality; it is a highly undesirable outcome. This project investigates the possible outcomes of wetland management (wetting and drying) on soil microbial diversity and community structure in acid sulfate soils. This was achieved via an extensive literature review, biochemical analyses of two wetland complexes, and DNA diversity analyses via Illumina Sequencing of the 27F-519R amplicon (16S; n = 24). Soil DNA analyses were conducted on freshly acquired and oxidised (8-12 weeks) samples from the Muthro Park and Spectacle Lakes wetland complexes to determine microbial diversity shifts caused by oxidation. The research has highlighted substantial knowledge gaps ranging from the ecologies of iron and sulfur cycling microorganisms through to best practice options for preventing sulfide accumulation and catastrophic oxidation of sulfidic materials. The findings from this study will inform management decisions on wetting and drying regimes in Murray-Darling wetland ecosystems.

11

Genome-resolved metagenomics of an autotrophic thiocyanate-degrading microbiome enriched from mine tailings

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Thiocyanate (SCN^-) pollution from mining and coking presents a problem worldwide. Here we experimentally investigated the capacity of an autotrophic microbiome sourced from mine tailings to degrade SCN^-. A bioreactor was inoculated with tailings, incubated autotrophically, and subjected to a range of environmental conditions. Genome-resolved metagenomics revealed that SCN^- hydrolase-encoding, sulphur-oxidizing autotrophic bacteria controlled SCN^-degradation. These bacteria supported metabolically-dependent non-SCN^--degrading sulphur-oxidizing autotrophs and non-sulphur oxidising heterotrophs. Microbiome structures varied spatially (planktonic versus sessile) and temporally (across changing environmental conditions), and shifted from *Thiobacilli* to novel gammaproteobacteria. Degradation of carbonyl sulphide (COS), a key intermediate in the global biogeochemical sulphur cycle, was attributed to plasmid-hosted $CS_2$and COS hydrolase genes associated with*Thiobacillus*, revealing the possibility for lateral transfer of this capability. In summary,

we show that native autotrophic microbiomes from mine tailings can be employed for SCN^- bioremediation, thus reducing the hydrological impact of mining.

12

Acid Saline Lakes as a Source of New Biomining Organisms.

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Biomining is a growing industry aimed at providing an economically feasible alternative, to extract valuable metals from low grade ores as compared to conventional extraction techniques. Acidophilic iron and sulfur oxidising microorganisms are currently used in this process however, these microbes are sensitive to chloride ions. Fresh water is therefore required for biomining processes which challenges the economic feasibility in countries where fresh water is scarce. Thus, this study aims to explore acid saline lakes, in search for iron and sulfur oxidising acidophiles that are chloride resistant. Lake Tyrrell, an acid saline lake in north western Victoria, harbours the niche environment in which these microbes would most likely be present, as it has low pH (~4), high salinity (>200 g.L-1 NaCl) and large iron and sulfur deposits. Water, sediment and biofilm samples were collected, and the diversity of the microbial populations determined using culture and non-culture dependant methods. Enrichment cultures were grown in Basal Salts media at both 35g/L and 105g/L NaCl at the pH of the sampling site (ranging from pH 4-5). Chloride tolerant acidophilic iron and sulfur oxidising microbes were isolated from ferrous iron overlay plates (Johnson & Hallberg 2007) inoculated with the enrichment cultures. From all samples, microbial growth was observed at both NaCl concentrations however, iron oxidation was only observed in enrichment cultures from biofilm samples at both NaCl levels. 16S rRNA gene amplicon analysis revealed the lake samples to be very diverse. The most abundant taxa present in the sediment and biofilm samples from the lake were from the families *Halobacteroidaceae*, *Balneolaceae*, *Deferribacteraceae* and *Salinisphaeraceae.*

This study has resulted in the isolation of an acidophilic iron oxidising microbes that may have an application in the biomining industry as well as giving an indication of the microbial diversity of Lake Tyrrell.

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13

Environmental and microbiome-driven factors influencing the efficiency of thiocyanate bioremediation

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Thiocyanate (SCN-) is a relatively stable contaminant in aquatic systems with both natural and anthropogenic origins. In gold mining, this compound is generated by a reaction between cyanide, which is commonly used for gold extraction, and sulphur compounds present in waste streams. Some microorganisms are capable of thiocyanate degradation as energy and/or carbon source and also to obtain nitrogen and/or sulphur. The inherent advantages of microbial consortia over single strains or co-cultures, especially when performed in large-scale bioremediation systems, necessitate detailed study of the effects of operational parameters on the efficiency of thiocyanate biodegradation.

Two field-scale treatment systems were implemented at a Victorian gold mine to remediate thiocyanate-contaminated tailings pondwater and groundwater. Each system was comprised of separate containment tanks dedicated to SCN- degradation, nitrification and denitrification. Samples were collected at regular intervals from each tank for metagenomic analysis using the Illumina Novaseq platform. Concentrations of SCN-, ammonium, nitrate, sulphate, dissolved oxygen, pH, and temperature were tracked over time.

Results showed that simple amendments of phosphate solutions could trigger the onset of microbial

thiocyanate biodegradation. However, tailings pondwater tanks showed considerable algal growth, as they were open to sunlight, while closed bioreactor tanks for groundwater treatment were dominated by prokaryotes (autotrophic bacteria). Variation in the type and relative contribution of thiocyanate degrading pathway (algal vs bacteria vs photochemical) in each treatment system are discussed in the context of changing environmental conditions and water quality.

14

Microbial iron cycling in the field and harnessed for a pilot-scale attempt at iron duricrust re-formation.

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High grade iron ore deposits in tropical areas are capped by a hard, erosion-resistant iron duricrust that protects the friable ore below [1]. Geochemical evidence suggests that biogeochemical cycling of iron has played a critical role in formation and ongoing evolution of these duricrusts even until the present day [2]. Hotspots for microbial activity in pools perched on iron duricrusts in Brazil indicates that extensive microbial iron cycling is occurring, with 5-25 ppm soluble iron typically measured, and up to 1000 ppm soluble iron detected in one instance. Novel iron reducing enrichment cultures have been obtained from microbial hotspots in the iron duricrust ecosystem and they are effective at goethite dissolution (up to 20% goethite reduction). One of these novel iron reducing microbial consortia was central in driving iron cycling and promoting iron duricrust re-formation from iron mine waste material in a 15-month pilot-scale experiment in Brazil. Effective microbial iron reduction was observed in all treatments during the pilot scale experiment. At end-harvest, the strongest aggregation of the iron duricrust fragments was observed in treatments that involved *in situ* microbial iron reduction (compared to *ex situ*). This technology holds promise for re-formation of iron duricrusts and accelerated remediation of iron ore deposits post-mining.

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2. 2. Monteiro HS, Vasconcelos, PM, Farley, KA, Spier, CA, Mello CL (2014) (U-Th)/He geochronology of goethite and the origin and evolution of cangas. Geochimica et Cosmochimica Acta, vol 131, pp 267-289.

15

Whole-community sequencing to identify new indicator bacteria for a tropical waste stabilisation pond

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Bacteria monitoring is a critical part of wastewater management and pond sanitation is generally assessed using the standard faecal indicator bacteria (FIB) *Escherichia coli* and enterococci. However, these bacteria are poor surrogates for pathogens, and provide no information about cyanobacteria. A focus on FIB misses the vast majority of resident pond bacteria and how they respond to environmental variables – particularly in tropical environments. Using 16S rRNA tag sequencing, we measured the bacterial community in 288 wastewater samples in a waste stabilisation pond (WSP) located in the northern suburbs of Darwin, northern Australia. Samples were collected during the wet and dry seasons over two years. Whole-community sequencing improved our understanding of the bacteria in the WSP, challenged common assumptions about the abundance of *E. coli* and cyanobacteria in the ponds, and revealed that wastewater-associated bacteria are spatially and temporarily dynamic even in 'simple' systems. Bacterial community changes were poorly explained by the standard physicochemical measurements such as conductivity, highlighting the need to expand monitoring variables. Cyanobacteria represented greater than 6% of the WSP bacterial population

regardless of sample timing and location. Faecal bacteria were abundant in the first (maturation) pond. However, in downstream ponds, these bacteria were less abundant, and instead, non-faecal bacterial taxa were common. For each pond, we identified a bacterial fingerprint that comprised both faecal and non-faecal taxa. These include new candidate bacterial indicators that closely track processes like nitrogen removal and human faecal waste. DNA-based detection allowed us to develop a multi-species approach to wastewater monitoring and to identify indicator families and potential surrogates that could be targeted in future to develop WSP-specific indicator probes. Combining the new indicators with standard *E. coli* and enterococci monitoring represents a locally relevant approach to wastewater monitoring and new tests for human faecal pollution for tropical climates.

16

A snapshot of diversity and function of microbes from a sugarcane bagasse pile

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Sugarcane bagasse is the residual material left after the stems have been crushed for sugar and is one of the most abundant agricultural wastes around the world. In Australia, it is usually incinerated to generate electricity at the mill or for the grid. However, it also has potential to be used as low cost feed for livestock and/or as a substrate in solid state fermentation to generate whole cell animal feed supplements. When bagasse is stored in piles in the open for long periods it develops into a unique ecological niche and is colonised by microbes originating from the sugarcane, the soil nearby or spores in the environment. Due to the microbial activity and the structure of the pile, it also develops into a variable environment with oxygen, temperature and pH gradients from the surface to the deeper layers. The gradient means that microorganisms will have location-specific features that allow growth in different environments. Such features may include a tolerance to low pH, high temperature, or low oxygen levels. The microbial community in bagasse piles is thus a potential resource for the discovery of useful and novel microbes and industrial enzymes. We have used traditional culturing and metabarcoding to understand the diversity of microorganisms found in a bagasse pile and develop a pipeline for the discovery of new enzymes. We now have a collection of 150 microorganisms displaying cellulase, xylanase, mannanase, lipase, phytase and protease activities. Apart from hosting biomass degraders, the bagasse is a suitable environment for the growth and maintenance of oleaginous yeasts and microbes with probiotic potential, that could be especially suited to inclusion in low cost fibrous animal feeds. We have sequenced the genomes of some of these strains and carried out preliminary poultry feed trials to test their effects *in vivo* compared to some commercial livestock feed supplements and link their genomic variation with probiotic function.

17

Title: Archaea community composition of an acidic mine Pit lake in the semi-arid tropics.

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Abstract

Archaea are known to be dominant in extreme environments such as acid rock drainage (ARD), which occurs when iron-sulfur minerals react with water and air. Worldwide, the main reservoirs of ARD are open pit lakes that are produced at the end of open pit mining operations in iron sulfur rich mining areas. Archaea promote ARD production by accelerating iron oxidation and they are involved in the cycling of elements at ARD sites.

We studied the archaea in an ARD mining pit lake (Batman Pit) located in the wet dry tropical climate of north Australia. The Batman Pit was 90m deep at the time of sampling and located in an area where extensive wet season rains bring highly acidic drainage to the Pit. This wet season drainage can significantly influence the physicochemical character and microbial population of the Batman Pit. We measured community composition using next generation sequencing and we also measured pit lake physicochemistry and climatic variables to identify key drivers of community change. The community composition and chemistry was measured once in the dry season and then at the end of the wet season.

The wet season chemistry of the water column was significantly different from that of the dry season. Elemental concentration of arsenic, selenium, iron and sulfur was significantly higher in the wet season samples. In the wet season, water temperature was higher and pH was lower compared to the dry season. Despite these significant changes in physicochemical parameters, the archaeal community composition did not change with season or depth. In this iron-sulfur rich environment, we found the ammonia oxidiser *Nitrosopumlis* was the dominant archaeon at both wet and dry season sampling times. The results also suggest that the archaea population in Batman Pit does not play a significant role in iron cycling, however they may have a significant role in nitrogen cycling.

18

Biofilm Ecology in the Premise Plumbing of Drinking Water Distribution Systems

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The supply of safe drinking water in buildings, from source to tap, is of paramount importance to public health. Premise plumbing is the portion of drinking water distribution systems (DWDS) beyond the service pipe. The plumbing is located within buildings and can be characterised by warm conditions, low water flows, poor levels of residual disinfectants, and high surface area to volume ratios. Consequently, these conditions may favour microbial growth, in both the flowing water and within biofilms on the pipe surfaces, constituting the drinking water (DW) microbiome.

It is estimated that only 5% of microbial growth within premise plumbing systems are in the bulk water, with the majority of biomass existing as biofilms. Furthermore, these DW biofilms have been implicated as the infectious source, supporting the complex ecology, persistence and increased pathogenicity of waterborne opportunistic pathogens like *Legionella pneumophila*. Such opportunistic pathogens may be transmitted through contaminated aerosols released by DWDS outlets to downstream users and susceptible individuals, leading to severe health implications. Thus, there is an urgent need to improve our understanding of the drinking water (DW) microbiome, to support the design of novel control strategies to remove opportunistic pathogens in these environments.

In this study, we characterised the DW microbiome of the 50 year-old Frank White Building at the University of Queensland, which was demolished in late June 2018. Prior to demolition, bulk water samples and a total of 15 m of ¾-inch copper pipes containing mature DW biofilms were obtained. The pipe was sectioned into 1 m lengths and DW biofilm and water sample communities were characterised using culture-dependent and culture-independent methods. Next generation sequencing (NGS) was used to assess bacterial, archaeal and protozoan community dynamics to evaluate the DW microbiome, and the implications of these findings are discussed. To our knowledge, this is the first comprehensive evaluation of such a length of plumbing biofilms and the findings shed light on and the complex ecology of DW microorganisms and various opportunistic pathogens.

19

Unusual DPANN Archaea from a radioactive legacy site

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During the 1960s, small quantities of radioactive materials were co-disposed with chemical waste at the Little Forest Legacy Site (Sydney, Australia) in three-metre-deep, unlined trenches. Prior research has investigated functional and population dynamics during a rainfall event using shotgun metagenomics. This revealed a broad abundance of candidate and potentially undescribed taxa in this iron-rich, radionuclide-contaminated environment. Thus, we explored the same dataset through genomic reconstruction and phylogenomics but focusing specifically in Archaea.

In total, we recovered 318 refined bins with ≥50% completeness and ≤10% redundancy, 37 of which belonged to Archaea. A concatenated protein tree was constructed using 44 highly conserved universal or Archaea-specific ribosomal proteins extracted from the archaeal MAGs plus > 200 non-redundant diverse genomes from public databases. Phylogenomic analysis revealed archaeal bins

corresponded to 10 different phyla, including 4 newly proposed lineages within the DPANN supergroup (LFWA-I to IV, 15 bins), Methanosaetaceae (3), Methanoperedenaceae (6), Thermoplasmata (1), Micrarchaeota *s. stricto* (1), Diapherotrites (2), Woesearchaeota (1), Pacearchaeota (6), Altiarchaeota (1) and Thaumarchaeota (1).

While most of the new DPANN lineages show reduced genomes with limited central metabolism similar to other typical DPANN, the candidate species from the proposed LFWA-III lineage show some unusual features.

- First, they have complete *de novo* nucleotide biosynthesis pathways.
- Second, they harbour all genes needed for the biosynthesis of both thiamine and riboflavin.
- Third, they present a partial TCA cycle.
- And most importantly, fourth, they contain highly connected amino acid pathways that can be tracked all the way to carbon source assimilation.

These features may suggest a lesser dependence on a host or symbiotic partner. In addition, LFWA-III seems to be a diverse in the sampled trench with 11 MAGs suggested to belong to 10 different genera in a single family based on ANI/AAI relatedness.

Future research will attempt to discover the possible symbionts of the diverse DPANN organisms present in the site.

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The Evolution of Molecular Methods in Microbial Ecology

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Although microbial life abounds on earth and has done so for billions of years, particularly the prokaryotic microbes defied an evolutionarily perspective until the 1980s. Archaea (formerly known as archaebacteria) were hardly known about then and many bacteria studied were human, animal or plant pathogens. Microbiologists studied bacteria by isolating them in pure culture on agar media (incubated under specific conditions of temperature, pressure and atmosphere) and defined their taxonomy by determining aspects of their phenotype (e.g. sole carbon sources for growth, acid production) and their genotype (e.g. mol% G+C of the genome).

How did the field of microbial ecology evolve? Development of methods exploring the 16S rRNA and its gene was crucial. PCR using “universal” 16S rRNA gene primers with DNA isolated from complex microbial communities, cloning and sequence analysis lead to the understanding that microbial diversity was tremendously greater than imagined and that bacterial taxonomy based on phenotype was flawed. Metagenomics was born and several complex microbial communities were subjected to this procedure. This led to some comprehension of resident microorganisms and genes in the community, to development of methods linking community structure with function and to discovery of organisms with novel metabolic pathways. Other “omics” and “meta” fields were spawned to address microbial community function. Microbial ecology is a field in evolution and the talk will highlight some of the procedures and findings that allows it to inform us about critical aspects of life on earth.

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What can the microbiome tell us about the future for marine ecosystems?

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TBC

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Diversity and function of microorganisms in microbial mat communities from Shark Bay, Western Australia

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Microbial communities play vital roles in biogeochemical cycles, however fundamental Carbon, Nitrogen, and Sulfur cycling processes catalysed by microbial communities are constrained spatially, temporally, and with regard to the different microbial groups involved (Carolan et al., 2015). Shark Bay offers a large distribution of microbial communities across a variety of intertidal plains that are attributed to a complex network of physicochemical factors (Prieto-Barajas et al., 2018). A range of studies have been conducted on these microbial communities from lipid analysis to metagenomics (Wong et al., 2018). Here we sampled microbial mat communities (i.e. smooth, pustular, etc.) occurring in different salinity ranges and during day/night cycles for metatranscriptomic and organic geochemical analyses to detect changes in taxonomic groups and their function. A preliminary investigation of electron accepting reactions using metatranscriptomic data with MG-RAST indicated changes in both bacterial diversity and function of a pustular microbial mat sampled during day and night intervals. In the day time, cyanobacteria are the prominent phyla utilising terminal cytochrome C oxidases, whereas at night time, proteobacteria are the prominent phyla utilising anaerobic respiratory reductase. This indicates an overall shift in community activity from aerobic to anaerobic respiration during a diel cycle. Ongoing work utilising metatranscriptomic and organic geochemical analyses will enable us to detect changes in element cycling taxonomic groups and potentially establish early diagenetic pathways of biomolecules such as the incorporation of sulfur into organic matter.

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Fermentative and respiratory bacteria are uncoupled in anoxic permeable sediments

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Permeable (sandy) sediments cover half the continental margin and are major regulators of oceanic carbon cycling. The microbial communities within these highly dynamic sediments frequently shift between aerated and anoxic states, and hence are less stratified than cohesive (muddy) sediments. A major question is therefore how these communities maintain metabolism during oxic-anoxic transitions. Here we show that molecular hydrogen (H_2) accumulates in silicate sand sediments due to decoupling of fermentative and respiratory bacteria following anoxia. *In situ* measurements show that H_2 is supersaturated by up to 250-fold in the water column overlying these sediments and has an isotopic composition consistent with fermentative production. Community and shotgun metagenomic profiling suggests that the sediments harbor diverse and specialized microbial communities with a high abundance of [NiFe]-hydrogenase genes. The hydrogenase profiles predict that H_2 is primarily produced by facultatively fermentative bacteria and can be consumed by aerobic respiratory bacteria. Consistently, we demonstrate through flow-through reactor and slurry experiments that H_2 is (i) rapidly produced by fermentation following anoxia, (ii) immediately consumed by aerobic respiration following reaeration, and (iii) only consumed by sulfate reduction during prolonged anoxia. We also detected high abundance and activity of hydrogenotrophic sulfur, nitrate, and nitrite reducers. In combination, these experiments confirm that fermentation dominates anoxic carbon mineralization in permeable sediments and, in contrast to cohesive sediments, is largely uncoupled from anaerobic respiration.

The frequent changes in oxygen availability in these sediments may have selected for metabolically flexible bacteria while excluding strict anaerobes.

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Holocene paleodepositional changes reflected in the sedimentary microbiome of the Black Sea

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While subsurface microbial communities are generally structured through *in situ*environmental conditions such as the availability of electron acceptors and donors, recent studies implied that a subset of these microbial taxa were present at the time of deposition and formed a genetic archive of bacterial species (i.e., a bacterial paleome) that can provide information about the paleodepositional environment. However, additional high sampling resolution records spanning key climate stages are required to verify this claim. Recent studies have shown that sediments underlying the permanently stratified and anoxic Black Sea contain well preserved photic zone-derived plankton DNA suitable for the reconstruction of past ecosystem-climate interactions. It is therefore to be expected that a subseafloor bacterial paleome is present in the Black Sea that will reveal a correlation with known paleoenvironmental changes in the region. To test this hypothesis, we used a shotgun metagenomic approach to study the taxonomic and functional diversity of the Black Sea's subsurface microbiome and compared community changes with the timing of Holocene climate shifts. Obligate aerobic bacteria made the largest contribution to the observed shifts in microbial communities associated with known Holocene climate stages and transitions and were likely seeded from the water column and did not undergo postdepositional selection. Presumably active anaerobic bacterial communities showed the most significant response to the timing of the establishment of modern-day environmental conditions 5200 years ago, previously shown to have resulted in a shift in planktonic communities and the type of organic matter being sequestered. No significant shift in the subseafloor microbiome was observed as a result of gradual environmental changes that occurred after the marine reconnection, 9 ka ago.

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The microbiome of lithifying and non-lithifying microbial mats from hypersaline lakes at Rottnest Island (Western Australia)

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Microbial mats are complex organosedimentary structures[1,2] organized as multilayered carpets of bacteria and archaea[3,4]. Within the microbial mat microenvironment, the occurrence of different metabolic processes can lead to alterations in local chemistry and induce carbonate precipitation[5,6]. The accretion of precipitated carbonate in lithifying microbial mats may induce microbialite formation[1]. Microbialite formation process are poorly understood but studies have suggested that taxonomic composition of lithifying microbial mats and their predominant metabolic pathways likely contribute[7,8,9,10]. In contrast to lithifying mats that potentially form microbialites, non-lithifying mats, that do not form microbialites, sporadically trap carbonate sand grains that are then actively bound to the microbial mat through the production of extracellular polymeric substances[11,12,13]. Both mat types are restricted in their occurrence to a few extreme environments, such as hypersaline lakes and geothermal springs[14,15]. Rottnest Island, located 18km off the coast of Perth (Western Australia) is home to both lithifying and non-lithifying mats; in contrast to other global locations these mats are poorly characterized. The aim of this study was to assess, via metagenome analysis, taxonomic diversity and functional capacity of lithifying (characterized as flocculent, blister, pustular) and non-lithifying (characterized as loosely cohesive and cohesive) microbial mats from 5 hypersaline lakes on Rottnest Island. Principal coordinate analysis revealed dissimilarities between mat types which accounted for 53% of taxonomic and 40% of functional variation (PERMANOVA, p=0.001). Proteobacteria were the dominant phylum across all mats (52% - lithifying mats; 41% - non-lithifying

mats). Bacteroidetes were more abundant in lithifying mats, whereas Cyanobacteria and Euryarchaeota were more abundant in non-lithifying mats. Rhodothermaceae and Rhodobacteraceae, known for their role in sulfur cycling and thus in microbialite formation, were identified as dominant families only in lithifying mats. Functional analysis identified numerous genes associated with sulfur reduction, photosynthesis and carbon cycling mechanisms; all known to increase the alkalinity of the surrounding microenvironment, thus promoting carbonate precipitation. Our results not only unravel significant information about the microbial mats at Rottnest Island; but also contribute to targeting the taxonomic groups and metabolic pathways involved in the process of microbialite formation.

26

Assessing the contribution of sediment microbes in mediating sulfide intrusion in seagrass ecosystems

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Sulfide is a potent phytotoxin and sulfide intrusion into seagrass tissues has been implicated in the declines of foundation seagrass species across the globe. To date, research has focused on understanding the physiological responses of individual plants and seagrass meadows to sulfide intrusion. The role of rhizosphere microorganisms in sulfide intrusion has not been thoroughly explored, despite the critical roles that microorganisms have in mediating marine sulfur cycling. We investigated if the composition of the sediment microbial community was linked to the vulnerability of seagrasses to sulfide intrusion by sampling sediments and seagrasses (*Posidonia sinuosa*) from six subtidal sites in Perth, Western Australia.

Seagrass $\delta^{34}S$ signatures varied significantly across the study area, and sites with highest signatures of sulfide intrusion also had long-term declines in seagrass density. Microbial communities also differed between sites. Microbial communities at sites impacted by sulfide intrusion had a high relative abundance of Chromatiaceae and Ectothiorhodospiraceae; 2 families of purple sulfur bacteria that have a dominant role in sulfur oxidation processes and thrive in environments low in oxygen and high in sulfides. These sites also had a high abundance of functional genes related to sulfur metabolism. The relative abundance of sulfate reduction genes was negatively correlated with seagrass $\delta^{34}S$, suggesting a quantitative link between sediment microbial communities and seagrass sulfide intrusion. Fluorescent in-situ hybridization revealed differential abundances of sulfide oxidizing cable bacteria on *P. sinuosa* roots along a sulfide intrusion gradient, suggesting that this taxon plays on important role in sulfur cycling in seagrass sediments.

We finish by providing recommendations for future research priorities that will help establish a quantitative and mechanistic understanding of the relationship between seagrass health, sulfur cycling, and rhizosphere microbial communities that will be valuable to the management and restoration of seagrass ecosystems globally.

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Unicellular cyanobacteria and eukaryotic phytoplankton communities around Australia's oceanic regions

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In the last decade a large collection of samples and associated metadata have been obtained from Australia's Integrated Marine Observing System network of national reference stations and various research voyages around Australia as part of the Australian Marine Microbial Biodiversity Initiative (AMMBI). These samples are enabling the systematic evaluation of the diversity, abundance and distributions of Australian marine microbial communities on an unprecedented scale. Here we report the analysis of phytoplankton communities in the highly productive, tropical marine environments of northern Australia. From previous studies using primarily microscopic and pigment analyses these waters have been characterised as being dominated by large diatoms, dinoflagellates and the nitrogen fixing, marine cyanobacterium *Trichodesmium*. However, using a combination of Illumina-based 16S and 18S ribosomal RNA amplicon sequencing and flow cytometry on surface samples collected during an oceanographic transect from the Arafura Sea through the Torres Strait to the Coral Sea, we found

that unicellular picocyanobacterial primary producers dominated the phytoplankton communities while picoeukaryotic phytoplankton formed a consistent, though smaller proportion. Major taxonomic groups displayed distinct biogeographic patterns. Unicellular picocyanobacteria dominated in both flow cytometric abundance and carbon biomass, with members of the *Synechococcus* genus dominating in the shallower Arafura Sea and Torres Strait, and *Prochlorococcus* dominating in the oligotrophic, low chlorophyll waters of the Coral Sea. Consistent with previous observations, sequence analysis indicated that a variety of diatoms exhibited high relative abundance in the Arafura Sea and Torres Strait, while dinoflagellates and prymnesiophytes were more abundant in the Coral Sea. Ordination analysis identified temperature, nutrients and water depth as environmental determinants of assemblage composition. Additionally, we found that the biggest contributor to the satellite-derived surface chlorophyll *a* signal was *Synechococcus* rather than the picoeukaryotic phytoplankton. Similar analyses of phytoplankton communities will be done on some of the other available AMMBI samples to better understand phytoplankton patterns in Australian marine waters and how they might respond to oceanic change now and in the future.

28

Microbial navigation in marine systems

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Ocean carbon cycling is driven by the concerted action of marine microbes, but the fine-scale interactions between these microbes and their physical and chemical environments remains elusive. Experimental investigations of bacterial navigation (chemotaxis) have been limited to highly simplified nutrient profiles, such as linear gradients. Moreover, mathematical frameworks which model the ecological interactions between microbial communities and their environment routinely overlook heterogeneities, considering bulk averages of bacterial densities and environmental conditions.

I will present current work (ARC DECRA 2018-20) which utilises a novel experimental platform for delivering sub-millimetre scale nutrient pulses, quantitatively mimicking those found in the ocean. Advanced video-microscopy is used to characterise microbial motion at the single cell level, and reveals the precise conditions under which bacteria can detect and climb dynamic nutrient gradients. New mathematical theory, based on the counting of individual molecules of dissolved organic matter, is in striking agreement with the experimental findings. From these quantitative foundations, we have developed a mechanistic framework for microbial motion, which directly unifies individual behaviour (cell motility, chemotaxis) with population-scale phenomena (collective nutrient uptake, competition between species). This provides a new path towards predicting ocean carbon cycling which is firmly based on microscale observations.

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Investigation of microbial communities in the 1st Gulf war affected, crude oil-contaminated groundwater in Kuwait

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Kuwait's only fresh water source was contaminated with crude oil in the First Gulf War (1991), where oils wells were destroyed, and seawater was used to extinguish oil fires. Limited research has focused on the effect of the contamination and microbial communities along the down gradient groundwater flow path. Here, the bacterial, archaeal and eukaryotic communities at 15 sites along the contaminated groundwater flow path were investigated. Characteristics of the hydrocarbon contaminants present along the transect were also examined for the first time. This study detected potential hydrocarbon degrading microorganisms such as *Hyphomicrobiaceae*, *Porphyromonadaceae* and *Eurotiomycetes,* and a significant correlation of the microbial community structures with total petroleum hydrocarbon (TPH) concentration and electric conductivity (EC) measurements. Anoxic conditions appear to be dominating the sites closer to the contamination source, with methanotrophs and anaerobic organisms present. The TPH consisted of a

prominent unresolved complex mixture (UCM), indicating the same persistent characteristics as identified in previous research. Lower concentrations further along the groundwater flow path probably due to dilution as indicated by correlations of TPH and EC, and contaminant characteristics. The findings contribute to understanding the groundwater pollution in Kuwait, and give further insight into designing potential future remediation efforts.

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Interactions among anthropogenic disturbance, an endemic disease and communities of mycorrhizal fungi

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A canker disease caused by the fungus *Quambalaria coyrecup* is devastating the keystone tree*Corymbia calophylla* throughout much of its native range in the southwest of Western Australia. These trees have significant economic, cultural and environmental importance. The talk will focus how anthropogenic disturbance such as forest fragmentation is predisposing these trees to disease by negatively affecting soil health and soil microbes needed to maintain healthy trees.

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Inter-strain interactions of *Rhizobium leguminosarum* influence field pea N nutrition

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Symbiotic rhizobial fixation provides an important source of nitrogen for legumes and soil partially replacing mineral nitrogen fertilizer inputs and adding more organic nitrogen into the cropping and pastures systems which stimulate beneficial soil microbial cycles such as decomposition. There is a capacity to further exploit rhizobial associations in Australian soils but this requires more knowledge of limitations for plant-rhizobial symbiosis due to various biotic and abiotic factors such as high temperature, low moisture content, soil acidity and having ineffective native rhizobial populations in soil. For instance, a single legume host can accommodate multiple rhizobial strains but the consequences of interactions among these strains for symbiotic outcomes are poorly understood. We tested the effects of single and multi-strain inoculations of *Rhizobium leguminosarum* on nodulation and on nitrogen fixation in field pea. Multi-strain inoculants differed in the degree of genetic similarity between paired strains and we hypothesized that genetically similar pairs would exhibit stronger competition and negatively affect nodulation and N fixation compared with genetically diverse pairs.

We observed that co-inoculation with rhizobial pairs with low genetic similarity resulted in significantly higher nodule numbers per plant compared to the responses of each isolate inoculated on its own. In contrast, highly genetically similar rhizobial pairs showed less nodulation in co-inoculated treatments compared to single inoculations. Furthermore, we observed a significant increase (~20%) in fixed nitrogen for co-inoculated treatments compared to the treatments inoculated with single rhizobial strains, however the degree of genetic similarity was not observed to affect the strength of this increase. Thus, interactions among strains during nodulation appear to result in synergistic effects on nitrogen fixation regardless of whether the interactions are competitive. Our work suggests that careful selection of multi-strain inocula may improve the outcomes for managing fertility of field pea and other legumes. Future work will address the consequences of these interactions for rhizobial fitness, particularly when coping with environmental stress.

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Understanding settlement: the role of common bacterial biofilm elements in the induction and metamorphosis of the polychaete *Hydroides elegans*

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Many benthic marine invertebrates are induced to settle and metamorphose as a result of interactions with bacterial biofilms. The identity of the cues that mediate these interactions, however, remain largely unknown. The serpulid polychaete *Hydroides elegans* is an excellent model organism for studies of larval settlement and metamorphosis in response to bacterial biofilms. Detailed investigations into the nature of the settlement cue produced by the bacterium *Pseudoalteromonas luteoviolacea* have previously established bacteriocin aggregates (or MACs) as the metamorphic cue. However, *H. elegans* can be induced to settle and metamorphose by a number of bacteria that do not produce MACs. An additional cue capable of inducing settlement in this species are associated with outer membrane vesicles (OMVs) produced by the bacterium *Cellulophaga lytica*. OMVs are produced ubiquitously by Gram negative bacteria and have been observed to mediate numerous interactions between bacteria and Eukaryotes including, but not limited to, cell-cell signalling, antibiotic resistance and virulence. The chemical composition of OMVs can be highly variable between species and can be composed primarily of proteins, phospholipids, lipopolysaccharide. OMVs from some bacteria include genetic material and or virulence factors. In this study we use enzymatic tests including DNase, RNase, Proteases and Lipases as well as chemical purification techniques to determine the component of these complex structures involved in the induction of metamorphosis in *H. elegans*. We show that induction of metamorphosis in *H. elegans* can be induced by a common bacterial component. The widespread presence and variation of this compound may explain the induction of metamorphosis of *H. elegans* by multiple bacterial biofilm species and improve our understanding of the role of biofilm bacteria in the induction of settlement in marine invertebrate larvae.

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Seagrass rhizosphere; understanding the bottom to manage the top

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Seagrass roots host a diverse microbiome that is critical for both their growth and survival. However, the components driving the interplay between seagrasses and their associated root microbiome are still largely unknown. Using a multifarious approach combining imaging techniques (confocal fluorescence *in situ* hybridisation, oxygen planar optodes and sulphide DGTs), with plant physiology and molecular sequencing, we have now built a more complete picture of the micro-environment of growing roots of seagrasses. By using this approach, we reveal that both root exudates and root oxygen loss play an essential role in shaping the structure of the root microbiome. We highlight that seagrasses appear to 'select' for their microbiomes, and that these microbiomes confer adaptive advantages for their host in both nutrient acquisition and protection against pytotoxic sulphides in the sediment. We also highlight the importance of above-ground light availability for seagrasses in regulating the structure of their root microbiomes, where light reduction can invoke a cascade of changes from alterations in root exudation and oxygen loss to a reduction in putative beneficial microorganisms and, ultimately, confirms the importance of the seagrass root environment – a critical, but often overlooked space.

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Drivers of mycorrhizal fungi and root pathogens in threatened woodlands of the Cumberland Plain

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In NSW, there is an ongoing effort to restore and rehabilitate native Cumberland Plain woodland (CPW) ecosystems, particularly those impacted by invasive species. Soil microbes likely play an important role in supporting growth of plants in these woodland soils due to their typically low nutrient availability. For instance, mycorrhizal fungi form associations with roots of many CPW plant species. These fungi are important drivers of ecosystem productivity and carbon storage, yet we lack an understanding of

what drives their distributions beyond broad generalisations of their roles in particular biomes. In addition, root pathogens are candidate drivers of dieback in many forest and woodland systems in Australia, with oomycetes receiving a significant amount of recent attention.

Field experimentation, combined with microscopic measures and high-throughput sequencing of soil and roots reveals patterns of changing abundance and species composition in mycorrhizal fungal and oomycete communities in response to soil properties and as trees age. In addition, our evidence from the *Eucalyptus* Free-Air Carbon Enrichment experiment

(https://www.westernsydney.edu.au/hie/facilities/EucFACE) suggests that these changes may be exacerbated under future atmospheric carbon dioxide concentrations. More data are needed to identify the best-integrated approach to improve soil health and CPW restoration outcomes, particularly in the presence of additional threats associated with climate change and human activities.

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Intraspecific variation in the gut communities of termites highlights their plasticity and potential for manipulation

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Termites successfully feed on various forms of lignocellulose and have optimised their cellulose-degrading consortia over evolutionary time, leading to the idea that their gut communities are highly conserved. Influences on the gut community over shorter time scales were investigated to tease apart vertically transferred and environmentally acquired portions of the gut community, using two endemic Western Australian termites with broad diets, *Tumulitermes westraliensis* and *Amitermes obeuntis*. We characterised intraspecific variation to show colony and caste differences and a strong effect of location on gut communities. A three week field study resulted in a significant effect of diet in both species, with minimal non-target impacts on the termites of interest. Finally, the inconsistent presence of protists in the gut was also supported by both observational and sequencing data, providing another factor shaping the gut community, perhaps seasonally.

Core communities made up of taxa shared by all samples included in the analysis are thought to play key roles in the gut and depending on the types of samples included, these can help draw conclusions about their source. A species level core community was described, made up of 56 and 116 taxa for *T. westraliensis* and *A. obeuntis* respectively, some or all of which are likely passed down from generation to generation. Taxa shared across species or specific to a diet or location were also determined and their functions inferred. Taxa exclusive to a location or feeding group are likely to be environmentally acquired, ingested with food or soil, particularly if these are shared across species. Together, these findings highlight the plasticity of the gut community and its potential as a model system for optimisation of industrial applications.

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Mycorrhizal fungi benefit ecosystem restoration, but how much depends on plant functional type, restoration context and time

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Mycorrhizal inoculation can enhance outcomes of ecological restoration but the factors that influence restoration outcomes are little explored. We searched the literature for field studies of mycorrhizal inoculation to identify factors that determine benefits to ecological restoration. We assessed 34 studies of plant growth (biomass) and plant species richness responses to mycorrhizal inoculation, and their dependence on six explanatory factors: plant identity (plant functional group), soil conditions (soil pH, concentrations of total N and plant-available P), disturbance history (severity of soil disturbance) and fungal identity (single species to whole soil inoculum). We also assessed the development of inoculation effects over time. Our analysis revealed that inoculation with mycorrhizal fungi enhanced

plant growth in restored grasslands, shrublands and woodlands spanning semi-arid to mesic sites around the world. The effect was substantial, increasing plant biomass by an average of 1.7 across the 26 studies that measured biomass, and tended to increase over time. A subset of studies examined plant species richness and similarity of restored species composition to the reference communities. In these studies, mycorrhizal inoculation increased species richness of restored plant communities by an average of 30%, and increased the similarity of restored communities to reference communities. Inoculation was most beneficial to plants with greater nutrient demand (woody nitrogen-fixers), plants with inefficient nutrient-uptake (C4-grasses) and plants growing on severely disturbed or nutrient-poor soils (i.e., low in total nitrogen or plant-available phosphorus). Overall, we conclude that mycorrhizal inoculation can promote plant growth and plant species richness in field conditions to improve the outcomes of ecological restoration projects.

Neuenkamp L., Prober, S.N., Price, J.N., Zobel, M., Standish, R.J. 2018. Benefits of mycorrhizal inoculation to ecological restoration depends on plant functional type, restoration context and time. Fungal Ecology (in press).

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Genome-wide screening for genetic factors important in microbe-plant and microbe-microbe ecological interactions

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The rate of generation of gene sequence information now vastly outpaces our ability to characterise genes and determine their contributions to physiology, metabolism and ecology. Saturation transposon mutagenesis techniques, such as Transposon Directed Insertion Sequencing (TraDIS) provide new information on gene essentiality, gene function and genetic interactions. TraDIS uses a dense library of randomly generated loss-of-function mutants combined with massive parallel sequencing to simultaneously study all non-essential genes in the genome and identify genes which are important in conditions of interest. Whilst initial applications of this technique were largely focused on characterising genes associated with pathogenicity, this approach can be used to identify genetic factors involved in a range of ecological processes.

The interactions between plant commensal bacteria, their hosts and co-occurring microorganisms are important to agriculture and ecosystem health, but many aspects of this are still poorly understood. Our group has applied TraDIS to identify genetic factors critical for plant surface colonisation and persistence. Using this approach, we have identified ~50 genes important in attachment to plant surfaces, many of these genes are associated with cell wall/membrane/envelope biogenesis. Conditionally responsive genes of interest are being investigated to elucidate functions and determine their role in attachment. We are currently investigating genes associated with rhizosphere persistence and in interactions with other soil microbes, including fungal pathogens. This work demonstrates the utility of TraDIS for probing complex ecological interactions.

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Effects of seasonal drought and host identity on endophytic fungal communities in forests of Cape York, northern Australia

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Fundamental questions

- Is there dynamism in the structure and diversity of fungal endophyte communities in response to wet and dry seasons in the monsoon tropics of northern Australia?
- Are communities of fungal endophytes associated with the phylogeny of their host plants?

Fungal endophytes provide ecological functions for their host plants, including promotion of plant growth, inhibition of plant pathogens and drought tolerance, which are all characteristics that have potential far-reaching agricultural use. However, the way in which endophyte communities across the major terrestrial plant lineages respond to seasonal water stress is a question that has not yet been examined in tropical endophytes using culture-independent methods. The lowland rainforests of the Iron Range in north-eastern Australia experience extreme seasonal fluctuations in water availability due to the monsoon cycle and unique regional geology. The aims of this study are to (a) establish how fungal endophyte diversity and community composition differs during the dry and wet seasons in the monsoon tropics, and (b) whether endophytes of host plants representing the major lineages in Embryophyta exhibit different community-level responses to seasonal water scarcity and abundance. This information has the potential to direct agriculture-focused bioprospecting efforts to isolate beneficial fungal endophytes from the Australian tropics, and to clarify the degree to which land plants have developed divergent microbiomes over deep evolutionary time. We compared the fungal microbiomes of plants in northern Australia sampled from wet and dry seasons, performing ITS metabarcoding on fungal communities in leaves and stems of 12 plant taxa, including angiosperms, monocots, magnoliids and bryophytes.

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Large Scale Microbial Ecology Using Citizen Scientists: Lessons from MicroBlitz

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Single cell viral tagging

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Viral discovery is accelerating at an unprecedented rate due to continuing advances in culture-independent sequence-based analyses. One important facet of this discovery is identification of the hosts of these newly characterised uncultured viruses. To this end, we have developed a single cell viral tagging approach. Fluorescently labeled anonymous virions are allowed to adsorb to unlabeled anonymous bacterial host cells which are then individually sorted as virus-host pairs, followed by genome amplification and high throughput sequencing to establish the identities of both the host and attached virus(es). We show the application of this method in the human gut microbiome including cross-tagging of viruses and bacteria between subjects.

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Soil microbial communities exhibit strong biogeographical patterns at fine taxonomic resolution

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Over the last decade significant studies have determined the community structure of soil microbial communities from the poles to the tropics. However, linking this structure to underlying niche and neutral drivers remains a significant challenge. In this study we addressed this by taking three cutting-edge approaches: (i) sampling according to a hierarchically nested design, (ii) analysis of taxa at fine taxonomic resolution using amplicon sequence variants, and (iii) application of the new multi-site diversity metric zeta diversity. We demonstrate that, at fine taxonomic resolution, soil microbial communities undergo a high rate of compositional turnover. They appear to be structured primarily by niche-based assembly processes driven by soil physicochemical factors. We also observed that broadening taxonomic resolution and filtering rare taxa causes a fundamental shift in the occupancy-frequency distribution, significantly slowing the rate of compositional turnover and causing false signals of stochastic community assembly. Our findings suggest that the interpretation of underlying niche and

neutral process driving microbial turnover is highly dependent on taxonomic resolution and filtering rare taxa. Moreover, these findings suggest that microbial communities exhibit much stronger biogeographical patterns than previously recognised.

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NanoSIMS for the microbial ecologist

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Mass spectrometry traditionally requires material to be extracted in bulk from samples, at the expense of information about the complex spatial relationships of the individual components. However, to understand physiology at the 'micro/nano' scale, a more sophisticated approach is essential. NanoSIMS (nanoscale secondary ion mass spectrometry) is an ion microprobe which combines high spatial resolution (50-100 nm) and high sensitivity. NanoSIMS is particularly well-suited to isotopic and elemental imaging, allowing the simultaneous detection of seven ion species with high mass resolution.

We will describe how combining stable isotope labelling with NanoSIMS allows us to directly visualise the distribution of labelled components within an experimental system, without changing the system's chemical nature.For example, ^{15}N and ^{13}C labels are commonly used in biological systems (nutrient tracking, drug delivery), however, there is potential for addition of any other stable isotope of interest (e.g. ^{34}S,^{41}K, ^{44}Ca, ^{57}Fe). Furthermore, isotopic labels can be conjugated to specific antibodies to identify proteins, or to oligonucleotides to identify specific species of bacteria.

NanoSIMS also allows interrogation of systems without addition of isotope tracers. For microbial ecology applications, this may be of use to investigate spatial partioning of more exotic elements – including those at very low concentrations. We anticipate applications in contaminated land rehabilitation as well as bio-prospecting.

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Finding keystone taxa in microbial networks

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Network analysis has been harnessed to elucidate microbial interactions and co-occurrence patterns in complex datasets across a variety of habitats. Ecological interpretation of these analyses has often been limited due to a lack of testable hypotheses and ecological theory that can be applied to correlation based analyses. This has been partially addressed by a series of metrics that determine which taxa are "keystones" in the network, defined as taxa that are central in determining microbiome structure and who's removal results in a fracturing of network topology. These taxa are potentially indicators of particular ecosystem states, targets for microbiome engineering and ideally suited to monitoring and incorporation into predictive models. Here we find keystone taxa across spatiotemporal gradients in three habitats: continental scale soil and marine surveys from the Australian Microbiome Initiative and clinical biofilms from limb wound infections. These taxa are keystones regardless of their abundance category, and are dynamic over gradients using balance-tree distribution modelling. Combined, they represent an inventory of taxa that can be targeted for experimental study and forecasting and who's population genomes will shed light on microbial functional potential in these habitats.

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Dynamic ocean provinces: An outlook for machine learning approaches in microbial ecology

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Microbes play a fundamental role in the health of all levels of marine ecosystems. Observing how microbial distributions vary over space and time can allow us to monitor the consequences of environmental change on ecosystem processes. However, our ability to forecast and mitigate the impacts of these changes requires novel approaches to project baseline observations onto broad-scale future climate scenarios. We assessed the utility of machine learning approaches to predict

microbial provinces of Australia's regional seas and oceans. Boosted regression trees (BRT) are correlative models recognised for their predictive power. We generated over 3,000 models for the most abundant microbial genotypes (Actual Sequence Variants) using extensive microbial community and environmental data collected as part of the Marine Microbes and Australian Microbiome projects. The performance of community models and predictions was evaluated with 10-fold cross validation and against independent datasets. Microbial communities were reconstructed from model predictions over 234,132 spatial points in the CSIRO Altas of Regional Seas (CARS09, 0.5˚ resolution) providing the first comprehensive spatial model of microbial communities for the Southern Hemisphere. Microbial community provinces, together with their transition zones and boundaries, were delineated using k-means and hierarchical clustering. Clear latitudinal oscillations were observed in the boundaries of microbial provinces (up to 5.0˚ in latitude) providing a powerful observation of the dynamics of microbial communities in the oceans. These species distribution models provide a framework to examine the trajectory of changes in microbial communities and processes in response to changing climate.

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Resolving proteomic responses to supercritical carbon dioxide exposure in *Geobacter sulfurreducens*

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The geological storage of CO_2 in a supercritical form is one of the essential tools required towards meeting the Paris accords. This research aims to determine how the deep subsurface microbial community may respond to large-scale geo-sequestration of CO_2. In-vitro experiments show that many microbes display limited growth and survive for short time periods when exposed to supercritical CO_2. However, field studies indicate that the whole community can survive and shift in response to supercritical CO_2 exposure. Understanding how a particular microorganism responds to supercritical CO_2 exposure will allow us to begin to understand the community shifts we see in field studies. BONCAT is a powerful tool that allows for the temporal selectivity of proteins in pulse tag experiments. Pulse times as low as a few minutes allow for rapid selection of newly created proteins. Here we investigated the potential for BONCAT tagging to provide insights into the proteomic response of *Geobacter sulfurreducens* exposed to supercritical CO_2. Preliminary findings indicate that the application of non-canonical amino acids to a *Geobacter sulfurreducens* containing growth media result in the production of endogenous BONCAT tagged proteins. Furthermore, the time frame for the incorporation of the non-canonical amino acids into endogenous proteins is relevant to supercritical CO_2 exposure. These findings suggest that further investigation of the application of BONCAT tagging on Geobacter *sulfurreducens* cultures under supercritical CO_2 conditions is a promising step towards understanding the proteomic response of microbes exposed to supercritical CO_2.

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Multivariate microbiome data analysis

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Our recent breakthroughs and advances in culture independent techniques, such as whole genome shotgun (WGS) metagenomics and 16S rRNA amplicon sequencing have dramatically changed the way we can examine microbial communities. But does the hype of microbiome outweighs the potential of our understanding of this 'second genome'? There are many hurdles to tackle before we are able to identify and compare bacteria driving changes in their ecosystem. In addition to the bioinformatics challenges, current statistical methods are limited to make sense of these complex data that are inherently sparse, compositional and multivariate.

I will discuss some of the topical challenges in 16S and WGS data analysis, including the presence of confounding variables and batch effects and some experimental design considerations. I will present our latest methodological developments to identify multivariate microbial signatures using Projection to Latent Structure (PLS) dimension reduction methods, and our recent advances in data integration for microbiome data, including longitudinal data. Our methods are implemented in our R toolkit mixOmics dedicated to biological (omics) data integration. I will illustrate these challenges and some proposed solutions on several microbial community studies from our network of collaborators.

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A proposal for a standardized archaeal taxonomy

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Culture-independent techniques such as metagenomics and single-cell genomics allow us to explore microbial diversity on the genome scale. This wealth of genomic data provides the opportunity to develop a comprehensive taxonomy based on evolutionary relationships. Here we propose a standardized archaeal taxonomy based on a concatenated protein phylogeny that conservatively removes polyphyletic groups and normalizes ranks based on relative evolutionary divergence. The robustness of the phylogeny was verified by inferring genome trees from concatenated marker gene sets using FastTree, IQ-TREE, and ExaML, and through comparisons to single-gene trees. Taxonomic curation followed the rules of the International Code of Nomenclature of Prokaryotes and recent proposals to use genome sequences as type material. From 1811 archaeal genomes, 11 phyla are described, including 3 phyla from major normalized monophyletic units from the Euryarchaeota, and a phylum resulting from the amalgamation of the TACK superphylum. The taxonomy is publicly available at the Genome Taxonomy Database (GTDB) website (http://gtdb.ecogenomic.org).

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Community profiling in the age of genomes

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Microbial community profiling has long been a primary focus in microbial ecology, and is often performed by amplification of the 16S rRNA gene and well established bioinformatic routines. The increasing use of shotgun metagenomics to characterise communities presents particular challenges to researchers, because reads cannot be easily grouped together to form operational taxonomic units (OTUs) as amplicon sequences can be. However, shotgun metagenomics is a much richer source of information for community profiling than traditional amplicon studies. Here we present three separate but related research projects that concentrate on different aspects of this difficult class of bioinformatic problems.

CoverM is a user-friendly tool for calculating community profiles from metagenomes in the context of a reference set of genomes. It uses established read mapping techniques, using an efficient algorithm to determine several coverage statistics e.g. mean coverage of a genome, or fraction of a genome covered by at least one read. Borrowing concepts from RNAseq bioinformatics, we then explore how alternative methods to direct read mapping based on de Bruijn graphs can be used for profiling. These techniques can, theoretically at least, calculate community profiles with a resolution that is able to separate two genomes with a single base pair difference. Finally, we present a scalable technique for community profiling in the absence of reference genomes, one that generates OTU table similar in many ways to amplicon-based profiles. The software (SingleM) has been applied to ~10,000 publicly available environmental metagenomes and will be made available as a website where sequence types recovered from a researcher's sample can be related to the increasingly vast set of public data.

These software are available at:

http://github.com/wwood/CoverM

http://github.com/wwood/SingleM

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The application of 3D printing and nutrient biomaterial microhabitats in microbial capture culture

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High-throughput environmental sequencing has revealed a microbial diversity much greater than that which we have brought into culture; it is estimated that the vast majority of microorganisms have yet to be succesfully cultured. A common explanation is that we often fail to mimic the environmental conditions that are needed by most microbial cells for growth. Even so, the microorganisms that have been successfully cultured so far has yielded humanity with a wealth of antibiotics, anti-cancer drugs, industrial enzymes and cultures, plant growth promoting bacteria and many other valuables. Bringing new microorganisms into culture may thus provide us with an even greater boon.

We have developed a range of new 3D printable lipid-based biomaterials for medical applications such as personalized implants and controlled drug release. While doing so, we found that the material could support the growth of a wide variety of cells, including human cells, pathogenic bacteria and soil microorganisms. It could also release nutrients that stimulated the growth of a soil inoculum. While continuing to pursue medical applications we are now also repurposing the biomaterial for environmental applications.

We are using 3D printing to manufacture a high-throughput system for capturing and culturing novel microorganisms from the environment. The goal is to encapsulate and release different nutrients from 3D printed microhabitats made from the new lipid-based biomaterials. These are then placed inside a 3D printed environmental array that then becomes capable of stimulating the growth of specific microorganisms from the environment based on their metabolism and nutrient needs. With this tool we hope to bring more microorganisms into culture, to discover new beneficial biomolecules and to shed light on the microbial diversity in nature.

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Comparing cats and dogs: Exploring 16S rRNA amplicon sequencing and metagenomic shotgun sequencing using a SKG mouse model

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Analysing microbial communities with next-generation sequencing (NGS) is unveiling diagnostic and therapeutic biomarkers for various diseases. The NGS approach 16S rRNA amplicon sequencing (16S) offers an economically viable option to characterise microbial community for these types of studies. However, 16S is limited by detail and accuracy for microbial taxonomic hierarchy and gene predictions. In contrast, metagenomic shotgun sequencing overcomes these limitations but at a far greater cost. Previous studies have compared 16S and shotgun techniques but were restricted by

small sample sizes (n=1), were conducted without biological context and were limited or without statistical analysis.

We aim to investigate the gut microbial response to a proposed Anti-Interleukin (IL)-23 treatment for Spondyloarthropathy (SpA) diseases. This study applied four different treatment regimens to 32 SKG mice and faecal samples were collected at three different time points during regime stages. These samples were analysed with 16S and shotgun sequencing techniques to examine how each approach represents gut microbial communities of the different treatment groups.

In this study we compare each treatment groups' microbial community as well as gene annotations using alpha-diversity, beta-diversity along with a statistical power assessment. More specifically we applied the spares Partial Least Squares Discriminant Analysis (sPLS-DA), which characterises microbial and gene signatures that discriminates treatment groups of different time points and generates graphical outputs. We also propose recommendation about sample size and statistical power for the two sequencing methods. Comparisons were conducted with 16S and shotgun sequencing approaches. Taxonomy classifications were conducted with QIIME2 and GraftM and gene annotations were generated with PICRUSt and MG-RAST.

Both sequencing approaches identified similar family ranking classification for high abundance OTUs. Although, 16S proved to have reduced detail and accuracy in taxonomy hierarchy and gene annotations. Overall, shotgun had greater taxonomy and gene resolution. The shotgun approach also performed better at identifying microbial and gene signatures that discriminated treatment groups and maintained a higher statistical power for both taxonomy and gene annotations.

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Development of new enzymes and microbial cells for lignin degradation and the enhancement of livestock feeds based on sugarcane fibre

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Sugarcane bagasse is one of the most abundant industrial by-products around the world. In Australia, it is often burnt to generate heat and electricity for sugar mills. Bagasse is typically stored uncovered in large piles for long periods prior to combustion. The microbial communities that grow in bagasse piles during storage can utilise lignocellulosic fibre for energy and growth and survive in this harsh environment that exhibits diverse ranges of pH and temperature. Thus these microbes and their enzymes may have potential for biotechnological exploitation. The use of microbes and their enzymes for the bioconversion of sugarcane bagasse into higher value-added products would offer economic and environmental benefits for the sugar industry. Sugarcane bagasse contains approximately 22-27% lignin which is considered the most difficult component of plant cell wall material to degrade and from which to derive value. Thus, in this study, we targeted microbes that express lignin degrading enzymes. Methods have been developed to screen isolated microbes in plate assays and decolourisation of synthetic dyes that are model compounds for lignin monomers in submerged cultures. Isolates displaying higher enzyme activities were selected and their enzyme production optimised using different media components and inducers. Strains were then assessed for their ability to degrade lignin using different analytical methods. Proteomic approaches were also used to identify new enzymes involved in lignin degradation. Preliminary results have revealed a variety of microorganisms displaying lignin degrading activity.

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Preferential accelerated weathering of Rare Earth Element hosting apatite and precipitation of REE minerals through bacterially mediated acid production.

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Rare Earth Elements (REEs) play an important role in our industrialised world, with their use expected to increase along with our technological needs. The extraction of REE are associated with a number of environmental issues, as the primary leaching technologies use multiple steps containing strong acids. The rate of REE extraction will no doubt continue to rise, and it is therefore necessary to begin the exploration for environmentally 'friendly' techniques. Because of the absolute requirement of phosphorous by the biosphere, a range of bioleaching studies have been carried out, examining the application of biogeochemical processes in REE solubilisation (Fathollahzadeh et al., 2018). In this study, the growth of *Acidithiobacillus thiooxidans* strain ATCC 19377 on thiosulphate was used to examine phosphorus acquisition, i.e., cell growth, and REE extraction from petrographic sections of Nolans Bore (NT) ore – a solid source of phosphorous. In this experimental system, individual *A.*

thiooxidans cells were observed growing as a biofilm on the petrographic sections and in the planktonic phase. Remarkably, *A. thiooxidans* was found to preferentially colonise the REE enriched phosphate-bearing grains, producing a dispersion halo of secondary REE phosphates, presumably resulting from pH neutralisation away from the metabolising bacteria. In addition to exopolymer, cells were found associated with elemental sulphur produced from the disproportionation of thiosulphate at the mineral surface and in the fluid phase. The resulting leaching efficiency of the system was low, with <1% of the leached REEs remaining in solution. When this data is coupled with targeted-weathering by bacteria, the rate of weathering increases significantly, as only a fraction of the rock specimen is enriched with REE and therefore has significant evidence of alteration. The preferential colonisation and leaching of REE enriched material suggest that a lower impact biotechnological approach to REE recovery might be possible.

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Growth Response of wheat to chemical and mineral fertiliser application influenced by multispecies microbial inoculant.

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Microorganisms play an important role in the acquisition and transfer of nutrients in soil and plants. They are involved in a range of processes that affect nutrient transformations and thus influence the subsequent availability to plant roots. But nutrient availability will also influence microbial diversity and function. Multispecies microbial inoculants may have dual functions as biostimulants and biocontrol agents and claimed agricultural benefits are influential for regulatory purposes. Biostimulants include commercial products containing substances or microorganisms that stimulate plant growth and can be involved in a range of processes that affect nutrient transformations in soil and thus influence nutrient availability, and types of fertilisers can influence soil microbial diversity and function. A glasshouse experiment was conducted at The University of Western Australia using wheat (cv. Wyalkatchem) to investigate the effects of different forms of mineral and chemical fertilisers along with application of a multispecies microbial inoculant. The experimental design was a randomised complete block with three replications of two distinct Australian Mineral Fertilisers (AMF1 and AMF2), and a chemical fertiliser with and without a microbial inoculant combination. The treatments were: control, mineral fertiliser (AMF1) without microbes, mineral fertiliser (AMF1) with microbes, mineral fertiliser (AMF2) without microbes, mineral fertiliser (AMF2) with microbes, chemical fertiliser (CF) without microbes, and chemical fertiliser (CF) with microbes. Despite an early reduction in plant shoot growth with chemical fertiliser + inoculant microbes, the microbial inoculant + mineral fertiliser treatments increased shoot growth compared to the control. At maturity, application of all fertilisers with or without the multispecies microbial inoculant increased grain yield compared to control. The fertiliser and inoculation of microbes influenced mycorrhizal root colonisation. Using 16S rRNA sequencing, the microbial inoculant and fertiliser treatments were shown to influence the diversity of rhizosphere bacteria. Overall, the multispecies microbial inoculant had beneficial effects on wheat yield when they were combined with the mineral and chemical fertilisers applied at the level recommended for on-farm use in south-western Australia.

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Constraint based metabolic flux analysis of *Acidithiobacillus ferrooxidans* under extreme environmental stresses

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Acidithiobacillus (*A.*) *ferrooxidans* is an important biomining microorganism which plays a role in the industrial extraction of metals from sulfide ores due to its ability to obtain energy from the oxidation of iron and sulfur. During industrial processes, *A. ferrooxidans* is subjected to extreme stresses, including

low pH, nutrient depletion, oxidative stress and exposure to toxic heavy metals, high temperatures and high salinity. Numerous studies have been carried out to identify the mechanisms used by *A. ferrooxidans* to survive these stresses. The sequencing of the genomes of members of the *Acidithiobacillus* species has provided crucial insights into the metabolic capabilities of these microorganisms. However, genome sequences alone are unable to predict physiological outcomes and metabolic capabilities of cells as a whole integrated system. Implementing constraint based reconstruction analysis can be a useful tool to overcome this challenge. Constraint based metabolic model reconstruction involves, i) systematically organizing information from genome annotations and 'omics' data sets in order to identify genes, proteins, reactions and metabolites that participate in important metabolic pathways, ii) setting constraints so that internal reaction rates (fluxes) of metabolites can be computed on the basis of a mathematical stoichiometric pathway model. Correlating metabolic pathways and the predictive outcomes of changes in metabolites and reactions, in turn, can be used to qualitatively and quantitatively elucidate biological information and allow better understanding of metabolic capabilities under different scenarios. Metabolic modelling and flux balance analyses have been extensively performed for a range of prokaryotes, eukaryotes and archaea used in medical, biotechnological and environmental applications, but only few studies have been performed on biomining microorganisms. Therefore, generating constraint based metabolic flux models for biomining microorganisms such as *A. ferrooxidans* under different environmental stresses provides a way of *in silico* determination of metabolic capabilities associated with the growth, survival and functionality of these microorganisms, which can ultimately help to inform enhanced extraction of metals from sulfide ores.

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Batch Effect Adjusting Methods for Microbiome Data

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Microbiome is defined as the collective genomes of an ecological community of microorganisms. Data analysis of microbiome data is challenged by the compositional and sparse nature of the data and the inherent dependency between microorganisms. In addition, microbiome studies suffer from unwanted sources of variation termed "batch effects" that may confound the effect of interest (e.g. treatment). Batch effects may be biological (e.g. diets, husbandry), technical (technician, sequencing) and / or computational (software, bioinformatics pipeline). They are often unavoidable in practice despite good experimental designs. We present different methods to remove or account for batch effects. These methods have different application types depending on their assumptions and the overall analysis aims. However, most were developed for microarray or RNA-Seq data. We present several strategies to choose the appropriate methods to adjust for batch effects and assess the efficiency of these approaches for batch effect removal in microbiome data.

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Developing microbial indicators for the health of aquatic systems

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Globally, the value of measuring and incorporating microbial diversity and community composition into environmental management plans is becoming more broadly accepted. Microbial diversity is now recognized as one of the most sensitive indicators to detect differences between disturbed and undisturbed soils in terrestrial ecosystems. However, the use of microbial indicators in the management of natural aquatic systems, both freshwater and marine, is still in development. Here, we present two case studies illustrating the way in which rapid and cost-effective measures of microbial diversity and composition (e.g. 16S rRNA amplicon sequencing) can be used for improved management in the Swan-Canning catchment of Perth, Western Australia. In the first case study, we show how the use of 16S rRNA sequencing of microbial communities across riparian soil gradients coupled with qPCR of functional genes, can be used to guide restoration and management of urban and degraded freshwater streams. In the second case study, we show how we can combine traditional seagrass health metrics (e.g. biomass and reproduction) with 16S rRNA sequencing of seagrass microbiomes to develop indicators for estuarine health. These case studies are exemplars of how an improved understanding of fundamental microbial (both free-living and host-associated) aquatic ecology (i.e. community composition) can facilitate the development of novel management and restoration strategies for aquatic systems spanning entire catchments.

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Accurately estimating a core community and using it as a tool to tease apart vertically and environmentally acquired components of the gut community of termites

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Our work on the variability of gut community structure and composition within a termite species has highlighted the need for extensive sampling and standardised techniques, to maximise data reproducibility and utility across research groups. Determining the core community has implications regarding taxa that may be vertically transferred from generation to generation and three aspects related to sampling for termite core microbiome studies were investigated: scale, the geographic distribution of sampling sites; scope, the caste (workers, soldiers, etc) composition of samples; and sampling intensity, the number of samples taken per unit of interest. Taxa shared by all castes are likely vertically transferred, whereas taxa exclusive to a location or feeding group are likely to be environmentally acquired, ingested with food or soil, particularly if these are shared across species.

We found that sampling intensity had the largest effect on the outcome of core community calculations, and is fortunately the easiest factor for researchers to control. We developed a simple random sampling method which future researchers can use on their own data sets to test whether sufficient sampling intensity has been reached. Scale and scope also had an impact and we make the following sampling recommendations to obtain an "accurate" core community:

Scope: All relevant sample types (castes in the termite context) should be included, for example only workers if that is the scope of the question, or all castes for a total species core community estimation.

Scale: The maximum relevant range should be included, which could be a single colony if that is the scope of the question, or multiple locations across the entire species range for a total species core community estimation.

Intensity: A minimum of 20 (ideally 30) samples per level of each unit of interest should be included. This means 20 samples total to estimate a species core microbiome, or 20 samples per treatment, location or colony as relevant to the scope of the research question.

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Improving DNA extractions from corrosion products

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Microbiologically Influenced Corrosion is the accelerated corrosion of a metal surface due to the presence of a biofilm. Metal corrosion typically result in formation and accumulation of corrosion products in the form of tubercles and rusticles. Rusticles are a corrosion product with an icicle-like morphology only found on deep water iron structures (>1000 m below sea level). A similar type of corrosion products with slightly different morphology are tubercles, commonly found on iron structures throughout the water column and in drinking water distribution systems. They are thought to be formed by a combination of iron oxidising/reducing and sulphate reducing microorganisms and the chelating properties of both the iron and the extracellular polymeric substances produced by said microorganisms. Both rusticles and tubercles are mainly composed of goethite and lepidocrocite (α-FeOOH and γ-FeOOH, respectively). MIC diagnosis and assessment typically involve isolation of DNA from corrosion products for identification of microbial consortium involved in metal deterioration. However, the extraction of DNA from rusticles and tubercles is problematic due to the co-extraction of iron and the subsequent interference with downstream processing. The fact that DNA adsorbs onto iron oxides constitutes a major problem for MIC studies and the DNA based techniques commonly used to diagnose, detect or monitor it. High concentrations of iron oxides inhibit the DNA extraction by interacting with its polyphosphate backbone, backbone that is also used to bind the DNA to the silica spin columns in popular extraction kits like the PowerSoil kit (Qiagen). Here we present a protocol to separate the adsorbed DNA and cells from the corrosion products and then the extracellular DNA from the microorganisms to reduce the bias from external sources of DNA.

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Circulation of tick-borne severe fever with thrombocytopenia syndrome (SFTS) Phlebovirus in the natural environments

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Tick-borne severe fever with thrombocytopenia syndrome virus (SFTSV) is a novel Phlebovirus in the family of Bunyaviridae and a causative agent of an emerging infectious disease in China, Japan, and the Republic of Korea (ROK). SFTS is mainly characterized by fever, leukopenia and thrombocytopenia in human. The purpose of this study is to investigate the circulation of SFTSV in the natural environments. The results of this study will provide the circulation and phylogenetic relationship among SFTS Phlebovirus ioslates.

To investigate the prevalence of SFTSV in the ROK, a total of 4,223 ticks were collected by flagging from Deogyusan National Park from 2015 to 2018. Animal sera were collected from domestic, wild and companion animals during 2013-2018. Viral RNA was extracted from ticks and sera using viral RNA extraction kit. One-step RT-nested PCR was performed to amplify the S segment of the SFTS virus. The sequence data were analyzed using Chromas and were aligned using CLUSTAL X. The phylogenetic analysis was constructed using the neighbor-joining method in MEGA7.

SFTSV was detected from all of the developmental stages, unfed lava, nymph and adult ticks. *Haemaphysalis longicornis* (3611, 85.5%) were the most abundant, followed by *H. flava* (502, 11.88%), *Ixodes nipponensis* (109, 2.5%), and *Ixodes ovatus* (1, 0.02%). The infection rate of SFTSV in total ticks was 5.8% (245/4,223), and the infection annual rate was 3.69% in 2015, 7.97% in 2016, 5.08% in 2017 and 4.68% in 2018. Three of 103 (2.9%) military dogs, 6 of 101 (5.9%) feral cats, 5 of 127 (3.9%) house cats, 32 of 1,005 (3.2%) black goats, 4 of 240 (1.7%) domesticated pigs, and 12 of 99 (12.1%) cattle were positive for SFTSV. Based on phylogenetic analysis, SFTSV is generally classified into Japanese and Chinese clades. In 36 sequences obtained from this study, 34 (94.4%) sequences were included in Japanese clade and only 2 (5.6%) sequences were included in Chinese clade.

These results indicates that SFTS Phlebovirus may have been circulated and distributed to several tick species and variety animals in the natural environments in Korea.

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Community structure and dynamics of petroleum-degrading microbes in subsurface environments of the North West Shelf, WA

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Subsurface petroleum environments, which include crude oil, natural gas, and unconventional oil and gas deposits, are indigenous habitats for anaerobic microbial communities that degrade petroleum compounds for their metabolic activities[1]. Petroleum reserves in the North West Shelf (NWS) of Western Australia are important energy resources for the Australian economy[2], but biodegraded crude oils complicate extraction and purification processes[3]. There have been extensive geochemical studies carried out on petroleum biodegradation in the NWS[4,5], but there is a lack of microbial ecology data pertaining to biodegraded petroleum in corresponding reservoirs. Reservoir temperature is the primary control on microbial growth and biodegradation rates, but salinity, fresh oil recharge rates, nutrient composition and availability, fluid migration, and build-up of intermediates also interactively influence microbial activity[6]. The microbial communities in subsurface petroleum environments work within syntrophic consortia that degrade petroleum compounds across varying metabolic and redox conditions[7], through which methane is discharged as a terminal product[8,9]. This study aims to characterise community structure and dynamics of petroleum-degrading microbes in the subsurface, which could be further applied to secondary crude oil extraction, as well as methane production from terminal crude oil degradation. Microbial diversity and function in relation to petroleum biodegradation are analysed by metagenomic and metatranscriptomic methods, while organic geochemical methods and compound-specific isotope analysis (CSIA) are used to characterise metabolite abundances and biodegradation pathways. Trace metal and inorganic components are also analysed to characterise influences of nutrients and other volatiles. Finally, anaerobic incubation experiments are being

conducted under various substrate and media inoculations to further characterise microbial community dynamics under controlled conditions, as well as their viability for microbially enhanced oil recovery and methane production.

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Distinct arabinoxylan degradative processes and fermentation end-products are exhibited by faecal bacterial communities with different cell-wall structures

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Plant cell wall polysaccharides are important dietary fibres in human diets. They are recalcitrant to digestion in the stomach and small intestine and are fermented by the colonic microbiota. The fermentation of dietary fibres provides *ca.* 10% of daily caloric intake, and is critical in maintaining colonic health. However, the mechanisms by which dietary fibres are degraded by the colonic microbiota are poorly understood. Arabinoxylans (AXs) are important dietary fibres in cereal grains. The complete degradation of AX into monosaccharides requires the concerted action of at least three types of enzyme: *endo*-β-1,4-d-xylanase, *exo*-α-l-arabinofuranosidase and *exo*-β-1,4-d-xylosidase. Under *in vitro* fermentation conditions, this study compared the enzymatic metabolism of water-soluble wheat AX between a porcine and a human faecal inoculum. Using both colorimetric substrates and AX solution, cellular localisation of the enzymes was investigated by assaying the enzyme activities before and after cell lysis. Results showed that activities of all the three enzymes were detected on the microbial cell-surface for both inocula. In addition, intracellular *exo*-acting enzymes were detected in the cell-wall and in the cytoplasm for fermentation with the porcine and human faecal inoculum, respectively. However, intracellular (in the cell-wall and/or cytoplasm) *endo*-β-1,4-d-xylanase was only detected in the human faecal fermentation. Metagenomic sequencing revealed that the microbial community produced from the porcine faecal inoculum promoted the growth of Gram-negative bacteria whereas Gram-positive bacteria dominated in those fermentations initiated with the human faecal inoculum. The different cell-wall structures between Gram-negative and Gram-positive bacteria explained the different enzyme locations by fermentation using different faecal sources. In addition, fermentation using the porcine faecal inoculum produced higher propionate whereas higher *n*-butyrate was produced from the human faecal inoculum. This study therefore demonstrated that bacterial communities with different cell wall structures exhibited distinct polysaccharide degradative processes, and favoured the production of different short chain fatty acids.

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Molecular mechanisms of bacterial virulence in phylogenetically diverse macroalgal pathogens

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Macroalgae are essential for the functioning of temperate marine ecosystems, but there is increasing evidence to suggest that their survival is under threat from diseases caused by microbial pathogens. *Nautella italica* R11, of the Alpha-Proteobacteria, and *Aquimarina* sp. AD1 and BL5, of the Bacteroidetes, have been recognised as etiological agents of bleaching disease in the model red alga, *Delisea pulchra.* However, there is a limited understanding surrounding the molecular mechanisms of pathogenesis in these phylogenetically diverse opportunistic pathogens. Using RNA-seq analysis, *N. italica* R11 exhibited a strong transcriptomic response to the presence of *D. pulchra*. In particular, functions related to central metabolism, carbohydrate metabolism and oxidative defences were found to be upregulated. Similarly, using a genomics approach, the *Aquimarina* strains were found to encode a high abundance of carbohydrate degrading enzymes and oxidative defence mechanisms suggesting a common role for these functions across algal pathogens. However, we also observed distinct differences between the strains, with virulence in *N. italica* R11 dependent on a LuxR-type transcriptional regulator. While in the *Aquimarina* strains, the presence of a type nine secretion system (T9SS) may facilitate the secretion of carbohydrate degrading enzymes which may result in disease symptoms. The outcome of this research reveals new functions important for the virulence of phylogenetically diverse algal pathogens and contributes to our greater understanding of the complex factors mitigating microbial diseases in macroalgae.

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***Kocuria marina* CE7 is salt tolerant, and alkali-producer**

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Human skin is the border with the external environment and, as such, is colonized by a diverse collection of microorganisms including bacteria. These microorganisms have been varying between the individuals as well as different parts of the skin. The skin surface pH is 4.8-6.0 and influences various factors for the growth of resident and pathogenic microorganisms. The acidic PH of the skin is one of the significant factors in making the skin less favorable for other foreign harmful bacteria. In this present study, we isolated a strain, CE7, from human skin with the high extracellular buffering property. The isolate is most close to *Kocuria marina* based on 16S rRNA gene sequencing. Genus *Kocuria* is gram-positive, coagulase-negative, and coccoid and belongs to a phylum Actinobacteria. *Kocuria marina* was initially isolated from marine sediment and showed to be salt tolerant. During growth on Nutrient broth, the pH of the medium is changed to alkaline 8.5-9.5, irrespective of an initial pH of the medium. The strain gives rise to the normal growth at pH 6~10, and the same growth level was attained at initial pH 5.0 for 72 h. Even in the latter case, the final pH was 8.5, indicating *K. marina* CE7 produces a buffering metabolite with a pKa of around 8.5. This strain also can grow at high NaCl concentrations up to 5% in the medium. Amino acid analysis of the supernatant of media following cell growth on mineral salts basal showed an accumulation of citrulline and valine. This result indicated that these metabolites and metabolism related to the biosynthesis of them might be related to the buffering capacity of the medium. Our complete the first genome sequence of *K. marina* showed that citrulline is biosynthesized by ornithine carbamoyltransferase (ArgF) which is encoded in a gene cluster *argCJBDFR*.

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***In vitro* fermentation of purified cereal and vegetable dietary carbohydrates using a human faecal inoculum promote distinct microbial communities**

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Arabinoxylan (AX) and galactoxyloglucan (GXG) are polysaccharides abundant in the primary cell walls of cereal grains and fruits/vegetables, respectively. In humans, these complex dietary fibres withstand digestion in the stomach and the small intestine, reaching the large intestine, where their fermentation by the resident microbiota is critical in maintaining colonic health. Yet understanding how the gut microbial community alters due to the fermentation of these complex cereal and fruit/vegetable dietary fibres is minimal. Here we investigated the fermentability of purified wheat and rye AX, as well as tamarind GXG using an *in vitro* batch culture approach, with a human faecal inoculum prepared by collecting human faeces from healthy male (n=3) and female (n=4) volunteers, consuming unrestricted diets. Fermentation proceeded up to 48h, and for each substrate, biomass for microbial community profiling was sampled at the start, then when most of the substrate had disappeared, at one time point in between, and at the end of fermentation. Shotgun metagenomics revealed that during fermentation, an unclassified Lachnospiraceae OTU was promoted by both WAX and GXG substrates. Moreover, for WAX, two additional OTUs (*Bacteroides plebeius* and a *Blautia* spp.) were promoted, while GAX fermentation promoted four more OTUs (an unclassified Bacteroidales, *Parabacteroides distasonis*, *Bacteroides uniformis* and an unclassified *Bacteroides* spp.). Ultimately, this study has revealed that differing bacterial communities are essential for the degradation of these cereal and fruit/vegetable complex dietary carbohydrates, and highlights the importance of consuming a varied diet to promote a diverse gut microbiota. Further bioinformatics is being conducted to investigate the functional capabilities of the bacterial communities promoted during the fermentation of these complex dietary carbohydrates.

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The mechanisms and impacts of superinfection by a filamentous phage in *Pseudomonas aeruginosa*

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Bacteriophage are numerically the most abundant organisms on the planet and it is therefore not surprising that they play significant roles in the ecology of bacteria. The best understood mechanisms by which phage affect microorganisms is through their lytic activity. In this capacity, phage play an important role in nutrient cycling as well as in modulation of microbial abundance. What is less well understood is the role of filamentous phage, which do not normally lyse their host cell during the replication cycle. In this study, we have focused on the role of a *Pseudomonas aeruginosa* filamentous prophage, Pf4, in mediating stress resistance, virulence and biofilm formation. The phage also plays a role in self-competition during mono-species but not during mixed species biofilm development. Our results clearly show that the phage plays an important role in biofilm development, resistance to surfactant stress, the generation of genetic variants as well as virulence in a mouse model of lung infection. We further show that these phenotypes are linked to conversion of the phage into a superinfective form that can induce host cell lysis. Further work links the development of superinfection to reactive oxygen-induced mutations in a single genetic locus in the phage. This locus, *repC*, has homology to immunity proteins from other bacteriophage. Using a combination of genetic deletions and complementation as well as RNA and ChIPseq, we show that the Pf4 encoded RepC can bind to

and regulate the expression of not only the Pf4 encoded genes, but virulence factor and stress associated genes in the P. aeruginosagenome. We have also generated crystal structures of the RepC gene to model how it binds to consensus sequences. These data show that the Pf4 phage, plays an important role in regulating host gene expression and may explain why filamentous phage are not eliminated by their host. These data also further blur the lines between host and parasite.

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Phylogenomic analysis of two recently recognised Firmicutes classes, TC1 and Dehalobacteriia

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The Firmicutes is one of the most widely recognised and best studied bacterial lineages, yet substantial uncultured diversity is still being revealed within the phylum courtesy of metagenome-assembled genomes (MAGs). Prominent examples include the two sister lineages TC1 and *Dehalobacteriia,* which were recently elevated to the rank of class under the rank normalised GTDB (Genome Taxonomy Database [1]) taxonomy. The GTDB has been recently created as part of an initiative to standardise microbial taxonomy based on genomic phylogeny. TC1 currently comprises only two MAGs, one of which is an endosymbiont of a fresh-water ciliate, *Trimyema compressum* [2], and the other from an n-alkene degrading enrichment culture. *Dehalobacteriia* comprises six MAGs and the genome of the first recognised cultured representative, *Dehalobacterium formicoaceticum,* after which the class is named. *D. formicoaceticum* utilises dichloromethane as its sole source of energy and carbon [3-4], while other members of this class were obtained from n-alkene- or naphthalene-degrading cultures. Metabolic reconstruction of the genomes belonging to these two classes has revealed an incomplete central carbohydrate pathway, including the tricarboxylic acid (TCA) cycle, indicative of a fermentative lifestyle in both groups. Pathways and key genes that are central to ecosystem function of the isolation source, such as methanogenesis and n-alkene and naphthalene degradation are also absent, suggesting that they scavenge organic compounds, including C1 compounds, from primary producers. All nine genomes possess complete or near-complete pathways for synthesising and degrading a number of amino acids, including arginine, serine and threonine. Some members of the two classes also contain syntrophic propionate pathway to oxidize propionate to acetate. Such pathway has previously been described in organisms such as syntrophic sulfate-reducer, *Syntrophobacter fumaroxidans* [5]. These findings therefore open new possibilities toward examining the metabolic potentials of uncultured bacterial diversities obtained from syntrophic communities.

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Genetic Diversity and Biotechnological Potential of Symbiotic Microbiome in Indonesian Marine Sponges revealed by Metagenomic Illumina Sequencing

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Microbiome in marine sponges have been recognized as important sources of many natural products with potent biological activities. These bioactive natural products play an ecological role as chemical

defense to protect sponge hosts from predators and microbial infections. From biotechnological point of view, they have become an interesting target in recent years for being developed towards clinically used medicines, such as anticancer, antiviral, and antimicrobial drugs [1]. However, the inherent difficulty in cultivating the majority of sponge-associated microbiome under normal laboratory conditions has hampered attempts to study their diversity and pharmacological potential [1]. In this present study, we applied a cultivation-independent approach by metagenomic 16S illumina MiSeq sequencing technology to investigate the genetic diversity of symbiotic microbiome associated with five species of Indonesia marine sponges (*Rhabdastrella* sp., *Theonella* sp., *Aaptos* sp., *Calyspongia* sp., and *Petrosia* sp.) in comparison with that of planktonic microbes present in the seawaters surrounding the sponge's habitat. We particularly compared the microbial composition of two *Theonella* sp. specimens living in geographically different locations (Kapoposang Island and Buton Island). The 16S-cloning approach indicated that Kapoposang *Rhabdastrella* sp. harbored "*Candidatus* Entotheonella" [2], as-yet uncultivable bacteria genus known as the producers of numerous polyketides and modified peptides previously reported from the Japanese sponges *Theonella* sp. [3–6]. We subsequently detected modular polyketide biosynthetic machinery called polyketide synthase (PKS) in sponge's microbiomes through the illumina sequencing of DNA regions encoding ketosynthase (KS) domain, a key component in PKS. Interestingly, diverse ketosynthase (KS) sequences were encoded on the microbial metagenome of Kapoposang *Rhabdastrella* sp., which showed similarity with those from type I PKS of known compounds. This suggests the potency of the symbiotic microbiome in Kapoposang *Rhabdastrella* sp. as a source of pharmacologically relevant polyketides.

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The role of phototrophy and heterotrophy in a laboratory model of Heron Island (southern Great Barrier Reef) beachrock formation through microbial carbonate precipitation

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Beachrocks are early diagenetic coastal sedimentary formations derived mainly from the precipitation of $CaCO_3$ cements in the intertidal zone. Natural beachrocks have attracted great attention as models to understand accelerated Microbial Carbonate Precipitation (MCP). Inspired by its natural capability to co-precipitate cement, sands, soils, minerals, metals, and to sequester CO_2, MCP is a promising technology with potential applications in the fields of Material Sciences, Climate Change, Geobiotechnology or Geobioengineering. MCP can occur as a by-product of numerous metabolic activities, such as, photosynthesis, ureolysis, denitrification, ammonification, sulphate reduction and methane oxidation. Although MCP has been reproduced with the aid of phototrophic bacteria in specific bioreactors, further experimental studies to optimize the synthesis of beachrock (e.g., the use of different microbial communities, geochemical water doping, sediment constitution) are challenging. In natural systems, heterotrophic bacteria are frequently associated with phototrophs and can increase dissolved inorganic carbon (DIC) concentrations, which promote carbonate precipitation. In sum, phototrophic and heterotrophic microbes have a greater potential to alter the micro-pore geochemistry and induce MCP. This work presents an experimental approach using natural carbonate sand, mixing of fresh-marine waters, and a naturally enriched autotrophic-heterotrophic microcosm to reproduce Heron Island (southern GBR) beachrock through accelerated MCP. Dense *Pisonia grandis* forest and thousands of birds producing tonnes of guano cover Heron Island. Those enrich groundwater in phosphates and nitrates, which reach the mixing zone at the beach, creating the perfect nutrient microenvironment for the beachrock microbial ecosystem. Dominant, epilithic cyanobacterial pink mats and an heterotrophic inoculum derived from leaves and guano were used in an experiment conducted over several weeks using active growth-conditions for these microorganisms and strontium-doped seawater to track MCP. Standard BG11 and hot-water extractable organic matter from leaves and guano were utilized, respectively as nutrient media. MCP was investigated based on water geochemistry and the FE-SEM analyses of new carbonate sub-products. Finally, DNA analyses were conducted by the amplification of primers 926f/1392r from bacteria and archaea.

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The microbiome of Australian abalone from aquaculture

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The digestive tract microbiome has a significant role in health, digestion and development. In healthy organisms, the microbiome is considered to improve health by promoting the uptake of essential nutrients for development, regulating the immune system, and helping to prevent pathogenic infection. On the other hand, shifts in the microbiome that lead to imbalance, known as a state of dysbiosis, are associated with a number of diseases and adverse health conditions. The importance of the digestive tract microbiome in abalone, a commercially important aquaculture species, has not been well established.

The aim of this research was to identify the core microbiome, in terms of diversity and plasticity, of Australian abalone throughout the aquaculture grow-out cycle. Metabarcoding approaches were used to sequence the intestinal microbiome of commercially cultured green lip (*Haliotis laevigata*) and hybrid (*Haliotis laevigata x H. rubra*) abalone. The community composition and plasticity of the microbiome were examined throughout the first year of development and during periods of optimal growth and temperature stress.

The intestinal microbiome adapted to experimental treatments (e.g., temperature and diet). However, there was a core group of genera, including *Vibrio* spp., *Psychrilyobacter* spp., and *Mycoplasma* spp., that consistently populated the intestines of adult and juvenile abalone. This research provides novel and practical information for researchers and farmers.

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Potential Mechanism of Subsurface Life Fuelled by Extracellular Electron Uptake

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Subsurface microbes constitute nearly half of total microbial cells on Earth and represent important bioresources[1], yet have remained mostly uncultivable due to their unknown energy sources. Identifying energy sources in the subsurface is therefore crucial for establishing novel cultivation methods to grow and isolate subsurface microbes. So far, abiotic hydrogen (H_2) generated in water radiolysis and water-rock reactions has been considered as the main energy source in subsurface environments. However, due to its low concentration (nM – μM) it is greatly outcompeted by that of electron acceptors (mM), and thus remains questionable to be sufficient for supporting the vast subsurface biosphere[2]. Here, we show that multi-heme cytochrome proteins, which enable cells to extract electrons from electrodes coupled with energy acquisition, are present in various sedimentary microbes. By performing genome sequencing and electrochemical analysis with a sulfate-reducing bacterium, *Desulfovibrio ferrophilus* IS5, which was isolated from marine sediments using iron coupons as the sole energy source[3], we identified two gene clusters encoding seven multi-heme cytochromes with extracellular and periplasmic localizations and two β-propeller proteins which potentially enable interactions between extracellular and periplasmic cytochromes forming a transmembrane electron-uptake conduit. Cells under organic starvation condition overexpress surface cytochromes, produce segmented nanowire structures covered by cytochromes, and become capable of electron uptake from electrodes. Whole-cell electrochemistry identifies the onset of electron-uptake potential at −0.3 V versus standard hydrogen electrode, which is very close to the redox potential of nicotinamide adenine dinucleotide $[NAD(P)^+/NAD(P)H = -0.32$ V], a ubiquitous energy carrier in cell respiration, indicating that electron uptake provides cells with a sufficient but minimum amount of energy for survival under organic-scarce conditions. Furthermore, identical genes encoding cytochromes are present in diverse sedimentary microbes across three phyla, *Proteobacteria*, *Thermodesulbacteria*, and *Aquificae*, suggesting that electron uptake widely fuels subsurface biosphere.

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Investigating the viral diversity in a western boundary current

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Viruses and phage impact upon the health of environments in unexpected ways. For example, they are important drivers of genetic diversity, they have been implicated in the acceleration of the biological carbon pump and their activity underpins the (re-)cycling of nutrients that support healthy marine ecosystems. However, there are very few studies of viral diversity for the Australian marine environments.

We carried out the first study of the genetic diversity of viral communities within the East Australian Current system and the adjacent Tasman Sea, two critical oceanographic systems that have a dramatic influence on the regional climatology of South-Eastern Australia. We analysed 60 metagenomes obtained from 24 different locations, that vary by sample depth and by fractionation method (0.2um, < 0.2um). Using both VirSorter[1] and bespoke analysis pipelines, we identified more than 20,000 viral contigs ranging from 2kbp to 190kbp, including more than 80 complete circular viral genomes, with the predominate group in the EAC genetically related to cyanophage. Preliminary analyses of viral diversity reveals four distinct groups, geographically clustered by surface water mass and depth. There was a clear distinction between the viral communities in the surface of the EAC and the colder Tasman Sea. This work provides an important baseline to support research to unravel the dynamics of microbial communities in a complex ocean system.

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Diversity dynamics and metabolic interactions of microbes living at the limits of life

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Lake Magic, located on the Yilgarn Craton in southern Western Australia, is a round lake with ~1km diameter and harbours one of the most unique environments on earth. It exhibits co-stressors of pH 1.6-4.5 and salinity of 32% TDS. The lake is known to have the highest concentration of dissolved aluminium, iron and silica in the world. Lake Magic undergoes stages of flooding, evapo-concentration and desiccation, depending on the local seasons. Thus, a large population of halophilic, halotolerant and acidophilic organisms interacting to drive key biogeochemical cycles are expected to reside in the lake, but even rudimentary understanding of the dynamics of microbial diversity during various lake stages is absent. Here, we used a temporal approach to understand prokaryotic diversity and metabolic interactions in lake water, sediment and salt mat through cultivation dependent and independent methods. The diversity dynamics were studied via high throughput 16S rRNA amplicon sequencing and flow cytometry. To further understand the survival strategies of microbes, their microbial interactions were studied in detail using culture dependent methods. We established a bottom up approach by using bacterial species isolated from Lake Magic in a well-controlled synthetic community. We studied the species metabolic interactions in all mono and pairwise cultures. The results indicated that the microbial diversity and composition are significantly affected by lake conditions. Furthermore, it was seen through the different time points that the sediment and salt mat communities evolved, becoming more specialized in buffering the increased acidity in the lake, as a strategy to survive.

The results from this study sheds light on the potential tactics to survive in this extreme environment. Studying these communities enables us to understand organisms living at the limits of life.

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Non-antibiotic pharmaceuticals contribute in dissemination of antibiotic resistance through mutation or horizontal gene transfer

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The spread of antibiotic resistance represents a global threat to public health, killing at least 700,000 people worldwide annually. Genetic mutation and horizontal gene transfer (HGT) are two dominant pathways to disseminate antibiotic resistance. It is commonly believed that antibiotics are major contributors in spreading antibiotic resistance through either mutagenesis or HGT. While pharmaceuticals are occurring in environments at increased levels, little is known whether non-antibiotic pharmaceuticals accelerate the spread of antibiotic resistance. We aim to fill the critical knowledge gaps in antibiotic resistance induced by non-antibiotic pharmaceuticals.

We first tested whether fluoxetine (an antidepressant drug) and triclosan (a common ingredient in tooth-paste and hand wash) could induce multi-antibiotic resistance on wild type *Escherichia coli*. Surprisingly, both fluoxetine and triclosan could cause significant resistance towards multiple antibiotics after 30-day exposure. The underlying mechanisms were revealed by a series phenotypic and genotypic experiments, including reactive oxygen species (ROS) production, genome-wide DNA sequencing, RNA sequencing and proteomic profiling. Fluoxetine or triclosan could induce ROS-mediated mutagenesis, trigger multi-drug efflux pumps, and further cause multi-antibiotic resistance.

We further investigated whether non-antibiotic pharmaceuticals (e.g. antiepileptic drug carbamazepine and triclosan) contribute in disseminating antibiotic resistance through HGT. Noticeably, these non-antibiotic pharmaceuticals with environmentally relevant concentrations non only promote conjugative transfer of multi-antibiotic resistance gewithin bacterial genera, but also enhance conjugation across bacterial genera. Similarly, the underlying mechanisms of the enhanced conjugation were revealed by combination of phenotypic and genotypic methods. Non-antibiotic pharmaceuticals induced a series of acute responses, including over-producing ROS, the SOS response; increasing cell membrane permeability, and pilus generation. Expressional levels of core genes related to these processes significantly up-regulated due to non-antibiotic pharmaceutical exposure.

Our study demonstrated that non-antibiotic pharmaceuticals not only induce multi-drug resistance via mutation, but also promote multi-antibiotic resistance gene transfer through horizontal gene transfer. Considering non-antibiotic pharmaceuticals are widely applied, our findings are wake-up calls to start re-evaluating the roles of non-antibiotic pharmaceuticals on inducing and disseminating antibiotic resistance.

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***Cyanothece* sp.CCY110, a promising bioremediation agent for potassium dichromate contaminated sites**

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Hexavalent chromium Cr(VI) compounds are highly toxic to the most life forms. Using cyanobacteria and microalgae as bioremediation agents can be a viable and cost-effective strategy to clean up chromium-contaminated sites without consumption of arable land and fresh water. Sixteen strains of cyanobacteria and microalgae were tested for tolerance against potassium dichromate ($K_2Cr_2O_7$) and for their ability to bioremediate the most toxic form of Cr(VI). Dichromate bioadsorption capacity was tested under different growth conditions (medium composition, temperature, and light intensity) in order to obtain higher biomass yield and higher bioremediation yield for dichromate ion. *Cyanothece* sp. CCY110, a marine cyanobacterium, presented remarkable features in which it was able to tolerate up to 10 mg/L of dichromate. While exposure to 1 mg/L dichromate showed no significant effect on the biomass yield, exposure to 5 and 10 mg/L dichromate resulted in about 20 and 40% decrease in the biomass yield, respectively. The bioadsorption yield at 1, 5 and 10 mg/L dichromate was 26.4±0.5, 22.8±0.3 and 16.8±1 percent, respectively. The optimization process revealed that changes in media composition, temperature, and light intensity significantly changed the

biomass production and bioadsorption yields, in which the highest bioadsorption and biomass production were achieved using ASNIII medium, 30°C, and 80 μEm^{-2}s^{-1} light intensity. This resulted in more than 50% increase in the biomass production yield (OD600 of 0.624±0.007), and more than 100% increase in the bioadsorption yield (46.4±1.1%). This suggests that the marine *Cyanothece* sp. CCY110 has a great potential for large-scale bioremediation of $K_2Cr_2O_7$ contaminated habitats.

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Shark bay microbial mat community responses to oil contamination a long-term lab-controlled experiment

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Shark Bay is considered as a unique assembly of environments and biota, and has been classified as a World Heritage site, thus granting its international protection. However, this does not exempt it from being subjected not only to a likely major petroleum spill from the exploration and production industry activities in the North of Western Australia, but also other hydrocarbon pollutants from recreational boats or commercial vessels crossing surrounding regions, i.e. Carnarvon or Exmouth. Therefore, making of Shark Bay a high-risk environmental region (AMSA, 2012).

Shallow pristine marine ecosystems in Shark Bay region have been well-studied lately (Allen, 2006; Pagès et al. 2014, 2015; Ruvindy et al. 2015; Plet et al. 2018). However, there is no information how these habitats might change to actual environmental threatens, e.g. predicted changes in seawater levels and oil spills. In order to contribute to a better understanding of their responses of such stressors, this study is focused on monitoring changes in the microbial composition of oil-polluted intertidal Microbial Mats (MM).

In our study, MM were collected from a hypersaline benthic environment, Nilemah embayment in Shark Bay, WA. These MM have been maintained in microcosms at 25°C with sterile hypersaline water, constant sterile air flux and diel regime by artificial light. The effect of oils that have been degraded to different extents has been subjected to smooth and pustular MM communities. The incubation experiments have been sampled before and after 30, 60, and 120 days of oil pollution. Samples of MM and oil traces in the water column were taken for organic geochemical analysis and, DNA and RNA were extracted in parallel for paired bacterial 16S rDNA vs.16S rRNA profiling. Therefore, it is not only expected to reveal those taxa that are present vs. metabolically active, but also identifying a range of metabolites involved in oil biodegradation (Bordenave et al., 2007; Aitken et al. 2018).

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Investigating the phylogenetic potential for bacterial mercury methylation

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Methylmercury (MeHg, CH_3Hg^+) is a potent neurotoxin and bioaccumulates in food webs. Microbial transformation of inorganic mercury produces most of the MeHg in the marine environment. The gene pair *hgcAB* encodes for Hg methylation, and this process has mainly been attributed to anaerobic bacteria. However, recent studies show the formation of methylmercury in aerobic water columns, although the mechanisms for non-anaerobic mercury methylation remain poorly understood. Our previous work found the marine microaerophilic bacterium *Nitrospina* as a potential mercury methylator within sea ice. Here, we found *Nitrospina*-like *hgcAB*genes were widely distributed in the global oxygen minimum zones (OMZs). We also identified a facultative anaerobe *Marinilabilia salmonicolor* carrying a putative *hgcAB* gene cluster that may enable Hg methylation. Furthermore, we found two aerobic *Alphaproteobacteria* carrying fused *hgcAB* genes which have very low identities (<40%) to any known fused *hgcAB* genes. We performed metagenomic analysis of seawater of Antarctic OMZs, to support culturing-based and culture-independent approaches to looking for new genes and pathways for potential non-anaerobic mercury methylation, and to refine existing models for mercury biogeochemical cycling.

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Isolation and detection of free living amoebae and associated bacteria from water storage tanks around Western Australia

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Free living amoebae (FLA) and amoebae resistant bacteria (ARBs) are a developing concern for water utilities globally. Several amoebae are resistant to chlorine and monochloramine disinfection in drinking water distribution systems (DWDS). In Western Australia (WA), FLA have been detected in the bulk water and biofilm of regional DWDS with sub-optimal disinfection residuals, however no work has been performed on the bulk water and sediment of drinking water storage tanks (DWSTs). This study investigated the presence and abundance of FLA and ARBs isolated from both bulk water and sediment samples from tanks in WA. Tanks sampled had a free chlorine residual above the required 0.5 mg/L and ranged from 0.57 – 0.96 mg/L. Viability and molecular testing was carried out using culture and quantitative polymerase chain reaction (qPCR) with melt curve analysis. Multiple viable amoebae were isolated from both bulk water and sediment samples. Molecular testing on total DNA isolates identified the presence of additional amoebae species and ARBs. This study shows that FLA and their associated ARBs are present in both the bulk water and sediment of well chlorinated tanks throughout WA. These findings are important to aid water utilities in monitoring of DWSTs to further protect consumers.

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A wolf in sheep's clothing: Protozoan expelled food vacuoles are a protective transmission vector for pathogens

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Vibrio cholerae, the causative agent of the acute diarrheal disease cholera, is an inhabitant of aquatic environments where it interacts with a wide variety of organisms, including heterotrophic protists (protozoa). Several species of these bacterial predators have been reported to release live, undigested bacteria in expelled food vacuoles (EFVs) when feeding on certain Gram-negative and Gram-positive pathogens. While the production of EFVs has been reported, their biological role as a vector for the transmission of pathogens remains unknown. Here we report that that protozoa release large numbers of EFVs when feeding on *V. cholerae*. The EFVs are stable, the bacterial cells within are protected from multiple stresses (low pH, antimicrobials and starvation) and vast numbers quickly escape when incubated at 37°C or in the presence of nutrients. We show that specific genes regulated by ToxR, play a significant role in the production of EFVs. Importantly, cells released from EFVs have a fitness advantage over planktonic cells both *in vitro* and *in vivo* and are highly infectious. Thus, results suggest that EFVs facilitate *V. cholerae* survival in the environment and as they move through the gastric environment, enhancing infectious potential and may significantly contribute to the dissemination of epidemic *V. cholerae* strains.

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Assessing the efficacy of continuous electrochlorination on the disinfection of drinking water

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Background

Disinfection is a vital step to produce safe drinking water eliminating the incidence of infectious diseases associated with water-borne pathogens. Traditionally, chlorine is the most commonly used disinfectant to treat drinking water. However, the use of chlorine gas or hypochlorite solution is associated with operational concerns especially from remote areas. Continuous electrochlorination (CEC) has gained a lot of attention as an alternative to the traditional use of conventional chlorination

technologies. CEC is a highly efficient and reliable mechanism to generate chlorine, however, its effects on surviving bacterial populations have not been yet sufficiently investigated.

Objective

Assess the efficacy of a CEC pilot unit including two successive electrolysis cells (i.e., pre and post oxidation) developed by Water Corporation of Western Australia on the disinfection of waters with different physicochemical characteristics.

Methods

The effects of the oxidants generated by the CEC on the disinfection of water has been tested on five source waters (ground waters and surface waters) located in Perth metropolitan area and regional areas of Western Australia.

Culture of common water-borne pathogens *Escherichia coli, Bacillus subtilis* and Bacteriophage *T4* were pumped into the feed line of the CEC pilot unit operated at flux ranging from 3 m^3/h to 30 m^3/h*;* Samples were collected immediately at the outlet of the pilot unit and quenched with sodium thiosulfate to remove any residual chlorine. The number of viable cells and chlorine residual were analysed by the spread plate method and DPD (N,N-diethyl-p-phenylenediamine) colorimetric method respectively, after both pre and post treatment.

Results

The CEC was successfully able to generate about 1.0 mg/L of free residual chlorine at the outlet whatever the initial chloride concentration of the source water. The oxidants generated were able to inactivate 6 log of each microorganism.

Conclusions

The CEC is an efficient and reliable on site generator of chlorine. The oxidants generated by the CEC can remove 6 log of *E. coli*, *B. subtilis* and bacteriophage T4.

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The influence of microbial biofilms and substrate materials on the settlement of marine invertebrates

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Seawall structures are an important ecological environment in any coastal setting. The protection of the land is a key role for these engineered structures, although they serve an alternate role as a habitat for many organisms from the marine environment. Therefore, it is vital to understand what features of these materials, typically natural stone and concrete, encourage or discourage settlement. Settlement may also be influenced by microbial communities, biofilms, that form on these structures. Thus, settlement may be a consequence of selection for a particular microbial community, driven by the physico-chemical attributes of the materials used. Alternatively, the microbial community may play little or no obvious role in larval settlement.

In this study, we monitored the initial settlement behaviour of the coral larvae *Pocillopora acuta* on substrates such as glass, ceramic tile, concrete and various stones (limestone, granite, marble and sandstone). We further compared settlement after first forming monospecies biofilms on these surfaces. Finally, we formed biofilm communities on these surfaces and correlated settlement efficiency with community and functional gene composition. The goal of these experiments was to determine what role the microbial inhabitants play in shaping the macro-inhabitants of the seawall substrates.

In line with previously published work, we showed that biofilms are a key agent in the induction of coral larval settlement. This was particularly evident for monospecies biofilms where the species used were genetically similar, but had striking differences in settlement efficiency. Our findings also suggest that the substrate is an important factor in coral larval settlement, where some substrates supported settlement and others either had no measurable effect or were toxic to larvae.

Thus, the settlement decision of larvae is likely to involve a complex process that accounts for the substratum properties as well as the microbial community that forms on that substratum. This

reinforces the need for further investigations to fully understand the relationships between substrate, biofilm and macro-settling organisms in a coastal environment.

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Secondary mineral formation during bioleaching of Merensky Reef materials: Implications for PGE migration

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The Merensky Reef is one of the world's iconic platinum group element (PGE) deposits, discovered by tracking PGEs that had weathered out from the platiniferous reef in the Bushveld Igneous Complex, South Africa. The bioleaching of Merensky Reef by *Acidithiobacillus ferrooxidans*, *Acidithiobacillus thiooxidans* and *Leptospirillum ferrooxidans* was studied to improve our understanding of PGEs migration and to identify any secondary minerals that might guide PGE exploration. The key materials comprising the Merensky Reef and targeted for bioleaching were chalcopyrite, pentlandite, pyrite and pyrrhotite, occurring within a matrix of clinopyroxene, orthropyroxene, chromite and norite, with the PGEs occurring as grains and distributed metals (Osbahr et al., 2013). Pyrrhotite and pyrite were shown to be important targets for bacterial weathering versus pentlandite and chalcopyrite, with dense bacterial colonization within 2 weeks, including iron phosphate and sulfur precipitation as secondary minerals. Pentlandite was oxidized in both abiotic and biotic systems, however, the colonisation of bacteria was only observed after 8 weeks bioleaching. Chalcopyrite was relatively inert to bioleaching in this silicate buffered system, which was consistent with the water chemistry. Orthopyroxene weathered easily under acid attack, and resulted in the release of silica and calcium. The chromite was found to be remarkably clean, and did not have any cells, i.e., any non-specific binding as observed with the silicates. No platinum or palladium was detected in the bioleaching solution, and no secondary, i.e., colloidal Pt or Pd was observed suggesting that weathering of PGE grains or distributed PGEs was not active. Most of the individual, micrometre-scale PGE grains identified at T=0 were found after leaching, demonstrating that while the matrix had deteriorated, most of the grains were still embedded in the ore specimen. Further mass wasting, including more active processes encountered during erosion are required before more active dispersion of grains, similar to PGEs migration in natural systems would occur.

1.Osbahr, I., Klemd, R., Oberthür, T., Brätz, H.,Schouwstra, R., 2013. Platinum-group element distribution in base-metal sulfides of the Merensky Reef from the eastern and western Bushveld Complex, South Africa. Mineralium Deposita. 48, 211-232.

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Biodegradation of detergent parameter on Bojongsoang Waste Stabilization Pond

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Bandung City is one of the most populous city in Indonesia with the percentage increase of population every year on average of 1.2%. The population density is causing environmental burdens as a buffer source of pollutants will be quite heavy. Detergent is one of the main parameters that is contained in the wastewater of the human population (domestic waste). High rates of detergent usage in community led to high detergent concentrations that are contained in the wastewater so it would be very disruptive to the sustainability of natural environment recovery process. At this time people commonly use detergent type of *Llinear Alkyl Sulfonate"*(LAS), which is environmentally friendly (easy to decompose in the environment). Detergent concentrations degrade through the processing with stabilization pond system. Specifically detergent degradation in the stabilization pond made through biodegradation by bacteria decomposers detergent.

Purpose of this study is to determine biodegradation detergent on stabilization pond. Degradation process of detergent contents in the wastewater carried through phases those are anaerobic process, facultative process, and maturation process as a whole could degrade levels of detergent by 92.52 %. Concentration of detergent in the domestic wastewater inlet channel by an average of 6.69 mg / l. Detergent concentrations after process by an average of 0.50 mg / l, the percentage of its reduction is 92.52 %. The highest degrade process occurred in facultative pond by an average of 77.57 %. From that degradation then carried out the identification of microorganisms' that was decomposing detergent in the facultative pond. Microorganism identification made by growth test of medium "minimum salt detergent" (DMS). The results of identification in facultative pond obtained seven types

of bacteria decomposers detergent. Those are *Xenorhabdus bovienii*; *Bordetella avium*; A*minobacter sp*; K*luyvera ascorbata*; *Acinetobacter sp; Enterobacter sakazaki; Plesiomonas shigelloides.*

From these studies it can be concluded that the content of detergents in domestic wastewater can be degraded significantly on biological process with help of certain bacteria that live naturally in the stabilization pond.

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Determination of phage host range by viral-tagging

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Publish consent withheld

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The role of bacteriophage in structuring microbial communities in wastewater treatment systems

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Bacteriophages can impact bacterial communities, not just through predation, but also through the regulation of host genes that can improve biofilm development. Bacterial communities are essential in the function of most ecosystems including engineered systems such as activated floccular sludges of water reclamation plants. In these systems, bacteria are responsible for nutrient removal and flocculation. Activated floccular sludge is often used to cultivate aerobic granules and the formation of such granules is normally achieved by selection through physical parameters. Although granule formation has been optimised by controlling these physical factors, granule instability and the long initiation times for their formation remain a challenge for their implementation in full scale water reclamation plants. As granules represent a suspended biofilm, it has been hypothesized that bacteriophages are important in the formation of granules. However, few studies have characterized the abundance and diversity of bacteriophages during aerobic granulation.

In this study, four reactors were operated for simultaneous nitrification, denitrification and phosphorus removal, and a metagenomics approach was used to monitor the abundance of bacteriophages and the bacterial communities of the granulating sludge. It was observed that there was an increase in abundance of Inoviridae during the initiation of granulation suggesting a role of filamentous phages in the formation of granules. Furthermore, there was also a shift in the abundance of lytic phages during granule maturation, suggesting that lysis of certain bacterial genera may also be important in granulation. Lytic bacteriophage may have selected for bacteria that made up denser sludge, and filamentous bacteriophage may be part of the granule structure. The changes in the abundance of bacteria, especially that of '*Candidatus* Accumulibacter', as well as physical factors such as settling time and biomass discharge are also important in this process.

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Comparison of DNA- and RNA-based 16S rRNA diversity profiling of the microbial community recovered from a Western Australian oilfield production facility

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Investigation of the microorganisms living in the fluids of oil production facilities is fundamental for an early determination of the presence of microbes that can potentially produce localised attack to the infrastructure; phenomenon acknowledges as microbiologically influenced corrosion (MIC). In the last decades, the microbiological characterisation has been mostly carried out by the implementation of traditional growth-based techniques. However, with advances in biotechnology, molecular microbiological methods (MMMs), which are culture-independent techniques, have begun to replace the conventional methods. Microbial diversity profiling based on DNA sequencing of the 16S rRNA gene is one of the methods being used by MIC researchers and oil operators to help identify and characterise the microorganisms present in the systems. DNA-based analysis has been found useful in the classification of the corrosive microorganisms, however, it is unable to differentiate among dormant, active or dead cells. Considering that only metabolically active microorganisms can cause

corrosion processes, other methodologically approaches are required for a better understanding of the implications of the microorganisms in the deterioration of metals. To assess the diversity and composition of the total and active microorganisms present in the produced water of a Western Australia oilfield, six samples were collected in different locations of the production facility. DNA and RNA were co-extracted from each sampling site, and 16S rRNA gene was sequenced by Illumina MiSeq platform. Bioinformatics analysis of the sequencing data revealed that the microbial community structure is similar along the facility. Comparison of the DNA- and RNA-based diversity profiling showed that the majority of the microorganisms present in the system were in an active state. Significant differences were found in the relative abundance of the microbial species associated with the *Euryarchaeota* and *Thermotogae* phyla, which were possibly linked to the changing environmental conditions in the facility. Our results highlight that the RNA-based 16S rRNA diversity profiling can significantly complement the information currently generated with the DNA-based methods for the study of MIC.

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Evaluating microbiologically Influenced corrosion using the wire beam electrode (WBE)

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The role of microorganisms in the corrosion of steels has been a topic of interest for decades which has major significance for Australian industry and implications for oil and gas and marine infrastructure. A bacterial strain isolated from an oil production facility in Western Australia was used to grow biofilms on carbon steel and conduct immersion corrosion tests under CO_2-conditions. Localised corrosion originated as a result of microbiologically Influenced corrosion (MIC) was investigated using a novel multi-electrode array system which provides spatial and temporal electrochemical information of metals in the presence of biofilms. Galvanic current/potential distributions maps at the WBE showed heterogeneous electrochemistry at the biofilm/steel interface. This novel approach will allow studying MIC mechanisms and its inhibition.

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An integrated biohydrometallurgical process for metal recovery from electronic wastes

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The treatment and recycling of electronic waste (e-waste) is a national priority in Australia. In Australia, only 10% of e-waste generated is recycled and the majority of the unrecycled waste is sent to landfill. This unrecycled e-waste contributes to 70% of the toxic chemicals in landfill, representing a large source of land and water contamination. This has led to environmental and human health concerns, especially as the volume of e-waste is growing faster than any other waste stream. Given its high metals content, e-waste is increasingly considered as a resource for metal values. It has been estimated that, in Australia the value of copper in e-waste is more than US$103 M annually. With a projected rise of 700% in the copper price over the next 25 years, recovering metal values from e-waste seems highly attractive. Currently, no economically feasible technology is available to facilitate their recovery in Australia. Largely due to the scattered population, which incurs a significant cost in transportation of e-waste. A decentralised treatment may be more suitable over centralised treatment for unlocking metal values from e-waste in Australia. This project aims to develop a novel, integrated biohydrometallurgical process that could be used as a decentralised treatment for recovering metals from e-waste. The process eliminates the use of high temperatures as microorganisms are used as catalysts. Further, other low-cost waste materials such as waste organics and sulfur will be employed to drive biogenic lixiviant generation, which can help reduce operating costs. This study will be the first of its kind to assess a complete flow sheet for biohydrometallurgical processing of e-wastes. The techno-economic feasibility of the process will also be evaluated.

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Efficiency and microbial ecology of a sulfate-reducing fluidized bed reactor treating mine water after hydrotalcite precipitation

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Acid mine drainage (AMD) typically consists of a suite of contaminants (e.g. sulfate, metals and acidity) prohibiting its direct release into the environment. On-site treatment is therefore required to reduce both the acidity and contaminant concentrations prior to discharge. The Virtual Curtain (VC) technology (licensed by CSIRO) can be applied to AMD treatment. Underpinning this technology is the formation of hydrotalcite, a layered double hydroxide mineral that can effectively remove a range of contaminants. However, the efficiency of the technology to remove sulfate is limited. Therefore, this study aimed to integrate biological sulfate-reduction (using fluidised bed reactor (FBR) process) with the VC process to maximise sulfate removal from AMD and characterise the microbial community in the FBR process. AMD from an Australian gold mine (pH ~4, sulfate 1500-1900 mg/L, with various metals including Al, Cd, Co, Cu, Fe, Mn, Ni, Zn) was first treated with the VC process. Subsequently, the effluent was treated in a laboratory-scale FBR with granular activated carbon as a biomass carrier, and ethanol as a microbial carbon source and electron donor at a hydraulic retention time of 1-2 days. The results showed that the VC process readily neutralised the AMD acidity (to pH ~7) and removed >99% of Al, Cd, Co, Cu, Fe, Mn, Ni, Zn and 10% sulfate. The subsequent FBR treatment increased the overall sulfate removal to ~80%. The microbial community in the FBR was characterised using next generation sequencing of 16S rRNA genes. The dominant families in the biofilm were *Anaerolineaceae* (40.9%), *Desulfomicrobiaceae* (17.9%), *Desulfovibrionaceae* (17.7 %) and *Lentimicrobiaceae* (11.2%), whereas the suspended microbial community was dominated by *Anaerolineaceae* (15.0%), *Desulfovibrionaceae* (25.6%) and *Spirochaetaceae* (12.5 %). Overall, this study confirmed that combining the VC process with a sulfate reducing FBR process was effective for AMD treatment.

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Application of plant-immune biosensors for screening and discovery of Actinobacteria biocontrol candidates against necrotrophic fungal pathogens

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Actinobacteria present a phylum of gram-positive bacteria, which can be terrestrial or aquatic. They form an essential part in our economics as agriculture and forests depend on their contribution to the soil system. They are of great interest as these bacteria can be applied in agriculture as plant growth promoting agents as well as in industry for their production of natural bioactive metabolites such as antibiotics and pathogen inhibiting compounds. Plant beneficial isolates are able to inhabit the plant root system without causing a disease and some are able to prime a plants immune response against certain pathogens. They are largely influenced by their surroundings where abiotic and biotic factors have impact on their metabolite production.

We have a collection of Actinobacteria isolated from south-west Western Australia. Our goal is to identify isolates that can prime a plants' immune response with potential to provide protection against a broad range of phytopathogens, but with a focus on protection against economically destructive necrotrophic fungal pathogens. This will be achieved through a novel approach, non-destructively

capturing visible plant immune-biosensor output (gene-based reporter systems) in real-time following inoculation with potential Actinobacteria biocontrol agents to identify isolates with novel plant immunity inducing properties. This way, plant microbe interactions can be monitored and involvement of Actinobacteria in plant defense signalling pathways can be determined. In combination, the Actinobacteria isolates are assessed for production of biocontrol compounds (e.g. antifungals) via routine *in vitro* pathogen growth inhibition assays. Using a combination of omics-guided approaches (genomics, transcriptomics, metabolomics), we aim to identify, extract and purify these compounds, and develop a biocontrol fungicide for agricultural disease control. In order to increase metabolite production in the selected Actinobacteria isolates, different culture conditions are being used to identify the optimal environment. By combining immune biosensor screening together with antifungal bioactivity assays, strong Actinobacteria strains can be selected with the potential of improving pest management for our agriculture.

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Phosphate precipitate formation during bioleaching of secondary copper sulfides

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The interaction of acidophilic iron and sulfur-oxidizing bacteria (*A. ferrooxidans*, *L. ferrooxidans* and *A. thiooxidans*) with a bornite-chalcocite ore specimen from the Salobo mine, Carajás, Brazil was examined at pH 4 and pH 2 to determine the effect of secondary mineral coatings on bioleaching / copper solubilization. In order to select for bacteria that could attach to mineral surfaces and promote colony formation (bacterial growth), polished sample chips were suspended in the bacterial consortium for 24 hr, and then transferred to fresh media for growth, providing a limited window for colonization. After 4 weeks growth, and using scanning electron microscopy, the bornite-chalcocite ore possessed colonies on all bornite-chalcocite surfaces, evenly scattered individual cells on the non-sulfidic minerals in the ore specimen, and secondary copper phosphate on the bornite-chalcocite ore at pH 4 versus iron phosphate in the pH 2 reaction systems. Viable cell counts in both systems were two orders of magnitude higher than the original inoculum by the end of the experiment indicating that bacteria are released from mineralized biofilms when growing on bornite-chalcocite surfaces. At pH 4, the lack of iron in solution and in the precipitates formed on the bornite-chalcocite ore sample, along with an examination of the mineral surface after removal of the copper phosphate precipitate with ion cleaning indicates a preferential leaching of chalcocite, with no significant solubilization of bornite. While secondary mineral formation, i.e., copper-phosphate formed at pH 4, can limit the recovery of copper, iron phosphate mineral coatings formed at pH 2 did not impact bacterial growth or copper recovery.

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Understanding soil microbial community shifts in response to fire and weed invasions in urban *Banksia* woodlands

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Urban bushland fragments in the Perth metropolitan area are under an ever-increasing amount of threat, with encroaching development and the changing climate being so severe, it has been classified as endangered [1]. How this ecosystem will be affected by these pressures is not well understood as parts of the community and their interactions have never even been described. For a 'global biodiversity hotspot' [2] not much is known about Western Australia's soil microbiota and how it influences the plant community, and therefore surprising outcomes could occur when environmental variables are changed. The difference in seasons is set to become even more stark with drier hotter summers and higher incidences of 'freak weather events' (such as droughts and wildfires) [3][4], so more knowledge is required to accurately manage the ecosystem for it to stay as diverse as it is. This study set out to quantify and describe the soil microbial community and whether it is influenced by Fire regime (Time since fire, fire type, ignition month, etc.) and how this affects (or is affected by) the plant community. Collecting soil samples and undertaking plant surveys would allow for a description of the soil microbial diversity (via PCR and DNA analysis) [5] and begin to fill a knowledge gap, but also quantify how fire regime changes the soil microbia composition and thus how this influences the prevalence of symbiotic plants species, with further implication for invasion risk from non-native plants.

However, there are chances to resist invasion, as if the native soil microbia are not compatible with the invasives, then they will be less fit and could be outcompeted by the native species [6][7].

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Bacterial communities on Black Perigord Truffles (*Tuber melanosporum*) during storage

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Truffles (*Tuber melanosporum*) are high value commercial fungi traditionally produced in the northern hemisphere. There is little understanding of the ecology of truffle bacterial microbiome and the role it plays in aroma development and spoilage during post-harvest storage. The aim of this study was to investigate the population structure and the functional potential of the bacterial communities associated with black perigord truffles from Spain using metagenomics and gas chromatography mass spectrometry. Black perigord truffle samples (N=14) were provided by Australian Truffle Traders, Manjimup, Western Australia during the Spanish truffle season. The fresh truffle samples were assessed at 3 time intervals, 0, 7 and 14 days to monitor the change in the bacterial population during storage. The V3-V4 region of 16s rRNA DNA was sequenced using the Illumina MiSeq Platform using paired end reads. Alpha-diversity of the Spanish truffles was low, with *Gammaproteobacteria* and *Alphaproteobacteria* dominating the populations. Operational taxonomic units relating to Enterobacteriaceae, *Rhizobiaceae*, and *Bradyrhizobiaceae* families were the most abundant sequences in the samples. Previous studies have also found Rhizobiales dominate across truffle species and geographic regions. The results in this study add further evidence to the possibility that truffles may form a mutualistic relationship with Rhizobiales, with the bacteria able to fix atmospheric nitrogen in the truffle fruiting bodies. Additionally, dimethyl sulphide compounds produced by *Enterobacteriaceae* may explain the high concentrations of DMS found in our GC-MS analysis of the truffles. Similar analysis is currently being performed with Australian black perigord truffles. It is hoped that by comparing data from the two regions this will allow truffle growers an insight into how the microbiome of the region may influence the growth and aromatic qualities of their truffles and the impact these microbes have on truffle spoilage.

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Microbial variations: Relationships between biogeochemistry, community composition and function across a 900 km aridity gradient

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Multiple soil functions will be affected by climate change, both through direct changes to the composition of soil microbial communities through changed rainfall and increased temperatures, and indirect and longer term changes to the composition and structure of associated vegetation communities. This study examined soil samples taken from 42 sites along a 900 km transect established under the Terrestrial Ecosystem Research Network (TERN) in South Australia. Microbial communities were analysed for community structure using 16S, 18S and ITS Illumina amplicon sequencing, and also by PLFA analysis to provide robust information on microbial community structure at time of sampling. To investigate microbial activity, rates of glucose mineralisation were quantified using ^{14}C-labelling approaches, and stable isotope probing using ^{13}C-glucose was conducted to identify the structure of the active microbial community. Beyond microbial measurements, comprehensive analysis of soil biogeochemistry including litter and soil organic matter chemistry by NMR was conducted. Combined with vegetation community structure and climatic information, these data were then analysed using multivariate statistical approaches to understand the strengths of relationships between vegetation, climatic, soil and microbial variability along the transect. We found that aridity, rather than SOM chemistry was the major driver, with bacteria and archaea (16S sequences) being influenced to a greater extent than fungi (ρ = 0.764 and ρ = 0.454 respectively, both $P \leq 0.001$). Though there was no relationship between fungal community structure and SOM chemistry, bacterial and archaeal community structure was also influenced by SOM chemistry (ρ = 0.269, $P \leq 0.001$). Ongoing work, particularly the finalisation of the ^{13}C-SIP activity, will help elucidate linkages between these datasets and their functional role in the environment.

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Diversity and function of phosphate solubilizing bacteria isolated from Mount Weld rare-earth mine Australia

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Currently the known high grade easily-acquirable reserves of rare earth element (REEs) containing phosphate minerals are depleting. The objective of this study was to enrich indigenous phosphate solubilising bacterial strains from various phosphate ores and evaluate their potential application in the bioleaching REEs from the ores. Bacterial communities were enriched from the highest grade known deposit of REEs in the world, in arid Western Australia. The dominant taxa enriched from the monazite concentrate were *Actinobacteria*, *Proteobacteria*, and *Firmicutes*. The consortium of indigenous bacteria solubilized REEs (Ce, La, Nd) up to a total final concentration of 0.836 mg L^{-1}.

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Microbially catalysed iron mobility within the critical zone at Salobo, Brazil

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The mobility of iron within the surficial zones of secondary metal deposits is well understood from a chemical processes perspective. However, here we examine the mobilisation of iron within the critical zone at the Salobo iron-oxide copper gold mine in Brazil from the perspective of iron-oxidising bacteria. The Salobo mine is an excellent study area due to a mineral assemblage exceptionally rich in iron (up to ~60 wt. %).A biogeochemical field study of weathered materials from the critical zone at Salobo was complemented by bench-top leaching experiments of fresh rocks, partly weathered rock and oxidised materials, using endemic, iron-oxidising acidophilic bacteria, e.g., *Acidithiobacillus ferrooxidans*. The bench-top study examined the interaction of iron-oxidising bacteria with ferrous iron-bearing minerals including sulphides, which are conventional substrates in supergene systems, as well as more refractory sources of energy, i.e., silicates and magnetite. The results are linked to detailed analyses

of textures and secondary mineral assemblages within the critical zone at Salobo and shed light on the role of iron-oxidising bacteria in the mobilisation of iron as well as the mobility of other elements, such as copper, manganese and phosphorus as a by-product of microbial activity.

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Urban bandicoots - a vector for ectomycorrhizal dispersal

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Mycophagy among Australian mammals is widespread and likely plays an important role in the dispersal and maintenance of fungi communities within the ecosystem. While many native mycophagous species have declined greatly over the past 200 years, the quenda (*Isoodon fusciventer*) has remained abundant across southwest Western Australia, persisting even in urban and peri-urban environments. As omnivores, quenda are known to feed opportunistically on fungi, but what type and how this changes with urbanisation has not yet been investigated.

Quenda scats were collected from foraging digs at thirteen bushland remnants on the Swan Coastal Plain south of Perth. Scats were analysed using high throughput sequencing to identify the fungi present. More than 800 fungal molecular operational taxonomic units (OTUs) were found across the 60 unique scats examined. There was an average of 46.1 OTUs per scat sample (range 8–120), with a significant negative correlation between the number of OTUs per sample and the distance from the urbanisation (Rs=-0.348, p=0.007). Scat fungal community was correlated with vegetation condition in bushland remnants, with a greater diversity of fungi from scats collected from natural bushland reserves compared to suburban remnants. Over half of the fungi OTUs detected (57.6%) putatively formed macroscopic fruiting bodies – fungi that would intuitively be most attractive to bandicoots, and a high proportion of the OTUs identified were known to be mycorrhizal (47.8%). Mycorrhizal fungi facilitate soil nutrients and water uptake for their host plant, playing an important role in healthy plant functioning. The consumption and dispersal of mycorrhizal fungi in quenda scat may have flow-on effects for plant health through mycorrhizal fungi dispersal.

This data gives us insight, not only into the fungal component of quenda diet, but also allow us to draw inferences about the fungal community found across the urban interface and the potential role of quenda in dispersing mycorrhizal fungi.

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Manure application promotes transmission of antibiotic resistance genes to vegetables

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The aggregation of antibiotic resistance genes (ARGs) in agro-ecosystems and their potential transmission into the food chain represents a serious threat to public health. However, we have very limited knowledge of the pathways and mechanisms for transmission of antibiotic resistance from the soil to vegetables following land application of animal manures. Here, we constructed a glasshouse pot experiment to explore the impacts of poultry or cattle manure application on the patterns of resistome and microbiome in the rhizosphere, endosphere and phyllosphere of the harvestedlettuce and cherry radish. In total, 145 ARGs and 10 MGEs were detected in all the samples. Manure application increased the ARG diversity and abundance in bulk soil, rhizosphere soil, phyllosphere and root endophyte for both lettuce and cherry radish. Moreover, phyllosphere and endophyte shared a large number of ARGs with manure and soil, suggesting that manured soil was an important source of ARGs detected in vegetable samples. Procrustes and network analysis suggested that the profile of ARGs was strongly affected by bacterial community compositions and four bacterial phyla were identified as potential hosts of ARGs.Our study suggests that vegetables grown in manure-amended soils are at risk of contamination by manure-borne antibiotic resistant bacteria.

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Screening of potassium solubilizing strains to develop different microbial consortium for wheat production

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Potassium (K) is an essential macronutrient, essential for regular biological functioning of plant and have significant impact on productivity but mainly present as insoluble form in soil. In this study, we have isolated, characterised and tested three soil bacteria which have shown potential to K-solubilization in addition to other plant growth promoting functional traits (IAA, P-solubilization, NH3). Based on 16S rRNA gene sequencing, these bacteria were identified; - *Burkholderia* sp., (KSB-1) *Pseudomonas* sp. (N55) - *Pseudomonas* sp. (P23). In a growth chamber experiment, we tested there capability in as a single isolate or in different consortia (KSB-1+ N55, KSB-1+ P23, N55+ P23, KSB-1+ N55+ P23). The controlled bio chamber experiment was conducted for wheat cultivar; Gazelle, to evaluate the impact of microbial inoculants on plant growth and productivity. We examined of shoot, root and grain yield response to inoculants treatments, which indicated that up to 29% (P23) and 16% (N55) increase root and shoot fresh weight, respectively over control. An increase of 15.76% (KSB-1) higher plant heights over control. For total productivity measured in terms of grain yield/plant, a noteworthy enhancement was recorded in all bacterial treatments in range of 46.57 to 16.43% over control. Future research will be focussed on identifying biotic (e.g. change in rhizosphere microbial communities) or abiotic (increase availability of nutrients) mechanisms that drives plant fitness and productivity gain and inoculants efficacy in field conditions with different wheat varieties.

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Assessing the microbial diversity and ecotoxicity in co-contaminated soil with aged Heavy Metals and Total Petroleum Hydrocarbons

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Globally, land contamination with petroleum hydrocarbons (PHCs) represents a significant challenge as they pose serious risks to natural ecosystems and human health if left untreated. The first step in removing PHCs from the environment is performed by the indigenous microorganisms, a process termed bioremediation. The ability of soil's indigenous microbial population to degrade PHCs offers a natural bioremediation approach with many advantages over traditional techniques. Understanding the changes dynamics of the microbial communities occurring following contamination is a crucial process since microorganisms are the backbone of any microbial degradation process. Many factors affect the biodegradation rate of the contaminant, in particular, the presence of co-contaminants (e.g. heavy metals) which makes the natural bioremediation a very complex process as heavy metals may have toxic effects on the microbiota.

The aim of this study was to evaluate the ecotoxicity associated with co-contamination in bioremediated soil samples as the exposure to multiple contaminants might increase the toxicity. In addition, this study aimed to improve knowledge of the dynamics of bacterial communities in the co-contaminated soil to enable the more appropriate design of bioremediation strategies for co-contaminated soils.

Contaminated soils were collected from former diesel power stations located in Marble Bar – Western Australia. Toxicity, as measured using the Microtox test, showed that the presence of both PHCs and heavy metals significantly ($(p \leq 0.05)$ elevated the ecotoxicity. Toxicity was correlated with the presence of lead (Pb), zinc (Zn) and PHCs (89, 60, 49%), respectively. 16S amplicon sequencing revealed a lack of dominant genera; however, despite the variation in soil type, several genera were present in most soil samples such as *Azospirillum* spp. which has been previously found in uncontaminated soils from hot and extreme arid regions. Likewise, many genera of hydrocarbon-degrading bacteria were identified in all soil samples, such as *Acinetobacter* spp. which has been associated with co-contaminated sites. This study concluded that PHCs and heavy metal co-contamination significantly elevated the associated toxicity.

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Canga lake-edge field characterisation: inspiration for iron ore mine remediation

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Iron ore provinces in tropical regions, including Brazil and Australia, are blanketed by an iron-rich duricrust, referred to as canga. To extract the iron ore, the canga must be removed, destroying the natural landscape and the associated biome. Iron ore remediation must therefore target the regeneration of the iron-rich blanket to provide a substrate for revegetation. Canga duricrusts are some of the longest-lived continuously exposed surfaces on Earth, evolving via the dissolution and precipitation of iron oxide minerals, mediated by the biome [1]. At a lake-edge near the S11D mine in the State of Pará, Brazil, canga appeared to form relatively quickly on a slope dipping 20 – 30°. Solutions flowing into the lake contained approximately 5 ppm $Fe^{2+}_{(aq)}$ and supported abundant microbial biofilms associated with fresh iron oxide precipitates. This highlighted the role of microorganisms in the dissolution of otherwise stable iron oxide minerals and the importance of water to transport renewable iron. These canga cements also contained microfossils, demonstrating microbial involvement in the formation of the ferruginous cements. Inspired by this natural system, the 'lake-edge' environment was replicated in the laboratory to optimise the conditions for biogeochemical iron cycling, which was then used to accelerate iron oxide cementation. For the treatment experiment, microbially-driven iron oxide dissolution was promoted in a reactor and the solutions from the reactor were allowed to flow over crushed canga. Water chemistry and the microbial biome for the treatment were monitored and compared with the ultrapure water-only control for the duration of the six month experiment before substrate hardness was measured after a two week drying period. This experiment provides promising results that microbially-driven iron oxide cycling may provide a novel biotechnological tool for iron ore mine remediation, accelerating slope stabilisation and the regeneration of a substrate akin to natural canga for revegetation programmes.

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Responses of comammox *Nitrospira* to four nitrification inhibitors in two agricultural soils

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The newly-discovered complete nitrification process by "comammox" bacteria, capable of oxidizing ammonium to nitrate in a single organism, is hypothesized to play a crucial role in the terrestrial nitrogen cycle. However, the responses of comammox bacteria to nitrification inhibitors and their relative contribution to nitrification in agricultural soils remain largely unknown. Here, we carried out a microcosm study to evaluate the impacts of four different nitrification inhibitors (acetylene, 1-octyne, nitrapyrin and DMPP) and N fertilizer ($(NH_4)_2SO_4$) on the abundance of comammox *Nitrospira*, ammonia-oxidizing archaea (AOA) and ammonia-oxidizing bacteria (AOB) in pasture and vegetable soils. We found that addition of $(NH_4)_2SO_4$ significantly increased the nitrate concentrations and addition of all these four nitrification inhibitors effectively reduced the production of nitrate in soil. Furthermore, the *amoA* gene abundances of comammox *Nitrospira* clade A, AOA and AOB significantly increased after the application of $(NH_4)_2SO_4$. The *amoA* gene abundances of comammox clade A were significantly inhibited by acetylene and nitrapyrin. AOB was significantly inhibited by all these four nitrification inhibitors though to varying degrees, and AOA was significantly inhibited only by acetylene. These results indicated acetylene could effectively impede the abundance of comammox *Nitrospira* and canonical nitrifiers, but 1-octyne only could inhibit the growth of AOB in agricultural soils. Our findings will provide valuable scientific basis for the efficiency of different nitrification inhibitors on the growth of comammox *Nitrospira* and canonical nitrifiers in agricultural management practices.

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The vineyard soil microbiome distinguish wine producing regions

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Regional variation in grape and wine quality characteristics is a critical feature of perceived product identity, with significant consequences for consumer preference and economic appreciation. Soil is a fundamental factor of viticulture, but the associated soil microorganisms have been largely ignored. In this study, we comprehensively investigated the microbial ecology of vineyard soils across southern Australia, suggesting soil-borne microbiome distinguish wine regions, especially fungal communities. Soils were collected from 14 vineyards (three sites per vineyard) in seven wine producing regions across southern Australia and also extensively sampled from one region to test the impact of vintage on soil microbial patterns. Next-generation sequencing of 16S ribosomal RNA and internal transcribed spacer ribosomal sequence was performed to build both bacterial and fungal distribution patterns. Results show that soil bacterial communities were dominated by *Actinobacteria* (32%) and *Proreobacteria* (31%), while fungal communities were less diverse, dominated by *Ascomycota* (70%). Species richness (α-diversity) of bacteria varied with regions but not fungi. Meanwhile, phylogenetic β-diversity of both bacterial and fungal communities segregates by region, based on principal coordinates analysis (PCoA) of weighted UniFrac distances. Random forest supervised learning models revealed that fungal models display a large degree of predictive power to distinguish soil samples coming from various regions. Furthermore, bacterial and fungal communities exhibit different responses to vintage and site-specific effects. Macroregion mainly drive vineyard soil bacterial community assembly, but fungi are more important for vintage effects. These findings will enrich understanding of vineyard microbial ecology, which is of great importance for regional origin of wine and agricultural systems and will enhance agricultural management and improve supply, consumer acceptance, and economic value.

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How can we better manage soil and plant microbiomes to increase crop production?

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The human population is predicted to reach 9 billion people by 2050[1] and there is a need to increase crop production on current agricultural land to meet the rising demand for food. Farmers routinely manage both plant and soil nutrition to increase crop yields. However, active management of soil microbiomes to stimulate crop growth is still in its infancy. Here, we present a patented technology to engineer soil microbiomes to increase crop production.

Bioprime[2] is a ferment of molasses that is routinely applied as seed coating, or as foliar and soil spray. It contains many diverse carbon compounds including 2,3-Butanediol and acetoin which directly stimulate plant growth[3]. Bioprime stimulates certain microbial taxa (e.g. Acidobacteria and Actinobacteria) while suppressing others (e.g. Alphaproteobacteria). Bioprime has been routinely applied to vegetables (e.g. carrots), strawberries, and table grapes in the horticulture industry for many years to control diseases (e.g. *Pythium, Fusarium, Sclerotinia,*) and has been trialled in broad-acre agriculture.

We conducted an on-farm potato trial in 2018 where Bioprime was applied (100 L/ha) into a soil previously amended with compost (40 t/ha in 2013) and into an unamended soil. Treatments containing Bioprime resulted in a significant increase in marketable potato yields (+33.8% for Bioprime and compost; and 33.2% for Bioprime $P \leq 0.039$) while compost on its own did not ($P = 0.214$). This was despite the historic compost application still being identifiable by soil chemical parameters (e.g. 30% more carbon, 100% more nitrate, and 11% more phosphate). The soil microbiome was also lastingly altered by both Bioprime and compost amendments as determined by ARISA and shown by principle component analysis for bacteria, archaea, and to a lesser degree fungi.

Bioprime on its own and combined with compost significantly increased potato yields highlighting the potential of this technology to improve crop production even on less fertile soils by engineering the soil microbiome and thus help feed the ever-growing world population.

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Evidence of primary surface colonizers on iron duricrust (canga) in Carajás, Brazil

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In the elevated plateaus of Carajás province in Brazil, ferruginous duricrusts, locally known as canga, protect the underlying weathered banded iron formations (BIF) - iron ore deposits. The processes that govern canga formation are still unclear but include recurrent partial dissolution and recrystallization of goethite through iron reduction and oxidation. Recent studies[1,2] suggest past and present biogeochemical cycling of iron within canga occurs at the surface or near-surface environment.

Field observations of exposed canga surfaces located near the banks of perched or ephemeral lakes identified surfaces encrusted with a mm-thick dark mat that, at first glance, could be mistaken for an oxidised coating. However, a combination of electron microscopy and metagenomics reveals the nature of the pervasive organic mat covering these goethite-cemented surfaces. A symbiotic community of cyanobacteria and fungi are acting as primary colonizers on this harsh, nutrient-poor substrate. In this mutualistic interaction, functionally resembling that of lichen; the hardy fungi shelters bacteria from UV damage, dry weather and exceptionally hot surface temperatures, whilst the cyanobacteria and their secretions provide a carbon source. The ‘thick’ bacterial biofilm might also act as an adherence agent for the larger heterotrophs.

This epilithic community seems to contribute to canga evolution in two opposing processes: (1) the bio-weathering of the surface through excretion of extracellular metabolites, such as organic acids; and (2) the bio-protection of weathered fragments through stabilization and trapping of fine particles. The weathering processes are both chemical and physical, with evidence of iron mobilized in the biofilm, and clear signs of cracking due to microbial activity. Underneath the live biofilm, multiple layers of fossilized bacteria are detected, implying an active growth front with a continuous weathering and formation cycle at the canga surface.

Understanding the contribution of microorganisms to the biogeochemical cycling of iron in canga is crucial when formulating post-mining biotechnological rehabilitation strategies, e.g., slope stabilisation, for global iron ore sites.

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Arbuscular mycorrhizal fungal diversity response to fertiliser, tilling, and crop rotation in wheat rhizospheres over multiple years

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Arbuscular mycorrhizal fungi (AMF) are soil microbes that colonize up to 90% of all land plants and offer benefits in the form of nutrient acquisition, water retention, and resistance to pathogens and pests. AMF diversity is thought to respond to a range of biotic and abiotic factors in soil management practices. This was studied in a multi-year wheat field trial incorporating variation in fertiliser application (N and P), tilling and crop rotation. The AMF diversity in each sample was analysed by PCR amplification with AMF-specific DNA primers that span the small sub-unit to the large sub-unit of the ribosomal RNA gene and the products were sequenced using Illumina MiSeq. These primers were chosen for this study because they provide greater coverage and depth of AMF sequences compared to traditional fungal primers. The AMF diversity of individual samples differed by treatment in fertiliser type and amount, tilled versus not-tilled, and previous crop rotations of canola or chickpea. Bioinformatic analyses showed a strong correlation between AMF diversity and field trial conditions,

particularly between the AMF genus *Funneliformis* and rhizosphere samples with no fertiliser or tilled treatments, and grown in conjunction with chickpea during rotation years. This indicates that treatments have a strong influence on AMF species diversity in agricultural trials, which may be explained by the filling of niches dictated by functional traits at the AMF genus or species level. Further research on functional traits will elucidate how AMF can be strategically applied for reducing chemical inputs and water use in agriculture.

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Microbial interactions between representative skin microbiome members in a mixed species microbial biofilm community

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The commensal skin microbiota plays an active role in maintaining skin health and function. Several clinical phenotypes are associated with an imbalance in the relative proportions of these microbiota including, atopic dermatitis, psoriasis, and dandruff. For example, dandruff, a scalp disorder that is characterized by abnormal flaking and irritation, is correlated with a higher incidence of *Malassezia restricta* and *Staphylococcus epidermidis*, and a lower incidence of *Cutiibacterium acnes, as* compared to normal scalp. It is not clear how the relative proportions of the microbial populations are either kept in balance (disease-free state), or lead to an imbalanced state, resulting in conditions such as in dandruff and atopic dermatitis. Understanding the mechanisms of interactions between members of the skin microbiome and their impact on skin health will lead to novel intervention strategies that would modulate the skin microbiome in favor of health.

To study the mechanisms by which skin microbes maintain stable communities, a robust and reproducible microbial community model was developed with three major skin microbial species: *S. epidermidis*, *C. acnes*, and *M. restricta*. Growth conditions were established to allow co-cultivation of all three microbes that form a mixed-species biofilm, and standardized methods were developed to label, quantify, and track their relative abundances within this community. Intriguingly, the mixed-species community produces significantly higher biofilm biomass than the mono-species biofilms. Further investigation revealed both cooperative and antagonistic interactions between different members of this community. Our data suggest that the relative proportions of each member may modulate the growth of the others and that such interactions dictate the microbiome balance/imbalance on the skin. Based on our *in vitro* model, we have defined the interaction network and gained a detailed understanding of the community dynamics of skin microbes. This *in vitro* model system forms the basis for the development of a fully characterized skin microbiota model, which could be further extended to mimic complex skin disease phenotypes and could help develop potential microbiota-targeted strategies.

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Environmental drivers of microbial community composition in shale-derived soils spanning a global climosequence

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Microbes are vital in driving mineral weathering and soil biogeochemical processes either directly or indirectly through their metabolic activities. Both composition and function of microbial communities are known to respond to gradients in environmental variables, which can be expected to change across soil horizons and consequently give rise to changes in weathering rates with depth. However, most studies of microbial community composition and function in soils to date have focused only on the upper 0-10 cm of soil, neglecting deeper horizons, where organic carbon and oxygen concentrations decrease and mineralogy shifts towards that of primary minerals. To identify environmental drivers of soil microbial community composition to depth, we collected soil samples from the surface to bedrock at five field sites spanning North America and Australia, all developed from shales. Geochemical,

mineralogical, and physical properties of the soils were analysed, and microbial community composition was evaluated through DNA extraction and 16S rRNA amplicon sequencing.

Across all sites and depths, total clay concentration and the mineralogical composition of these clays emerged as significant environmental drivers of microbial community composition. Relative abundances of taxa known to exhibit primarily aerobic and/or heterotrophic metabolisms (*Actinobacteria*, *Acidobacteria*, *Planctomycetes*, *Verrucomicrobia*) tended to decrease with depth; whereas relative abundances of taxa known to exhibit primarily fermentative or anaerobic metabolisms (*Firmicutes*, *Bacteroidetes*, *Crenarchaeota*) tended to increase with depth. Environmental drivers produced distinct microbial community compositions between the North American and Australian sites despite their similar parent material. SIMPER analysis identified *Acidobacteria*, *Actinobacteria*, and *Bacteroidetes* as accounting for a combined 25% of average community dissimilarity between the North American and Australian sites. The clear depth-related changes in microbial community composition in our study emphasise the need to understand microbial community composition and function in subsurface soil horizons and hence improve quantification of their roles in weathering and nutrient cycling processes.

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Bio-mineral fertilizer shows potential in wheat production

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Rock phosphate (RP) is a phosphorus fertilizer source and insoluble in soil[1], and is being depleted at an increasing rate while the demand for food production increases[2,3]. Microbes play an important role to mineralize RP and release plant available phosphate[4]. Consequently, utilization of microorganisms to increase the availability of P in soil is an attractive proposition for developing more economically and environmentally sustainable agriculture. Arbuscular mycorrhizal fungi will colonize plants better if phosphate becomes sparingly available[5]. So, these bacterial and mycorrhizal interactions with RP are important phenomena in phosphorus utilisation efficiency (PUTE) for crop production. Novel Troforté bio-mineral fertilizer technology (mineral fertilizer and inoculated multi-strain suite of beneficial bacteria and fungi) can effectivity enhance soil fertility through a microbially-mediated controlled nutrient release mechanism.

Glasshouse and *field experiments* were conducted to investigate the effects of the mineral fertilizer and inoculated microbes combined with a P dose-response curve (0, 1.5, 3.0, 6.0, 9.0 and 12.0% P) on growth and PUTE of wheat (*Triticum aestivum* L. var Mace). *Under glasshouse conditions*, mineral fertilizer + microbes produced significant increases in mycorrhizal colonisation, wheat root and shoot biomass, shoot P uptake compared to control (no fertilizer no microbes) or mineral no microbes. Shoot P uptake was decreased at *higher rates* of 9-12% P (excess). *Under field conditions*, mineral fertilizer + microbes with RP increased grain yield and grain quality significantly over control (no fertilizer no microbes), mineral fertilizer no microbes no extra P, and mineral fertilizer + microbes with P as soluble triple superphosphate (TSP).

This research trial demonstrates that for a typical wheat program in Western Australia, a Troforté bio-mineral (mineral fertilizer/microbe) based system can be highly efficient on use of low inputs of RP (optimal rate of 3% P), and grain yield *when compared to P from TSP.* Further, the RP mineral program delivers an increase in gross margin >11.4% compared to TSP – demonstrating potential for economic and environmental sustainability.

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Mining the genomes of soil and endophytic Actinobacteria for new biopesticides

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Many beneficial soil and plant endophyte microbes of the Actinobacteria phylum are known for their ability to produce bioactive secondary metabolites. For example, this includes metabolites with antibiotic properties against other microbes such as phytopathogens (1,2,3). However, genome sequencing has revealed these bacteria have far greater potential to produce bioactive compounds than was previously thought based on traditional *in vitro* bioactivity assays (3,4). It is predicted that these "undetected" compounds may account for up to 90% of Actinobacteria chemical potential (1). This vast hidden potential can be tapped for the discovery of new biopesticides which are needed for the replacement of an increasing number of chemical pesticides that are no longer used owing to 1) toxicity, 2) increasing regulation or 3) increased incidence of resistance in pathogen and pest populations. A collection of Actinobacteria isolated from soils and plant roots from south-west Western Australian environments was curated (5,6). A subset of the collection was screened for inhibitory activity against a diverse panel of fungal phytopathogens with a focus on necrotrophic phytopathogens for which no or limited host resistance has been described. These phytopathogens included members of the *Fusarium*genus, *Verticillium* genus, and the broad host range pathogens *Rhizoctonia solani* and *Sclerotinia sclerotiorum*; causal agents of *Rhizoctonia* root and hypocotyl rot, and *Sclerotinia* stem rot respectively. Coupled with whole genome sequencing and prediction of biosynthetic gene clusters (7), diverse chemical potential was discovered within the selected Actinobacteria strains. Metabolomics and transcriptomics approaches are being combined with the genomics outputs to identify potentially new antifungal secondary metabolites for biopesticide applications.

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Microbial community dynamics under concrete environments in search for novel alkalophiles for potential application of Biocement

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Microbially Induced Carbonate Precipitation (MICP) has recently emerged as a potential technology for generation of low energy, self-healing and sustainable cement with applications in remediation and restoration of building materials. This bio-based process has been developed from the principle of biomineralization wherein microbial metabolic activities lead to cementation of natural structures as corals, beach rocks, caves etc at ambient temperature conditions making the whole process highly energy efficient. This bacterially induced mineralization has been mimicked in laboratory conditions for creation of Biocement which offers the benefits of formation at ambient temperature conditions, is highly sustainable, durable, self-healing and recyclable. Most of the applications of Biocement in engineering sector till date have utilised ureolytic, alkalophilic strain *Bacillus pasteurii* which offers optimal biocementation under moderately alkaline environments of pH 9 and low salinity but actual concrete environments are much harsh with pH upto 13 and exposure to marine conditions with salinity upto 5%. Under actual harsh concrete environments, the viability and metabolic activity of standard lab strains has been found to decline tremendously which is quite challenging for successful applications. In the current study we have made an attempt to understand microbial dynamics under highly alkaline concrete environments and then isolate extremophilic strains with higher viability, metabolic activity and Biocement formation in order to improve the applications of this sustainable technology in actual concrete environments. For this study, we have selected three alkalophilic sites including cement, calcareous soils and microbialites to enrich ureolytic alkalophilic strains under concrete simulated environments reaching upto pH 13 and salinity upto 5% and low nutrient conditions. Significant changes in the community dynamics were recorded from naturally alkaline to concrete simulated environmental conditions. Few extremophilic (ureolytic, alkalophilic) strains were successfully isolated with potential to produce Biocement under actual concrete environments. Further studies are being carried out to understand the molecular mechanisms involved in survival and

metabolisms of these strains to endure such extreme environments in order to improve our knowledge along with efficacy of self-healing Biocement for Civil and Geotechnical sector.

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Microbial lanthanide remobilisation in highly weathered Australian soils

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Microbes are hypothesized to constrain the form, distribution, and abundance of lanthanides in granitic soils. In weathering, primary minerals are broken down, mobilising lanthanides into solution. Solubilized lanthanides precipitate with dissolved phosphate to form secondary minerals. Previous work has shown high microbial abundance correlates with lanthanide depletion, including observations of microbial cells attached to secondary lanthanide phosphate minerals. We have identified a Victorian granite soil profile exhibiting regions of lanthanide enrichment and depletion. SEM and trace element data revealed the formation of secondary lanthanide phosphate minerals less than 5um in size throughout the profile, including characteristic zones of enrichment and depletion. Our preliminary results have shown the common soil bacterium *Pseudomonas putida* KT2240, which contains a lanthanide dependent dehydrogenase, is capable of growing on synthesized lanthanum phosphate nanocrystals analogous to the secondary lanthanide phosphate minerals found in our site. By focusing on the interactions between soil microorganisms and lanthanide-phosphate minerals, our work seeks to understand how microbes break down these highly insoluble secondary minerals in the environment.

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Distribution patterns of antibiotic resistance genes in urban greenspaces

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Urban green spaces are closely related to the activities of urban residents and thus provide a potential route for the transmission of antibiotic resistance genes (ARGs) from environmental bacterial communities to human commensals and pathogens through skin-surface contact. However, we still have very limited understanding of the prevalence of ARGs in urban greenspaces and the factors that influence their distribution patterns. Here we profiled the resistome of a wide spectrum of ARGs from soil and turf-grass phyllosphere samples from 40 urban greenspaces across Greater Melbourne, Australia through high-throughput quantitative PCR. A total of 252 and 273 ARGs conferring resistance to eight classes of antibiotics were detected in soil and turf-grass phyllosphere samples, respectively. We proposed the concept of “core urban resistome” which represent the most dominant ARG subtypes in an urban environment. For both soil and turf-grass phyllosphere, the core resistome was mainly affiliated into multi-drug resistance. For both sample types, the relative abundance of both total ARGs and core resistome were significantly and positively correlated with the relative abundance of mobile genetic elements (MGEs). For soil samples, the relative abundance of total ARGs and number of ARGs detected were significantly and positively correlated with soil salinity and cation change capacity (CEC). Taken together, the results indicate that in urban environment high soil salinity and CEC could potentially act as stress factors for the prevalence of ARGs. As urbanization is rapidly happing around the world, it is important to improve the awareness of the role of urban greenspaces as reservoirs of ARGs which could potentially pose risks of ARGs transmission to human microbiome through skin-surface contact.

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