MMEG 2022 Abstracts

Thursday, Dec 1st, 2022

09:15 - 10:00

Keynote Speaker: The importance of volatile compounds in microbial interactions

Dr Paolina Garbeva (Netherlands Institute for Ecology)

The ability of microorganisms to release volatile compounds has attracted research attention only in the last decade. Recent research has revealed that microbial volatiles are chemically very diverse and play important roles in long-distance interactions and communication. Microbial volatiles can diffuse fast in both gas- and water phase and hence, mediate swift chemical interactions. Next to the constitutively emitted volatiles, microbes can emit induced volatiles that are triggered by microbial interspecific interactions. For example, the antimicrobial volatile compound 2,5-bis(1-methylethyl)-pyrazine is produced as a result of interaction between the Gram-positive Paenibacillus sp. AD87 and the Gram-negative Burkholderia sp. AD24. The unusual terpene sodorifen, is produced by Serratia plymuthica PRI-2C in response to the fungal pathogen Fusarium culmorum. In my talk, I will highlight the importance of microbial interactions for the production of volatile compounds. Furthermore, I will present and discuss recent insights into the natural functions of microbial volatiles and their role in inter-specific and inter-kingdom interactions.

10:00 - 11:30
Session: Chemical Ecology & Environmental Chemistry.
Co-chairs: Dr Katherine Duncan & Dr. Darren Scobie (University of Strathclyde)

Ainsley Beaton[1], Tom McLean[1], Matt Hutchings[1]
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Understanding the Regulation and Ecological Functions of Natural Product Antibiotics

Introduction: *Streptomyces* are ubiquitous in Nature and produce many specialised metabolites, but their advantages for production is not well understood. Understanding their regulation in response to the environment could be key to the discovery of novel specialised metabolites. Two-component systems (2CS) sense and respond to environmental conditions.
Key results: The CutRS 2CS is highly conserved in *Streptomyces* and is known to affect the production of specialised metabolites, with its deletion increasing production of the redox-active antibiotic actinorhodin in *S. coelicolor*. ChIP-seq and proteomics suggest a link between secretion stress and CutRS. The only conserved residues in the extracellular sensor domain of all CutS homologues are two cysteines which may be important in sensing disulphide bond formation and correct folding of secreted proteins. Substitution of these leads to a change in function of the CutRS system that suggests it is essential for CutRS functionality. We hypothesise that loss of CutRS induces actinorhodin production because this molecule can oxidise cysteine residues and rescue the defect in protein folding.
Concluding statement: We propose that actinorhodin, although typically described as an antibiotic, is produced as part of the host secretion stress response.

Key words: Regulation, Secondary metabolites, signal detection

Seasonal stability and species specificity of two marine sponge microbiomes

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Introduction

Marine sponges are globally distributed and functionally important members of marine and freshwater systems, with well-established microbial associations which can account for over a third of the sponge biomass. Many species harbour an abundant, complex microbial consortia which is often host-specific, but in other species can be demonstrative of a transitory associations with the surrounding water column. Environmental changes, such as light, temperature and pH fluctuations, have been shown to result in alterations to the sponge holobiont.

Key Results

Next-generation sequencing of two marine sponges native to the U.K. (*Hymeniacidon perlevis, Suberties massa*) was performed at two temperatures. Diversity within *S. massa* was dominated by one sponge-enriched family, *Terasakiellaceae*, with remaining communities showing a similar community composition to the surrounding seawater. In comparison a number of sponge-enriched and sponge-specific families were detected in *H. perlevis*, demonstrating a more complex and diverse microbiome than *S. massa*. Both sponges showed stable, consistent microbial associations across seasons.

Concluding Statements

A core microbiome was identified in each sponge species which was host-specific despite close geographical associations and was not disrupted by temperature changes. Further work is underway to examine microbial metabolic pathways active in the sponge holobiont.

Key words: marine, sponge, microbiome, microbiome-stability

Ecosystem-scale patterns in the diversity and seasonal abundance of algicidal bacteria in the Western English Channel

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Diatoms are a major group of photosynthetic microalgae, which often dominate phytoplankton communities and are widely recognised to have global-scale impacts on marine biogeochemical cycles. The co-existence of diatoms and bacteria in marine environments has resulted in a complex web of interactions ranging from synergistic to antagonistic. Of particular interest are antagonistic interactions between diatoms and algicidal bacteria, which are shown to influence diatom physiology, hence shaping phytoplankton communities. However, a lack of systematic reviews of the diversity and abundance of algicidal bacteria in nature means we have a limited understanding of their ecological significance.

Here, we combine laboratory and field-based approaches to characterise antagonistic diatom-bacteria interactions in the Western English Channel at an ecosystem scale. By employing a plaque assay method over an annual cycle, we examine the diversity and seasonal abundance of diatom algicidal bacteria in this highly productive ecosystem. We reveal previously unreported diatom-algicidal activity in 14 novel putative diatom pathogens, spanning ten orders of bacteria. Furthermore, we uncover the seasonal persistence of a broad range algicidal bacterium, *Ponticoccus alexandrii,* and identify a 'nutritional switch' underlying its algicidal activity.

Algicidal bacteria, diatoms, microbe-microbe interactions, plaque assay, pathogen

Environmental microbial genomics as a potential new tool to trace the origin of falsified antimicrobials

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Falsified antimicrobials are a threat to human health, representing 40% of illegally produced medicines reported globally. These products contain microbial contaminants because they are manufactured in unhygienic environment. Moreover, falsified antimicrobials may contain suboptimal concentrations of the active pharmaceutical ingredient, which facilitates the emergence of antimicrobial resistance. Eradicating falsified antimicrobials from the market is impeded by difficulties in tracing their geographic origin, necessary to tackle their illicit trade routes. Here, we present a series of experiments in which we are evaluating the use of 16S rRNA amplicon sequencing to unveil the pharmabiome of falsified antimicrobial products of known geographic origin. We aim is to test whether the microbial signature of the products can serve to trace their geographical provenance. Furthermore, we want to evaluate the potential of metabarcoding to pinpoint antimicrobial-inhabiting microflora that could facilitate antimicrobial resistance. These investigations are part of the interdisciplinary Wellcome-funded project FORESFA (FORensic Epidemiology and impact of Substandard and Falsified Antimicrobials on public health), whose ultimate goal is to integrate genetic, physico-chemical, social network analysis and modelling methods into common tools for countering the global criminal trade of falsified medicines.
Keywords: falsified antimicrobials, antimicrobial resistance, metabarcoding, microbial forensics

Microbial degradation of polystyrene microplastics in seawater analysed by stable isotope tracing techniques

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Introduction: Microplastics, which range from 0.001 – 5 mm in size, are an urgent and growing threat to the marine environment, with negative impacts across all trophic levels of the ecosystem. Polystyrene, which is widely used to produce disposable food containers, dining ware, and packaging foam, is one of the most dominant polymers identified amongst the millions of tonnes of plastic debris in the ocean. While some studies have demonstrated bacterial degradation of polystyrene in terrestrial environments, very few have considered biodegradation by marine microorganisms.

Key results: In this study, we took seawater from the northeast Atlantic and amended it with ^{12}C- or ^{13}C-labelled polystyrene microparticles (~150 μm diameter) to demonstrate biodegradation. Microbial taxa directly involved in polystyrene metabolism were identified using DNA-based Stable Isotope Probing (DNA-SIP). Complementing this, we also identified breakdown products via gas chromatography coupled with mass spectrometry, and isotope ratio mass spectrometry. A time series over three months provided insight into the rate of biodegradation.

Concluding statement: Polystyrene is a recalcitrant pollutant that is difficult to biodegrade. Our results can contribute to models for predicting the fate and longevity of polystyrene microplastic particles in the ocean.

Keywords: microplastics, polystyrene, biodegradation, marine, stable isotope probing

Interactions between microbial communities and plastics in freshwater and estuarine environments

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Plastics in rivers and estuaries are rapidly colonised by diverse microorganisms. However, knowledge on the diversity and function of plastic-associated microbial communities, and the biogeochemical alterations they might cause, is limited.

We carried out a field-based survey where we sequenced the 16S rRNA gene to investigate the microbial communities colonising plastic polymers and sediments from six rivers and three estuaries in the UK.

Our results showed significant differences between plastic-associated and sediment-dwelling microbial communities in both riverine and estuarine environments. In estuaries, the plastic-associated microbial composition was significantly enriched with Desulfosarcinaceae, Desulfatiglandaceae, and Sulfurimonadaceae compared to the sediment's communities. In rivers, plastic-associated bacteria had twice less observed species and lower Shannon and Simpson indices compared to sediment's communities. The river sites' beta-diversity showed that the communities grouped by sample type hence, having markedly distinct communities between plastics and sediments. Plastics in rivers were significantly enriched in Methylomonadaceae and Methylophilaceae.

Overall, our results suggest that plastics found in rivers and estuaries are colonised by distinct microorganisms enhanced with certain functional groups such as sulfate-reducers, sulfur-oxidisers and methanotrophs. Therefore, plastic pollution in rivers and estuaries could alter sulfur and carbon cycles.

Plastisphere, rivers, estuaries, biogeochemical cycles

Using “microbial-baits” to study seasonal microbial community dynamics in an arsenic-impacted aquifer in Cambodia

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Millions of people worldwide are at risk of chronic illnesses due to long-term exposure to trace levels of arsenic (As) in drinking water. Microbial processes have been implicated in As release from sediments into groundwater, possibly through the microbially-mediated reductive dissolution of Fe(III)/As(V) mineral assemblages. However, the precise mechanism of As release and the microbes involved remain under debate. Here we combined the *in situ* deployment of Fe(III)-coated “microbial-baits”, with geochemical and microbiological techniques to study the impact of seasonal changes in environmental conditions on the groundwater microbial community in an As-impacted aquifer in Cambodia. The collected aquifer water showed reducing conditions and high As concentrations, dominated by As(III). Our data suggest that seasonal perturbations may impact the groundwater biogeochemical cycles and change the microbial structures within the aquifer, which affects As-containing Fe mineral phases, resulting in As adsorption/desorption. These data illustrate that “microbial-baits” can be a useful approach for studying microbial community dynamics in remote environments, particularly during periods with restricted access to study sites.

Lower-order interactions dominate bacterial responses to complex chemical mixtures

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Our understanding of how microbes respond to pollutants is almost wholly based on single-species responses to individual chemicals. However, in natural environments, microbes are bathed in complex cocktails of pollutants and the response of microbes to mixtures of chemicals may not be readily predictable based on their responses to pollutants in isolation. Here we developed a novel framework to test interactions among multiple chemical stressors. We assayed the growth of model and non-model strains of bacteria in all combinations of 8 chemicals, allowing us to identify the high-order interactions that arise among chemicals. We found inconsistent responses of different bacteria to stressor mixtures, which could not be predicted from phylogeny. Bacterial responses to increasingly complex chemical mixtures were more likely to deviate from our null model predictions. However, net interactions were mainly explained by lower-order interactions among a small number of chemicals, with limited evidence of high-level “emergent” interactions. These results provide the basis for predicting microbial dynamics in perturbed ecosystems and may form the basis for a new generation of eco-toxicology.

Pollutant, Stressor, Interaction, Bacterial growth.

Culture and Metagenomic analyses of solvent contaminated sites

Thomas Hender [1], Steve Cartman [2], Leighton Pritchard [1], Nicholas Tucker [1]
University of Strathcylde[1] Mitsubishi Chemical[2]

The biosynthesis of industrial platform chemicals has become a promising route for a sustainable alternative to petrochemicals. Limitations due to toxic effects on production strains are currently a major bottleneck. Organisms from contaminated environments which have built a natural resilience to a variety of industrially relevant compounds may hold the key to a sustainable plastics industry.
The set design department of the Royal Conservatoire of Scotland (RCS) use solvents, paints and dyes that are frequently passed through the drainage system provided an alternative to an industrial. Here we demonstrate that mixed culture-metagenomics of RCS samples can be used to screen for tolerant-organisms to certain industrially relevant compounds. We show a dramatic increase in Pseudomonas, including a potentially novel species in enrichment cultures treated with a methacrylate ester involved in the production of acrylic plastic. Assembles of a conjugative-plasmid predicted to encode drug resistance mechanisms of the ATP binding cassette super family, the resistance nodulation cell division superfamily and mercery transport proteins MerP, MerT. These assemblies have been confirmed using PCR and full genome sequencing of the isolated organism. The organism RCS09A shows increased tolerance when compared to industry standard *P. putida.* This mega-plasmid could be used to improve production strain tolerance and increase yields.

Keywords: Metagenomics, Pseudomonas, Industrial Environments, Biotechnology, Mega-Plasmid

12:30 - 14:00
Session: Advanced Methods and New Techniques in Microbial Ecology. Co-chairs: Dr Liam Rooney (University of Strathclyde) & Dr Sophie Holland (Heriot-Watt University)

Developing a rapid high throughput test to detect biodegradation of environmentally relevant chemicals.

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Career stage of presenting author: Fifth year PhD student

Pharmaceuticals are present in the aquatic environment as a consequence of their use by society, and their effects on the biota present are of growing concern. Determination of the persistence of these pharmaceutical chemicals in aquatic systems is crucial, with standardised OECD tests in place to assess this. However, these tests are low throughput, do not characterise the microbial inocula, and their stringent standardisation does not always accurately reflect real, variable environmental conditions, such as temperature.

We developed a high-throughput, replicable method of measuring chemical biodegradation, using inocula concentrated by tangential flow filtration and microcentrifugation, to overcome the biodegradation lottery seen in degradation studies using environmental samples with low microbial biomass. This methodology was utilised in a year-long, seasonal assay, which assessed the microbial cell number, microbial cell viability, time to 50% degradation (DT_{50}) of *p*-Nitrophenol and Q10 at five time points.

Results show that the degradation rate of *p*-Nitrophenol showed less variability, a smaller DT_{50} ($P<0.0001$), and lower Q10 ($P=0.018$) with concentrated samples, compared to un-concentrated samples, demonstrating that microbial cell number and incubation temperature exert significant impacts on the outcomes of biodegradation and persistence tests, with the methodologies established allowing for subsequent testing on multiple environmentally relevant pharmaceutical chemicals.

Analysis of the microbial communities showed significant ($P > 0.05$) differences in community composition across the five months sampled in both natural river water, and concentrated inocula, but no significant differences in the relative abundance of bacterial classes between natural river water and cells concentrated by tangential flow filtration at any sampling time.

Electrochemical impedance spectroscopy testing of antibiofilm secondary metabolites

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Deaths from AMR are projected to increase to more than 10 million *per annum* by 2050. Bacteria within biofilms have shown resistance to 100-fold higher concentrations of antibiotics than planktonic cells. This is due to entering a dormant-like state, reducing their growth rate. As many antibiotics target mechanisms of active metabolism, they are less effective. New antibiofilm-metabolites are needed to inhibit formation and target established biofilms. Bacteria from the marine environment are a rich, untapped source of novel bioactive metabolites, many of which have not been tested for antibiofilm properties. We have isolated 12 actinomycetes from Scottish marine sediments and extracted their metabolites. These extracts have been quantified for their ability to prevent *Pseudomonas aeruginosa* (PA14) biofilm formation on sensors using Electrochemical Impedance Spectroscopy (EIS); validated using crystal violet staining. *P. aeruginosa* control samples measured with EIS showed significant decrease in ohms (1.06 – 0.709 Ω), while those exposed to 1 mg/mL of actinomycete metabolite extract showed no significant change (0.815 – 0.813 Ω), indicating biofilm formation. Our results show successful isolation of a *P. aeruginosa* antibiofilm agent, quantifiable by EIS. Next, *P. aeruginosa* biofilms will be formed on medically-relevant materials and the biofilm formation on these quantified with EIS.

Keywords: Biofilm; Pseudomonas aeruginosa; impedance; secondary metabolite; AMR

THE CULTIVABLE FRACTION OF THE OAK MICROBIOTA HAS SUPPRESSIVE PROPERTIES AGAINST ACUTE OAK DECLINE-ASSOCIATED PATHOGENS.

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Brenneria goodwinii is a key bacterial plant pathogen that causes tissue necrosis in oak trees affected by the decline-disease termed Acute Oak Decline (AOD). Microbial culture collections and other culture-based methods are essential to study and leverage the host-protective properties of the plant microbiota against pathogen attack. In our research, we generated and characterised a microbial culture collection of over 20,000 bacterial and fungal isolates from the above- and below-ground compartments of 150 healthy oak trees located across Britain. We found that two different isolation methods – agar plating and dilution to extinction – were required to capture different fractions of the oak microbiota. This was evidenced in the single-gene community profiling analysis of the isolates, where only 16% of the Amplicon Sequencing Variants overlapped between the two isolation methods. An *in vitro* plate-based screening of this collection showed that 1,065 bacterial isolates can supress the growth of the AOD pathogen *B. goodwinii*. These results demonstrate the disease-suppressive potential of the oak microbiome. In further work, we will test random synthetic communities of suppressive isolates in microculture plate assays and microbiome transplant experiments for tree disease suppression.

Key words: Plant disease suppression, Acute Oak Decline, culture-based approaches.

Sedimentary DNA records long-term changes in a lake bacterial community in response to varying nutrient availability

Amy C. Thorpe[1,2], Amy Anderson[3], Tim Goodall[1], Stephen J. Thackeray[3], Stephen C. Maberly[3], James A. Bendle[2], Hyun S. Gweon[4] and Daniel S. Read[1]

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Microbial communities play important roles in lake ecosystems and are sensitive to environmental change. However, our understanding of how bacteria respond to long-term change is limited because traditional palaeolimnological techniques (pigments and microfossils) are restricted to organisms with well-preserved structures. Sedimentary DNA (sedDNA) is a promising technique to reconstruct past communities using DNA preserved in lake sediments, but the ability of sedDNA to accurately record historical bacterioplankton dynamics is unknown. We sequenced and quantified the 16S rRNA gene in sediment cores from Esthwaite Water (English Lake District) which has concurrent long-term monitoring data. sedDNA revealed distinct shifts in bacterial community composition throughout the 113-year sediment core record, which included an episode of accelerated eutrophication. The record was validated using long-term microscopy-based monitoring of cyanobacteria in surface waters, and known pelagic bacteria were detected in the sediment. However, the quantity of DNA declined with depth, indicating some degradation may have occurred. These results suggest that sedDNA has significant potential as a record of past bacterial communities, but an improved understanding of DNA deposition and degradation is required to further the application of sedDNA in palaeolimnology.

Keywords: Lake, sedDNA, bacteria, cyanobacteria, palaeolimnology

Specialism and Generalism in Fluctuating Environments

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Environmental fluctuations are known to be key to maintaining population diversity. However, the influence of the temporal scale of these fluctuations has been understudied. We used an experimental evolution approach to study the effect of different temporal scales of between-generation environmental variation on microbial evolutionary trajectory, with a specific focus on the adaptation of specialists and generalists. We imposed a range of fluctuations by varying the environment at scales ranging from 1 day to 2 weeks and then used multiple estimates of microbial fitness to determine the population response to these changes. We predicted that the shortest scales of variation would give rise to a population of generalists adapted well to both conditions, while the longest temporal scale of variation would become sequential homogeneous environments, causing the evolution of sequential specialists adapted to each environment. Intermediate scales were predicted to lead to a population containing a mixture of specialists and generalists. The fitness of the population was estimated from the growth rate, the yield and the shape of the growth curve with particular focus on analysis of the 'Jack-of-all-Trades, Master-of-None' hypothesis. Initial analyses show that differential adaptation is best shown by the maximum yield.

[Keywords] experimental evolution, environmental variation, specialists, generalists

Know your river – rivers as a resource for all

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Rivers are a valuable resource for leisure, agriculture, and economic activities and can support extensive, dynamic ecosystems. Discharges from wastewater treatment plants, particularly during heavy rainfall, can cause pollution, damage ecosystems, and raise the risk of antibiotic bacteria surviving in waterways. Know your river is a citizen science project aimed at understanding recreational river use and river microbial communities. We invited members of the public to complete a survey on river use and separately collect samples from their local waterways, returning these to us for analysis. We performed chemical analysis, using Triple Quad LC-MS, to detect antibiotics and microbiology to enumerate *E. coli* and other coliforms and determine their resistance profile. 99% of samples contained coliforms and 33% *E. coli*, some with extensive multi-drug resistance. We found the most resistant *E. coli* came from a small number of the waterways, which also often had high levels of *E. coli* and coliforms. Two antibiotics, trimethoprim, and sulfamethoxazole were found in most rivers. Separately, we found that boating, swimming, and walking were the most common leisure activities, with those involved in boating being the most likely to report sickness associated with river use.

Intra-colony channel morphology in *Escherichia coli* biofilms is governed by nutrient availability and substrate stiffness

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Intra-colony channels have been recently identified in mature *Escherichia coli* biofilms, where they facilitate nutrient transport [1]. We show that substrate composition and stiffness have an effect on channel morphology.
We imaged live *E. coli* biofilms with fluorescence mesoscopy, which provides single-cell resolution within the whole biofilm volume [2], and we designed an image analysis pipeline using the image processing software FIJI and a Python script to quantify channel morphology. We found that channel width was proportionally larger at the edge of the biofilm with respect to the centre, irrespective of nutrient availability. Channels forming on carbon-limited substrates were on average 25% wider than those forming on nitrogen-limited substrates. Substrate stiffness variation led to a change in channel density inside biofilms grown on rich medium, with soft substrates leading to narrower and more densely packed channels than hard substrates.
This work demonstrates the first quantitative analysis of intra-colony channels in *E. coli* biofilms. Thanks to its simplicity, our image analysis pipeline can be easily adapted for the study of internal patterns in a diverse range of biofilms.

[1] Rooney LM, Amos WB, Hoskisson PA, McConnell G. Intra-colony channels in E. coli function as a nutrient uptake system. ISME J. 2020 Oct;14(10):2461-2473
[2] Gail McConnell, Johanna Trägårdh, Rumelo Amor, John Dempster, Es Reid, William Bradshaw Amos (2016) A novel optical microscope for imaging large embryos and tissue volumes with sub-cellular resolution throughout eLife 5:e18659

Keywords: *E. coli* biofilms, fluorescence microscopy, image analysis

Validation of High-throughput QPCR array and subsequent profiling of antibiotic resistance genes from Thai septic tanks

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AMR, *intI*1, Wastewater, HT-QPCR array

Antimicrobial resistance (AMR) poses serious global public health threat, and Wastewater treatment continues to be a major contributor of AMR genes and bacteria to the environment and is exacerbated in the global South where antibiotic use is unregulated. Recently developed high-throughput QPCR array technologies permit monitoring of hundreds of AMR genes and selected mobile elements simultaneously. We used the array to profile and quantify AMR and integrase genes from influent, effluent, and sludge within Thai septic tanks associated with household and healthcare units and subsequently, investigated the link between *intI*1 and overall AMR abundance. First, we validated the QPCR array by selecting subset of array primers (16S_*rRNA* and *intI*1 primers) and quantified Thai septic tank wastewater samples in-house and on the array.

Optimised array QPCR primers had 92.7-95.68% standard curve efficiency. A log-fold difference in Ct between array 16S_*rRNA* and optimised 16S_*rRNA* primer was observed. *intI*3 relative gene abundance significantly correlated (*p-value* < 0.001) with majority of targeted AMR genes, including those not associated with integron, compared to *intI*1. Slightly lower AMR gene abundances were observed in healthcare septic tank compared to household. Lower *intI*1 copies/ng DNA quantified with Array *intI*1 primers compared to previously selected optimised *intI*1 primer sets.

Biosynthetic Gene Cluster Distribution is Conserved Amongst Actinomycetes

Authors

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Affiliations

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Introduction

The specialised metabolites produced by actinomycete genera, most famously *Streptomyces*, are of critical importance to society by finding clinical use as antibiotics, immunosuppressants, and anti-cancer drugs. Despite this, there is a dearth of understanding as to how the development of these metabolites has played a role in the evolution of actinomycetes. In particular, how and if chromosomal location plays a role in the evolution of specialised metabolism.

Key Results

To answer this, we compare the chromosomal loci of specialised metabolite biosynthetic gene clusters (BGCs) in high-quality whole-genome assemblies of multiple actinomycete genera. By doing this, we have identified that multiple genera have genus-specific distributions of BGC, although there is a general pattern of BGCs accumulating in the origin-distal region of the chromosome. The distance of BCGs from the origin is partially determined by the nature of that BGC – for example, in *Micromonospora* NAGGN and T3PKS clusters accumulate towards the origin of replication whilst T1PKS and NRPS accumulate in the *ori*-distal region.

Concluding Statement

The chromosome is a molecular habitat inhabited by genes under constant selection pressure. This work demonstrates that at the BGC level, selection occurs based on chromosomal loci.

14:30 - 16:00 Session: Biogeochemical Cycling. Co-chairs: Dr. John Moreau & Dr. Fabien Cholet (University of Glasgow)

Methanotrophs co-oxidise isoprene to epoxyisoprene but do they constitute a sink for isoprene in the environment?

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Isoprene is a climate-active trace gas which is predominantly emitted by terrestrial plants in quantities similar to that of methane (~500 Tg(C) per year). Due to its volatility and reactivity, it has a major, but multifaceted, effect on atmospheric chemistry. An important biological sink for isoprene is oxidation by specialised isoprene-degrading bacteria that can use isoprene as a sole source of carbon and energy. These bacteria initiate isoprene degradation by oxidising isoprene to epoxyisoprene using isoprene monooxygenase (IsoMO), a multi-component enzyme and a member of the soluble diiron monooxygenase (SDIMO) family which includes soluble methane monooxygenase (sMMO), alkene and aromatic monooxygenases.

SDIMOs can typically co-oxidise a broad range of substrates. We report that sMMO-expressing methane oxidising bacteria (methanotrophs) produced epoxyisoprene from isoprene at comparable rates to that of the isoprene degrader, *Rhodococcus* sp. AD45. An important question which we are examining is if methanotrophs also constitute a sink for isoprene in the environment. *Sphagnum*-dominated peatlands host free-living methanotrophs, and they are potentially a significant source of isoprene. Using specific inhibitors of IsoMO and sMMO we investigated if isoprene uptake by *Sphagnum* moss was due to isoprene consumption by isoprene degraders or by non-specific co-oxidation by methanotrophs.

Isoprene, SDIMO, methanotrophs, *Sphagnum*

Nitrification in Intertidal Mudflat Driven by a Low Abundance AOB Cluster

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Nitrogen cycle in coastal ecosystems is largely driven by microbial activities. Nitrification plays a central role by controlling the concentration of available substrate for denitrification, leading to nitrogen loss and the production green-house gases. On the Montportail-Brouage mudflat (French Atlantic coast) the physical arrangement of the mudflat in ridges and runnels significantly affects microbial nitrification with higher rates measured in runnels compared to ridges.

We shown that, despite being more abundant in ridges, ammonia-oxidizing bacteria (AOB) are more active in runnels, explaining the higher nitrification rates. Sequencing of the *amoA* gene from co-extracted DNA and RNA revealed the presence of a low-abundance but high activity AOB cluster that likely drives nitrification in this ecosystem whereas the most abundant AOB cluster was inactive.

In conclusion, this study shows that *i)* terrain spatial heterogeneity can significantly affect rates of biochemical transformations and therefore need to be considered when accessing global biogeochemical budjets; *ii)* Nitrification in this costal environment is likely driven by small proportion of the AOB community that displays high nitrification activity whereas *iii)* the majority of AOB were inactive, illustrating *iv)* the importance of robust transcriptomics studies in environmental microbiology.

Keywords: Nitrification, transcriptomics, estuarine, nitrogen-cycle, *amoA*

Towards a better understanding of nitrogen cycling in soils across landscapes

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Volatile reactive nitrogen oxides (NO_y) are significant atmospheric pollutants, including NO + NO_2 (NO_x) and HONO + HNO_3 + NO_3(NO_z). Biogenic sources, including soil, account for over 50% of natural NO_y emissions to the atmosphere. Despite their importance, NO_y emissions from soils are generally not included in atmospheric models due to a lack of mechanistic data.

Spatial heterogeneity amongst landscapes and across population gradients likely influences NO_y fluxes due to differences in atmospheric deposition rates and anthropogenic soil modifications – likely altering microbial and abiotic cycling of NOy; however, this has not been explored in mechanistic detail. Here, we link nitrifying and denitrifying soil microbes, across a gradient of urbanisation and land-use, to NOy gas fluxes from soil. To resolve temporal discrepancies, soil sampling (0-10 cm) occurred seasonally.

Results show significant changes in relative abundances of microbial orders associated with nitrification, denitrification and nitrogen fixation across a human population gradient, and between land-use types, suggesting anthropogenic impact on soil microbiology. From structural equation modelling (SEM), we see NOy and NO fluxes significantly affected by land-use type. Soil physicochemistry is a major influence on other measured variables and is therefore likely to be an important predictor of soil NOy fluxes.

Keywords: Soil, Biogeochemical cycling, Anthropogenic impacts

Seasonal variability in microbial and phytoplanktonic DMSP cycling in temperate shelf seawaters

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Dimethylsulfoniopropionate (DMSP), an abundant organosulfur compound and source of climate-cooling gases, e.g., dimethylsulfide (DMS), is thought to be predominantly produced by marine phytoplankton. Increasing evidence of bacterial DMSP production in diverse marine environments challenge this. However, few environmental studies consider the bacterial contribution to total DMSP production. Here, we explored the dynamic associations between bacterial/phytoplanktonic communities, their genetic potential and DMSP/DMS production in shelf seawaters from March to July 2021. We observed two large DMSP spikes in April 20 and June 28, which coincided with high expression of the Dinoflagellate *DSYB* gene and an unpublished cyanobacterial DMSP synthesis gene termed *dsyC*, respectively. The predicted contribution of phytoplanktonic and cyano/bacterial DMSP production showed temporal shifts in dominance, with the former dominating from March to May and the latter from May to July. Similar shifts were also observed for DMSP catabolic genes, but here bacteria were always the major protagonists. Overall, our results highlight that cyano/bacteria and phytoplankton are both important and mutually complementary in DMSP production, but bacteria are likely the major drivers of DMS production in surface oceans.

Marine sulfur cycling, Seasonal DMSP cycling, climate-active gas production

Investigating phosphorus-calcium signalling in the marine diatom, *Phaeodactylum tricornutum*

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Marine diatoms are ubiquitous microalgae that contribute around one fifth of global CO_2 fixation. Phosphorus, an essential element needed for all living organisms, is often found in scarce supply in many marine ecosystems and can thus limit diatom growth and productivity. As diatoms compete with other phytoplankton, they have evolved metabolic mechanisms to cope with prolonged periods of phosphorus limitation. In addition, diatoms can rapidly sense when the phosphorus supply increases and regulate their metabolism accordingly. Recent evidence has identified that diatoms can sense phosphorus using a calcium-dependent signalling pathway, however, the molecular machinery underpinning this pathway remains unknown.

Here, we investigated the role of calcium-dependent protein kinases (CDPKS), which are upregulated during phosphorus limitation, in governing recovery responses of phosphorus-starved diatom cells to phosphorus resupply. Employing CRISPR-Cas9, we generated gene knockout lines of *PtCDPK1* in the marine diatom, *Phaeodactylum tricornutum*. Mutants show evidence of enhanced physiological stress during phosphate limitation compared to WT cultures. Phosphoproteomics analysis will also shed light on downstream CDPK protein targets. This work aims to provide new insights into the molecular mechanisms enabling this important group of marine algae to thrive in regions of pulsed nutrient supply.

Key words: *Phaeodactylum tricornutum*, nutrient sensing, phosphate, calcium-signalling

A flair for phosphonates; *Roseovarius nubinhibens* obtains phosphorus from (R)-1-Hydroxy-2-aminoethylphosphonate via a novel PbfA-PhnYA metabolic pathway

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Phosphonate breakdown factor A (pbfA) is an ammonia lyase recently shown to expand the substrate scope of the characterized phosphonoacetaldehyde hydrolase (phnX) pathway. pbfA acts on (*R*)-1-Hydroxy-2-aminoethylphosphonate (OH2AEP), a derivative of the most common biogenic phosphonate 2-aminoethylphosphonate (2AEP), converting it to phosphonoacetaldehyde in which the carbon-phosphorus bond is cleaved by *phnX* releasing inorganic phosphate for assimilation. Here we conducted a sequence homology search and gene neighborhood analysis of 3090 bacterial type-strain organisms and determined the presence of *pbfA* in 39 bacterial gene clusters encoding *phnWYA*, a pathway for the Pi-independent degradation of 2AEP).
We now show that *Roseovarius nubinhibens* metabolises OH2AEP via a novel PbfA-PhnWYA pathway as the sole phosphorus source. Within cells of *R. nubinhibens* regulation of aminophosphonate metabolism is substrate inducible, rather than PHO regulated, being under control of a LysR transcriptional regulator with gene expression occurring under ambient Pi concentrations. *R. nubinhibens* taxonomically belongs to the major clade of marine bacteria Roseobacteraceae. This clade represents 20% and 3-5% of bacterial cells in coastal and oligotrophic surface waters respectively. The versatility of phosphonate metabolism pathways in *R. nubinhibens* suggests that microbial species accessing phosphonate phosphate under LysR regulation may have important implications to understanding of the marine phosphorus-cycle.

Keywords: Phosphonates, marine, bacteria, *pbfA*

Investigations into Mistreated Microbes.

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The UK has a rich history of industrial activities dating back centuries; however, this comes with a legacy of disturbances to the landscape through mining and quarrying but also the disposal of waste to landfill. These anthropogenic impacts have a direct impact on the microbial communities resident within these environments through the changing of geochemical conditions such as: O_2 levels, pH or exposure to metals. The environmental microbiology research group at the University of Huddersfield focuses on the understanding of the microbial communities that emerge from these activities. In County Durham, where alkaline leachates mix with the local sediments, we have used metagenomic analysis to look at the changing community and metabolic functionality resulting from the pH stress. Whilst some microbial taxa are negatively impacted by increased pH, the generation of carbonate tufa presents a carbon capture event that can lead to methanogenesis, which could be harnessed for commercial production. Finally, in several soughs in Derbyshire, we have used a combination of metagenomics and geochemical analysis to investigate biofilm formation, where carbohydrates seem to act as a diffusion barrier to oxygen to enhance sulphur cycling, whilst other organisms work together to utilize iron.

Anthropogenic, methanogenesis, speleotherm, geochemical cycles.

Dead or alive? Bon Jovi's guide to microbial eukaryotes behind the Southern Ocean's biological carbon pump.

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The Southern Ocean is critical in the global carbon cycle, with air-sea exchange and sequestration via the biological carbon pump (BCP) responsible for 40% of carbon uptake. Microbial production and degradation of organic material (OM) influences carbon transport and storage, yet knowledge of microbial eukaryote activity in the BCP is limited, limiting understanding of their role in marine carbon cycling. We used high-volume *in situ* sampling combined with DNA (total) and RNA (potentially 'active') assessments of microbial eukaryote diversity along repeated transects across a key junction of global ocean circulation in the Southern Ocean. Across transects, surface and deep communities were spatially and temporally distinct, with apparent increasing dissimilarity of DNA and RNA-determined communities with depth. Exploring community dissimilarity based on 18S rRNA/rRNA gene ratios at amplicon sequence variance (ASV) resolution, we identified ASVs with reduced 'activity' at depth who we suggest contribute to sinking OM. We also identified ASVs which remain stable or show increased 'activity' at depth, which we propose undertake active degradation and remineralisation of OM. This study highlights the understudied role of microbial eukaryotes in the globally significant Southern Ocean.

Keywords: marine, eukaryotes, carbon, biogeochemical, Southern Ocean.

Site-seeing in ammonia monooxygenases

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Introduction

Ammonia oxidising microorganisms (AOMs) facilitate ammonia oxidation, the first step of nitrification and an integral component of the global biogeochemical cycle. However, their activity also siphons ammonia-based fertiliser from agricultural environments and directs it into the nitrogen cycle. This generates climate-active gases and threatens food security. To reduce the loss of fertiliser and balance the nitrogen cycle, it is essential to enhance our understanding of AOM-mediated ammonia oxidation.

Bacterial and archaeal AOM both use ammonia monooxygenases (AMO) to oxidise ammonia, but historical issues with purification have limited study of AMO structure and function. In particular, the location and architecture of the active site remains elusive. The advent of AlphaFold2 represents an avenue for investigating the active site in the absence of crystal structures.

Key results

1. Structural differences between bacterial and archaeal AMO revealed by AlphaFold2.
2. Putative active sites identified via molecular docking simulations.
3. Alkyne inhibitors could be used to experimentally validate the active site.

Concluding statement

Here we demonstrate an *in silico* approach to gain insight into the active site architecture of the challenging and biologically relevant AMO proteins, and present a proteomics-based methodology for testing those insights *in vitro.*

Keywords: ammonia monooxygenase, ammonia oxidation, nitrification, nitrogen cycling, alphafold

Niche specialisation of sponge-associated microbes

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The comparative diversity and biogeography of free-living marine and sponge-associated microorganisms has been studied, but the mechanisms of niche specialisation and host specificity of sponge endosymbionts is still unclear. Among crucial sponge-associated microbes, ammonia-oxidising archaea (AOA) likely play a key role in nitrogen metabolism in the host. Here, we analysed the diversity, host-specificity and phylosymbiosis of AOA in light of the total microbial diversity using a dataset of globally distributed sponge species, covering most orders of the class Demospongiae. The diversity of AOA did not reflect the diversity of the whole microbiome, suggesting their host-specialisation. Using comparative phylogenetic analysis of both host phylogeny and AOA diversity, we defined specialist and generalist AOA, on the basis of their host niche distribution. A strong host-specificity was observed for some host species, as well as for some host orders. These specialist AOA are restricted to a few phylogenetic clusters, suggesting a strong metabolic specialisation and selection. Analysis of the phylosymbiosis between sponges and AOA is currently performed to elucidate existence of coevolutionary history between the two symbiotic partners.

niche specialization, endosymbiont, sponge, archaea, *amoA*

Microbial-induced calcite precipitation under sub and supercritical CO_2 conditions for soil remediation

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Abstract: Chemical and biological carbonation processes offer the potential for simultaneous carbon sequestration and soil remediation over long time scales. In both approaches, isomorphous substitution of Ca^{2+} by toxic divalent cations (e.g., Cu^{2+}, Sr^{2+}) in calcite ($CaCO_3$) and precipitation of pure carbonates (e.g., $CuCO_3$) are facilitated by alkaline environmental conditions and presence of CO_2. Microbial-Induced Calcite Precipitation (MICP) via urea hydrolysis is a biogeochemical process, in which ureolytic bacteria catalyse hydrolysis of urea into CO_2 and NH_4, provided sources of calcium (Ca^{2+}), urea, and simple carbon nutrients are present. In chemical carbonation, alkaline industry products (e.g., cement, slag, basalt quarry fines) source Ca^{2+} through chemical weathering of oxide and silicate minerals. Accelerated carbonation can be achieved by increasing CO_2 concentration and pressures. Although recent research has demonstrated viability of ureolytic bacteria under high CO_2 and sub/supercritical CO_2 conditions, the effectiveness of this method for soil remediation purposes remains unexplored. Thus, this research project aims to investigate the potential of MICP that also involves the use of alkaline industry by-products as a source of calcium for the bio-remediation of soil heavy metals.

Keywords: MICP, accelerated carbonation, soil remediation, heavy metals

COMPARATIVE METABOLOMIC ANALYSIS OF PHYLOGENETICALLY DIVERSE ACTINOMYCETES

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Bacteria within the order Actinomycetales (often called actinomycetes) are a rich source of novel bioactive metabolites, yet the correlation of ecological niche to both biological and chemical diversity is not well understood. Rare actinomycetes, are all genera within this order except for the genus *Streptomyces* and are named due to their less-frequent study. As such, rare actiomycetes from underexplored habitats represent an exciting opportunity to focus on natural product discovery. Here, preliminary data will be presented focused on the isolation rare actinomycetes from marine habitats in Scotland and the Pacific Ocean. A future goal is to study the effects of abiotic parameters relating to their environmental, such as temperature, on their produced chemistry. We hypothesize that culturing strains under environmentally-relevant conditions will influence microbial chemistry and build knowledge that will aid future biodiscovery efforts from diverse ecological niches.

Actinomycetes, specialized metabolites, environmental microbiology.

Uncovering Associations Between Anthropogenic Pollution and Antimicrobial Resistance

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To tackle the growing threat of antimicrobial resistance (AMR), a greater understanding of its causes and prevalence is required. AMR genes (ARGs) may be co-selected for alongside resistance genes for environmental stressors such as potentially toxic elements (PTEs) and polycyclic aromatic hydrocarbons (PAHs). This means that anthropogenic pollution could impact the spread of AMR in the wider environment, beyond clinical settings. Knowledge of how contaminants cycle in the environment and associate with ARGs could enable the use of biogeochemical mapping for the identification of AMR hotspots.

Wastewater treatment plants (WWTPs) can act as a hub for anthropogenic pollutants and therefore represent an ideal environment to explore a vast array of parameters. In this study, we analyse sediments upstream and downstream of Indian and Scottish WWTPs and through the evaluation of geochemical and microbiological parameters, we aim to identify links that could explain the prevalence of AMR. Geochemically, our work will include measurement of physicochemical parameters and the use of ICP-AES/MS and GC-MS. Microbiology will include culturing of bacteria and the protist *Acanthamoeba*, susceptibility testing, and metagenomics. We will then carry out statistical analysis to uncover any correlation between ARGs and anthropogenic contaminants.

Keywords: antimicrobial resistance, environmental contamination

Planktonic marine yeasts – towards a functional trait understanding

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Marine yeasts are heterotrophic plankton which remineralise organic matter in the water column. Yeasts are widespread in the open ocean, yet their environmental drivers are unknown. To investigate how marine yeast activity varies across chemical gradients, isolates from the Atlantic Ocean were cultured under a range of nutrient conditions.

Unicellular marine yeast *Naganishia diffluens* decreases in cell size and growth rate under decreasing nitrogen concentrations. At environmentally relevant nitrogen concentrations of <10µM, cell size and growth rate are significantly lower with nitrate as the sole nitrogen source, compared with ammonium or urea.

Polymorphic yeast *Aureobasidium pullulans* changes morphotype in response to nutrient concentration. Under low nutrient conditions, only the single-celled yeast form exists. Under high nutrients, *A. pullulans* populations simultaneously demonstrate multicellular filamentous growth.

The phenotypic plasticity of marine yeasts in response to resource availability may contribute to their persistence throughout diverse marine environments. These observations build towards a framework of the key functional traits of marine fungi. Identifying common traits measurable across diverse plankton groups, regardless of taxonomy, is an important step towards understanding pelagic ecosystem structure and function.

Keywords: plankton, nutrients, morphology, functional diversity

Future Oak: a microbiome-wide association study of acute oak decline severity in Quercus robur

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Acute Oak Decline (AOD) is a complex Decline disease of oak in which weeping stem lesions are caused by a multi-species bacterial "pathobiome", leading to tissue necrosis and often tree death. AOD is spreading across the UK, causing concern about the future of oak trees, but to date there are no effective means of management. One target may be the oak microbiome, which may contain microbial taxa that provide protective effects against the activity of these pathogenic species, either through direct inhibition or resource competition. To locate such microbial taxa, we are conducting a microbiome-wide association study, characterising the leaf, stem and rhizosphere microbiomes of 300 Quercus robur trees across the range of AOD severity at 30 sites across the UK. To date, we have constructed ~600 MAGs associated with the oak phyllosphere. These MAGs provide key insight into the composition and function of the microbiome with changing disease severity, as well as the physiological changes occurring in the tree following disease onset. Future work will aim to identify microbial taxa across the whole tree associated with reduced disease severity.

Keywords: microbiome, forest pathology, mwas, metagenomics, community ecology

Investigating the relationship between plant host DMSP production and cycling by symbiotic rhizobia

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Dimethylsulfoniopropionate (DMSP) is an anti-stress molecule produced by marine algae, bacteria and some plants. Once released into the environment, DMSP is a major nutrient for marine microbes, is key in global carbon and sulphur cycling, and is the main precursor for the climate-active gas dimethyl sulphide (DMS). Globally, most DMS is thought to result from the microbial catabolism of DMSP in marine settings. However, some terrestrial bacteria of the rhizobia group including *Rhizobium* sp. NGR234 and *Burkholderia cepacia* contain DMSP lyase enzymes and generate DMS. DMSP degradation by symbiotic rhizobia is still poorly studied. Here, we explored the distribution of DMSP lyases and their functionality in rhizobia.

Bioinformatic analyses showed that many rhizobia contained candidate DMSP lyases spanning majority of known families of these enzymes (DddD, DddW, DddL, DddQ, DddK, DddP, DddX). Furthermore, functional analyses with the Rhizobia containing predicted DMSP lyases were shown to catabolise DMSP to produce DMS.

These results show that DMSP lyases genes are widespread in the rhizobia family and imply that some leguminous plant hosts of these bacteria may produce DMSP as an antistress compound and/or to aid interactions with specific rhizobia.

Key words: DMSP, Rhizobia, DMSP lyases

The impact of drought and nutrient stress on oak tree microbiome composition and severity of Acute Oak Decline

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Acute Oak Decline (AOD) is a polymicrobial decline disease affecting mature oak trees in the UK. Trees affected by AOD show symptoms of bark tissue necrosis that can eventually lead to tree death within 5 years of symptom onset. The necrosis is thought to be caused by a pathobiome consisting of, but not limited to, the bacteria *Brenneria goodwinii*, *Gibbsiella quercinecans* and *Rahnella Victoriana*. Previous studies have shown a shift in the microbiome of trees affected by AOD and certain abiotic stressors such as drought and nutrient stress are thought to be strong predisposing factors of AOD. This project will use 144 oak trees from a woodland field plot to investigate how drought stress and nutrient stress influence the microbiome composition of sessile (*Q. petraea*) oak trees and whether this shift in microbiome increases the oaks susceptibility to AOD. Leaf, bark, and rhizosphere samples have been collected at three timepoints and will undergo microbiome analysis using 16S rRNA gene and ITS community profiling.

Keywords: Acute Oak Decline (AOD), tree microbiome, 16S rRNA gene and ITS community profiling, *Brenneria goodwinii*

First things first, primary metabolism in *Streptomyces.*

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To combat the antimicrobial resistance crisis there is a need to develop new antibiotics while increasing the production of existing antimicrobials. Pyruvate kinase (Pyk) has proved to be a good target for metabolic engineering to increase antibiotic production. Pyruvatephosphate dikinase (PPDK) usually catalyses the opposite reaction to Pyk but in contrast is reversable and occurs in a three-step process. There are two copies of Pyk in the majority of *Streptomyces* and two copies of PpdK, therefore four potential biochemical routes for converting pyruvate to PEP. There are currently no studies involving the role of PPDK in actinobacteria, however previous work has shows one of the Pyk enzymes in *S. coelicolor* is upregulated 30-fold preceding antibiotic production.

We investigate the role of PPDK in *Streptomyces* by creating knockdown mutants using CRISPRi /dCas9 technology and over-expressing of the proteins in WT strains. These strains exhibit both growth and developmental phenotypes. We hope this enables us to better understand the role of PPKD in streptomycetes primary metabolism and carbon flux around the pyruvate-phosphoenolpyruvate-oxaloacetate node of central metabolism under a range of conditions to understand how this impacts the availability of precursor molecules for specialised metabolite production.

Key words:
Primary metabolism, AMR, Carbon utilisation.

Methylation silent plasmid vector for the genetic manipulation of industrially important *Streptomyces* species.

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Integrating plasmids are widely used in *Streptomyces,* exploiting bacteriophage-derived integrases to insert into specific sites in the genome of *Streptomyces spp.* One unusual feature of many *Streptomyces spp.* is the presence of strict methyl-specific restriction enzyme system which efficiently degrades foreign methylated DNA. Therefore, conjugation of plasmids into many *Streptomyces spp.* is performed using *Escherichia coli* ET12567: a strain which lacks the *dam*/*dcm* methylation systems. This is problematic as ET12567 is slow growing, inefficiently transformed, and mutagenic so must be transformed with minimal delay before conjugation and is not appropriate for long term plasmid storage. To overcome this problem, we have modified the *Streptomyces* integrating plasmid vector pSET152 to inactivate most of the Dam/Dcm sites by mutation. Initial testing showed our methylation silent vector can be conjugated by DH5α directly into the model strain *Streptomyces coelicolor* with high efficiency. A codon optimised fluorescently tagged gene was also successfully integrated and expressed using the new vector. We are hopeful that this new plasmid will allow easier introduction of DNA into the genomes of industrially relevant, antibiotic-producing *Streptomyces* and hence, simplify future research.

Keywords: Molecular tool, Methods, *Streptomyces*, Conjugations, Methylation.

Cleaning up our act: applying anammox to the treatment of landfill leachate

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Ammonium-rich leachate is a major issue in decommissioned landfill sites, with treatment and haulage costing £240,000 annually in Norfolk alone. Constructed wetlands provide a sustainable alternative by treating leachate *in-situ* using microbial anammox (anaerobic ammonia oxidation). Unlike conventional treatment, the anammox pathway does not emit the greenhouse gas N_2O and is more cost effective. The aim of this study was to assess the diversity and activity of anammox bacteria in a vertical flow constructed wetland fed with landfill leachate and planted with *Phragmites australis.*

Bacterial diversity was assessed by 16S rRNA gene amplicon sequencing. Anammox species from the genera *Kuenenia* sp. and *Brocadia* sp. were detected in addition to a potentially novel anammox species and an aerobic nitrifier, *Nitrospira sp*.. Anammox activity was measured using the isotopic tracer $(^{15}NH_4)_2SO_4$ and GC-MS. The abundance of hydrazine oxidoreductase (*hzo)*, a gene unique to anammox, was quantified using qPCR. The abundance of anammox bacteria present in the constructed wetlands was at least comparable to successful anammox-driven wastewater treatment facilities.

The greenhouse gas emissions from the system will be monitored to further the sustainability of this leachate management strategy.

Key words: anammox, leachate, ^{15}N, *hzo*

Investigating the antibiotic properties of specialised metabolites produced by actinomycetes isolated from the Porcupine Abyssal Plain.

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Actinobacteria are prolific producers of specialised metabolites with antibiotic properties, yet the rediscovery of known chemistry is a challenge that needs to be overcome in the era of antibiotic resistance. One strategy is to target underexplored environments for new biological diversity and in turn, new chemical diversity. Deep-sea environments are vastly underexplored for microbial specialised metabolites. These are classed as extreme due to cold temperatures, high pressure, limited light, and anoxic conditions. Therefore, it is hypothesised that microorganisms isolated from extreme environments, may produce novel specialised metabolites.

In this work, bacterial strains were isolated from abyssal sediment for the Porcupine Abyssal Plain within the North Atlantic Ocean at depths of over 4600m and their ability to kill clinically relevant pathogens was assessed. In future work, bioactive isolates will be identified through 16S rRNA gene sequencing. We also plan on conducting whole genome sequencing (WGS) as well as metabolomic analysis of produced metabolites, using Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) and molecular networking.

Word Count: 177

Keywords: Actinobacteria, metabolites, deep-sea, bioactivity.

Investigating *Streptomyces clavuligerus* Linear Replicons for Improved Clavulanic Acid Production

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Streptomyces clavuligerus (Sclav) produces clavulanic acid and is composed of four giant linear plasmids (GLPs) and its chromosome. Various genes essential for the maintenance of linear replicons, such as *tap* and *tpg* which encode telomeric terminal proteins, are found on three out of four GLPs. We will investigate plasmid-chromosome interactions to determine the role of *tap-tpg* and aim to cure GLPs for decreased metabolic burden and increased clavulanic acid production. Previous work demonstrated a circularised chromosome and loss of plasmid after cutting the largest GLP, pSCL4, potentially due to the absence of *tap-tpg*. To determine the role of *tap-tpg* in chromosomal and plasmid linearity, we tested their inactivation using CRISPR-dcas9 multiplexing, targeting $tap\text{-}tpg_4$ on pSCL4, $tap\text{-}tpg_3$ on pSCL3 and $tap\text{-}tpg_2$ on pSCL2. *Sclav* colonies were screened for the loss of replicon telomeres. Illumina sequencing showed loss of plasmids and telomeres in multiplexed mutant strains. Future work will focus on determining GLP copy numbers in the mutants and elucidating the mechanisms of telomere replication in these multi-replicon organisms to eventually create a plasmid-free strain.

AMR, CRISPR-dCas9, terminal proteins

CALCIUM-MEDIATED CELLULAR STRESS SIGNALLING IN MARINE DIATOMS IS DEPENDENT ON CELL DENSITY

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Calcium signalling is used ubiquitously in all eukaryotic organisms to perceive and respond to environmental stimuli. However, very little is currently known about the mechanisms that marine diatoms use to detect and respond to stress conditions in their surrounding environment. With the rapid improvement of imaging techniques, we are now able to investigate diatom signalling in much greater detail, and discern the involvement of calcium signalling in specific stress responses. We used the model diatom *Phaeodactylum tricornutum* to investigate how cell density affects the calcium dependent signalling mechanisms of marine diatom cells. Our results suggest that calcium elevations observed in response to hypo-osmotic stress are dependent on cell density, with cells at a low density often exhibiting no response to a hypo-osmotic shock. These results are consistent over a range of hypo-osmotic shock strengths. We will now investigate whether there is a change in the strategy used for osmoregulation in marine diatom cells as density increases.

KEYWORDS
Diatoms, Calcium signalling, Osmotic, Density, *Phaeodactylum tricornutum*

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Traces of microbial life and activity in Arctic endolithic habitats

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Arctic endolithic (rock-dwelling) microbial communities are a key interface between biology and geology. Despite subzero temperatures, nutrient shortage, and a lack of liquid water, these microbes thrive beneath the surface of translucent rocks. They are vital for our understanding of the parameters for life in extreme environments and what biosignatures are suitable to target in future astrobiological missions. However, their diversity, distribution, metabolic capabilities, and biosignatures remain vastly unexplored. The aim of this study is to investigate the microbe-mineral interactions and biosignatures associated with microbial communities in Arctic endolithic habitats. Eight samples of gypsum with endolithic microbial communities, collected from Petuniabukta Bay, Svalbard in July 2019, were prepared as thin-sections and imaged via optical microscopy. The imaged thin-sections contained potential biosignatures including ooids, organic matter, and microbialites. MicroRaman spectroscopy further indicated the presence of organic matter within carbonate-gypsum oolitic structures. DNA was extracted from the gypsum endolith samples, yielding 2 - 6 ng/µL from 0.3 g of rock powder per extraction. Ongoing work including DNA sequencing and metagenomics will help to elucidate endolithic microbe-mineral interactions and produce a profile of mineral and biochemical macromolecule biosignatures to target in extraterrestrial environments.

extremophiles, biosignatures, endolithic, polar microbiology

Investigating the infection biology and ecology of *Gymnopus fusipes*, a fungal root rot pathogen of woodland trees.

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Gymnopus fusipes (syn. *Collybia fusipes*) is an understudied basidiomycete fungus common to woodlands across temperate regions, identified as a primary pathogen with strong links to episodes oak decline in Europe. A systematic literature review revealed that *G. fusipes* is known to cause severe root rot on young and mature trees, both broadleaf and coniferous, sometimes resulting in complete destruction of host root systems. Orange lesions on large central roots are distinctive signs of infection, but above ground, decline symptoms (such as presence of fruiting bodies and poor crown condition) are not always correlated with infection status, leading to poor detection and diagnosis. We aimed to develop a diagnostic tool for accurate detection of *G. fusipes* in environmental samples, and to investigate mechanisms of infection. Species-specific qPCR primers were designed for the *18S rRNA* gene, and were accurate in detecting *G. fusipes* in fruiting bodies and infected woody tissue. Ongoing work aims to investigate infection at a molecular level using transcriptomic analysis of *G. fusipes*. These results are crucial to understanding the spread, ecology and infection biology of this important root rot pathogen.

Keywords: *Gymnopus fusipes*; root rot; qPCR diagnostic; transcriptomics; oak decline

Metagenomic guided bioprospecting of CO_2-driven metabolism in geothermal springs for novel bioprocesses

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As a result of the deepening climate crisis there is a growing necessity for the responsible management of anthropogenic CO_2 emissions. Carbon capture and utilization (CCU) presents an attractive means to minimize the CO_2 subsurface storage burden whilst creating waste from wealth. However, current bioprocesses rely mainly on photosynthetic CO_2 conversion pathways via single strains. We propose to find new nature-based solutions for CO_2 utilisation by leveraging CO_2-driven microbial communities that inhabit geothermal springs. Carbon cycling in these environments is largely driven by efficient, light-independent CO_2 fixation processes. This CO_2 fixation supports a network of interconnected metabolic processes which, together, represent attractive pathways for efficient CO_2 bioconversion to useful products. Here, we developed a computational pipeline for reconstructing community metabolic networks to explore the metabolic potential of geothermal communities to convert CO_2 to value-added products. Using experimentally acquired and publicly available geothermal metagenomes, we performed comparative metagenomic analyses, which revealed key taxa responsible for carbon fixation and knock-on metabolic processes. We identify key primary and secondary metabolites that could be targeted through microbiome engineering of enriched and synthetic communities.

metagenomics, CO_2 utilisation, bioprospecting, geothermal, environmental biotechnology

New tools for visualising transcription and translation in tandem in *Streptomyces*.

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The bacterial genus *Streptomyces* are responsible for the production of clinically relevant antimicrobial agents and understanding control of their biosynthesis will help addressing the challenge of antimicrobial resistance. The bald (*bld*) mutants in *Streptomyces* are blocked at an earlier stage of development and are unable to erect aerial hyphae, with many mutations in *bld* loci pleiotropically blocking antimicrobial production. Perhaps the most severe *bld* phenotype is found in the *bldA* locus, encoding the rare leucyl-tRNA, where mutations result in complete loss of morphological development and antimicrobial production.

In our attempts to create new molecular tools for studying *Streptomyces*, we have been utilizing the 'vegetable aptamer' systems to develop genetic probes that allow for quantifying and imaging of transcription and translation simultaneously in live *Streptomyces*. As proof-of-concept for these tools, we have exploited the Broccoli aptamer and coupled this to a TTA-leucine codon containing the mCherry fluorescent protein reporter to create a system for simultaneously visualizing *bldA* transcription and translation to revisit the role of *bldA* in antimicrobial biosynthesis control. Here, we discuss a novel genetic for *Streptomyces,* which will assist in the development of current and future antimicrobials.

Keywords: *Streptomyces*, *bldA*, transcription, translation, aptamer.

Friday, Dec. 2nd, 2022

09:15 - 10:00

Keynote Speaker:Towards linking metabolites to their likely microbial hosts

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Vancomycin-resistant *Enterococcus faecium* (VRE) is an emerging antibiotic-resistant pathogen. Strain-level investigations are beginning to reveal the molecular mechanisms used by VRE to colonize regions of the human bowel. However, the role of commensal bacteria during VRE colonization remains largely unknown. We employed amplicon 16S rRNA gene sequencing and metabolomics in a murine model system to try and investigate functional roles of the gut microbiome during VRE colonization. First-order taxonomic shifts between *Bacteroidetes* and *Tenericutes* within the gut microbial community composition were detected in response to pretreatment using antibiotics and subsequent VRE challenge. Using neural networking approaches to find cooccurrence profiles of bacteria and metabolites, we detected key metabolome features associated with butyric acid during and after VRE colonization. These metabolite features were associated with *Bacteroides*, indicative of a transition toward a preperturbative naïve microbiome. This study shows the impacts of antibiotics on the gut ecosystem and the progression of the microbiome in response to colonization with VRE. Our results offer insights toward identifying potential nonantibiotic alternatives to eliminate VRE through metabolic reengineering to preferentially select for *Bacteroides*.

Microbiota, metabolomics, gut colonisation,

10:00 - 11:30
Session: Applied & Geo-microbiology. Co-chairs: Dr Anastasiia Kostrytsia (University of Glasgow) & Dr. Naji Bassil (University of Manchester)

Anaerobic Biodegradation of Citric Acid in the Presence of Ni and U at Alkaline pH; Impact on Metal Fate and Speciation

Natalie Byrd[1], Jonathan R Lloyd[1], Luke T. Townsend[1], Joe S Small[1], Frank Taylor[2], Heath Bagshaw[3], Christopher Boothman[1], Lewis Hughes[1], Katherine Morris*[1]

Citrate is a key decontaminant, and complexing agent, in the nuclear industry and here we explore its biogeochemical fate in the presence of Ni and U under conditions relevant to low level radioactive waste (LLW) disposal. Citrate is expected to increase Ni solubility via complexation, even under alkaline pH levels expected in cementitious repositories for L/ILW. By contrast, U-citrate complexes are unlikely to form at elevated pH, however citrate can be utilised as an electron donor to fuel microbial reduction reactions (e.g. U(VI) bioreduction). Microbial metabolism may influence the metal speciation and therefore their solubility. Accordingly, understanding the fate of Ni and U in the presence of citrate informs radioactive waste management practices. Enrichment and resting cell experiments were used to explore citrate biodegradation in the presence of Ni and U under nitrate- and sulfate-reducing conditions at pH 9-10. A multi-technique approach was adopted to characterise the aqueous geochemistry (pH, IC, ICP-MS, and ESI-MS), solid phase mineralogy (SEM/TEM with EDS/SAED), and bacterial communities (16S rRNA gene sequencing) in each system. Overall, findings from this work suggests that citrate will be microbially degraded under LLW repository conditions, and that degradation will support microbe-metal interactions that support the immobilisation of contaminant species.

Key words: radioactive waste disposal, citrate, alkaline

Secreted proteins predicted from activated sludge metagenomes illuminate distinct capabilities to interact with the external environment

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Wastewater treatment plants (WWTPs) process large quantities of waste material via complex microbial communities that transform and take-up diverse organic and inorganic molecules. Secreted proteins are critical because many are the first to interact with and/or degrade external molecules. Here, we performed a community-wide, genome resolved analysis of predicted secreted proteins from 1083 high-quality metagenome-assembled genomes (MAGs) from Danish WWTPs. Major phylogenetic clades had clear differences in numbers and types of predicted secreted proteins, indicating strong phylogenetic signals for propensities to secrete proteins. Secreted catabolic enzymes for different classes of macromolecules also had patterns among major phylogenetic groups, which likely manifests in niche partitioning and distinct community functions. MAGs from many abundant Betaproteotobacteria have limited secreted hydrolases, indicating much of the community is reliant on relatively simple molecules, possibly made available from other populations. Diverse secreted multi-heme cytochromes suggest capacities for extracellular electron-transfer by various taxa, including some Bacteroidota that encode massive, undescribed multi-heme cytochromes with >100 heme-binding motifs. Overall, this study provides a new perspective on the functioning of WWTP microbiota and their capacities to interact with the external environment.

Key words: wastewater, secreted-proteome, macromolecules, cytochromes, metagenome.

Understanding the role of electron donors and microbial consortium in arsenic release from iron minerals

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Millions of people in South and Southeast Asia are at risk of chronic arsenic exposure [1]. Within the aquifers of these regions, it is widely accepted that arsenic release occurs via microbial-reduction of iron (oxy)hydroxide minerals [2]. However, the identity of the microbes and electron donors in this process remains poorly understood. Historic studies have suggested the importance of bioavailable organic matter [3]; more recent studies have suggested other electron donors including methane [4].

In this work, we collected the groundwater-microbes (from boreholes in Kandal Provice, Cambodia) on simplified Fe(III)/As(V) mineral coatings, followed by long term (~9 month) incubations with contrasting electron donor treatments. Amendment with acetate and lactate resulted in consistent reduction of Fe(III)/As(V) (and sulfate) phases, likely by metal(oid) reducers (e.g. *Geobacter* and *Geothrix*). Amendment with methane resulted in sporadic redox responses, by possible symbioses between proteobacterial methanotrophs (e.g. *Methylomonas*) and metal(oid) reducers; these mechanisms were further investigated in subculture incubations. This work introduces new techniques for the study of subsurface biogeochemistry, and expands the range of potential mechanisms for microbial-mediated arsenic release in aquifers.

arsenic, electron donors, organics, methane

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Chemical, taxonomic and metabolic characterisation of Western Sahara salt plains

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Oligotrophic environments such as sandy deserts are studied to understand the boundaries of life on Earth. In this study, salt crystals, water and sediment samples were collected from the Western Sahara salt plains. The chemistry of the samples was determined by ion chromatography and inductively coupled plasma optical emission spectroscopy, the results of which showed a dominance of sodium and chloride, confirming that halite was the dominant salt phase within the sampled locations. The taxonomic diversity was determined through sequencing of 16S rRNA gene amplicons, which showed that halophilic and extremely halophilic Bacteria and Archaea were most abundant across all sample types. Metagenomic sequencing was then used to identify the functional diversity of the microbial communities. High-quality metagenome assembled genomes (MAGs) of halophilic Archaea, including Candidatus species, were recovered from the samples. The results of this study add to the existing knowledge about microbial life in deserts and show that despite the oligotrophic conditions microbial life is abundant. This has implications for the search for extinct life on Mars because the Western Sahara salt plains are a novel analogue for the Noachian-Hesperian transition that occurred ~3.7 Gya on Mars.

Hypersaline; Metagenomics; Chemistry; Mars

Valorization of waste iron substrates for the production of functional Fe(II) nanomaterials

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The present study demonstrated a biotechnological approach to utilize waste iron residues from drinking water production for synthesizing biovivianite (a potential fertilizer) by the Fe(III)-reducing bacterium, *Geobacter sulfurreducens.* The waste iron residue consisted of non-pelletized iron sludges from 2 groundwater treatment facilities (Huijbergen (H) and Spannenburg (S). In addition, 6 sets of pelletized iron sludges (dry pellets – FerroSorp (FS), P-loaded FS, Noordbergum (NB), P-loaded NB, and Shwertmannite (SHM), P-loaded SHM) were also used. The influence of P adsorption on the biotransformation of Fe(III) materials was investigated using batch microcosm experiments. The extent of bioreduction was higher in the pelletized Fe(III) materials than in the P-loaded counterparts. The production of Fe(II) in the pelletized Fe(III) materials was dependent on its crystallinity. Thus, Fe(III) material dominated by 2-line ferrihydrite (FerroSorp) according to XRD analysis, showed a higher bioreduction extent compared to those with Schwertmannite as the main composition. For the non-pelletized Fe(III) materials (wet sludge), higher P concentrations supported higher rates of Fe(II) production (Fe/P 1 > Fe/P 1.5 > Fe/P 3.3). XRD analysis showed the formation of vivianite in the non-pelletized sludges.

Keywords: Waste iron, vivianite, Fe(III)-reducing bacteria.

Title: Impact of pathogen burden and management practices on potato blackleg disease prevalence and soil microbial community dynamics

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Submission Theme: Applied and Geo-Microbiology

Abstract:
Potatoes, a critical food crop, are highly susceptible to pests and pathogens but management practices to limit disease are a balancing act. For example, dry conditions favour common scab (*Streptomyces* spp.) development, while wet conditions favour blackleg (*Pectobacterium* spp.) disease. Disease susceptibility is thus altered by watering regimes but it isn't known if these interventions perturb the soil microbiome and whether this affects crop yields. Using field trials, we aimed to determine how the pathogen load of potato seed stocks (at zero, low, and high levels of *Pectobacterium* spp) and subsequent irrigation management (unirrigated and three regimes that differed in the timing and level of watering) impacted: i) crop yields; ii) disease development (blackleg or common scab); and the iii) associated microbial community dynamics (through amplicon sequencing of soil before planting and the rhizosphere at harvest). Irrigation increased blackleg symptoms in the low and high stocks (~11.3%) but not in the zero stock (~1.4%). Not irrigating led to increased common scab symptoms (~5%) and reduced crop yields by approx. 100 tubers/HA. Irrigation alone did not impact the soil microbiomes, but it was altered in the plots with a high blackleg pathogen burden, showing increased *Planctomycetota*, *Chloroflexi* and *Acidobacteria* species compared to the low and zero treatments. We conclude that the initial pathogen burden on seed tubers could substantially affect soil community dynamics more than the irrigation regime.

Key Words: Agriculture, Soil Microbiome, Management Practices, Blackleg

Coupling genomic and culture-based approaches to unearth the microbiology of hydraulic fractured Bowland shale

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Microbial communities inhabiting fractured shales can cause corrosion, gas souring and biofouling. Understanding their ecology can prove useful for effective management and microbial control. Here we expand the knowledge of fractured shale microbiomes by characterizing the microbiology and geochemistry of flowback fluids from exploratory wells in the Bowland Shale, UK. We coupled (meta)genomic and culturing techniques to gain insight into the microbial ecology. Compared with previously studied fractured shales, Bowland shale fluids are characterized by relatively low salinity and high microbial diversity. Taxonomic analyses revealed the presence of lineages known to degrade hydrocarbons, including *Shewanellaceae*, *Marinobacteraceae*, *Halomonadaceae* and *Pseudomonadaceae*. Community-level metabolic profiling showed the presence of genes responsible for hydrocarbon, nitrogen, and sulfur metabolisms and biocide resistance. Lastly, we report the isolation and genomic characterization of a strain of *Marinobacter lipolyticus,* which has the capacity to utilize diverse organic carbon sources including aliphatic and aromatic hydrocarbons. Taken together these findings suggest that in the fractured Bowland Shale, where chemically lean input fluids were used for fracturing, hydrocarbons derived from the formation might play a key role supporting the microbial community that results.

[hydraulic fracturing, deep biosphere, metagenomics, carbon cycling, hydrocarbon metabolism]

Contribution of manganese oxide sand and its associated microbiome on organic micropollutants removal in water treatment

Baptiste A.J. Poursat[1], Jinsong Wang[2], Nora B. Sutton[2], William Sloan[1], Cindy Smith[1]

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Keywords: Organic micropollutants, Sand filter, drinking water, Manganese oxidizing bacteria

The presence of organic micropollutants (OMPs), such as pharmaceuticals and pesticides, in water is a threat to drinking water production and wastewater treatment. Even though there is an apparent progress in developing new technologies to treat organic micropollutants, the challenge of finding cost effective processes remains, especially in decentralized systems. Manganese oxides (MnOx), that accumulate on sand in filters, are one of the strongest oxidants occurring in water. Our previous results showed that they can participate to a wide range of redox reactions with organic compounds, including pharmaceuticals and pesticides. Furthermore, biogenic oxides (BioMnOx), produced by microbial activity, normally yield more oxidative power than chemogenic ones. This study aims to further investigate the removal processes and mechanisms of organic matter, such as humic acid and OMP, by MnOx in small scale sand filter. For this, the oxidative power of manganese oxides and associated microbiome, will be investigated in column experiment by combining analytical chemistry, molecular biology and microbial ecology. This project will help to develop small scall drinking water treatment for a decentralized application on the Scottish isles.

The potential impacts of CO_2 injection and storage on deep biosphere communities

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Society relies heavily on the subsurface for waste storage and resource recovery, and this reliance will only increase as we look to the subsurface to store CO_2 emissions. However, little consideration is given to the microbial life residing within the subsurface until microbial processes become problematic for engineering. We now know that microbial communities in the subsurface are highly diverse and often active. As such, any engineering intervention will impact on these communities, and in turn these microorganisms are likely to impact on our engineering efforts, for good or for bad. Here we present research results into the CO_2 fixation potential and activity of subsurface communities that reside in formation types targeted for permanent geological CO_2 storage. These include deep granite-, sandstone- and basalt-hosted saline aquifers and depleted hydrocarbon reservoirs. In particular, we highlight the abundance of CO_2-fixing pathways encoded in these communities, and the net potential to consume vs produce CO_2. In surveying deep biosphere baseline capabilities for CO_2 utilisation, we can start to predict which formation types might respond positively or negatively to CO_2 injection, better design lab-based simulation experiments, and target potentially useful processes for biotechnological development.

Deep biosphere, subsurface, carbon cycling, CO_2 fixation, biotechnology

Bioreduction of precious metal ions Au (III) and Ag (I)

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Gold mining industries produce residues such as aqueous waste, waste rock and tailings, which may contain unrecovered gold in relatively low abundances, as well as other heavy metals, toxic chemical substances such as mercury and cyanide, or even radioactive elements that can negatively influence the environment. Microorganisms can offer passive and sustainable approaches for the bioremediation of land and contaminated water. In addition, metal-reducing bacteria are able to convert high oxidation state precious metal ions in waste solutions, to nano-scale zero-valent metal precipitates, offering a potentially inexpensive metal recovery approach. In this study, we investigated the biorecovery of gold and silver as metallic nanoparticles using the metal-reducing bacterium *Geobacter sulfurreducens*. Inductively coupled plasma mass spectrometry ICP-MS showed that > 96% of Au (III) and Ag (I) were reduced to zero-valent nanoparticles, with H_2 a more effective electron donor than acetate (especially for Au (III)). In addition, X-ray diffraction and transmission electron microscopy confirmed the formation of Au and Ag nanoparticles. These findings suggest that the use of *G. sulfurreducens* and hydrogen could be a good potential option for the biorecovery of Au and Ag from mine effluents, and this hypothesis is being tested against materials collected from Saudi mining sites.

precious metal ions, biorecovery, *G. sulfurreducens*, nanoparticles

Longitudinal Analysis of the Gill Microbiota in Farmed Atlantic Salmon in Scotland

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Atlantic Salmon aquaculture is a global industry and Scotland a major producer of farmed salmon supporting 10,000 jobs, and valued at £601 million (Kenyon and Davies, 2018). However, gill disease is a growing challenge for the industry, in particular Complex Gill Disease. As the role of the microbiome is increasingly understood to influence host health, it has become apparent that the gill microbiome of farmed Atlantic Salmon through production and disease outbreaks is not well described. To this end a longitudinal study of the gill microbiome of farmed salmon through a production cycle at sea was undertaken. Eight fish from two pens at a single site were sampled monthly from April 2019 to February 2020, (n=104 fish). Gills underwent 16S rRNA Illumina MiSeq amplicon library preparation and analysis to characterise the microbiome.
Alpha diversity, measured by species richness, varied over time reducing to its lowest value, 178.84+/-58.69, in May 2019 and peaking at 483.78+/-104.92, November 2019. Beta diversity varied very little over the thirteen months at sea, with no difference in Bray-Curtis Dissimilarity (R2= 0.120, p= 1), but difference in the unweighted Unifrac (R2 =0.131, P >0.001) over time. Phylum Proteobacteria dominated all gills while Bacteriodota, Acintobacteriodota and Firmicutes were also highly abundant throughout.

Key Words
Microbiome, Atlantic Salmon, 16S rRNA, Aquaculture

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Method optimisation for the extraction and analysis of biofilm from drinking water treatment plant biofilters

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Rapid gravity filtration is commonly applied in conventional drinking water treatment plants, and the filter medium (e.g., sand) serves as a growth substrate for a diverse microbial community [1]. Flow cytometry (FCM) was the proposed method to rapidly quantify microbial cells in biofilters; a protocol for the extraction of microbial cells from sand samples for subsequent quantification of total cell counts (TCC) on the FCM has been developed [2]. However, due to the membrane-destroying properties of the surfactant used during cell extractions, intact cell counts (ICC) could not be evaluated. We optimized the cell extraction protocol for ICC and found that not only the process of extracting the cells but also preparation of sand samples as well as storage time and condition have a significant influence on the TCC and ICC recovered. Pre-treatment with 1% Glutaraldehyde prior to sample storage at -20°C, -80°C or flash freezing significantly reduced the loss of TCC during sample storage. A significant loss of ICC within one day of sample storage was observed for all treatments and ranged between 59% (flash freeze, no fixative) and 82% (-20°C, no fixative).

Water biofiltration, Flow cytometry (FCM), Biofilm, Cell extraction, Glutaraldehyde

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Identification of novel catabolic pathways for lignin fragment degradation in Pseudomonas putida KT2440 and Paenibacillus sp

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There is considerable interest in finding new sustainable biocatalytic routes to fuels from renewable carbon sources, such as lignin and its industrial use is eagerly anticipated to achieve sustainable development. The scientific knowledge of the metabolic pathways by which lignin is converted into monocyclic aromatic intermediate is still incomplete. To facilitate the screening of molecular and enzymatic processes, a biosensor was developed in *P. putida* KT2440 to detect lignin-derived substrates. The positive response of this biosensor when screened in presence of catechol indicated a previously unreported carboxylation reaction of catechol to protocatechuic acid in *P. putida* KT2440.
5,5'-Dehydrodivanillic acid (DDVA) is widely used as a model compound for the degradation of biphenyl lignin components. The lignin-degrading strain Paenibacillus sp. was observed to grow on this model compound. Bioinformatic analysis revealed that the genome does contain *mhq* genes that could be involved in the conversion of DDVA to vanillic acid. Similar genes were also found in other lignin-degrading strains. The expression of these genes in the presence of DDVA was confirmed using RT-qPCR, validating the hypothesis for a new DDVA degradation pathway in Paenibacillus sp.

lignin, biodegradation, *Paenibacillus*, biosensor, pathways

Genome-centric spatial and temporal investigation of biological functioning within drinking water biofilters

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Granular activated carbon (GAC) biofilters are used for removing pollutants from drinking water via adsorption and microbiological activity. The strategy of customizing microbial communities has been previously suggested to enhance biofilters performance. For this a clear understanding of who is present, and their functional roles is needed. Twelve bench-scale GAC columns (H=30cm, D=3cm) were run at 21±1°C for six months and fed at 1 ml/min with reservoir water (Patsehill-UK). At weeks 6, 10, 13 and 24, a set of biofilters triplicates was deconstructed with depth resolution (at 0-2, 6-8, 10-15 and 15-30cm) to analyse the GAC filter-bed microbial communities. Following DNA extraction (FastDNA-Spin Kit for Soil), 32 metagenomic libraries were prepared (KAPA-HyperPlus kit), and sequenced with IIlumina NovaSeq (Earlham Institute) generating 2.4B reads. Metagenomic data processing pipeline included MEGAHIT assembler, METABAT2 initial binner and CheckM bin refiner. It generated **512** Metagenome Assembled Genomes (MAGs) (>75% completeness, <5% contamination), with **148** MAGs being unknown. Lower numbers of taxa and functions were observed with biofilter depth (alpha diversity), with 15-30cm community being most dissimilar one (beta diversity). *Proteobacteria* was most dominant phylum, with highly abundant bins belonging to *Burkholderiales* order.

Keywords: Metagenomic-assembled genome, drinking water, biofiltration.

Microbial impacts on colloid-radionuclide interactions in legacy spent nuclear fuel ponds

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The legacy spent nuclear fuel ponds (SNFPs) at Sellafield house a diverse inventory of waste from the early Magnox reactors. These reactors used uranium metal as a fuel encased in a magnesium non-oxide cladding. Corrosion of the cladding results in the release of radionuclides, primarily uranium, and the formation of brucite ($Mg(OH)_2$) phases which are present both in the corroded Magnox sludge at the base of the pond and suspended in the water column as colloids[1]. Colloids have the potential to mobilise insoluble phases providing an important pathway for radionuclide migration. The SNFPs are maintained at high pH to minimise corrosion of the cladding, however significant corrosion has still occurred.

Despite the seemingly inhospitable conditions in SNFPs, numerous studies have found microorganisms capable of surviving in SNFPs[2,3,4]. Previous work has demonstrated increased abiotic sorption of strontium to brucite in the presence of organic matter derived from *Pseudanabaena catenata*[5], which dominates algal blooms in the ponds. In this study we focus on uranium interactions with colloidal brucite in the presence of microbes adapted to high pH environments under conditions relevant to the SNFPs at Sellafield.

Legacy spent fuel pond, alkaliphile, colloid

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The effect of filter bed length on the microbial communities within GAC biofilters and treated effluent water

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The supply of clean drinking water to remote rural communities can be more challenging than to major conurbations. At present rural communities rely on energy and chemical intensive water treatment plants. Thus, alternative, lower energy methods of water treatment are being sought. A potential solution may be found in granular activated carbon (GAC) biofiltration as a point of use treatment system. However, the biological mechanisms of removal must be better understood before a scaled down point of use filtration system becomes viable. Here we investigate the spatial and temporal changes which occur within the communities of lab-scale GAC biofilters from start up to 23 weeks. Furthermore, we investigate the performance and communities of three biofilter bed sizes, long (90cm), medium (60cm) and short (30cm). We found that biomass, activity, and richness increased over time and decreased through the depth of the filter bed. Significant variance between communities was found to be explained by both depth and time. The most abundant taxa in deeper sections of the filter bed showed more variation with time than communities at the top of the filter. Filter bed length was also shown to be a significant factor explaining variance between developed communities at week 23.

biofiltration, granular activated carbon, water treatment

Controlling and modelling the impact of bentonite microbial communities in disposal of radioactive wastes

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Disposal in a deep geological facility (GDF) is the preferred route for the world's growing inventory of nuclear wastes. Bentonite clay is a common component of the engineered barrier system, serving to isolate and stabilise the canisterized high heat-generating wastes in the geosphere [1].

Bentonites naturally contain sulfate-reducing bacteria (SRB), which in the presence of the correct substrates, produce H_2S that is highly corrosive to metals. Sulfate, the electron acceptor, is present in most groundwaters, and the corrosion of steel produces hydrogen, an electron donor [2]. Compacting bentonite on deposition can restrict GDF microbial activity, since swelling pressures upon saturation, restrict the available porosity for microbes [3]. Additionally, groundwater chemistry and salinity [4] may impact bentonite mechanical properties (e.g., swelling capacity), and the energetics of bacterial metabolism [6].

This work uses bentonite SRB enrichment cultures to assess parameters that control microbial metabolism and bio-corrosion of steel cannisters in bentonite systems under realistic GDF conditions. This will provide evidence to underpin lines of argument and models used in the safety assessments for geological disposal. Experiments described include microcosm incubations containing bentonite slurries, and pressure cell bioreactors.

Microbiology, Bentonite, Radionuclides, Remediation, Groundwater

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Understanding the relationship between the sizes of dissolved organic carbon (DOC) and their bioavailability for a biofilter microbial community

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The ecological mechanisms that drive the microbial communities at different depths of drinking water biofilters to preferentially degrade different DOC fractions are still poorly understood. Previous studies have found a close link between the sizes of DOC fractions and their bioavailability[1,2]. In this experiment, we assessed the bioavailability of different model DOC fractions in a batch experiment; the test was performed using a microbial community homogeneously developed on glass beads and monitored for 7 days. The model DOC fractions were citrate, glycogen, fulvic acid or humic acid at an organic carbon concentration of 2 ppm, thus representing a wide range of sizes of DOC. DOC measurement revealed that the concentrations of citrate and glycogen decreased to below the detection limit within 3 days, and such a decrease was accompanied by an increase in cell counts from 10^5 to 10^7 cells/mL based on flow cytometric analyses. Humic and fulvic acid concentrations were stable by the end of the experiment, despite an increase in cell counts from 10^5 to 10^6 cells/mL. Our results highlighted a positive correlation between the sizes of DOC and the substrate utilisation rate/ growth rate of a biofilter microbial community.

Keywords:
Dissolved organic carbon (DOC); Biofilters; Bioavailability.

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