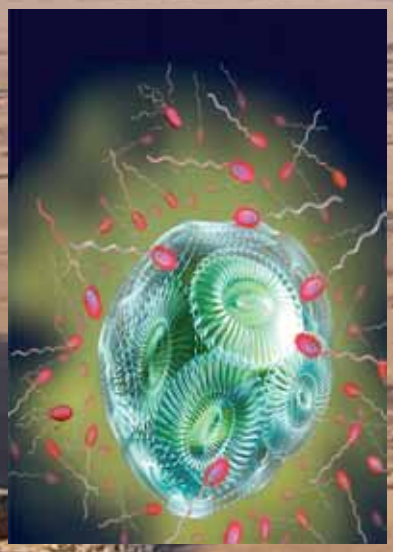
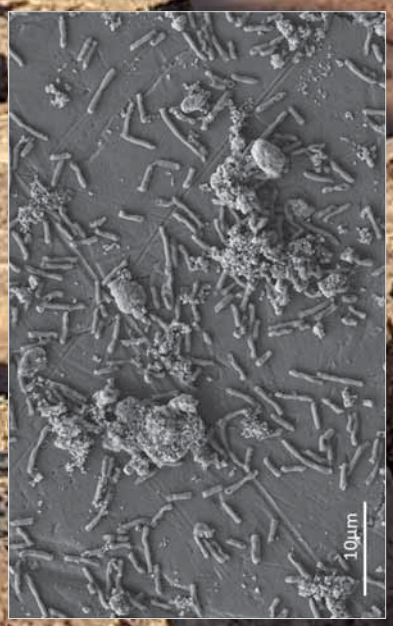
# Microbiology AUSTRALIA

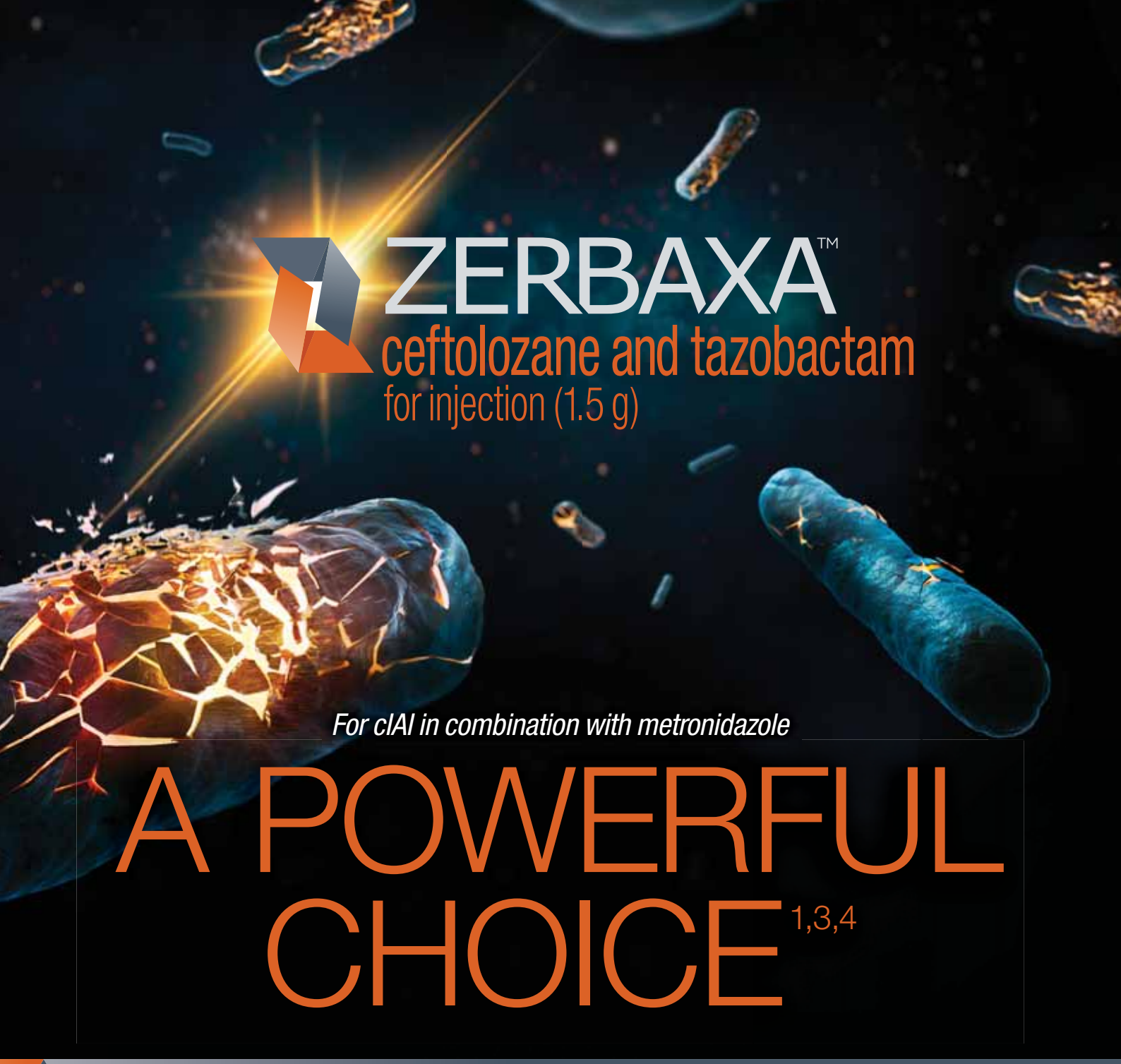
OFFICIAL JOURNAL OF THE AUSTRALIAN SOCIETY FOR MICROBIOLOGY INC.

Volume 39 Number 1 March 2018


# Environmental Microbiomes







# ZERBAXA™

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*For cIAI in combination with metronidazole*

# A POWERFUL CHOICE[1,3,4]

## ZERBAXA + metronidazole had a high level of antimicrobial activity against *P. aeruginosa*[1,2,4]

In cIAI, all patients in the ME population with *P. aeruginosa* infection at baseline had a 100% clinical cure rate with ZERBAXA plus metronidazole (n=26/26) vs. 93.1% with meropenem (n=27/29) (subgroup analysis; p values not stated).[4]

## ZERBAXA has a low potential for development of resistance in *P. aeruginosa*.[1,2]

**ZERBAXA is stable against common *P. aeruginosa* resistance mechanisms *in vitro*^ including:**[2]

- Overexpression of AmpC
- Increased express of efflux pumps
- Reduced expression of carbapenem-specific porins (OprD)

^*in vitro* activity does not necessarily predict clinical efficacy

cIAI=complicated intra-abdominal infection; CI=confidence interval; MITT=microbiological intent-to-treat; ME=microbiologically evaluable

In cIAI, clinical cure rate for ZERBAXA + metronidazole (n=389) was non-inferior to meropenem (n=417) in the MITT population at test of cure, 83% vs 87.3% (weighted difference, -4.2%; 95% CI, -8.91, 0.54; p value not stated).[3]

Study design in cIAI: A total of 993 adults hospitalised with a cIAI were randomised in a multinational, double-blind, phase 3, non-inferiority trial comparing ZERBAXA (ceftolozane 1000 mg and tazobactam 500 mg) plus metronidazole (0.5 g) intravenously every 8 hours with meropenem (1 g) intravenously every 8 hours for 4–14 days. Complicated intra-abdominal infections included appendicitis, cholecystitis, diverticulitis, gastric/duodenal perforation, perforation of the intestine, and other causes of intra-abdominal abscesses and peritonitis.[3]

Primary endpoint, clinical cure, was defined as complete resolution or significant improvement in signs and symptoms of the index infection at the test of cure visit (24–32 days from start of therapy), using a non-inferiority margin of 10%. The primary efficacy analysis population was the microbiological intent to-treat (MITT) population which included all patients who had at least one baseline intra-abdominal pathogen regardless of the susceptibility to study drug.[3]

Secondary endpoint was the same end point in the microbiologically evaluable (ME) population, which included all protocol-adherent MITT patients.[3]

## Before prescribing, please review the Product Information. The Product Information is available upon request from MSD or can be accessed at www.msdinfo.com.au/zerbaxapi

PBS Information: This product is not listed on the PBS.

**ZERBAXA (ceftolozane/tazobactam 1000 mg/500 mg): Indications:** Treatment of infections in adults suspected or proven to be caused by designated susceptible microorganisms: complicated intra-abdominal infections in combination with metronidazole; complicated urinary tract infections, including pyelonephritis. **Contraindications:** Hypersensitivity to any component of this product; known hypersensitivity to members of cephalosporin class or other members of ß-lactam class. **Precautions:** Hypersensitivity; *clostridium difficile* - associated diarrhoea; severely immunocompromised patients receiving immunosuppressive therapy; renal impairment; fertility effects, use in pregnancy, lactation; children <18 years. **Pregnancy Category:** B1. **Interactions:** Drugs that inhibit OAT1 or OAT3 (e.g., probenecid, diclofenac, cimetidine) may increase tazobactam plasma concentrations. **Adverse Effects:** Most common (5% or greater in either indication) were nausea; diarrhoea; headache; pyrexia; others, see full PI. **Dosage:** *Complicated intra-abdominal infections:* 1000 mg ceftolozane/500mg tazobactam (with metronidazole 500 mg IV) every 8 hours by IV infusion (over 1 hour) for 4-14 days; *complicated urinary tract infections, including pyelonephritis:* 1000 mg ceftolozane/500mg tazobactam every 8 hours by IV infusion (over 1 hour) for 7 days; for dosage in renal impairment, see full PI. **Based on PI dated 7 August 2017.**

**References: 1.** ZERBAXA Product Information. 4 November 2015. **2.** Zhanel GG, Chung P, Adam H, Zelenitsky S, Denisuik A *et al.* 2014. Ceftolozane/ Tazobactam: A Novel Cephalosporin/ß-Lactamase Inhibitor Combination with Activity Against Multidrug-Resistant Gram-Negative Bacilli. *Drugs*, 74(1):31–51. **3.** Solomkin J, Hershberger E, Miller B, Popejoy M, Friedland I *et al.* 2015. Ceftolozane/Tazobactam Plus Metronidazole for Complicated Intra-abdominal Infections in an Era of Multidrug Resistance: Results From a Randomized, Double-Blind, Phase 3 Trial (ASPECT-cIAI). *Clin Infect Dis*, 60(10):1462–71. **4.** Miller B, Popejoy MW, Hershberger E, Steenbergen JN, Alverdy J. 2016. Characteristics and Outcomes of Complicated Intra-abdominal Infections Involving *Pseudomonas aeruginosa* from a Randomized, Double-Blind, Phase 3 Ceftolozane-Tazobactam Study. *Antimicrob Agents Chemother*, 60(7):4387-90.



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# Call for nominations for the position of VP Corporate Affairs

The Vice-President Corporate Affairs plays an important role in overseeing the management of the Society's finances. The current VP Corporate Affairs is Cheryl Power, who has served her term, and we are seeking someone to replace her. The position is for 3 years, which can be extended to 5 years. The successful nominee will serve one year as VP-elect before taking up their role. During this period, they will work with Cheryl. In addition to overseeing their specific portfolio area, the successful nominee will be a member of the executive team. In this role she or he will interact with the other VPs and the ASM President to guide all aspects of the Society's operations.

We now call for nominations for the position of VP Corporate Affairs. Candidates for election to this position shall be members of the ASM. They must be proposed and seconded by Society members and provide a short statement as to how their particular skills will contribute to the Society.

Nominations must be received before the 31st March, 2018. Please send completed nominations by email to the ASM National Office (admin@theasm.com.au).

**Nomination for the position of VP Corporate Affairs of The Australian Society for Microbiology Inc**

We the undersigned wish to nominate _____

of _____

for the position of VP Corporate Affairs of The Australian Society for Microbiology

***Proposer*** *(FASM / MASM / SASM / Honorary Life Member)*

Name _____

Signature _____

***Seconder*** *(FASM / MASM / SASM / Honorary Life Member)*

Name _____

Signature _____

***I accept this nomination for the position of VP Corporate Affairs of the ASM***

Name _____

Signature _____

Date _____

***Statement by nominee (no more than 100 words):***

# The Australian Society for Microbiology Inc.

9/397 Smith Street
Fitzroy, Vic. 3065
Tel: 1300 656 423
Fax: 03 9329 1777
Email: admin@theasm.com.au
www.theasm.org.au
ABN 24 065 463 274

For *Microbiology Australia* correspondence, see address below.

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ISSN 1324-4272
eISSN 2201-9189





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**Volume 39 Number 1 March 2018**


## Contents



*Cover image: Background: Maktesh Ramon, a geological landform situated between the arid and hyper-arid region of the Negev Desert, Israel. Photo taken by Sean Bay. Upper inset: Phycosphere (J Seymour and J-B Raina). Lower inset: SEM of bioleaching microbe,* Enterobacter aerogenes *(M Corbett and E Watkin).*

*Roy Robins-Browne*
**President of ASM**

Dear fellow microbiologists

As this is my first communication with you this year, I think it's not too late to wish you a happy new year and all the best for 2018.

As I've mentioned previously, ASM aims to give our members maximum value for their membership. Some new initiatives for 2018 include an annual teacher's travel award, valued at $4000, to attend the American Society for Microbiology Conference for Undergraduate Educators (AMSCUE), which is the world's premier microbiology teachers' conference. We also have instituted 100 travel awards, each valued at $200, to make it easier for members within 10 years of attaining their highest qualification to attend our Annual Scientific Meeting. This year's meeting is in Brisbane from 1–4 July (http://asmmeeting.theasm.org.au/). The scientific and social programs are shaping up beautifully, and I know you will be fired up and reinvigorated by attending. Please enter the dates in your diary now.

We also have new awards that allow undergraduate students to undertake a research project in an approved laboratory during the summer vacation. Contact your State Branch for more details.

Apart from our Annual Scientific Meeting, ASM is sponsoring the MMM (Molecular Microbiology Meeting) in Sydney this year from 11–12 April. This meeting provides a wonderful opportunity for biological scientists who see potential translational applications for their research, and for clinical scientists and clinicians who want to hear about recent advances in biology and biotechnology. For more information, visit http://sydney.edu.au/medicine/criticalinfection/mmm/index.php.

ASM Council has also resolved to underwrite the biennial BacPath meeting from 2019 onwards. Next year's meeting will be in Western Australia at a 4-star venue near Perth, and like all 14 meetings before it will be collegial, exciting and informative.

I am delighted to announce the appointment of Associate Professor Priscilla Johanesen as our inaugural Student and Early Career Researcher (ECR) Engagement Co-ordinator. Priscilla has long been involved in arranging Students' Day at our annual conference and will now take on the added responsibility of fostering the development of student and ECR members of our Society. The main purpose of her role is to engage students and ECRs to improve their overall experience by providing resources, activities and opportunities that to bring them together and promote their professional development. Please encourage your students to attend our meetings and become involved in these activities. Students who attend just one meeting a year will recoup more than their annual subscription.

Some other ongoing initiatives involve strengthening our ties with microbiological societies in our region, including the New Zealand and Singapore microbiology societies. If you are interested in attending the annual meetings of either of these societies, you can do so at local members' rates. We are also looking at strengthening our ties with other biological societies within Australia, such as the Australian Society for Biochemistry and Molecular Biology, the Australian Society for Antimicrobials and the Australian Society for Infectious Diseases. This may include holding a joint or overlapping conference with one or more of these societies.

Finally, I want to remind you that the **closing date for our annual awards and prizes is 31 March** (http://www.theasm.org.au/awards/). Please encourage anyone you know who may be eligible for one of these awards to apply as soon as possible.

# Environmental microbiomes

*Linda L Blackall*
Email: lblackall@swin.edu.au

The March 2015 issue of *Microbiology Australia*[1] was devoted to 'Mammalian microbiomes' and this March 2018 issue on 'Environmental microbiomes' complements that previous one. Additionally, authors of articles in the current issue were largely chosen from oral presenters at the inaugural ASM-sponsored Australian Microbial Ecology (AUSME2017) conference held a year ago in Melbourne. That 3-day conference in February 2017 celebrated the field of Microbial Ecology.

Like the various compartments of mammals covered in the March 2015 *Microbiology Australia* issue, a complex suite of microbes including prokaryotes (Bacteria and Archaea), microbial eukaryotes, and viruses are found in the majority of niches on Earth. These 'environmental microbiomes' are vital to all global nutrient cycles, pollution biodegradation and bioremediation, in host-associations (e.g. of all non-mammals and in plant nutrient provisioning) and in ecosystem health (including in the built environment). The generation of metagenome sequences, of metatranscriptomic, metaproteomic and metametabolomic information and their data analyses and interpretation continue to be major drivers in microbiome application.

In a recent Nature Microbiology consensus statement article[2], which catalogued global microbiome research, 'Microbiomes' were defined as '. . .host-, ecosystem- or habitat-associated communities of microorganisms', and 'microbiome research' was defined as '. . .those studies that emphasise community-level analyses using "omics technologies" '. In the non-human related microbiome field, major national but mostly international collaborative programmes have titles like Microbial Observatories, the International Census of Marine Microbes, the International Soil Metagenome Sequencing Consortium 'TerraGenome', and the Earth Microbiome Project. Philanthropic (e.g. Gordon and Betty Moore and W. M. Keck Foundations) and Governmental agencies (many nations) have come together to co-fund these overarching microbiome research initiatives whose goals are ambitious like understanding causes of climate change in forest, grassland, and permafrost ecosystems and constructing a microbial map of planet Earth. The consensus article concluded that the dominant microbiome research activities were related to human niches and/or they focussed on basic biology research themes. The following were identified as significant future needs: computational biology; biorepositories for data; development of high throughput tools; and longitudinal, functional and interdisciplinary research topics. The practical application of microbiome research to Earth's sustainability and for improved human livelihood will involve deeper industry and commercial involvement[3].

Projections for future microbiome studies have been reported in recent outlook manuscripts. A recent non-peer reviewed perspective piece[4] proposed that distinct microbiome characteristic types including microbial processes, microbial community properties, and microbial membership should be linked to each other and to higher level system processes that they impact. In another perspective piece, Xu *et al.*[5], argue that over the next 10 years, microbiome research will be propelled by changes in our thinking and in technology. It ambitiously described how microbiome research will move to determining the state, function and interactions of microbes by developing imaging and visualisation methods, individuals rather than consortia will be probed, and we will move to data science from data analysis. The cardinal feature in many viewpoint papers is the fact that method development has been and will continue to be a major stimulus for studying, comprehending, and manipulating microbiomes. An essential part of this development is standardisation of protocols and use of controls[6].

Although human-associated ecosystems might dominate the microbiome field[2], environmental microbiome investigations have revealed staggering microbial biodiversity and unprecedented biochemical transformation scope – a few specific examples are given here. In 2016 Hug *et al.*[7] dramatically expanded and reformatted the tree of life, and described a whole Candidate Radiation Phylum. Anantharaman *et al.*[8], reported many new sub-surface sourced microbial genomes and discovered metabolic handoffs in simple consortia. In late 2017, Parkes *et al.*[9] reported 7903 bacterial genomes from public metagenome data submissions inflating the

bacterial and archaeal phylogenetic diversity by an amazing >30%. Great contributions to environmental microbiomes continue to be provided by several Australian research groups[9–13], as well as groups with articles in this *Microbiology Australia* issue.

The articles in this *Microbiology Australia* issue cover a broad range of environmental microbiome studies, largely from Australian-based researchers – demonstrating the vibrancy of the field. The environments covered include marine (water and host-associated), terrestrial (soil), bioremediation (wastewater treatment, bioleaching, mining), and cultural artworks. Papers cover microbial processes (chemotaxis, nitrogen cycling, synergism, life without water), biotechnological advances and opportunities, microdiversity, and microbial and trait-based ecology. Numerous microbial groups including Bacteria, Archaea, Fungi and Viruses are topics from different contributors. It comprises a panorama of subjects within the field of Environmental Microbiomes.

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## Biography

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

# The importance of resolving biogeographic patterns of microbial microdiversity

*Alexander B Chase*
Department of Ecology
and Evolutionary Biology
University of California
Irvine, CA, USA
Email: abchase@uci.edu

*Jennifer BH Martiny*
Department of Ecology
and Evolutionary Biology
University of California
Irvine, CA, USA
Email: jmartiny@uci.edu

**For centuries, ecologists have used biogeographic patterns to test the processes governing the assembly and maintenance of plant and animal communities. Similarly, evolutionary biologists have used historical biogeography (e.g. phylogeography) to understand the importance of geological events as barriers to dispersal that shape species distributions. As the field of microbial biogeography initially developed, the utilisation of highly conserved marker genes, such as the 16S ribosomal RNA gene, stimulated investigations into the biogeographic patterns of the microbial community as a whole. Here, we propose that we should now consider the biogeographic patterns of microdiversity, the fine-scale genetic diversity observed within a traditional ribosomal-based operational taxonomic unit.**

Biogeography investigates how ecological and evolutionary processes influence the distribution of biodiversity and the structure of contemporary communities[1]. Historically, biogeographic patterns of plants and animals are studied at the species level and describe large-scale patterns of species' distributions. In contrast, the vast majority of microbial biogeographic studies investigate patterns by sampling the entire community at broad taxonomic designations. Typically, these studies define operational taxonomic units (OTUs) using a highly conserved ribosomal marker gene, usually the 16S rRNA gene for bacteria and archaea. However, the decision of which genetic region to target, and in particular the genetic resolution of that region, can influence the biogeographic patterns observed[2]. While these conserved regions can capture a large breadth of the microbial community, these regions, by their very nature, limit the detection of finer-scale genetic variation. By resolving diversity within the OTU designation, we can detect ecological and evolutionary processes occurring at this fine taxonomic scale that might otherwise be overlooked.

## What OTU-based biogeography can and can't tell us

It is now well established that microbial communities assayed by traditional OTU designations display distinct biogeographic patterns over space and time. These patterns have been identified in environments ranging from marine[3], to terrestrial[4], and to human-associated systems[5]. Combined with abiotic and biotic data from the sampled environment, such patterns can provide initial hypotheses about the ecological processes shaping microbial community assemblages[6]. Thousands of microbial studies now demonstrate that OTU-based patterns primarily reflect the importance of selection of environmental conditions based on correlations between microbial composition and the environment (Figure 1*a*). These patterns indicate that OTUs comprising each microbial community vary in their ability to tolerate various abiotic and biotic conditions, suggesting partitioning of environmental resources and niche spaces among taxa in the community.

While environmental variables explain much of the variation in microbial composition, many studies also find that some variation is correlated to the geographic distances between communities[6]. This observation can be illustrated with a distance-decay curve, or a negative correlation between the similarity in microbial composition with geographic distance between pairwise samples[7] (Figure 1*b*). If this negative relationship holds after accounting for environmental variation, then the pattern suggests that ecological drift, caused by stochastic fluctuations in demographic patterns, contributes to variation in community composition[8,9]. Further,

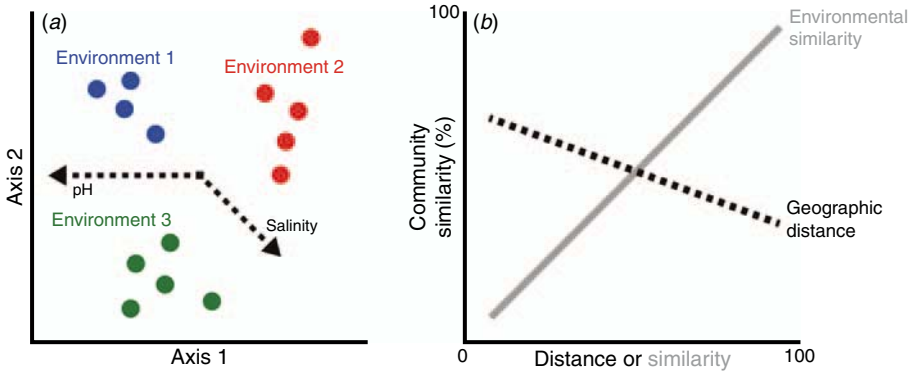


Figure 1. Hypothetical community analyses from OTU-based studies. (*a*) An ordination plot depicting community composition across three environments with the main environmental factors driving compositional differences indicated with dashed arrows. Each point represents a sampled microbial community, with points closer to one another indicating higher similarity in community composition. (*b*) Community similarity among a collection of samples is often positively correlated to environmental similarity (grey line) and negatively correlated with geographic distance (black dashed line, also called a distance-decay curve). The influence of strong environmental selection on the community is reflected in the positive correlation with increasing environmental similarity, while the influence of ecological drift is reflected in the negative correlation with increasing geographic distance between samples.

since ecological drift depends on restricted dispersal, the pattern gives insight into the degree of dispersal limitation between the sampled communities. A caveat to such studies is that it is impossible to completely account for environmental variation, and the environment is spatially autocorrelated. However, such OTU-based studies suggest that the ecological processes of both environmental selection and ecological drift contribute to biogeographic patterns at this broad genetic resolution[7].

In contrast to ecological processes, biogeographic patterns of OTU-based analyses are unlikely to detect patterns shaped by evolutionary processes. This limitation is due to the broad resolution of conserved marker genes. Variation in these genetic regions capture relatively distant evolutionary divergences, especially when clustered at 97% sequence similarity. Indeed, a 3% sequence divergence in the 16S rRNA gene, the most common level of OTU clustering, represents roughly 150 million years of evolutionary history[10], or before the origin of modern birds[11]. In other words, biogeographic patterns for birds at this taxonomic level would mask all diversification within the group. Similarly, the use of such conserved marker genes for microbes will generally preclude detecting biogeographic patterns emerging from evolutionary processes, such as endemism and niche conservatism, as observed for macroorganisms assessed at the species or population level.

## What is microbial microdiversity

Studies based on 16S rRNA sequences have been instrumental in identifying ecological patterns and their underlying processes at relatively broad genetic resolutions. However, it is increasingly clear that there is extensive genetic diversity within 16S-based OTUs, so-called microdiversity, in environmental habitats[12,13]. For example, a natural population of the bacterioplankton *Vibrio splendidus* contained >1000 distinct genotypes, even when clustered at >99% 16S rRNA sequence similarity[14]. Based on their very nature, conserved marker genes lack the variability to resolve fine-scale diversity within an OTU. Even with the implementation of exact sequence variants (ESVs), the 16S rRNA gene simply cannot resolve the fine-scale variation among closely related microbial lineages[15]. Thus, different approaches are needed to investigate the biogeographic patterns of this vast genetic diversity.

Beyond identifying genetic microdiversity, a key question is whether this genetic variation is phenotypically relevant[16]. Investigations into microdiverse marine bacterial taxa suggest that they vary in physiological traits including preferences for particular abiotic conditions[13,17]. Further, some of this trait variation within OTU-based taxa appears to be phylogenetically conserved within microdiverse clades[18,19], although resolving the phylogeny of such closely related strains is often difficult with 16S rRNA sequences (Figure 2*a*). Instead, taxon-specific marker genes or, ideally full genome sequences, can often resolve microdiverse clades and reveal which traits are shared among particular phylogenetic clades (Figure 2*b*). For example, an analysis of strain diversity of an abundant leaf litter bacterium, *Curtobacterium*, exhibited extensive variation in the degree of polymeric carbohydrate degradation and temperature preference among microdiverse clades[20]. Thus, more resolved genetic and physiological studies can help to establish the phylogenetic distribution of traits.

## What biogeographic patterns of microdiversity can tell us

The presence of trait variation among microdiverse clades suggests that microdiversity will exhibit distinctive biogeographic patterns. If this trait variation corresponds to different ecological preferences, then the environment should select for specific clades under variable conditions. Indeed, different bacterial ecotypes,

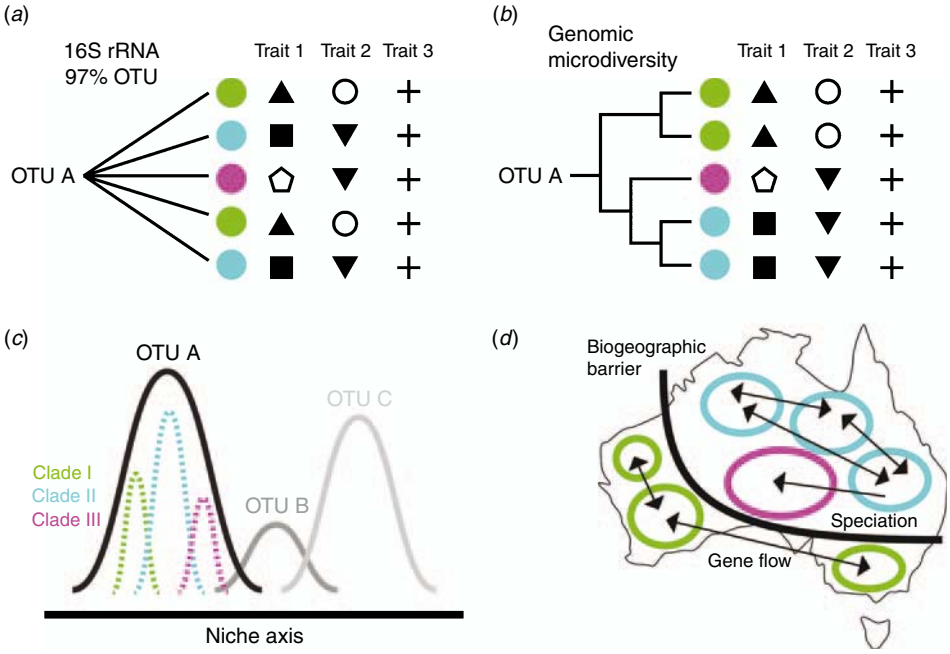


Figure 2. Detection of ecological and evolutionary processes within OTU A with microdiverse Clades I (green), II (blue), and III (pink). (*a*) The 16S rRNA gene often cannot resolve phylogenetic relationships within a 16S-based OTU and, subsequently, the distribution of traits among clades. (*b*) Genomic sequences or multi-locus sequence analyses (MLSA) of marker genes can resolve phylogenetic relationships at a finer-scale revealing, in this hypothetical example, that strains within clades share more similar traits. (*c*) Trait variation within microdiverse taxa can promote resource partitioning in the environment leading to fine-scale niche differentiation among clades (represented in colored dashed lines) that would otherwise be masked at the OTU level (black line represents the total niche for OTU A). (*d*) Investigating genetic differentiation within OTUs is more likely to reveal dispersal limitation (measured by gene flow between clade populations) and the presence of biogeographic barriers that contribute to microbial diversification. In this hypothetical example, black arrows represent gene flow between populations of microdiverse clades, where limited gene flow (no arrows connecting green with the blue and pink populations) suggests the presence of biogeographic barriers.

or ecological populations, have repeatedly been shown to vary in their spatial distribution. Thus, closely-related clades appear to partition niche space in the environment that would normally be masked at the OTU level (Figure 2*c*). For example, at the OTU level, the globally distributed cyanobacterium, *Prochlorococcus*, shows a broad preference for low-nutrient and warmer waters[21]. However, microdiverse clades of *Prochlorococcus* exhibit distinct spatial distribution patterns shaped by additional environmental factors, including light availability and temperature[12,17,22]. Thus, biogeographic patterns of microdiversity can elucidate the importance of key environmental parameters governing niche differentiation that may not be identifiable at the OTU designation.

Perhaps even more importantly, a focus on microdiversity can reveal evolutionary processes that would otherwise be masked at a broader genetic resolution. Thus far, few environmental studies have targeted microbial diversity at a fine enough scale to investigate how evolutionary mechanisms, such as mutation and genetic drift, can lead to differential biogeographic patterns[18,23]. Those examples that do exist find evidence for evolutionary processes contributing to spatial patterns. In one such example, reduced dispersal between hot spring populations of the archaeon thermophile *Sulfolobus*, restricted gene flow to allow diversification to occur among geographic regions[24,25]. Similarly in terrestrial soils, dispersal limitation at regional spatial scales structures bacterial populations of *Streptomyces* along a latitudinal gradient[26]. With the increased availability of computational tools to study population genomics[27] and the incorporation of gene flow networks[28], we expect that more studies will consider the spatial distribution of microdiversity. Such studies are likely to illuminate the effects of evolutionary processes on microbial diversity in the environment, including the presence of biogeographic barriers and the degree of microbial endemism[29] (Figure 2*d*).

## Conclusions

Future progress in microbial biogeography necessitates moving beyond the OTU designation. While OTU-based studies will continue to play an important role in microbial biogeography, an intensified focus on finer-genetic diversity will uncover thus-far unidentified ecological and evolutionary patterns. However, these studies will require targeted sampling of particular microbial taxa rather than the entire community. Generally, this effort will require moving beyond targeting the 16S rRNA gene; even ESVs of this region will not be able to distinguish microbial populations at a fine enough genetic scale. And while extensive shotgun metagenomic and targeted amplicon sampling can reveal co-occurrence of novel microdiversity associated with distinct environmental conditions[30], these studies are dependent on the interpretation of genomic potential for ecological diversity. Therefore, there is still a need

to link the genomic variation to functional traits that will define ecotypes. The return to isolation-based studies to gather relevant genetic and physiological information will better inform environmental metagenomic studies investigating microbial microdiversity. By expanding the focus to microbial microdiversity and implementing targeted environmental studies, we can better understand the ecological and evolutionary processes generating microbial biogeographic patterns as macroecologists have done for decades.

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## Biographies

**Alexander B Chase** is a doctoral student at the University of California, Irvine under Dr Jennifer Martiny. His research interests include applying his previous training in ecological theory, where he worked under Dr Stephen Hubbell at the University of California, Los Angeles, to microbial systems. He utilises bioinformatics to wrangle genomic and metagenomic data to investigate the ecological and evolutionary processes affecting fine-scale diversity in environmental bacterial taxa.

**Jennifer BH Martiny** is a Professor of Ecology and Evolutionary Biology and Director of the Microbiome Initiative at the University of California, Irvine. She is a fellow of the Ecological Society of America and the American Academy of Microbiology. Her lab's research aims to uncover fundamental principles of the generation and maintenance of diversity in microbial communities.

**Corrigendum**
In *Microbiology Australia* (Volume 38, Issue 4, pp. 204–205), the organisation name 'World Federation of Culture Collections' should be 'World Federation for Culture Collections' throughout.

# Viruses in corals: hidden drivers of coral bleaching and disease?

*Patrick Buerger*

CSIRO Land and Water
Clunies Ross Street
Canberra, ACT 2601, Australia
School of BioSciences
University of Melbourne
Parkville, Vic. 3010, Australia
Email: patrick.buerger@csiro.au

*Madeleine JH van Oppen*

School of BioSciences
University of Melbourne
Parkville, Vic. 3010, Australia
Australian Institute of Marine Science, PMB #3
Townsville, Qld 4810, Australia
Email:
madeleine.van@unimelb.edu.au

**Marine viruses are the largest, but most poorly explored genetic reservoir on the planet. They occur ubiquitously in the ocean at an average density of 5–15 × $10^6$ viruses per mL of seawater, which represents abundances an order of magnitude higher than those of bacteria. While viruses are known agents of a number of diseases in the marine environment, little is known about their beneficial function to corals. Herein, we briefly introduce the topic of viruses as potential drivers of coral bleaching and disease.**

## Increasing prevalence of coral bleaching and disease

Corals form a symbiosis with microscopic algae (*Symbiodinium* spp.), which are the primary carbon source of their host through translocation of photosynthates. The loss of these intracellular symbionts is referred to as coral bleaching, causing the coral tissue to pale and resulting in a vulnerable state of the coral animal[1].

In recent years, coral bleaching and diseases have increasingly contributed to coral mortality for a number of reasons. First, warm seawater temperature anomalies that lead to mass bleaching events have increased in frequency and have left corals less time to recover[2]. Such temperature anomalies have been associated with higher disease incidence, possibly due to increased activity of pathogenic bacteria at elevated temperatures combined with reduced immunocompetence of stressed corals[3]. Second, the growing spatial scale of anthropogenic impacts on coral reefs such as reduced water quality[4] and tourism activities[5] have also been linked to higher disease prevalence. For example, up to 15-fold higher coral disease prevalence was reported on reefs in the Great Barrier Reef that had tourist platforms compared to those without[5]. Third, the frequency and severity of cyclones and crown-of-thorns starfish predation have increased; these disturbances cause breakages and injuries to corals and provide entry points for pathogenic microorganisms[6,7]. Despite the increase of coral disease occurrence, the tools required for rapid diagnostics are still lacking and management strategies to prevent and mitigate coral disease outbreaks are largely inadequate[8]. Of prime concern is that causative agents have not been identified for the majority of the described coral diseases. While a few known scleractinian coral pathogens are bacteria[9], the role of viruses in coral health and disease has barely been examined.

## Virus diversity in corals

Coral-associated virus communities are highly diverse and comprise bacteriophages, archaeal and eukaryotic viruses[10–12]. Despite this diversity, only a smaller subset of taxonomic groups are commonly found in corals, including bacteriophages belonging to the order of the *Caudovirales*, and eukaryotic nucleocytoplasmic large DNA viruses (NCLDVs) belonging to the families *Phycodnaviridae*, *Mimiviridae*, *Poxviridae* and *Iridoviridae*, as well as *Polydnavridae* and *Retroviridae*[10–12]. The coral-associated viral diversity shows that viruses could infect all cellular members of the coral holobiont, i.e. the coral animal, algal symbionts and all of its other microscopic and macroscopic symbionts.

## Eukaryotic viruses in coral disease and bleaching

Although over 20 coral diseases have been described, none of them are unequivocally shown to be caused by a eukaryotic virus that directly infects the coral animal or symbiotic algae (Figure 1A). For example, yellow band/blotch disease (YBD) causes degradation

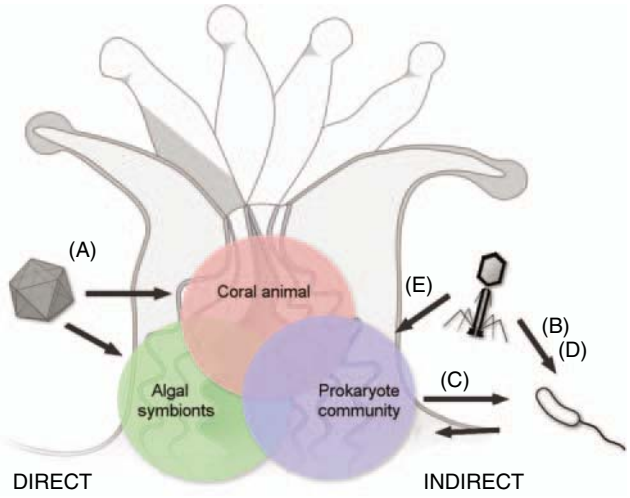


Figure 1. Viruses in coral health and disease. Viruses could contribute to or interfere with disease pathogenesis, for example, through direct (labelled A) and indirect (labelled B–D) processes. (A) Direct processes include eukaryotic viruses that target either the coral animal or *Symbiodinium*, e.g. as suggested in the case of virus-induced coral bleaching and yellow blotch disease, the virus on its own would cause the disease, therefore a direct interaction. (B–D) Indirect processes include bacteriophages that interact with the prokaryote community, which then have a secondary influence on the coral animal or algal endosymbionts (*Symbiodinium* spp.). (B) A bacteriophage might increase the virulence of an infected bacterium though horizontal gene transfer of virulence genes, which then causes a disease in the coral. In addition, bacteriophages may infect and lyse pathogenic bacteria, reducing the impact of a disease (C) as part of the coral microbiome, or (D) external from the coral holobiont, e.g. applied manually in phage therapy. (E) A bacteriophage may also interact with the coral prokaryote community and lyse a probiotic bacterium, which could open up a niche for a coral pathogen. Individual images publicly available for reuse with modification.

of *Symbiodinium* cells and has tentatively been linked to the abundance of virus-like particles (VLPs)[13]. Similarly, corals affected with white plague disease in the Caribbean have shown increased numbers of single-strand DNA viruses[14]. For both diseases, associated viruses still need to be isolated to investigate their causality using methods such as Koch's postulates.

Viral lysis (disintegration of infected cells) of *Symbiodinium* may be responsible for some instances of coral bleaching (Figure 2). A distant cousin of the dinoflagellate *Heterocapsa circularisquama* RNA virus, first detected with metatranscriptomics[16], has recently gained attention for its potential role in coral bleaching (reviewed in Thurber *et al.*[17] and Sweet and Bythell[18]). Transcripts of the ssRNA virus were shown to be present at high abundance in a heat-sensitive *Symbiodinium* culture, while they were barely detectable in a conspecific heat-tolerant culture, suggesting *Symbiodinium* and perhaps coral thermal tolerance is linked to the presence of this virus[19]. In order to progress the research in the field, PCR primers have been designed to assess presence and diversity of the ssRNA virus; these primers can potentially be modified for virus quantification during *in situ* coral bleaching events[20].

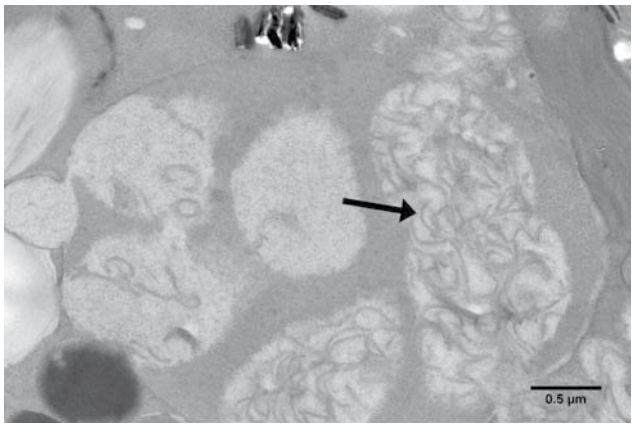


Figure 2. Transmission electron microscopy image of a cultured *Symbiodinium* cell, thin sectioned. The chromosomes of an untreated control strain of *Symbiodinium* C1 were degrading while showing the presence of unknown filamentous virus-like particles. Chromosomes in the image are light grey circular shaped. Filamentous virus-like particles are indicated by the arrow. *Symbiodinium* strain was cultured at the Australian Institute of Marine Science. Image courtesy: Karen Weynberg[15].

## The potential roles of bacteriophages in coral disease

The mechanisms by which lysogenic and lytic bacteriophages interfere with or contribute to coral disease pathogenesis are primarily indirect, i.e. bacteriophages on their own do not influence the coral animal or *Symbiodinium*, but infect bacteria, which then secondarily influence coral health.

After infection of a target bacterium, the lysogenic stage refers to the integration of the bacteriophage genome into the bacterial host genome as a prophage. Bacteriophages may increase the virulence of a bacterial pathogen after establishing lysogeny and transferring new genetic material into the host bacterium. For example, the pathogenicity of the bacterium *Vibrio cholerae* primarily depends on infection by a lysogenic bacteriophage (CTXphi). The bacteriophage transfers genes that encode for one of the primary virulence factors, in this case the cholera toxin (CT), and converts *V. cholerae* from a non-pathogenic to a pathogenic strain[21]. Lysogenic conversion has been suggested to also increase the virulence of *Vibrio coralliilyticus* (Figure 1*B*), because parts of the bacterium's virulence factors that are linked to the coral disease white syndrome and coral bleaching are arranged similarly to the pathogenicity islands of the *V. cholerae* prophage[22].

Other lysogenic bacteriophages persist over extended periods of time until a trigger induces a lytic cycle, e.g., an increase in temperature or UV radiation. The lytic stage is characterised by the replication of bacteriophages within the bacterial host, which results in lysis of the host cell and release of newly produced bacteriophages[23]. For instance, traces of bacteriophages were detected in the CRISPR arrays within the genomes of cyanobacteria,

*Roseofilum reptotaenium* and *Geitlerinema* sp., two species dominating the black band disease mat in terms of biomass[24]. These findings suggest the cyanobacteria are regularly infected by bacteriophages and that phages may play a role in the disease development[24].

Viruses may also have positive effects on coral health, such as purely lytic bacteriophages[25]. Specific bacteriophages that target pathogenic bacteria may form part of the natural coral microbiome and confer some disease resistance by preventing bacteria from excessive proliferation (Figure 1*C*)[26]. Lytic bacteriophages have been applied successfully in lab-based phage therapies for the treatment of several bacterial coral diseases, e.g., white syndrome caused by *Vibrio coralliilyticus* strains[27,28]. The promising potential of phage therapy to treat a coral disease has been showcased, for instance, through the effective mitigation of white plague-like progression and transmission to other corals, during both a seven-week field experiment[29] and a 21-day laboratory experiment[27].

## Conclusion and progress

Although viruses might contribute key aspects to coral bleaching and diseases, our understanding of this field of research is still scant. Even less is known about the functional contribution of viruses to coral health[18]. The current scarcity of coral virus-related studies can be linked to scientific challenges associated with environmental virus research and the difficulty to distinguish between causality and correlation of viruses with a coral disease. In order to overcome these issues, future research should establish coral host-virus model systems and consider versatile research approaches. For instance, relevant hosts for establishing virus cultures are *Symbiodinium* to investigate coral bleaching, *R. reptotaenium* for black band disease virulence models, and *V. coralliilyticus* for white syndrome virulence models. Multifaceted research approaches should include (1) viral metagenomics to characterise and describe virus communities in field-collected corals[30], (2) flow cytometry for virus enumeration[31], (3) liquid and plaque assays to isolate bacteriophages[32], and (4) bioinformatic pipelines that are designed for virus sequence data[33]. Research over the next decade will likely solve some of these issues and shed more light on the ecological importance of viruses in coral holobiont functioning. This will hopefully provide new ways to manage coral diseases on the reef.

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## Biographies

**Dr Patrick Buerger** is a postdoctoral researcher with CSIRO's Synthetic Biology Future Science Platform and The University of Melbourne, Australia. He completed his PhD research on bacteriophages in the coral black band disease at the Australian Institute of Marine Science in Townsville, Australia.

**Professor Madeleine van Oppen** holds positions at The University of Melbourne and the Australian Institute of Marine Science (Townsville, Australia). Her research focuses on assisted evolution, coral reef restoration, and microbial symbionts of corals.

# Swimming in the sea: chemotaxis by marine bacteria

*Justin R Seymour*

Climate Change Cluster (C3)
University of Technology Sydney
NSW 2007, Australia
Email: Justin.Seymour@uts.edu.au

*Jean-Baptiste Raina*

Climate Change Cluster (C3)
University of Technology Sydney
NSW 2007, Australia

**Like many organisms, bacteria regularly inhabit environments characterised by spatiotemporal heterogeneity in the availability of resources required for growth and energy generation, meaning they must either tune their metabolism to prevailing conditions or have the capacity to migrate to favourable microenvironments[1]. To achieve the latter, bacteria measure their resource landscape and suitably direct their locomotion using a behaviour called *chemotaxis*, which is the ability to guide movement up or down chemical gradients. The capacity to perform chemotaxis is widespread across the bacterial domain, although most of our understanding of this phenotype is derived from enteric bacteria[2,3]. In the ocean, marine bacteria are often motile[4], and in fact capable of much higher swimming speeds[5] and chemotactic precision[6] than these enteric models for chemotaxis[2]. Here we discuss the underlying motives and purposes for bacterial chemotaxis in the ocean, by noting that marine bacteria experience a surprisingly heterogeneous chemical seascape[7,8], whereby chemotaxis can provide substantial fitness advantages and even influence large-scale processes including marine ecosystem productivity, biogeochemical cycling and disease.**

## Chemotaxis

Motile bacteria propel themselves by rotating helical flagella driven by molecular motors[2,3]. Chemotaxis by these motile cells is achieved through the constant measurement of local chemical concentrations using trans-membrane chemoreceptors, while a complex signal transduction network interprets this information, allowing cells to detect chemical gradients and regulate motility accordingly[1–3]. This chemotactic behaviour ultimately allows bacteria to swim toward favourable chemicals and away from noxious substances (Figure 1). Chemotaxis is one of the best-described sensory systems in biology, with a highly developed understanding of this behaviour acquired from well-defined model organisms, such as *Escherichia coli*[2]. The importance of chemotaxis is typically

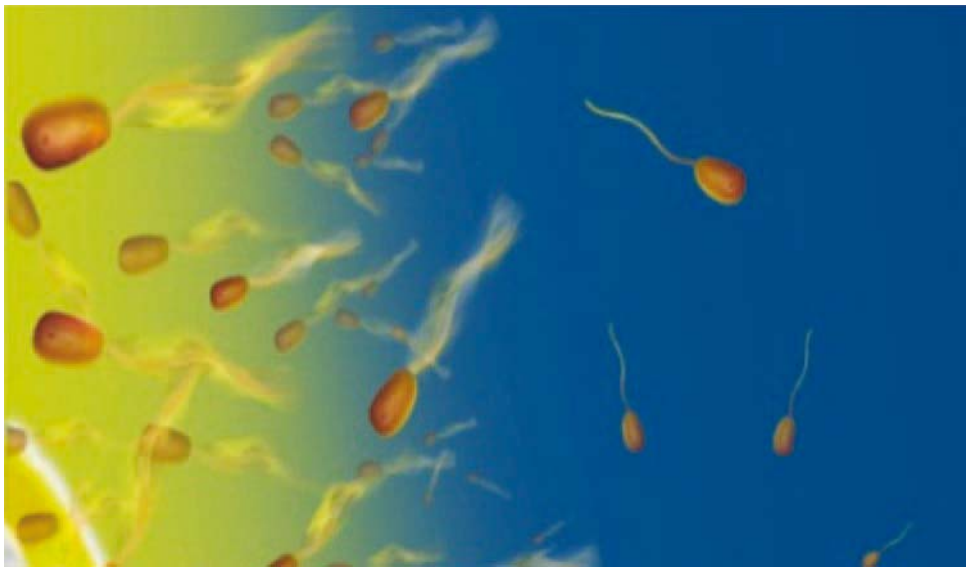

Figure 1. During chemotaxis, motile bacteria sense chemical concentrations using transmembrane chemoreceptors allowing them to direct their movement up or down chemical gradients, often resulting in accumulation of cells near to the source of a chemical attractant.

considered within the context of highly structured microenvironments, where chemical gradients are strong and stable, such as within biofilms or in association with the internal and external surfaces of plant and animal hosts[3]. However, growing evidence suggests chemotaxis is a ubiquitous phenotype across a wide range of environments, even within the ostensibly well-mixed and homogenous ocean, where it is employed by diverse groups of marine bacteria for a variety of functions including resource acquisition and host infection[9].

## Marine bacteria use chemotaxis to exploit a heterogeneous seascape

On average, every litre of seawater contains 1 billion bacteria and several thousand different bacterial 'species'[10]. These marine bacteria control most of the oceans' major chemical cycles (e.g. carbon, nitrogen, and sulphur)[7,11], support food-web productivity by acting as the principal conduit for the transfer of energy and matter from the oceans' large pools of dissolved organic matter to higher trophic levels[12], and can exhibit important symbiotic and pathogenic interactions with many species of marine animals and plants[13,14]. However, the role of chemotaxis among marine bacteria has been widely over-looked, largely because biological oceanographers have traditionally measured microbiological processes over large spatiotemporal scales, and incorrectly presumed that the microscale chemical seascape experienced by planktonic bacteria inhabiting the ocean's water column is homogenous.

Rather than comprising a dilute homogenous soup, from the perspective of a chemotactic bacterium seawater is awash with microscale chemical features that contribute to a complex microspatial architecture[7,15] that will often afford significant fitness advantages to chemotactic cells[16]. Large pools of suspended and sinking organic particles provide rich localised resource hotspots for bacteria that can colonise them[17], while the lysis, egestion, excretion, and exudation of other marine organisms results in a patchwork of microscale chemical gradients in the water column[8,15].

Importantly, the fluid dynamics operating at these very small scales are extremely different to those experienced at larger scales, meaning that, perhaps counter-intuitively, microscale chemical gradients are not significantly mixed or dispersed by ocean turbulence. Below a minimum length scale of generally a few millimetres, viscosity becomes dominant and turbulent energy is lost as heat. The only physical force acting on chemical gradients at this scale is molecular diffusion, which slowly dissipates gradients rather than mixing and erasing them[15]. It is also noteworthy that this lack of true mixing at microbial-scales means that no matter the bulk levels of ocean turbulence, the passive movement of a bacterium remains in synchrony with nearby features, so the cell will not be 'washed past' or 'washed away' from a gradient, particle or microbial associate that is suspended in the same water column[9]. Hence, the physical dynamics operating at the ocean's microscale accommodate the persistence of chemical gradients and the utility of chemotaxis in the water column.

Motile marine bacteria use chemotactic receptors to detect and target a wide range of microscale chemical features in the water column. Large pools of particulate organic carbon associated with suspended and sinking particles, ranging from micrometre-sized colloids to millimetre-sized marine snow aggregates are derived from a number of sources, including the flocculation of dead

phytoplankton biomass and zooplankton fecal pellets. These particles typically contain concentrations of organic molecules that exceed background concentrations by 2–4 orders of magnitude[18]. Chemotactic bacteria colonise these particles at substantially higher rates than non-motile bacteria[17].

The first evidence for the potential importance of chemotaxis among marine bacteria came from the observation that many are highly chemotactic towards the chemical products of phytoplankton[19]. This led to the hypothesis that marine bacteria will use chemotaxis to colonise the 'phycosphere' – the region immediately surrounding a phytoplankton cell that is enriched in exuded organic substrates[20] (Figure 2). The potential capacity of bacteria to use chemotaxis to take advantage of this microenvironment is notable given that marine heterotrophic bacteria obtain a large fraction of their carbon demand directly from phytoplankton, consuming up to 50% of phytoplankton-fixed carbon[21]. Recent studies using microfluidic experiments[22] and direct microscopic observations of chemotactic bacteria aggregating around phytoplankton cells[16] support predictions that bacterial chemotaxis will enhance bacterial uptake of phytoplankton derived carbon, as well as potentially underpinning the establishment and maintenance of important phytoplankton-bacteria relationships in the ocean[20].

In addition to colonising particles and phycospheres, there is also evidence that marine bacteria can use chemotaxis to take advantage of ephemeral microscale nutrient patches arising from the lysis and excretion of other microbes[8]. While these patches often only span a few tens to hundreds of micrometres and persist for less than 5–10 min, the ability of chemotactic marine bacteria to very rapidly home in on and subsequently exploit these abundant localised sources of organic substrates has been predicted to significantly enhance organic matter remineralisation rates in the pelagic ocean[1].

## Chemotaxis at the sediment-water interface

Compared to the complex, 3-dimensional and often short-lived chemical microenvironments occurring in the pelagic environment, the chemical landscape of the sediment-water interface at the bottom of the water column is often much simpler. Elevated concentrations of organic matter present on the seafloor promote high levels of microbial activity, which in turn generates steep vertical oxygen and nutrient gradients that are relatively stable through time. The surface sediments are often dominated by sulphur-oxidising bacteria, including *Thiovulum majus*, the fastest swimming bacteria recorded (swimming up to 600 $\mu m\ s^{-1}$)[23], which uses chemotaxis to form dense aggregations in the narrow region where optimal concentrations of oxygen and hydrogen sulphide co-exist.

## Host associations

The surfaces of benthic (e.g. corals, sponges, seaweeds) and pelagic macro-organisms (e.g. fish) are also characterised by strong chemical gradients, resulting from exudation of organic and inorganic compounds, which in some cases can attract specific

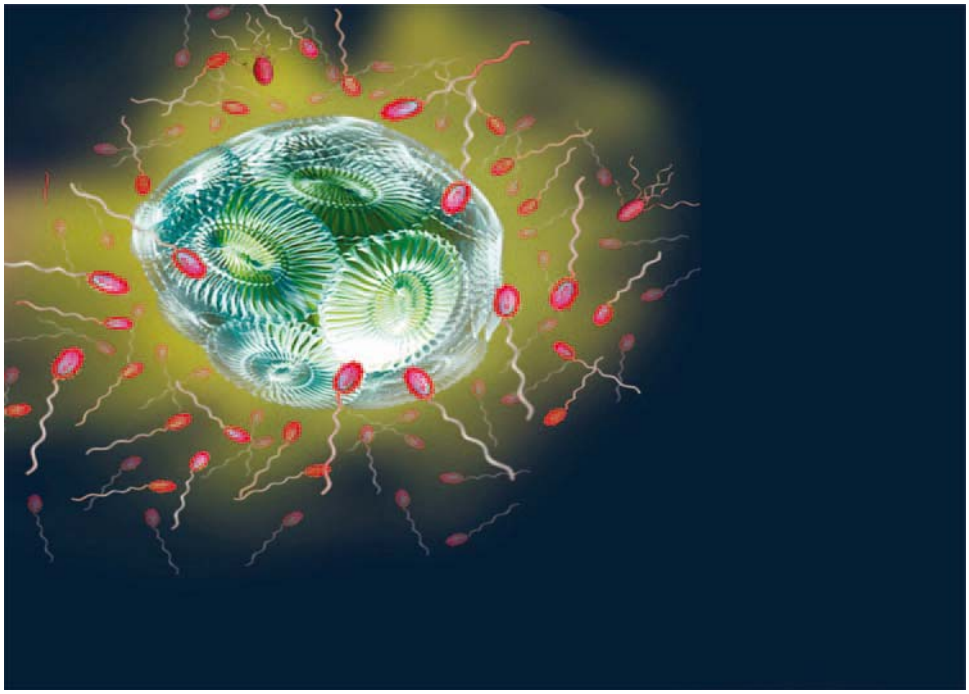

Figure 2. The phycosphere is the region surrounding an individual phytoplankton cell that is enriched in dissolved organic substrates exuded by the cell into the surrounding water. Chemical gradients within the phycosphere are utilised by chemotactic bacteria to colonise this microenvironment in order to gain increased access to phytoplankton-derived organic carbon and/or establish more specific metabolic interactions with the phytoplankton cell.

bacterial partners[24]. Indeed, chemotaxis is sometimes central to the establishment of host-microbe symbiotic interactions and infection by pathogens. One of the best-studied examples is the chemotaxis-mediated symbiosis between bobtail squid and the light-emitting bacterium *Vibrio fischeri*. Very low densities of *V. fischeri* are present in the water column, but they exhibit chemotactic migration towards chitin oligosaccharides, which are released from the squid's light-organs and act as a signal to trigger their colonisation[25]. Conversely, other vibrios are pathogens that use chemotaxis towards fish intestinal mucus (*V. anguillarum* and *V. furnissii*)[26], or coral mucus (*V. coralliilyticus* and *V. shiloi*)[27] to initiate the infection process.

## From the lab to the field

The capillary assay was the first method used to quantify chemotaxis and is still widely used today. It uses a glass capillary filled with a specific concentration of a chemoattractant, which is immersed in a homogenous bacterial suspension[28]. The compound subsequently diffuses out of the capillary and chemotactic cells respond by migrating into the capillary. A wide variety of chemotaxis assays have since been developed, but the most recent and sophisticated techniques employ microfluidic devices[22]. These platforms enable the generation of carefully controlled microgradients, which when coupled with high-speed video microscopy, permit tracking of the chemotactic swimming behaviour of individual cells. However, until recently, all available chemotaxis assays have shared a common limitation: their use is restricted to laboratory conditions and cultured microorganisms. We recently developed a new device that enables assessment of chemotaxis assays among natural microbial communities of bacteria within the environment[29]. Results derived from this new *in situ* platform have confirmed that natural assemblages of pelagic marine bacteria exhibit strong chemotaxis towards amino acids[29], that bacteria in the vicinity of reef-building corals are attracted by common coral exudates such as dimethylsulphoniopropionate[30], and that the acquisition of specific bacterial associates by marine sponges is not solely passive, but can also be mediated by chemotaxis[31].

## The importance of chemotaxis in the marine environment

Besides providing a competitive advantage to motile cells, chemotaxis among marine bacteria has the potential to impact a number of large-scale ecological and biogeochemical processes. The initiation of symbiotic interactions that underpin the ecological success of some of the most productive marine ecosystems (e.g. coral reefs, hydrothermal vents, seagrass meadows) often relies on chemotaxis[13,30]. This behaviour is also involved in pathogenicity and disease outbreaks among a wide range of marine species[32,33], both in natural habitats[27] and in aquaculture settings[34], sometimes with dire ecological and economic consequences. In the pelagic environment, chemotaxis plays an often pivotal role in all major biogeochemical cycles, by affecting the rates and directions of key chemical transformations[1,9,20]. For example, chemotaxis-mediated particle colonisation influences controls the amount of carbon that either sinks to the deep sea or is respired in the upper ocean, ultimately influencing global carbon budgets[35].

## Summary

Rather than the homogenous microscale seascape long assumed by oceanographers, marine bacteria inhabit a surprisingly heterogeneous environment, with a plethora of localised microniches likely to persist in the water column, across spatial scales commensurate with the movement of motile bacteria. The capacity to employ chemotaxis to navigate this environment and successfully exploit microscale resource gradients will therefore provide a significant fitness advantage to some marine bacteria within a number scenarios. Many examples of highly effective chemotactic capacity among both cultured marine bacteria and natural communities are consistent with this notion, with several lines of evidence suggesting that these microscale bacterial behaviour's will likely have a number of important ocean-scale implications.

## Acknowledgements

This work was supported by an Australian Research Council (ARC) Discovery Project DP140101045 and Gordon and Betty Moore Foundation Grant GBMF3801 to JRS. JRS is supported by an ARC Future Fellowship FT130100218 and J-BR is supported by ARC DECRA Fellowship DE160100636.

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## Biographies

**Justin Seymour** is an ARC Future Fellow and Associate Professor at the University of Technology Sydney, where he leads the Ocean Microbiology Group. His research is focused on the ecology of marine microorganisms and among his interests is how marine bacteria use behaviours typically considered most important in highly structured and confined environments – namely motility and chemotaxis – to exploit a patchy resource landscape within the ocean's water column.

**Jean-Baptiste Raina** is an ARC DECRA Fellow at the University of Technology of Sydney. His research focuses on marine microbial symbioses and in his current project he is developing new tools to visualise and quantify the interactions occurring between marine microbes at the micrometre-scale.

# Emerging microbiome technologies for sustainable increase in farm productivity and environmental security





*Brajesh K Singh*[A,B,E], *Pankaj Trivedi*[C], *Saurabh Singh*[D], *Catriona A Macdonald*[A] *and Jay Prakash Verma*[A,D]

[A]Hawkesbury Institute for the Environment, Western Sydney University, Penrith, NSW, Australia

[B]Global Centre for Land-Based Innovation, Western Sydney University, Penrith, NSW, Australia

[C]Bioagricultural Sciences and Pest Management, Colorado State University, Fort Collins, CO, USA

[D]Institute of Environment and Sustainable Development, Banaras Hindu University, Varanasi-221005, U.P. India

[E]Tel: +61 2 4570 1329, Email: b.singh@westernsydney.edu.au

**Farming systems are under pressure to sustainably increase productivity to meet demand for food and fibre for a growing global population under shrinking arable lands and changing climatic conditions. Furthermore, conventional farming has led to declines in soil fertility and, in some cases, inappropriate and excessive use of chemical fertilisers and pesticides has caused soil degradation, negatively impacting human and environmental health. The soil and plant microbiomes are significant determinants of plant fitness and productivity. Microbes are also the main drivers of global biogeochemical cycles and thus key to sustainable agriculture. There is increasing evidence that with development of appropriate technologies, the plant microbiome can be harnessed to potentially decrease the frequency of plant diseases, increase resource use efficiencies and ultimately enhance agricultural productivity, while simultaneously decreasing the input of chemical fertilisers and pesticides, resulting in reduced greenhouse gas emissions and promoting environmental sustainability. However, to successfully translate potential to practical outcomes, both fundamental and applied research are needed to overcome current constraints. Research efforts need to be embedded in industrial requirements and policy and social frameworks to expedite the process of innovation, commercialisation and adoption. We propose that learning from the advancement in the human microbiome can significantly expedite the discovery and innovation of effective microbial products for sustainable and productive farming. This article summarises the emergence of microbiome technologies for the agriculture industry and how to facilitate the development and adoption of environmentally friendly microbiome technologies for sustainable increase in farm productivity.**

The global population is expected to reach 9 billion by 2050 and an increase of 70–100% in farm productivity is needed to meet the demand for food and fibre. This increase in agriculture productivity needs to be met from a shrinking arable land area due to multiple

demands (e.g. food, fuel, fibre and climate mitigation) and land-degradation. Current farming practices that use chemical fertilisers and pesticides have contributed significantly to increase farm productivity but have also contributed, in some cases, to chemical contamination, soil degradation, loss of biodiversity and compromised soil and water quality, which together impact overall environmental sustainability and can impact human health. Agriculture productivity faces additional major challenges including structural decline in soil fertility (i.e. increase in inputs does not result in proportional yield gain) and negative impact of climate change including extreme weather events[1,2]. Emerging microbiome approaches have potential to address most of these challenges as a complimentary approach to conventional farming.

The plant microbiome, which consists of microbiota associated with all plant compartments (e.g. root, stem, leaves, flowers, seed), many of which have a wide and beneficial impact on plant fitness and productivity, if exploited appropriately, can boost agricultural productivity and environmental outcomes. The plant microbiome affects host physiology and productivity by improving resistance to biotic (e.g. disease and pest attack) and abiotic (e.g. nutrient and water limitation, heavy metal contamination) stresses[3]. The plant microbiome is immensely diverse and comprises mainly mutualistic partners where microbes receive carbon and habitats in return for supply nutrients and defense provision against plant pests and pathogens. Manipulating the plant microbiome has great potential to increase farm productivity by enhancing resource (e.g. water and nutrients) use efficiency and reducing the impact of disease and pest incidences. Because plant and microbial associations have evolved together for millions of years, they have well-developed mutual recognition, association and communication mechanisms[4]. Identifying drivers of microbial assembly and communication molecules can therefore significantly advance our ability to manipulate microbiomes for better outcomes.

## Concept of core and hub microbiome

There is growing evidence that different plant species harbor distinct microbiomes that are significant determinants of their survival and fitness[3]. However, not all plant microbiota are beneficial. In fact, a significant proportion are opportunistic microbes which are there only to exploit available nutrients and a small number are plant pathogens[3,5], which may dominate under certain environmental conditions and limit crop productivity. Therefore, identifying beneficial microbes is a critical first step to harness them to sustainably increase farm productivity. In this regard, applying the core microbiome (persistent members of microbiota that appear in all communities associated with a particular crop or plant species under different environments and management practices) approach is gaining scientific attention[6,7]. The core microbiome is considered a critical component for essential functions for holobionts (i.e. plant plus microbiota) as they are enriched, selected and inherited by evolutionary steps[6]. The core microbiome of a number of crops including maize, barley, rice, soybean, lettuce, and sugarcane have been reported[7–9] with some taxa present in most of the studied crop hosts. However, few biogeography studies have questioned the universal distribution of taxonomic core microbiome under various environmental conditions. It has been suggested that the microbiota recruited by a given plant genotype in different environments seems to share greater functional than taxonomic similarity[6]. According to this view, elucidating a functional core microbiome either by directly looking for functional attributes (using metagenomics such as shotgun sequencing) or indirectly through taxonomic information (using methods such as PICRUSt and Tax4Fun) will provide better understanding of the role of the microbiome in plant performance and health that can be harnessed for improving farm productivity for multiple crops.

The identification of a core microbiome of various crop species will help to identify plant-associated microbes that should be prioritised for further research, inclusion in culture collections, and manipulative experiments to improve crop productivity further. Fast moving omics technologies (genomics, metagenomics, metatranscriptomics and metaproteomics) can provide the information on key functional roles of uncultivable microbes within the plant microbiome[10] and identify those that are adaptive to environmental pressures. Comparison of the core microbiomes between plant species and genotypes within a species reveals host-driven differences in microbiome assembly. The mechanisms by which hosts assemble microbial community are not fully understood, although plant biochemical traits such as hormones, secondary metabolites, cuticle composition, root length and exudates, and plant defences (immunity) have been identified as important determinants. Because the crop microbiome, plant phenotype, and environment interact to affect yield, comparing the microbiomes of plants grown in contrasting environments can potentially provide key insights of the microbial role in plant fitness. Microbes that are especially common in challenging environments are more likely to protect yield under biotic and abiotic stresses. The core microbiome still contains hundreds of microbial 'species' and therefore, it is logistically difficult to manipulate the systems. To address this challenge, a 'hub microbiota approach' has been used[11]. This approach is based on the concept that microbiomes are a complex and inter-connected network where different populations have different roles and some 'keystone or hub species' are crucial for maintenance of the functioning network[7,11,12]. The finding that the

effect of host and abiotic factors can cascade through communities via 'hub' microbes is important to understand the fluctuations in community structure and functions that can be linked to plant performance. Theoretically, these hub species are highly interconnected and centre of the microbial network and therefore, changing any of the hub microbiome can have a significant impact on the core and overall microbiome of plant species. Thus hub microbiota are prime targets for *in situ* manipulation of the crop microbiome for better productivity and environmental outcomes.

## Current status and challenges

Use of microbes for agriculture has been practiced for several decades, mainly in the form of bio-fertilisers and bio-pesticides. These are mainly one-species products that either provide nutrients, particularly nitrogen (e.g. use of symbiotic rhizobia or free-living *Azotobacter*), mobilise phosphorus (e.g. *Penicillium* species) or protect against pests; insect (e.g. *Bacillus thurengenesis*) or fungus (e.g. *Trichoderma viride*). In recent years, a number of start-up companies (e.g. Indigo Ag, Chr-Hansen, NewLeaf Symbiotics, Growcentia) and large multi-national companies (e.g. Bayer Ltd, Nufarm, Monsanto BioAg) have commercialised microbial products for enhancing farm productivity. In fact, microbial products are one of the fastest growing start-ups and are expected to have global market of $6.4 billion by 2022. It is estimated by 2020, there will be more bio-pesticides in the European market than chemical pesticides and within the next few years, microbial products will have complementary markets of chemical pesticides ($55 billion[1]). This projection is based on the fact that current microbial products are based on a small proportion of cultivable species (~5% of the total microbiome) and biochemical characterisation of whole microbiomes for agriculture products is in its infancy. Further, the majority of cultivable microbes have yet to be explored for their plant beneficial activities. For example, more than 50% of human medicines come from natural resource[13] but only 11% of pesticides have biological origin, suggesting that most pesticidal properties from microbes are yet to be discovered. These possibilities have attracted significant investments from both government agencies and private companies. However, to realise the full potential, a number of technical, regulatory and social challenges need to be addressed.

The technical challenges are significant. First, we are unable to cultivate most environmental microbes (>95%) and that means most microbial metabolisms involved in plant health are not yet characterised. This heavily constrains our ability to harness them for agriculture productivity. Second, the performance of microbial products in field conditions has been mixed and in some cases effective performance in greenhouse studies was not replicated in field conditions. In several cases microbial products were not able to colonise plants or were outcompeted by indigenous microflora. Sustaining the efficiency of microbial products for the duration of the crop cycle is another major challenge. Activities of several microbes are influenced by abiotic (e.g. soil types, pH), climatic (e.g. drought) and biotic (e.g. competition with indigenous microflora, recognition of host-microbial interactions) conditions. Microbes not only need to survive but colonise crop plants and maintain activities for at least the duration of the crop cycle. In other words, microbial products, in several cases, perform short of the gold standard for industry, i.e. works in all environmental conditions and at all crop stages. These are serious challenges that need to be overcome if microbial products can be used alongside or as a substitute to agrochemicals. In addition to these technical challenges, different collection and analysis techniques, reagents, and parameters may introduce variations in microbiome results further compounding the biologically relevant role of the microbiome in practical settings[14]. Equally important, with the present 'microbiome potential', it should be emphasised that the structure and function of the microbiome are only one component in the multi-trophic cascades that determines host response. Thus, only an integrative multivariable approach, which integrates the physiology and genetics of both host and microbiome, as well as other environmental variables (including stress such as drought), may ensure that microbiome-based approaches are implemented to their fullest potential to influence plant production and health.

## Two key approaches for harnessing the plant microbiomes

A simplified approach for harnessing plant microbiomes (we used this term both for isolated consortium and *in situ* microbiome) is outlined in Figure 1. First, characterisation of plant beneficial microbes can be achieved by the isolation from the rhizosphere, phyllosphere or endosphere. Isolates can be screened for their plant growth-promoting properties, and interspecific interaction assessed. Through selection of those microbes that demonstrate synergistic interactions between each other and with plants (as opposed to those that are antagonistic), a core microbiome that leads to plant and environmental benefits can be identified and harnessed directly.

## Improvised traditional approaches

Traditional methods of microbial screening and isolation and their use in agriculture have provided some success both in nutrient supply and pest management. However, improvement in isolation

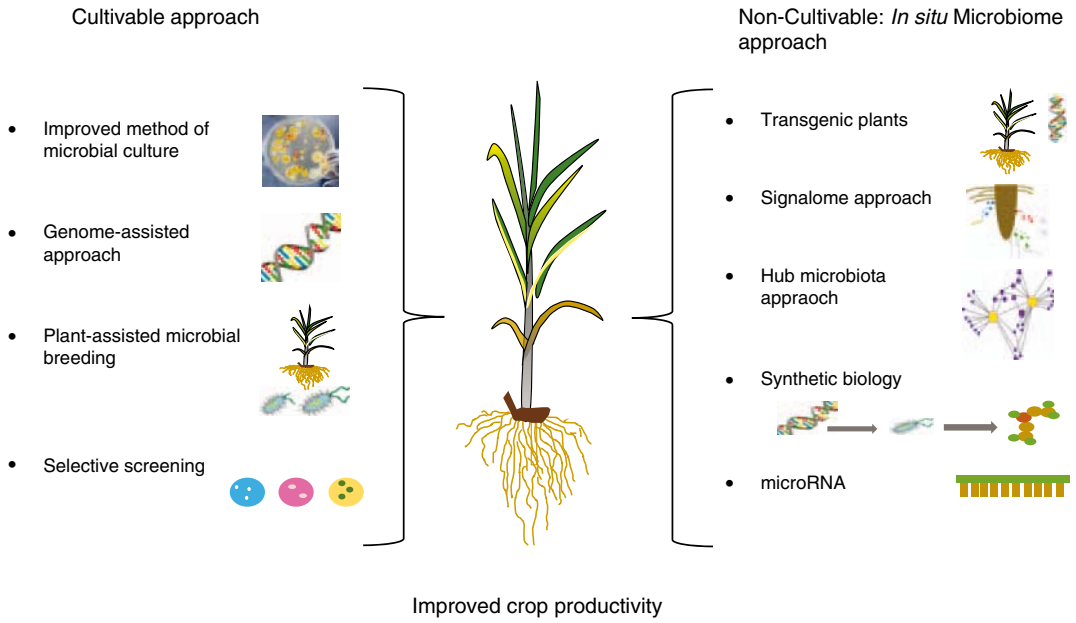


Figure 1. A schematic representation of current and potential cultivable and in situ microbiome approaches to increase crop productivity.

and screening methods has been frustratingly slow and we are still not able to culture the vast majority of microbes. Some progresses have been made, for example in formulating optimised media (e.g. shell vial procedure)[15], automated sorting and imaging techniques[16], and use of helper strains[17] to cultivate novel microbes from complex environmental settings. Emerging technologies such as genomics have allowed the cultivation of previously uncultivable microbes by identifying special nutritional or co-factor requirements[18]. Bai *et al*.[19] have shown that through selective screening protocols a culture collection of microbes can be generated that represents the majority of bacterial species that are reproducibly detectable by culture independent community sequencing. As the number of genomes obtained from binning of metagenomics sequence data is rapidly growing, this genome-assisted cultivation approach has potential to significantly improve microbial cultivation fields. Success of microbial products in field conditions can be enhanced either by the improvement of strains or using local microflora which are adapted to a particular region. Furthermore, plant-assisted microbial breeding can improve the mutual recognition of host and microbial products that can help the colonisation in field conditions.

Use of endophytes provides another avenue for better efficacy, particularly if the endophyte can colonise in the early stage of crop development. In such scenarios, the competition with indigenous microflora is minimised, which improves their ability to maintain activities. For example, Mitter *et al*.[20] discovered that the introduction of beneficial endophytes to the flower of parent plants can drive its inclusion in the progeny seed microbiome, thereby inducing vertical inheritance to the offspring generations. There is growing evidence that the use of consortia provides better performance than single species products and future products should target this approach where multiple species can be harnessed[1,21]. However, it is important to examine the synergy of survival, lifestyle and activities of individual members for successful outcomes under field conditions. The success of these and probiotic approaches (see below) depends on addressing key fundamental questions, i.e. identification of requirements of recognition, colonisation, persistence, and continuous activities of introduced microbiota. This is a critical knowledge gap that needs to be addressed in order to provide consistent efficacy of microbial products. Framing 'invasion ecological theory' in creating unique niches for the introduced microbes can be useful to address this challenge.

## Emerging approaches to manipulate plant microbiome *in situ*

A number of approaches are being currently developed and trialled to harness the whole plant microbiome without a need to culture, including use of transgenic or more conventional approaches. For example, using engineered plants with root traits that stimulate beneficial microbes such as mycorrhiza; nitrogen fixers, phosphorous, potassium and zinc solubilisers; siderophore and phytohormone producing microbes can have direct positive impacts on farm productivities[22]. However, given the public perception of transgenic plants, use of this approach for food crops remains limited. Other non-transgenic approaches which can manipulate the microbiome *in situ* are gaining more attention. These include:

(1) exploiting plant-microbial communication molecules: plants and microbes both produce a number of communication molecules to communicate their requirements to their partners. For example, when a plant is starved of phosphorus,

it produces signal molecules which rhizosphere microbes respond to by upregulating their phosphorus-mobilising genes[23]. Similar signaling mechanisms are also evident for attack of pathogens and pests[4]. Plants also communicate with each other through volatile organic compounds (VOCs) to induce responses that facilitate colonisation with beneficial microbes[24]. However, given the extremely low quantity of signal molecules produced, only a few such molecules have been characterised. If the detection and characterisation of signal molecules can be improved, it will provide an important tool to introduce a directional change in microbial activities which is beneficial for plant performance.

(2) use of microbial cocktails, which does not have direct beneficial impacts on plants, can increase the activity of indigenous plant-beneficial microflora. These cocktails mainly contain microbes with high amounts of signal molecules.

(3) identification of hub microbiota of crop species, and their role in microbiome assembly and activities, provides an important tool to manipulate the whole microbiome *in situ*.

(4) synthetic biology provides another important tool to engineer novel and predictable functions in crop probiotics, which upon release on a plant can enhance the activities of beneficial microbes in a predictable fashion.

(5) *in situ* genome engineering tools[25] can be used to directly engineer the genome of the *in situ* microbiome. Here mobile genetic materials (e.g. plasmid) can be delivered to indigenous microflora where they promote desired and directional functions.

(6) plants have micro-RNA (miRNA) which is responsible for regulating the structure and function of plant-microbe interactions and the rhizosphere microbiome. This has been utilised to restore healthy digestive systems[26] and could be a significant tool to target beneficiary microbes for enhanced crop production.

We envisage that the use of microfluidics-based technologies will be instrumental in providing unique insights into the microscale plant-microbiome interactions in complex root microenvironments by allowing dynamic imaging of these interactions. This technology is likely to enhance the current rate of discoveries in the field of microbiome research[27] with tremendous applications towards harnessing beneficial interactions in large field settings. All above emerging technologies in combination with ecological engineering (use of management tools such as crop rotation, non-tillage) and plant breeding (e.g. the integration of the plant breeding with a particular microbiome) has a significant potential to manipulate host microbiomes to enhance the efficiency of controlling plant diseases and increasing farm productivity[2]. However, these technologies are still in their infancy and need to be further tested for their efficacy under field conditions as well as for any non-intended impact, for example, negative impact on overall environmental outcomes.

## Learning from the human microbiome and future perspectives

The fundamental principle of microbial assembly in humans and plants is identical and is based on selection enrichment and evolutionary processes and there are important similarities between plant and human microbiomes in their functional roles. There is growing evidence that human microbiomes play an essential role in physiological, emotional and evolutionary aspects and therefore overall health and fitness of humans. Plant microbiomes have a similar role for overall plant fitness and health (Figure 2). Learning from the advancement in human microbiome research (which is at a significantly more advanced stage) can significantly expedite discovery and innovations in agricultural microbiology because there are striking similarities in the functional role between human and plant microbiomes (Figure 2). For example, there is increasing evidence that dysregulation of

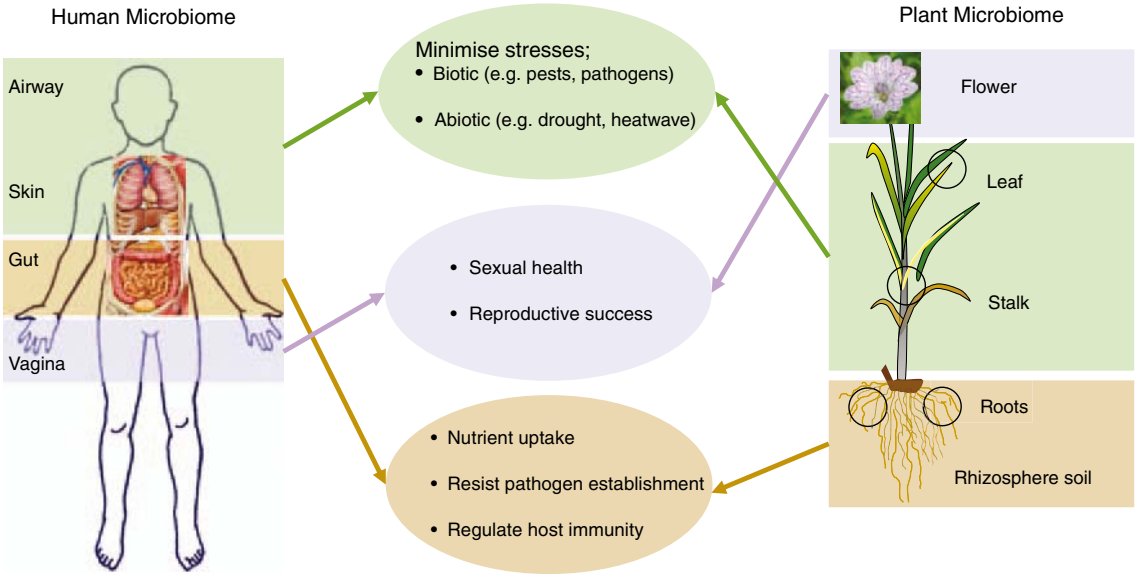


Figure 2. Functional similarity between human and plant microbiomes. All aspects of human and plant heath are influenced by resistant microbiomes. For example, the gut microbiome is known to play important role in nutrient uptake, resistance against pathogen invasion and regulation of immunity in humans. Rhizosphere microbiomes carry out almost identical functions for the plant. Similarly, there is increasing evidence that microbiomes of reproductive organs play a critical role in sexual health and reproductive success both in humans and plants.

human and microbiota is associated with multiple human diseases including diabetes, colorectal cancer, liver cirrhosis[28]. The essential role of the gut microbiome in effectiveness of cancer chemo- and immunotherapy has been found and recent studies suggest that humans can be grouped to responsive and non-responsive groups of therapy based on their intestinal microbiomes. More importantly, transfer of microbiomes from responsive to non-responsive groups can improve the efficacy of the cancer medicines[29], indicating the direct role of microbiota. A similar framework can be developed to identify the mechanisms of pesticide resistance in weeds, insects and pathogens and intervention can be developed (e.g. pesticide + responsive microbiota) for effective pest control with minimal use of chemical or biological pesticides. Similarly, the role of gut and genital microbiomes in enteric and HIV infections is well documented[30], and if the key (core and hub) microbiota of a crop species that protects or promotes immunity against pathogens can be identified, an appropriate intervention (e.g. microbial cocktails, probiotics, microbial transplant) can be developed to minimise the rate of infections and hence improve farm productivity. In human microbiome research, the next avenue is the utilisation of the microbiome information to assist personalised diagnostic assessment, risk stratification, disease prevention, and treatment-decision-making[14]. Once this concept is developed and successfully implemented, it can also be used for tailor-made microbiome interventions in context based situations for increased plant performance and health.

## Conclusion

The microbiome approaches provide significant opportunities to increase farm productivity in an environmentally sustainable way. However, plant microbiome research is still in its infancy and further research is needed to advance both theoretical and experimental frameworks in order to convert potential into reality. Systematic and concerted efforts are required to identify core and hub microbiota of important crop species and how they respond to biotic and abiotic stresses. Although use of microbial products has been growing rapidly, transformational changes in the industry will come from our ability to manipulate the whole microbiome *in situ*. There are a number of technologies being developed but a major challenge will be efficacy of these technologies in field conditions. Integrating effective microbiome approaches with emerging precision agriculture, synthetic biology, satellite, big data and genomic approaches can provide a strong framework to realise the true potential of plant microbiome technologies in agriculture and environmental sectors. With these challenges met, incorporating microbiome-related interventions for increasing plant productivity in an environmentally sustainable way, by promoting resilience/resistance to abiotic and biotic stresses may emerge as an integral part of modern agriculture.

## Acknowledgements

BKS acknowledge funding from Australian Research Council (DP170104634). JPV is grateful to DBT for providing the fellowship of Indo-Australia Carrier Boosting Gold Fellowship for working on microbiome research at Hawkesbury Institute for the Environment, Western, Sydney University, Penrith, NSW, Australia. PT acknowledges funding from Cutrale Citrus Industry and National Institute for Food and Agriculture, USA.

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## Biographies

**Professor Brajesh Singh's** research interests encompass soil biology and ecosystem ecology. His research identifies the quantitative relationships between biodiversity and ecosystem functions and how natural/anthropogenic pressures such as land-use and climate change affect these. His applied research harnesses the knowledge gained in fundamental research to achieve sustainable development, environmental protection and food security.

**Dr Pankaj Trivedi** is an Assistant Professor in the Bioagricultural Sciences and Pest Management at Colorado State University, USA. He is a microbial ecologist and his research program addresses the assembly, fitness and roles of plant, insect, and soil-associated microbiomes; how these are influenced by various biotic and abiotic factors; and what are their consequences on plant productivity, agro-ecosystems sustainability, and local and global level ecological processes.

**Dr Catriona Macdonald** is a research lecturer at the Hawkesbury Institute for the Environment at the University of Western Sydney. She has >10 years of experience in the field of soil microbiology and nutrient cycling. Her research interests are focussed on understanding microbial-mediated function under environmental change and interaction with plant communities, and how we can utilise the microbial community to overcome associated constraints on productivity and ecosystem function.

**Mr Saurabh Singh** is a PhD student at Banaras Hindu University India and his research interests include environmental microbiology, and plant-microbe interaction, phosphate solubilisation.

**Dr Jay Prakash** is an Assistant Professor at Banaras Hindu University India, and his research interests include use of microbes for climate resilient sustainable agriculture, biofertiliser, soil fertility management, remediation of agrochemical, and cellulose degradation for bioethanol production.

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# Manipulating the soil microbiome for improved nitrogen management

*Hang-Wei Hu*

School of Agriculture and Food
Faculty of Veterinary and Agricultural Sciences
The University of Melbourne
Vic. 3010, Australia
Tel: +61 3 9035 7639
Email: hang-wei.hu@unimelb.edu.au

*Ji-Zheng He*

School of Agriculture and Food
Faculty of Veterinary and Agricultural Sciences
The University of Melbourne
Vic. 3010, Australia
Tel: +61 3 9035 8890
Email: jizheng.he@unimelb.edu.au

**The soil microbiome, including bacteria, archaea, fungi, viruses, and other microbial eukaryotes, has crucial roles in the biogeochemical cycling of nitrogen (N), the maintenance of soil fertility, and the plant N use efficiency (NUE) in agro-ecosystems[1]. Recent advances in omics-based technologies (e.g. metagenomics, metatranscriptomics, and metaproteomics) have expanded our understanding of the soil microbiome and their controls on specific N-cycling processes[1–3]. Given the growing N-based fertiliser consumption and continuous land degradation, innovative technologies are needed to manipulate the soil microbiome to improve crop NUE, reduce N losses and increase N reservation in soil. This article discusses the research directions to facilitate the development of microbiome-manipulating technologies for sustainable management of N transformation processes**.

The crop nitrogen use efficiency (NUE) in modern agro-ecosystems is notoriously low, as more than 50% of N fertiliser applied is lost to the environment through ammonia volatilisation, nitrate leaching, and emissions of nitrous oxide ($N_2O$), the third most important greenhouse gas[4,5]. These losses are mostly driven by a myriad of N-cycling processes (in particular, nitrification and denitrification) that can be modulated by a broad range of soil microorganisms (Figure 1)[6,7]. Conventional agricultural practices mainly rely on agronomic measures and chemical inputs to improve NUE, which could lead to soil degradation and loss of biodiversity, with detrimental consequences for soil health and ecosystem functioning[8]. For example, long-term use of synthetic fertilisers, herbicides, and pesticides can negatively influence bacteria and fungi that create organic matter essential to plants. To meet the increasing food demand of a global population of more than 11 billion by 2100, there is an urgent need to discover new intervention points to manage N-cycling microorganisms for improved NUE and sustainable agricultural production.

Propelled by the evidence in manipulating gut microbiomes for improved human health, there are growing interests focused towards the manipulation of the soil microbiome to reduce soil erosion, to enhance plant growth and disease resistance in agro-ecosystems, and to promote the remediation of heavy metal-contaminated soils[1,3]. In this article, we discuss the currently-used technologies and emerging microbial biotechnologies that can manipulate the soil microbiome *in situ* to mitigate the processes of agricultural N loss and improve crop NUE, leading to both enhanced crop yield and positive environmental and social outcomes.

## Physicochemical approaches to manipulate the soil microbiome

Physicochemical approaches have been put forward to reduce agricultural N losses through manipulating the abundance, structure and activities of soil N-cycling microorganisms or controlling the amount of N resources available to microorganisms (Figure 2). Some practical tools utilised in agro-ecosystems to improve NUE include: (1) use of synthetic nitrification inhibitors (e.g. DMPP and DCD) to inhibit the activity of ammonia oxidisers and reduce the N loss through $N_2O$ emission and nitrate leaching[9]; (2) use of urease inhibitors (e.g. N-(n-butyl) thiophosphoric triamide (NBPT)) to inhibit the expression of genes encoding ureases that catalyse urea hydrolysis[10]; (3) manipulation of soil properties (e.g. soil pH, C:N ratio, and moisture) by agrochemical amendments and agronomic

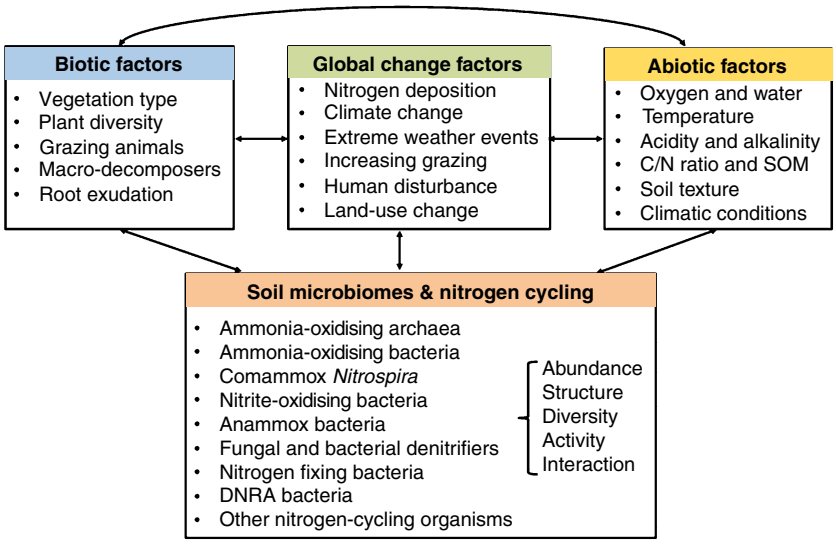


Figure 1. The soil microbiome components involved in nitrogen transformation processes are influenced by a wide range of abiotic, biotic, and emerging global change factors as well as their interactive effects.

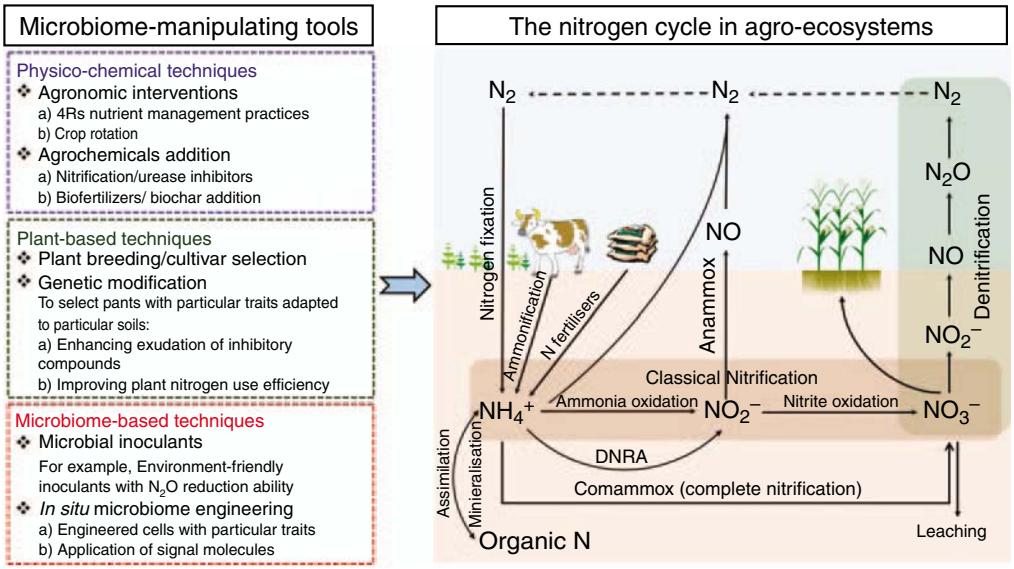


Figure 2. Schematic overview of the microbiome-manipulating tools that can be used for managing the nitrogen cycling processes in agro-ecosystems. DNRA, dissimilatory nitrate reduction to ammonium; Anammox, anaerobic ammonia oxidation.

practices to indirectly reshape the abundance, diversity and structure of soil microbiomes; (4) incorporation of plant residues to enhance microbial N immobilisation and reduce the amount of inorganic N available to soil microbes[11]; and (5) use of precise nutrient management practices and high-efficiency fertilisers to better synchronise N supply and crop N demand and reduce N available to soil microorganisms. Tools (1) and (2) are direct practices that impact soil microorganisms while the other three tools are indirect practices.

The outcomes of these physicochemical technologies are variable across soils, primarily owing to their largely unknown impacts on soil microorganisms. For example, the nitrification inhibitor DMPP could effectively inhibit nitrification and $N_2O$ emissions in alkaline soils through influencing the abundance and metabolic activity of ammonia-oxidising bacteria, but had no significant effects in many acidic soils probably due to the fast degradation of DMPP[9,10]. Other drawbacks of synthetic inhibitors include difficulties in application, rapid degradation, increased ammonia volatilisation, and migration into the food system[5,12]. In addition, long-term use of chemicals has detrimental environmental impacts, resulting in the accumulation of residues in fields, loss of beneficial microorganisms, and disruption of the plant and soil microbiota association[13]. An improved understanding of the key functional genes, enzymes and regulatory mechanisms of N-cycling processes, and their responses to interactions between different climatic, soil and biotic properties (Figure 1), should be essential to improvement of physicochemical strategies.

## Plant-based approaches to manipulate the soil microbiome

Plant physiological traits can be selected by plant breeding (cultivar selection) or genetic modification techniques to secrete specific compounds or signalling molecules for the direct manipulation of the soil microbiome *in situ*[8,11]. Plants have developed intimate relationships with their interacting soil microbiomes and the environment (termed as the 'phytobiome')[14]. Some plant and crop roots (e.g. *Fallopia* spp. and *Brachiaria humidicola*) can exudate organic compounds to inhibit the ammonia monooxygenase (enzyme capable of oxidising $NH_3$ to $NH_2OH$) and hydroxylamine oxidoreductase (enzyme capable of oxidising $NH_2OH$ to $NO_2^-$) of ammonia oxidisers[15], or to inhibit the metabolic activity of denitrifiers[16]. Screening agricultural crops with similar traits may greatly enhance our ability to improve crop NUE by using them directly for *in situ* microbiome engineering. A conventional plant breeding programme, however, rarely takes into account the interactions within phytobiome[14], which may result in loss of beneficial microbiota, disruption of symbiosis associations, and unknown consequences for other ecosystem processes[13]. Future plant-based strategies should integrate the knowledge of the phytobiome into the programme, by which specific N-cycling microorganisms are manipulated *in situ* without compromising beneficial microbiota and other ecosystem functions[3].

## Emerging microbial biotechnology approaches to manipulate the soil microbiome

Microbial biotechnologies have shown enormous potential in reducing N losses via $N_2O$ emissions in soybean root systems where denitrifiers harbouring $N_2O$ reductase, enzyme capable of reducing $N_2O$ to $N_2$, were amended[17]. These is evidence that the application of organic fertilisers inoculated with $N_2O$-reducing denitrifiers decreased $N_2O$ emissions in agricultural soils at field scales[18]. However, the persistence and functionality of these inoculated microbiota are uncertain, as most of them are unlikely to persist in soil due to the strong competition from indigenous microbiota. When using specific bacterial or mycorrhizal inocula as a strategy to manipulate the soil microbiome, there is an urgent need to modify the mode of delivery to increase their colonisation potential. Some approaches[8] include: (1) use of consortia of multiple compatible microbes, rather than single-strain formulations, to better compete with indigenous microbiota; (2) use of synbiotics to provide support for colonisation of the inoculated strains; (3) use of slow release systems for inocula to provide continual inoculation under field conditions; and (4) using chemical pesticides or predators for the indigenous microbiota to create new niches for the introduced microbiota. The combination of these approaches might help to achieve maximum benefits and improved crop NUE.

Emerging microbial biotechnology tools are proposed to precisely manipulate the soil microbiome *in situ*, by adding or withdrawing chemicals[19], to regulate N transformation processes under various conditions. Multidisciplinary approaches, especially genome engineering and synthetic biology, by fully taking advantages of microbiome knowledge, are needed for maximising the contribution of microbiome-based biotechnologies to sustainable management of the N cycle. Here, we highlight the key opportunities and research priorities to harness the soil microbiome to manage N transformation processes:

(1) Exploration of the core soil microbiome components involved in N cycling processes and their signalling compounds (or their inhibitors) for chemical conversations, and how they are impacted by plants, climate, soil properties, and agronomic practices (Figure 1). These efforts will lead to the identification of a set of functional taxa that should be prioritised for further research, and provide new ways through direct manipulation of the microbiome activities or via genetically engineering the native microbiomes *in situ*. Microbiome-based approaches targeting at reducing rates of nitrification and denitrification (pathways leading to N losses) and increasing rates of dissimilatory nitrate reduction to ammonium (DNRA, the pathway capable of reserving N in soil), would have multiple benefits such as reduced $N_2O$ emissions, increased farm productivity, reduced water contamination, and higher farm profitability through reduced use of fertilisers.

(2) Technological improvements are needed to decipher the 'dark matter' of microbial chemistries, as current metabolomics studies can only match a small fraction of data to known chemical compounds and biochemical pathways[20]. Quorum sensing signals have been found to regulate the communication between ammonia oxidisers and nitrite oxidisers, and to regulate the production and consumption of N oxide gases in a model nitrite oxidiser[21]. We are just beginning to recognise the diversity and specificity of signalling molecules, with the advancement of integrated metabolome and proteome technologies[14], and thus becoming more reliable to develop microbiome-engineering strategies that could utilise the natural signalling channels of the N-cycling microorganisms.

(3) Harnessing the emerging synthetic biology and genome editing tools to directly engineer the genomes and metabolic pathways of indigenous soil microbiome mediating N-cycling processes *in situ* with high specificity and efficacy[19]. We need a comprehensive knowledge of the gene regulation frameworks and modelling tools (through integrating various components of microbiome datasets, soil parameters, weather data and new computational methods) to predict the effects of microbiome manipulations *in situ* and reliably monitor the engineering outcomes. Precision tools such as sequence-specific gene editing using CRISPR/Cas9 delivered by phage or conjugative elements[22], and synthetic microbial consortia engineered to disrupt or replace existing communities, are needed for modifying microbiota and their genes *in situ*.

(4) The emerging *in situ* microbiome-manipulation tools (in particular, use of genetically modified organisms) in the natural environment are subject to regulatory requirements and societal concerns[13]. Coordinated efforts and multidisciplinary networks of policy makers, industry stakeholders, engineers, public and private partners, and agricultural communities will

consolidate and translate new microbiome-related innovations into practical solutions for farmers and ensure that risks associated with microbiome research are properly addressed. In addition to traditional agency-specific requests for proposals, strategic funding investments by national-level interdisciplinary initiatives (e.g. USA National Microbiome Initiative) could ensure availability of sufficient resources for developing broadly applicable microbiome-based tools[2,19,23].

## Concluding remarks and future perspectives

Although there are a range of ways in which crop NUE and agricultural productivity could be improved by the management of the soil microbiome, this is an area of great challenge which requires advances in multi-omics technologies, systems biology, synthetic biology, data analytics, standardised protocols, and modelling, as well as new collaborative efforts among scientists, engineers, agribusiness professionals and agricultural communities. Therefore, utilisation of existing physicochemical technologies will be the major approaches to manipulate the soil microbiome in short or medium terms. Over a longer term, we envision the innovation in *in situ* genome engineering technology will offer precise microbiome management approaches to sustainably increase agriculture productivity. These technologies will show enormous potential in managing N transformation processes and can be integrated into next-generation precision agriculture for site-specific management. Under a context of global change and a growing human population, harnessing the capabilities of Earth's microbiomes will potentially lead to reduced chemical inputs, improved soil and water health, and increased productivity and sustainability of global agro-ecosystems.

## Acknowledgements

This work was financially supported by Australian Research Council (DE150100870, DP160101028 and LP160101134).

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## Biographies

**Dr Hangwei Hu** is an ARC DECRA Fellow in the School of Agriculture and Food, Faculty of Veterinary and Agricultural Sciences at The University of Melbourne. His research is focused on soil nitrogen cycling processes, environmental microbiomes, and soil antibiotic resistance genes.

**Dr Jizheng He** is Professor of Soil Ecology at the University of Melbourne. His research interests focus on the soil microbial biogeography, microbial processes of carbon and nitrogen cycles, soil fertility and nutrients management in agricultural ecosystems.

# Life without water: how do bacteria generate biomass in desert ecosystems?



*Sean Bay*[A], *Belinda Ferrari*[B] *and Chris Greening*[C]

[A]School of Biological Sciences, Centre for Geometric Biology, Monash University, 25 Rainforest Walk, Clayton, Vic. 3800, Australia.
Tel: +61 4 1355 5721, Email: sean.bay@monash.edu

[B]School of Biotechnology and Biomolecular Sciences, UNSW Sydney, Biosciences South E26, Kensington, NSW 2052, Australia.
Tel: +61 2 9385 2032, Email: b.ferrari@unsw.edu.au

[C]School of Biological Sciences, Centre for Geometric Biology, Monash University, 25 Rainforest Walk, Clayton, Vic. 3800, Australia.
Tel: +61 3 9905 1692, Email: chris.greening@monash.edu

**Many of the world's most arid deserts harbour surprisingly diverse communities of heterotrophic bacteria. These organisms persist in surface soils under extreme climatic conditions, despite lacking obvious energy inputs from phototrophic primary producers. A longstanding conundrum has been how these communities sustain enough energy to maintain their diversity and biomass. We recently helped to resolve this conundrum by demonstrating that some desert communities are structured by a minimalistic mode of chemosynthetic primary production, where atmospheric trace gases, not sunlight, serve as the main energy sources. These findings are supported by pure culture studies that suggest atmospheric trace gases are dependable energy sources for the long-term survival of dormant soil bacteria. We predict that atmospheric trace gases may be a major energy source for desert ecosystems worldwide.**

Deserts are one of the largest biomes. They cover one-fifth of the planet's terrestrial land surface ($33.7 \times 10^6$ km$^2$) and occupy latitudinal ranges along the tropics, Arctic, and Antarctic. Deserts are defined as having a precipitation to evapotranspiration ratio (P/ET) of less than 1 and can be classified as sub-humid (0.5–0.65), semi-arid (0.2–0.5), arid (0.05–0.2) and hyper-arid (<0.05)[1]. With exception of hyper-arid deserts, these regions are collectively recognised as drylands and are critical for human development. However, the productivity and biodiversity of these regions is being increasingly threatened by anthropogenic land degradation and climate change.

## Microbial community structure in desert soils

Organisms inhabiting arid and hyper-arid desert ecosystems face multiple physicochemical pressures, including water and organic carbon deficit, UV radiation damage, and often extreme temperature variations. Despite these stressors, these ecosystems host a surprising abundance and diversity of microorganisms[2–4]. Culture-independent surveys show microbial communities inhabiting both hot and cold deserts are similar on a phylum level to those inhabiting mesic soils, but are highly specialised at the species level and strongly shaped by physicochemical factors[3,5,6]. Aerobic heterotrophs from the Terrabacteria superphylum (including Actinobacteria and Chloroflexi) are particularly dominant in desert soils, with Proteobacteria, Acidobacteria, and Bacteroidetes phylotypes also common (Figure 1)[7–13]. It is thought that these communities are integral for supporting ecosystem services in desert regions, including nutrient turnover and fixation of carbon and nitrogen[14].

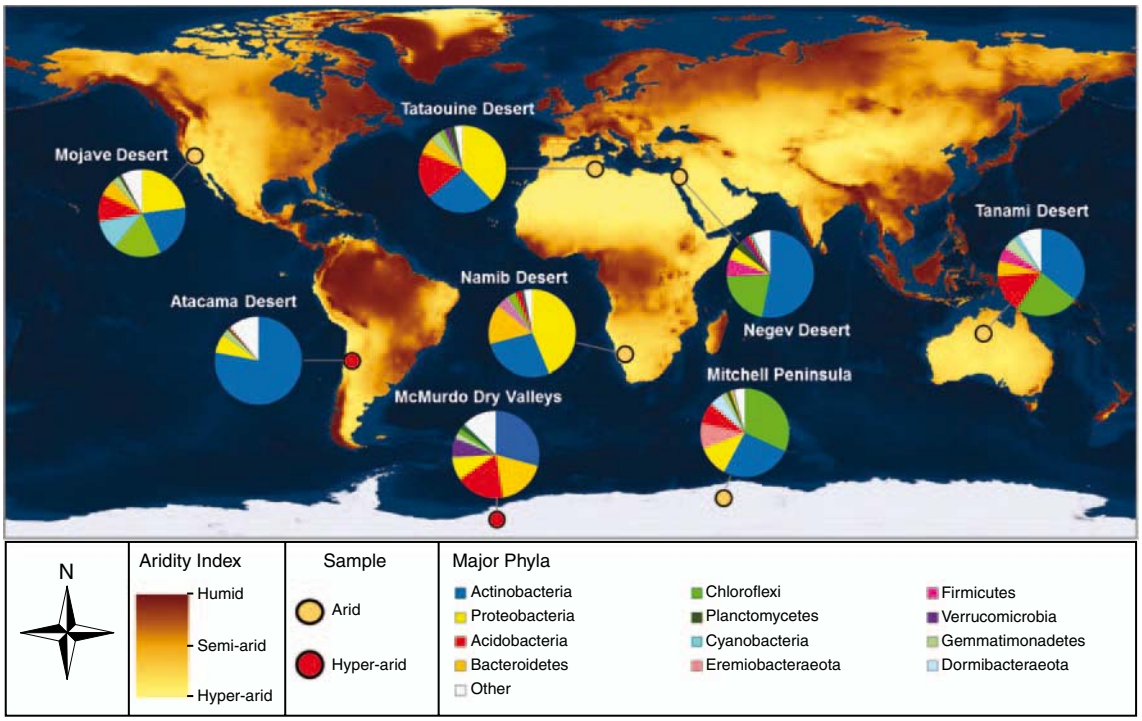


Figure 1. Basemap showing microbial community structure of desert ecosystems in different continents. Pie charts represent the relative abundance of major bacterial phyla of three cold[7–9] and five hot deserts[10–13], as determined by 16S rRNA gene amplicon sequencing. The Negev Desert chart shows unpublished data collected by Sean Bay. The map is shaded by Global Aridity Index (AI), the ratio of precipitation availability over atmospheric water demand[1]. Calculations are based on mean annual precipitation (MAP) and mean annual potential evapotranspiration (MAE) data from 1950–2000 and are displayed as a grid layer at a spatial resolution of 30 arc-second (~1 km at the tropics).

The relative abundance and diversity of microbial taxa in desert ecosystems shows considerable variation across multiple spatial scales. This reflects both the influence of climatic factors and the inherent heterogeneity of surface soils in terms of physical structure, chemical composition, and nutrient bioavailability[3,14]. In desert communities, soil moisture and organic carbon content are thought to be particularly important factors driving niche processes. This reflects that organic carbon derived from photosynthetic primary production is a major energy source for the heterotrophic microorganisms that generally dominate these communities[2–4]. However, the combined effects of water deficit and damaging UV radiation inhibit photosynthetic processes and in turn limit primary production in arid and hyper-arid desert ecosystems. On a global scale, plant biomass per unit area is two to threefold less in drylands (6 kg km$^{-2}$) compared to temperate ecosystems (10–18 kg km$^{-2}$)[15].

To withstand the physiochemical pressures of desert ecosystems, some photosynthetic bacteria (e.g. Cyanobacteria) and algae (e.g. Chlorophyta) have evolved cellular mechanisms to withstand the physicochemical pressures of desert ecosystems. Notably, many phototrophs can efficiently colonise cracks and fissures of translucent rocks and biological soil crusts[2,16,17]. These environmental refugia provide desiccation buffers and protection from UV radiation, allowing these specialised producers to fix carbon and nitrogen at sufficient rates to support associated heterotrophic communities. As a result, phototrophs are dominant primary producers in dryland ecosystems worldwide[3,16]. However, culture-independent studies indicate both arid and hyper-arid deserts, such as those in Atacama, Negev, and Antarctica, often harbour diverse communities of putative aerobic heterotrophic bacteria, despite very low abundances of Cyanobacteria and other phototrophs[2,6,7,18]. A longstanding conundrum has been how these heterotrophic bacteria sustain energy and biomass in the absence of obvious primary producers.

## A minimalistic mode of primary production

Lacking obvious organic carbon inputs, most microorganisms within hyper-arid desert communities seemingly persist in various dormant states, where energy is directed towards persistence rather than growth[19]. While dormancy offers microorganisms a bet-hedging strategy to survive chemically and physically challenging conditions, it is not a cost-free state, as some maintenance energy is required for basic cellular functions such as macromolecular repair[19,20]. Through recent studies, we have provided evidence that some desert surface soil communities are structured by a minimalistic mode of primary production, where atmospheric gases, not sunlight, serve as the main energy source[21].

We analysed the surface soil microbial communities of two coastal ice-free desert sites in Eastern Antarctica, Robinson Ridge and Adams Flat. Both sites had limited capacity for photosynthesis and

were extremely low in organic carbon content. Despite this, they harboured diverse communities of bacteria belonging to the superphylum Terrabacteria, including Actinobacteria, Chloroflexi, and two candidate phyla, WPS-2 (*Candidatus* Eremiobacteraeota – desert bacterial phylum) and AD3 (*Candidatus* Dormibacteraeota – dormant bacterial phylum). To understand the metabolic potential of this community, shotgun metagenomics and differential coverage binning were used to construct 23 draft microbial genomes. Genes supporting energy conservation were widespread, with the majority of the bacteria encoding high-affinity lineages of the enzymes [NiFe]-hydrogenase and a carbon monoxide dehydrogenase[21]. Pure culture studies on multiple organisms have shown that these enzymes facilitate trace gas scavenging to support persistence of heterotrophic bacteria under organic carbon starvation[22–25]. Gas chromatography measurements confirmed that aerobic soil microcosms aerobically scavenged $H_2$ and CO at rapid rates[21]. For dormant bacteria, atmospheric trace gases are favourable energy sources, given their ubiquity throughout the troposphere, low redox potential, and high diffusivity[26].

In addition, we found that bacteria from the Actinobacteria, Eremiobacteraeota, and Dormibacteraeota clades encoded the genes for autotrophic $CO_2$ fixation via the Calvin Benson-Bassham (CBB) cycle. We validated that the soil communities encoded and expressed type IE RuBisCO enzyme[21], a recently discovered clade of the $CO_2$-fixing enzyme that supports hydrogenotrophic growth in some Actinobacteria[27] but is absent from known phototrophs. The co-occurrence of these genes with high-affinity hydrogenases and carbon monoxide dehydrogenases suggested that these communities were able to fix $CO_2$ into biomass using atmospheric trace gases, rather than solely relying on exogenous inputs from photosynthetic organisms. To test this, we traced assimilation of $^{14}C$-labelled $CO_2$ by these samples in microcosm experiments. We were able to demonstrate that, under $H_2$-enriched conditions, chemosynthetic $CO_2$ fixation increased up to tenfold. In contrast, no significant stimulation was observed following light illumination[21]. Based on these findings, we propose that, in desert ecosystems where photosynthetic organisms are excluded due to aridity, dormant bacterial communities are sustained by atmospheric chemosynthesis: members maintain energy and carbon needs by aerobically respiring atmospheric $H_2$ and CO and, in some cases, using these gases to fix $CO_2$ into biomass (Figure 2).

## Aerobic gas scavengers

Pure culture studies have provided insights into the physiological role and biochemical basis of trace gas scavenging[26,28]. For example, our research has recently helped to resolve the biochemical basis and physiological significance of atmospheric $H_2$ oxidation[22,24,29]. In the lower troposphere, $H_2$ occurs at trace amounts (~530 ppbv) and is rapidly cycled between sources (e.g. methane photolysis, fossil fuel combustion) and sinks (i.e. bacterial scavenging, hydroxyl radical oxidation). Atmospheric $H_2$ scavenging, in addition to being ecologically important, is of major biogeochemical significance given it is the primary sink in the global $H_2$ cycle[26,30].

To harness the energy of $H_2$, bacteria employ specialised metalloenzymes called hydrogenases to catalyse the reversible reaction $H_2 \rightleftharpoons 2H^+ + 2e^-$[31]. Historically, hydrogen metabolism was thought

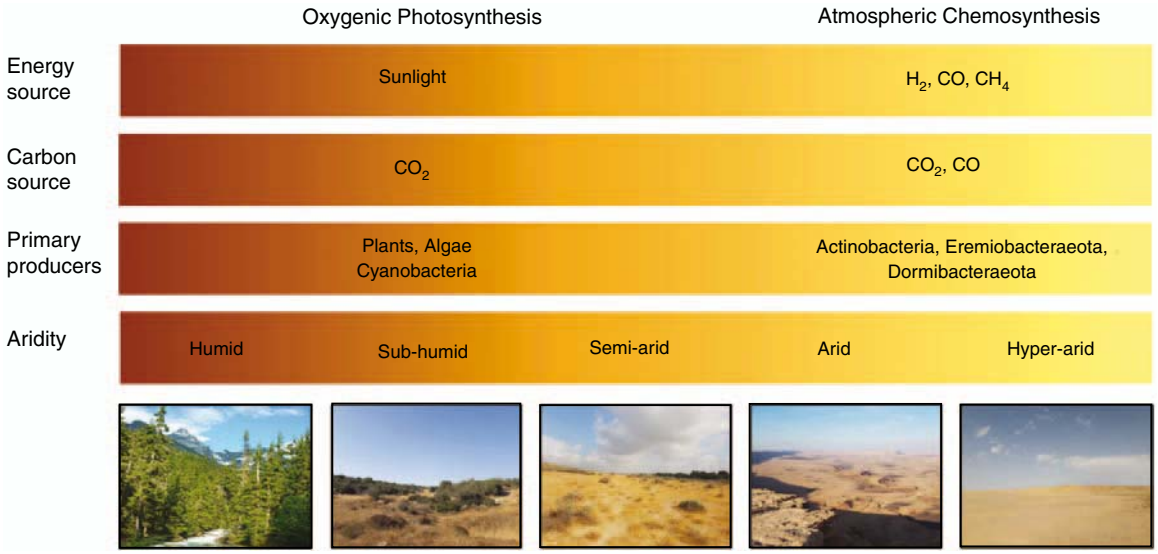


Figure 2. Schematic showing the predicted interactions between photosynthetic and chemosynthetic primary production strategies along an aridity gradient. As aridity increases, photosynthetic primary producers become less abundant relative to specialised bacteria that use atmospheric trace gases to generate biomass. Pictures correspond to five climatic zones from humid to hyper-arid. From left to right: Coniferous forest near Vancouver, Canada; Shrubland near Tel Aviv Israel; Grassland near Be'er Sheva, Israel; Mitzpe Ramon, Negev Desert, Israel; hyper-arid site near Eilat, Negev Desert, Israel. Photos taken by Sean Bay.

to primarily occur in low $O_2$, high $H_2$ environments such as oceanic sediments, gastrointestinal tracts, and hydrothermal systems. Reflecting this, the first isolated hydrogenotrophs had low affinities for $H_2$, and the first structurally characterised hydrogenase enzymes were highly $O_2$-sensitive[31]. However, recent studies have demonstrated that diverse soil bacteria can aerobically respire $H_2$ even at atmospheric concentrations[22,24,32]. We now know of four [NiFe]-hydrogenase lineages (group 1h, 1d, 1f, 2a) that support aerobic respiration and have biochemical adaptations to function in the presence of $O_2$[33,34]. Of these, the group 1h [NiFe]-hydrogenase is a high-affinity enzyme that primarily mediates atmospheric $H_2$ scavenging and is widely distributed in aerobic soil bacteria[32,35,36], including those in Antarctica[21].

Our recent pure culture studies have shown that the survival of bacteria belonging to dominant soil phyla such as Actinobacteria and Acidobacteria is enhanced by aerobic respiration of $H_2$. For example, the model soil organism *Mycobacterium smegmatis* upregulates the expression of two high-affinity hydrogenases under carbon starvation and persists by oxidising $H_2$ below atmospheric levels. Mutant strains, lacking the genes encoding hydrogenase structural subunits, have a 40% reduction in survival in carbon-limited batch and continuous cultures[22,23,37]. The physiological role of atmospheric $H_2$ scavenging was further tested with a thermophilic isolate from an oligotrophic volcanic soil, namely *Pyrinomonas methylaliphatogenes K22*. Following the transition from exponential to stationary phase, this acidobacterium upregulated the expression of an eight-gene operon of the high affinity group 1h [NiFe]-hydrogenase. Depletion of its carbon sources triggered the transition to a non-replicative persistent state supported by atmospheric $H_2$ scavenging[24]. Furthermore, pioneering work led by the Constant group has demonstrated that exospores of *Streptomyces* species express homologous enzymes and use them to support long-term survival[25,32,35].

## Conclusions and future directions

It is indisputable that microbial persistence requires energy. However, atmospheric substrates such as $H_2$ and CO have long been overlooked as potential energy sources. We now have evidence that aerobic respiration of these gases is widespread and have a rationale for the adaptive advantage this offers to dormant bacteria living in conditions where persistence is favoured over growth. While trace gases serve as energy sources for bacteria in aerated soil ecosystems worldwide, they are particularly important for microbial communities in soils with low water and carbon content, where phototrophs are excluded. We have confirmed that trace gases serve as the primary energy sources supporting two Antarctic desert sites[21]. Moreover, there is evidence that the enzymes mediating atmospheric chemosynthesis are also encoded in other oligotrophic ecosystems, including the hyper-arid deserts of the Atacama[38] and volcanic deposits of Hawaii[39].

Our recent findings in the Antarctic, as well as ongoing research into trace gas scavenging, will form the basis of future investigations. We are particularly interested in answering how significant this process is in explaining microbial biodiversity and primary production in other desert ecosystems such as the Negev Desert, Israel and the Atacama Desert, Chile. A key question is how does the balance between photosynthetic primary production and chemosynthetic primary production change along aridity gradients. These ecological studies are being supported by ongoing work focused on understanding the physiology and biochemistry of trace gas scavenging using pure bacterial cultures and purified enzymes.

## Acknowledgements

This work was supported by a Monash University PhD Scholarship (awarded to SB), ARC Future Fellowship (FT170100341; awarded to BF), ARC DECRA Fellowship (DE170100310; awarded to CG), and Australian Antarctic Science project grant (4406; awarded to BF and CG). We thank Steven Chown, Melodie McGeoch, Osnat Gillor, Mukan Ji, Don Cowan, Philip Hugenholtz, Paul Sunnucks, Dustin Marshall, Linda Blackall, and members of the Greening and Ferrari laboratories for helpful discussions.

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## Biographies

**Sean Bay** is a second year PhD student under the primary supervision of Dr Chris Greening at Monash University. He is investigating how microbial biodiversity and primary production strategies are influenced by aridity.

**Dr Ferrari** is an Associate Professor and ARC Future Fellow at UNSW Sydney. Her major interest is understanding the microbial diversity of soil bacteria, particularly in underexplored regions of Antarctica.

**Dr Chris Greening** is a Lecturer and ARC DECRA Fellow at the School of Biological Sciences and Centre for Geometric Biology, Monash University. He is a biochemist-turned-microbiologist investigating the metabolic basis of bacterial persistence.

# Rock-art microbiome: influences on long term preservation of historic and culturally important engravings




*Deirdre B Gleeson*[A,C] *Matthias Leopold*[A,D] *Benjamin Smith*[B,E] *John L Black*[B,F]

[A]UWA School of Agriculture and Environment, The University of Western Australia, Crawley, WA 6009, Australia

[B]Centre for Rock Art Research and Management, The University of Western Australia, Crawley, WA 6009, Australia

[C]Email: deirdre.gleeson@uwa.edu.au

[D]Email: matthias.leopold@uwa.edu.au

[E]Email: benjamin.smith@uwa.edu.au

[F]Email: jblack@pnc.com.au

**The Burrup Peninsula in north-west Western Australia is home to one of the most substantial collections of rock engravings, or petroglyphs, in the world. These petroglyphs are carved through the dark coloured patina, commonly referred to as rock varnish, into the weathering rind of the local parent rock. Rock varnish is essentially a thin layer of manganese (Mn) and iron (Fe) oxides and hydroxides with embedded clay minerals, the formation of which is relatively poorly understood. It is generally considered to be a hostile environment for microorganisms due to extreme environmental conditions including low nutrient availability, lack of water, exposure to extreme ultraviolet radiation and intense seasonal and diurnal temperature fluctuations. However, despite these environmental extremes, microorganisms have been found on and in rock varnish and have been reported as playing a significant role in the formation of rock varnish. Given this, it is likely that any change in local environmental conditions will influence the types and activities of microorganisms found in and on rock varnish and associated rock art. This article focuses on the major influences on the microbiome of culturally important rock art in the Burrup Peninsula and the implications of any environmental change on the rock art itself.**

The Burrup Peninsula (see Figure 1) is estimated to contain over 1 million petroglyphs. These form one of the longest sequences of art in the world, extending back probably as much as 40 000 years. This makes the area one of the most significant rock art regions in the world[1]. The engravings are diverse in form and include those of many animal and bird species including extinct animals (e.g. *Thylacines*, the Tasmanian tiger)[2]. The petroglyphs (see Figure 2 as an example) on the Burrup Peninsula are carved into the rock varnish of the parent granophyre and gabbro igneous rocks. Rock varnish usually forms very slowly, at a rate of 1–10 µm per thousand years, particularly in arid desert environments where rainfall is low[3,4]. At its most basic rock varnish can be described as a dark coloured coating with thickness that seldom exceeds 200 µm that is composed mostly of Mn and Fe oxides that are cemented to clay minerals in a laminated structure[3]. The Mn and Fe present in rock varnish comes from a range of sources likely including the atmosphere, precipitation, dust and from surrounding soils[5,6]. Mn and Fe concentrations can vary greatly but in general Mn is enriched

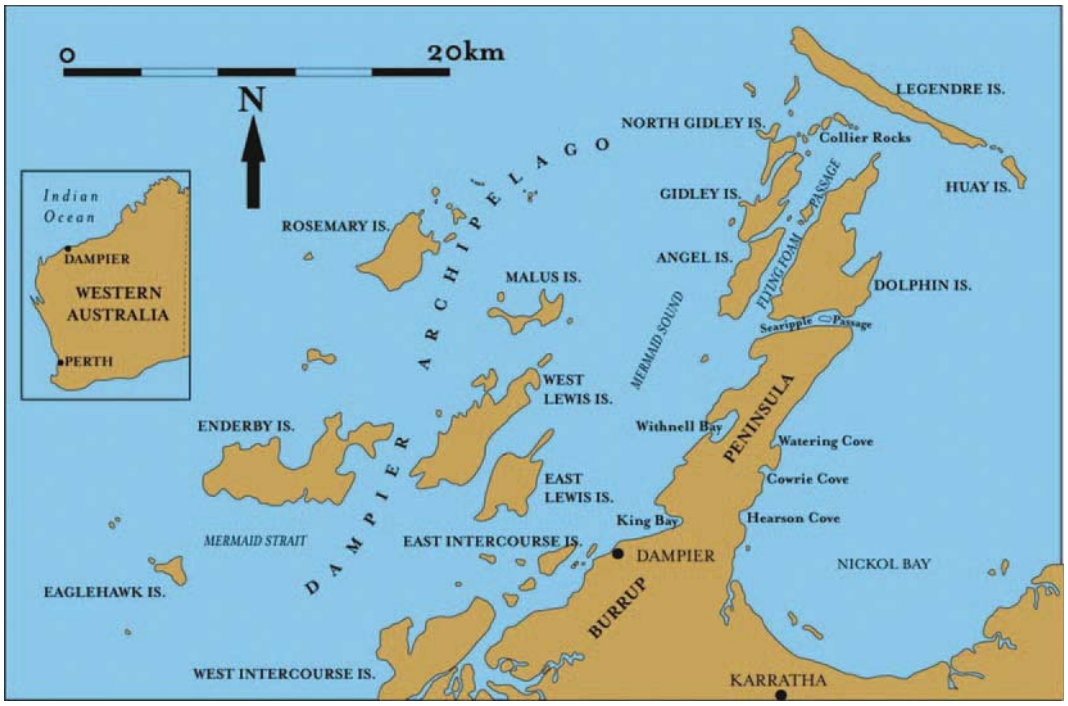


Figure 1. Map showing the Dampier Archipelago and adjacent mainland, Pilbara region Western Australia.

Figure 2. Burrup rock with petroglyph. Image by Mike Donaldson, reproduced with the permission of Murujuga Aboriginal Corporation.

over Fe relative to their natural distribution with Mn oxides usually accounting for around 20% of the total oxides[7].

There is some evidence suggesting that the microlaminations in rock varnish correlate with alterations in environmental conditions[3]. Liu *et al*.[8] hypothesise that Fe-rich layers are formed during dry conditions whereas darker Mn-rich layers are formed during periods of wet[3]. Precipitation and solubility of Mn is generally controlled by pH and Eh conditions with low Eh and pH usually leading to dissolution of Mn whereas high Eh and pH promotes precipitation. The ratio of Mn to Fe in the varnish thus varies with climatic conditions[9]. It is likely that when conditions are moist this promotes the activity of bacteria and fungi which in turn produce organic acids that work to decrease local pH. Field observations by Northup and colleagues at the Black Canyon (New Mexico, US) reported a strong correlation of visibly more substantial Mn varnishing on preferential water pathways flowing on rock surfaces and cliff faces, and in ephemeral rock pothole pools[10].

## Rock varnish formation

The mechanism of rock varnish formation and growth is still under discussion with both biotic and abiotic processes being postulated. It has been proposed that if abiotic processes were responsible for producing rock varnish that rates would be much more rapid than empirical measurements suggest[11]. Evidence of a biological role has been mounting[5] with significant interest being generated around likely biological mechanisms. For example the formation of β-alanine and δ-butyric acid by enzymatic carboxylation is indicative of biological activity[12]. There is also significant evidence that suggests that microorganisms can directly or indirectly control Mn precipitation[13,14] with biomineralisation of Mn being proposed in a wide range of environments including hot springs[15] and soils[16]. The elevated concentration of Mn that is well above that of the geological background also suggests that the formation of rock varnish is likely a biological process. This is because Mn and Fe compounds concentrated in bacteria and fungi become chemically bound in the crystalline structure and external coating of the clay minerals that cements the clays to rock surfaces[17]. The hypotheses that microbial communities play a role in the formation of rock varnish are also supported by the fact that a number of microbial metabolic pathways use Fe and Mn as electron donors[18,19].

## The role of the microbiome

Many different bacteria and fungi have been isolated and characterised from rock varnish[10,20–22] but those with the ability to oxidise and precipitate Mn and Fe[13,19] are of particular interest. Within this group budding bacteria, for example the genera *Hyphomicrobium* and *Pedomicrobium*, have been extensively studied as they have the ability to encrust Mn and Fe oxides within their cells[23,24]. These types of budding bacteria have been identified growing on rock varnishes found in warm deserts[5,25] and are known to produce Mn-rich deposits on rocks present in mountain soils[26], acid mine drainage[19], and caves[27,28]. Krinsley *et al*.[11] reported the first evidence of budding bacteria present *in situ* within rock varnish that were directly enhancing Mn and Fe. The authors investigated a site at Erie Barge Canal (New York) where rock varnish had completely coated the quarried sandstone over the course of approximately 100 years[11]. This site has rock varnish that is approximately 15 µm thick and the authors conclude that only one or two budding bacteria encrusting Mn and Fe oxides each year would be needed to generate the rock varnish[11]. By extrapolating from this data the authors speculate that it would take only one budding-bacterium every 400 years to explain a 20 µm thick, 10 000-year-old warm desert varnish similar to that found in the Burrup Peninsula[11]. We can therefore hypothesise that unique and rarely occurring environmental conditions are required to promote rock varnish formation by specific budding bacteria, for example, optimum moisture, UV and solar radiation exposure.

## Industrial expansion: what does the future hold?

Recent expansion of industry in the Burrup Peninsula may potentially upset the delicate balance of environmental conditions that led to rock varnish formation. Acid rain and nitrogen deposition as a result of industrial expansion has the potential to stimulate microorganisms that may not be compatible with rock varnish formation and or which may produce organic acids that could be detrimental to the survival of the rock varnish[29,30]. The combined influence of acid rain and microbial organic acid production will decrease the pH of the rock art environment potentially resulting in dissolution of the Mn and Fe within the rock varnish ultimately leading to deterioration and consequent destruction of the rock art. Research is required to identify the specific organisms responsible for rock varnish formation and to assess the impact of pollution on the rock art microbiome and likely impacts on rock varnish so that we can better understand how to protect the culturally significant rock art present in the Burrup Peninsula.

## Acknowledgements

The authors acknowledge and thank the Murujuga Aboriginal Corporation.

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## Biographies

**Dr Deirdre Gleeson** is a microbial ecologist focussing on both natural and managed ecosystems across terrestrial and aquatic environments where she studies the microbiomes of agricultural systems, contaminated landscapes, microbioliates and seagrass and mangrove rhizospheres. She is particularly interested in microbe-mineral interactions and how these relationships relate to rock art preservation in the Burrup Peninsula.

**Dr Matthias Leopold** is a soil geomorphologist focusing on soils as geoarchives. He integrates knowledge from pedology and geoscience to study and understand near surface processes at various scales and environments. Matthias has broad experience in geoarchaeology and has worked with archaeologists on rock art at the Burrup Peninsula for the past three years.

**Professor Benjamin Smith** is Associate Dean (Research) in the Faculty of Arts, Business, Law and Education and Professor of World Rock Art at the University of Western Australia. He is President of the ICOMOS International Committee on Rock Art.

**Dr John L Black AM** is a Research Management Consultant specialising in the management of large programs for the animal and veterinary industries. He worked in the CSIRO Division of Animal Production for 23 years studying comparative physiology and nutrition across animal species and in the development of computer simulation models before becoming Assistant Chief of the Division. In recent years, he has been investigating, *pro bono*, the impacts of industrial emissions on petroglyphs on Murujuga.

# The geomicrobiology of mining environments

*Talitha C Santini*

School of Earth and Environmental Sciences
Room 325, Steele Building
St Lucia Campus
The University of Queensland
Brisbane, Qld 4072, Australia
Email: t.santini@uq.edu.au

*Emma J Gagen*

School of Earth and Environmental Sciences
Room 218, Richards Building
St Lucia Campus
The University of Queensland
Brisbane, Qld 4072, Australia
Email: e.gagen@uq.edu.au

**As the global population increases, so does the demand for minerals and energy resources. Demand for some of the major global commodities is currently growing at rates of: copper – 1.6% p.a.[1]; iron ore: 1.4% p.a.[2]; aluminium – 5% p.a.[3]; rare earth elements – 7% p.a.[4], driven not only by population growth in China, India, and Africa, but also by increasing urbanisation and industrialisation globally. Technological advances in renewable energy production and storage, construction materials, transport, and computing could see demand for some of these resources spike by 2600% over the next 25 years under the most extreme demand scenarios[5]. Coupled with declining ore grades, this demand means that the global extent of mining environments is set to increase dramatically. Land disturbance attributed to mining was estimated to be 400 000 km$^2$ in 2007[6], with projected rates of increase of 10 000 km$^2$ per year[7]. This will increase the worldwide extent of mining environments from around 500 000 km$^2$ at present to 1 330 000 km$^2$ by 2100, larger than the combined land area of New South Wales and Victoria (1 050 000 km$^2$), making them a globally important habitat for the hardiest of microbial life. The extreme geochemical and physical conditions prevalent in mining environments present great opportunities for discovery of novel microbial species and functions, as well as exciting challenges for microbiologists to apply their understanding to solve complex remediation problems.**

Major habitats in mining environments can be divided into two main groups (Figure 1): mine sites, where ore is excavated and crushed, including waste storage sites for *overburden* (rock and soil materials removed to access the ore body), and *waste rock* (sub-economic rock surrounding the higher grade ore body); and processing/refinery sites, where the ore is upgraded or purified to separate the target element or resource, including waste storage sites for by-products from either aqueous (*tailings*) or high temperature smelting (slags) refining techniques, and *wastewaters* from these processes. Not covered in this article are mining-affected environments around mining and refinery sites, which receive inputs from mine sites in the form of dust (ore, overburden, tailings, and the resource product), surface water and groundwater discharges (wastewaters), or even solid wastes (tailings, waste rock) which, in some cases, are exported by riverine or marine disposal. The severity of impacts and disturbance is far lower in mining-affected environments around the site than within the mining or refinery sites that may generate offsite impacts, and we have therefore excluded them from the primary mining environments (mines and refineries) to be discussed here.

Ore bodies are, by their definition, geochemical and mineralogical anomalies, containing target resources at elevated concentrations compared to the average in continental crusts. It should be no surprise, then, that the excavation of these ores and exposure to water and air generates unusual geochemical environments for microbial communities to inhabit and modify. Even more extreme geochemical and physical environments are created in the tailings and wastewater streams produced from ore processing and refining activities (Figure 1) as a result of the elevated temperatures and pressures used in processing and refining, and the chemical reagents added to enhance resource recovery. The processing conditions effectively sterilise tailings and wastewater streams, and the extreme geochemical and physical conditions then impose strong selection pressures on future microbial colonisers. pH values tend to be $\leq$4.5 or $\geq$8.5, due to the use of acidic or alkaline refining conditions, and/or the reaction of ore or process-generated minerals producing acidity (e.g. oxidation of sulphides;


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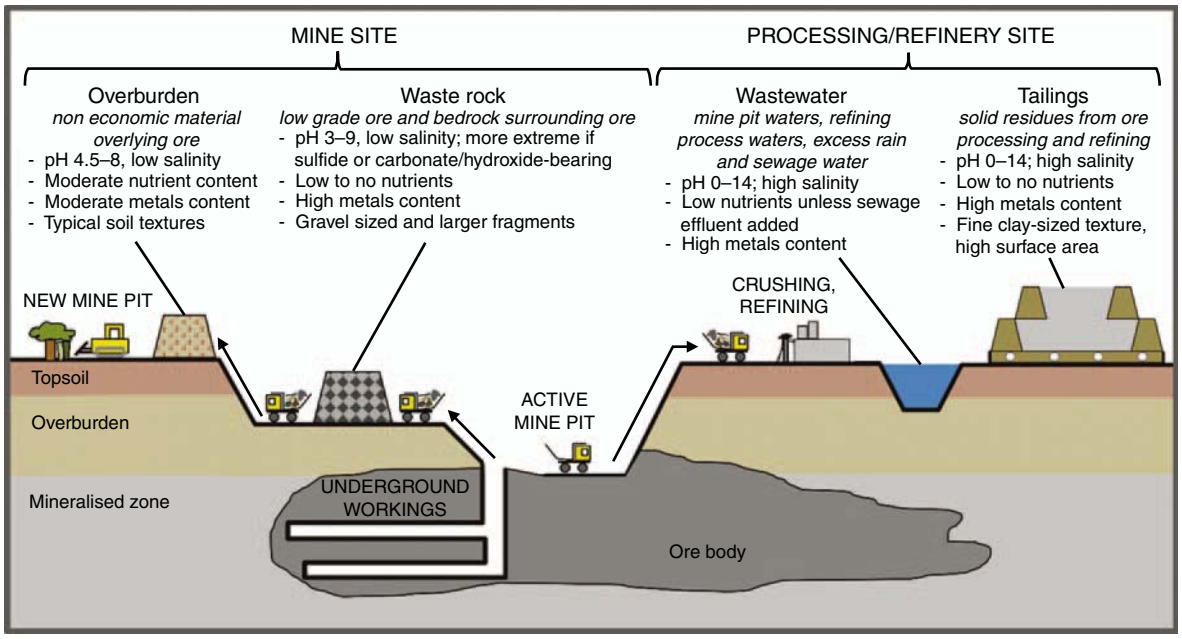


Figure 1. Major materials and environments within mine and refinery sites, with brief descriptions of typical geochemical and physical conditions prevalent in these environments and illustrations of the two major modes of ore excavation (open pit and underground). Note that *in situ* ore extraction (which can be used for copper and uranium) is not presented here, and that in some cases, the ore body is exposed at the ground surface rather than overlain by soil and overburden. This is particularly common in sulphide deposits affected by supergene processes.

Equation 1) or alkalinity (e.g. dissolution of hydroxides or carbonates; Equation 2).

$$4FeS_{2(s)}+15O_{2(g)}+14H_2O_{(l)}\rightarrow 4Fe(OH)_{3(s)}+8H^+_{(aq)}+8SO_4{}^{2-}{}_{(aq)} \quad (1)$$

$$Ca(OH)_{2(s)}\rightarrow Ca^{2+}{}_{(aq)}+OH^-{}_{(aq)} \quad (2)$$

Salinity, and particularly sodicity, is usually high enough in tailings to inhibit growth of even the most salt-tolerant plant species (>4 mS $cm^{-1}$) and classifies wastewaters as brackish to brine (1–35 g $L^{-1}$ salt), due to the addition of various (often sodium-based) reagents during refining processes. Major biological nutrients (C, N, K, P) are present in low to negligible concentrations, because the depth at which ores are excavated during mining, and their low surface area *in situ*, does not allow for significant microbial colonisation and fixation of atmospheric carbon and nitrogen, and does not expose the ore to near-surface weathering processes that release K and P from minerals (commonly feldspars of the general formula (K,Na,Ca)$(Al,P,Si)_4O_8$, micas of the general formula $(K,Na,Ca)(Al,Mg,Fe)_{2–3}(Si,Al,Fe^{3+})_4O_{10}(OH,F)_2$, and apatite $Ca_3(PO_4)_2$). Crushing of ore to enhance reaction kinetics during refining creates tailings materials that are prone to waterlogging, largely anaerobic, and exhibit rapid mineral weathering rates (both chemically and biologically driven) due to the large particle surface areas. The extreme pH and high mineral weathering rates release heavy metals (Pb, Hg, Cd, Co, Sn), metalloids (As, Se, Sb, B), and other elements at concentrations typically considered to be toxic for most plant and microbial life.

And yet life persists! Although generally low biomass and low diversity[8–11], active microbial communities appear to be present across all mining environments. Dominant phyla tend to be those known to host lineages tolerant of one or more of the challenging environmental conditions present in mining environments, such as pH, salinity, high metals/metalloid concentrations, and lack of organic carbon. For example, acid mine drainage and sulphidic waste rock are dominated by *Gammaproteobacteria*, *Betaproteobacteria*, *Actinobacteria*, *Nitrospira*, and *Firmicutes*[8,12], and alkaline tailings are dominated by *Gammaproteobacteria*, *Firmicutes*, *Actinobacteria*, and *Bacteroidetes*[11]. However, community composition diverges within mining environments at lower taxonomic levels, where the influences of site specific factors like ore type, environmental conditions, and process chemistry play a greater role[11].

Cultivation and isolation of novel species from mine sites has yielded fundamental insights into processes of element cycling (e.g. arsenic[13]; silver[14]; gold[15]; rare earth elements[16]; thiocyanate[17]), the mechanisms and origins of pH, salt, and metal tolerances (e.g. acid and chloride tolerance[18]; gold[19]), and microbe-mineral interactions[20]. Some of these novel species are genetically tractable, e.g. *Marinobacter subterrani* from an iron mine, and are thus invaluable tools for fundamental investigations into microbial physiology and metabolism[21]. Others are becoming useful tools in biotechnology; for example, an *Acidithiobacillus thiooxidans* strain isolated from a copper mine that is now being used in industrial bioleaching[22]. Metal-tolerant organisms from mines also

hold promise amongst the range of organisms being considered in approaches for recovery of metals, a process known as biomining, including eukaryotic microbes e.g. *Euglena mutabilis* and *Chlorella protothecoides* isolated from a copper mine[23]. The heavy metal tolerances of these eukaryotes also makes them useful bioindicators for metal contamination in aquatic systems. Such discoveries are facilitated in mining environments, which provide selection pressures of sufficient strength to promote the proliferation of species with these tolerances and capabilities.

At a community level, the restricted diversity present in mining environments has proved ideal for development of new bioinformatics tools, such as metagenomics from a study of microbial communities in acidic mine wastewaters[24]. Understanding processes of microbial community succession, and metabolic interdependencies between species is also vastly easier at low levels of microbial community diversity[25] (although increasing metagenomic sequencing breadth and depth can assist for more diverse communities; *cf* Wrighton *et al*.[26]), and both are emerging fields of fundamental research in mining environments and environments impacted by mining and refining activities. The high concentrations of elements which are on average present at low concentrations in the Earth's crust, and the lack of organic carbon to support alternative (higher energy yielding) metabolic pathways makes mining environments fertile ground for the discovery of novel metabolic pathways, which at present are only hypothesised by theoretical bioenergetic calculations for these reactions.

Already, insights from the geomicrobiology of mining environments have improved our understanding of the Earth's geological past and likely future, as well as supporting advances in industrial capabilities across sectors as diverse as food processing and preservation, agriculture, mineral processing, astrobiology, pharmaceuticals, and human health. Given that this article is focussed on mining environments, we will provide a couple of examples from our research groups on application of these insights to the remediation of mining environments, for two of Australia's largest mineral commodities, iron ore and bauxite (aluminium ore).

## Accelerating iron cementation for iron ore mine site remediation

In tropical areas, iron ore that has been formed by the long-term weathering of banded iron formations (BIFs) is often capped by a hard, well-consolidated iron duricrust that hosts a unique plant ecosystem adapted to survive only in the harsh duricrust (background, Figure 2*a*). The iron duricrust exists as an extensive blanket covering the relatively soft iron ore below, and because it is extremely resistant to erosion, it often defines the landscape in these regions as ridges and plateaus. The duricrust itself is a ferricrete comprised of fragments of iron ore and BIF cemented together by goethite. Effective post-mining rehabilitation strategies of these iron ore areas relies on re-formation of the duricrust, which to date has not been achieved due to a lack of understanding about how the duricrust formed, and therefore how to re-establish it. Geochemical and microbial fossil evidence suggests that biological cycling of iron has contributed to the evolution of the duricrusts throughout geologic history, particularly the dissolution and reprecipitation of goethite[27,28]; thus, potentially, present-day biological iron cycling could be harnessed to 're-form' this duricrust on a

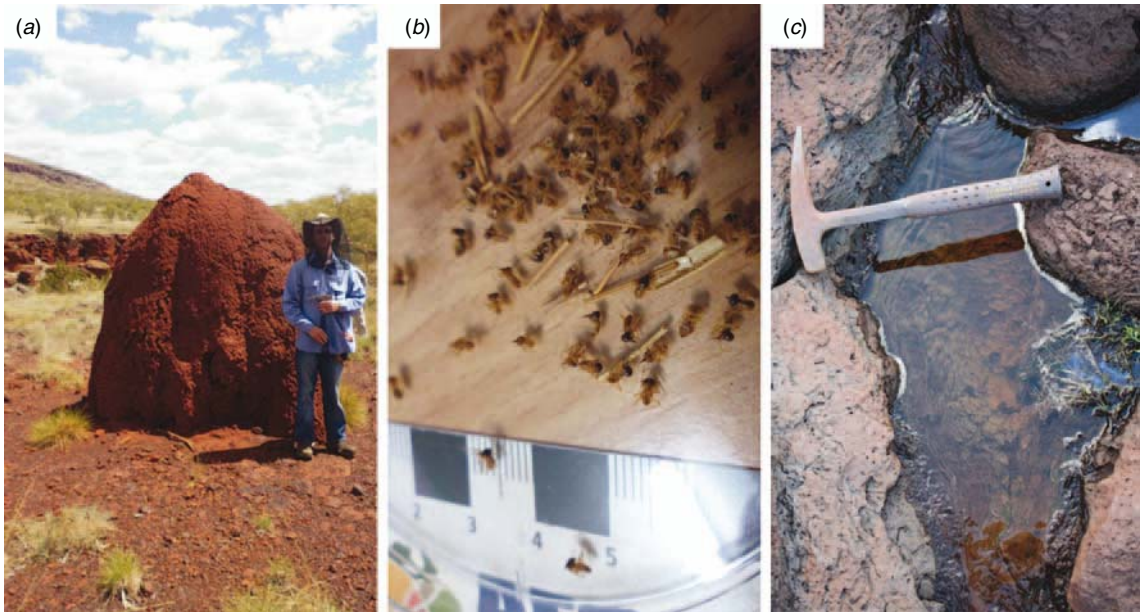


Figure 2. Cultivating microorganisms responsible for iron cycling in banded iron ore formation mining environments, such as (*a*) northwestern Western Australia, underpins strategies to re-form the duricrust caprock on vastly reduced timescales. Cultivation targets include (*b*) termite gut microbiota, and (*c*) microbial mats present in perched water pools. Photos courtesy E. Gagen.

shorter time scale. Exploring the microbiomes associated with iron duricrusts has revealed that lakes, ponds and puddles perched on the duricrust are a source of both iron oxidising and iron reducing microorganisms (Figure 2*c*), probably working in tandem under natural conditions and actively cycling iron. The gut of termites that penetrate into the duricrust (Figure 2*b*) and build their nests on and in it has also proven to be a novel source of microorganisms capable of reducing some of the more crystalline iron oxides in the duricrust effectively in consortia via fermentation (unpublished data). Given the novelty of this process and its potential application biotechnologically, metagenomic approaches are being used to reconstruct the main genomes from this consortia and elucidate the mechanisms of goethite reduction. Field-scale trials using microorganisms from the iron duricrust associated ecosystems are also currently underway to test the concept of 're-forming' the duricrust through accelerated biological iron cycling.

## Neutralising pH in alkaline alumina refining (bauxite) residue for tailings remediation

Aluminium is produced from bauxite (aluminium ore) by a two stage process, involving an alkaline hydrothermal digest (Bayer process) to release aluminium as aluminate, which is then precipitated as alumina ($Al_2O_3$), followed by an electrolysis step to recover pure aluminium metal from the alumina. The tailings produced in the first step are known as bauxite residue, and are typically discharged into tailings storage facilities (Figure 3*a*) at pH 11–13. One of the key goals of tailings remediation is to decrease pH to values $\leq$ 8.5–9. Previous work focussed on addition of chemical amendments to achieve this: carbon dioxide (atmospheric, or process-derived); weak acids; and seawater. These amendments are expensive and often most effective when completed prior to tailings discharge, making remediation of existing tailings storage areas difficult. Field work across bauxite residue storage facilities up to 40 years old suggested that microbial fermentation of organic carbon (driven by *Firmicutes*, a dominant phylum in bauxite residue communities) was likely playing an important, but neglected, role in neutralising pH[11]. Building on insights from field work characterising the structure and function of microbial communities in bauxite residues before, during, and after remediation, our research group has now developed microbially driven approaches for pH neutralisation in bauxite residue that will enable remediation of both existing and future alkaline tailings and wastewater streams[29,30]. These approaches have been successful at laboratory (Figure 3*b*) and glasshouse scale (Figure 3*c*), and in early 2018, will be tested in an industry-first field scale trial in Western Australia.

In summary, mining environments present unusually harsh conditions for biology with their extremes of pH, salinity, metals concentrations, and nutrient availability. However, microbial communities still thrive; and in many cases, often drive geochemical cycling under these conditions. The consequences of this can be negative (e.g. acid mine drainage, mobilisation of heavy metals) or positive (e.g. fermentation to neutralise alkaline wastes, iron cycling to stabilise and re-form surface duricrusts). With the rapidly expanding mining sector, it is important that we as microbiologists continue to strive to better understand the role of microbes in

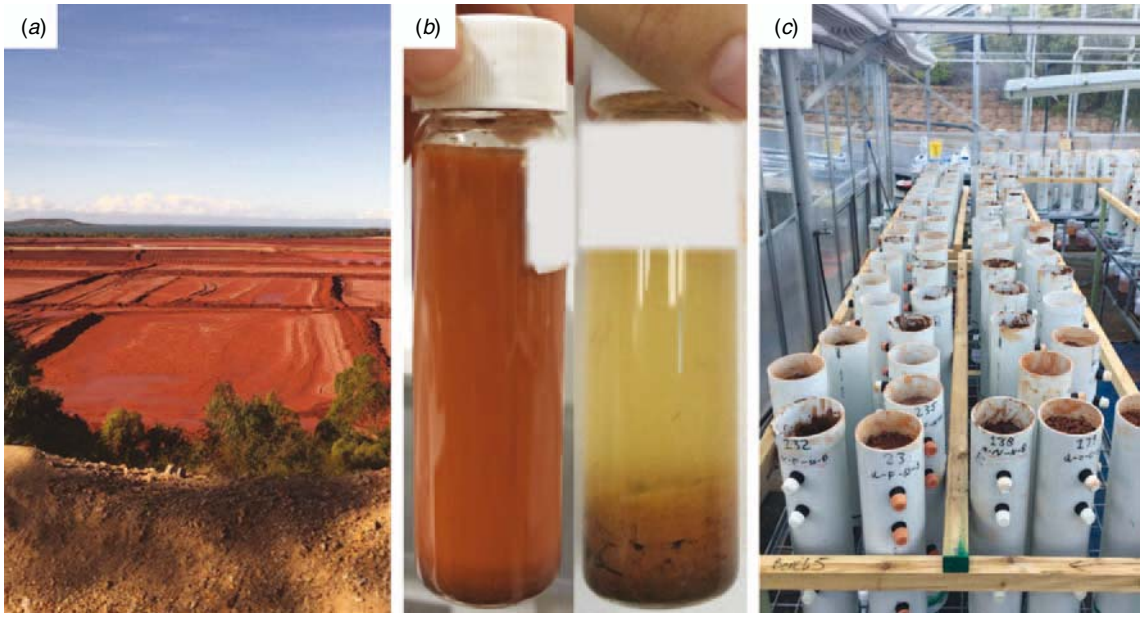


Figure 3. Identification of salt- and alkali-tolerant *Firmicutes* and other putative organic carbon fermenters in (*a*) weathered bauxite residue (alumina refining tailings) paved the way for development of a microbially driven pH neutralisation technique offering significant advantages over abiotic approaches. This technique has now been successfully implemented at (*b*) laboratory and (*c*) glasshouse scale, and will soon be tested at full field scale. Photos courtesy T. Santini and L. Malcolm.

geochemical cycling in these natural and anthropogenically generated systems, and to seize opportunities to harness the novel microbial potential available to us from these unique ecosystems. This will not only expand our understanding of microbial diversity, evolution, and functional capacity, but enable us to contribute to solving some of the most urgent challenges facing the mining industry, by developing new microbially driven technologies for ore extraction, ore processing, and environmental rehabilitation. This will become even more important as the mining industry continues to explore unconventional resources such as deep seafloor and sub-seafloor deposits, and new modes of extraction such as *in situ* leaching.

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## Biographies

**Talitha Santini** is a Senior Lecturer in the School of Earth and Environmental Sciences at the University of Queensland, where she leads a research group in microbial ecology and biogeochemistry. A major focus of this group is understanding the links between biotic and abiotic mineral weathering and nutrient cycling processes. Much of Dr Santini's research work is supported by industry partners, which include Alcoa, South32, BHP, Rio Tinto, Rusal and Newmont.

**Emma Gagen** is a Postdoctoral Research Fellow in geomicrobiology in the School of Earth and Environmental Sciences at the University of Queensland. Her primary research project focuses on the role microorganisms play in iron cycling in geologic systems and is largely supported by Vale S.A. Other projects she contributes to relate to microbial colonisation of meteorites, bacterial degradation of anhydrite, microbiology of sulphur-rich environments and formation of seafloor iron-manganese crusts. Emma's research interests extend to all areas of environmental microbiology and she is fascinated by the role microorganisms play in geochemical processes.

# Establishing microbial baselines to identify indicators of coral reef health




*Bettina Glasl*[A,B,C,F] *David G Bourne*[A,B] *Pedro R Frade*[D] *Nicole S Webster*[A,E]

[A]Australian Institute of Marine Science, Townsville, Qld 4810, Australia

[B]College of Science and Engineering, James Cook University, Townsville, Qld 4811, Australia

[C]AIMS@JCU, Townsville, Qld 4811, Australia

[D]Centre of Marine Sciences, University of Algarve, 8005-139 Faro, Portugal

[E]Australian Centre for Ecogenomics, University of Queensland, Brisbane, Qld 4072, Australia

[F]Tel: +61 7 4753 4444, Fax: +61 7 4772 5852, Email: b.glasl@aims.gov.au

**Microorganisms make a significant contribution to reef ecosystem health and resilience via their critical role in mediating nutrient transformations, their interactions with macro-organisms and their provision of chemical cues that underpin the recruitment of diverse reef taxa. However, environmental changes often cause compositional and functional shifts in microbial communities that can have flow-on consequences for microbial-mediated processes. These microbial alterations may impact the health of specific host organisms and can have repercussions for the functioning of entire coral ecosystems. Assessing changes in reef microbial communities should therefore provide an early indicator of ecosystem impacts and would underpin the development of diagnostic tools that could help forecast shifts in coral reef health under different environmental states. Monitoring, management and active restoration efforts have recently intensified and diversified in response to global declines in coral reef health. Here we propose that regular monitoring of coral reef microorganisms could provide a rapid and sensitive platform for identifying declining ecosystem health that can complement existing management frameworks. By summarising the most common threats to coral reefs, with a particular focus on the Great Barrier Reef, and elaborating on the role of microbes in coral reef health and ecosystem stability, we highlight the diagnostic applicability of microbes in reef management programs. Fundamental to this objective is the establishment of microbial baselines for Australia's coral reefs.**

Coral reefs represent one of the most diverse ecosystems on the planet, providing home for an estimated 25% of all known marine species[1]. Each year Australia's iconic Great Barrier Reef (GBR) attracts millions of tourists from all over the world and provides $6.4 billion dollars to the Australian economy[2]. However, reefs globally are facing unprecedented pressures[3]. During the past three decades, the GBR has also been severely impacted by the combined effects of climate change, crown of thorns starfish outbreaks, coral disease, overfishing and declining water quality[3–5]. Back-to-back bleaching events were experienced in 2016 and 2017 on the GBR, resulting in over 80% mortality of corals in some regions and an estimated loss of 29% of corals across the GBR system[3,6]. In addition to global pressures related to climate change, coral reefs are also affected at local scales[7]. For example, the GBR is locally affected by the run-off from 35 river basins, draining an area of over 424 000 km$^2$ [8]. Intensified agricultural land use in the GBR catchment area has caused an increase of sediments, nutrients and pesticides associated with terrestrial runoff, resulting in a

significant decline in water quality, which poses ongoing chronic and periodic acute threats to the health of the GBR[9].

Coral reef monitoring and management initiatives are well-established in Australia. For example, since the early 1980s the Australian Institute of Marine Science (AIMS) has assessed the health of Australia's coral reefs via its Long-Term Monitoring Program (LTMP). The Great Barrier Reef Marine Park Authority (GBRMPA) has managed the GBR area for over 40 years under the *Great Barrier Reef Marine Park Act 1975*. In 2015, the Australian and Queensland governments released the Reef 2050 Long-Term Sustainability Plan, outlining concrete measures to manage and protect the GBR over the next three decades. However, despite the focus on coral reef monitoring and management initiatives across all levels of government and strong community engagement in many areas, the coral reefs surrounding much of the Australian coastline, like other parts of the world, have demonstrated concerning declines in recent years[3,5]. One aspect that is poorly understood yet fundamental to coral reef functioning and ecosystem resilience is the contribution of microorganisms. Here we highlight that incorporating microbial based monitoring approaches into coral reef management initiatives will increase our understanding of reef ecosystem health and inform potential options for increasing reef resilience (Figure 1).

## Importance of microbes in coral reefs

Microorganisms play an essential role in coral reef ecosystem processes and form diverse symbiotic relationships with benthos-dominating macro-organisms such as corals, sponges and algae[10–12] (Figure 2). The functional role of microbes in coral reefs include biochemical cycling of nutrients, degradation and remineralisation, host nutrition, vitamin synthesis, production of secondary metabolites and host defence via the production of antimicrobial peptides[10,11]. Microbes often form specific and stable associations with their host species[13] and can assist them to acclimate to the prevailing environmental conditions[14,15].

Environmental variations, such as seasonal run-off or anthropogenic-induced fluctuations in water quality are known to alter the composition and function of the reef microbiome[16,17]. Numerous studies have shown a clear shift in microbial community composition and function in coral reef waters and associated with dominant benthic life forms (such as corals) as the health of the ecosystem declines[18,19] (Figure 3). However, despite the recognised influence microbes have on coral reef health[10,20], a holistic understanding of their dynamics in coral reef ecosystems remains elusive[21]. Establishing microbial baselines that characterise the temporal and spatial microbial dynamics in coral reefs is urgently needed to underpin rapid and sensitive assessments of declining

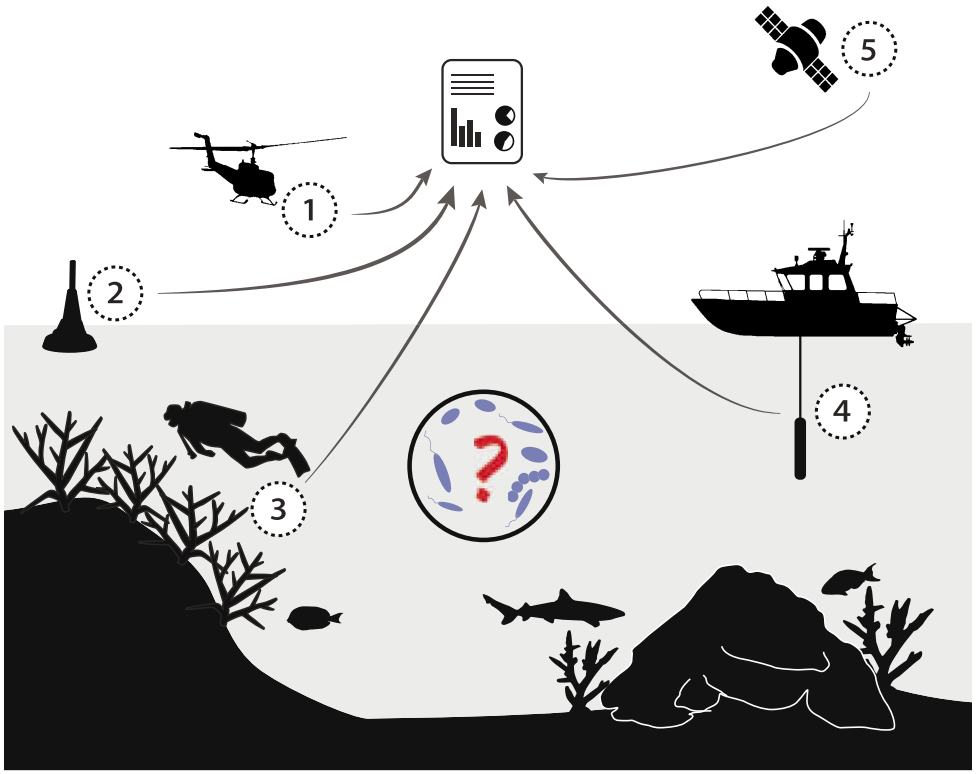


Figure 1. Implemented management strategies under Australia's Reef 2050 Long-Term Sustainability Plan are guided by an integrated monitoring approach including 1) large scale aerial surveys, 2) mooring systems and weather stations that provide data on surface (e.g. wind, precipitation, barometric pressure, temperature) and subsurface conditions (e.g. temperature, conductivity, chlorophyll fluorescence, turbidity, oxygen, light transmission and photosynthetically active radiation), 3) assessment of coral cover, coral recruitment, coral community composition and coral–macroalgae ratios on a reef, 4) comprehensive water quality assessments and screening for pesticide concentrations and 5) near surface concentration measurements of chlorophyll *a* and total suspended solids based on remote sensing technologies. Currently, this integrated monitoring framework lacks a microbial approach and hence, excludes a considerable part of the coral reef biodiversity.

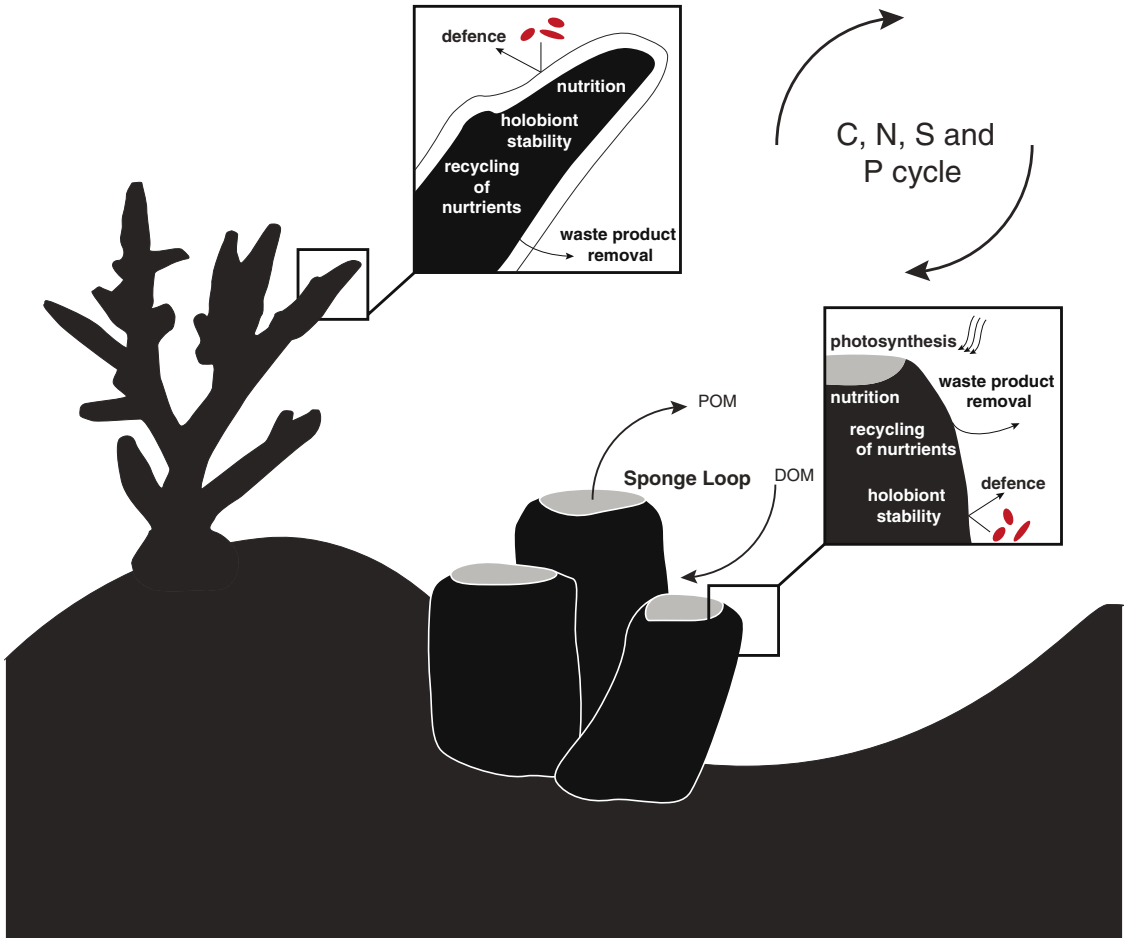


Figure 2. Simplified overview of microbial functions in a coral reef ecosystem. Microbes play a fundamental role in all major biogeochemical cycles (Carbon, Nitrogen, Sulfur and Phosphorus) in the coral reef ecosystem and contribute to their host's nutrition, waste product removal, pathogen defence and holobiont stability.

reef health and make predictions about the consequences of future environmental changes[10,22].

## Australia's initiatives to establish microbial baselines

Recent advances in next generation sequencing technologies combined with an increased recognition of the crucial ecosystem roles played by microorganisms, has resulted in a heightened commitment to understand spatial and temporal microbial dynamics in Australian ecosystems. For example, the BASE project (Biomes of Australian Soil Environments) is the first Australian soil microbial diversity database, providing amplicon sequencing data alongside contextual data for more than 900 sites across Australia[23]. Another example is the Australian Marine Microbial Biodiversity Initiative (AMMBI), which was the first standardised microbial ocean observatory program undertaken at a continental scale. AMMBI aims to provide long-term microbial sequencing data from seven different pelagic sites around Australia, providing important baseline data on microbial composition and function in Australian off-shore waters. This is linked to extensive physicochemical and oceanography data derived from the Integrated Marine Observing System (IMOS) reference stations (www.imos.org.au), allowing both hindcasting and forecasting of microbial responses to environmental conditions. Recently the Marine Microbes (MM) project (www.bioplatforms.com/marine-microbes/) was established as part of the larger AMMBI initiative to sample microbial communities associated with corals, sponges, seaweeds, seagrasses, seawater and sediment from benthic sites across Australia, including sampling locations in the GBR, Perth and Sydney. The MM project aims to provide the first holistic microbial baseline for coral reefs in Australia.

## Microbes as indicators of coral reef health

Indicator organisms are used to effectively monitor habitat conditions and environmental changes[24]. Biological indicators are a well-established monitoring tool for estuarine and freshwater ecosystems[25,26] and also find application in coral reef ecosystems[27]. In the context of public health, microorganisms are extensively used as indicators to monitor drinking water supplies and the quality of recreational waters in order to prevent gastrointestinal illnesses[28,29]. Furthermore, recent advances in human microbiome research have led to an increase of microbial based diagnostic and


Figure 3. Coral reef ecosystems are increasingly affected by the intensification of environmental pressures emerging from land-use changes, overfishing, crown-of-thorns starfish outbreaks, coral diseases and climate change. Degradation of coral reefs and a shift from coral to macroalgae dominated benthic communities (from left to right) has been observed globally. As the health of the coral reef ecosystem changes, microorganisms rapidly respond. The microbiome of healthy reefs is dominated by beneficial and symbiotic microbes (blue), but as ecosystem health declines the microbiome shifts to an unbeneficial community, dominated by pathogens and opportunists (red).

therapeutic approaches[30]. Despite the emerging predictive power of the microbiome in human disease diagnostics[30,31], the use of microorganisms as sensitive indicators of environmental stress in coral reef ecosystems or as predictive markers for water quality in marine systems has remained relatively unexplored[10,22]. Microbialisation scores are among the few attempts to monitor coral reef ecosystem declines based on the metabolic rates of microbial communities and reef-associated fishes[32]. Incorporating microbial monitoring tools into current coral reef health assessment programs will confer significant advantages as microbes are known to rapidly respond to changes in their environment, allowing for early diagnosis of changing water conditions and host physiological states.

Despite many potential advantages, microbial systems for monitoring coral reefs are still very much in their infancy and considerable additional research and validation would be required before microbial based monitoring approaches could be applied. Additional technical considerations that remain to be addressed include: (1) How frequently should sampling occur? (2) How and what should be sampled (e.g. seawater, sediment, microbiomes of benthic organisms such as corals or seaweed)? (3) What types of samples and analyses would be necessary (e.g. community profiling, targeted screening for particular microbial indicator taxa and/or functions)? and (4) How to minimise costs and increase efficiency of a microbial based monitoring system to ensure real-time assessment of reef health?

## Conclusion

The important role of microbes in coral reef ecosystem functioning and their contribution to the resistance and resilience of coral reefs has become widely accepted[20,22]. However, although Australia is at the forefront of coral reef studies and coral reef monitoring operations, to date, microbes have not been considered in large-scale monitoring approaches. The past few years have seen increased interest in understanding microbial dynamics in Australia's ecosystems, which has led to holistic sampling efforts to establish the first microbial baselines for soils and marine environments. We argue that the establishment and ongoing assessment of such microbial baselines will be crucial to understanding microbial dynamics in response to broad ranging anthropogenic impacts. The inclusion of microbial monitoring approaches alongside our current coral reef monitoring framework will improve our ability to rapidly detect changes occurring in Australian coral reefs resulting in improved protection and management of these ecologically and economically unique ecosystems.

## Acknowledgements

We thank Megan Huggett for her feedback and scientific input. Funding was provided through the AIMS@JCU PhD Scholarship, GBRMPA Science Management Research Award and the Advance Queensland PhD Scholarship to BG. PRF was supported by the Portuguese Science and Technology Foundation (FCT) through fellowship SFRH/BPD/110285/2015.

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## Biographies

**Bettina Glasl** is currently undertaking her PhD research at James Cook University in collaboration with the Australian Institute of Marine Science. Bettina has recently been awarded a prestigious Advance QLD PhD Scholarship by the Queensland Government. Her PhD research focuses on the temporal dynamics of coral reef microbiomes and the resistance and resilience of coral and sponge microbiomes upon environmental disturbances.

**David G Bourne** is a Senior Lecturer at James Cook University and a Principal Research Scientist at the Australian Institute of Marine Science (AIMS). His training is in the area of molecular microbial ecology with his research focused on investigation of microbial diversity, structure and function in complex ecosystems. His research is divided essentially into two areas, the first investigating the normal microbial communities associated with corals and their functional roles in maintaining coral fitness. The second research focus is to elucidate pathogens and mechanism of disease onset in corals and the implications this has on a stressed reef ecosystem in light of climate change being a major driver of coral reef degradation.

**Pedro R Frade** is a postdoctoral researcher at the Centre for Marine Sciences (CCMAR) at the University of Algarve (Portugal). His research focuses on the microbial ecology of coral reef ecosystems, in particular the functional role of coral-associated microbial communities belonging to the three domains of Life and their contribution to niche diversification and adaptation of corals. Pedro uses field and experimental approaches to investigate the microbiome's functional diversity in auto- vs heterotrophic corals, across shallow vs mesophotic reefs, and for tropical vs temperate coral hosts.

**Nicole S Webster** is a principal research scientist at the Australian Institute of Marine Science where she undertakes research into how microorganisms contribute to reef ecosystem health. Nicole also holds a joint appointment as Principal Research Fellow at the Australian Centre for Ecogenomics at the University of Queensland. In both positions Nicole uses experimental and field-based ecological research to explore multiple facets of coral reef microbiology and symbiosis.

# Engineering biological nitrogen removal in wastewater treatment via the control of nitrite oxidising bacteria using free nitrous acid

*Andrew Elohim Laloo*

Advanced Water Management Centre
University of Queensland
Brisbane, Qld, Australia
Email: a.laloo@awmc.uq.edu.au

*Philip L Bond*

Advanced Water Management Centre
University of Queensland
Brisbane, Qld, Australia

**Nitrogen compounds need to be removed or captured from wastewater streams before disposal to protect our aquatic environments from eutrophication. Particular bacteria facilitating the biological removal of nitrogen during wastewater treatment include ammonia oxidising bacteria (AOB), nitrite oxidising bacteria (NOB), denitrifiers, as well as anaerobic ammonium oxidising (Anammox) bacteria. Manipulating these microbial communities can improve efficiency in nitrogen removal. Bypassing nitrate production by selectively inhibiting NOB reduces the need for oxygen and the addition of external carbon for the nitrogen removal. Various approaches to selectively inhibit NOB in the nitrification process are available. Here we present an approach using the biocide, free nitrous acid (FNA) to selectively suppress NOB growth thereby improving the efficiency of the nitrogen removal process.**

## Improving efficiency of nitrogen removal

The principal forms of nitrogen species in wastewater are ammonium, nitrite and nitrate as well as organic nitrogen. Removal from wastewater is essential before release into aquatic environments to prevent nitrogen build up that may lead to eutrophication and endanger aquatic life[1]. For wastewater treatment, biological nitrogen removal is favoured over physical-chemical processes due to efficiency and cost benefits[2].

Conventional biological nitrogen removal in wastewater treatment (WWT) plants involves a 2 step biological process: autotrophic nitrification followed by heterotrophic denitrification. These steps result in nitrogen gas being released from the system (Figure 1*a*). The nitrification step includes the oxidation of ammonia to nitrite via ammonia-oxidising bacteria (AOB) and then oxidation to nitrate through the activity of nitrite oxidising bacteria (NOB). Nitrification is then followed by heterotrophic denitrification where nitrate is reduced to nitrite and finally to nitrogen gas[3]. Here, the inhibition of NOB activity can be beneficial for achieving lowered operational costs for WWT.

Recently, a novel autotrophic nitrogen removal process, i.e. deammonification, has been developed. This consists of a partial nitrification or nitritation, in which approximately half the ammonium is converted to nitrite by AOB. This is then followed by an anaerobic ammonium oxidation (anammox) process, governed by anammox bacteria, wherein the remaining ammonium and nitrite is converted to Nitrogen ($N_2$) (Figure 1*b*)[4]. The anammox process has gained much research traction and has been applied extensively in Europe. It requires less energy through reduced aeration and requires no input of organic carbon compared to the conventional nitrification-denitrification WWT process[5–7]. Nitritation, the partial conversion of ammonium to a 50 : 50 mixture of ammonium and nitrite, is favoured as a feed for anammox (Figure 1*b*). Thus, a reduction in the activity of NOB is necessary to achieve this favoured feed and obtain energy efficiency and reduced costs.

Hence inhibiting NOB is beneficial for achieving a more cost and energy efficient WWT process. This applies to both the conventional nitrification-denitrification and the anammox process (Figure 1).


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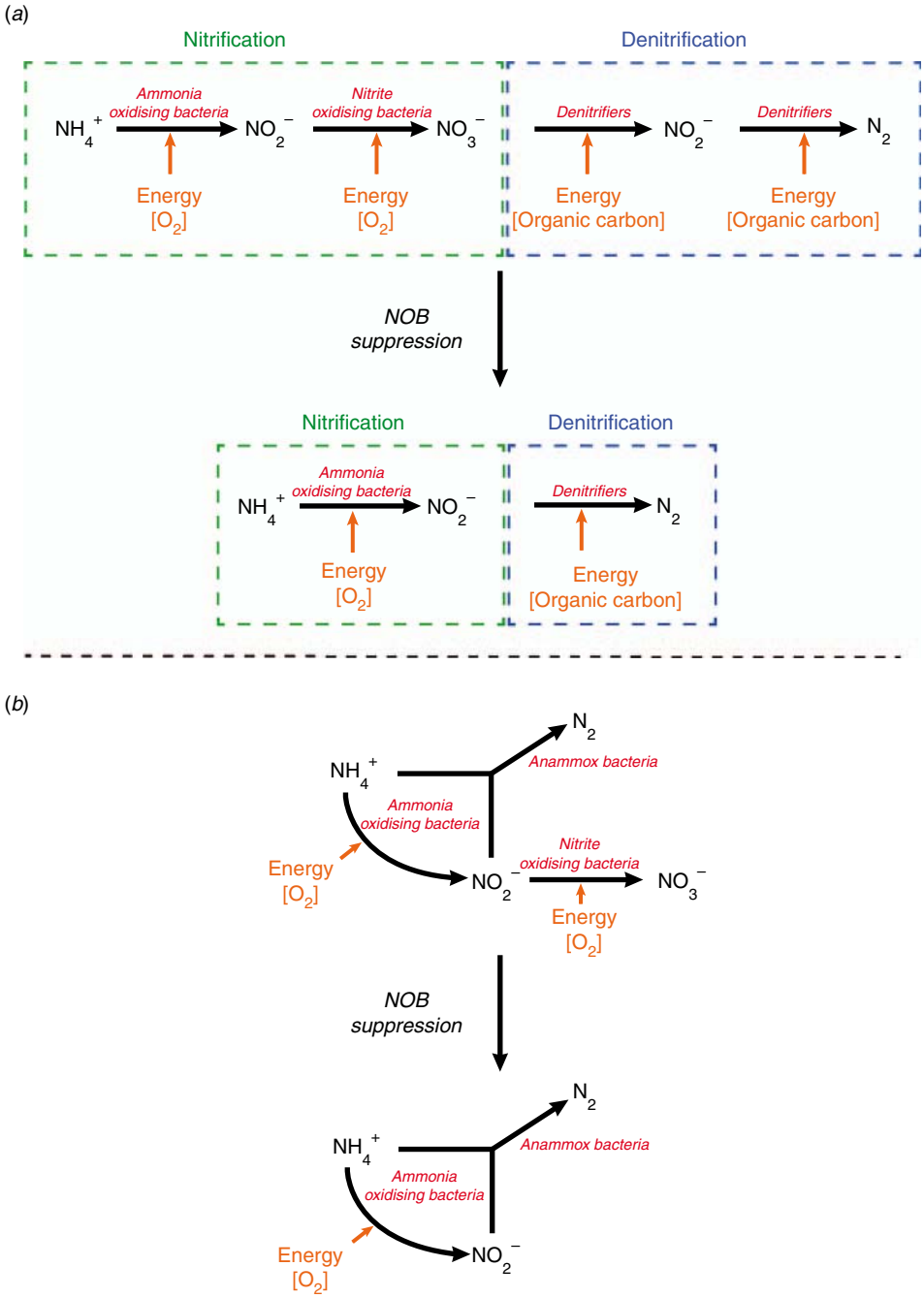


Figure 1. A schematic overview of the selective suppression of NOB in both (*a*) the conventional nitrification-denitrification process and in the (*b*) anammox process, which would allow for improved energy saving during the biological nitrogen removal in WWT.

## Achieving selective NOB suppression

Several approaches have been applied for controlling the NOB activity in both conventional nitrogen removal and the anammox process. A challenge for these processes is the selective inhibition of NOB activity and/or growth while retaining the presence of AOB (Figure 1). Both these groups have similar slow growth kinetics but require large substrate turnover and respiratory rates[5]. Various physical and chemical methods are proposed for suppression of NOB growth in bioreactors and include running bioreactors at limiting dissolved oxygen (DO) concentrations, increasing the temperature in combination with low solid retention times, and the use of intermittent aeration. However the relatively low nitrogen concentrations and low temperature in mainstream wastewater make it difficult to selectively inhibit NOB while allowing AOB to grow[5]. Interestingly, the addition of free nitrous acid (FNA) is successfully shown to selectively suppress NOB activity and growth in conditions otherwise favourable for nitrification[5].

## Free Nitrous Acid (FNA) suppression of NOB growth

The protonated form of nitrite, FNA is a biocide that is bacteriostatic in parts per billion and bacteriocidal in parts per million levels[8].

However, different types of microorganisms exhibit varying tolerance to FNA[8]. It turns out that AOB have substantially higher tolerance to FNA compared to NOB, and this provides opportunity to selectively inhibit NOB[9,10]. Indeed, it is seen that a FNA treatment of sludge, combined with low oxygen levels, selectively inhibit NOB and partially inhibit AOB. This treatment achieved partial nitritation and produced a water composition ideal for anammox nitrogen removal[5].

## Biocidal mechanisms of FNA action on various bacteria

Improved control of such bacterial populations will be achieved through understanding the antimicrobial effects of FNA and by determining the counteracting responses of the different organisms. Transcriptomic responses recently studied in *Pseudomonas aeruginosa* PAO1 and *Desulfovibrio vulgaris* reveal that FNA causes severe disruption to the bacterial energy conserving mechanisms[9,11]. Additionally, *Desulfovibrio vulgaris* shows multiple responses to oxidative stress during FNA exposure.

The higher tolerance of AOB to FNA is intriguing given that NOB have additional metabolic pathways to deal with high levels of nitrite compared to AOB. Both AOB and NOB have the nitrite detoxifying protein nitrite reductase (*nirK)* that converts nitrite to nitric oxide (NO)[12]. However, NOB have additional nitrite reductase (*nirBD)* and nitrite oxidoreductase (*norA/B)* genes, and thus possess the potential to convert nitrite to ammonia and nitrate, which could better alleviate toxic nitrite levels. Recently, a metaproteomic investigation revealed that FNA induced oxidative stress upon a nitrifying community. However, AOB was able to tolerate elevated levels of FNA compared to NOB due to a superior oxidative stress response[13].

## Concluding remarks

Determining the mechanisms of FNA action in both AOB and NOB is important to establish a deeper understanding of the difference in tolerance of these 2 groups of highly relevant nitrogen removal bacteria. Such understanding is significant for optimising strategies for improved reactor performance and for reducing the operational costs involved in the nitrogen removal WWT process.

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## Biographies


**Andrew Elohim Laloo** is a PhD student at the Advanced Water Management Centre, University of Queensland. His research focusses on studying wastewater microbiomes particularly those associated with nitrogen removal process using *-omic* technologies.

**Phil Bond** is a Senior Research Fellow at the Advanced Water Management Centre, University of Queensland. He studies various microbial ecosystems that include wastewater treatment, sewer corrosion, water supply systems, anaerobic digestion and acid leaching environments.

# Microbial cooperation improves bioleaching recovery rates

*Melissa K Corbett*

School of Pharmacy and Biomedical Sciences
CHIRI Biosciences
Curtin University
Perth, WA, Australia

*Elizabeth LJ Watkin*

School of Pharmacy and Biomedical Sciences
CHIRI Biosciences
Curtin University
Perth, WA, Australia
Tel: +61 2 9266 2955
Email: e.watkin@curtin.edu.au

**Whilst bioleaching is primarily used to recover minerals from low-grade ores, the increasing demand for Rare Earth elements combined with supply chain concerns is opening up new avenues of extraction from mine tailings, waste products and recyclable materials. Exploration of new, novel and economically viable techniques are required to manage the coming shortage and volatility of global markets with more environmentally sound alternatives to traditional mining operations holding the key.**

The exploitation of microbes in the industrial application of bioleaching has been underway since the 1950s[1] due to their ability to mobilise minerals from ore bodies, with either heap leaching implemented for the recovery of Cu, Zn, Ni[2] or stirred tank reactors for U or Au[3]. With fewer discoveries of large high grade mineral deposits occurring[4] it is anticipated that demand for raw minerals will outstrip reserves for not only these elements, but also for Rare Earth Elements (REEs). REEs are fundamental components of mobile phones, lasers, electric batteries and superconductors[5]. With dwindling supplies of high grade REE stocks, ever increasing demand for new technologies and a push for the mining industry to 'go green', the processing of lower grade ores, recycling of electronic waste and treatment of discarded mining by-products using bioleaching applications is proving attractive. Due to this the use and application of bioleaching techniques is expanding as they are more cost efficient, less energy intensive and employ more eco-friendly techniques[2].

REEs (15 elements with atomic numbers ranging from 57 to 71)[6] are located amid carbonates, placer deposits, pegmatites and marine phosphates[7]. However, current bioleaching applications utilise the autotrophic oxidation of ferrous and reduced sulphur compounds for mineral release and subsequent recovery, which are found in low amounts in REE ore bodies. Nevertheless, studies of REE mineral extraction from phosphate ores by bioleaching are in their infancy[8–10]. These bioleaching activities utilise acidophilic and heterotrophic phosphate solubilising microorganisms (PSMs), those often employed to increase soluble phosphate levels in agricultural settings. Species currently identified with the potential to recovery REEs from phosphate laden ores include *Pseudomonas*, *Acinetobacter*, *Bacillus, Microbacterium, Aspergillus*, *Penicillium* and *Cladosporium*[10]. Primarily, the focus of REE bioleaching investigations have utilised pure cultures on sterile ore and have resulted in varied rates of recovery depending on both the microbial species employed and mineralogical characteristics of the ore used.

For example, the fungal species *Penicillium tricolor* was shown to leach 30–70% of available REEs from red mud[9] compared to *Bacillus megaterium*, which leached less than 1% from monazite[11]. Industrial operations with ferrous and sulphide ores often involve two or more species due to the leaching chemistry requirements, size of the processes and the inability to maintain sterility. It has been shown that mixed acidophilic populations increase recovery rates of copper[12] compared to pure cultures. Our research initially conducted with pure cultures[13] (Figure 1) demonstrated low recovery rates of REEs from a concentrated Western Australian monazite, whereas when bioleaching was performed using non-sterile ore complete with the native population and an introduced PSM (Figure 2), REE leaching rates increased tenfold with some species[14]. These leaching rates were much greater than those recorded with either pure cultures or the native consortia alone.

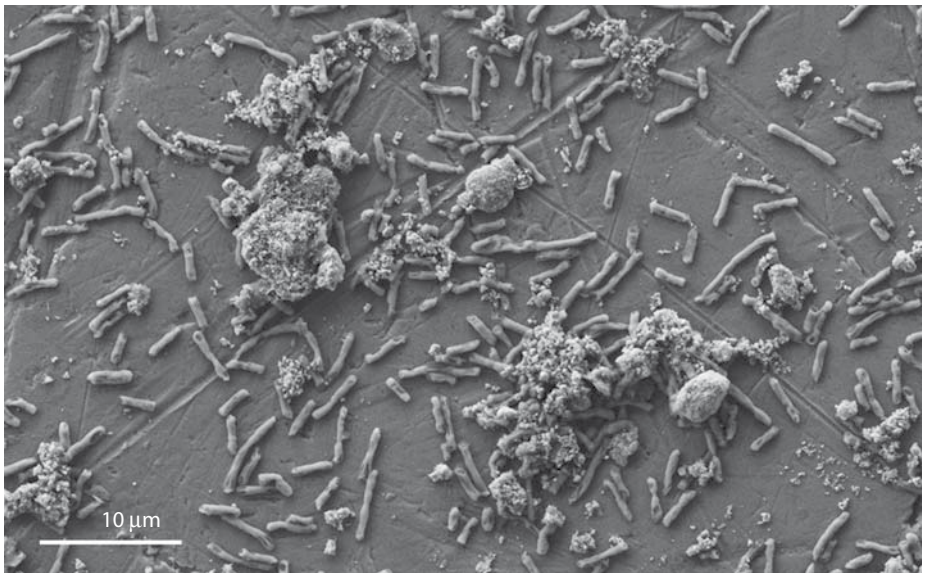


Figure 1. Scanning Electron Microscopy of pure cultures of *Enterobacter aerogenes* employed during bioleaching trials of sterile Mount Weld Monazite concentrate for the recovery of REEs. No indigenous microbes were identified during this leaching process.

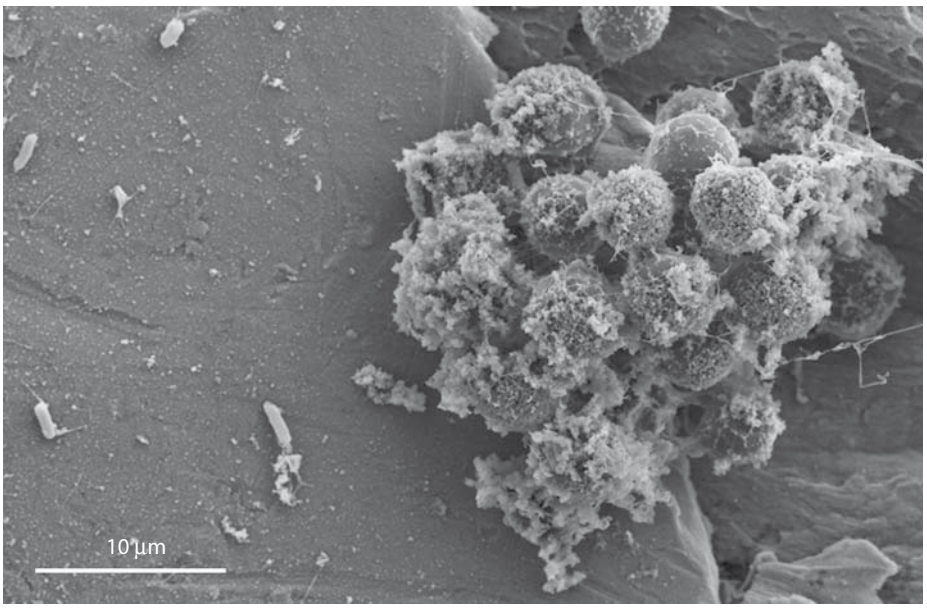


Figure 2. Scanning Electron Microscopy of non-sterile monazite bioleaching experiment inoculated with a starter *Penicillium* sp. After an 8-day incubation, establishment of native bacilli species and production of exopolysaccharide substances were detected along with fungal matter adhered to the monazite.

Cultures with a fungal starter organism such as *Penicillium* sp. had higher leaching rates than those commenced with a bacterial isolate such as *Enterobacter* or *Pseudomonas* sp. As the indigenous microbial population present on the ore is expected to be limited in number[2], the addition of a heterotrophic PSM aids in initiating leaching processes during the early operating phases.

Unlike the chemolithoautrophic pathways employed by the microbial consortia for growth during ferrous and sulphide leaching operations, heterotrophic leaching of REE phosphates requires the addition of a carbon source, usually in the form of glucose, which can be cost prohibitive on a large scale. The provision of molasses, a waste product generated from sugarcane refining is a financially more viable possibility that will meet microbial growth needs for optimum ongoing leaching. Fermentation of glucose by an introduced heterotroph to the non-sterile leaching environment results in the manufacture of numerous ligands, predominately organic acids including acetic, citric, formic, oxalic and pyruvic depending on the PSM employed, which drives a significant portion of the REE leaching process. This initial consumption of glucose by the introduced species and the resultant availability of secondary metabolites can enable the growth of heterotrophic, mixotrophic and acidophilic microorganisms already existing on the ore, with the presence of native *Firmicutes* notably increasing leaching rates[14]. In this symbiotic association, with the generation of secondary carbon compounds, a lowered pH environment is

Figure 3. Stirred tank bioleaching reactors set-up for optimisation of REE recovery from phosphate bearing ores.

established and as the system stabilises with increased numbers of indigenous species, the need to add further glucose is reduced. As it has been demonstrated that the native consortia alone are capable of REE leaching albeit at very low levels, initiation of indigenous activity appears to require one or more unknown metabolites that arise as a result of the inoculant species fermenting glucose.

The uptake of bioleaching as a viable alternative to traditional methods for the recovery of REEs has been slow due to the inherent unknowns in a biological based system and the uncertainty in value for money returns. In Australia there are currently no commercial REE bioleaching projects using heterotrophic or mixotrophic microorganisms despite Australia having one of the largest REE deposits in the world[15]. To encourage more mining corporations to opt for a more environmentally friendly approach to REE recovery, extensive research needs be undertaken to determine not only the best PSM to 'prime' the system, but also to examine the complex interactions occurring between the introduced PSM and native consortia. Armed with this evidence, optimisation of REE bioleaching operations (Figure 3) is an obtainable goal with improved leaching rates likely to allow the construction of long term reactor systems with decreased operating costs and lower environmental impacts.

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## Biographies


**Dr Melissa Corbett** is a lecturer in microbiology at Curtin University within the School of Pharmacy and Biomedical Sciences. Dr Corbett's post-doctoral research has focused on the use of microorganisms for bioleaching in saline conditions as well as investigating how to recover rare earth elements from radioactive sources using microbial action. For the past 5 years she has also lectured undergraduate students in the field of Microbiology.

**Elizabeth Watkin** is Professor of Microbiology at Curtin University. The overarching theme of her research is the microbial ecology of environmental systems and covers the fields of mining biotechnology and mineral resource recovery, microbial induced corrosion, and microbial fouling of water (particularly within mining systems).

# Understanding microbiomes through trait-based ecology

*Jennifer L Wood*

Applied Environmental Microbiology
Department of Physiology
La Trobe University
Bundoora, Vic. 3086, Australia
Email: Jen.Wiltshire@latrobe.edu.au

*Ashley E Franks*

Applied Environmental Microbiology
Department of Physiology
La Trobe University
Bundoora, Vic. 3086, Australia
Email: a.franks@latrobe.edu.au

**Ecology is the study of the interactions amongst organisms and their environment[1]. In microbial ecology, a major goal is to understand how environmental microbiomes impact ecosystem health and function. This desire to mechanistically link micro and macro processes is increasingly highlighting the importance of functional ecology, which aims to develop an understanding of relationships using functional traits, as opposed to species names. A functional trait may be any morphological or physiological trait that influences the performance or fitness of an individual in a given environment, such as regeneration time, size, antibiotic production or motility[2]. Although it is not possible to measure a given trait for each individual within an environmental microbiome, community-level functional traits can be derived from the community metagenome either directly via shotgun sequencing or predictively (for bacteria) from 16S rRNA profiles[3]. In understanding environmental microbiomes, functional traits have unique properties that can be utilised to (1) compare microbiomes using an ecological framework, (2) understand processes governing community assembly, and (3) build predictive ecological models.**

## Functional comparisons of environmental microbiomes

Functional traits are not necessarily conserved across phylogenetically closely-related taxa, but rather are conserved amongst organisms with similar life strategies. As such, trait-based comparisons of environmental microbiomes can be used to elucidate repeated ecological patterns across microbiomes even if they are taxonomically distinct. For example, if one was attempting to understand ecological similarities amongst geographically dispersed microbiomes from comparable ecosystems (e.g. wetland microbiomes), one may find that the communities contain vastly different suites of species. In this instance species names alone cannot be used to identify ecological trends uniting these microbiomes. However, because the microbiomes are from similar ecosystems, it is likely that within each microbiome different species will employ similar life strategies to survive and thus exhibit similar functional traits. In this way, functional traits can identify meaningful ecological patterns across taxonomically distinct microbiomes.

## Linking micro to macro: understanding of processes governing community assembly

The concept that environmental filters act on traits – not species – can be used to interpret how environmental parameters alter microbiomes in an ecologically meaningful way. The twin-filter hypothesis proposes that a two-step filtering process acts on local species pools: a primary 'ecological filter' increases the trait similarity within a community by selecting for similar life strategists (i.e. environments characterised by severe nutrient stress will select for traits that produce stress-tolerant life strategists); secondary 'proximal filters' then select against traits which affect survival but are not integral to the broad life strategy (e.g. variation in tolerance to environmental toxins or resistance to local pathogens and predators), creating dissimilarity within the local subset of species and generating the final community structure (Figure 1)[4]. By examining which traits are enriched by a given environment, or environmental parameter, we can begin to hypothesise how a community is experiencing that environment and why different communities diverge in their ecology. For example, Wood *et al*.[5] demonstrated that the community-level changes induced by the

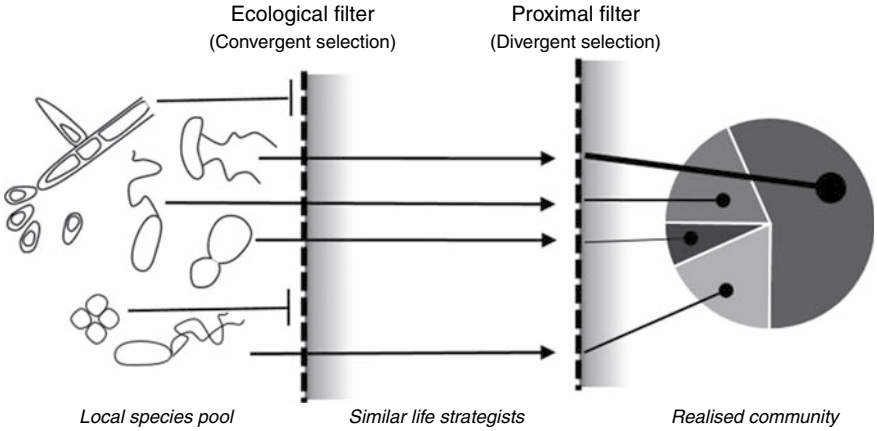


Figure 1. Schematic representation of the twin-filter hypothesis[4]. The local species pool provides a source of species that can potentially enter a community. An ecological filter acts on life-history associated traits, selecting for species that exhibit similar life strategies (e.g. stress tolerators, competitors, colonisers). Secondary 'proximal filters' such as toxins, predators or the type of carbon present determine final community composition.

Table 1. CSR theory definitions used to classify traits as competitive, stress-tolerant or ruderal, adapted from Grime and Pierce[4]. Selected macro and microbiological functional traits are given as examples of traits that can be associated with each C, S or R definition.

| Trait definition | Macro (plant) example | Micro example |
|---|---|---|
| **Competitive trait:** Traits facilitating the monopolisation of local resources | High chlorophyll concentration<br>Large leaves<br>Large canopy<br>Allelochemical production<br>Large root spread | High membrane transporter density<br>Siderophore production<br>Biofilm formation<br>Production of antimicrobial compounds<br>Filamentous growth |
| **Stress-tolerant trait:** Traits facilitating survival in chronically underproductive environments | Slow growth<br>Mechanical defenses (spines)<br>Chemical defenses<br>Detoxification mechanisms<br>Production of free radical scavengers | Slow growth<br>Altered membrane chemistry<br>Melanin/pigment production<br>Increased DNA repair ability<br>Production of free radical scavengers |
| **Ruderal trait:** Traits facilitating the rapid re-establishment of a population | Short life cycle<br>High photosynthetic capacity<br>High seed number<br>High seed dispersal ability | Rapid growth<br>Increased capacity for central metabolic flux<br>Overwintering structures (e.g. sclerotia)<br>High spore dispersal ability<br>Motility |

presence of a plant rhizosphere were due to the selection of traits linked to microbial competition for resources (i.e. antibiotic production, siderophore production). Similarly, DeLong *et al*.[6] demonstrated the presence of contrasting ecologies between communities from the phototrophic zone and from near-ocean floor depths, with foraging traits selected for in the phototrophic zone, whilst survival (stress tolerance) traits were characteristic of communities at depth.

## Building trait-based predictive models

Functional traits can be incorporated into ecological classification frameworks which aim to predict how environmental microbiomes will change over time. Grime's CSR theory is an ecological classification framework that groups traits in terms of three broad life strategies: competitive, stress tolerance and ruderal (colonisation) life strategies[6]. Each life strategy group is an umbrella term that encompasses multiple functional traits which achieve the same outcome (Table 1). For example, stress tolerance traits may be defined as any trait that constitutes an investment in the maintenance of organismal biomass. In plants this may be the production of thorns or chemical compounds to deter herbivory. In an environmental microbiome this may manifest as an increase in the prevalence of DNA repair pathways or genes involved in the production of free radical scavengers.

The CSR theory proposes that organisms face a three-way resource trade-off between the investment in C, S or R life strategies, which is governed by the levels of stress (due to resource availability) and disturbance present in an environment[4]. The theory predicts that when stress and disturbance are minimal, the investment of resources into competitive traits confers a selective advantage that outweighs the loss in fitness due to reduced investment in other adaptive strategies, such as stress-tolerance or colonisation

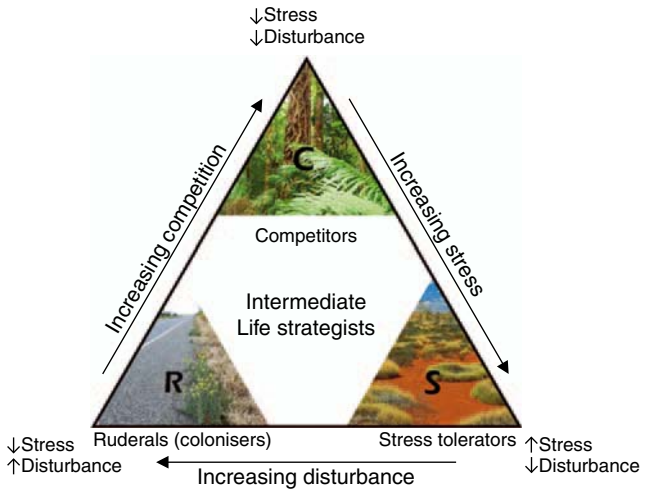


Figure 2. Diagrammatic representation of the CSR theory - Competitive, stress tolerant and ruderal life strategies (and their associated functional traits) form a three-way resource trade-off triangle. The selective advantage of each life strategy – and consequentially the amount of resources allocated to it – are governed by levels of environmental stress and disturbance. Where: **Stress** = external constraints limiting the rates of resource acquisition, growth or reproduction; **Disturbance** = an event causing the partial or complete destruction of cellular material. Images depict plant communities that represent typical examples of C, S or R dominated communities.

potential (Figure 2). In these communities, competitive interactions – and organisms with traits that contribute to a competitive life strategy – will prevail. Conversely, the theory predicts that stress-tolerance traits and life strategies will prevail when stress is high (i.e. resources are limited) but disturbance is low. Stress tolerant life strategists tend to be slow growing and are adapted to retaining resources. Finally, when stress is low but disturbance is high the theory predicts that ruderal traits, which pertain to re-colonisation potential, will confer a selective advantage and ruderal life strategists will prevail.

The use of ecological theories, such as the CSR hypothesis, presents a clear route towards developing predictive models which could be incorporated into ecosystem-level conservation and management practices. Indeed, even though CSR theory has its roots in plant ecology, the core principals are recognised as being applicable to microbial communities[7–9]. A current barrier to developing predictive trait-based models is that ecological interpretations of microbial traits often rely on prevalent opinions from the literature, rather than on empirical data. For example, the production of antimicrobial metabolites is generally considered to be a competitive trait[10]. However, compounds recognised for their antibiotic activity *in vitro* have been shown to influence biofilm formation in *Bacillus subtilis* suggesting their primary role may be cell–cell communication[11].

Future research using controlled microcosms with defined gradients of resource availability (stress) and disturbance are needed to confirm ecological assumptions about functional traits. Trait screening using controlled conditions can also be used to identify core predictor traits that can routinely and robustly discriminate between environmental microbiomes with contrasting ecologies. Ultimately, the development of broad ecological theories that facilitate the classification and comparison of microbiomes from disparate environments will assist in realising the full potential of large-scale collaborative initiatives, such as the TerraGenome project[12], the Earth Microbiome project (EMP)[13] and Australia's Biomes of Australian Soil Environments (BASE)[14] project.

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## Biographies

**Dr Jennifer L Wood** is post-doctoral fellow in the Applied and Environmental Microbiology Laboratory at La Trobe University. She has recently completed her doctoral research which investigated the utility of functional approaches in understanding soil microbiomes for which she was awarded the 'Nancy Millis award for theses of exceptional merit'. Her broad research interest is in developing trait-based approaches to interpret and predict how environmental and anthropogenic changes impact microbial community ecology and function. Currently she is working with the

Defence Science Technology Group investigating functional changes associated with microbiologically influenced corrosion in marine environments.

**Associate Professor Ashley E Franks** is head of the Applied and Environmental Microbiology Laboratory at La Trobe University. He conducted his doctorate research as part of the Centre of Marine Biofouling and Bioinnovation at the University of New South Wales by investigating antifungal compounds produced by marine bacteria in biofilms. During his PhD he spent 4 months at the University of Exeter in the UK on an Adrian Lee Fellowship to develop dual bacterial/yeast biofilm systems. On graduating he moved to the Biomerit Research Centre in Cork, Ireland to work on bacterial plant interactions as a Government of Ireland Fellow in Science Technology and Engineering. This research looked at how to use bacteria to help plant growth. He then took a position as a Senior Scientist and Research Professor within the Geobacter Project at the University of Massachusetts Amherst in the USA where he worked on microbes that make electricity. He currently serves as the Chair of the Awards Committee for the International Society of Microbial Electrochemical Technology and was previously the Secretary of Synthetic Biology Australasia.

# Incorporating fungal community ecology into invasion biology: challenges and opportunities

*Eleonora Egidi*

Department of Physiology,
Anatomy and Microbiology
La Trobe University
Kingsbury Drive
Bundoora, Vic. 3083, Australia

*Ashley E Franks*

Applied Environmental
Microbiology
Department of Physiology
La Trobe University
Bundoora, Vic. 3086, Australia
Email: a.franks@latrobe.edu.au

**Recently, the role of the plant-associated mycobiome (i.e. the fungal community) in influencing the competitive success of invasive plant species has received increasing attention. Fungi act as primary drivers of the plant invasion process due to their ability to form both beneficial and detrimental relationships with terrestrial plant species. Here we review the role of the plant mycobiome in promoting or inhibiting plant species invasion into foreign ecosystems. Moreover, the potential to exploit these relationships for invasive plant control and restoration of native communities is discussed. Incorporating fungal community ecology into invasion and restoration biology will aid in the management and control of invasive plant species in Australia.**

Alien invasive plant species represent an ever-increasing worldwide problem. The expansion of invasive species in non-native ranges can dramatically alter the structure and population dynamics of the invaded community, with the negative impact of invasive plants on ecosystem structure and function resulting in changes to native vegetation composition and productivity, nutrient cycling, soil characteristics, and even human well-being[1].

Many factors regulate exotic species naturalisation and invasion success, including the ability to rapidly access resources, allelopathy, and the modification of ecosystem processes (reviewed in Levine *et al.*[2]). However, an increasing body of evidence suggests a pivotal role for the plant-associated mycobiome (i.e. the fungal community) in influencing the competitive success of invasive species[3–6]. Fungi are important terrestrial ecosystem components, acting as mutualists, pathogens, decomposers, and food sources. Because of their primary role as drivers of many ecosystem functions and their ability to establish intimate relationships with terrestrial plant species (e.g. mycorrhizal fungi or leaf endophytes) (Figure 1), fungal communities can critically influence plant fitness and survival and, hence, their colonisation and invasion patterns[5,7].

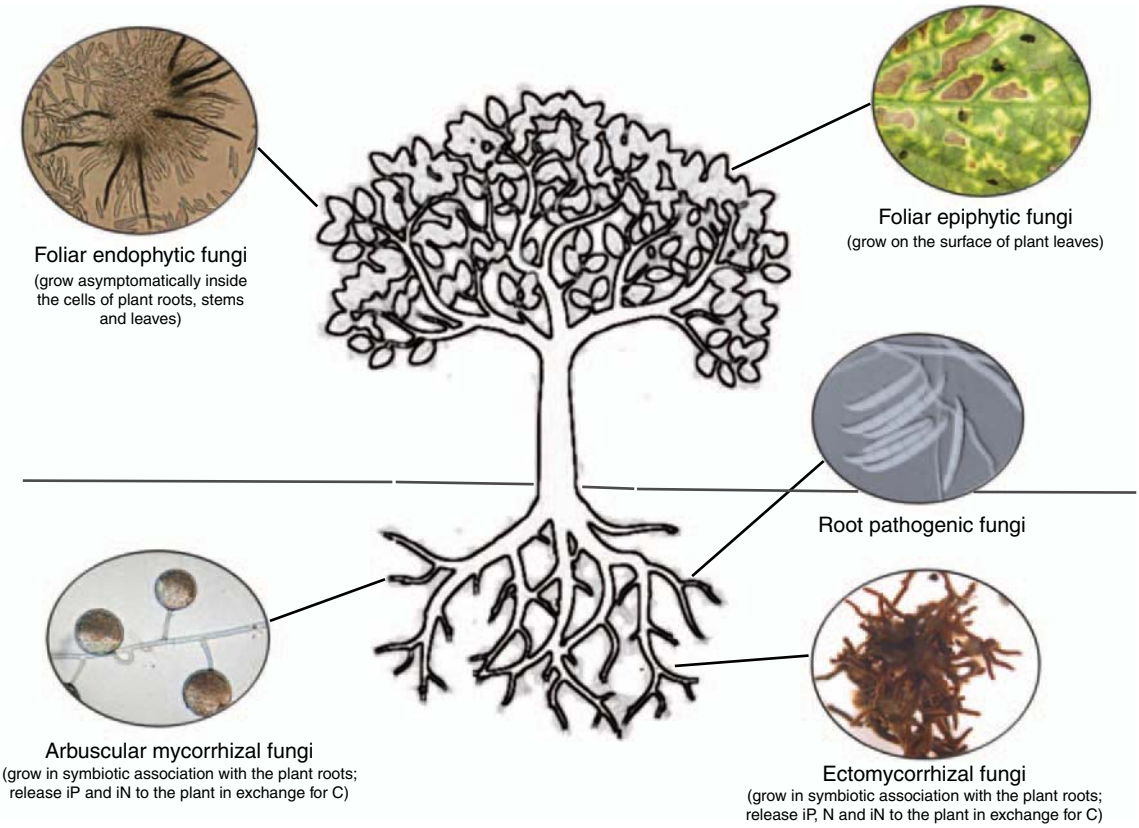


Figure 1. The plant-associated mycobiome. Fungi live in close association with many plant compartments, where they carry out different functions (see Peay *et al.*[7]). iP, inorganic phosphorous; N, nitrogen; iN, inorganic nitrogen; C, carbon.

Understanding the mechanistic interactions underpinning the invasion process is predicted to be particularly important for Australia, where invasive species pose not only a threat to the biodiversity of its unique vegetation, but also an economic problem[8]. Following accidental and deliberate introductions after European colonisation of the continent, approximately 2700 exotic plants species are now considered established within the continent, with almost 250 having been declared harmful or being under some form of legal control measure[9]. In terms of economic burden, the control of alien plant species is calculated to cost approximately AU$4 billion annually for agriculture alone[10]. The cost associated with managing native plant communities, although difficult to estimate, is predicted to also be considerable.

Given the environmental and economic implications of invasive plant species control, the need to find effective strategies for the prevention, early detection, and eradication of invasive species now represents a priority in many economic and scientific agendas. Here we discuss the role of the plant mycobiome in promoting or inhibiting invasive plant species incursion into foreign ecosystems, and propose ways through which these relationships can be exploited for invasive plant control and native communities restoration.

## Mechanisms and effects of fungi-plant interaction during invasion

*Pathogenic relationships*: The interactions between invasive species and their fungal communities are complex, with invasion success often being defined by the nature of such relationships. For example, during invasive plant establishment, pathogenic fungi can form novel associations detrimental to the invasive plants[11]. This possibility is usually amplified if the invasive and native plants are not phylogenetically related, resulting in specific targeting of the invasive species, and thus prevention of invasion through inhibition (Figure 2). In contrast, successful invasion is often promoted by the loss of detrimental microbes, such as pathogenic fungi, particularly when they are not present in the newly colonised habitat[12]. Invasive species can also act as reservoirs for microorganisms that are pathogenic for the invaded community, with a consequent enhancement of the negative impact of invasion on the native vegetation, as observed during the invasion of non-native *Spartina alterniflora* and its fungal pathogen (*Fusarium palustre*) on native Chinese saltmarsh plants. This instance implies the ability of the invader population to resist or tolerate native pathogens within the invaded areas, causing an increase in pathogen loads detrimental to the native community[13].

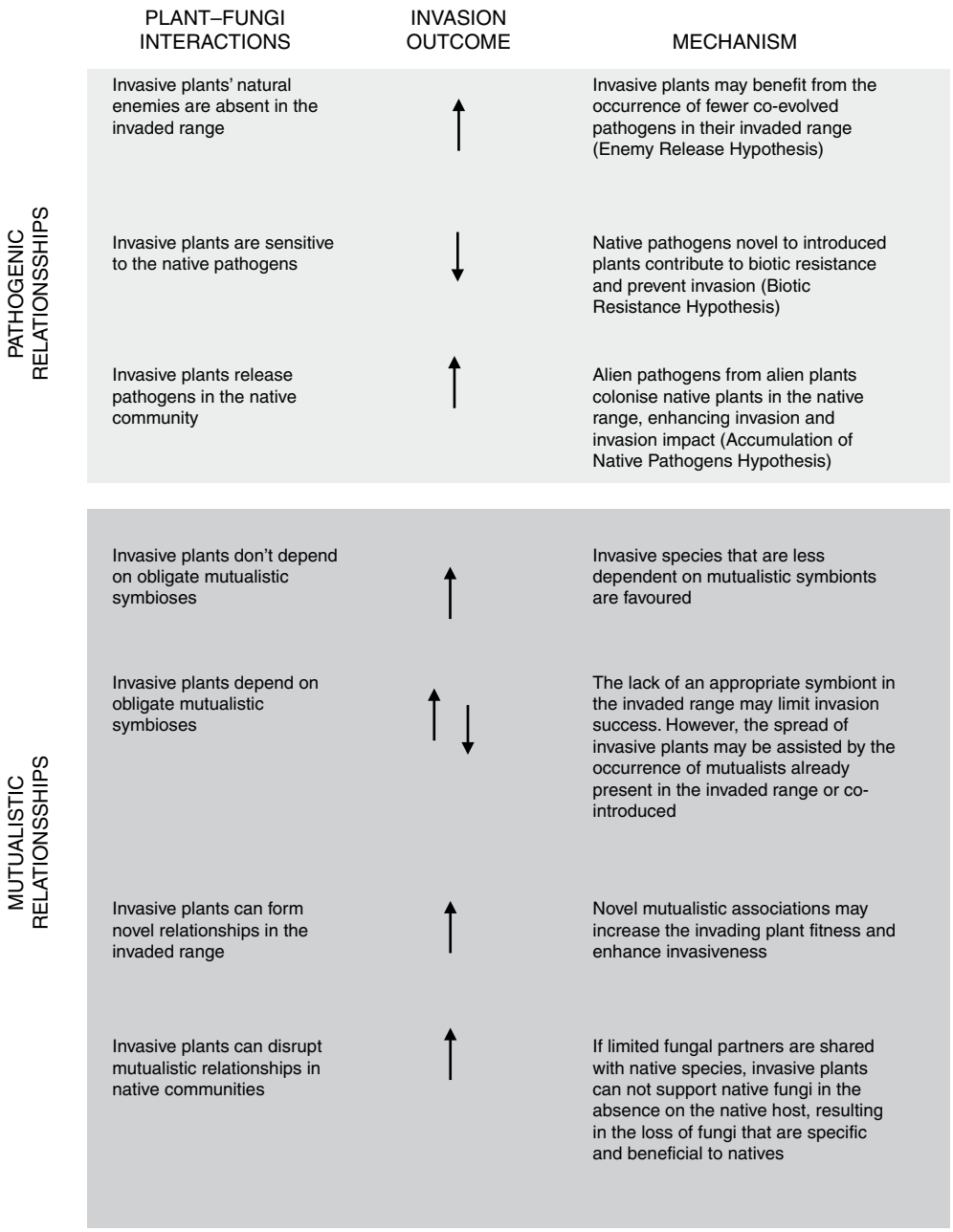


Figure 2. Role of the plant mycobiome in facilitating and/or constraining plant invasion success. The outcomes of the invasion process are represented by arrows pointing up or down, indicating favourable or disadvantageous conditions for the establishment of alien plants, respectively.

*Symbiotic relationships*: An equally important aspect of the plant-fungi relationship during the invasion process pertains to the role of symbiotic interactions, such as mycorrhizal interactions. Mutualism preservation is often vital for the invaders, and the occurrence of such beneficial interactions in the invaded habitat can be enhanced by the presence of native arbuscular mycorrhizal fungi with low endemism and low specificity in their range of associations[14]. Invading plants able to form novel symbiotic associations are also typically more successful. This strategy is particularly relevant for arbuscular mycorrhizal plants, which tend to establish novel associations in their exotic range, resulting in increased fitness and enhanced invasiveness[15], such as in the case of the invasive North American species *Ambrosia artemisiifolia*. Alternatively, the co-introduction of associated symbionts in the colonised habitat may favour the invasive plants[5]. Co-introduction is usually due to the transport of infected plants or propagules, or from the translocation of contaminated soil. The presence of a suitable symbiotic partner, either native or alien, can significantly enhance invader fitness in the newly colonised habitat, as observed in the case of non-native willows introduced with their native fungal symbionts in Southern Australia riparian systems[16].

In some instances, co-introduced symbionts may also be able to modify the native microbial community structure, including

substantial loss of belowground diversity and native mutualists, with detrimental repercussions for the native host's fitness. Such invasive processes can result in negative modifications of the functional attributes of the invaded system[5]. For example, the invasion of non-ectomycorrhizal communities by ectomycorrhizal plants causes both a decrease in soil carbon and a co-release of nutrients from the soil[17]. Plant-fungus co-invasion may also cause shifts in the total plant biomass; a change either enhanced by the increase in mutualistic fungi[18] or decreased by the pathogenic invaders[19,20]. Both the biomass shift and the plant compositional modification can critically alter important ecosystem processes in the native community invaders[19,20].

## Implications for management

A better understanding of plant-fungi interactions can have important repercussions for the mitigation of negative impacts of invasive plants and the design of better restoration strategies[5]. Novel microbe-mediated approaches for invasive plant control may include the inhibition of fungal symbiotic relationships that provide competitive advantages to invasive plant species (e.g. through the introduction of pathogenic microbes or inhibition of beneficial fungi)[4,21]. In particular, the co-introduction of native pathogens has been proposed as an effective strategy to manage alien plant species. An example of the successful application of fungi as biocontrol agents is represented by *Phragmidium violaceum*, used to control invasive blackberry trees in Australia[22]. Similarly, the rust fungus *Uromyces pencanus* has been proposed as a promising biocontrol agent to reduce the spread of *Nassella neesiana* (Chilean needle grass), a grass species invasive to the southern hemisphere[23].

In addition to the invading plant management, the plant-mycobiome relationship can be harnessed to mitigate the legacies of disrupted fungal communities resulting from processes such as plant-fungi co-invasion. Effective restoration strategies may be represented by manipulations targeted to increase the fitness of native plants. Particularly, the effectiveness of using native inocula to improve the establishment, growth and diversity of plants in their native range has been demonstrated in many instances (e.g. see Maltz and Treseder[24]), offering an alternative approach to the native community re-establishment. In this sense, national initiatives such as the 'Biomes of Australian Soil Environments' (BASE) project[25] may offer a useful baseline to explore the Australian microbial biodiversity and map the occurrence of suitable microbial partners in potential reintroduction ranges, thus facilitating the selection of reintroduction locations that support a fungal community similar to the native plant community of origin[26].

## Conclusions

In the past decade, a mounting body of evidence from ecological studies has contributed to unravelling the pivotal contribution of the plant-fungi interaction in mediating the successful plant invasion. Much of this novel insight converged to recognise that invasion dynamics cannot be understood or predicted without a thorough characterisation of the relationships occurring between invasive and native plants together with their respective microbiomes[5,21]. We therefore believe that approaching the plant-fungus relationships during invasion ecology studies in a mechanistic framework represents a fundamental prerequisite to better understand the processes underpinning the competitive success of invasive plant species, and thus design effective and long-lasting management and restoration strategies. Particularly, the characterisation of the microbial diversity and temporal variability, including endophytes and rhizosphere microbes, relevant to both the invasive and native plants at different growth stages, as well as the identification of their functional role and their effect on plant growth, development and tolerance[21], will represent the foundation to design effective microbiome manipulation strategies and predict possible invasion outcomes. Exploring new venues to protect and restore native vegetation communities may be particularly relevant for Australia, a continent where many terrestrial systems are experiencing a rapidly increasing environmental pressure related to climate change, habitat loss and fragmentation, and growing human populations. Further research focused on elucidating the impact of linked plant-fungal invasions in the context of ecosystem-level and community assembly processes holds promise to provide a solid scientific framework to predict invasion trajectories, and thus improve the outcomes of alien plant invasion management approaches.

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## Biographies

**Dr Eleonora Egidi** is mycologist interested in different aspects of fungal research, including fungal ecology, biology and phylogeny. Eleonora has an international background and has been involved in collaborative projects for the identification of new fungal barcoding regions, study of fungal communities from extreme environments, and use of fungi in phytoremediation. She is currently a postdoc in the Applied and Environmental Microbiology Laboratory at La Trobe University, where she is investigating the biodiversity of Australian fungi. Her scientific interest focusses on the role of fungi as drivers of plant diversity in Australian natural ecosystems, as well as understanding how these communities respond to environmental changes.

**Associate Professor Ashley E Franks** is head of the Applied and Environmental Microbiology Laboratory at La Trobe University. He conducted his doctorate research as part of the Centre of Marine Biofouling and Bioinnovation at the University of New South Wales by investigating antifungal compounds produced by marine bacteria in biofilms. During his PhD he spent 4 months at the University of Exeter in the UK on an Adrian Lee Fellowship to develop dual bacterial/yeast biofilm systems. On graduating he moved to the Biomerit Research Centre in Cork, Ireland to work on bacterial plant interactions as a Government of Ireland Fellow in Science Technology and Engineering. This research looked at how to use bacteria to help plant growth. He then took a position as a Senior Scientist and Research Professor within the Geobacter Project at the University of Massachusetts Amherst in the USA where he worked on microbes that make electricity. He currently serves as the Chair of the Awards Committee for the International Society of Microbial Electrochemical Technology and was previously the Secretary of Synthetic Biology Australasia.

# 2017 ASM Tri-State Scientific Meeting

*Paul Sideris,* Conference Chair

The 2017 ASM Tri-State Scientific Meeting was held in conjunction with the ASM Parasitology and Tropical Medicine SIG Parasitology Masterclass during September 2017 at the Novotel City in Darwin, NT.

The ASM Tri-State Scientific Meeting has been initiated and supported by the SA and NT and WA ASM branches for over 25 years. It is held every 3 years and organised alternately between SA/NT and WA. This year it was SA/NTs turn to organise the meeting. The aim of Tri-State is to effectively bring ASM to the Australian Top End providing NT ASM members and colleagues with an informative meeting on scientific and clinical Microbiology uniquely relevant to northern and central Australia incorporating local and indigenous issues. The format of the meeting is always relaxed, casual and intimate with the opportunity to also provide delegates with networking opportunities they otherwise may not have.

In order to attract more delegates, it was decided this year to combine the Tri-State meeting with another meeting. As parasitology is also relevant to the Top End, the Parasitology and Tropical Medicine SIG were approached and were more than happy to form with us to provide back to back meetings.

The LOC consisted of the following: Paul Sideris (SA), Chris Ossowicz (SA), Peter Traynor (SA), Harsha Sheorey (VIC), Stephen Kidd (SA/NT Branch Chair), Phil Giffard (NT), Rebecca Wake (WA), Pam Smith (NT) and Brooke Taylor (NT).

The international speakers included Professor Harvey Rubin from the University of Pennsylvania USA, Dr Richard Bradbury from CDC in the US and Dr Bert Mulder from The Netherlands. The Scientific Program for Tri-State included sessions on Mycobacteria, Scabies, Bacterial Skin infections in the Top End, STDs, Antimicrobial Sensitivity Testing, Microbiology in the Congo, Australia's Preparedness for Emerging and Exotic Vector-borne Diseases and the Energise the Chain global project. All the speakers were excellent and relevant and the audience was engaged and inspired.

Both meetings were well attended; 56 delegates attended the Tri-State Meeting and 54 attended the Parasitology Masterclass (PMC). The majority attended both meetings. All in all it was an excellent and very successful meeting enjoyed by the delegates and presenters alike.

Local ABC Radio requested an interview with Harvey Rubin. The interview discussing the Energise the Chain project can be heard at the following link: http://www.abc.net.au/radio/darwin/programs/afternoons/phone-towers-saving-vaccines-and-children/8986070

The LOC together with SA/NT ASM are extremely grateful for funding provided by ASM to hold this meeting. We are also extremely grateful for the sponsorship provided by ThermoFisher Scientific and Cell Biosciences. Without this contribution of funds it would not be possible to hold meetings such as this that benefit our members enormously.

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