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Cover image: Acropora loripes at Saxon Reef, Great Barrier Reef, Australia (Justin Maire and Ashley Dungan).

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Vertical Transmission

Mark Schembri
President of ASM

A belated Happy New Year everyone! The year is rolling on quickly and no doubt most of us are back to our busy schedule. As travel becomes more normal again, this year promises to bring many changes and we look forward to renewing friendships with international collaborators and welcoming international students back to our university campuses. That said, the uncertainty of COVID-19 infection remains, with many of us due to be offered a fifth dose of a vaccine that we never even knew of ~3.5 years ago – a constant reminder to us all of the power of science and the importance of microbiology!

One of the first things on this year's agenda is ASM Hour, which will start again in March. Our theme leaders have assembled a great list of topics and there are fantastic talks lined up. A change from previous years is that we will use the same link for all ASM Hour sessions. Hopefully this will make these talks more accessible to all of our membership. I would also like to remind you about our Communication Ambassador Program. Our ambassadors contribute to ASM's communication channels by sharing content on ASM events, showcasing their work and serving as public advocates for microbiology. We provide all ambassadors with professional communication training covering social media strategies, fostering a personal brand online, and becoming an online influencer in science and beyond. If you are an early career researcher (ECR) and are interested in joining this program, check out the application on our website (closes 31 March).

Life continues to throw up challenges, and I would like to extend my sincere condolences to any of our members whose family have been affected by the devastating earthquakes in southern Türkiye and Syria, or the severe flooding in New Zealand. Our thoughts are with you at this difficult time, and I hope you can reach out to your support networks if you need help. As microbiologists, we are acutely aware of the terrible diseases such as cholera and tetanus that can follow such disasters, and we hope these regions can avoid such potentially devastating infections.

Our 2023 Annual Scientific Meeting in Perth is approaching fast and registrations are open. I encourage everyone planning to attend the conference to book your travel early, as the cost of flights may rise quickly. We are also offering fifty $200-discount registrations for the conference to students and ECRs with up to 5 years of equivalent experience post their highest qualification. We have a fantastic list of International Plenary speakers, as well as highlight presentations from our own Prof. Julian Rood (Rubbo Orator) and Prof. Dena Lyras (just announced as our Distinguished Orator Award winner) – congratulations to both of you! There is a link on the ASM Homepage that will take you directly to the ASM Conference website. I hope to see many of you there!

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GUEST EDITORIAL
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Microbiology Australia

AusME 2022 Melbourne conference

Vanessa R. Marcelino and Christina Birnbaum

This issue of Microbiology Australia contains contributions from AusME 2022 presenters who were invited by Guest Editors, Chris Greening, Zahra Islam, Christina Birnbaum and Steve Petrovski. The contributions showcase some of the opportunities in microbiology to enhance sustainable development. These articles span using microbiology to mitigate climate change, enhance agricultural production, degrade pollutants and protect vulnerable species.

Microbial ecologists across Australia rejoiced with the opportunity to meet, share latest science and catch up at the latest Australian Microbial Ecology Conference (AusME 2022). The conference was held from 7 to 9 November 2022 at Melbourne Connect (The University of Melbourne) – a beautiful new venue that seeks to support interdisciplinarity and innovation, as does AusME. The conference is supported by The Australian Society for Microbiology and was designed as a biannual meeting to bring the diverse microbial ecology community together. The first meeting was held in 2017 in Melbourne, followed by AusME 2019 in Perth. The pandemic impeded AusME from happening in 2021, and the community was eager to finally meet face-to-face after a long hiatus.

The exciting scientific program of AusME 2022 covered topics ranging from aquatic to terrestrial and host-associated ecosystems, presented through a diverse selection of plenary and invited speakers, oral presentations and posters. AusME 2022 also offered two workshops: 'Introduction to Metabarcoding using QIIME2', led by Ashley Dungan (The University of Melbourne), and 'Introduction to Galaxy', led by Simon Gladman (Australian BioCommons). The scientific program contained six sessions: Terrestrial Microbiology, Aquatic Microbiology, Human Microbiology, Industrial and Food Microbiology, Symbiosis, and the Microbial Toolbox. We were joined in person by 171 delegates, and the majority of presentations were given by students. There were six plenary speakers whose research topics ranged from how to define what a healthy microbiome is, feeding habits of gut microbes, signalling in the legume rhizosphere, communications and interactions in microbial communities and the use of targeted therapeutics to improve health. All plenaries were thought-provoking and shed a light on recent advances on microbial ecology in diverse contexts.

The first plenary speaker was Prof. Maureen O'Malley (University of Sydney), who challenged us to re-think healthy *v*. dysfunctional microbiomes. Following this very stimulating plenary, we proceeded with the presentations focussing on terrestrial microbiology. Dr Hang-wei Hu (The University of Melbourne) presented novel findings illustrating that soil protists are an important factor to the development of antibiotic resistance in bacterial communities. Archaeal ecology was discussed by multiple speakers, highlighting how little we know about archaeal diversity and functions despite their importance. Although we are still only uncovering the total terrestrial microbial diversity, some presentations showed how microbial communities can be utilised in ecological restoration. For example, Dr Náthali Machado de Lima (University of New South Wales and Loam Bio) showed that biocrust cyanobacteria can improve the performance of native plants in extreme environmental conditions.

Microbial ecology inherently deals with associations between organisms, and so it came as no surprise that many presentations at AusME 2022 focussed on symbiosis. Plenary speaker Prof. Scott Rice (CSIRO) discussed the emergent properties of multi-species biofilms, showing that they can be more resistant to stress than the ones formed by a single species. Cross-kingdom symbiotic interactions were addressed by several speakers in the symbiosis session. For example, Dr Adam Frew (Western Sydney University) discussed how mycorrhizal fungi affect plant defences against insect herbivory. Prof. Ulrike Mathesius (Australian National University) and Prof. Damien Maher (Southern Cross University) both discussed the ecology of nitrogen- bacteria, albeit in different environments. Prof. Mathesius presented on genetic modification of isoflavonoids synthesis and exudation in the model legume *Medicago truncatula*, which altered rhizobial interaction in legume roots improving symbiosis and reducing parasite infection. Prof. Maher opened our eyes to the weird and wonderful world of bark-dwelling bacterial communities, including nitrogen fixers, trace gas oxidisers and other microbes usually associated with soils rather than bark.

Several studies on symbiosis presented at AusME 2022 focussed on aquatic hosts, highlighting a treasure trove of discoveries in marine habitats. Assoc. Prof. Suhelen Egan (University of New South Wales), for example, delved deep into the ecological roles of *Roseobacter* spp. for their seaweed hosts. Prof. Elizabeth Dinsdale (Flinders University) indulged the audience in her plenary talk with a video showing how they have sampled the microbiome of sharks and rays. The brave efforts of Prof. Dinsdale's team allowed them to reconstruct Metagenome Assembled Genomes (MAGs) of the microbiome associated with cartilaginous fishes, and uncover patterns of host–microbiome co-evolution.

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This year's AusME featured multiple talks focussed on human microbiology. Plenary speaker Prof. Georgina Hold (University of New South Wales) reflected on the use of microbial therapeutics to improve health. In an effort to move the field towards a mechanistic understanding of the human gut microbiome, Prof. Hold's team developed an artificial gut system to perform carefully controlled experiments. Assoc. Prof. Sam Forster (Hudson Institute of Medical Research) discussed how advances in culturing anaerobic microbes is helping to make causal links between specific microbial strains and Inflammatory Bowel Disease. Dr Hajara Aslam (Deakin University) provided an overview of the links between the human gut microbiome, diet and mental health, and Dr Erin Shanahan (University of Sydney) discussed how the gut microbiome and diet can affect cancer immunotherapy.

The explosion of new insights in the microbial ecology field is largely due to recent technological advances, and AusME 2022 presenters highlighted the development and application of exciting new tools for microbiology. Plenary speaker Prof. Rob Edwards (Flinders University) is a pioneer in the development of bioinformatic tools to analyse microbiome data. His tools allowed the discovery of a massive diversity of phages from metagenome data, many years before they were cultivated. Dr Ben Woodcroft (Queensland University of Technology) presented a set of bioinformatic tools for high-throughput analyses of genomes and metagenomes, and their beautiful logos featuring Australian animals did not go unnoticed. Some AusME22 participants are leading the way in integrating multi-omics datasets to better understand the ecology of microbial systems. For example, Dr Cheong Xin Chan (University of Queensland) showed how an integrated multi-omics dataset is helping us understand the ecology and evolution of blooming behaviour in microalgae. Plenary speaker Prof. Phil Pope (Norwegian University of Life Sciences) discussed how the integration of multi-omics and phenotyping data is taking our understanding of microbial feeding habits to the next level. Prof. Pope also presented an innovative method to study the spatial arrangement of host-associated bacteria and their interactions in three dimensions. These and many other technological advances highlighted at AusME 2022 are certain to benefit agricultural, food and other industrial sectors. Dr Chris Rinke (University of Queensland), for example, presented how they are uncovering bacterial metabolic pathways related to polystyrene degradation, which they found in a worm that eats styrofoam. It is exciting to imagine where the next steps will take us.

Last but not least, congratulations to all the prize winners! Best oral People's Choice Presentation award went jointly to early career researchers (ECRs) Dr Rachael Lappan (Monash University), Dr Simon Law (CSIRO) and PhD student Talisa Doering (The University of Melbourne). Dr Laura Rood (Tasmanian Institute of Agriculture) and Dr Cami Plum (Monash University) jointly won the best ECR Poster Prize and Jiasui Li (University of New South Wales) and Calloway Thatcher (James Cook University) jointly won the Best Student Poster award. The Federation of European Microbiological Societies awarded the best People's Choice Poster award to Cecilie Gotze (The University of Melbourne).

Beyond the exciting science, AusME 2022 provided a great opportunity to reconnect, meet new colleagues and catch up with old ones. The informal evening function at the Inner North Brewery provided further opportunities to socialise while admiring the red moon outside. We hope that these connections will persist, and that AusME 2022 was a source of inspiration to continue the great microbial ecology work full steam ahead.

Acknowledgements. We are extremely grateful to Chris Greening for chairing AusME 2022 with dedication and enthusiasm, and to our colleagues in the local organising committee for their invaluable help with all aspects of the conference planning: Zahra Islam, Kate Howell, Ashley Dungan and Steve Petrovski. We thank the ASM state branches for supporting their local members with awards to attend AusME 2022. Previous AusME chairs Linda Blackall and Deirdre Gleeson provided invaluable advice to guide the 2022 meeting. We also thank the ASM Executive, the local VIC branch of the ASM and ASN Events for their support. AusME counted with generous sponsorship from the ARC Research Hub for Smart Fertilisers, Australian Centre for International Agricultural Research, Australian Centre for Ecogenomics, The University of Melbourne, The University of Queensland, UNSW Ramaciotti Centre for Genomics, Merck, MP Biomedicals and the Federation of European Microbiological Societies.

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Microbiology Australia

Friends to the rescue: using arbuscular mycorrhizal fungi to future-proof Australian agriculture

Meike Katharina Heuck[A,*], Christina Birnbaum[B,C] and Adam Frew[A,C]

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ABSTRACT

With a rising global population and the challenges of climate change, there is an increasing need to find solutions to maintain crop yields in an ecologically sustainable way. Although many studies have focussed on this issue, comparatively few are conducted in the southern hemisphere. This is worrisome because the geographical and geomorphological conditions within Australia differ greatly from the northern hemisphere. To ensure food security, approaches can rely on conventional agricultural methods as well as commercial arbuscular mycorrhizal (AM) fungal inoculants. Both approaches lack the capacity to be successful in the long term or could have unknown negative effects on the naturally occurring microbial communities. We advocate for a sustainable and holistic approach that combines the effective management of functionally diverse AM fungal communities with precision farming techniques while integrating landscape elements into agricultural fields. In addition, landowners and scientists should collaborate and communicate their work with industry and government to take forward the shift to a more-sustainable agriculture. In this way, we will be better able to secure our food production while restoring our soil ecosystems.

Keywords: arbuscular mycorrhizal fungi, Australia, conventional agriculture, food security, multifunctionality.

The agriculture which feeds us currently faces several formidable threats. Owing to population growth, we are required to produce more food in a reliable way.[1] Further compounding the issue is climate change that will cause longer droughts or extreme heat and will therefore likely reduce agricultural productivity.[2] The urgency to find solutions is reflected in the abundance of scientific papers published on the subject, which has considerably increased since 2005.[3]

In efforts to address these challenges, considerable attention has been given to the arbuscular mycorrhizal (AM) fungi. AM fungi are a group of soil organisms that are found in almost all terrestrial ecosystems and form a symbiotic relationship with ~80% of land plants.[4] AM fungi are widely recognised to have vast potential in sustainable agriculture as they can provide plants with water and nutrients, mainly phosphorus, and in return receive carbon in the form of sugars and lipids from their host plant.[4] Furthermore, AM fungi are functionally diverse and contribute to many important soil functions.[5] These include nutrient cycling,[6] reducing soil nutrient loss[7] and decomposition.[8] They also have the ability to increase plant resistance to biotic stressors such as herbivores and pathogens, as well as abiotic stressors including drought or high temperatures.[9,10]

Conventional farming techniques often include tillage as well as the use of synthetic fertilisers and pesticides. Although these methods can ensure high yield production in many contexts, they are becoming increasingly expensive and restricted in their use. Furthermore, they often have negative environmental impacts,[11] which include a reduced soil organic matter content, long-term soil acidification[12] as well as a negative effect on AM fungi (see Fig. 1).[13–15]

Soil acidification has been shown to reduce AM fungal diversity[16] and high soil fertility due to fertilisation can reduce plant dependency on the symbiosis with AM fungi, which may cause plants to lose the remaining benefits of symbiosis, such as pest and drought resistance. Furthermore, the use of certain pesticides can inhibit enzymatic pathways and stop the protein production of AM fungi.[17] Pesticides can also inhibit hyphal growth and thus several physiological processes including the uptake and transport of metabolites and nutrients between the fungus and the plant.[15] Additionally, common agricultural practices may shape the functional composition of AM fungi, for

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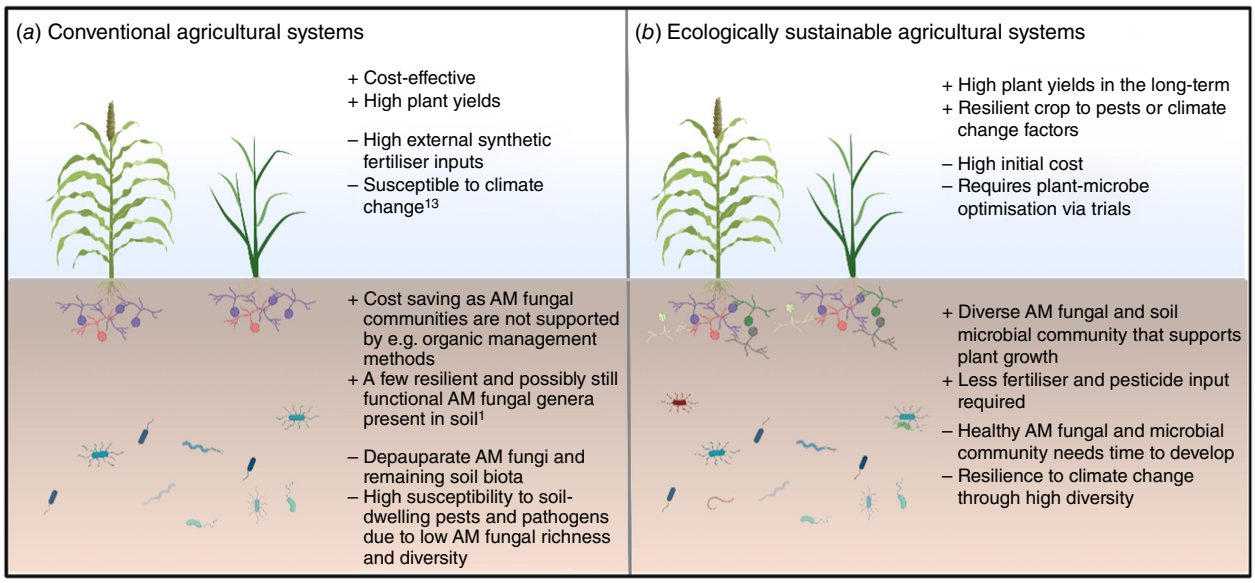

Fig. 1. Positive and negative effects of conventional (*a*) and ecologically sustainable (*b*) agriculture on plants and the soil microbial community. Created with BioRender.

example, it may select for taxa that are more-aggressive competitors for plant carbohydrates, resulting in a net cost to the plant.[18,19] Along with the use of synthetic fertilisers and pesticides, the tillage regime can disrupt the symbiosis between plants and the fungi. For example, tillage can physically disrupt plant access to the fungal network,[20] and bare fallow areas after tillage can leave AM fungi without a symbiotic partner and consequently the next generation of plants experiences lower colonisation rates.[21]

Moreover, the use of synthetic fertilisers and pesticides leaves residues in the soil that negatively affect AM fungi even for years after the field has been converted to a sustainable method. This is, for example, reflected in a lower rate of colonisation of the roots by the fungi.[15]

Notably, research focusing on AM fungi is mainly limited to the northern hemisphere.[22] This is concerning because the distinct geography and geomorphology of Australia hampers our ability to apply our current knowledge from the North to the Australian continent.

The climate in Australia is diverse, considering that the continent extends from 10° to 43°S, as well as being located between 153° and 113°E. Therefore, the climate ranges from arid to tropical and cool montane,[23] which, in conjunction with the bedrock, results in many different soil types and agricultural systems. Owing to Australia's history, a variety of agricultural methods were used in a reasonably short time, which is a contrast to the agricultural industrialisation history in Europe or Asia.[24] Before British colonisation of Australia, the soils were mainly used in a low-intensity way by the Indigenous peoples. This type of agriculture meant only small impacts on the nutrient cycles and low soil disturbance, and therefore was less disruptive to the natural ecosystem.[25] From 1800 onwards, the land was intensively used by the settlers and many native landscapes were converted into agricultural fields, which was mainly cultivated using conventional techniques.[26]

The constant degradation of the soil and thus, the negative impact on microorganisms is problematic, since Australia's soils generally have a low nutrient availability – phosphorus content being particularly emphasised here – because of the absence of glacial overprinting, and have a reasonably thin A horizon (topsoil).[27] Such characteristics only amplify the crucial need for adequate food security solutions that are suited to the Australian landscape.

A strategy that is employed in Australia and worldwide to address the adverse effects of conventional agriculture on soil organisms, including AM fungi, is to directly inoculate the soil with soil microbiota. Various distributors offer commercial AM fungal inoculants.[28] Providing access to such inoculants does advance greater awareness of the importance of soil microbiota and AM fungi to the public and practitioners. However, such inoculants typically contain a single fungal species, or a small group of cosmopolitan species.[29] As AM fungi are functionally diverse, it is unlikely that single species can provide the necessary functions that a more-diverse AM fungal community could offer.[30] Moreover, there is a risk that the species introduced into the soil will disrupt the naturally occurring community. If the introduced AM fungal species do not naturally occur in the local ecosystem, this may have unintended consequences and even displace the native community, jeopardising soil health in the long run.[31] However, there is insufficient evidence to show that an introduced species will be able to persist in a new environment, one that presumably already has an established AM fungal community.[32] Furthermore, the viability of the inoculants currently available is questionable as a recent study demonstrated, showing that most of the tested Australian inoculants failed to support mycorrhizal root colonisation, although most of these inoculants contain AM fungal species known to colonise a broad range of host plants and soil types.[28]

The aforementioned issues highlight that we need to keep our crop yields high in the long term while simultaneously establishing healthy soil ecosystems. Therefore, we propose an ecologically sustainable and holistic approach. Since AM fungal communities are functionally diverse and may assume

partially similar roles of pesticides and fertilisers, the approach involves supporting the local, naturally occurring, AM fungal communities. Emam found that live soil inoculum containing the original AM fungal community is more effective at supporting plant growth than using a commercial inoculum.[33] The notion that live soil inoculum is more effective has also been shown for plant resistance to pests.[34] Given the extensive diversity of soil types in Australia and their generally limited nutrient availability, it is essential to adopt site-specific management solutions to promote ecologically sustainable agriculture. We need to shift from methods motivated by short term productivity to broader incorporation of ecological indicators of sustainable agricultural functioning, exploiting the potential benefits of soil microorganisms as effectively as possible in a way that can significantly reduce the need for synthetic inputs.

Conventional farming practices have been critical in food security and ensuring livelihoods of people around the world and in Australia. We recognise that for many, working with a focus on soil ecology is not feasible due to barriers such as costs or accessibility. Despite being a world leader in organic agriculture in terms of the area certified between 2000 and 2018, only 8.8% of Australia's agricultural land is certified as organic.[35] To encourage adoption of organic agriculture in Australia, the government could take several steps, including: enhancing information dissemination through research and extension services, developing more effective market-based tools for environmental performance, and addressing the current institutional bias against organic agriculture.[36] Where possible, we also suggest broader accessibility to technologies such as precision farming, an approach that analyses specific crop needs by using *inter alia* remote sensing and GPS. With this approach, it is possible to apply fertilisers in small doses as required by the crops,[37] which may mitigate any negative effects on the mycorrhizal symbiosis. Furthermore, the integration of landscape elements such as small forest patches, field margins or hedgerows as well as cover crops can be beneficial. These not only serve as a biodiversity pool for AM fungi and other soil microorganisms, but also protect the soil from erosion.[21,38] Moving away from monocultures could also be an important method, as polycultures have been shown to double AM fungal richness.[39]

For a rapid rethink in agriculture, we suggest scientists work together synergistically with industry and farmers to deliver healthier soils that will support plant growth while reducing costly external inputs. On one hand, it will be important to map and successfully monitor AM fungi across agroecosystems in Australia, which is currently being undertaken by a national research project called DigUpDirt (see https://www.digupdirt.net/). On the other hand, it is increasingly important to test the effect of resident AM fungal species on crops and how this differs between AM fungi derived from different management practices. We encourage scientists to communicate their work with the industry and respond to needs of the farming community. Close collaboration and knowledge sharing between scientists and land managers is what is needed to advance the shift to sustainable agriculture.

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Engineering biodegradable coatings for sustainable fertilisers

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ABSTRACT

With the pressures of a changing global climate and ever-growing population, the need for sustainable agricultural practices that increase crop yields while decreasing greenhouse gas emissions are critical. Currently used practices to increase yields can often be problematic due to low nitrogen use efficiency or a potential overreliance on agrichemicals that can alter the community composition of a given ecosystem, although this is typically system and situation dependent. As such, the next generation of enhanced efficiency fertilisers that combine chemical, materials engineering and biological components are likely to be a game changer. Integral to their success is a better understanding of how plant–soil microbiomes interact with the new enhanced efficiency fertilisers, and how we can best tailor the fertilisers to suit different plant–soil combinations. In particular, the biodegradation properties of new fertiliser coatings must be given careful consideration so as to not further burden agricultural soils with microplastics or cause ecotoxicity problems. This perspective proposes novel, interdisciplinary strategies to generate highly efficient, biodegradable fertiliser coatings for use in the agricultural sector.

Keywords: agriculture, biodegradation, biotechnology, fertilisers, plant–microbiome interactions, polymers, soil microbiology, sustainability.

The challenges of feeding the world on finite agricultural land

With global populations set to reach 10 billion by 2050,[1] there is increasing pressure to match food production within existing agricultural lands in the face of a changing global climate. Integral to global food security is an increasing reliance on synthetic fertilisers to improve crop yields,[2] while simultaneously reducing their negative environmental impacts.[3] Though there have been recent shifts towards designing fertilisers with enhanced efficiency[1,4,5] including those that have been coated with a polymeric substance such as metal–phenolic networks,[6,7] these have not been widely adopted by the global agricultural industry. In addition, strategies to further increase crop yields, such as the deployment of pesticides, herbicides and enzymatic inhibitors,[8,9] may also lead to disruptions in the balanced plant holobiont (i.e. the collection of microorganisms such as bacteria, fungi, archaea and protists, that form close associations with the plant host).[10,11] Thus, the design of new generation fertilisers must take into consideration sustained and tailorable release profiles, the degradation of coatings and ecotoxicity potential, as well as the potential benefits of incorporating probiotic microorganisms into engineered coatings to enhance plant performance.

Among the major design challenges for the development of new fertilisers is the composition of coatings that cannot only slow the release of the internal nitrogenous compound, but also can be completely biodegraded by indigenous soil microorganisms. This remains an understudied challenge within both the fertiliser and agricultural industry, as the microorganisms that comprise plant holobionts are often host or soil specific,[12] and may not be shared among different crop types.[13,14] As such, innovative microbial solutions are required to ensure that newly developed biopolymeric fertiliser coatings can be degraded by a wide range of microorganisms native to different soil types and plant species. In addition, ensuring that the polymers are completely degraded and do not generate microplastics[15–17] is paramount to ensure that ecosystems are not further burdened. We thus outline a cross-disciplinary strategy combining materials engineering and plant–soil microbiology approaches to generate innovative hybrid chemical–biological fertilisers for use in Australian agricultural systems.

Current state of agricultural practices and potential innovation strategies

Current practices within the agricultural industry are heavily weighted towards the usage of conventional fertilisers that are typically applied in liquid form or as uncoated granules.[4]

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As Australia possesses one of the highest nitrogen footprints in the world (~47 kg of nitrogen per capita per year), with food production comprising the largest component,[18] it is of great importance to develop new products to reduce the environmental and socioeconomic impacts of fertiliser use.[1] It is well established that intensive and improper use of nitrogen-based fertilisers can lead to numerous undesirable effects including mining of soil nitrogen in low-rainfall cropping areas,[18] nitrate leaching into waterways causing eutrophication,[19] and nitrous oxide and ammonia emissions into the atmosphere, contributing to global warming.[20] With the current cost of developing, producing and deploying enhanced efficiency fertilisers up to 10 times higher than that of commercial fertilisers within the agricultural sector,[5,21] the use of these commercial fertilisers will continue to be widespread and are unlikely to decrease unless next-generation fertilisers are comparably priced and are higher efficiency.

Within the agricultural sector, three major approaches are currently used to mitigate excess nitrogen loss within cropping lands as well as increase crop nitrogen use efficiencies: (1) addition of chemical urease and nitrification inhibitors[4,10,11]; (2) utilisation of the 4R Nutrient Stewardship concept (right source of nutrients, at the right rate, at the right time and in the right place)[22]; and (3) use of physical barriers to slow the release of fertilisers.[7,23] Although the benefits of urease and nitrification inhibitors have been well documented,[5,11] the use of polymer-coated fertilisers has been comparatively less studied. The effectiveness of polymer-coated fertilisers specifically was demonstrated to have negative to negligible positive effects on drylands and grasslands, and was highly influenced by soil pH.[5] This is a concerning phenomenon for translation into the Australian agricultural sector, which is predominantly grasslands and drylands.[24,25] Additionally, the type of polymer used in the coating needs to be carefully selected to ensure that it is capable of natural degradation, meets national biodegradability standards and by-products do not have ecotoxicological effects. It is important to note that currently there are no global standards governing the biodegradation parameters of fertiliser coatings. More work is required to understand the effects of additives on polymer degradation by microorganisms, as numerous reports have highlighted deleterious effects of microplastics on soil organisms and functions.[26] Subsequently, the question remains, can enhanced-efficiency polymer-coated fertilisers be generated for use within the Australian agricultural industry, taking into consideration the unique properties of Australian soils?

Upcoming multidisciplinary approaches to engineering biodegradable fertiliser coatings

The effectiveness of controlled release fertilisers could potentially be improved by the incorporation of biological additives, such as plant-growth promoting bacteria (PGPB) as well as polymer-degrading microorganisms (PDMs). Biofertilisers, or microbial inoculants, can be split into two major classes, rhizobia-based inoculants that are primarily applied to legumes, and non-rhizobia based inoculants to non-legumous crops. Non-rhizobia biofertilisers in the form of peat or liquid supplements have been demonstrated to increase the yield of numerous crops including soybeans, maize and rice, though positive effects can vary greatly across different applications.[27,28] Although biofertilisers have been implemented for decades,[28] they are scarcely used within the Australian agricultural sector aside from in forage legumes.[27] In particular, the uptake of these biofertilisers has been sporadic in wheat-producing nations and has had inconsistent results between countries, indicating that species-specific interactions between plant subtypes and microbial inoculants might be critical to consider.[27]

Similarly, the discovery and characterisation of PDMs is rapidly growing in response to the overuse of plastics worldwide, though their efficiencies in different ecosystems remains understudied.[16] A recent review by Gambarini *et al.* determined that, although the degradation capacity for microorganisms is taxonomically widespread, experimental evidence of this has been minimal so far.[29] Some of the better-characterised PDMs include *Ideonella sakaiensis*, which has been shown to degrade polyethylene terephthalate, numerous species within the order Bacillales, which are capable of polypropylene and polystyrene degradation, and species from the *Amycolatopsis* genus, which have been shown to degrade polylactic acid polymers.[29] In particular, the conditions within which polymers are partially or completely degraded can differ extensively between different polymer types, with synthetic polymers derived from fossil fuels (e.g. polyethylene terephthalate, polypropylene and polystyrene) typically only degraded by microorganisms under specific conditions.[15,29] Conversely, coatings developed from biopolymers, polymers that are made from renewable resources (e.g. polyhydroxybutyrate) would likely be better candidates as they have a greater biodegradation potential than synthetic polymers.[29] Thus, careful consideration of polymer type as well as the *in situ* degradation capacity of the agricultural soil tested must be at the forefront of the generation of fertiliser coatings. Soil properties, such as pH and organic carbon content, in conjunction with the plant species grown must also be considered as these can drastically alter the composition of microorganisms within the rhizosphere.[12] As such, the testing of multiple soil types and incubation conditions on the same polymer type must be carried out to assess the generalisability of degradation across different agricultural systems. It is likely that multiple polymer and microbial combinations need to be generated for each plant–soil combination due to specific nature of plant–soil–microbiome interactions.[13] Thus, an ongoing challenge is finding microbial combinations that will promote the growth of crops, remain in the soil long term and are able to be incorporated into existing fertiliser delivery strategies such as coatings.

Our strategy for developing next-generation smart fertilisers is to use a multidisciplinary approach, combining complimentary microbiological, chemical and materials engineering strategies. As demonstrated by the schematic in Fig. 1, we aim to combine culture-independent and culture-dependent microbiological techniques with materials engineering to develop economically viable smart fertilisers capable of increasing yields and reducing nitrogen losses. Determination of soil physicochemical properties as well as overall microbial community structure could potentially

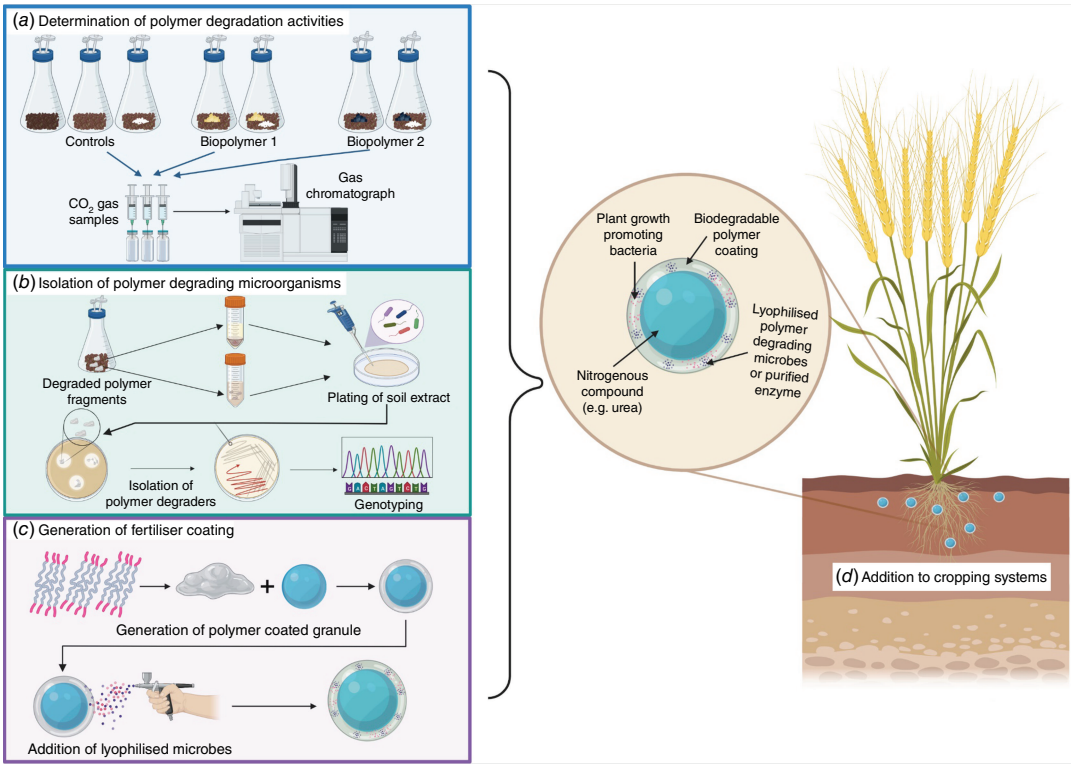

Fig. 1. Schematic highlighting the multidisciplinary strategy to generate, test and implement new biodegradable fertiliser coatings. Our approach to designing new fertiliser coatings includes the following: (*a*) *in situ* determination polymer degrading capacity of chosen polymer and potential polymer-degrading microorganisms using a combination of gas chromatography and visual observation techniques, (*b*) isolation of polymer degrading microorganisms using traditional culturing methods and genome-informed culturing methods, (*c*) materials engineering approaches to generate new biodegradable polymers and coating of granulised fertiliser, followed by external application of lyophilised polymer degrading and plant-growth promotion microorganisms or crystallised polymer degradation enzymes, and (*d*) deployment of smart fertiliser into cropping systems such as wheat fields. These smart fertilisers will be customised for each major soil type as well as plant species to maximise efficiency. Figure created using BioRender.

enable genome-informed cultivation strategies to target both PGPB and PDMs specific to each major Australian crop species–soil combination (Fig. 1*b*).[30] *In situ* biodegradation studies, assayed by gas chromatography (GC) of CO_2 production and scanning electron microscopy (SEM), using biodegradable polymer candidates will also inform downstream coating design, with candidates able to be degraded by multiple soil types and in multiple conditions prioritised (Fig. 1*a*). The direct measurement of degradation by GC and SEM will be accompanied by additional, complementary analytical techniques, such as complete soil physiochemical analysis and Fourier-transform infrared spectroscopy. Partially degraded polymers will then be used as the starting inoculum for PDM isolation using minimal media with fresh polymer as the sole carbon source (Fig. 1*b*). Isolates will then be phenotyped to determine the mechanism by which polymer degradation was occurring, with the potential to purify degradation enzymes. Within a coating, it is theoretically possible to include urease and nitrification inhibitors[1] as well as a microbial cocktail of lyophilised PGPB and PDM or purified enzyme (Fig. 1*c*). This would allow for controlled release of the encapsulated chemical fertiliser (e.g. urea) because of the degradation effects of the PDM, the inhibition of major enzymatic pathways leading to nitrogen losses and the delivery of PGPB directly to the rhizosphere (Fig. 1*d*).

Concluding remarks

With an increasing global population and a changing global climate, addressing food scarcity through innovative microorganism-forward agriculture is paramount. Only through a deep understanding of plant–soil–microbiome interactions and using multi-disciplinary approaches can new biodegradable polymer coatings for chemical fertilisers be generated. This new generation of enhanced efficiency fertilisers should be tailored to specific plant–soil combinations to obtain the best yields and nitrogen use efficiencies while also being a viable economic alternative to currently used chemical fertilisers.

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Monitoring the viable grapevine microbiome to enhance the quality of wild wines

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ABSTRACT

Grapevines that are used for winemaking host a diverse range of microorganisms that make up their microbiome. The microbes that inhabit the grapevine have been used by winemakers to produce wine for centuries, although modern wine producers often rely on inoculated microorganisms such as *Saccharomyces cerevisiae*. In the Australian wine industry, there is a movement towards returning to the utilisation of the microbiome for wine fermentation. With the recent increase in the understanding of the role of the grapevine microbiome in grapevine health, fermentation and subsequent wine sensory traits, the microbial world offers a new level of complexity that can be harnessed for winemaking. In order to develop and maintain a desired vineyard micro-biodiversity, extensive microbial monitoring is required. Here we discuss the utilisation of a viability selection dye in order to distinguish between microorganisms that are live and associated with the host, and relic signals generated from non-living sources.

Keywords: fermentation, metagenomics, micro-biodiversity, microbiome, microbiota, wild, wine.

Fermentation and the grapevine microbiome

The process of producing wine from grape juice was discovered to be the result of microbial organisms in the nineteenth century by Louis Pasteur. With advancements in biochemistry and microbiology, the understanding behind this complex fermentation process has been investigated extensively. As such, winemaking is one of the oldest human utilisations of microorganisms through the fine-tuned control of these complex fermentation reactions. The fermentation process is mostly completed during 'primary fermentation', typically by inoculated yeasts, wherein most of the alcohol content is produced by the alcoholic fermentation of glucose and fructose into ethanol. This is followed by 'secondary fermentation', which leads to microbial stability and advance the wine's sensory profile (its flavour and aroma) through processes such as malolactic fermentation.[1] Two categories of microorganisms, fungi (predominantly yeasts) and bacteria, are recognised as the driving force of the primary and secondary fermentation processes, as well as the more recently recognised 'spontaneous fermentation' process performed by wild yeasts and bacteria, which originate from the plant's microbiome.[2–6]

The grapevine microbiome, similar to other, complex microbiomes such as the human gut microbiome, is separated into distinct compartments with each hosting its own diversity of microbes. The main subdivisions of the grapevine microbiome are the rhizosphere microbiome (the area surrounding the roots), the endosphere microbiome (the area within the plant's tissues) and the phyllosphere microbiome (the surface of the aerial portion of the plant). Of these, the rhizosphere has been the main focus since its coinage[7]; however, current literature is beginning to recognise the importance of the phyllosphere and endosphere microbiomes because of their role in vine health as well as wine fermentation.[8] Both phyllospheric and endospheric microorganisms inhabit the grape berry tissues, collectively known as the carposphere microbiome, and as such act as diverse pools of fungal and bacterial taxa that can be utilised as wild inoculations to be utilised during spontaneous fermentation[9,10] (Table 1).

The movement towards wild wines

Currently, within the Australian wine industry, a movement is underway with some wineries adopting traditional practises for winemaking through the production of 'wild wines'. Wild wines are fermented exclusively through spontaneous fermentation, using wild

Table 1. Top 10 most abundant bacterial and fungal genera present in the grape carposphere microbiome identified across multiple regions globally.[10]

Top 10 fungi	Top 10 bacteria
Alternaria	*Bacillus*
Aureobasidium	*Blastococcus*
Botrytis	*Enterobacter*
Cladosporium	*Erwinia*
Cryptococcus	*Gaiella*
Davidiella	*Massilia*
Guehomyces	*Methylobacterium*
Penicillium	*Micrococcus*
Sporobolomyces	*Pseudomonas*
Rhodotorula	*Sphingomonas*

microorganisms derived from the grapevine microbiome to ferment wines to completion as opposed to exogenously inoculated *Saccharomyces cerevisiae* cultures (Fig. 1). By utilising the grapevine microbiome for wine fermentation, winemakers are capable of producing complex wines that have unique and diverse sensory traits.[6,11] Furthermore, many methods of maintaining optimal micro-biodiversity in vineyards, such as bio-dynamic and low-input approaches, align with the Australian wine industry's promotion of 'organic' and pesticide-free products for health-conscious target consumers and more sustainable viticultural practises.[12]

Although wild wines are becoming favourable, there is a multitude of challenges that must be overcome to successfully complete fermentation and produce wines with palatable characteristics. Two methods of producing wild wines currently exist. The first utilises vine-borne microbial isolates that can be inoculated into wine ferments to drive and support the complex fermentation reactions.[13–15] Although this method does not use specialised microorganisms such as *S. cerevisiae*, it also does not completely depend on the endogenous grapevine microbiota, and thus does not require the involved viticultural processes, such as monitoring, which come along with wild ferments. The second, and more-traditional method, utilises the vineyard's own micro-biodiversity, which is cultivated on the grapevine that makes its way into the wine ferment during the crushing process. This can produce complex sensory traits that vary between seasons and regions depending on a variety of different factors (natural, human-derived and environmental).

As with most organisms, a healthy grapevine microbiome is one that holds a richness and diversity specific to its host organism,[16] with the opposite typically being an indicator of a disease state within the host vine (e.g. one pathogenic taxon dominating the niche).[16–18] As the health of a plant can be dictated by that state of its microbiome, as well as the outcomes of wild fermentation, suitable micro-biodiversities must be maintained within vineyards for effective wine production. Although the occurrence of each taxon is largely host-dependent,[10,18] these microbial communities are also affected by various stressors from the environment that shape the structure of the grapevine microbiome. The frequency of each taxon is also dependent on season, geographic location, water availability, UV exposure and human intervention.[16] Controlled cultivation of these specific communities is crucial to the host plant's development; however, for wild winemaking, effective monitoring of the micro-biodiversity is also of great importance in order to encourage the development of beneficial species and hinder the growth of unwanted microorganisms such as those related to wine spoilage.

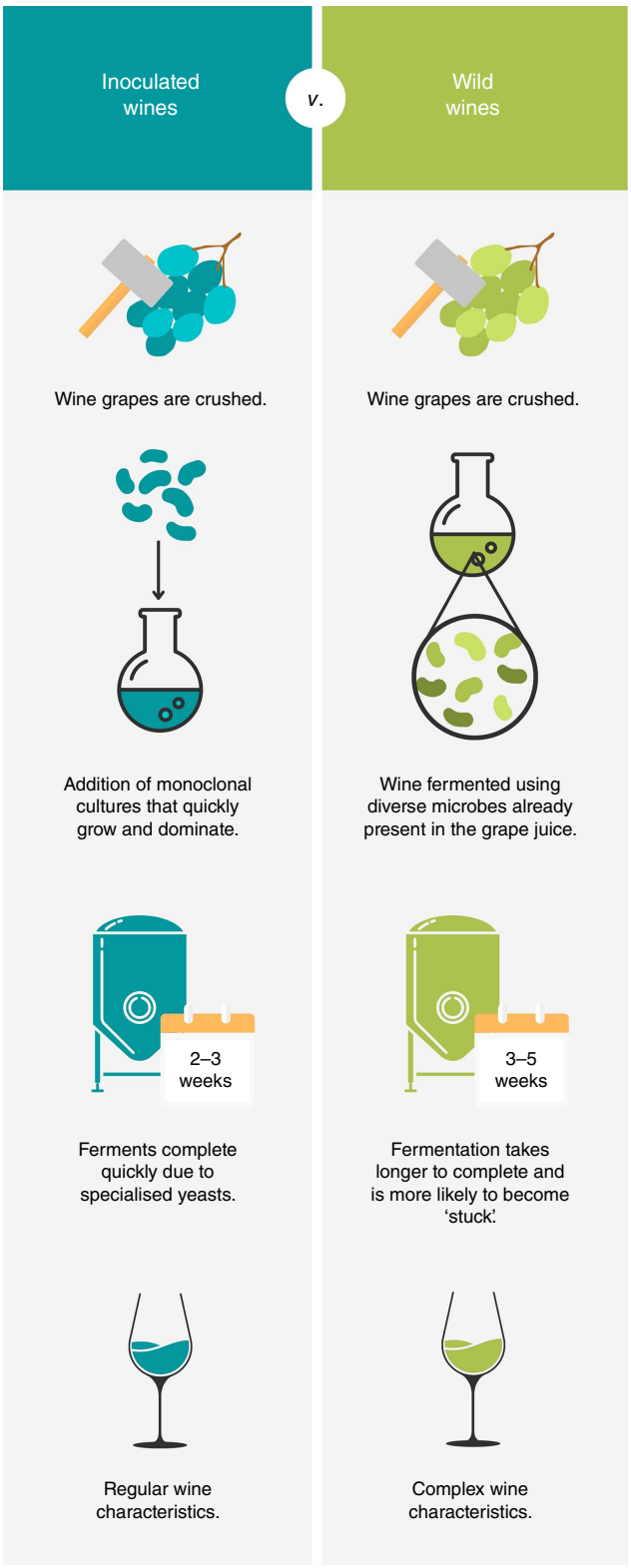

Fig. 1. Inoculated wines *v.* wild wines.

Dead or alive? Monitoring the viable grapevine microbiome

Metagenomic monitoring methods, including many of the PCR-based quantifications and DNA sequencing technologies that are typically applied to microbiome research, must be utilised to properly ensure vineyards are maintaining a healthy micro-biodiversity. The introduction of these methods would allow for incredibly efficient characterisation of the microbial richness and diversity present on a vineyard's grapevines. Understanding which microorganisms are present in the grapevine microbiomes of a vineyard will allow grape growers to understand what involvement is necessary to cultivate or preserve a healthy microbiota. However, one of the most significant downsides to current microbiome analyses is the inability to discern which organisms were 'alive', at the time of sampling.[19,20] Relic DNA makes up all DNA from non-living sources, such as dead cells, compromised cells or environmental DNA (eDNA),[21] and can be just as easily amplified and sequenced as DNA extracted from viable cells. This indiscriminate nature of PCR can provide false positives to vineyard biodiversity monitoring, which, with the wrong intervention, could lead to undesired impacts on the wild microorganisms and downstream outcomes.

Discrimination between live and dead cells is an important milestone that must be overcome to allow grape growers the ability to determine which taxa are present and associated with their host vines. To combat this, we have utilised the viability selection dye, propidium monoazide (PMA),[20–23] which is capable of covalently binding to DNA when exposed to light. The binding of the PMA dye to DNA prevents it from being amplified by PCR, and thus, cannot be observed. However, the dye cannot cross the intact membrane of live cells, allowing it to only bind to relic DNA from compromised cells or environmental sources. We tested this dye using leaf phyllosphere microbiome samples collected from *Vitis vinifera*

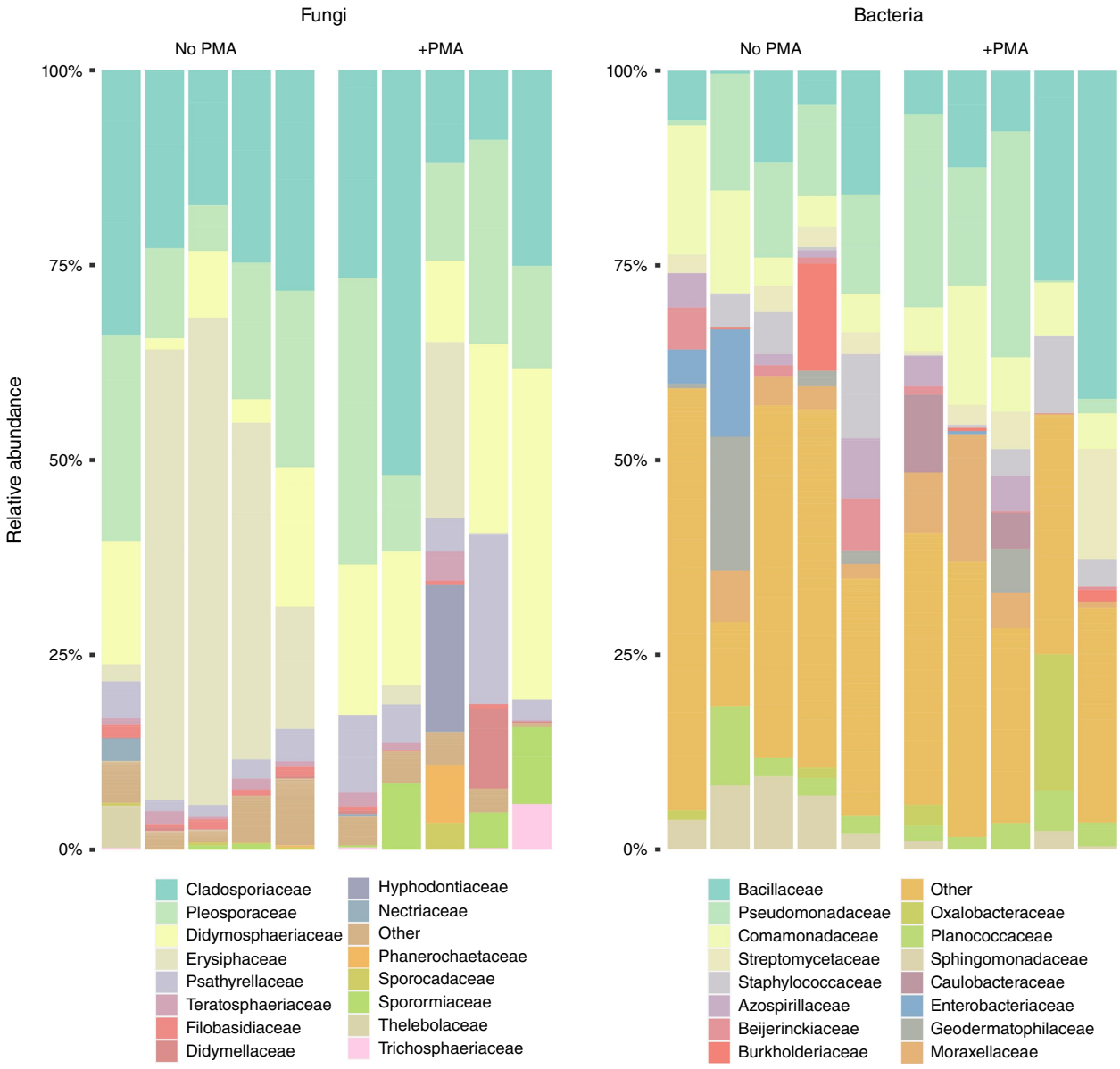

Fig. 2. Stacked bar charts showing the top 15 families of fungi and bacteria collected from leaf phyllosphere samples. Samples were either treated with PMA dye to remove relic DNA or left untreated (representative of the total DNA).

'Syrah' (Shiraz) grapevines and were able to gain sequencing data representative of the living phyllosphere microbiome.

Our PMA-Seq results utilised both *16S* and *ITS* rRNA gene amplicon sequencing to observe the bacterial and fungal communities respectively. Sequencing was conducted on two sets of grape leaf swab samples; both sets were prepared identically with one being treated with PMA (representative of the viable communities) and the other left untreated (representative of the total DNA within the sample) prior to DNA extraction and amplification (B. Welsh, unpubl. data). DNA sequencing of these samples demonstrated the PMA dye's ability to effectively remove relic DNA from grapevine microbiome samples without sacrificing the quality of the data (Fig. 2). For example, from the untreated sequencing data, we observed a high relative abundance of the fungal family Erysiphaceae, a taxon responsible for powdery mildew (a common grapevine infection); however, this abundance was lower in the samples treated with PMA. This result suggests that the observed Erysiphaceae taxa were, in fact, false positives, and the DNA responsible for those sequences likely came from non-viable sources. In a vineyard setting, if these metagenomic monitoring practices were taking place without the use of PMA viability selection, these results would have suggested a disease instance within the vines, resulting in fungicidal sprays that could affect the established structure of the grapevine microbiome. Responses to false positives could be catastrophic to the micro-biodiversity of vineyards resulting in unfavourable sensory characteristics in wild wines.

Conclusions

With the growing movement towards wild wines within the wine industry, new viticultural and oenological practices will be required. To produce grapevines with sufficient micro-biodiversity for the fermentation of wine, metagenomic analytical techniques must be adopted for sufficient monitoring of the grapevine microbiome. With the inclusion of metagenomic monitoring in vineyards, considerations must be taken to ensure these microbial communities are observed with extreme scrutiny to account for potential false positives such as those demonstrated in our results. By specifically monitoring the living microbiome, the wine industry can begin to discover and adopt practices for cultivating microbial communities which produce healthier grapevines for more sustainable practices, as well as higher quality wild wines for the current growing market.

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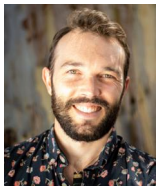

Dr Raphael Eisenhofer is a postdoctoral researcher at the Globe Institute, University of Copenhagen, Denmark, and an adjunct assistant lecturer at the University of Adelaide. His research focus is studying the microbiomes of native Australian mammals, though he applies his metagenomic expertise to diverse study systems.

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Dr Stephen Kidd is a research group leader at the University of Adelaide. His group studies the bacterial response to environmental stress, specifically the adaptation over long periods of time and the impact on the functionality of the microorganisms. This has extended to the responses of microbial communities, such as the grapevine microbiome.

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Growing soil organic carbon in dryland agricultural systems

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ABSTRACT

Soil organic carbon (SOC) plays a crucial role in dryland agricultural systems, improving resilience, productivity and delivering a range of ecosystem services including carbon (C) sequestration and broader ecosystem health. Although the net primary production (NPP) is the principal source of C inputs to soil, plant–microbe interactions can help increase NPP and stimulate plant C inputs to the soil through a variety of mechanisms. Additionally, the soil microbial community plays a crucial role in the loss (CO_2 respiration) and stabilisation of SOC. With improved understanding of soil microbiomes and plant–microbe interactions, there are new emerging strategies in which microorganisms may be harnessed either directly or indirectly to increase the amount of C added and stabilised in dryland soils.

Sources of carbon, the role of microorganisms and the impacts of management practices

The primary source of carbon (C) inputs to soil is net primary production (NPP) by photosynthetic fixation of atmospheric CO_2 to plant biomass and exudates[1] (Fig. 1). Although exogenous inputs in the form of organic amendments, such as manures, composts and biochars, may be locally relevant they are not typically available in regionally or nationally significant quantities, and typically involve the diversion of C that has already been removed from the atmosphere; thus they are not the concern of this paper. In dryland systems that account for ~45% of global land area[2] and typically receive <500 mm of annual rainfall, water availability is the governing factor that limits NPP and thus flows of C to the soil organic C (SOC) pool. Soil microorganisms process C inputs from plants – famously described by David Jenkinson as 'The eye of the needle'[3] – with a proportion of the C lost through microbial respiration to CO_2, and the remainder retained in microbial biomass. This partitioning is termed carbon-use efficiency (CUE).[4] In turn, microbial biomass is itself subsequently further cycled upon cell death.

Over the past three decades, our understanding of the processes involved in SOC stabilisation have evolved greatly. The traditional view of chemical recalcitrance driving persistence has been largely replaced by multiple lines of evidence that point towards physical and chemical protection of kinetically unstable compounds within aggregates and upon clay particles.[5] Although emerging research suggests that direct stabilisation of plant C inputs may have been underestimated,[6] it is understood that the soil microbial community plays a pivotal role in both stabilisation and loss of C in soils. Indeed, in low CUE situations, over 90% of C from fresh inputs may be respired[7] (Fig. 1).

Soil organic C does not exist in isolation. It is a component of soil organic matter (SOM), and has been shown to broadly conform to Redfield's stoichiometric ratio, having fairly consistent proportions of C:N:P:S.[8] Plant C inputs typically contain far greater C:nutrient ratios than microbial biomass or stabilised SOM. Thus, in order to build SOC, nutrients are also required, and the availability of nutrients in ratios broadly representative of SOM typically increases microbial CUE,[4] although clay content and the size of the extant microbial biomass can be more important drivers.[9]

Modern management practices in dryland systems often seek to maximise water availability for the target crop through the adoption of zero tillage, stubble retention as a mulch and the control of summer weeds. Each of these practices can have a direct or indirect effect on the inputs to and losses of SOC. Additionally, in dryland cropping systems such as those predominantly found across the Australian wheatbelt, nitrogen (N) is frequently a limiting factor for crop production because of conservative application rates that target individual season profitability and may 'mine' mineralised N from SOM,[10] resulting in increased losses of SOC by mineralisation.

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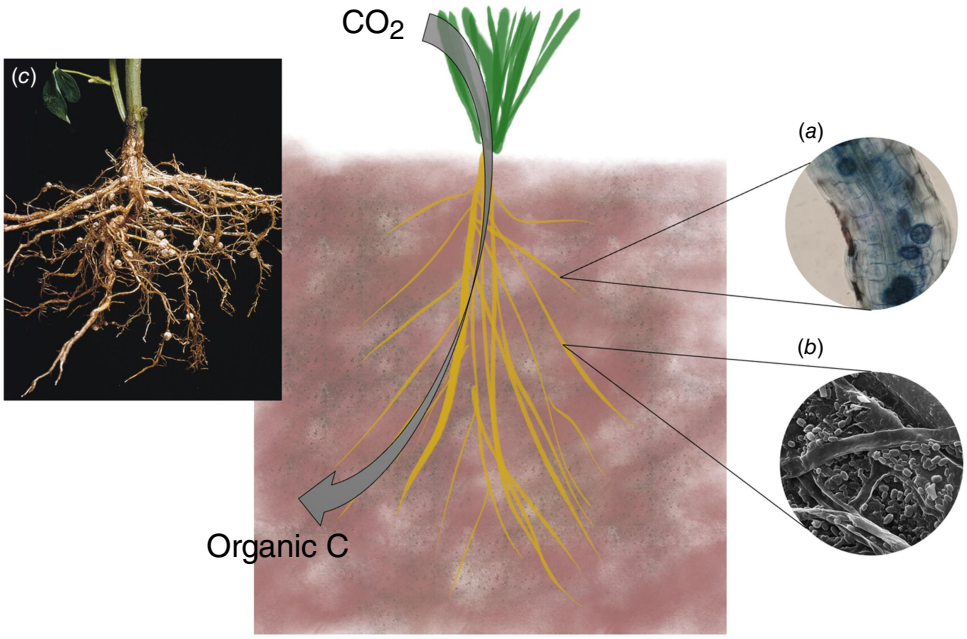

Fig. 1. Conceptual diagram of carbon flow from the atmosphere by the plant to the soil. Photosynthesis fixes atmospheric CO_2, which then enters the soil by above- and below-ground litter and rhizodeposits. Symbiotic fungi such as mycorrhizae (*a*), plant growth promoting rhizobacteria (*b*) or, in the case of legumes, rhizobia (*c*) modulate plant C inputs to the soil by facilitating greater root exploration, N_2 fixation, greater plant productivity and, especially in the case of mycorrhizae, the deposition of their own necromass.

Against this background, a number of practices are emerging that seek to deliver more C to the soil through increasing the proportion of time a living plant is present in the system (e.g. by using cover crops in otherwise fallow periods[11]), harnessing plant traits that increase C delivery below ground,[12] or manipulating the soil, rhizosphere or root microbiome to increase the stabilisation of plant C inputs.

Progress to date

The most widespread type of biological amendment in regular use in dryland agriculture are rhizobia inocula – N_2 fixing symbionts of legume crops. Tightly optimised for legume crop type and often soil properties, rhizobia can fix 20–25 kg N Mg dry matter^{-1} year^{-1} in dryland systems.[13] Because of the lower C:N ratio of legume-derived organic matter inputs, higher CUE and thus greater retention of legume-derived C may be expected.

However, rhizobia are, by and large, the exception. Although there are a multitude of biological amendments available, including both active inocula and biostimulant products, consistency of results remains poor.[14] Unlike the highly specific and well-understood legume–*Rhizobium* symbiosis that is harnessed to perform an exclusive function, many commercially available microbial inocula have more generalist target outcomes. In a lot of cases, including those with reported aims of increasing SOC, mechanisms and modes of action have often remained unclear or poorly verified. Additionally, there is a need to improve mechanisms of application that protect microbial inoculants from the harsh soil environment prior to their colonisation of the root and rhizosphere. It is necessary to develop strategies for effective inoculation methods, so that single species or consortia of microorganisms of interest can gain an advantage in colonisation efficiency over native microbiomes in the field environment. This may present a significant challenge given that native microbial communities are highly adapted to their environment.

An important approach to potentially improve the efficacy of microbial inocula and biostimulants in dryland agricultural systems is to develop a clearer understanding of the limits to crop productivity and thus C inputs. Aligned with this, a better understanding of interactive effects of multiple management interventions across several seasons and rotations on plant C inputs by NPP and microbial stabilisation of C inputs is required. Hallama *et al.*[11] concluded that cover crops increased mycorrhizal abundance, leading to improved colonisation of the main crop and subsequent provision of nutrients. However, although appropriate application of fungicides may control crop disease and increase crop yield and C inputs of an existing crop, fungicides may also negatively affect non-target fungal functions,[15] including potentially beneficial mycorrhizae, and this is particularly the case for anti-fungal seed coatings.

A promising future?

Recent plant root research has improved our understanding of the diversity of root traits and their contribution to plant and ecosystem functioning.[16] Coupled with this is the progress in understanding root–microbiome interactions both in terms of the drivers of microbiome function and the potential

consequences of manipulating microbiomes to stimulate root growth, health and biogeochemical processes – including C turnover and stabilisation.[17] It is suggested that root architectural traits known to increase below-ground plant-derived C inputs are important drivers of microbial community structure and biomass, which in turn contribute to turnover and stabilisation of freshly added C.

A number of microbial genera and species generally referred to as plant-growth promoting rhizobacteria (PGPR; e.g. *Pseudomonas*, *Azospirillum*, *Bacillus* spp.) have been shown to produce phytohormones (e.g. auxins, gibberellins and cytokinin) and other quorum sensing molecules[18] that promote root growth (lateral root formation, root hair development), thus expanding rhizosphere and rhizodeposit C addition. PGPR may also benefit root growth and functioning through establishment of rhizobial and mycorrhizal symbioses, protection from soil-borne root pathogens and by eliciting plant defences including induced systemic resistance. Beneficial effects from such interactions, in particular from introduced organisms, depend upon the correct microbe–plant combination and the expression of the required functional traits (e.g. producing optimal concentrations of hormones at the appropriate time). Therefore, harnessing this type of root–microbe interaction requires a deeper understanding of the complex feedback mechanisms associated with the abundances of specific microbial species, the types and concentrations of chemical signals, and genetic and environmental controls for functional expression.[18]

Despite extensive research demonstrating potential benefits from the symbiotic associations between arbuscular mycorrhizae and agricultural crops, practical applications of these associations are yet to be utilised, largely because the complex influences of edaphic and environmental factors in field environments are not well understood. In Australian dryland agricultural soils with low SOM concentrations, arbuscular mycorrhizae can make a significant contribution to improving plant C inputs and turnover processes through their extensive hyphal networks combined with enzymatic, metabolic and nutrient acquisition capabilities and effects on C translocation below-ground.[16]

Plant genotype may play a significant role in the recruitment, assembly and activities of the rhizosphere microbiome. The composition of highly diverse rhizosphere microbiomes is largely selected by the host plant but primarily modulated by soil type.[19] For example, a core root microbiome dominated by a restricted group of bacterial taxa has been found in multiple phyla growing in close proximity. This suggests shared functionality relating to traits in the core root bacterial communities and opportunities for a targeted approach to manipulate root growth across a broad spectrum of plant types.[20] Crop-based variations in rhizosphere microbiomes have been well documented, with diverse rotations being shown to increase disease suppression and avoid negative legacies.[21] Recent evidence has shown that structural or taxonomic diversification of rhizosphere-associated microbial communities exists within crop varieties and between wild and domesticated accessions of many crops (barley, wheat, maize, pearl millet and arabidopsis) – that is, root-associated microbiomes have greater heritable variation.[22] Such differences in root traits and microbiomes could contribute to variability among cultivars for soil C cycling and SOC sequestration potential. A strong relationship between phylogenetic distance and rhizosphere microbiome dissimilarity is reported for different species in both monocotyledons and dicotyledons.[23]

As mentioned earlier, N availability is the greatest constraint to Australian dryland wheat production. Engineering crops and synthetic plant–microbe symbioses to introduce N_2 fixation machinery to cereals and other non-legume crops is decades away. Therefore, designer plant–diazotrophic combinations are an attractive option to remove N constraints to production and to increase C inputs in agricultural systems.[24] The ecological significance of free-living or associative diazotrophic N_2-fixing bacteria in agricultural soils is becoming more appreciated. A diverse diazotrophic community exists in soils and in below- and above-ground plant parts, and a growing body of evidence suggests that they could be a significant contributor to cereal crop N budgets and thus C inputs.[25] This presents an opportunity to harness their capacity in cereal dominated cropping systems.

Although improvements to plant production have been facilitated through breeding and targeted gene manipulation of agricultural crops, there is growing evidence that microorganisms associated with crops can impart positive outcomes on biomass production and biogeochemical processes including C turnover in the vicinity of plant roots. Thus, by understanding the relationship between plants and microbes, more-efficient agricultural systems, particularly in dryland or rainfed cropping regions, could be developed through selection of improved plant genotype–microbiome combinations. Another recent approach is the direct crop application of microbe-to-plant signal molecules, such as isoflavonoids to improve crop tolerance to stresses and enhance plant growth, thus avoiding the constraints related to the successful introduction of microbial inoculants.[26]

In conclusion, clear potential pathways are emerging by which microorganisms may be harnessed either directly or indirectly to increase the amount of C added and stabilised in dryland soils. These will come from both wider rotation and system-based changes, such as appropriate adoption of cover crops and their feedback effects, and also by targeted plant–microbe interactions and introduction of PGPRs, etc. However, all are underpinned by sustaining or enhancing C inputs from the cash crop, and thus there will be occasions when management to maximise this may involve the targeted use of agrochemicals as part of a sustainable agricultural system.

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Data availability. Data sharing is not applicable as no new data were generated or analysed during this study.

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Dr Gupta Vadakattu investigates on aspects of genetic and functional diversity, functional capability and resilience of soil biota in agricultural soils. Special interests include: genetic, functional and environmental regulators of biological disease suppression in soils, phenotypic and functional diversity of microbiota in the rhizosphere systems, diversity and functional capacity of diazotrophs in annual and perennial crops and turnover to carbon, nutrients and biological health of soils.

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Mitigating greenhouse gas emissions from waste treatment through microbiological innovation

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ABSTRACT

The emission of greenhouse gases (GHGs) from the treatment of municipal, agricultural and industrial waste occurs in virtually every city on our planet. This is due to various microbial activities at different stages of waste treatment. Traditional treatment methods have a significant environmental impact, producing methane, carbon dioxide and nitrous oxide emissions, in addition to demanding high energy input and having low treatment efficiencies. To address these issues, the Australian water and waste sectors are shifting towards the adoption of next-generation, carbon-neutral treatment options. Here I discuss our current knowledge gaps in mitigating GHG emissions from waste streams, with a focus on wastewater treatment plants. I highlight the application of real-time genomics to identify sources of GHG emissions, monitor mitigation efforts, assist process operation and guide plant operations. I also emphasise recent innovations of microbial processes that capture GHG from waste and upgrade them into higher value products. Ultimately, combined effort across disciplines is required to proactively mitigate the global threat of climate change.

Greenhouse gas emissions from waste streams

The first systematic quantification of methane and carbon dioxide emissions from wastewater treatment plants (WWTPs) dates back to 1993, at a full-scale wastewater treatment plant serving 12 500 inhabitants in Durham, NH, USA.[1] Aeration is an essential part of modern wastewater treatment, which provides oxygen to support the respiration of aerobic microorganisms to degrade organic carbon and nitrogen compounds. The activated sludge process (Fig. 1), which uses microbial flocs or granules to remove pollutants such as carbon, nitrogen and phosphorus from wastewater, depends on this aeration.[2] This is a crucial process to safeguard public and environmental health,[2] but it also leads to the emission of carbon dioxide through the respiration of heterotrophic microorganisms and nitrous oxide (N_2O) through activities of nitrifying and denitrifying microorganisms. Anaerobic digestion (Fig. 1) is the biological treatment of waste sludge in the absence of oxygen to stabilise organic matter while producing biogas, containing methane and carbon dioxide.[3] Anaerobic digestion has become a mature technology widely applied in WWTPs in Australia and across the world. Disposal and land application of digested sludge also results in carbon dioxide emissions. It is estimated that methane emission from wastewater treatment and landfills (Fig. 1) accounted for 21% of global methane emissions in 2021.[4] Established sewage networks and wastewater treatment facilities in nearly every city make WWTPs among the largest point sources of GHG emissions and also ideal entry points to mitigate GHG emissions (Fig. 1).

Pinpointing sources and regulators

Microorganisms in wastewater treatment play key roles in regulating GHG emissions, so it is critical to understand their physiology and ecology to effectively reduce these emissions. The respiration of microorganisms in standard treatment technologies, such as the anaerobic–anoxic–oxic (AAO) process or sequencing batch reactors (SBR), leads to direct carbon dioxide discharge into the atmosphere. Anaerobic digestion is the major source of methane emission based on estimation from eight Austrian WWTPs[5]; and it also produces carbon dioxide to a less extent.[6] More recently, it has been recognised that much nitrous oxide is being released from WWTPs at significant rates and can contribute

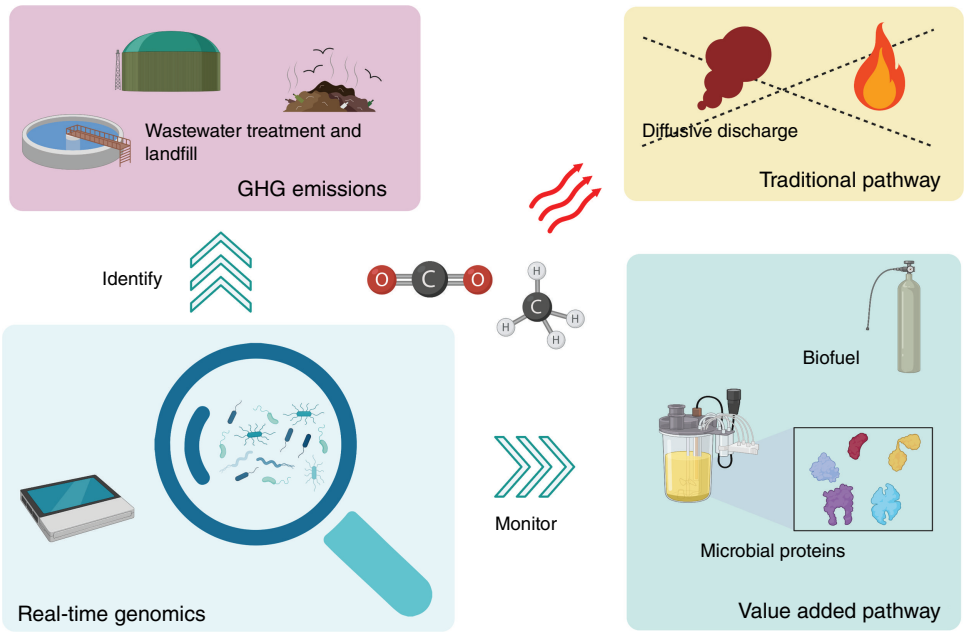

Fig. 1. Conceptual illustration of applying real-time genomics to identify hotspots of GHG emission at wastewater treatment and agricultural settings, and to monitor microbial waste valorisation processes. Created in Biorender.com.

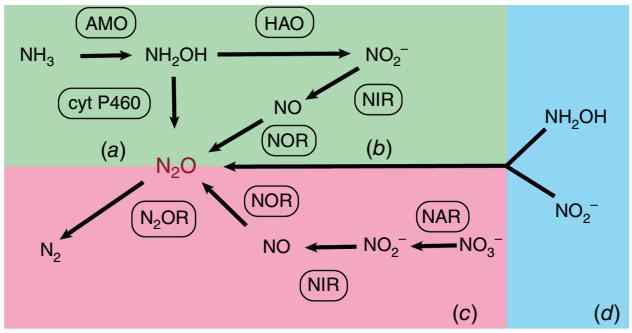

Fig. 2. Pathways of nitrous oxide emission encoded in ammonia oxidisers (green), denitrifiers (pink) and the abiotic codenitrification pathway (blue); subprocesses include nitrification and denitrification by ammonia oxidisers (*a*, *b*), denitrification by heterotrophic microorganisms (*c*), and codenitrification (*d*). Key enzymes for nitrogen compound transformation are indicated in circles, including AMO, ammonia monooxygenase; HAO, hydroxylamine oxidoreductase; cyt P460, cytochrome P460; N_2OR, nitrous oxide reductase; NOR, nitric oxide reductase; NIR, nitrite reductase; and NAR, nitrate reductase.

up to 83% of the emissions footprint of WWTPs expressed as CO_2 equivalents.[7] Both nitrifying and denitrifying microorganisms mediate the release and consumption of nitrous oxide through four known processes: (a) as a by-product of hydroxylamine (NH_2OH) oxidation by nitrifiers; (b) through nitrifier denitrification during the reduction of nitric oxide (NO); (c) denitrification by heterotrophic microorganisms; and (d) codenitrification, where one nitrogen atom in N_2O originates from hydroxylamine and the other from nitrite[8,9] (Fig. 2). Compared to carbon dioxide and methane, we are still in the preliminary stage of understanding nitrous oxide regulation pathways, and require integrated research efforts of the microbiology, biochemistry and biogeochemistry underlining these processes.

We currently lack microbiology-based prediction tools for GHG emissions. Isotopic techniques have been developed to quantify the contribution of nitrous oxide production and consumption from different regulatory pathways, but they are labour-intensive and hindered by the presence of unidentified microbial pathways.[9] Additionally, these methods require elaborate laboratory procedures and equipment, making them difficult to operate remotely. The mobile tracer gas dispersion method can estimate major sources of GHG emissions on a plant level based on the dispersion patterns of methane and nitrous oxide in the atmosphere,[10] but it does not allow for long-term and continuous monitoring and is heavily dependent on stable wind patterns.[11] By contrast, process unit quantification using the standard floating hood technique has methodological limitations in addressing spatial variability of GHG emissions or inaccuracies caused by mass transfer alterations inside the hood, as well as practical limitations in terms of difficulties in being deployed at foaming and turbulent wastewaters or treatment units with obstacles such as surface aerators.[11] These limitations make it challenging in extrapolating GHG emission estimation from floating hood measurements to larger areas and capturing the spatial dynamics of GHG emissions at the plant level.[11] The main drawback of these methods is that they fail to uncover the microbial regulatory processes, lack the ability to consider metabolic diversity, and are not able to predict greenhouse gas emissions with optimal precision on a spatial and temporal scale. These models rely on generic pathways to describe overall nitrous oxide emission; they do not address the inner workings of microbial communities; and therefore, cannot discern the contributions of individual pathways as described above (Fig. 2).

Predictive understanding of GHG emissions from the waste sector depends on better understanding the microbial mediators and mitigators of this process. This requires systematic investigation of the physiology of GHG cycling

microorganisms, including methanogens, nitrifiers, denitrifiers and heterotrophic bacteria, found in all locations of wastewater treatment plants (WWTPs) known for GHG emissions, including aeration tanks of the AAO process or SBR reactors, anaerobic digesters, sludge drying lagoons, clarifiers and disinfection units.[12,13] Culturing is the definitive way to characterise the capacity of these microorganisms to produce or consume GHGs and understand how this cycling varies depending on environmental conditions. Rapid advancements in sequencing technologies have significantly decreased the cost of sequencing, with increased throughput and higher accuracy using less genetic material. The use of portable sequencers, such as the Oxford nanopore minion (ONION), coupled with cloud computing, has allowed specialists to observe shifts in methanogenic communities on-site at WWTP operations in near real time.[14] This can provide early warning of microorganisms that have potential to increase GHG emissions, therefore inform mitigation strategies such as pH alteration to suppress the growth of these organisms. By integrating physiological data of these microorganisms with real-time genomics techniques, biogeochemical measurements and supervised machine-learning approaches, it will be increasingly possible to develop predictive models for GHG emissions across space and time. However, this requires further calibration based on operational data such as temperature, dissolved oxygen and nutrient load.

Using microbiology to reduce and recycle emissions

Real-time genomics to guiding treatment operations

There are several options to use real-time genomics to guide GHG mitigation and plant operation. Nitrous oxide reductase (N_2OR, Fig. 2) is the only known enzyme responsible for reducing nitrous oxide emissions.[15] By metagenomics and metatranscriptomics, the activity of microorganisms expressing *NosZ* can be estimated at hotspots of GHG emissions through the identification of microorganisms encoding the *NosZ* gene and their mRNA transcripts. This information can be used to identify optimal operational conditions (such as carbon and nitrogen load, pH, dissolved oxygen level, and temperature) at which N_2OR activities are the highest in real time. Additionally, monitoring the expression of cytochrome P460 or nitric oxide reductase (cyt P460 and NOR, Fig. 2) at different treatment conditions can reveal metabolic dynamics of nitrous oxide emission, which can guide process operation to reduce GHG emissions.

Moreover, real-time genomics can assist optimisation of treatment processes. Traditional treatment methods have drawbacks such as high energy consumption, the need for a carbon source, fugitive GHG emissions, low energy recovery efficiency and excessive sludge production.[16] Recent advances in combining anaerobic methane-oxidising archaea and bacteria with existing treatment techniques have the potential to overcome these barriers by utilising methane as a carbon and energy source to enhance nitrogen removal.[17] However, one limitation of using these anaerobic microorganisms is their slow growth (doubling time >10 days); it can take longer than a year before performance improvement is visible.[16] Genomic sequencing and real-time analysis can greatly aid in optimising reactor configuration and operational conditions by providing immediate feedback on the ecological (such as abundance) and physiological states (expression of genes related to growth, adaptation and stress response) of these anaerobic methanotrophs.

Furthermore, real-time genomics can provide early warnings of unwanted microorganisms. The stable performance of wastewater treatment processes relies on the healthy composition of microbial communities. For instance, the notoriously long-standing problem of poor sludge settleability in clarifiers (also known as 'sludge bulking') is caused by filamentous bacteria.[18] Partial nitritation coupled with the anammox process (anaerobic ammonia oxidation) is a highly promising treatment method as it reduces aeration costs by up to 60% aeration costs and eliminates the need for organic carbon dosing.[19,20] However, the appearance of nitrite oxidising bacteria, which competes with anammox bacteria for the substrate nitrite, can impede the process. The emergence of these unwanted microorganisms can deteriorate the treatment processes, but they can only be detected when they have already established themselves. This leaves treatment specialists in a reactive position, as current methods such as physiochemical measurements cannot detect them early. Real-time genomics can predict the emergence of these unwanted microorganisms through early detection by genome-resolved metagenomics or marker gene-based sequencing combined with physiological data. This enables targeted response strategies, such as lowering dissolved oxygen and increasing ammonia concentration to suppress nitrite oxidising bacteria.[21] These genomics solutions require refinement and testing for improved robustness, increased accuracy and reduced cost before full-scale implementation.

Converting methane to value-added carbon

One promising way to mitigate GHG emissions from the waste sector is to convert methane produced by methanogens into value-added carbon. By using the metabolic capacities of methanotrophic microorganisms,[22] it is possible to convert methane into higher-value products such as methanol, polyhydroxyalkanoates (PHA), biopolymers and single-cell proteins (SCPs).[23] The first step in these conversions is methane oxidation to methanol catalysed by the soluble form of methane monooxygenase (sMMO) or its particulate form (pMMO) (Eqn 1).[24]

$$CH_4 + O_2 + 2e^-/2H^+ \rightarrow CH_3OH + H_2O \qquad (1)$$

where $\Delta G^0 = -111\ kJ\ mol^{-1}$.

The most well-known aerobic methanotrophs for biotechnological exploration include *Methylococcus capsulatus* Bath, *Methylomonas* spp. and *Methylosinus trichosporium* OB3b. However, aerobic conversion requires an oxygen input of at least a 1:1 methane to oxygen ratio. Although oxygen is widely available, it costs from US$98.4 to $123.0 per tonne depending on the cost of energy,[25] which increases the total cost.[26] Anaerobic methanotrophic (ANME) archaea

and '*Candidatus* Methylomirabilis' (NC10) bacteria are promising candidates for methane biorefinery through anaerobic or intra-aerobic oxidation,[27] with significant research and development to understand their metabolism and ecology.

Innovative CO_2 capture

By 2050, microbial proteins are estimated to replace between 10 and 19% of conventional crop-based animal feed protein demand, depending on socio-economic development and microbial protein production from GHGs.[28] This can substantially decrease global cropland expansion, GHG emission and nitrogen pollution. Carbon dioxide produced at WWTPs is conventionally perceived as waste carbon, but it can be an ideal stock feed for a variety of novel carbon dioxide capture technologies because it is produced at a point source. In addition, aerobic hydrogen oxidising microorganisms such as *Cupriavidus necator* are considered to be 'powerful microbial actuators' due to their ability to use hydrogen to conserve energy (Eqn 2) and fix carbon dioxide into cellular material,[29] producing single-cell proteins with increased value and high yield.

$$H_2(g) + \tfrac{1}{2}\,O_2(g) \rightarrow H_2O(l) \qquad (2)$$

where $\Delta G^0 = -237.1\ kJ\ mol^{-1}$.

Examples include *Sulfuricurvum* that was shown to provide stable performance over nearly 100 days in a laboratory scale study, converting carbon dioxide, ammonium and hydrogen into high quality microbial proteins.[30] Also, purple phototrophic bacteria are anoxygenic phototrophs capable of using a variety of organic (volatile fatty acids) and inorganic (hydrogen gas, hydrogen sulfide and ferrous iron) substrates for anaerobic photoheterotrophic or photoautotrophic growth.[31] Their unique capacity for near-infrared light absorption by bacteriochlorophylls or visible light absorption by carotenoids highlights their suitability for carbon dioxide capture and single-cell protein production in Australia, where solar radiation is abundant.[32] Additionally, carbon dioxide capturing through microalgae to produce biodiesel offers another interesting avenue to harvest solar energy to fix carbon dioxide with high biomass yield.[33] Finally, it is also possible to produce biochemicals such as ethanol, methane, hydrogen and propanediol through microbial electrosynthesis with renewable electricity as input.[34]

Conclusion

Climate change poses an imminent existential threat to humanity. Innovative microbiological technologies such as real-time genomics can provide crucial information to mitigate GHG emission at treatment plants. Microbiology also offers a range of solutions for capturing and valorising GHGs (Fig. 1). As microbiologists, we strive to reframe the current paradigm of intensive resource exploration and waste discharge into valorisation of waste and GHG mitigation with improved treatment processes. To make this happen, we need combined expertise in molecular and structural microbiology, process engineering, mathematical modelling. We also need to foster collaboration among waste management, academic research, and renewable energy sectors to limit global temperature increase to below 1.5°C in the next two decades.[35]

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Biography

Dr Gaofeng Ni is a Postdoctoral Research Fellow and Industrial Microbiology Team Leader at the One Health Microbiology Laboratory led by Professor Chris Greening at Monash University. His expertise ranges from biotechnological innovation in industrial wastewater treatment to the molecular physiology and metabolism of microorganisms living at extreme conditions.

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Microbial conversion of waste gases into single-cell protein

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ABSTRACT

Climate change and food security are two of our most significant global challenges of our time. Conventional approaches for food production not only produce greenhouse gases but also require extensive land and water resources. An alternative is to use gas fermentation to convert greenhouse gases as feedstocks into microbial protein-rich biomass (single-cell protein). Aerobic methanotrophic (methane-oxidising) and hydrogenotrophic (hydrogen-oxidising) bacteria, which produce biomass using gases as their energy and carbon sources, are ideal candidates for single-cell protein production. However, multiple innovations are required for single-cell protein production to be economical and sustainable. Although current technologies rely on conversion of purified single gaseous substrates, the potential to directly use mixed gas streams from point sources remains reasonably unexplored. In addition, there is much potential to increase nutritional and commercial value of single-cell protein through synthetic biology. In this perspective, we discuss the principles, approaches, and outlook for gas fermentation technologies aiming to significantly reduce greenhouse gas emissions and enhance food security.

Background

The International Panel on Climate Change 2022 report has warned the world to limit global warming to 1.5°C within the next two decades.[1] Global warming is a result of anthropogenic greenhouse gases (GHGs) such as carbon dioxide and methane, which are primarily produced by the energy, waste, transport, and agriculture and food industries. There is a paramount need to reduce and recycle these emissions given climate change is leading to environmental catastrophes and increasing human health risks. A circular economy would employ economically viable processes to convert emissions into products, thereby mitigating their impact. Gas fermentation technologies offer several advantages by producing various end-products such as high-value chemicals, biofuels and protein feed.[2,3] In contrast to typical gas conversion processes by thermochemical catalysis (e.g. Fischer–Tropsch reactors), biological processes can occur at close to ambient temperatures and pressures. Toxic gases also poison chemical catalysts, whereas some bacteria tolerate and even utilise these compounds.[4]

The global population is expected to rise to 9.8 billion by 2050 (UN 2019 Revision of World Population Prospects[5]), increasing global food demand. Current practices for animal-derived protein production lead to significant release of GHGs, and may not be feasible for increasing consumption levels of a growing population. A recent study found that food production generated ~35% of the world's anthropogenic GHG emissions from 2007 to 2013.[6] There is much industry interest in transforming the food production system and, at the same time, minimising further climate impacts and biodiversity loss. Rising public awareness and ecological factors are encouraging consumers towards more sustainable products. Some microbes can convert GHGs into single-cell protein, reducing the climate's adverse impacts on food production. Single-cell protein (SCP) is derived from bacteria, algae, yeast or fungi and has high protein content. SCP therefore has the potential to replace traditional protein sources such as fish and soybean products in human and animal feed.[7] Currently, SCP occupies a reasonably small market for human nutrition, though SCP is likely to become a significant alternative amid rising global demand for protein and increasing need for sustainable food production.[8] Advantages of SCP include reduced production time, water and carbon footprints, and biodiversity impacts, e.g. extensive use of Antarctic krill in aquaculture feed is driving concerns of

Southern Ocean ecosystem collapse.[9] Further, SCP can be produced at any time of the year, avoiding the risk of seasonal and climatic variations, as well as biotic factors such as pathogens or pests.[10] Thus, SCP production can be a more efficient and sustainable solution than traditional agriculture.

Bacteria are particularly promising SCP producers, given they can use inorganic feedstocks and produce 50–80% protein by dry weight.[11] Bacteria can produce SCP using a wide range of feedstocks, including waste gases. Waste gases are produced in abundance by various industries and released into the atmosphere. Methane is the second most abundant GHG after carbon dioxide and has contributed ~30% of global emissions to date.[12] Aerobic methane-oxidising bacteria, also known as methanotrophs, consume significant amounts of methane before it is emitted into the atmosphere and also serve as the primary biological sink of atmospheric methane (~30 Tg year^{-1}).[13] There is much ongoing research and development on using methanotrophs to produce methane-derived SCP.[14] Methane-derived SCP has a promising nutritional profile: it is reported to be rich in essential amino acids such as histidine, valine, phenylalanine, isoleucine, leucine, threonine, and lysine,[15] with notable amounts of vitamins, minerals, and essential fatty acids.[16] In addition to methanotrophs, aerobic hydrogen-oxidising bacteria (HOB), also known as Knallgas bacteria, have emerged as promising candidates for SCP production. HOB fix carbon dioxide using hydrogen as the electron donor and oxygen as the electron acceptor. Hydrogen is oxidised by hydrogenases and carbon dioxide is fixed for biomass production by the Calvin–Benson–Bassham cycle under aerobic conditions.[17,18]

Commercial advancements in SCP production

The need for sustainable feed solutions is increasing the demand for single-cell proteins. Though SCP production has gained increased attention in recent years, it has been under investigation since the 1960s. Pruteen is an example of SCP that was first commercialised by Imperial Chemical Industries using the methanotrophic bacterium *Methylophilus methylotrophus*.[19] Despite the high protein content of Pruteen, the production was discontinued because of poor economics. Since then, SCP production is becoming more economically favourable and methane is gaining renewed attention as a cheap feedstock while reducing GHG emissions.[20] Innovators include Calysta, an American company that uses methanotroph, *Methylococcus capsulatus*, to convert methane from natural gas into the protein feed, 'FeedKind'. FeedKind is reported to use 100 times less water and 1000 times less land when compared to soy-based meal.[21] Similarly, Unibio in Denmark produces methane-based SCP to feed pigs and pets.[22] Other companies such as String Bio and Circe Biotechnologie GmbH are developing SCP focusing on aquaculture industry.[23,24]

In recent years, several companies have also explored the use of HOB to produce food and feed ingredients. Solar Foods, a Finnish company founded in 2017, has developed human-grade SCP, 'Solein', and has submitted to the European Commission for approval for safe human consumption.[25] Similarly, other companies pioneering mass production of hydrogen-based SCP are Novonutrients, Kiverdi and Deep Branch Biotechnology.

It should be noted that SCP must not only have nutritional value, but should also be safe for human and animal nutrition. The limiting factor with bacterial SCP for human consumption is the presence of nucleic acids (~8–12%).[26] This concentration of nucleic acids can cause human health issues such as kidney stones and gout.[27] However, nucleic acids can be removed during food processing, for example by nuclease treatment.[28] Another important aspect to consider in SCP production is from an economic point of view regarding feedstock availability, scalability and processing. Therefore, further advancements are important for producing cost-effective SCP.

Future directions

To improve the economic viability of SCP production, mixtures of waste gases should be used as substrates. So far, SCP production relies on single gaseous substrates typically derived from fossil fuels. The advantages of using waste gases are that they are cheap, available and have a lower environmental footprint. One notable example is Lanzatech, a key technology developer in gas fermentation, which uses steel mill off-gas or syngas to produce carbon-negative products by anaerobic acetogenic bacteria.[29]

We now have evidence that most aerobic bacteria are mixotrophs and can use multiple substrates to enhance growth and survival.[30,31] The metabolic versatility of aerobic bacteria can provide a platform to exploit mixed waste gases as a substrate to produce protein-rich biomass. Our current work aims to assess whether metabolic versatility will enable mixotrophic bacteria to convert mixed waste gases from different sources, such as steel mill gas, biogas and syngas to maximise the benefits offered by mixotrophic bacteria for the production of animal feed or human food (Fig. 1). To do this, we are assessing growth, gas conversion and metabolite production of a panel of hydrogenotrophs and methanotrophs. For example, we have discovered that the verrucomicrobial methanotroph *Methylacidiphilum* sp. RTK17.1 optimally grows using a combination of hydrogen and methane simultaneously as energy sources, carbon dioxide as the carbon source and oxygen as the final electron acceptor.[30] This depends on the activities of its uptake [NiFe]-hydrogenase, particulate methane monooxygenases, and RuBisCO-dependent Calvin–Benson–Bassham cycle. It is therefore ideal to use a range of flue gas and biogas streams. *Hydrogenophaga pseudoflava* is a promising candidate for aerobic syngas conversion. This bacterium grows by simultaneously oxidising hydrogen and carbon monoxide to support aerobic respiration and carbon fixation, a process that depends on the activities of oxygen- and carbon monoxide-tolerant hydrogenases and carbon monoxide dehydrogenase.[32] Another hydrogenotrophic model organism, *Cupriavidus necator* H16, has been intensively studied and grows rapidly in the presence of hydrogen and carbon dioxide, while accumulating high titres of biomass.[33]

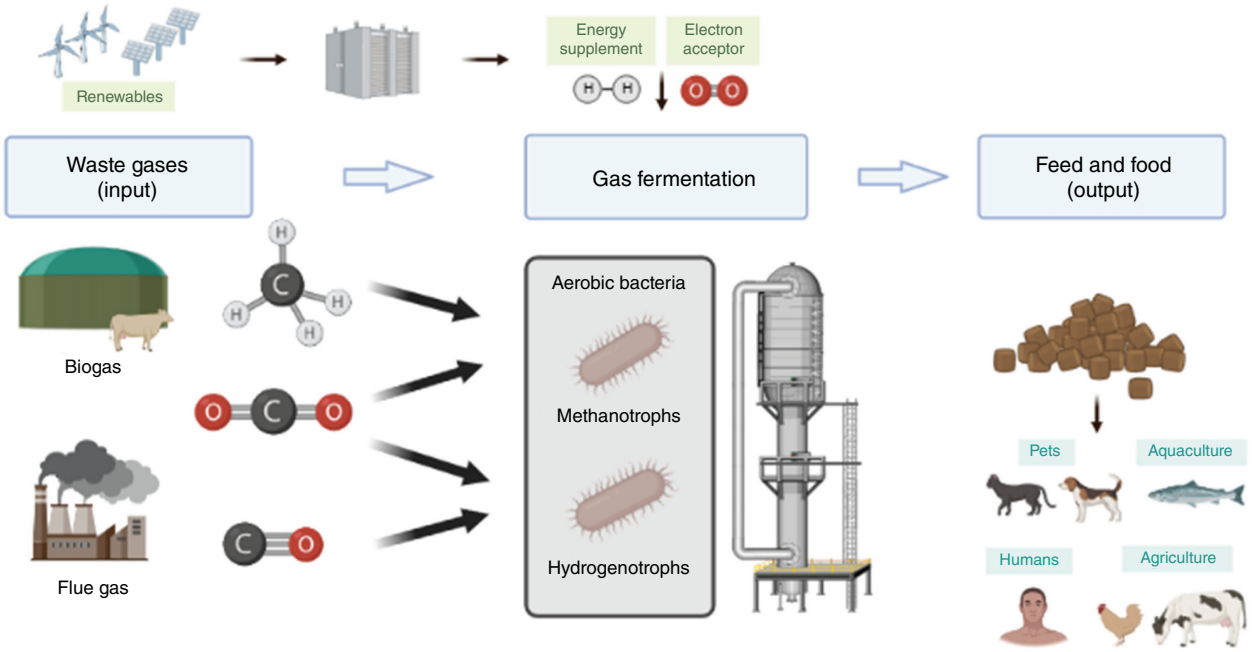

Fig. 1. General process overview for SCP production using bacterial gas fermentation. Figure was created using BioRender.com.

The ultimate aim is to produce high-value SCP suitable for pets, livestock and aquaculture feed and eventually food-grade protein by lowering nucleic acid content. With the rapid development of genetic engineering, SCP can be leveraged in the food and feed industry. The process can be optimised using systems engineering (e.g. changing substrate inputs, bioreactor design), adaptive laboratory evolution and synthetic biology. Gas-consuming bacteria can be genetically engineered to produce desired products such as carotenoid colourants and omega-3 fatty acids, potentially assisting the replacement of wild-catch fishmeal with sustainable fish feed. Astaxanthin, a keto-carotenoid, improves salmon colouration and omega-3 fatty acids are a nutritional supplement for consumers. The well-characterised biosynthesis genes for these metabolites can be synthesised, transformed and induced in the bacterium of interest. Equivalent approaches could achieve the opportunity for producing other high-value chemicals.

Summary

Industrial processes such as fuel refining and waste treatment produce large quantities of mixed waste gases. At the same time, the demand for feed for agriculture, aqua-culture and pets is growing. Sustainable feed production is needed to alleviate land use change, biodiversity loss and climate change impacts. Therefore, a solution is to use mixotrophic bacteria to produce nutritionally rich animal feed from waste gases. Mixotrophic bacteria provide an opportunity to use mixed waste gases as feedstock, given their mixotrophic capability for utilising diverse gases contributing to alleviating climate change. Although such approaches are scientifically sound, efficient bioprocesses are needed for economic implementation and scalability.

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Data availability. The data that support this study are available in the article.

Conflicts of interest. Woodside Energy has interest in commercialising waste gases into SCP in feed or food industry. J. Joshi is an employee of Woodside Energy.

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Gas fermentation for microbial sustainable aviation fuel production

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ABSTRACT

The challenge of limiting global warming to below 1.5°C requires all industries to implement new technologies and change practices immediately. The aviation industry contributes 2% of human-induced CO_2 emissions and 12% of all transport emissions. Decarbonising the aviation industry, which relies heavily on high-density liquid fuels, has been difficult to achieve. The problems are compounded by the continued reliance on so-called sustainable aviation fuels, which use first-generation agricultural feedstocks, creating a trade-off between biomass for food and feed and its use as a feedstock for energy generation. Decarbonising aviation is also challenging because of problems in developing electric aircraft. Alternative feedstocks already exist that provide a more feasible path towards decelerating climate change. One such alternative is to use gas fermentation to convert greenhouse gases (e.g. from food production and food waste) into fuels using microbial acetogens. Acetogens are anaerobic microorganisms capable of producing alcohols from gaseous CO, CO_2 and H_2. Australia offers feedstock resources for gas fermentation with abundant H_2 and CO_2 production in proximity to each other. In this review, we put forward the principles, approaches and opportunities offered by gas fermentation technologies to replace our dependency on fossil fuels for aviation fuel production in Australia.

Keywords: acetogens, aviation fuel, carbon footprint, *Clostridium autoethanogenum*, gas fermentation, greenhouse gases emissions, isobutanol, sustainable aviation fuel.

Background

Tackling the climate crisis is a defining challenge of our times. The Intergovernmental Panel on Climate Change (IPCC) has warned that climate change is occurring faster than previous modelling had anticipated. This calls for large step change driven by technological developments, requiring adoption at scale through substantially changed practices, particularly by industry, to meet this existential threat. As we recover from the COVID-19 pandemic, exacerbated by the geopolitical disturbances wrought by the Russia–Ukraine war, preliminary data suggest that global CO_2 emissions from fossil fuels have just set a new record of 36.6 billion tonnes in 2022, an increase of 1% compared to 2021. This increase is because of the demand for crude oil growth, especially in the aviation sector as demand for air travel increases.[1]

Among transportation, aviation is considered the fastest-growing industry.[2] Aviation fuel consumption by commercial airlines reached 57 billion gallons ($\sim 215 \times 10^9$ L) in 2021 while transporting 2.3 million passengers globally.[3,4] The International Air Transport Association (IATA) forecasts global air passengers to reach 4.0 billion in 2024, exceeding pre-COVID-19 levels.[5] Although international air travel was almost non-existent in the Asia-Pacific due to COVID-19 outbreaks and strict travel restrictions in 2021, solid growth in the region was observed in October 2022, when passenger traffic increased by 440.4% compared to October 2021.[6] In Australia, for the 12 months between September 2021 and August 2022, 84.7 million passengers travelled to domestic and international destinations from local airports, an increase of 86% from the previous year.[7]

In general, the demand for global commercial aviation is increasing rapidly. As a result, the aviation industry already accounts for 2% of global CO_2 emissions and is responsible for many other pollutants, including large solid waste generated during commercial flights.[8] In alignment with the Paris Agreement's ambitious goal to decarbonise the aviation industry, the IATA have set objectives to achieve net-zero emissions by 2050, indicating that ~21.2 billion tonnes of CO_2 must be abated.[9] Batteries are often

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held up as a transport solution to emissions. Electrification indeed presents a pathway for many industries to decarbonise, in which electricity generation relies on renewable or carbon-neutral processes. Although advances in battery technology have introduced the concept of electric aviation, its industrial feasibility remains unclear. Batteries are likely to be feasible for larger road and rail vehicles, and even small aircraft within a decade; however, the weight and range of batteries will preclude their feasibility for large, long-haul transport (primarily commercial and military aviation) without a currently unforeseen technological disruption. To underscore the point, an Airbus A380 has a fuel capacity of 320 000 L of energy-dense, non-oxygenated, hydrocarbon-based liquid fuel.[8,10] In a recent calculation of flying an electric Airbus A320, improvements in battery energy density would have to reach up to 800 Wh kg^{-1}, compared to 100–265 Wh kg^{-1} currently available in electric vehicle batteries.[11,12] As such, a realistic strategy to reach net-zero aviation emissions will include an initial switch to sustainable aviation fuels (SAFs), followed by a transition to carbon-neutral energy sources, such as electric power, to propel larger aircraft. Hence, for the foreseeable future, in terms of a transition from fossil fuels to electrification, improving the economics of SAF production remains a viable path to cut CO_2 emissions. However, the current SAF supply is limited and highly expensive due to infrastructure challenges and the stringent policy framework regulating the production and use of SAFs in current aircraft engines.[13]

Current methods for sustainable aviation fuel production

Unlike petroleum-based fuels, SAFs are alternative fuels produced from renewable sources such as biomass and waste products and therefore have a smaller carbon footprint.[8] Novel methods for producing SAFs include alcohol-to-jet (ATJ), a technically feasible process that supplies commercial-scale aviation biofuels. It involves several catalytic steps such as dehydration, oligomerisation, hydrogenation and fractionation of C_2–C_5 alcohols produced through biochemical fermentation processes, ultimately producing paraffinic kerosene hydrocarbons used as jet fuel products.[14] Isobutanol and ethanol are the only ATJ alcohols certified for commercial use in the US by the ASTM D7566 Standard Specification for Aviation Turbine Fuels Containing Synthesised Hydrocarbon in 2016 and 2018, with up to a maximum blending ratio of 50%.[14,15] Isobutanol is superior to ethanol as jet fuel due to its chemical structure and higher energy density of 33 MJ kg^{-1}, as well as lower vapour pressure, hygroscopicity and flammability.[16]

Moreover, the upgrading process from alcohol intermediate to the final hydrocarbon jet fuel in ATJ processes has a 34% lower conversion cost when using isobutanol instead of ethanol.[15] Therefore, a recent industrial shift to ethanol-free biofuels has been observed. For example, Gevo, Inc.'s biorefinery in Texas has been producing renewable isobutanol for commercial jet fuel using ATJ processes since 2011[17] (Fig. 1). Their production technology, named Gevo Integrated fermentation technology (GIFT), relies on corn waste biomass fermentation using a yeast strain engineered to produce high-yield isobutanol. The bioprocess is coupled to a product recovery system, continuously removing isobutanol as it forms.[15,17]

Current microbial isobutanol production uses yeast strains, in particular *Saccharomyces cerevisiae*. Yeast offers numerous advantages such as its native alcohol production, ease of engineering, low contamination risk and innate tolerance to short-chain alcohols.[18,19] Small concentrations of isobutanol are natively produced in *S. cerevisiae* as a by-product of valine degradation by the Ehrlich pathway. In the last steps of this pathway, 2-ketoisovalerate (KIV) is converted to isobutyraldehyde by 2-keto-acid decarboxylases (Kdc)[19] (Fig. 2*b*). However, isobutanol production in yeast is limited by the spatial separation of two different cell compartments as KIV is synthesised from pyruvate in the mitochondria and

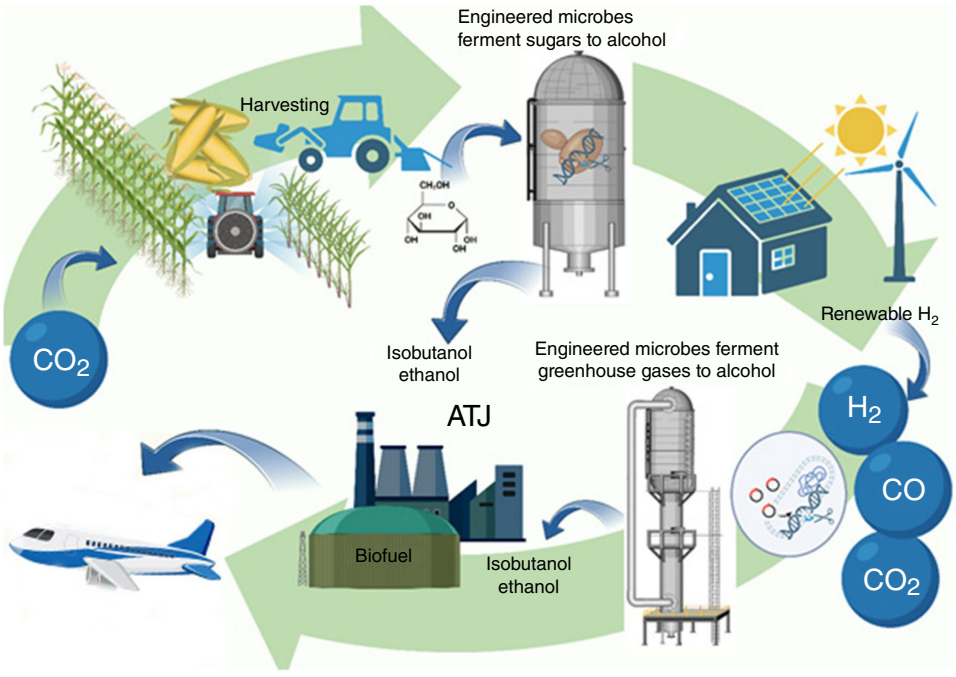

Fig. 1. Biofuel production using engineered microorganisms. Current ethanol and isobutanol production methods using engineered microorganisms such as yeast involve biomass and sugar feedstocks, which compete with food and use of arable land. More sustainable biofuel production methods include using renewable or waste sources, leading to carbon-neutral or carbon-negative processes. Our work aims to explore biofuel production alternatives using the acetogen *Clostridium autoethanogenum* to produce isobutanol from waste $CO/CO_2 + H_2$ gases.

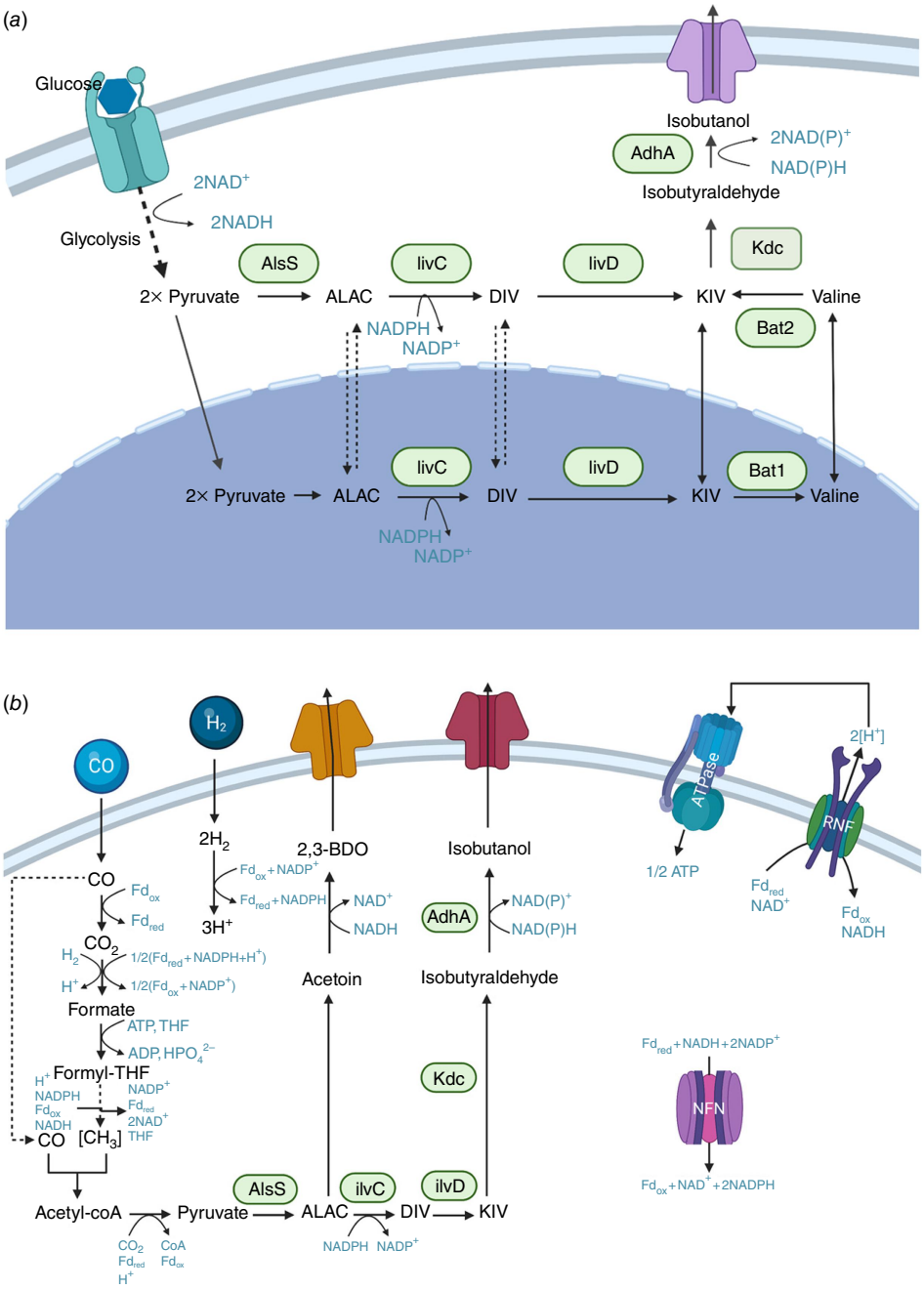

Fig. 2. Microbial isobutanol production from pyruvate. THF, tetrahydrofolate; coA, coenzyme A; 2,3-BDO, 2,3-butanediol; Fd_{ox}, Ferrodoxin oxidised; Fd_{red}, Ferrodoxin reduced; ALAC, 2-acetolactate; DIV, 2,3-dihydroxyisovalerate; KIV, 2-ketoisovalerate. Including ATPase, NFN and RNF complex. (*a*) Native isobutanol production from glucose in yeast. Isobutanol synthesis results from valine degradation by the Ehrlich pathway in the cytosol, whereas valine is formed from pyruvate in the mitochondria. This compartment separation is one the main limiting factors for high-yield isobutanol production. (*b*) Isobutanol synthetic pathway design for *Clostridium autoethanogenum*. Similarly to yeast isobutanol production, isobutanol synthesis derives from pyruvate by the Ehrlich pathway. However, metabolic engineering is needed to catalyse the reaction from 2-ketoisovalerate to isobutyraldehyde by 2-keto-acid decarboxylases (Kdc).

then needs to be transported to the cytosol for its conversion to isobutanol.[20] Furthermore, the availability of NADPH and NADH combined with the channelling of pyruvate towards other compounds might also limit the native productivity towards isobutanol. Importantly, yeast uses sugars, starch, or lignocellulosic biomass-renewable feedstocks from arable land that may compete with food production. Therefore, research focusing on other technologies to produce SAF precursors, for instance from non-biomass feedstocks, are needed to minimise environmental, social and economic aspects.

Gas fermentation is a novel approach to SAF production

An alternative to sugar and biomass-based production of alcohols for ATJ is gas fermentation. Gas fermentation is a cost-competitive technology that uses low-carbon feedstocks (C1), including waste gases derived from steel mills, biomass, and agricultural or municipal waste.[21] Microorganisms known as acetogens can use CO, CO_2 and H_2 to generate biomass and SAF intermediates. As such, acetogens are considered attractive microbial platforms for industrial gas fermentation. Acetogens grow in anaerobic conditions and fix gaseous, inorganic carbon into valuable compounds such as ethanol, 2,3-butanediol (2,3-BDO) and acetate.[22] Acetogens from the genus *Clostridium* use the Wood–Ljungdahl pathway to reduce CO or CO_2 and H_2 into acetyl-CoA and other products by fixing CO or CO_2 into cell biomass[23] (Fig. 2*b*). LanzaTech has commercialised large-scale ethanol production from waste gas fermentation and is currently producing acetone and isopropanol at pilot industrial scale.[24] The carbon-negative-produced ethanol is converted into ATJ in a process optimised by LanzaJet, a sister company near to

completing their both commercial facility in Georgia, USA, and the first ATJ production plant in Europe in partnership with SkyNRG.[25] However, expanding the product portfolio of acetogens to more valuable compounds, such as isobutanol, requires metabolic engineering.[26]

The process currently employed by Lanzatech to produce ethanol relies on CO-rich gases from industrial and solid waste. However, to ferment CO_2, acetogens need H_2. H_2 has historically come from methane steam reforming, a process with inherent carbon emissions. Considerable effort is currently underway to reduce the cost of renewable hydrogen generation.[27] For instance, large-scale green hydrogen production capacity should be developed in Queensland, Australia by 2025. In collaboration with the Queensland Government and Orica, a mining and infrastructure services provider, the H_2-Hub Gladstone complex will produce green hydrogen by water electrolysis using renewable energy from solar and wind.[28] In Gladstone, there are numerous CO_2 sources from refining natural gas. The availability of CO_2 in proximity to the new H_2-Hub offers an excellent opportunity for gas fermentation and, thus, the production of net-zero biofuels made in Australia, where the reliance on a steady supply of imported aviation fuel is a concern for national sovereignty. Australia is uniquely positioned to develop a thriving gas fermentation industry to produce sustainable hydrocarbons using renewable gas feedstocks.

Since recombinant isobutanol production in acetogenic *Clostridium* may overcome the challenges of pathway compartmentalisation and energy cofactor limitations we, in collaboration with LanzaTech, are currently developing a *C. autoethanogenum* strain to produce isobutanol (Fig. 2*b*). Although the Wood–Ljungdahl pathway from acetogens is net neutral in ATP production, it is the most efficient, most straightforward and only known linear pathway for synthesising acetyl-CoA from CO_2, involving several enzymatic complexes generating redox cofactors that could be utilised for reactions involving isobutanol biosynthesis[29] (Fig. 2*b*). Compared to yeast glucose metabolism, *C. autoethanogenum* can generate NADPH from the Nfn transhydrogenase complex and NADH from the membrane-bound, oxidoreductase Rnf complex.[30] Hence, our research aims to explore net-zero isobutanol production in acetogens that could potentially use Australian renewable feedstocks and eventually contribute to sustainable aviation fuel production in Australia.

Conclusion

Australia currently imports most of its jet fuel, which is derived from fossil fuels and harms the environment. As the demand for aviation increases, alternatives to develop SAFs at industrial scale are needed. Implementing an Australian jet fuel infrastructure for manufacturing sustainable aviation fuel and establishing a reliable supply chain is not easy, but implementing such capability is in the national interest. The delivery of a new Australian SAF infrastructure will require support and approvals by the government and the aviation industry at all levels. Using acetogenic Clostridium as catalysts, gas fermentation offers a unique opportunity to overcome current bio-isobutanol production methods using yeast. The emergence of the H_2 industry and the production of waste gases from the national energy industry can provide feedstocks for gas fermentation and position Australia at the forefront of SAF production.

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Advancing coral microbiome manipulation to build long-term climate resilience

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ABSTRACT

Coral reefs house one-third of all marine species and are of high cultural and socioeconomic importance. However, coral reefs are under dire threat from climate change and other anthropogenic stressors. Climate change is causing coral bleaching, the breakdown of the symbiosis between the coral host and its algal symbionts, often resulting in coral mortality and the deterioration of these valuable ecosystems. While it is essential to counteract the root causes of climate change, it remains urgent to develop coral restoration and conservation methods that will buy time for coral reefs. The manipulation of the bacterial microbiome that is associated with corals has been suggested as one intervention to improve coral climate resilience. Early coral microbiome-manipulation studies, which are aimed at enhancing bleaching tolerance, have shown promising results, but the inoculated bacteria did generally not persist within the coral microbiome. Here, we highlight the importance of long-term incorporation of bacterial inocula into the microbiome of target corals, as repeated inoculations will be too costly and not feasible on large reef systems like the Great Barrier Reef. Therefore, coral microbiome-manipulation studies need to prioritise approaches that can provide sustained coral climate resilience.

Keywords: assisted evolution, coral bleaching, coral microbiome, microbiome manipulation, probiotics.

The threat of climate change to coral reefs

Tropical coral reefs are biodiversity hotspots, protect our coastlines from floods and storms, are socioeconomically important because they provide employment and support several industries, and have high cultural and spiritual value. However, tropical coral reefs are disappearing due to the impacts of climate change, which is causing a gradual increase in sea surface temperatures (SSTs), and also an increase in the frequency, intensity and duration of summer heatwaves. Higher-than-usual SSTs in combination with high irradiance levels, which often occur during these extreme summer events, are the main cause of mass coral bleaching.[1] Coral bleaching is the loss of dinoflagellate photosymbionts (Symbiodiniaceae family) from coral tissues. Since Symbiodiniaceae provide corals with most of their energy, bleaching is often followed by coral starvation and death, and reef degradation.[2] On the Great Barrier Reef (GBR), seven large-scale bleaching events have occurred since 1998, and <2% of the GBR has never bleached.[3] Effective bleaching mitigation and restoration approaches that will buy time for coral reefs until global warming is curbed are therefore urgently required. Accelerating evolutionary processes to enhance coral bleaching resilience through assisted evolution[4] is being explored as one option, which includes the manipulation of the coral bacterial microbiome.[5]

The coral host together with its associated microorganisms, including bacteria, archaea, Symbiodiniaceae and other protists, viruses and fungi,[6] are referred to as the coral holobiont. Coral-associated bacteria are diverse and believed to play important roles for the holobiont such as cycling nutrients,[7] producing essential vitamins and amino acids, regulating the bacterial community and warding off pathogens.[8] The composition of coral-associated bacterial communities is sometimes correlated with heat tolerance of the coral host,[9] suggesting a bacterial role in the coral heat stress response.

There are currently three hypotheses that explain the cellular mechanisms underpinning coral bleaching. The oxidative stress theory posits that high SSTs and irradiance impair the Symbiodiniaceae photosystem, triggering an overproduction of toxic reactive oxygen species (ROS) that leak into coral cells where they cause a cellular cascade that

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results in the separation of Symbiodiniaceae from the host.[10] The second hypothesis suggests bleaching is triggered by an accumulation of ROS and damage to the Calvin–Benson–Bassham cycle, due to the host not meeting the CO_2 demands for the faster-growing algal endosymbionts under elevated temperatures.[11] The third hypothesis poses that heat stress heightens host catabolism, which increases available ammonium for Symbiodiniaceae, fuelling algal growth and carbon usage.[12] This results in carbon limitation of the host and phosphorus limitation of Symbiodiniaceae, causing damage to the algal photosystem and its membranes[13] and, again, leading to the overproduction of ROS, which triggers bleaching. Thus, potentially relevant bacterial traits for microbiome manipulation to boost thermal bleaching resilience include the neutralisation of ROS by antioxidants or the supply of carbon to the host to minimise its starvation.

Microbiome manipulation as a tool to enhance coral climate resilience

Here, we define microbiome manipulation as the directed alteration of the microbiome by humans, with the overall goal to provide host health benefits. This can be achieved by approaches such as probiotics or the transplantation of microbial communities. Microbiome manipulation is a common approach in medicine, including, for instance, the use of fecal microbiome transplantation to treat *Clostridium* infections.[14] However, microbiome manipulation in corals is still at an early stage. Generally, its feasibility has been demonstrated by showing that the composition of coral-associated bacterial communities can be modified through bacterial inoculation.[15] Initial coral microbiome-manipulation studies have focussed on disease treatment, such as white pox disease,[16] or on the bioremediation of oil pollution.[17] Recent microbiome-manipulation studies to enhance coral bleaching resilience are promising, although clear correlations between the presence or abundance of inoculated bacteria and coral bleaching tolerance have not been proven yet. One study determined that bacteria isolated from corals and surrounding seawater had putative beneficial functions such as antagonistic activity against a common coral pathogen, activity of the ROS-scavenging enzyme catalase, and potentially contributed to sulfur and nitrogen cycling (by determining the presence of genes involved in the pathways).[18] Inoculating the coral *Pocillopora damicornis* with this bacterial cocktail partially inhibited thermal coral bleaching and pathogen infection.[18] A similar study selected candidate probiotic bacteria from *Mussismilia hispida* by screening for the same attributes as above, and showed that administering the probiotic mix to *M. hispida* reduced bleaching and improved recovery after thermal stress.[19] An additional study showed short-term bleaching mitigation of previously heat-sensitive corals after they were inoculated with a microbiome obtained from heat-tolerant corals from the same species.[20] Even though all three studies applied no-inoculum controls, increased bleaching tolerance might stem from the bacterial cocktail acting as a source of nutrition for the coral host growing heterotrophically. Integrating non-beneficial or dead bacteria as an additional negative control that might act as a food source without providing any other benefits may help decipher the impact of heterotrophic feeding *v*. microbiome manipulation on thermal tolerance. A recent study inoculated the coral model sea anemone *Exaiptasia diaphana* with either a consortium of bacterial strains with high ROS-scavenging abilities, a negative control that contained closely related bacterial strains with no ROS-scavenging abilities (to control for the effect of heterotrophic feeding), or a no-inoculum control.[21] The inoculated bacteria were lost from the *E. diaphana* host prior to heat stress application precluding any conclusions on their impact on bleaching resilience to be drawn.

Overall, these studies showed limited and short-term uptake of some of the inoculated bacteria,[20,21] or restructuring of the bacterial community composition following inoculation.[18,19] However, no study has demonstrated long-term uptake and temporal stability of the inoculated bacteria, although divergent microbiome communities were observed in coral juveniles 4 months after a single microbiome transplant from each of four different species of adult corals, including one adult coral that was conspecific to the larval recipients.[15] To provide long-term benefits and to create a sustainable solution to build coral bleaching resilience, putative beneficial coral bacteria need to form a temporally stable association with the coral host, thereby limiting the need for repeated inoculations across vast geographical scales.

Using stably associated bacteria for long-term benefits

Several aspects need to be considered to ensure uptake and persistence of inoculated bacteria by the host to guarantee long-term beneficial effects on holobiont performance (Fig. 1). First, we recommend focussing on bacteria that are stably associated with the host. Although corals associate with a high portion of ephemeral bacteria, there is a smaller portion that forms a more temporally stable symbiosis with the coral host.[22] Stable members of the coral microbiome are more likely to be found in the coral tissues,[23] where some are described to form bacterial aggregates,[24] and sometimes they are vertically transmitted to coral offspring. A successful example from another biological system is the use of the vertically transmitted, intracellular bacterium *Wolbachia* to reduce the spread of the viral disease, dengue. When introduced into *Aedes aegypti* mosquitoes, *Wolbachia* provides arboviral protection to mosquito hosts and spreads quickly and efficiently through wild populations following the release of infected mosquitoes, without the need for further intervention.[25] This is despite *A. aegypti* not being a natural host for *Wolbachia*. Therefore, temporal stability of bacteria within the host microbiome should be studied over longer timescales (e.g. months and longer), as most previous experiments have tested a duration of 24 h,[20] 5,[19] 11[18] and 35 days,[21] except for one study testing over 4 months,[5] and over multiple generations.

Second, we propose to source bacteria from coral microhabitats where beneficial functions are required. One of the key mechanisms in bleaching involves the overproduction of ROS by Symbiodiniaceace in host gastrodermal cells. Therefore, ROS-scavenging bacteria that closely associate with

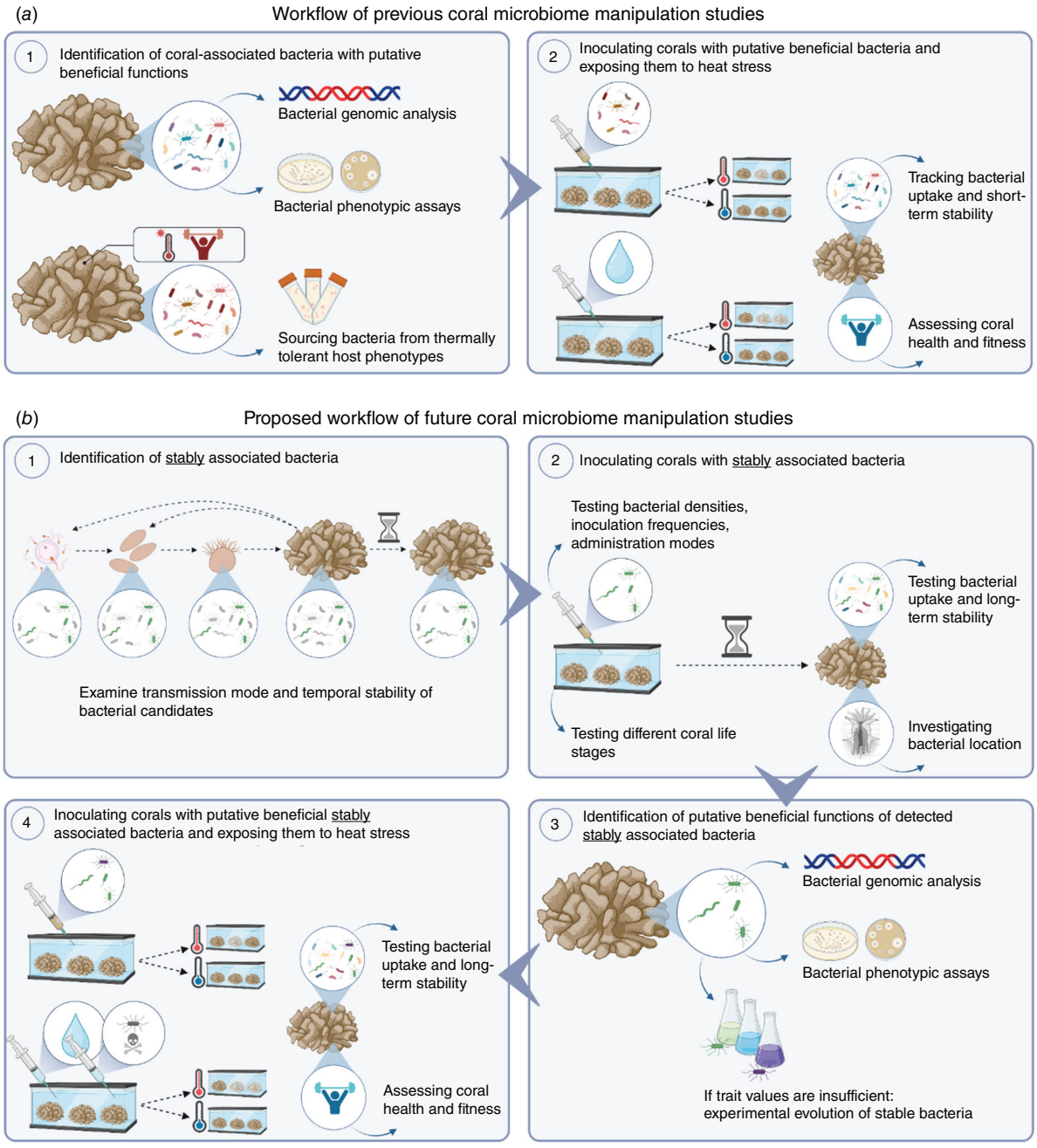

Fig. 1. Conceptual figure describing (*a*) the workflow of previous coral microbiome-manipulation studies that aimed to enhance coral bleaching resilience and (*b*) our proposed workflow for future studies. Previous coral microbiome-manipulation studies (*a*) started with (1) the identification of coral-associated bacteria with putative beneficial functions by performing bacterial genomic and phenotypic analyses, or by sourcing putative beneficial bacteria from thermally tolerant coral host phenotypes. This was followed by (2) inoculating corals with identified putative beneficial coral-associated bacteria or filtered seawater (negative control), exposing treated corals to heat stress or ambient temperatures and examining bacterial uptake and short-term stability, as well as determining coral holobiont health and fitness. We propose (*b*) for future coral microbiome-manipulation studies aiming for long-term coral bleaching resilience to start with (1) identifying stably associated bacteria by examining the transmission mode of bacterial candidates throughout different coral life stages and their temporal stability within adult corals. Subsequently, we propose to (2) inoculate corals with stably associated bacteria to test bacterial uptake and long-term stability, as well as investigating the location of the bacterial candidates within the coral holobiont. Here, we also propose to test the effect of different bacterial densities, inoculation frequencies, administration modes and coral life stages on the bacterial uptake and stability. Afterwards, we propose to (3) identify putative beneficial functions of stable coral-associated bacteria by bacterial genomic and phenotypic analyses. If functions of interest of stable bacteria are insufficient, we recommend experimental evolution to enhance their functional potential. If multiple strains are chosen, we also propose to test for interspecific interactions including effects on growth rates and the efficiency of the putative beneficial functions of interest. Finally, we advise to (4) inoculate corals with putative beneficial stable coral-associated bacteria, filtered seawater (first negative control), and heat-killed bacteria (second negative control), expose them to heat or ambient temperatures, assess coral health and fitness and track the uptake and temporal stability of inoculated bacteria within the coral holobiont. Created with BioRender.com.

Symbiodiniaceae or are present in the gastrodermis would be relevant. Some bacteria co-localise with Symbiodiniaceae in culture and *in hospite*[26,27] and may play a role in Symbiodiniaceae and coral health.[28]

Once stable members from coral microhabitats are identified, the next critical step for candidate selection will be to understand their functions, especially with regards to coral bleaching and climate resilience. This can be achieved through genomic analyses and phenotypic assays. If candidates of interest exhibit limited functional ability, such as low ROS-scavenging abilities, laboratory evolution experiments may be used to enhance their abilities, for example through long-term exposure to oxidative stress conditions.[29]

In summary, the selection of bacterial candidates based on their temporal stability and location within the coral holobiont, followed by their bleaching mitigation functions are critical steps in developing microbiome-manipulation techniques that aim to build long-term bleaching resilience. Since recent models predict that environmental conditions will become unsuitable for coral reefs by 2035,[30] the next decade will be crucial to curb greenhouse gas emissions and develop effective and sustainable conservation methods to buy time for corals.

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Dr Justin Maire is currently a postdoctoral fellow at the University of Melbourne. He obtained his PhD in 2018 from Institut National des Sciences Appliquées de Lyon, France, on symbiotic interactions between insects and bacteria and their impact on host immunity and development. In 2019, he moved to the University of Melbourne, where his research is focused on coral–bacteria associations. He specifically focuses on closely associated symbionts, e.g. intracellular, vertically transmitted symbionts, and their potential use as bacterial probiotics and microbiome engineering as an approach for the mitigation of coral bleaching and coral reef conservation. This includes characterising the taxonomy, localisation and functions of symbionts of interests.

Prof. Madeleine J. H. van Oppen is an ecological geneticist with an interest in microbial symbioses and climate change adaptation of reef corals. Her early career focused on evolutionary and population genetics of algae and fish, and subsequently corals. Currently, her team is using bioengineering approaches aimed at increasing coral climate resilience and the likelihood that coral reefs will survive this century. These interventions include coral host hybridisation and conditioning, directed evolution of microalgal symbionts and bacterial probiotics.

Prof. Linda L. Blackall is an environmental microbial ecologist, who has studied many different complex microbial communities ranging from host associated through to free living in numerous environments. One of her research fields is the microbiota of corals and sponges. The numerous methods she develops and employs in her research allow elucidation of microbial complexity and function in these diverse biomes.

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Pouch bacteria: an understudied and potentially important facet of marsupial reproduction

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ABSTRACT

Australia is home to a rich biodiversity of marsupials that are found nowhere else. Unfortunately, many of these species are currently threatened with extinction due to introduced feral predators and other anthropogenic factors. There is growing recognition that host-associated microorganisms can play important roles for animal health, with billions of dollars currently being invested into human gut microbiome research and the development of microbiome-based therapeutics to improve human health. Can microorganisms also be harnessed to stem the tide of marsupial extinctions? In this review, we provide an overview of some of the challenges facing Australia's marsupials, and our current understanding of the microbiology of the marsupial pouch. We also propose outstanding research questions pertaining to the marsupial pouch, which, if addressed, may provide actionable knowledge and novel microbial therapies that could help stem the tide of marsupial extinctions in Australia.

Introduction

The island continent of Australia is globally significant for its unique biodiversity, and is home to the most distinctive terrestrial mammal fauna on Earth. Australia is particularly notable for harbouring the world's richest diversity of extant marsupials, with over 200 species found only in Australia, across habitats ranging from arid deserts to tropical rainforests.[1] These include several iconic taxa such as kangaroos, koalas, and wombats, many of which hold great cultural significance to Australians.

Marsupials diverged from eutherian ('placental') mammals *c*. 160 Ma during the early Jurassic period,[2] with Australian lineages evolving largely in geographic isolation following separation from the Gondwanan supercontinent *c*. 40 Ma. Unsurprisingly given this divergence time, several anatomical and physiological differences exist between marsupials and eutherians – the most notable being reproductive strategy. Marsupials give birth after a short gestation period to undeveloped young (hereafter 'joeys'), which crawl toward and latch onto a teat located in a maternal pouch (marsupium) and continue development *ex utero* through lactation. This means that, unlike eutherian neonates, marsupial joeys are exposed to the external environment prior to developing a functional immune system and are thus highly vulnerable to microbial infections.[3] Despite this risk, however, *ex utero* development is highly advantageous for survival in Australia's adverse and often unpredictable environments, as it allows for increased maternal control of reproductive effort during unfavourable conditions.[4]

Challenges facing Australia's marsupials

Despite harbouring much of the earth's mammalian diversity, Australia currently reports the highest mammalian extinction rate on earth, with 39 mammal species confirmed to have become extinct in the ~200 years since European colonisation.[5] These extinctions represent >10% of all global mammal extinctions during this period, and amount to a major loss of global marsupial diversity.[1] With 52 Australian mammals currently listed as Endangered (incl. 9 Critically Endangered) and 58 listed as Vulnerable, further mammalian extinctions are likely in coming decades without substantial intervention.[5] The primary drivers of population declines among Australian mammals are predation by introduced species such as feral cats (*Felis catus*) and foxes (*Vulpes vulpes*), and habitat loss due to extensive land clearing.[1] Several endangered marsupial species, including

quolls, koalas and Tasmanian devils, have also experienced significant population declines due to disease, the spread of which is exacerbated by increased habitat fragmentation.[6–8] Additionally, Australia's mammals are vulnerable to increased frequency of extreme weather events such as bushfire, floods and drought resulting from climate change.[9]

Given the complexities associated with managing these threats, establishing healthy insurance populations *ex situ* through captive breeding is essential for preventing further mammal extinctions in Australia. These captive populations are an invaluable resource for reintroduction and repopulation programmes, as well as furthering research into threat and disease adaptation. Captive breeding is also beneficial for expanding and maintaining genetic diversity through the application of selective breeding and recent advances in artificial insemination.[10] This is particularly useful for the genetic rescue of increasingly fragmented mammal populations by targeted translocation.[11]

Despite these benefits, however, the successful management and breeding of endangered marsupials in captivity presents several challenges. For instance, the reproductive success of several species can be hindered by low fertility and conception rates. This can occur because of several factors, including breeding incompatibility among captive stock, behavioural or social shifts in captivity, and dietary changes.[12] Overall breeding outputs in some species are also affected by high rates of neonatal and juvenile mortality, which can occur because of infections, behavioural stress caused by environmental modification in captivity and other environmental factors that remain poorly understood.[13–15] The issue of neonatal mortality during early development is of particular concern in captive koala colonies, where seasonal mortality rates among pouch young can exceed 50%, predominantly due to bacterial infections.[13]

Recent research into host–microbiome associations has shown that microbes play important roles in animal health and evolution.[16] Monitoring and modulating the gut microbiome is being proposed as a tool for improving conservation outcomes for endangered animals.[17] Comparatively less is known about host-microbiome associations in the context of the mammalian female reproductive tract (FRT; e.g. vagina, urogenital tract, milk, pouch), especially for endangered species. However, growing evidence suggests that FRT-associated microbial communities may play important roles in several host functions essential for reproductive and developmental success.[18] Extending this logic to marsupials, we propose that microbial communities in the maternal pouch may represent an important and overlooked factor of successful reproduction (Fig. 1).

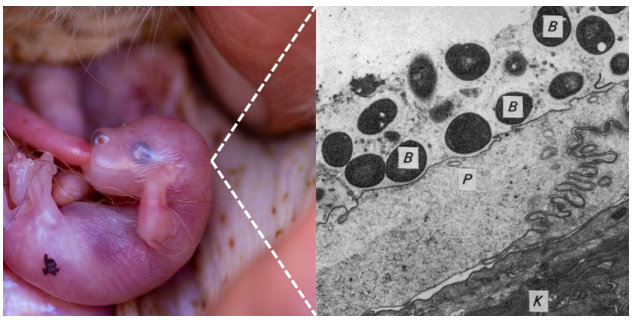

Fig. 1. (Left) A yellow-footed rock wallaby joey with fingernail for scale (source: Raphael Eisenhofer). (Right) Electron micrograph (12 000×) section from the skin of a newborn opossum from Krause *et al.*[3] Bacteria (*B*) can clearly be seen coating the surface of the periderm (*P*). *K* is the forming keratinising layer of epidermis.

Pouch microbiology – what is known

To date, microbiological research into the marsupial pouch has received little attention, with fewer than ten studies being published since the first in 1972 by Yadav, Stanley and Waring on the quokka (*Setonix brachyurus*).[19] This and the three other cultivation-dependent studies found substantial reductions in, or a complete lack of, culturable bacteria from koala, tammar wallaby, brushtail possum and quokka pouches prior to and immediately following birth.[19–22] The first use of cultivation-independent techniques to study microbes in the marsupial pouch was in 2004 by Deakin and Cooper.[23] Using a mixture of cultivation-dependent and -independent methods on common brushtail possums (*Trichosurus vulpecula*), they found similar trends to the prior cultivation-dependent studies.[23] Similar trends were also observed in a cultivation-independent study on tammar wallaby (*Macropus eugenii*) pouches.[24] Two further cultivation-independent studies using high-throughput *16S* rRNA gene sequencing on Tasmanian devil pouches (*Sarcophilus harrisii*) found differences in composition, but not diversity, between lactating and non-lactating females.[25,26] Overall, the trend in these pouch microbiology studies is a shift in diversity or composition associated with the reproductive status of the host, generally with a reduction in bacterial diversity close to and immediately following joey birth.

However, it is increasingly recognised that DNA contamination can compromise *16S* rRNA gene studies – particularly those targeting samples with low microbial biomass.[27] Recently, Weiss *et al.* applied a robust experimental framework, including the collection and sequencing of numerous negative control samples and the quantitative estimation of sample biomass using qPCR, to demonstrate that the southern hairy-nosed wombat (SHNW) (*Lasiorhinus latifrons*) pouch does indeed harbour resident bacteria.[28] Weiss *et al.* analysed multiple sample types from 26 wild SHNWs to show that the pouch of reproductively active females is compositionally distinct from other body sites and is dominated by a handful of Gram-positive bacteria.[28] The closest match for three of the five dominant pouch bacteria were to pouch bacterial isolates from tammar wallabies,[24] with *16S* rRNA gene sequence divergence consistent with the estimated divergence time between tammar wallabies and wombats – offering tantalising (albeit preliminary) evidence for co-speciation between pouch bacteria and marsupials.

Characterisation of pouch-associated microbial communities has also highlighted a potential link between bacterial composition and reproductive failure. This was demonstrated in a recent study (Maidment) of 38 captive koalas (*Phascolarctos cinereus*), where females who lost pouch young exhibited a significantly different pouch microbial compositional profile to females rearing to full term.

Interestingly, although both animal groups showed similar decreases in microbial richness between mating and parturition, the pouch microbiota of successful mothers re-diversified in the months following parturition, whereas unsuccessful mothers remained dominated by *Enterobacteriaceae* until loss of young occurred 5–7 months post-partum.[29] Taken together with similar findings in koalas by Osawa *et al.*[21] and O'Callaghan,[13] these recent findings add further weight to the hypothesis that dysbiosis of microbial communities in the marsupial pouch may be associated with neonatal mortality.

Unknowns and future directions

The past decade has seen a rapid accumulation of evidence demonstrating that the gut microbiome can play important roles in host health,[16] and some have suggested that the gut microbiome should be considered for animal conservation.[17] We wish to extend this idea and hypothesise that some pouch bacteria are beneficial to the reproductive success of marsupials. One beneficial function that pouch bacteria could bring to their hosts is the competitive exclusion of potentially harmful microorganisms from the pouch. The exact mechanisms are unknown, but bacteria are known to use a range of antagonistic tools to gain an advantage over heterospecific bacteria.[30] Such bacteria could have co-speciated with their marsupial hosts and are thus likely well adapted to surviving endogenous host antimicrobial defences. Perturbations of the 'natural' pouch microbiome could therefore have detrimental impacts to the reproductive success of marsupials by increased inflammation or joey mortality caused by opportunistic microorganisms. Such disruptions to the pouch microbiome could be caused by various factors present in captivity (which can affect the gut microbiome[31]), such as horizontal transfer of pathogens from humans, antibiotic treatment, or the lack of vertical transmission of pouch bacteria from mother to joey (in cases where an underdeveloped joey is rescued from a dead mother's pouch).

Our current understanding of marsupial pouch microbiology is limited. More experiments are needed to confirm a link between pouch bacteria and the reproductive success of marsupials, and to identify factors that may influence the composition of the pouch microbiome across marsupials. Some key outstanding questions that we think should be addressed are:

1. Are captive pouch microbiomes different from wild?
2. For a given marsupial species, is there a 'healthy' pouch microbiome? If so, can diagnostic tests be developed to aid breeding programs?
3. Can pouch bacteria competitively exclude opportunistic pathogens? If so, by which mechanisms?
4. How do pouch bacteria evade endogenous host defences?
5. Can pouch bacteria influence the immunological profile of the pouch? If so, how?
6. Do pouch bacteria influence the development of the joey's immune system? If so, what are the implications for the joey's future health?
7. Have pouch bacteria been co-speciating with their marsupial hosts?
8. Can the pouch microbiome be manipulated by probiotics or pouch-microbiome transplants?

Such knowledge could be applied in a conservation management context, leading to the development of novel tools and therapies to improve the captive breeding success of marsupials and help stem the tide of marsupial extinctions in Australia.

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Bioprospecting for and the applications of halophilic acidophiles in bioleaching operations

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ABSTRACT

The economic recovery of metals from sulfide ores has become a topic of increasing interest due to the escalating demand for critical minerals and the reducing grade of available ores. Bioleaching is the use of acidophilic iron and sulfur-oxidising microorganisms to facilitate the extraction of base metals from primary sulfide ores and tailings. One significant issue limiting the use of bioleaching is the availability of freshwater due to the sensitivity of these microbes to chloride. The use of saline tolerant acidophilic iron- and-sulfur oxidising microorganisms will go a long way to addressing this issue. There are three possible means of sourcing suitable microorganisms; adaptation, genetic engineering and bioprospecting, with bioprospecting showing the greatest possibilities. Bioprospecting in search of native organisms for bioleaching operations has led researchers to numerous locations around the world and the isolation of iron- and sulfur-oxidising acidophiles that are capable of tolerating high levels of salinity has been of particular interest in these investigations.

Keywords: acid saline lakes, *Acidihalobacter*, acidophiles, bioleaching, bioprospecting, genetic engineering, halophiles, saline drains.

As the global transition to, and the goal of governments for a zero emissions energy system are embraced worldwide, the demand for critical minerals such as cobalt, copper, nickel, lithium and rare earth elements is outstripping current availability.[1] Compounding the lack of materials are issues of low-grade ores (uneconomical using current recovery processes), water consumption and downstream co-contamination of unwanted elements such as uranium during traditional extraction processes. Commercial success of mining operations often includes reclaiming and recycling mine wastes with some sites operating bioleaching heaps as an inexpensive alternative to the traditional pyro-metallurgical methods for the extraction of metals from low grade sulfide ores.

Even though bioleaching has been employed successfully for decades, lengthy leaching cycles and low recovery efficiencies have hindered large scale investment and adoption of the process. The demand for critical minerals is increasing and the resulting upwards trajectory of mineral commodity prices showing no signs of slowing.[2] As a result, investigations into optimising bioleaching practices for the reclamation of elements (re-processing) from fresh or abandoned tailings is gathering interest, as is the application of this technology to lower grade primary ores. To optimise bioleaching processes, numerous avenues are being explored to not only increase mineral yield from these practices, but also to decrease operating, environmental and maintenance costs of the complex systems, tackling different parameters of the procedures involved. Prospective strategies for advancing bioleaching operations include deliberate adaptation of organisms to extreme conditions, genetic engineering and bioprospecting.

The microorganisms currently applied to bioleaching of sulfide ores are consistently a combination of acidophilic bacteria and archaea whose ability to prosper in low pH conditions enables the dissolution of iron and inorganic sulfur by way of metabolism and acid generation, thus liberating recoverable minerals. Under mesophilic conditions, bioleaching populations are dominated by genera such as *Acidithiobacillus*, *Acidiphilium*, *Acidiferrobacter* and *Leptospirillum*,[3] whereas increasing temperatures (up to 60°C) alters the taxa to favour *Sulfobacillus, Acidimicrobium* and *Ferroplasma.* Communities comprising multiple species are more adept at undertaking a variety of tasks than a single species alone,[4] but all can be adversely affected by changes in numerous parameters including water potential, ore porosity and surface area undesirably resulting in decreased retrieval efficiencies.

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These bioleaching microorganisms were originally isolated from some of the most inhospitable regions on earth[5] so adaptive culturing of both pure and mixed populations to extreme conditions (high compound metal concentrations, acidity, salinity) has seen some successes. Subculturing under situations of continuous or accumulative ore concentrations has been adopted by many researchers as a way to enhance bioleaching performance and increase element recovery.[6] However, it can be a difficult and lengthy process maintaining discrete population numbers overtime in these adapted cultures. An alternative approach to the adaptation of microbial populations is to utilise a naturally occurring microbial consortium. A consortium of microbes can have superior benefits over cultures of pure isolates as a broader array of multifaceted functions including inter-species biofilm formation occur that have demonstrated a greater rate of mineral solubilisation[7] than when pure cultures were applied.

Advances in proteomic and metabolomic analysis of microbial systems has enabled researchers to understand how microorganisms can tolerate extreme conditions. With this information, genetic manipulation and modification of existing bioleaching microorganisms to improve on leaching efficiencies has shown promise when applied to the recovery of rare earth elements.[8] The incorporation of metabolic pathways involved in the degradation of organic compounds from heterotrophs such as *Acidiphilum* or *Sulfobacillus* into autotrophic iron and sulfur oxidisers (*Acidithiobacillus*) could reduce issues of organic toxicity that organics often bring to bioleaching operations.[9] Designing and manufacturing synthetic microbial consortia for bioleaching applications is an emerging area of research, however, their application for *in situ* bioleaching (in particular heap leaching) remains to be seen because of strong environmental release laws. As microbial diversity, complete with wide metabolic potential, is influenced not only by the variable environment but also with the interactions between competing microorganisms, the genetic analysis of organisms often conducted on pure cultures does not reflect real world situations and should be taken into consideration if designing a synthetic population.

Bioleaching processes at high temperatures ($>45^{\circ}C$) has seen success with the application of various thermophilic microbial species (*Sulfolobus*, *Metallosphaera*, *Acidianus*)[10] for the recovery of copper, uranium and gold. Owing to the higher running costs of these operations (stirred tank reactors), this process is often restricted to high value minerals. This, nevertheless, has fuelled the search for more thermophilic organisms capable of solubilising iron–sulfide rich ores in locales such as hydrothermal vents[11] (which is now an emerging deep-sea biotechnology industry), volcanic areas[12] and hot springs.[13]

For bioleaching operations to be economically and environmentally sustainable, water consumption must be tightly controlled as it is essential for ore processing and recovery. Implementation of untreated groundwater sources for bioleaching applications runs the risk of tapping into sources with high levels of total dissolved solids, and the use of sea water requires understanding how chloride ions can affect mixed microbial communities.[14] The mesophilic acidophiles listed earlier all demonstrate extreme sensitivity to chloride. Their cell membrane is permeable to the chloride ion, which, on entry to the cell, results in the negation of the positive membrane potential.[15] Consequently, proton entry follows resulting in acidification of the cytoplasm, disturbing the proton motive force and eventually cell death. Microorganisms capable of surviving conditions of low pH, high salt and utilising Fe/S for energy are few in number as environments where these stresses co-exist are extraordinary unique. Therefore, bioprospecting for microorganisms that thrive in these conditions rapidly narrows the number of locations across the globe in which they may be found.

Our initial explorations took us to the acidic saline drains in the Yilgarn Craton of Western Australia in 2008 where we isolated a potential candidate known as F5.[16] F5 effectively released base metals from pyrite, pentlandite and chalcopyrite under leaching conditions with up to 30 $g\,L^{-1}$ of chloride ions present, and following genomic sequencing was named *Acidihalobacter prosperus*.[17] This organism was then further re-classified as *A. yilgarnensis* due to genomic sequence comparisons with related species demonstrating a clear difference between it and an already existing *A. prosperus*.[18] *Acidihalobacter prosperus* (originally named *Thiobacillus prosperus*) was isolated from a shallow geothermally heated seafloor on the Aeolian Islands, Vulcano, Italy.[19] Subsequently a further two halotolerant acidophiles of this genus, *A. prosperus* V6 and *A. ferrooxidans* V8, were isolated from mixed shallow acidic marine pools, also on the Aeolian Islands. These isolates have subsequently been renamed *A. prosperus*, *A. aeolianus and A. ferrooxydans*,[20,21] all of which are capable of growth at low pH (1.8–2.0) as well as tolerating high salt and oxidising pyritic compounds. The *Acidihalobacter* species may in the future be applied to bioleaching operations where concentrations of salt in the water render the usual consortium unviable.

Even though the organisms that currently make up the *Acidihalobacter* genus were isolated in locations greater than 13 000 km apart, similar environmental pressures have resulted in the conservation of genes essential for survival. Genome exploration of these organisms has revealed that the low pH and high salt tolerances evolved separately[22] with the halophilic organism gaining genes for acid tolerance through horizontal gene transfer. Armed with this information, *in vitro* modelling of genetic alterations made to bioleaching organisms (*Acidithiobacillus ferrooxidans*) can allow us to predict the success and applicability of organisms for mineral recovery in high salt, low pH conditions.

As the Yilgarn Craton of Western Australia is dotted with numerous acidic saline lakes, it provides an ideal opportunity for further prospecting[23] while utilising the information gained from molecular modelling to target specific environments with the greatest chance of hosting prokaryotes suitable for bioleaching in regions where saline water is of concern. Another hypersaline lake in Australia whose pH fluctuates with the seasons is Lake Tyrell (a shallow, salt-crusted depression) in Victoria, a location where numerous bioprospecting expeditions have been conducted[24,25] in attempt to isolate organisms for biotechnological applications. A global search of other hypersaline lakes with low pH (<4) for iron–sulfur-oxidising halophiles to either adapt to

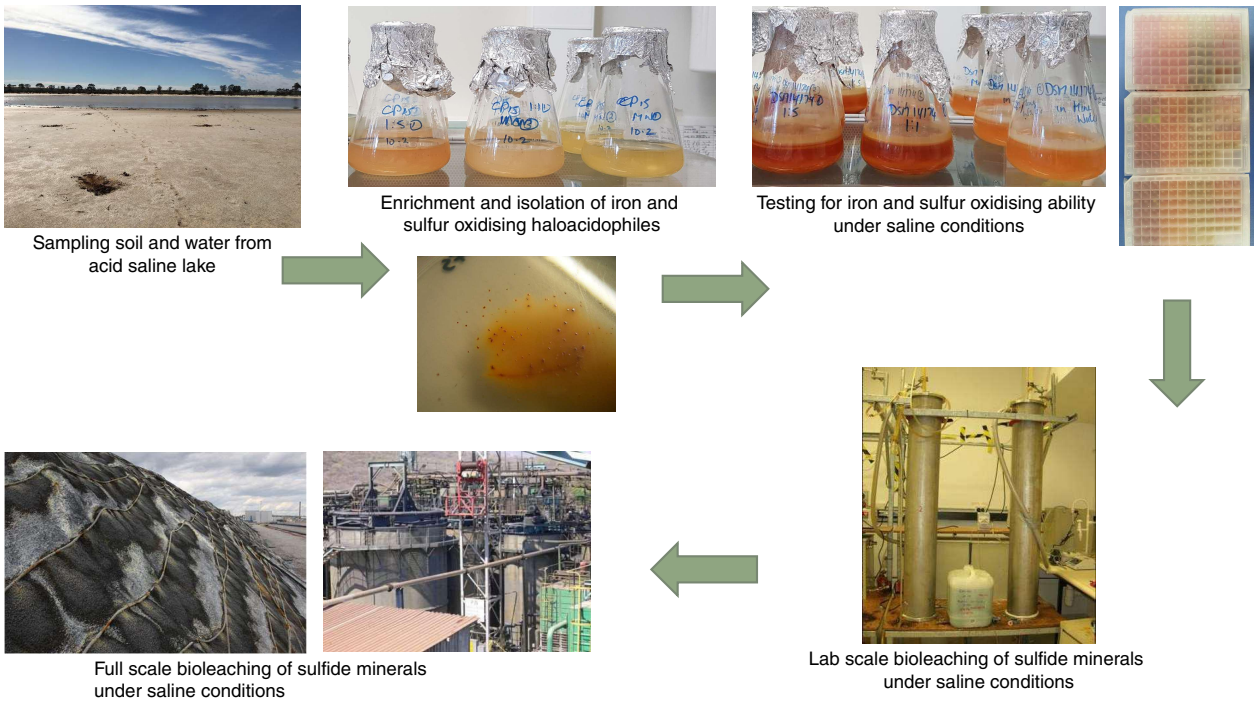

Fig. 1. Life cycle of bioprospecting for halophilic iron- and sulfur-oxidising acidophile for use in the application of saline water bioleaching.

acidic conditions or genetically modify to contain acid resistance mechanisms could allow for the construction of a consortia suitable for bioleaching in regions with salt contaminated waters (Fig. 1).

By understanding the microbial diversity, proteomic, lipidomic and genetic contributions, adaptations and pressures in the unique high salt, low pH environments, advances in the efficiency of bioleaching process could be accomplished. Although a range of approaches such as adaption and genetic modification could be applied to answering this problem, bioprospecting is the approach most likely to provide a successful solution.

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Elizabeth Watkin is Associate Dean (Science and Mathematics) and Professor of Environmental Microbiology in the School of Science, at Edith Cowan University. She has over 20 years of experience in the field of microbial ecology of environmental systems and covers the fields of mining biotechnology, mineral resource recovery and microbial fouling of water (particularly within mining systems). Her research team investigates biotechnological processes for environmental and industrial applications and approaches to mitigate microbially caused problems such as biocorrosion, biofouling and bioclogging. Prior to 2005, her main area of research was symbiotic nitrogen fixing bacteria in both agricultural and native legume species. She maintains a minor research interest in this area.

Microbiology Australia updates

Microbiology Australia (MA) continues to gain greater recognition globally, now being listed by the *Directory of Open Access Journals* (DOAJ), an online directory that indexes and provides access to high-quality, open access, peer-reviewed journals. Inclusion in DOAJ has the benefits of raising visibility of MA content and, importantly, demonstrates best practice editorial and publishing standards (which also helps distinguish MA from the plethora of predatory open access journals out there). More information on the benefits of indexing in DOAJ can be found at: https://doaj.org/apply/why-index/#why-your-journal-should-be-indexed-in-doaj

Other databases listing MA include Scopus and Clarivate's Emerging Sources Citation Index (ESCI). MA is a member of COPE (Committee on Publication Ethics) and requires authors to list conflicts of interest and sources of funding.

Most importantly, MA aims to meets the needs of members of *The Australian Society for Microbiology*. Melinda and Tess (pictured here) were proud to receive the print copy of the most recent issue.

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Microbiology Australia

Microbiology in sustainable remediation of contaminated sites

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ABSTRACT

The release of chemicals that have negative human or environmental health impacts has been rife around the world for a century. Approaches to contaminated site remediation have evolved over this time to address environmental contamination. Over the past 15 years there has been an increasing focus on sustainability in remediation. Bioremediation has emerged as a remediation technology of choice based on sustainability credentials. Research on pollutant biodegradation, including the discovery and characterisation of microbes responsible, underpins biological remediation applications.

Keywords: bioremediation, chemical contamination, mine sites, organohalide respiring bacteria, reductive dechlorination.

Chemical contamination and contaminated site remediation

Research into the biotransformation of chemical compounds by microorganisms underpins environmental biotechnologies such as wastewater treatment, anaerobic digestion and bioremediation of contaminated sites. Such technologies are well established and arguably essential in our collective quest to minimise our impact on human and environmental health globally. Sustainable remediation technologies such as bioremediation have especial pertinence given the rate at which society has been polluting the environment over the past century.[1]

International conventions on pollution compel nations and states to develop guidelines, legislation and regulations limiting production and use of harmful chemistry and stipulating trigger values for contaminated site clean-up action. The most common contamination events are the leakage of petroleum hydrocarbons from underground storage tanks, the release of chlorinated organics (organochlorines) from dry cleaning and mechanical facilities, the inappropriate disposal of asbestos and mishandling of heavy metals. The widespread use of fluorinated organics (e.g. perfluoroalkyl substances, PFAS, in fire-fighting foams) has also risen to prominence in recent years.[2]

Less common but more serious in scale and impact are contamination events arising from chemical manufacturing facilities. Examples include the Botany Industrial Park in Sydney and the Altona Chemical Complex in Melbourne. Industrial-scale chemical production and handling has resulted in massive soil, groundwater and surface water contamination that can lead to human exposure through direct dermal contact, ingestion and inhalation.[3]

Acute exposure to toxic chemicals can have dramatic impacts on human health leading to rapid cardiac or respiratory failure. Such incidences are rare, however. More common, and therefore concerning, is the long-term exposure of humans to low concentrations of environmental contaminants. This can lead to increased incidences of cancer, liver failure, reduced reproductive success and immune system malfunction. Beyond impacts on human and environmental health, contaminated sites can cause massive disruption in large public and private infrastructure development projects. Examples include the impact of PFAS on the West Gate Tunnel project in Melbourne and the impact of coal tar on the Barangaroo development in Sydney.

Australia has a strong international reputation in common-sense environmental consulting and contracting in the contaminated-site remediation industry. This is rooted in a risk-based approach to ensure potential harm to sensitive environmental or human receptors is minimised while encouraging sustainable efforts to clean up contaminated sites. Sustainability in this sense encompasses social, economic and environmental considerations.[4]

When chemical contamination of the environment is discovered (e.g. chlorinated solvents from dry cleaning operations or petroleum hydrocarbons from petrol stations), iterative rounds of site characterisation are used to develop a conceptual site model enabling development of a risk-based remediation strategy as stipulated in the National Environment Protection (Assessment of Contaminated Sites) Measure (1999) for contaminated sites.[5] Remediation technology options are then assessed through cost, benefit and sustainability analyses to ensure viable approaches to managing risk are economically feasible, supported by various

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stakeholders and have minimal negative environmental impacts. Finally, a remediation action plan is developed and executed.

The Australasian Land and Groundwater Association (ALGA) supports the Sustainable Remediation Forum (SuRF-ANZ), which is a member of the International Sustainable Remediation Alliance. The SuRF-ANZ envisions the principles of sustainable remediation to not only be applied but be recognised as a necessary part of developing a site remediation and management strategy. Additionally, they envision having the principles written into formal regulatory requirements to be a normal part of responding to site contamination.

Bioremediation as a sustainable remediation technology option

The National Environment Protection Council of Australia publishes National Environment Protection Measures that specify national standards for contaminated site remediation. The National Environment Protection (Assessment of Contaminated Sites) Measure (1999)[5] provides remediation practitioners (environmental consultants and contractors) with a hierarchy of preferred remediation options. Ideally, contamination is treated *in situ*. If this is not possible, the preference is for contamination to be extracted and treated on site. The least preferred option is for contamination to be removed from the site and treated or disposed of elsewhere. It is up to state and territory governments to legislate requirements for site remediation.

First and foremost, a remediation technology for any given site must have the ability to contain, extract or transform the pollutant in question from the matrix it is contaminating. Containment of contaminant mass using physical barriers or restricting access to particular sites is often a cost-effective approach to mitigating immediate risk, but leaves contaminants *in situ* for future generations to manage.

Removal of contaminated materials from a site using heavy-handed engineering approaches such as 'dig and dump' and 'pump and treat' relieves the need for future management of a site, but risks the spread of contamination and often just moves the risk to another location. As sustainability has risen in importance as a selective criterion, these approaches have lost favour given that they are generally costly, energy intensive with associated greenhouse gas emissions, and disruptive to the environment in the case of large-scale excavation or groundwater extraction.[4]

This century has seen the development and widespread application of more-nuanced remediation technologies that transform pollutants *in situ* to benign products with greatly reduced or unmeasurable impacts on human or environmental health. An example is *in situ* chemical oxidation using strong oxidising agents to mineralise contaminants.[6] This can be cost effective but comes with risks in application and effectively sterilises the contaminated matrix. Another example is *in situ* chemical reduction using reducing agents such as zero-valent iron to chemically reduce contaminants to reduce toxicity.[7] There are, however, extremely challenging sites for which it is not feasible to remediate with any of the aforementioned technologies based on sustainability.

The estuarine sediments of Homebush Bay, adjacent to the formerly heavily industrialised Rhodes Peninsula, is one such example. Chemical manufacturing during the 20th century resulted in heavy dioxin contamination of the harbour sediments, resulting in a commercial fishing ban west of the Sydney Harbour Bridge.[8] Although the most heavily contaminated sediments were successfully excavated and treated using *ex situ* physical and chemical approaches by *c*. 2010, there is no viable remediation option for treating the remaining contamination, which continues to be a source of dioxin contamination throughout Port Jackson. This is glamorous Sydney Harbour's dirty secret and bioremediation might just be the solution.

Anaerobic biodegradation research underpins subsurface bioremediation applications

Our approach to addressing organochlorine contamination in anaerobic environments is to discover and characterise novel bacteria that can transform these toxic compounds into harmless or less-harmful derivatives. For example, we have discovered two bacteria, *Dehalobacter restrictus* strain UNSWDHB and *Formimonas warabiya* strain DCMF, that can work together to transform chloroform (a common toxic groundwater pollutant) to dichloromethane (less harmful than chloroform) and then acetate (harmless).[9–11] Another example is the discovery of a *Dehalobium* species that can reductively dechlorinate the most toxic dioxin found in Sydney harbour sediments (2,3,7,8-tetrachlorodibenzo-*p*-dioxin) to the much less toxic trichlorodibenzo-*p*-dioxin congener.[12]

Discovery of novel organochlorine-degrading bacteria is achieved by using sediment or water from an organochlorine-contaminated environment as an inoculum in anaerobic microcosms. Typically, under this condition we expect to observe organochlorine respiration, where the organochlorine of interest is used as the respiratory terminal electron acceptor resulting in the removal of chloride from the organochlorine. Obligate organochlorine-respiring bacteria (ORB) are heterotrophs that use hydrogen as the electron donor and acetate and bicarbonate as organic and inorganic sources of carbon respectively. Therefore, these substrates are supplied in the anaerobic growth medium. After the initial organochlorine pulse has been depleted it is immediately resupplied. This cycle is repeated and at each iteration *16S* rRNA gene amplicon sequencing is performed to follow the change in community profile, and to identify ORB involved in degrading the organochlorine. Several serial transfers with ~1% inoculation of the parent culture can result in enrichment of the desired ORB to ~90% of the microbial population.

For use at an organochlorine contaminated site, laboratory-scale microcosms in the order of 100 mL must be scaled up by ~1000-fold (i.e. 100 L). We have found that beer kegs are ideal for this purpose as they can maintain anaerobic conditions, are solvent resistant and are cost effective. ORB cultures that are grown in 20-L kegs can then be deployed at contaminated sites in existing groundwater monitoring wells. Once the cultures are *in situ*, tracking their activity in a dynamic system such as a subsurface aquifer can be challenging. This is because environmental factors can cause large fluctuations in contaminant concentrations. To overcome this challenge a number of steps are taken to functionally characterise ORB so that more than contaminant concentration can be used to confirm *in situ* degradation of the target organochlorine.

Firstly, the gene encoding the functional enzyme (i.e. the reductive dehalogenase; Rdase) is elucidated so that *in situ* functional cell numbers can be correlated with contaminant or degradation product concentrations. The Rdase is discovered using native polyacrylamide gel electrophoresis (PAGE)-coupled–liquid chromatography–mass spectroscopy (LCMS) and biochemical activity assays. Proteins expressed during cell growth by organohalide respiration are extracted under anaerobic conditions and separated by electrophoresis in their non-denatured and therefore functional form. Discrete protein bands are excised from the gels and tested for activity in anaerobic activity assays that contain a range of organohalides. When the active protein band is identified, the amino acid sequence of the reductive dehalogenase is determined by liquid chromatography–tandem mass spectroscopy (LC-MSMS). The amino acid sequence is then *in silico* reverse translated to its corresponding nucleotide sequence, which can then be retrieved from the ORB genome.[10,13] From here qPCR primers can be made for targeted *in situ* tracking of ORB alongside contaminant depletion.

The isotope enrichment factor is a unique signature associated with different chemical reaction mechanisms and can therefore be used for confirming or differentiating between different contaminant degradation pathways. The isotope enrichment factor for the ORB-facilitated attenuation of a specific organochlorine is determined by gas chromatography–combustion–isotope ratio mass spectroscopy (GC-C-IRMS). GC-C-IRMS ascertains the stable carbon or chlorine isotope ratios of individual organochlorines after GC separation. The mathematical relationship between the changes in isotope ratio *v*. change in organochlorine concentration provides the unique isotope enrichment factor for the *in situ* assessment of ORB activity.[14,15]

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Mike Manefield is an environmental microbiologist who teaches environmental science and engineering in the School of Civil and Environmental Engineering at UNSW. His primary research interest is in pollutant biodegradation. He has published over 130 articles and supervised over 45 PhD, Masters and Honours candidates to completion. He is founder of Novorem Pty Ltd (https://novorem.com.au) supporting Australian industry with environmental microbiology expertise.

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Facing our plastic waste crisis: biorecycling as a promising solution

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ABSTRACT

We are in a global plastic waste crisis. Plastic production has steadily increased over the last half century, while recycling rates remain as low as 9% in some nations, including Australia. Most plastic waste ends up in landfill or the environment as a lost resource, triggering the production of more virgin plastic to satisfy demands. Shifting away from this wasteful, linear economy towards a circular economy, where waste products are treated as a valuable resource and are recycled, will require considerable innovative advancements to our current plastic recycling methods. Biological recycling (biorecycling) has emerged as a promising solution, with several advantages over mechanical and chemical recycling. Using enzymatic reactions, long plastic polymers are cut into monomers without the need for high temperatures or chemical catalysts, and without affecting product quality. Biorecycling allows sustainable, commercially viable and near-infinite recycling of synthetic polymers. In this paper, we discuss reasons for our current plastic waste crisis, compare plastic recycling methods with a focus on biorecycling and explore commercial ventures of enzyme-based recycling technologies. We present recent developments in enzyme discovery, enzyme characterisations and protein engineering. Finally, we propose a strategy to move towards a circular plastic economy, by embracing biorecycling.

Keywords: biorecycling, enzyme discovery, plastic recycling, protein engineering, sustainable development.

Background

Finding a sustainable solution to deal with the ever-increasing amount of plastic waste has emerged as one of the main environmental challenges of our time. Major contributing factors to the global plastic waste crisis are the high popularity of short-lived plastic products in combination with low recovery and recycling rates. Global plastic production has steadily increased since the 1950s, resulting in an annual production of 460 million tonnes of plastic in 2019.[1] This trend is likely to continue, and is predicted to exceed 1 billion tonnes by the year 2050.[2] Most plastic products are inexpensive to manufacture[3]; however, the true costs are revealed once they reach the end of their life. For over one-third of all plastic products, such as packaging material, this happens after only a single use.[2] The majority of this waste (~79%) ends up in landfill or the environment,[4] where it can persist for several years to centuries.[5] Discarded plastics accumulate in our oceans, creating over US$2.2 trillion (~A$3.23 trillion) in environmental and social damage per year.[6]

Tackling this plastic waste crisis requires a combined effort of the public, industry and government. We need to reduce our plastic consumption, find more sustainable alternatives to plastic products whenever possible, and improve recovery and recycling of plastic waste. Recycling rates are low in many nations, including Australia and the USA, which recycle only ~9% of their plastic waste.[7,8] Ideally, close to 100% of all plastic waste would be recycled. High recycling rates are essential to transition from the current linear economy, whereby fossil fuel-derived plastic products are discarded after use, toward a circular economy, in which plastic is a resource that can be recycled indefinitely (Fig. 1). A deciding factor in achieving this goal is the development of efficient, sustainable and scalable recycling methods. Currently, plastic recycling approaches include mechanical, chemical and biological recycling. Mechanical recycling is the most common commercial method,[9] and has the benefits of a simple and inexpensive process, and a low demand on energy and resources.[10] However, mechanical recycling is usually a 'down-cycling' process,

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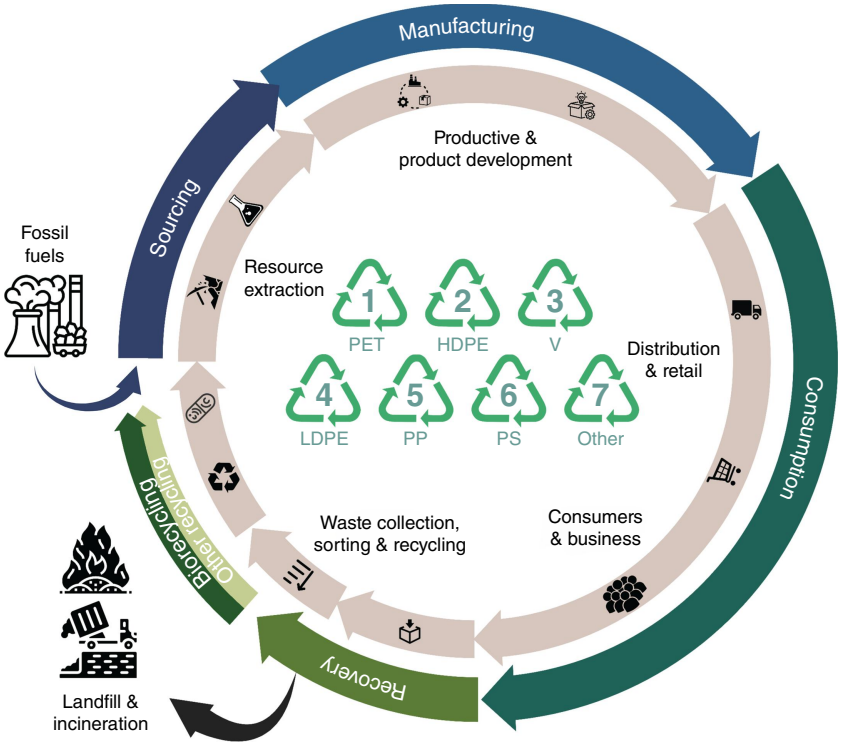

Fig. 1. Transitioning to a circular plastic economy. Initially, the sourcing of feedstock for plastic production will contain crude oil (fossil fuels; blue arrow), and plastic waste recovery will be incomplete, with resources diverted for use in waste-to-energy approaches (incineration) or lost in landfill (black arrow). Post transition to a circular economy, plastic waste recovery will take advantage of biorecycling, and potentially other recycling methods such as chemical recycling, to convert 100% of the recovered plastic waste into feedstock for plastic production. Abbreviations: PET, polyethylene terephthalate; HDPE, high density polyethylene; V, vinyl (also known as polyvinyl chloride, PVC); LDPE, low density polyethylene; PP, polypropylene; PS, polystyrene; Other, other plastic types. Created with BioRender.com Image source of recycling symbols 'Green Vectors by Vecteezy', https://www.vecteezy.com/free-vector/green.

resulting in an end product of lesser quality and lower value.[11] Chemical recycling aims to recover plastic compounds using chemical catalysts and has lower purity requirements for plastic waste feedstock.[10,12] Chemically recycled plastics are of a quality comparable to virgin plastics, allowing these plastics to be recycled multiple times.[10] However, chemical recycling requires high infrastructure investments, uses costly chemicals, has high energy demands,[10,13] and can create toxic gaseous products and wastewater.[14,15]

The latest addition to the plastic recycling toolbox is biological recycling, or biorecycling, using natural and engineered enzymes to depolymerise plastic waste into its building blocks (monomers). Biorecycling has several advantages over mechanical and chemical approaches. Using enzymatic reactions, the long plastic polymers are cut into monomers, without degrading the material, allowing the repolymerisation into virgin-grade plastic.[16,17] Enzymatic reactions can occur at standard temperatures and ambient pressure, limiting required energy demands.[18,19] When biorecycling reaches its full potential, a cocktail of diverse enzymes could be employed to target and recycle a wide range of plastics, including contaminated and mixed plastic waste. This will eliminate strict requirements for pre-processing, such as washing and sorting, will reduce costs and will speed up the recycling process. Overall, biological recycling promises to become a widely applicable and cost-effective process that is also environmentally friendly. Most plastic-depolymerising enzymes reported to date belong to the family of carboxylic ester hydrolases (CEHs; EC 3.1.1), including lipase, esterase and PETase. CEHs catalyse the hydrolysis of ester bonds, which are part of the backbone of hydrolysable plastics, i.e. polyamides such as nylon, and polyesters, such as polyethylene terephthalate (PET). Biodegradation of PET, a plastic commonly used to make soft drink and water bottles, has been the focus of several research projects in the last 5 years, and has recently been explored for commercial plastic biorecycling.[20] By contrast, plastics with a carbon–carbon backbone, such as polyethylene (PE), polypropylene (PP), polystyrene (PS) and polyvinyl chloride (PVC), do not contain hydrolysable groups and are considered to be more recalcitrant to biodegradation. However, over the last decade, several bacteria and fungi have been reported to degrade C–C plastics, including PE and PS,[21] PP,[22] PVC,[23] and recently two PE-degrading enzymes have been characterised.[19] Companies working on biorecycling of PET and other polyesters have the first-mover advantage in commercial plastic biorecycling, however, the next targets will be the more recalcitrant C–C plastics.

Commercial advancements in biorecycling of plastic waste

Industrial applications of enzyme-based plastic recycling technologies are still in their early stages, and most methods will require time to mature and scale up to a commercial level. However, the plastic biorecycling space has seen a steep increase in partnerships and funding announcements over the last 2 years. Public funding remains essential for technology development, and subsequent industry partnerships are key for process optimisation and construction of pilot plants to upscale enzyme depolymerisation workflows. Examples of commercial enterprises include:

Carbios (France) has developed a biorecycling process based on the enzymatic depolymerisation of PET. The approach is promoted as an industrial process that allows the recycling of all types of PET waste without loss of quality. The enzyme, an engineered PET hydrolase, achieves a minimum of 90% PET depolymerisation into monomers over 10 h.[17] Carbios received €3 million of public funding in 2021,[24] and launched an industrial demonstration plant

in the same year with a depolymerisation reactor capable of processing 2 tonnes of PET, which is equivalent to 100 000 bottles, per cycle.

Samsara Eco (Australia) was launched in 2021 and focuses on enzymes to recycle PET and other polyester resins, with the goal to produce plastics with the same properties as virgin resin. Raising over A$54 million in 2022, the company has started to develop their first commercial recycling plant in Melbourne, Vic., Australia. This facility will be designed to treat ~20 000 tonnes of plastic per year, starting in 2024, with the long term goal to recycle over 1.5 million tonnes of plastic waste by 2030.[25]

Other commercial ventures in the biorecycling space include *Epoch Biodesign* (UK), *Protein Evolution* (USA), and *Enzymity* (Latvia). All three companies design candidate enzymes using artificial intelligence (AI), followed by enzyme synthesis and variant testing in the laboratory. Information about their targeted plastic types is not currently available. Another company, *Birch Biosciences* (USA) is targeting PET and polyurethane plastics, focusing on AI, i.e. complex machine learning, to re-design naturally occurring proteins for an improved hydrolysis of plastic polymers under industrial, scalable conditions. Birch Biosciences is currently backed by the United States National Science Foundation, Department of Energy and by private investors (J. Kers, pers. comm.). Last but not least, *Plasticentropy* (Spain), a spinoff from the Spanish Research Council, targets the enzymatic degradation of PE and other polyolefin plastics (F. Bertocchini, pers. comm.), utilising enzymes produced by waxworms.[19] The company currently lists the characterisation of enzyme activities, and enzyme optimisation as their main goals.

Recent achievements and future directions of biorecycling

Accelerating the field of biorecycling in the coming years will require considerable efforts in enzyme discovery, microbial culturing, enzyme characterisation and protein engineering. Enzyme discovery is well underway, and recent studies found genes of plastic-depolymerising enzymes in a wide range of bacterial and fungal lineages[26] and in ocean and soil samples from around the globe.[27] Our ongoing work supports these results, e.g. we found that abundances of genes encoding synthetic polymer-degrading enzymes increased with depth in the world's oceans, and that these genes are readily transferred between microbial hosts (C. Rinke, unpubl. data). Insect larvae and their gut microbiomes have been another treasure trove for the recovery of potential plastic-degrading enzymes. Waxworms, mealworms, superworms and other insect larvae have been investigated for the biodegradation of several plastics.[28] Our work focusing on superworms, the larval stages of the darkling beetle (*Zophobas morio*), confirmed that the insects can survive and even gain weight on a sole diet of PS foam.[29] Applying metagenomics, a method to recover and sequence nearly all DNA in a sample, allowed us to infer several enzymes with PS-degrading capabilities in bacterial genomes recovered from the superworm gut. We concluded that the insects and their gut microbiome are an ideal combination to tackle recalcitrant plastics such as PS. First, the insect host shreds the plastic, introducing hydrolysable groups into the polymer, and then, the gut microbes break down these polymers into styrene monomers.[29] Styrene, a naturally occurring substance, can then be imported into the bacterial cells and further metabolised.[30]

Once plastic-degrading enzymes are discovered, the computationally predicted functions of these enzymes need to be experimentally verified in the laboratory. Biodegradation of PET is currently the best examined depolymerisation pathway, involving two validated enzymes working sequentially to break down PET into its two monomers, ethylene glycol and terephthalic acid,[18,31] which can be used to produce new PET resin. Functional validations of plastic-degrading enzymes for polymers with a C–C backbone have only been reported for PE. Two PE-degrading enzymes recovered from the saliva of wax moth larvae demonstrated the ability of naturally occurring enzymes to depolymerise these recalcitrant plastics.[19] This discovery also highlighted that enzymatic PE degradation can occur after only a few hours at room temperature without the need for an abiotic oxidation pre-treatment.[19] We predict that more experimental validations and detailed enzyme characterisations will follow in the coming years, generating a large arsenal of enzymes, accelerating biorecycling approaches. Our aim is to add PS-degrading enzymes to this list, and we are currently bringing gut bacteria from PS-fed superworms into culture. We will then validate bacterial enzymes and pathways involved in PS degradation using CRISPRi-based gene silencing.[32] Subsequent enzyme characterisations will focus on specificity and catalytic efficiency, and the latter will benefit from downstream protein engineering.

Plastic biodegradation in natural environments happens at a slow pace, e.g. PE bottles need at least several decades to degrade in the ocean.[5] Protein engineering of naturally occurring enzymes will be essential to speed up the degradation process by generating optimised, specific and highly active enzymes. A recent machine learning-guided protein engineering approach resulted in an enzyme, termed FAST-PETase, with superior PET-degrading abilities. The engineered enzyme could degrade untreated, post-consumer PET products nearly completely within 1 week.[16]

Over the next decade, we can expect many more reports of well-characterised and engineered enzymes targeting a wide range of synthetic polymers. This diverse arsenal of plastic-degrading enzymes will further encourage commercial applications and will lead to a valorisation of end-of-life plastics. Instead of being treated as waste, these plastics will be considered a valuable resource, bringing us one step closer to the desired circular plastic economy (Fig. 1).

Conclusion and outlook

Plastic waste now pollutes every corner of our planet. We need to address this crisis urgently by reducing plastic production while increasing recycling efforts. The federal government has recently taken the initiative by joining an international agreement to recycle or reuse 100% of all plastic waste by 2040.[33] Achieving this ambitious goal will require (1) strong legislation to reduce plastic consumption,

e.g. a uniform, federal ban of single-use plastic products such as packaging, (2) targeted investments in research and development of plastic recycling technologies, such as biorecycling, and (3) upgrades of the current recycling infrastructure to go beyond mechanical recycling.

The collapse of Australia's largest soft plastic recycling program, REDcycle,[34] has dramatically emphasised that current recycling methods struggle with mixed plastic waste and are challenging to scale up to cope with the large amount of plastic waste produced in Australia.[35] Plastic biorecycling is on track to provide an economically viable and sustainable solution. The advantage of processing contaminated and mixed plastic waste, in combination with low energy requirements and good scalability, favour this new approach. However, considerable investments in enzymatic plastic biodegradation research are necessary to mature, scale up and commercialise biorecycling over the next years. Initially, government support and extended producer responsibility (EPR) schemes can play a key role to supply the necessary funds. Implementing EPR will require regulations that legally oblige plastics manufacturers to pay for recycling and disposal of their products, instead of passing the responsibility to the consumer. In summary, it is possible to transfer to a circular plastic economy, where 100% of all plastic waste is biorecycled, but it will require a combined effort by the government, researchers, industry and the public to make it happen.

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Data availability. The data that support this study are available in FigShare at https://doi.org/10.6084/m9.figshare.22207567.

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Restoring Australia's long-term innovation requires investment in basic research

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ABSTRACT

It is well established that basic research underpins long-term innovation outputs such as transformative drugs and medical devices. However, Australia may be hobbling its long-term innovation capabilities by decreasing investment in basic research. Although there has been recent growth in our local venture capital and biopharmaceutical business sectors (that typically fund applied research and experimental development), both are comparatively undersized. Initiatives are needed to ensure long-term commitment to basic research at globally competitive levels and to ensure that long-term innovation capabilities are supported.

> Science enables new discoveries; it is the fuel for transformed industries … [but] … have we done all that we can to ensure that great Australian science, put to new industrial uses, is set up not just to survive but thrive in this country? [Ed Husic MP, National Press Club, November 2022].

Research and development (R&D) in the medical, biotechnology, and pharmaceutical sectors ('biomed' sector for brevity), is unique among industries. Distinguished by very high costs, long product development times and high risks, biomed has one of the highest R&D intensities of any industry.[1] For example, a novel oncology treatment typically requires 10–15 years and costs US$2.7 billion to advance from basic research to licensure, yet with only a 3.4% chance of successfully launching on the market.[2] Given the R&D intensity, multiple stakeholders are often required to finance and deliver biomed innovations as they progress through their technology readiness levels (TRLs).[3] In Australian biomed industries, as well as in many other jurisdictions globally, governments typically finance basic, and some applied research, from TRLs 1–3 through higher education investment in research and development (HERD), venture capital and private equity, whereas business typically finances later-stage applied R&D activities, such as preclinical and clinical trials from TRLs 4–9, through business investment in research and development (BERD). However, without well-developed venture capital funds, or a culture of private equity and business investment in research, governments are often required to invest in applied R&D activities with the objective of ensuring that basic research technologies advance to market.

Over the last two decades, significant effort has been made in Australia to develop and attract biomed research funding, encourage both private and business investment, and progress basic research technologies through their TRLs. The Commonwealth Government funds basic research primarily through the National Health and Medical Research Council (NHMRC), the Australian Research Council (ARC) and the newly established (2015) Medical Research Future Fund (MRFF). Both the ARC and NHMRC funds have schemes in place to encourage business investment, translation and advancement of basic research towards later TRLs (notably the ARC Linkage scheme and NHMRC Partnership Projects scheme). The MRFF strategically focuses on translation of research outcomes into policy and practice, as well as commercialisation of new technologies.[4] The Australian venture capital sector has been growing in both absolute size of assets under management and as a percentage of gross domestic product (GDP) (see Fig. 1); in 2013, venture capital investment represented 0.016% of GDP and increased 1.8 fold in the 5-year period to 2018.[5] But, has the increase in funding across all TRLs been enough to support Australia's biomed sectors and encourage long-term innovation success?

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Australia's innovation output

The recent 2022 Global Innovation Index (GII) ranked Australia 25th among 132 countries for its innovation capabilities.[7] Our ranking has been steadily declining over the past 5 years, down from 23rd in 2020, 22nd in 2019 and 20th in 2018. More concerning is that we rank even lower for our innovation outputs, with no sign of improvement over the last 3 years (31st in 2020, 33rd in 2021 and 32nd in 2022).[7] Our innovation output score and corresponding global rank has been consistently described as a weakness.[7] Innovation output measures include knowledge and technology outputs such as intellectual property (IP), financial returns on that IP, numbers of scientific journal articles and their H-indices, and numbers of new businesses, to name just a few; widely accepted as important measures of industry growth and returns to investment in R&D.[6] Further, Australia's innovation productivity score, that is our innovation output score divided by our input score, is less than 60% and has been steadily declining since 2013. This suggests we are not effective at 'translating costly [basic research] innovation investments into quality output. Australia produces fewer innovation outputs relative to its level of innovation investment'.[7] Leading biomed innovation economies such as the United States of America and Switzerland, and emerging economies such as China, are far more effective at translating innovation investments into outputs; China and Switzerland's innovation productivity scores are above 90% and have been consistently so over the last 5 years, and, although the USA's score is just below 80%, its overall innovation index has consistently ranked in the top five economies.[7] Given our strong government investment in research and growing venture capital sector, why is Australia performing so poorly in comparison to other leading economies?

Australia's innovation input

The answer may lie in the quantity and *type* of investment into our innovation process across TRLs. In terms of later TRLs, although Australia's venture capital investments have grown, the rate has been lower than the other leading biomed innovation economies mentioned above: Switzerland's venture capital investment increased 2.3 fold and the US's

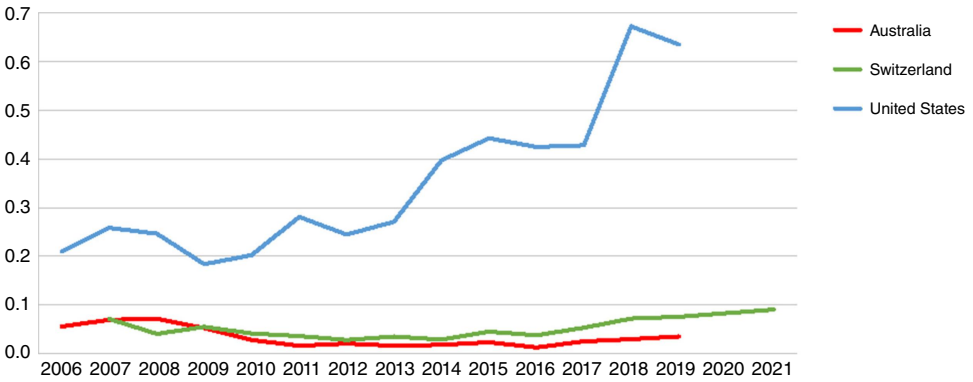

Fig. 1. Venture capital investment as a percentage of national gross domestic product (GDP). Source: OECD.[6] Note: data from China were not available from the OECD.

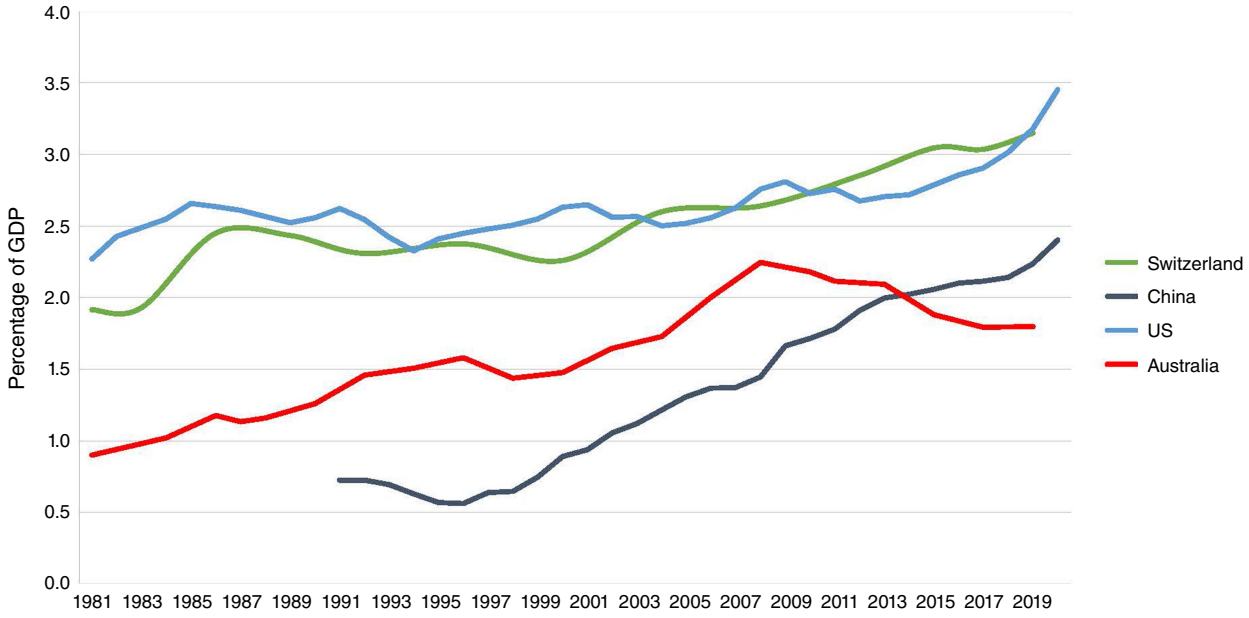

Fig. 2. Gross domestic expenditure on research and development (GERD) of select countries since 1981 (or as available). Source: OECD.[7]

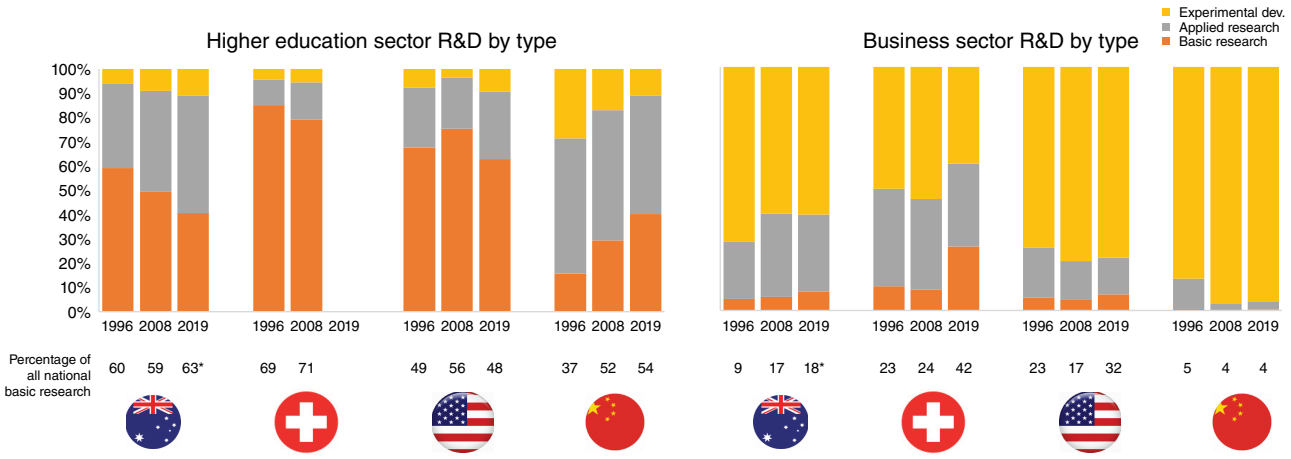

Fig. 3. Left: higher education sector by type of R&D performed. Right: business sector by type of R&D performed. The percentage of all national basic research numbers marked with an asterisk (*) are estimates. Australia's 2019 data were not complete for all sectors in the OECD database; authors combined 2018 and 2019 sector-level data; Source: OECD,[6] accessed August 2022.

venture capital investment increased 2.6 fold (see Fig. 1).[6] In terms of earlier TRLs, Australia has also been underinvesting in R&D relative to GDP compared to the US, Switzerland and China. From the 1980s until 2008, Australia was increasing R&D investment as a percentage of GDP at a similar rate to other large economies (see Fig. 2).[6] However, for the most recent 15 years, Australia has decreased R&D investment as a percentage of GDP, while other global economies have continued to increase their R&D investment over the same period.

In terms of type of R&D, we observe that the higher education execution of basic research in Australia has been steadily declining relative to applied research and experimental development (see Fig. 3, left).[6] Notably, Australia's higher education sector has decreased execution of basic research by 18% since 1996 while prioritising applied research (+14%) and experimental development (+5%). In 1996, nearly 60% of Australia's HERD was executing basic research, but in 2019 basic research only accounted for 40% of total HERD. This has not been the case for the US or China, both of which have increased their proportions of HERD in basic research. Indeed, with the expansion of the Chinese economy, China's basic research execution as a proportion of total HERD has increased from 15 to 40% over the last 15 years (Fig. 3, left).[6] Although Switzerland decreased the proportion of HERD execution of basic research between 1996 and 2008, it still maintained an overall relative execution of basic research at ~70% of HERD (the highest of the three comparison economies). Further, execution of basic research by Switzerland's business sector (BERD), relative to applied and experimental development, increased to 26% in 2019, up from 10% in 1996 (see Fig. 3, right).[6]

The importance of basic research

After World War II, President Franklin D. Roosevelt commissioned a report to distill the lessons learnt from wartime to inform investment in the future US economy. The report made the case for Government support of basic research, defining basic research as that which is 'essential for combatting disease, ensuring national security, and increasing the standard of living, including supporting new industries and jobs … [that] results in general knowledge and an understanding of nature and its law'.[8] Five years later, US Congress created the National Science Foundation, which, in collaboration with the National Institutes of Health, underpins the USA's investment in basic research. With a focus on pharmaceutical drug development, Spector *et al.*[9] further highlighted that the most transformative and successful medicines exist today because of fundamental, basic research that was carried out decades before their eventual launch on the market. Without this basic research, conducted without an explicit, short-term goal of drug discovery, medicines such as the ACE inhibitor captopril, cancer treatment imatinib, or cardiovascular treatment lovastatin would not have existed to benefit patients, nor have contributed to the subsequent creation of trillion-dollar markets.

Improving Australia's biopharmaceutical sector

In view of the clear importance of basic research investment for longer term innovation outputs, juxtaposed with declining investment in total R&D and basic research funding, a relatively undersized venture capital industry, and comparatively limited BERD, how can Australia's medical, biotechnology and pharmaceutical sectors advance discoveries through their TRLs, ensure efficient innovation outputs and remain globally competitive?

One solution is to increase our investment into basic research in the higher education sector thereby reversing the decline in investment in basic research relative to applied research. In Australia, however, ARC and NHMRC funding has not substantially increased over the past decade, whereas the MRFF, whose activities arguably fall outside the basic research TRLs, grew to A$20 billion in July 2020.[4,10,11] Indeed none of the seven guiding principles of the MRFF mention acquisition of greater knowledge or improving fundamental understandings of phenomena; in the words of Vannevar Bush, supporting research 'that results in general knowledge and an understanding of nature and its law'.[8]

Although the guiding principles focus on very important outcomes, a focus on funding basic research for the sake of fundamental knowledge is the crucial foundation. It is vital so that our innovation pipeline is well supported at its origins.

Another solution could be to increase Australia's total R&D investment to globally competitive levels, as was the case prior to 2008. The authors welcome the announcement of the A$15 billion National Reconstruction Fund intended to strengthen Australia's medical science, among other areas. Government support for innovation is absolutely critical, but investment from other stakeholders is also critical. For example, leading innovative countries have either been able to attract foreign investment into their economies or have a healthy and growing local business, venture capital and private equity sector responsible for funding primarily later-stage applied biopharmaceutical R&D. Compared to other countries, foreign direct investment inflow to Australia has recently decreased from 3–5% of GDP in 2009–19 to 1–2% in 2020–21, and this foreign investment is highly concentrated in mining and quarrying (35% of all FDI inflow positions in 2019), financial and insurance (11%) and real estate (11%), with no data available for the biopharmaceutical sector.[5] In comparison, the US's pharmaceutical sector is one of their largest beneficiaries of foreign direct investment representing 12% of positions.[6]

Australia is also under-represented in venture capital, which is a critical enabler of transitioning basic research into more applied and experimental development efforts. Venture capital investment correlates very strongly with success of countries in developing emerging sectors like therapeutic stem cell research.[12] According to the Preqin database,[13] the global venture industry has ~A$1.7 trillion in assets under management (as of March 2021),[13] where the Australian venture capital sector represents ~A$12 billion (as of June 2021)[5] or 0.7%. Although the total number of both venture deals and deal size in Australia has increased over the last decade, investments were predominantly in IT, consumer discretionary and financial services start-ups, rather than the healthcare sector.[5]

Based on the clear evidence of the crucial dependence of biomedical breakthroughs on fundamental research that precedes them by decades, the National Reconstruction Fund's Reference Group should commit to Australia's basic research capability and contribute to restoring Australia's investment to long-term innovation. Without a commitment to funding basic research at globally competitive levels, Australia will see its innovation ecosystem and bench of well-trained scientists further diminished over the next decade. We suggest that the National Reconstruction Fund's Reference Group should develop mechanisms to encourage venture capital and potentially foreign investment into Australia's *basic* research in higher education settings, our immense talent pool, as well as applied and experimental development research. These mechanisms could include new or more effective incentives for international business investment, repatriating and importing talent with international experience into the Australian ecosystem (often bringing capital with them), and strengthening our links into global life science networks.

Although more needs to be done to understand the current barriers to global business investment into Australian R&D, the recent success stories such as the Inflazome acquisition by Roche, ResApp acquisition by Pfizer and AstraZeneca's deal with Starpharma demonstrate that some foreign investors see the opportunities and appreciate the outcomes of Australian basic research projects that began in higher education settings. The COVID-19 pandemic reminded us of the importance of investment in basic discovery research, particularly in the field of microbiology. Without the foresight of the Coalition for Epidemic Preparedness Innovations (CEPI) in investing in the basic research performed by companies such as Moderna, it is doubtful whether a novel mRNA covid vaccine candidate would have been in clinical trials within 2 months of the outbreak, a timeframe that was substantially faster than for novel vaccine candidates during other epidemics such as the 2009 H1N1 swine flu or 2014 Ebola outbreaks.[14]

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Data availability. The data that support this study are primarily publicly available in the sources cited in the references section, or were obtained from Prequin by subscription. Data will be shared upon reasonable request to the corresponding author with permission from Prequin.

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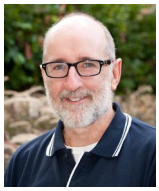
Biographies

Dr Rosemary Harrison is a Biotechnology executive with nearly 20 years of global experience working on pharmaceutical corporate strategy, portfolio management and business development. She is currently Chief Business and Strategy Officer at TCR2 Therapeutics (NASDAQ: TCRR) in Boston, USA, responsible for driving a range of activities including strategy, operational planning, corporate partnerships and long-term growth opportunities. Dr Harrison holds a Bachelor of Biotechnology in Drug Design and Development (Hons, UQ Medal) and a PhD from The University of Queensland.



Emeritus Professor Ross Barnard, FASM, was director of the biotechnology program at the University of Queensland from 2000 to 2019. Prior to that he was at Panbio Ltd (as program leader for nucleic acid diagnostics development) and the Cooperative Research Centre for Diagnostic Technologies. He has worked on diagnostics development for a variety of infectious agents. In 2005, he undertook a sabbatical at the Australian Animal Health Laboratory in Geelong, Vic., Australia (now the Australian Centre for Disease Preparedness), during which time he collaborated on the development of a broad-spectrum, RT-PCR-based Influenza A diagnostic. He was a NHMRC C. J. Martin Fellow at the University of Queensland and the University of California, Santa Cruz.

Dr Lisette Pregelj is a Lecturer in Biotechnology at The University of Queensland (UQ). Her research and teaching focuses on commercialisation of biotechnology and pharmaceutical innovations, specifically precision medicines and medical devices and the effect of regulation and innovation capabilities on their speed from discovery through to market. She previously held Postdoctoral Research Fellowships with UQ's Business School and Australian Institute of Business and Economics.

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Microbiology Australia

Bacpath 16

Timothy Wells and Ulrike Kappler

This year, the 16th ASM Bacterial Pathogens (Bacpath) conference was held in Brisbane between 27 and 30 September 2022 using venues located at the University of Queensland and the Emporium Hotel near South Bank Parklands. After a year's delay due to COVID-19, it was fantastic to be able to hold a face-to-face meeting, giving the 175 participants from all over Australia an opportunity to (re)connect with colleagues, and to meet new researchers in this exciting research field. The scientific program was diverse and stimulating with leading and emerging microbiologists from around Australia presenting an exciting array of science, research and the advances in the field of bacterial infection and pathogenesis.

The conference kicked off with a welcome reception at the Emporium Hotel. As it had been 3 years since the last physical Bacpath, it was an excellent opportunity to catch up with old friends and network with new colleagues in the stylish surrounds of the Emporium Hotel Piano bar that features a self-playing grand piano.

The scientific program commenced on the Wednesday morning with the ASM Bacpath Oration presented by Prof. Dena Lyras. The talk, 'From plasmid biology to pathogenesis: a series of fortunate events', was a fascinating walk-through of the research, from her early work on fundamentals of plasmid biology to her current studies in bacterial virulence.

Perhaps inspired by Prof. Lyras' presentation, recent research on *Clostridioides difficile* was a real highlight of the conference with award-winning talks from Ashleigh Rogers, Diana Lopez-Urena, Christine Ong and Dr Yogitha Srikhanta. These talks covered a range of diverse virulence functions discovered in *C. difficile*, including impediment of host colonic repair, effects on the enteric nervous system and links between sporulation and antibiotic resistance.

Wednesday continued with high-quality presentations across a variety of areas including 'host–pathogen interactions', 'characterising virulence determinants', 'gene regulation' and 'structure and function of virulence factors'. Research on the antibiotic resistance and persistence of urinary tract infections was a highlight with award winning presentations by PhD students Sophia Hawas and Chyden Chang. The final session of the day began with an excellent talk by Dr Stephanie Neville on the structural basis of bacterial manganese import. Finally, we concluded the day back at the Emporium Hotel for networking and poster session.

Thursday saw a change in focus with our second keynote speaker, Prof. Michael Jennings, discussing the importance of studying glycan-based host–pathogen interactions to develop diagnostics, preventatives and cures for infectious diseases. The day continued with sessions on 'surface factor biology', 'antibiotic resistance', 'detection, treatment and vaccines' and 'metabolism and virulence'. Award-winning talks by Winton Wu on 'Uncovering the role of the functional small RNA interactome in antibiotic tolerance in *Staphylococcus aureus*' and Maoge Zang on 'The multifunctional roles of membrane-integrated phosphatases in *A. baumannii* envelope biology' were a further highlight. The day's final session included our third keynote speaker, Prof. Deborah Williamson, detailing novel approaches to the detection, prevention and control of bacterial sexually transmitted pathogens. Thursday evening was again spent at the Emporium Hotel for a fantastic poster session, before the majority of the networking moved up to the rooftop bar, the Terrace, with fantastic views over Brisbane.

On Friday, Prof. Tim Stinear presented his Keynote talk on 'Resistance, persistence and *Staphylococcus aureus*'. The conference concluded at lunchtime, following two final sessions on 'host–pathogen interactions' and 'regulation of bacterial virulence'. Throughout the 3 days, our delegates enjoyed the fabulous science, stimulating discussions and great catering that was provided, and we are all already looking forward to the next Bacpath, which will be held in NSW and will provide more opportunities to showcase new, high-quality research on bacterial pathogens.

The award winners from Bacpath.

Acknowledgements. Bacpath 16 was kindly sponsored by BMG Labtech, Griffith University, The University of Queensland, Menzies Health Institute Qld, Doherty Institute and BioCyc. We are extremely grateful for their very generous and continued support. Finally, we thank the ASM Executive, in particular Mark Schembri and Kate Seib, as well as the Local Organising Committee (Mark Walker (Chair), Timothy Wells and Ulrike Kappler (Venue, ASN liaisons), Freda Jen and Mathew Sullivan (Sponsorship), Makrina Totsika, John Atack and Nick West (Program organisers), Johanna Kenyon and Chris Day (Poster session organisers), Yaramah Zalucki (Registration), Olivia Jessop, Sarah Cahill and Evgeny Semchenko (Social), Stephan Brouwer and David De Oliveria (Virtual Conference Mirror Coordinators) for making this fantastic event happen. We also to thank ASN Events (especially Kara Barker and Tim Hancock) whose support was invaluable for the smooth running of the conference.

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Microbiology Australia

CliniCon

Belinda McEwan and Mikayla Kingston

The Australian Society for Microbiology (ASM) held its inaugural CliniCon meeting following the completion of the ASM annual scientific meeting on the beautiful Darling Harbour in Sydney from 14 to 15 July 2022, with a focus on diagnostic and clinical microbiology. The meeting was held in a hybrid style with delegates catching up face to face or joining virtually.

The meeting commenced with a light lunch where delegates and trade were able to catch up with each other after the previous 2 years of COVID-19.

The first session kicked off with three great presentations from three well-known Australian parasitologists. A fascinating presentation given by the charismatic Dr Richard Bradbury (Federation University) on basic (yet tricky) microscopy of parasites, followed by an enlightening presentation of diagnosis of blood parasites presented by Francesca Azzato (VIDRL) and then Dr Harsha Sheorey (SVH) with some unique case studies to share with us.

Following on from these three presentations was a case study on *Echinococcus* sp. and *Helicobacter pylori* presented by Mikayla Kingston (NPV) and to round out the first session of CliniCon Robert Norton (Path Qld) gave a presentation on '*Burkholderia pseudomallei* – laboratory identification in the MALDI-TOF era and safety'.

At the afternoon tea intermission many delegates were catching the speakers from the first session to ask further questions while juggling a drink and biscuit.

The second session of CliniCon commenced with talks on Mycobacterium, where Maria Globan (VIDRL), Caroline Lavender (VIDRL) and Taryn Crighton (NSW Path) gave in-depth informative presentations around identification, susceptibility testing and Buruli ulcers.

The second session was rounded out with presentations on '*Neisseria gonorrhoeae* – the rise of the susceptible clones' from Ella Trembizki (UQCCR) and the final presentation for the day was by Dazhi Jin (HMC), discussing the development of a novel multiple cross displacement amplification assay for *Clostridium difficile* as a point of care test.

Delegates were then delighted to have the opportunity to network once again at the welcome function held in the trade hall with the trade participants. There were many happy faces catching up over food and drinks and updating each other on the past 2 COVID years.

The second day commenced with a session dedicated to the area of mycology, where Charlotte Webster (NSW Path) and Evanthia Tambosis (NSW Path) led us through the world of fungi; however, in reality, they only touched the surface of mycology. They laid down foundations for the rest of us to help with identification of some of the simpler fungi.

Following morning tea on day two we were enlightened by three comprehensive presentations. The first presentation was on Monkeypox by Linda Hueston (NSW Path), followed by a presentation by Shane Byrne (S&N Path) with his experience navigating COVID-19 and the challenges of testing from the laboratory perspective – wow, what a crazy roller coaster ride that was, and I'm sure many other laboratories around Australia had similar experiences.

Continuing on with the COVID-19 theme, Wayne Dimitch (NRL) then updated delegates on their evaluation of COVID serology kits available in Australia and their performance. Finally, Rickyle Balea (USC) completed the session before the lunch break with a brief presentation of his work on the detection of Zika virus using rapid isothermal tests as an alternative to RT-PCR.

The closing session of CliniCon 2022 commenced with Tony Jennings (S&N Path) who discussed and highlighted the issues and current challenges within the clinical pathology industry, a topic many delegates could relate to.

Derek Holzhauser (RCPA) informed delegates on the reasonably new area for diagnostic microbiology laboratories on digital microscopy in the clinical and diagnostic microbiology laboratory. Will this be the way of the future?

Jacob Tickner (UQ) gave a short presentation on rapid molecular diagnostics for drug-resistant *Salmonella typhi*, followed by Emma Sweeney (UQ) presenting on individualised treatment of *Mycoplasma genitalium* infections. The final speaker was Christopher McIver (SGH), who presented on the elusiveness and significance of clinical isolates of cysteine requiring *Escherichia coli*.

The ASM thanks all those involved in making the meeting a huge success. And a special thanks are given to the speakers, session chairs and delegates. Our goal was to create a more-specific clinical-based meeting and networking forum. We hope the meeting was informative and looking forward to seeing you at our next CliniCon meeting in Perth in 2023!

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Microbiology Australia

Obituary: Peter William Robertson (7 July 1945–6 July 2022)

William D. Rawlinson, Mark J. Ferson and Peter C. Taylor

In July 2022, 1 day before his 77th birthday, Associate Professor Peter William Robertson sadly passed away. Peter was a world leader in diagnosis using serology, particularly in the areas of human immunodeficiency virus (HIV) and other blood-borne virus diseases. Peter worked on improved diagnosis for common diseases including respiratory viruses at a time when before we had heard of SARS-CoV-2 and were more concerned with influenza. He undertook applied research into serological diagnostics, and development of national quality assurance programs that to this day are used by diagnostic laboratories around Australia. Peter's particular expertise was in trying to bring old and new technology to new clinical problems. Peter was appointed Principal Scientist as head of Area Serology, a Conjoint Associate Professor at UNSW, and was a postgraduate student supervisor and lecturer at UNSW. Trainee scientists and pathologists would regularly spend time in Peter's laboratory, and many of them would participate in research projects leading to peer-reviewed publications.

His early work was in Eastpath, subsequently, the South Eastern Area Laboratory Service (SEALS), and most recently the Area Serology Laboratory of SAViD, New South Wales Health Pathology Randwick. Peter was appointed to the Department of Microbiology, The Prince of Wales Hospital, in 1970 to develop and enhance serological diagnosis of infectious diseases. He had a much broader role, and was instrumental in positioning this reference laboratory at the centre of diagnostics for blood-borne viruses, infections of pregnant women, EBV characterisation, as well as much of diagnostic serology. He was an early adopter of new technology. He undertook studies in techniques no longer used routinely, such as column fractionation of immunoglobulin classes to establish IgM and IgA assays to identify primary infections *in utero* and of IgA assays to be able to demonstrate active adult pertussis the cause of whooping cough in infants. Peter's expertise in the diagnosis of congenital rubella and toxoplasmosis was sought by clinicians and courts alike, and his work on diagnostic serology of measles, pertussis and meningococcal disease, among others, were highly valued by the public health community in New South Wales and more broadly.

The demand for widespread testing for blood-borne infections increased with new tests for HIV but also with the increasing array of hepatitis viruses. Blood-borne virus testing is fundamental to the public health programs to reduce infection with hepatitis B and C, as well as HIV. Peter's work personally in developing these studies, for example on HIV (published in the *Medical Journal of Australia* in 1985) at the time when HIV (then called HTLV-3) diagnostics was in its infancy, has saved many lives. Peter has been a mentor to the majority of Australians working in serology in the late 20th century in one way or another. He founded the first serology quality assurance program for the RCPA in 1973 when this was sent out from Prince of Wales Hospital as part of the national microbiology survey.

Peter's work in HIV included working closely with government, national bodies such as the NRL, and in the Area Serology Laboratory at Randwick. He continuously improved assays for HIV and used these assays during the 1980s to test at risk individuals and produce improved confirmatory assays (such as Western Blot in 1992) for serological diagnosis of HIV. His work on congenital infections, including congenital cytomegalovirus (CMV) and rubella, started in the 1970s on the back of his research published in 1969, on anti-Rh antibody testing. As principal scientist of the Area Serology Laboratory, he continued to research and publish in translation of better ways of diagnosing congenital CMV, and congenital toxoplasmosis, through collaborations around Australia.

Peter's education was initially a BSc in microbiology in 1967, followed by a MSc in 1968 and a PhD 5 years later under Prof. Kevin Marshall, on the immune responses in intestinal lymphoid tissue. He initially started as a training medical technician at the Kanematsu Institute at Sydney Hospital in 1963, 2 years later becoming a science student and then moving to the New South Wales blood transfusion service where his passion for serology and diagnosis led him to his later position as principal scientist of what was then SEALS. He was honoured with admission as a founding fellow of the Faculty of Science at the Royal College of Pathologists of Australasia in 2012 (FFSc), and received commendations from the New South Wales Government with the Premiers Public Sector Award Commendation in 2010, and a Meritorious Service Medallion in 2012. He became production manager of a Therapeutic Goods Administration-licenced donor screening laboratory in the early 2000s, and continued to supervise students in medicine and science, as well as being an invited speaker at meetings around the country.

Peter married Susan van Gelder in 1967, and together they raised three children, Michael, Jennifer and John. He is very much missed by them, and by his colleagues. He was above all a man of honesty, passion, vision and belief. The world is smaller for his absence, but we are all slightly greater for having known him. *Requiescet in pace.*

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ASM Ambassadors:
connecting members with conferences and each other

L: Aditi Aiyer (Didi)

R: Mahjabeen Khan

R: Sarah Revitt-Mills

Above: Hannah Nugent

Below: Sicilia Perumalsamy

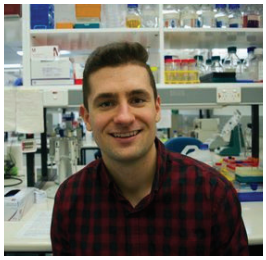
REBECCA LE BARD

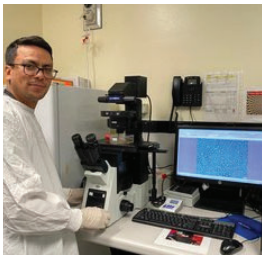
VICE PRESIDENT COMMUNICATIONS

Above: Arthika Manoharan

Below: Adrianna Turner

R: Miljan Stupar





L: Binod Rayamajhee

R: Rhys White

Top L: Jennifer Hosmer

L: Melinda Ashcroft

L: Claudia Stocks

Join the team!

THEME LEADERS

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MOLECULAR MICROBIOLOGY

JAI TREE

PETER REVILL

VIROLOGY

ADAM TAYLOR

KATE HOWELL

LINDA BLACKALL

MICROBIAL ECOLOGY & ENVIRONMENTAL MICROBIOLOGY

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