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Unravelling the Seagrass Microbiome

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Abstract

Seagrass meadows are marine angiosperms critically important for carbon sequestration and fauna nursery grounds. The global decline of seagrass habitats is associated with coastal nutrient pollution. The seagrass microbiome influences plant health under changing environmental conditions. The response of microbial interactions to nutrient overload are poorly understood. This study used a novel high throughput workflow to map interactions within the seagrass microbiome and evaluate the effect of nutrients on these interactions. Twelve bacterial strains isolated from seagrass seeds were used, creating pairwise co-cultures using robot-assisted colony printing. These bacterial co-cultures were grown and monitored on control and nutrient-enriched media (nitrate, phosphate, iron and copper) using automated imaging and image analysis. Use of these novel technologies both in bacterial pinning and growth monitoring, allowed detection of pronounced differences in growth with mono-cultures of *Lysobacter luteus* S6 reaching a colony size of 12 mm^2 whereas *Psychromonas arctica* S11 showed minimal growth reaching 4 mm^2. Across nutrient addition treatments the microbial growth and co-culture interactions showed dramatic shifts with multiple strains being inhibited while other strains developed mutualisms to combat the nutrient stress. For example, *Pseudoalteromonas spiralis* S5 when paired with *Rhodococcus cerasti* S3 in high phosphate concentrations showed 827.9 ±14.3 mm larger growth whereas *Rhodococcus cerasti S3* showed the highest growth curves with *Pseudoalteromonas spiralis* S5 than it did with any other condition. Additionally, four fungal species were isolated and identified in this research by extraction from seagrass meadows. These including endophytes (*Pyrenochaetopsis leptospora, Pseudeurotium zonatum*) potentially contributing to blade health and epiphytes (*Penicillium commune, Cladosporium halotolerans*) linked to root-associated defence. This research demonstrates high throughput technologies can successfully be used for large scale microbial interactions cross-examinations to understand partnerships and ideal growth conditions. These could be utilised towards probiotic-based approaches to improve seagrass health, germination and resilience to anthropogenic stress.

Lay abstract

Seagrasses are marine plants highly important for marine life and maintaining a healthy ocean. The seagrass forms underwater meadows which are rapidly disappearing due to anthropogenic effects. Sewage pollution causes large amounts of macronutrients such as nitrogen, phosphorus & micronutrients; iron and copper which pollutes the meadows, causing damage to the plants and the microorganisms living in the sediment. These microorganisms are important influencers of seagrass plant health. However, it is not fully understood how well these microorganisms can survive in the increasing nutrients associated with sewage pollution and their potential to help the seagrass mitigate the stress.

Twelve bacteria were grown in controlled concentrations of nitrate, phosphate, iron and copper to examine nutrient resilience. The use of new technology allowed growth curves to be measured, comparing how well microbes collaborated under different nutrient loads. As the bacteria cultures were grown next to neighbouring strains, the relationship of the 'co-cultures' could be categorised as either helpful, harmful or neutral. In control media there were 29 neutral, 25 negative, and 15 positive co-culture interactions which significantly shifted across the different nutrient conditions with some showing no resilience to the nutrient load. Some combinations collaborated to stay resilient to the high nutrient strain, for example *Pseudoalteromonas spiralis* S5 when grown with *Rhodococcus cerasti* S3 in high phosphate concentrations grew 827.9 ±14.3 mm larger growth whereas *Rhodococcus cerasti S3* showed the highest growth curves with *Pseudoalteromonas spiralis* S5. Four species of fungi were isolated and identified. Evidence showed both bacteria and fungi indicated potential for new drugs. However, this was beyond the scope of this research paper.

The project demonstrated greater clarity on how the bacteria important to seagrass react to sewage pollution. Future work could trial integrating bacterial strains that work well together to improve seagrass health and resilience to anthropogenic influences.

Declarations

This work has not previously been accepted in substance for any degree and is not being concurrently submitted in candidature for any degree.

Signed..... .....[redacted].....

Date.....30/9/2025.....

This thesis is the result of my own investigations, except where otherwise stated.

Other sources are acknowledged by footnotes giving explicit references. A

bibliography is appended.

Signed..... .....[redacted].....

Date..... 30/9/2025.....

I hereby give consent for my thesis, if accepted, to be available for electronic sharing

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Date..... 30/9/2025.....

The University's ethical procedures have been followed and, where appropriate, that

ethical approval has been granted.

Signed..... .....[redacted].....

Date..... 30/9/2025.....

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Abbreviations

Abbreviations	Definition
AUC	Area Under Curve
BGC	Biosynthetic Gene Cluster
DNA	Deoxyribonucleic acid
F2	Guillards F2 media
MA	Marine Agar
MB	Marine Broth
MEA	Malt Extract Agar
MEAA	Malt Agar Extract & Antibiotics

Introduction

The Importance of Seagrass

Seagrasses are marine angiosperms covering 17 million hectares of global oceans providing a wide range of ecosystem services (Waycott et al., 2009). Seagrasses are known to form extensive meadows in healthy shallow marine environments. They provide crucial ecosystem services such as food production, carbon sequestration, and nursery grounds for various commercially important fish species such as Cod (Lima et al., 2023). They play a significant role in mitigating climate change and preserving biodiversity (Vasco et al., 2022; Unsworth et al., 2018). They are known to be associated with a high biodiversity and strong connectivity to neighbouring ecosystems such as mangroves, kelp forests and coral reefs. While the importance of seagrass for coastal ecosystems and carbon sequestration is widely acknowledged, seagrass meadows are suffering significant decline from fishery trawl, storm damage and increased stress from nutrient pollution (Orth *et al*., 2006; Waycott *et al*., 2009; Short *et al*., 2011). Seagrasses form foundational habitats in coastal ecosystems, where their associated microbiomes play a pivotal role in maintaining ecosystem functioning (Cúcio et al., 2016). The microbiome supports essential processes such as seed germination, nutrient availability, and defence against pathogens (Tarquinio et al., 2019). Recognising the importance of the seagrass holobiont is crucial for restoration efforts and for assessing the health of seagrass ecosystems (Tarquinio et al., 2021; Ugarelli et al., 2017).

Seagrass microbes

The importance of plant microbiomes is widely recognised with wide ranging benefits of microbe-microbe interactions for the host plant (Trivedi et al., 2020). The seagrass microbiome is highly diverse, comprising not only bacteria but also fungi, protists and microalgae (Hurtado-McCormick et al., 2019). Recent studies show that bacteria are major constituents, but fungi and microalgae are also components for processes such as seed germination, nutrient cycling, and pathogen defence (Tarquinio et al., 2019). Distinct microbial communities inhabit different seagrass microenvironments, with bacteria showing stronger microenvironmental preferences compared to fungi (Hurtado-McCormick et al., 2019). Bacterial communities, form a holobiont with seagrasses and are crucial for plant development and survival. Specific bacteria inhabit leaves, roots, and rhizomes, with certain taxa such as Actinobacteria, Proteobacteria and Cyanobacteria potentially beneficial to seagrass blades (Egan et al., 2013; Rabbani et al., 2021; Yan et al., 2021). Endophytic bacteria in seagrass seeds may promote growth, fix nitrogen, and enhance resistance to disease, thereby improving seedling survival

and supporting restoration (Tarquinio et al., 2021). These communities exhibit varied levels of health and function across seagrass communities in different environmental conditions (Rabbani et al., 2021; Yan et al., 2021). A global study of the *Zostera marina* microbiome revealed a core fungal community with many unclassified taxa, underlining the need for further research on marine fungi (Ettinger et al., 2020). Notably, plant blades are colonised by fungi from orders such as Lobulomycetales, Glomerellales, and *Colletotrichum* – some associated with decomposition and infection – while beneficial fungi such as *Penicillium* and *Cladosporium* may contribute to detoxification and plant growth (Ettinger, 2020).

Functionally, the seagrass microbiome underpins key biogeochemical processes. Microbes mediate carbon, nitrogen, phosphorus, and sulphur cycles, directly sustaining marine productivity (Millan, 2022; Cifuentes et al., 2000). For example, oxygen loss from seagrass roots generates microenvironments favouring beneficial sulphur-oxidising bacteria, thus mitigating toxic hydrogen sulphide in anoxic sediments (Mejia et al., 2016; Cúcio et al., 2016; Fahimipour et al., 2017; Ugarelli et al., 2017). Symbiotic bacteria can also detoxify metals and excess phosphate, aiding plant growth and resilience in varied habitats (Martin et al., 2019; Valdez et al., 2020). Nitrogen-fixing microbes further connect primary production with higher trophic levels, as communities of seagrass present with nitrogen-fixing microbes are healthier producing more biomass (McRoy and Goering, 1974; Kirchman et al., 1984). Moreover, many symbionts metabolise organic carbon exuded by seagrass and synthesize phytohormones that influence host development (Tarquinio et al., 2019; Crump et al., 2018). Some evidence suggests that the bacterial communities benefit seagrass indicating they could be used as a probiotic enhancement to build resilience (Ransome, 2024). As these communities shift through the seagrass life stages, understanding of the communities from seeds to late-stage plants is required (Matsapume Detcharoen et al., 2024). Seagrass microbes and their interspecies interactions are affected by eutrophication, heavy metal contamination, and excessive nutrient inputs. These may disrupt microbiome functions such as denitrification, sulphide detoxification, and disease suppression leading to ecosystem destabilisation and loss of services (Ugarelli et al., 2017; Liu et al., 2018).

Threats to seagrass

Current ongoing anthropogenic activities show that the seagrass holobiont is vulnerable to multiple threats from human activities and environmental stressors. The microbiome responds rapidly to such changes, making it a potential ecological indicator (Conte et al., 2021). For instance, nutrient surpluses

stimulate heterotrophic bacteria, increasing sulphide concentrations and precipitating die-offs (Ugarelli et al., 2017). Nonetheless, some symbionts may still provide resilience by detoxifying sulphide and buffering the effects of eutrophication through the production of exocellular enzymes (Ugarelli et al., 2017; Pita et al., 2018). Despite growing recognition of these microbial associations, knowledge gaps remain. Many seagrass-associated bacteria and fungi are poorly characterised, and the dynamics of microbe-microbe interactions under environmental stress are not fully understood (Ettinger et al., 2020; Lima et al., 2023). Many studies use metagenomics and metabolomics to research microbial diversity and functioning. However, cultivation-based approaches are vital for experimentally validating these interactions (Shakya et al., 2019). Therefore, this research also aims to address the gap using novel high throughput approaches that enables an examination of the vast extent of microbes that are present in the seagrass microbiome. Further research is critically needed to understand how changes in environmental conditions, such as nutrient loading, affect microbiome structure and function. This understanding is essential for developing effective conservation and restoration strategies where controlled use of seagrass bacteria could be used as a probiotic to regions facing regional decline (Tarquinio et al., 2019; Yan et al., 2021).

Aims of this research

The overarching aim of this study is to build an understanding of the dynamic nature of the bacterial seagrass microbiome isolated from *Z. marina* seeds (Jones., 2024) under controlled environmental conditions.

Specifically, the objectives are to:

(i) Explore the pairwise interactions in the seagrass microbiome utilising and advancing novel high throughput technologies

(ii) Create a microbial interaction network based on this data transformed using R studio software

(iii) Simulate natural and eutrophic marine nutrient conditions assessing how nutrient pollution may affect the nature of the bacterial interactions

(iv) Identify microorganisms with a higher tolerance to high nutrient load in mono-culture or co-culture

(v) Isolate and identify seagrass fungi from seagrass for future investigation of their bioactive potential that may affect seagrass systems.

Materials and Methods

Bacterial strains and cultivation

The bacterial strains were selected from the inventory of seagrass bacteria stored at Swansea University from previous work (Jones, 2025). As the bacterial colonies were isolated by vortexing the seagrass seeds, it is unknown which species are endophytes and which are epiphytes. However, their association with *Z. marina* seeds should be studied to identify any significant relationships which could be important for germination and early growth (Tarquinio et al., 2021). Many of the strains are recognised for their importance to the microbiome. Examples include *Vibrio hispanicus* which are known to assist in nutrient mineralisation, or *Variovorax paradoxus* which is evidenced to stimulate early plant growth in seed cotyledon (Jones, 2025; Sun et al., 2024). The strain *Lysobacter luteus* S6 and S7 was taken from separately isolated colonies of the same species to observe any difference in growth or behaviour between strains. Furthermore, the selected strains had similar growth responses to f2 media and would be ideal strains for analysis in the high throughput pipeline.

Bacterial strains (Table 1) were isolated in a previous study from seeds of *Z. marina* or associated sediment in September 2023 (Jones., 2024) by plating on Marine Agar (MA; Marine Broth (MB; Millipore) + 1.5% agar (Formedium)) and F2 (Sigma) + 2% instant ocean salts (IO; aquarium systems) + 1.5% agar with additives of ammonium sulphate (Thermo Scientific) at 2.02 mM final concentration and sucrose (0.2% final concentration, as used by Cifuentes et al., 2003, Thermo scientific). The pure isolates were stored in 30% glycerol and 70% MB at -80 °C. at -80 °C.

Bacteria were inoculated on MB + 1.5% agar from the freezer stock and incubated at 25 °C for 14 days. To adapt bacteria to the experimental conditions, they were grown in F2 + 2% IO supplemented with 11 nM glucose (Thermo scientific) and 50 nM NH_4Cl (Thermo scientific) (F2+IO+glu+N) with or without 1.5% agar at 25 °C and 150 rpm.

Media preparation

Marine broth media: 3.74g of Marine Broth (Millipore) and 1L of deionised water. For solid agar 1.5g of Agar (Formedium) was added before autoclave.

F2 media: 20 ml of Guillards nutrient media (Culture collection of algae and protozoa), 20 g of instant ocean salt (aquarium systems), 2 g of glucose (Thermo Scientific), 0.267g of Ammonium chloride (Thermo Scientific), 15g Agar (Formedium) with 980 ml of deionised water.

MEA: 50 g Malt extract agar (Formedium), 20 g instant ocean salt (aquarium systems) with 1L of deionised water

MEAA: 50 g Malt extract agar (Formedium), 20 g instant ocean salt (aquarium systems), with 1L of deionised water, 1.5ml of both Ampicillin (100 µg/mL) and Chloramphenicol (35 µg/mL) (Thermo Fisher), added after autoclave when cooled to 60 °C

Table 1: List of bacterial species used in this investigation.

Species number	Strain ID (Jones 2024)	Species based on 16S rRNA gene	Phylum
S1	*St2h*	*Vibrio hispanicus*	Proteobacteria
S2	*St2a*	*Phycrobacter pulmonis*	Proteobacteria
S3	*St3a*	*Rhodococcus cerasti*	Actinomycetota
S4	*St3c*	*Pseudoalteromonas fulgina*	Proteobacteria
S5	*St1a*	*Pseudoalteromonas spiralis*	Proteobacteria
S6	*St1e*	*Lysobacter luteus*	Proteobacteria
S7	*St1h*	*Lysobacter luteus*	Proteobacteria
S8	*Sd2b*	*Marinomonas primoryensis*	Proteobacteria
S9	*St2b*	*Chrysobacterium indolthethicum*	Bacteroidetes
S10	*Sd3a*	*Variovorax paradoxus*	Proteobacteria
S11	*Sd3g*	*Psychromonas arctica*	Proteobacteria
S12	*St3h*	*Rhodococcus yunnanensis*	Actinomycetota

Species identification and genome sequencing

For 16S rRNA sequence analysis, first, a tiny amount of bacterial biomass was lysed in 200 µL dH_2O in 1.5 mL tubes through heating in a water bath to 95 °C for 180 mins before being centrifuged for 3 mins at 20,000 x g. 25 µL of PCR reaction was prepared for each strain using universal primers (16S rRNA 27F_16s_F & 1492R_16S_R (0.2 µM, (Ludwig, 2007)) and DreamTaq (Qiagen) and supernatant from cell lysate. The PCR involved denaturation at 95 °C for five minutes, then 29 cycles of denaturation at 95 °C 30s seconds, annealing at 55 °C for 30 seconds and extension period of 72 °C for 30 seconds before a final extension of 72 °C for five minutes. As proof of successful PCR, the product was then checked using 2% agarose gel electrophoresis with a 1Kb ladder as expected PCR products were not predicted to be greater, before being sent for sequencing at Eurofins. Amplicons were purified using the Promega PCR purification kit and then sent off to Eurofins for Sanger sequencing. The 16S rRNA sequences were submitted to NCBI BLAST using the 16S rRNA database limited to type material searching for highly similar sequences to identify their taxonomic classification of strains being used as per manufacturer's instructions.

Genomic DNA of the strains *Vibrio hispanicus* S1, *Phycrobacter pulmonis* S2, *Pseudoalteromonas spirlais* S5, *Lysobacter luteus* S6, *Lysobacter luteus* S7, *Chrysobacterium indolthethicum* S9, *Variovorax paradoxus* S10 and *Psychromonas arctica* S11 was extracted using Nucleospin Tissue, DNA RNA and protein purification kit. Whole genome sequencing was performed using Oxford Nanopore (Oxford Nanopore Technologies, 2021). With the help of a fellow student Hongli Wang, the reads were quality checked and filtered using Nanofilt and assembled using Nextdenovo or Fly. Phylogenetic trees were created using MEGA12) . Phylogenetic trees were constructed using autoMLST by inputting the assembled genome FASTA files. The autoMLST, identified and concatenated conserved housekeeping genes and generated a multiple sequence alignment, which was used to infer a maximum-likelihood phylogeny with IQ-TREE using 1,000 ultrafast bootstrap replicates. Closely related type strains from NCBI were included for reference. The resulting tree file was exported in Nexus format and visualised and formatted using MEGA12. Genomes were additionally analysed using antiSMASH (2025) to identify biosynthetic gene clusters associated with secondary metabolite production. Furthermore, the extracted genomes were analysed using antiSmash (antiSMASH, 2025) to identify biosynthetic gene clusters responsible for producing secondary metabolites.

Co-cultivation setup

The ROTOR+ HDA robot (Singer Instruments) was utilized to create mono-cultures and co-cultures on agar plates (Figure 1). Pre-cultures of all strains grown in liquid MB for one week were diluted to an OD600 of 0.4 in F2 media. Two 96 well microtiter source plates (Thermo Fisher Scientific, 167008) were prepared contain 100 µL of diluted pre-culture per well: one plate with strains organized in columns to represent the first strain of each pair, and the other plate with strains arranged diagonally to represent the second strain (Table A1). This setup enabled bacterial co-inoculation, plates that were then incubated at 25 °C in the Reshape Biotech Imaging System with images being taken every 2 hours for 7 days. The imaging system consists of an incubation chamber with controlled lighting along with a camera capturing the top and bottom of the agar at controlled time points to monitor colony growth as a time lapse. Images are automatically analysed within the Reshape software using AI driven colony characteristic measuring technology, this means that at regular time points, any co-cultured colony size can be measured.

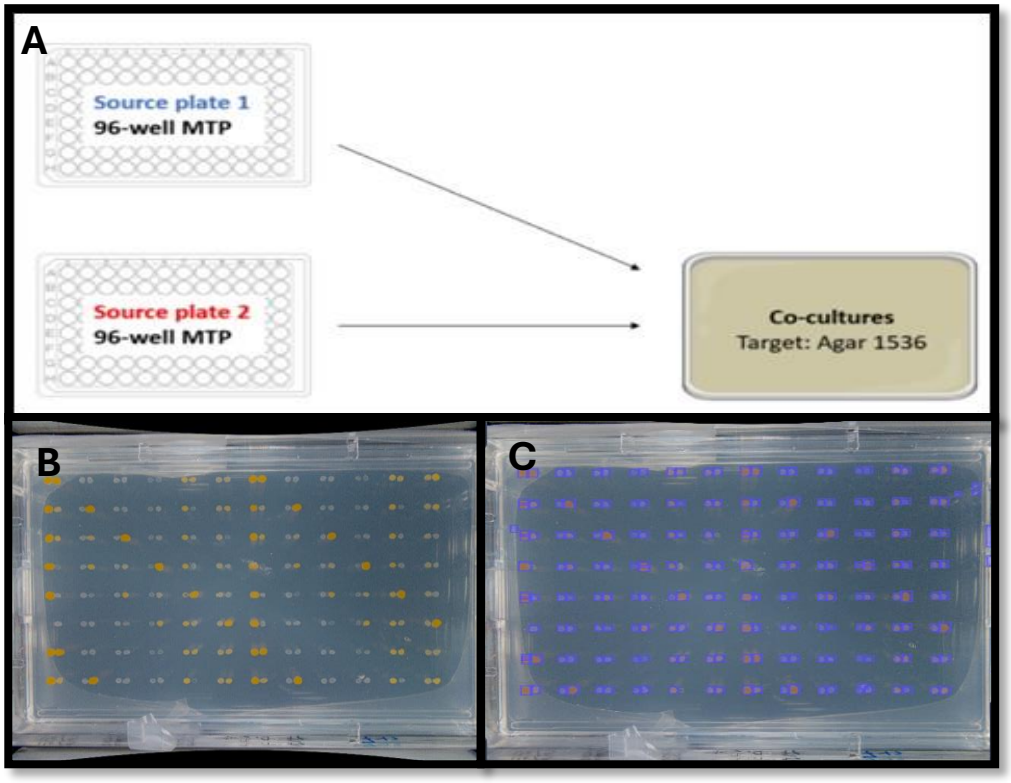


Figure 1: A) Overview of the assembly of co-cultures using the ROTOR+ pinning machine. B) Photographs taken in the Reshape machine showing co-culture growths on F2 media with 2% glucose carbon source. C) Identification of bacteria 'object' tracked by the Reshape labelled as object ID and measured at controlled intervals

Growth conditions

To evaluate the effect of environmental stressors on microbial interactions, co-cultures were monitored on F2 supplemented with additional nitrate, phosphate, iron or copper (Table 2) along with the original standards of F/2 media as listed in Appendix 5, resulting in nine different media nutrient conditions tested including the control without nutrient addition. To simulate eutrophic conditions, a medium and high concentration for each nutrient was tested. The medium nutrient concentrations of nitrate, phosphate and iron correspond to a yearly average nutrient content of the UK coastline measured by Copernicus satellites (Copernicus, 2020). The high concentrations are double the medium concentrations, which was decided due to lack of accurate measurements available in literature. Generally, the high concentrations exceed the environmental annual average, therefore would likely be associated with high concentrations attributed to nutrient pollution. Copper is not being measured in Copernicus datasets; therefore, values were taken from a study assessing which intermediate and extreme copper concentrations bacteria from the seagrass *Cymodocea nodosa* are tolerant to (Llagostera et al., 2016).

Table 2: Concentrations of additional nutrients added to F2 to simulate nutrient stress. The medium and high columns correspond to the nutrient additves added to the media where as the final concentration column are the final concentration of chemicals when factoring in the nutrients previously present in the f/2 nutrient media (Appendix 5)

Compound	**Chemical formula**	**Concentration (mmol/L)**			
		Medium	Final concentration	High	Final concentration
Sodium nitrate (Thermo scientific)	$NaNO_3$	5.00×10^{-3}	$2.26x10^{-2}$	1.00×10^{-2}	$2.76x10^{-2}$
Sodium phosphate (Thermo scientific)	Na_2HPO_4	5.00×10^{-4}	$1.22x10^{-3}$	1.00×10^{-3}	$1.72x10^{-3}$
Copper chloride (Acros organics)	$CuCl_2$ (dihydrate)	1.47×10^{-2}	$1.47x10^{-2}$	2.93×10^{-2}	$2.93x10^{-2}$
Ferrous sulphate (Thermo scientific)	$FeSO_4,7H_20$	1.35×10^{-5}	$2.47x10^{-4}$	2.70×10^{-5}	$2.6x10^{-4}$

Data analysis

Initially, data from the reshape machine was formatted in excel to prepare for analysis, this involved assigning the correct co-culture to the Object ID label given by the Reshape. Data analysis was performed in R studio where the bacterial growth data received by the Reshape Ecosystem was plotted as a curve using the R packages ggplot2, dplyr, tidyr, stringr, forcats, randomcoloR, tidyverse, ggraph, tidygraph (Pedersen, 2024; Pedersen & RStudio, 2022; Hadley Wickham, 2019a; Wickham, 2019b; Wickham, 2020; Wickham et al., 2020; Wickham & Henry, 2020; Wickham & RStudio, 2023; Wickham & RStudio, 2019). Growth curves for each bacterial strain were plotted at six-hour intervals. The area under the curve (AUC) was calculated as an average of the replicates along with standard error values. These AUC values were taken further for interaction network analysis and statistical analysis. The statistical analysis involved multiple T-tests comparing the AUC values for co-cultures to the appropriate mono-culture. Previous studies examining bacterial interaction networks compare relationships within interaction networks by using qPCR to estimate cell numbers as a proxy for biomass along with change in colony size at endpoint value (Blasche et al., 2021). However, the equipment used in this research project allowed to measure bacterial growth in co-cultures over time. Therefore, the potential for the AUC values to provide more accurate data for interpretation and network analysis is possible. This is because growth rates only provide the comparison of colony size between the start and end points. The growth curves allow the calculation of the precise growth rates from the regular six-hour intervals of each co-culture. The AUC values were converted into interaction score ranging from -1.0 to 1.0 which symbolises negative interactions and positive interactions. The interaction score was calculated using the following formula: $\frac{(co\text{-}culture\ AUC)}{(monoculture\ AUC)} - 1$. These interactions scores were further classified with values between -0.1 to 0.1 are neutral with negative values between -0.1 to -1 and positive values between 0.1-1.0. This assumption was made due to the shortage of studies analysing bacterial interactions through comparisons to mono-cultures in this manner. Similar studies have calculated interaction scores from inferring an interaction matrix from a single microbial sample, using a dissimilarity-overlap analysis (Yogev Yonatan et al., 2024). However, the collection of mono-culture and co-culture data allows for a simpler approach using the previously stated assumptions. Any co-culture combination that did not have a mono-culture to be compared to were left out of the interaction plots to ensure there were no false results from incorrect calculations. All code is available at the GitHub repository: https://github.com/PEllwood-Swansea/Masters-thesis-data-analysis/tree/82885392621c0688280959e46497163cab076ad4. To begin characterising baseline interactions between bacterial co-cultures, the growth data obtained from the Reshape

Ecosystem was configured to associate the tracked 'object ID' of each colony with its strain ID and the co-culture that the strain was grown in. The growth data was arranged to plot mean bacterial growth which is then grouped by the co-culture that the strain is in. Due to occasional absence in bacterial growth, some growth curves are based off limited data resulting in high standard error bars, however the data was checked to ensure the tracked object IDs were assigned to the correct co-culture. Analysing the results of the growth curves will involve reviewing the primary strain in the co-culture, e.g. *V. hispanicus* S1, and reviewing its growth when cultured with the second strain, then written as S1-S2. This will view the effect that *Pb. pulmonis* S2 has on the growth of *V. hispanicus* S1. Analysing the results of the growth curves will involve reviewing the growth of a strain, e.g. S1, in mono-culture and comparing it to its growth when co-cultured with a second strain, e.g. S1-S2. This will identify the effect that S2 has on the growth of S1.

Fungal isolation and identification

To obtain seagrass-associated fungi, blades, roots, root sediment and surrounding sediment were collected from Llanelli mudflats (51°40'42.6"N 4°10'52.2"W , on the 11/04/2025). Collection involved gently unearthing the sediment with a spade to not disturb or damage the plants. Seagrass was taken and placed into Ziplock bags and transferred to the laboratory within 2 hours for processing. Further seagrass samples were obtained from a seagrass specific aquarium maintained at Swansea University. For fungal isolation, seagrass samples were washed with sterile water to remove sediment, disinfected with 95% (5% deionised water) ethanol for ten seconds, 70% (30% deionised water) ethanol for one minute and then 10% (90% deionised water) bleach for one minute. The blades and roots of the seagrass were then separately printed onto MEAA to transfer any epiphytic fungi to the plate and then placed onto a separate MEAA plate to isolate endophytic fungi. Plates were incubated at 20 °C for 14 days. Any residual epiphytic fungi living on the seagrass surface not destroyed from the ethanol sterilisation or endophytic fungi growing on MEAA were re-streaked to obtain single colonies before DNA extraction and sequencing. Genomic DNA was extracted using the DNeasy plant mini kit (Qiagen). The ITS region was amplified using ITS1 and ITS4 primer (Thermo Fisher) Phusion polymerase and buffers from the Phusion PCR kit (Thermo Fisher). Denaturation occurred at 98 °C for five minutes, and then 35 cycles of 98 °C for 10 seconds, an annealing temperature of 60 °C for 20 seconds and an extension period of 72 °C for 30 second, before. As proof of successful PCR, the product was then checked using 2% agarose gel electrophoresis before being sent for Sanger sequencing at Eurofins. The sequences were compared to the NCBI ITS database using BLAST limited to type material. Multiple

strains were found and photographed, however only a few were identified to species level due to time constraints.

Results

Bacterial Growth in Control media

To assess the high throughput workflow and establish baseline data, the bacterial growth in control media was plotted as a curve from the formatted Reshape data. This generated growth curves of the bacterial strains by measuring the colony area every six hours. In mono-culture, all 12 strains (*Vibrio hispanicus* S1*, Phycrobacter pulmonis,* S2*, Rhodococcus cerasti* S3*, Pseudoalteromonas fulgina,* S4*, Pseudoalteromonas spiralis* S5*, Lysobacter leutus* S6*, Lysobacter leutus* S7*, Marinomonas primoryensis* S8, *Chrysobacerium indolthethicum* S9, *Variovorax paradoxus* S10, *Psychromonas arctica* S11, *Rhodococcus yunnanensis* S12) showed consistent growth in control F2 (Figure 2). Some of the largest colonies observed were for *L. luteus* S6 reaching 11-12.5 mm^2 ±7.5 mm^2 after one week of growth. This is larger than growth of other strains such as *P. arctica* S11 which showed minimal growth varying between 2.5-4 mm^2 ±1.5 mm^2, which is an increase over the week growth by ~1.5 mm^2. This is further shown in the AUC values where there is a clear difference in growth curves between the strains (Figure 3). *R. cerasti* S3 had the fastest initial growth rate with the majority of growth occurring within the first two days whereas *Ps. fulgina* S4 grew modestly from 0.1 mm^2 to 2.5 mm^2 ± *3.2* mm^2. Furthermore, strains such as *M. primoryensis* S8 and *V. paradoxus* S10 have fluctuating growth curves between the mono-cultures and co-cultures (Figure 2).

When grown in co-culture, the effects of pairing different bacterial strains produced pronounced differences, examples include *L. luteus* S7 which grew four times smaller in co-culture than it did in mono-culture. Multiple inhibitory pairings of *L.luteus* as both S6 & S7 were identified: S6–S3 ($p = 4.8 \times 10^{-5}$), S6–S5 ($p = 0.029$), S7–S5 ($p = 0.011$). More inhibitory pairings were identified via t-tests; S2–S7 ($p = 0.014$), S12–S9 ($p = 1.4 \times 10^{-7}$), and S12–S11 ($p = 1.5 \times 10^{-4}$) & S5-S3 ($p = 0.010$). Overall, 32 of the 77 pairings were inhibitory interactions (Table A3) indicating that inhibitory interactions are the most abundant in media with minimal nutrient enhancement. . Some co-cultures showed large variances in AUC values, when *R. cerasti* S3 was co-cultured with *M. primorysensis* S8 and *C. indolthethicum* S9 it showed much higher variance in growth sizes revealing its most consistently similar growth curves

were in mono-culture. In the control media there were also 21 positive, out of 77 interactions observed. *Py. arctica* S11 showed higher growth with Ps. *spiralis* S5 & *L.luteus* S6, S11–S5 ($p = 0.028$) and S11–S6 ($p = 3.5\times10^{-7}$). Indicating that while *Py. artica* S11 can form symbiotic relationships with strains under nutrient limited control media. Another positive interaction was observed between *Ps. fulgina* S4 and C. *indolthethicum* S9 ($p < 0.05$). An asymmetric relationship was observed for *Py. arctica* S11 and *R. yunnanensis* S12. *Py. arctica* S11 grew significantly better in co-culture with *R. yunnanensis* S12 than in its mono-culture ($p = 0.011$), however the reciprocal interaction (S12–S11) was negative, i.e. *Py. arctica* S11 grew less in co-culture with *R. yunnanensis* S12 then in its mono-culture ($p = 0.041$).

Overall, the control condition successfully shows a baseline of bacterial mono- and co-cultivation with a wide range of growth phenotypes. While some strains, such as *Ps. spiralis* S5 and *L. luteus* S6, consistently inhibited partners. Others, such as *Py. arctica* S11, showed facilitative relationships with both *L. luteus* S6 and *Ps. spiralis* S5. There is also a large abundance of neutral interactions as 24 of the 77 interactions had no significant difference between the mono- and co-cultures. The production of growth curves allows simple comparisons of the bacterial co-cultures to the mono-cultures. Representing this for all the nutrient conditions is simpler when summarised to area under curve values (Figure 3).

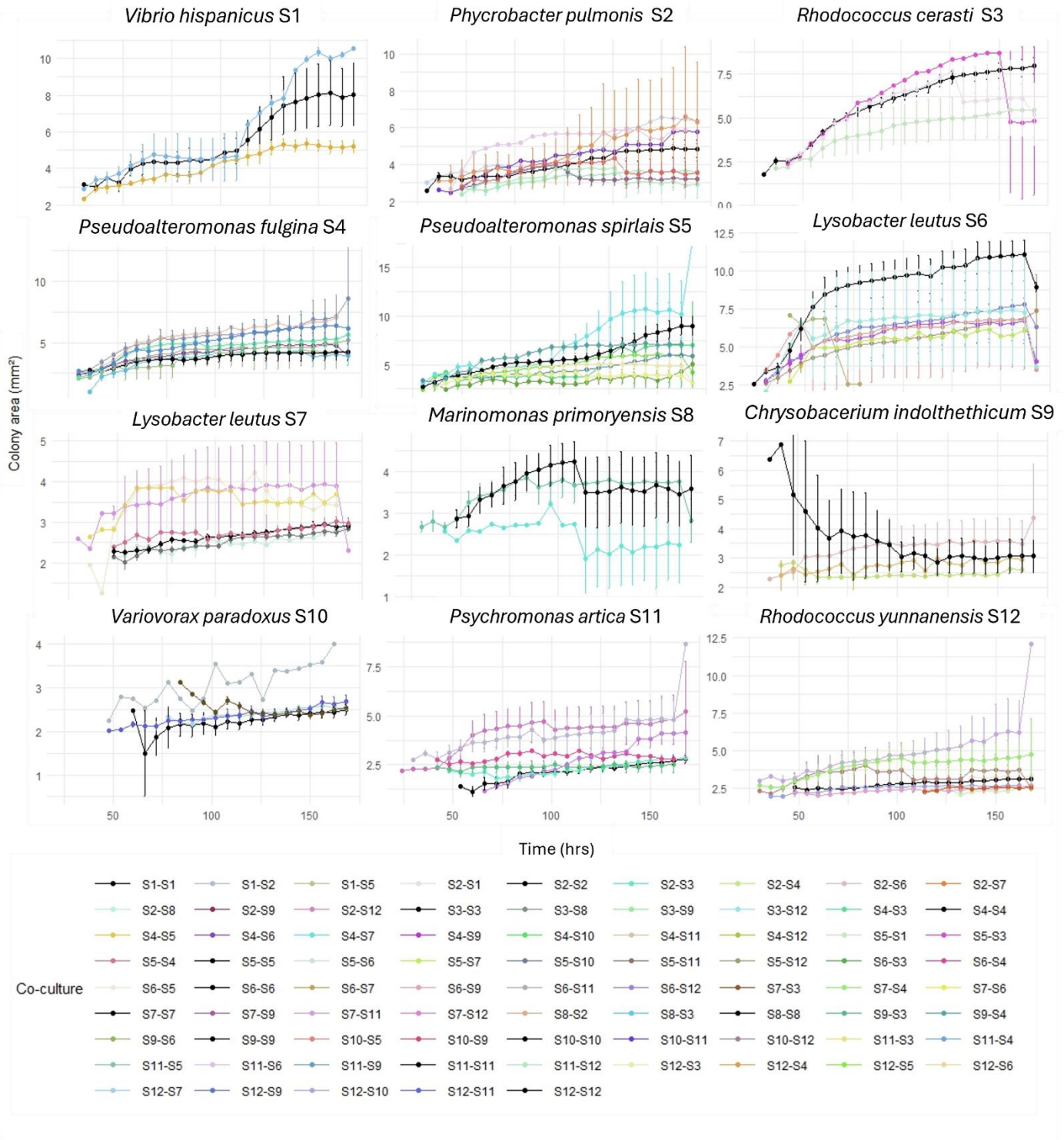


Figure 2 : Growth curves of all bacterial strains (*Vibrio hispanicus* S1*, Phycrobacter pulmonis*, S2, *Rhodococcus cerasti S3, Pseudoalteromonas fulgina*, S4, *Pseudoalteromonas spiralis* S5, *Lysobacter leutus* S6, *Lysobacter leutus* S7, *Marinomonas primoryensis* S8, *Chrysobacerium indolthethicum* S9, *Variovorax paradoxus* S10, *Psychromonas arctica* S11, *Rhodococcus yunnanensis* S12) in all mono- and co-cultures on F2 agar (control condition) represented by average colony area (mm^2) over time (hrs) at 6 hour intervals. Error bars represent standard errors across replicates (n = 5; statistical analysis available in Appendix table 3. Mono-cultures are represented by black lines.

Effects of nitrate on co-cultivation

Increasing nitrate concentration in the medium resulted in various responses of the co-cultures with both facilitative and inhibitory interactions being observed (Figure 3). No co-cultures involving *Ps. spiralis* S5 or *L. luteus* S6 grew under medium nitrate, suggesting a strong intolerance to this nutrient stress. Medium nitrate addition caused five of the twelve strains to benefit significantly in mono-culture compared to the control, including *L. luteus* S7, *C. indolthethicum* S9, *V. paradoxus* S10, *Py. arctica* S11, and *R. yunnanensis* S12. For example, *V. paradoxus* S10 increased by +191 mm^2 ± *64.5* mm^2 *(*p = 0.016), while *L. luteus* S7 grew +166.8 mm^2 ± *70.63* mm^2 (p = 0.036). However, these improvements were largely restricted to mono-cultures, as most co-cultures under medium nitrate did not grow significantly better than their controls. However, mono-cultures of *Ps. spiralis* S5 and *L. luteus* S6 were inhibited, reductions of –288.5 mm^2 ± *93.92* mm^2 *(*p = 0.0037) and –532 mm^2 ± *195.9* mm^2 *(*p = 0.013). When the nitrate concentration was doubled, some mono-cultures were inhibited in comparison to the control medium with only the mono-culture of *C. indolthethicum* S9 showing marginally higher (non-statistically significant) growth. Strains that showed inhibition such as *Ps. spiralis* S5 (–384.1 mm^2, ± *72.24* mm^2 p = 2.3×10^{-13}) and *R. cerasti* S3 (–219.2 mm^2, ± *57.4* mm^2 p = 0.0014). Meanwhile others thrived; co-cultures such as *R. yunnanensis* and *C. indolthethicum* S12–S9, which grew more in high nitrate (180.68 mm^2 ± 79.77 mm^2, p = $4.29\text{x}10^{-2}$) than in the medium nitrate condition (133.94 mm^2 ± 37.06 mm^2, p = $2.4\text{x}10^{-2}$). Overall, nitrate enrichment revealed a polarised community as certain strains benefitted; however, many strains, including (*R. cerasti* S3, *Ps. spiralis* S5, *L. luteus* S6) were strongly suppressed. This suggests that nitrate availability strongly benefits some strains while inhibiting others (Table A3).

Effects of phosphate on co-culture growth

At medium phosphate, three strains, namely *Ps. fulgina* S4, *Ps. spiralis* S5 and *Pb. pulmonis* S2, displayed significantly improved growth compared to the F2 control medium (Figure 3). The *Ps. spiralis* S5 increased by 1,104 mm^2 ±*103.8* mm^2 (p = 1.6×10^{-13}) in mono-culture. However, when phosphate levels were further increased in the high phosphate condition, *Ps. spiralis* S5 showed reduced growth in mono-culture in comparison to the control (–715 mm^2 AUC, ± 72.15 mm^2 p = 2.2×10^{-9}). Other strains such as *Ps. fulgina* S4 & *Py. arctica* S11 showed similar trends with higher growth in the medium condition and being unable to grow in the high phosphate condition. In high phosphate there was a reduced diversity with only three strains (*L. luteus* S5, *R. cerasti* S3, and *R. yunnanensis* S12) being able to grow. Many of the co-cultures followed a similar trend to the mono-cultures. For example, *Ps.*

spiralis S5 grew +827.9 mm^2 ± *146.86* mm^2 ($p = 7.8\times10^{-5}$) in co-culture with *R. cerasti* S3 in medium phosphate, while *R. yunnanensis and Py. arctica* S12–S11 also achieved higher growth (+179.6 mm^2, ± 24.26 mm^2 $p = 2.5\times10^{-4}$) than either mono-culture of the two strains, suggesting facilitation under moderate phosphate enrichment.

Effects of iron on co-cultivation

Iron enrichment supported many successful co-cultures to grow, however not many colonies had significantly larger AUC values than the co-cultures in the control media. At the medium iron concentration, several mono-cultures grew significantly better than in the control media, including *L. luteus* S7 (+282.42 mm^2, ± 74.91 mm^2 $p = 4.6\times10^{-6}$) and *Py. arctica* S11 (+292.71 mm^2 ± 106.09 mm^2, $p = 1.62\times10^{-02}$). However, iron additions showed lower growth sizes than the control for many co-cultures , examples such as, *Ps. fulgina and L. luteus* S4–S7 showed significant inhibition in the *L. luteus* S7 strain (–301.3 mm^2, ± 113.51, $p = 1.82x10^{-2}$), a trend consistent with its behaviour in control media, where *L. luteus* S7 failed to grow when paired with others. The only co-cultures of *L. luteus* S7 when compared to monoculture were when paired with *Py. arctica* S11 and *R. yunnanensis* S12 which showed higher AUC values by +100 and +300. When iron concentrations are doubled, some co-cultures showed greater positive relationships than when in medium concentration although the overall trend was still biased toward inhibition when compared to the same co-cultures in control media. Positive examples included *R. yunnanensis* and *C. indolthethicum* S12–S9 (145.10 mm^2 ± 41.62, $p = 1.07x10^{-2}$) and *R. yunnanensis* S12 mono-culture (51.43 mm^2 ± 25.27, $p = 4.23x10^{-2}$). Several mono-cultures including *V. paradoxus* S10, *Py. arctica* S11, and *L. luteus* S7 showed high growths in high iron ranging from 300–500 mm^2, all $p < 0.05$ when compared to the same combination in control media), but their co-cultures did not show the same growth response, suggesting that under the iron enrichment amplifies the competitive inhibition of the co-cultures.

Effect of copper on bacteria co-cultivation

The addition of copper tested produced the largest change in bacterial interactions compared to the control media (Figure 5). The most significant of which are, *Py. arctica S*11–S11 which grew +67.5 mm^2 ± 24.42 mm^2 ($p = 0.0187$) compared to control media. However, when *R. yunnanensis* S12 is paired with *Py. arctica* S11, the co-culture showed even larger colony areas (+126.2 mm^2, ± 26.26 mm^2 $p = 2.5\times10^{-4}$), which is the largest copper-driven boost observed when compared to the mono-culture in the same media. The medium copper concentration produced the swarming of the colonies on agar (Figure 4) as well as positive growths between *R. yunnanensis and Py. arctica* S12-S11, which is a large

variation in responses as, while some bacteria indicated stress from the swarming, other co-cultures showed copper resilient growth.

Despite some bacteria growing successfully on the medium copper concentration, many strains including monocultures of *R. cerasti* S3, *Ps. spiralis* S5, *L. luteus* S6, *C. indolthethicum* S9 and co-cultures of *L. luteus and Py. arctica* S6-S11, all showed significantly lower growth compared to the control media with an average reduction of ~400 mm^2 (Table A2). At the high copper concentration, growth was almost entirely suppressed with only the monoculture of *C. indolthethicum* S9 showing any sign of growth (234.2 mm^2 ± 102.9 mm^2 $p = 2.71x10^{-2}$), however less than on the control medium.

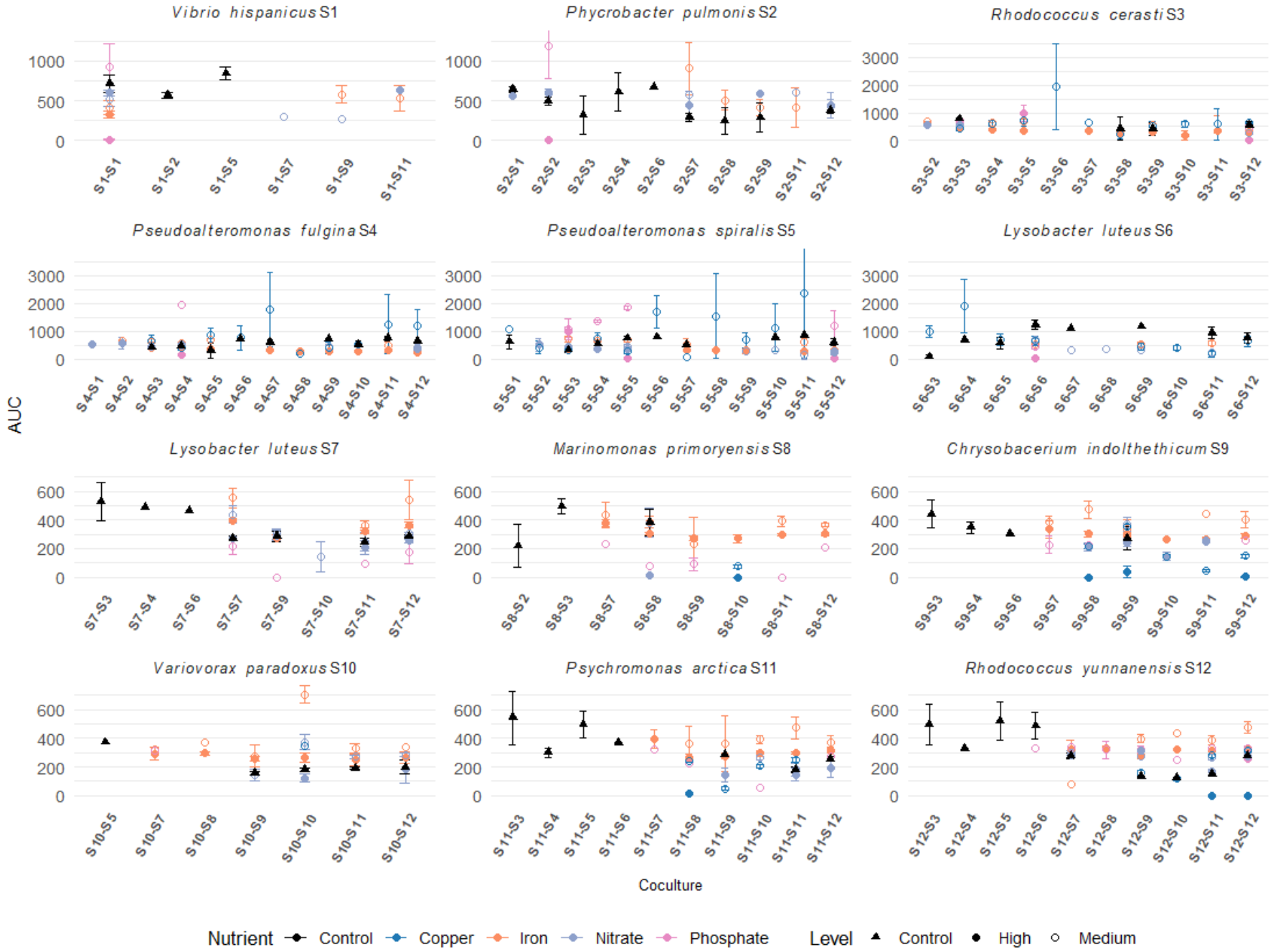


Figure 3: Area Under Curve (AUC) values of all cocultures grouped by starter strain. Across eight different nutrient conditions: Control, Medium Iron, High Iron, Medium Phosphate, High phosphate, Medium Phosphate, Medium Nitrate, High Nitrate and Medium & High Copper. Each dot represents the mean AUC of a coculture with ±SE

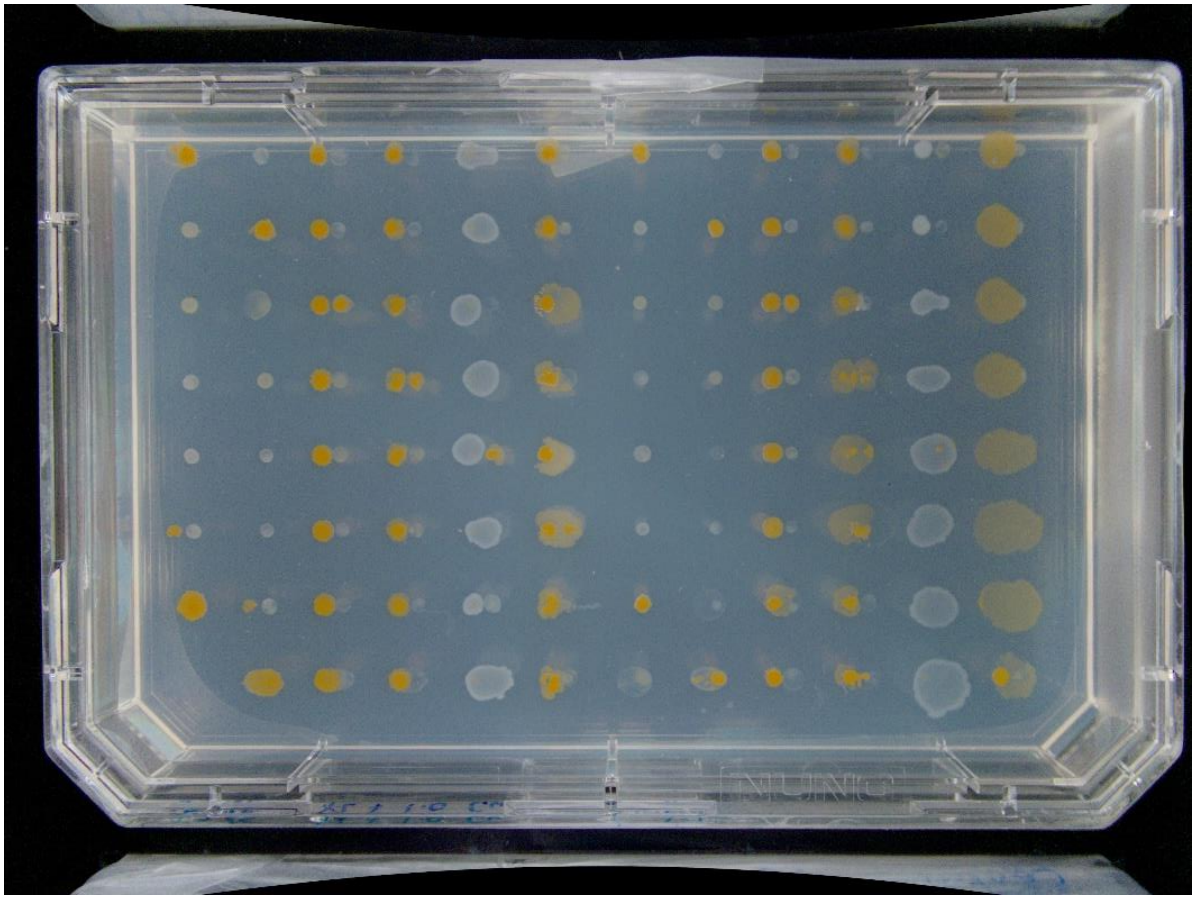


Figure 4: Image of the omni tray used in the medium copper concentration co-culture experiment showing bacterial strains swarming .

Effects of nutrients on bacterial interactions

In the control condition there were 29 neutral, 25 negative, and 15 positive co-culture interactions (Figure 5). There were also variations in how different strains of the same species behaved, the two *L. luteus* isolates S6 and S7 behaved differently, with *L. luteus* S7 engaging in largely neutral interactions while *L. luteus* S6 showed widespread inhibition. Varied interactions were also observed as *V. paradoxus* S10 promoted *P. spiralis* S5, whereas *Ps. spiralis* S5 inhibited *V. paradoxus* S10. Such directional imbalances were also seen between *C. indolthethicum* S9 and *L. luteus* S6, *L. luteus* S6 and S11, and even between the two *L. luteus* strains themselves (S6–S7). These examples highlight how interactions in control conditions were partner specific, however the high abundance of interactions seen provide a foundation to compare the effects of media with nutrient additions.

The interaction networks of bacterial co-cultures in medium nitrate (Figure 5), interactions showed signs of inhibition, though some positive relationships persisted, particularly involving *Pb. pulmonis* S2, *L. luteus* S7, *C. indolthethicum* S11, and *R. yunnanensis* S12. Doubling the nitrate concentration creates large shifts in interactions as negative interactions fell by 23 to 5 and many previously inhibitory relationships became neutral or positive. Therefore, nitrate enrichment at medium concentrations caused many inhibitory interactions between the co-cultures. When the concentration doubled, although some co-cultures such as R. *yunnanensis* and *R. cerasti* S12-S3 or *L. luteus and V. paradoxus* S7-S10 don't grow, the network of interactions shows an overall decrease in inhibitory interactions as it shifts to more positive and neutral interactions.

In medium phosphate, interactions were largely neutral with six negative and only a few positive interactions seen between *C. indolthethicum* S9, *Py. arctica* S11, *R. yunnanensis* S12. When phosphate was doubled, the network collapsed almost completely showing that high phosphate conditions are too toxic for bacterial growth. Only three strains (*Ps. spiralis* S5, *R. cerasti* S3, and *R. yunnanensis* S12) continued to grow, with all others inhibited. Interestingly, under this stress a new mutualism between *Ps. spiralis* S5 and *R. cerasti* S3 emerged, which was absent under medium phosphate, suggesting that phosphate toxicity may induce cooperative behaviours as a survival mechanism. The phosphate media also produced a visual observation of clearing halo around *Ps.spiralis* S5 (Figure 6) where *Ps. spiralis* S5 could be metabolising phosphate.

The medium iron condition produced more successful growths than any other media, when examining the interaction type of medium iron, most interactions were negative or neutral, with only a few positives (notably involving *V. hispanicus* S1, *L. luteus* S6, and *C. indolthehicum* S9). When iron concentrations are doubled, the network contracted further into dominant inhibition, with only a single positive edge observed (*L. luteus* S7 promoting *Py. arctica* S11). Increases in iron enrichment intensified competition between strains producing increasingly hostile networks. However, a diverse range of co-cultures successfully grew in the iron nutrient media in comparison to the Nitrate, phosphate and copper. Therefore, although it creates a high abundance of inhibitory interactions, it still is a universally important nutrient for bacterial growth.

The microbial network at medium copper contained the highest number of positive interactions across all treatments, with 18 positive connections involving nearly every strain except *V. hispanicus* S1 and *M. primoryensis* S8. Medium copper promoted widespread facilitation between co-cultures and high copper erode nearly all interactions. At high copper concentrations, the network collapsed

dramatically as only five strains grew, most interactions were neutral, and the only negative edge observed was between *R. yunnanensis* S12 and *C. indolthethicum* S9.

Across all nutrient conditions, medium concentrations sustained more complex networks with a mixture of positive, negative, and neutral relationships, while high concentrations consistently decreased or even collapsed the network. Iron enrichment drove communities towards greater competition whereas high phosphate resulted in extremely few bacterial growths with few stress-tolerant mutualisms. Nitrate showed a shift from inhibition to facilitation between many strains at high concentrations, and copper uniquely supported the most facilitation at medium levels before collapsing under high stress. These contrasting patterns demonstrate that nutrient stress can have a variety of effects on microbial relationships and concentrations of these nutrients can shift the nature of these interactions between inhibitory and facilitative.

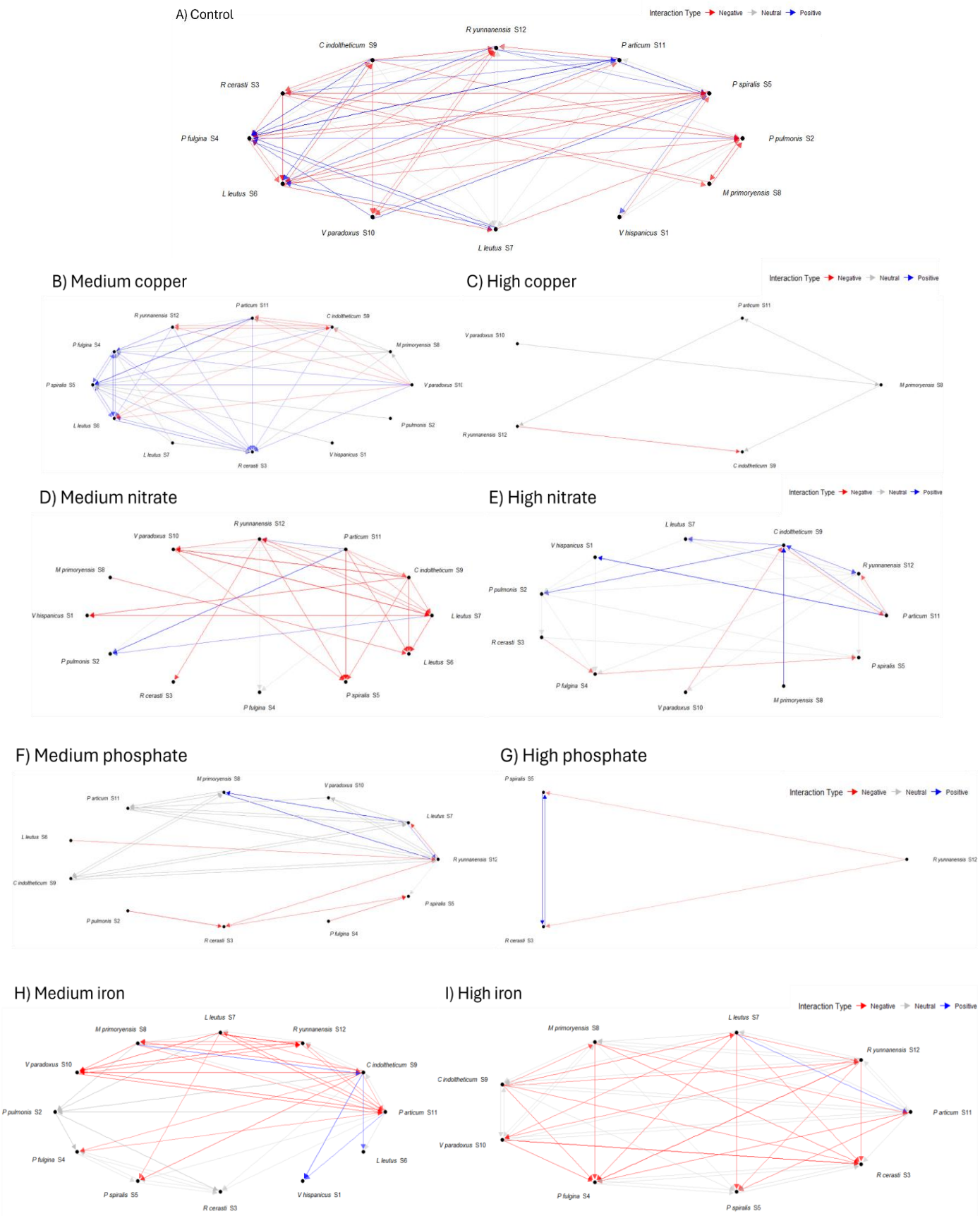


Figure 5: Interaction network reviewing bacterial interactions based off AUC values. Interaction scores were created through comparisons of co-culture AUC value to the monocultures. The direction of the arrow should be interpreted as the effect a strain has on another strain.

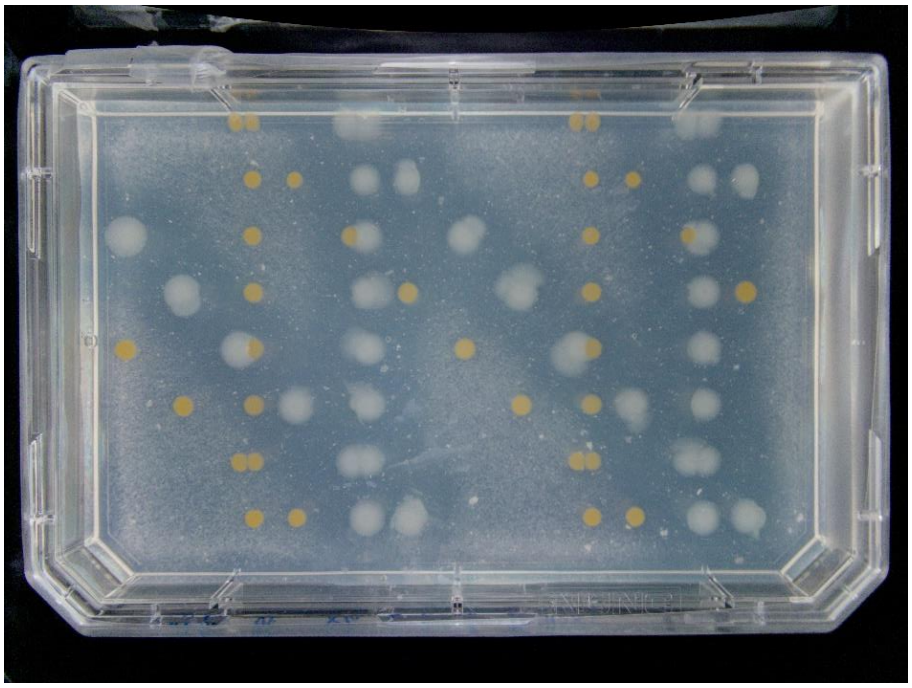


Figure 6: Bacterial co-cultures in high phosphate media, the circular grey growths are *Ps. spiralis* with a clearing zone surrounding it. This clearing zone is clear as the phosphate in the media (shown as white dots) have disappeared surrounding S5 growths showing some form of metabolic activity.

To identify possible mechanisms of interactions, genome sequences of *V. hispanicus* S1, *Pb. pulmonis* S2, *Ps. spiralis* S5, *L. luteus* S6, *L. luteus* S7, *C. indolthethicum* S9, *V. paradoxus* S10, and *Py. arctica* S11 could be produced and demonstrate a wide genetic diversity with *L. luteus* S6 and S7 being the most similar. Both strains of *L. luteus* S6 & S7 are distantly related to *Ps. spiralis* positioned on the distant branch of the phylogenetic tree. There are also different species with close branches such as *Py. arctica* and *V. paradoxus*.

Genomic analysis (Table 3) demonstrated the variety and abundance of secondary metabolite pathways identified in the bacterial genome. Most of the genomes carry between 2-3 BGC's, whereas *V. hispanicus* S1 has 24 BGC's. Some of the most frequently detected classes included RiPP -like (Ribosomally synthesized and post-translationally modified Peptides), NRPS (Nonribosomal Peptide Synthetases) and Terpene-precursors. However, some strains showed unique classes such as *V. hispanicus* S1 containing isocyanide and ectoine, *L. luteus* S6 & S7 containing arylopolyene and *C. indolthethicum* S9 containing betalactone.

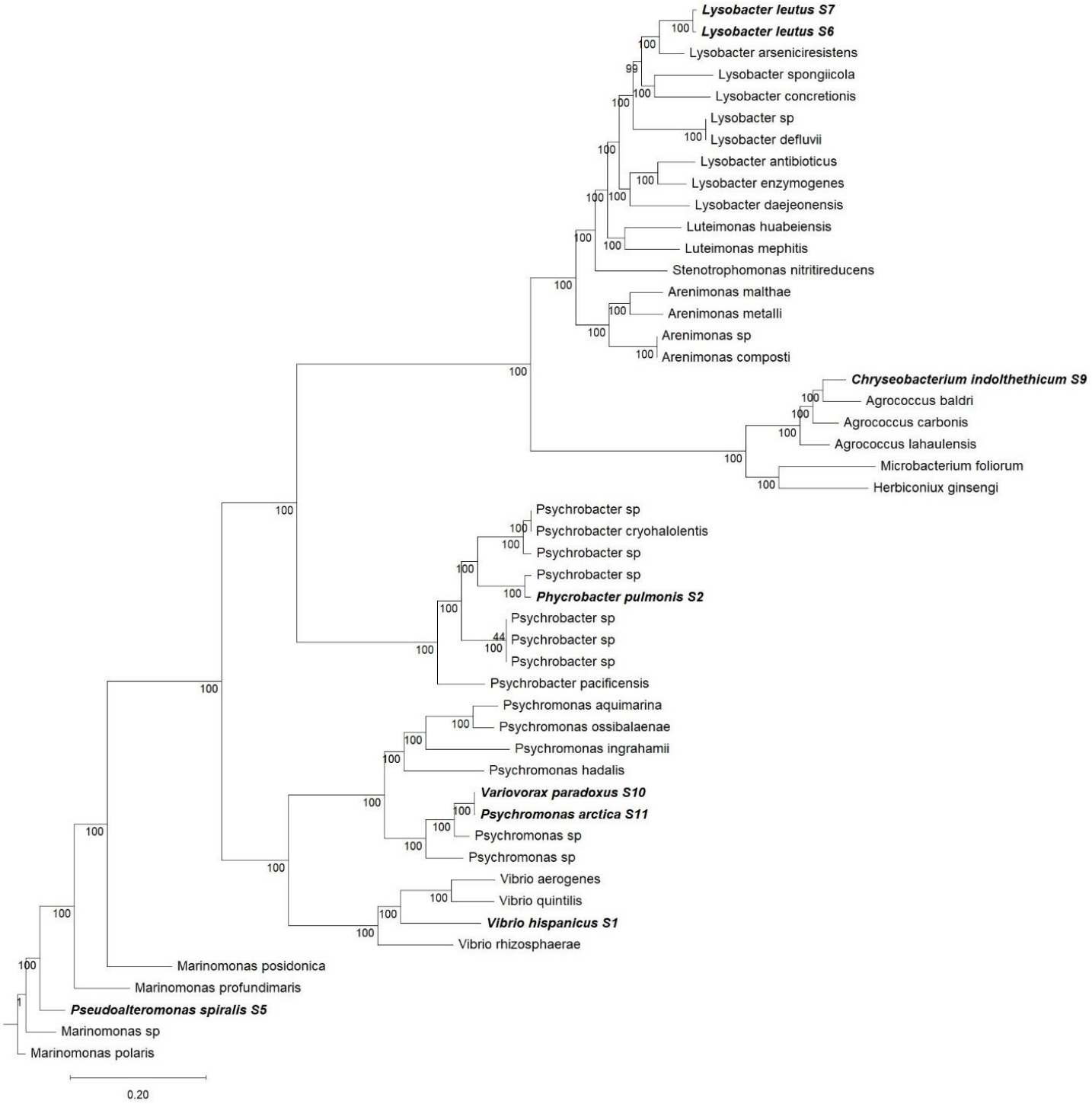


Figure 7: Maximum likelihood multi-local sequence based tree generated by alignments (1000 bootstrap replicates) from extracted genomic DNA sequences with NCBI sequences from closely related strains. The tree was generated in created in AutoMLST as edited in MEGA12. The tree is rooted with *Marinomonas polaris*. The distance scale is shown to the bottom left of the phylogram. Containing genomic DNA of strains *L. luteus, V. hispanicus, C. indolthethicum, Pb. pulmonis, V. paradox, Py. arctica* ,of the seagrass bacteria in bold and closely related species sequences taken from NCBI and included by AutoMLST . Not all species studied in this investigation are included in the tree.

Table 3: The predicted biosynthetic gene clusters identified by antiSMASH (reference) listing the total number of BGC's detected and the most similar known BGC's for *V. hispanicus* S1, *Pb. pulmonis* S2, *Ps. spiralis* S5 , *L. luteus* S6 &S7 *C. indolthethicum* S9, *V. paradoxus* S10, *Py. arctica* S11 each strain. NRPS (non-ribosomal peptide synthetase), PKS (polyketide synthase) types I–III, prodigiosin, hydrogen cyanide, cyanobactin, RiPP-like (ribosomally synthesized and post-translationally modified peptides), terpene-precursors, betalactones, redox cofactors, arylpolyenes, PUFA (polyunsaturated fatty acids), and ectoine. Only gene clusters with medium or high confidence levels were included in the table.

Strain ID	Species	Total no. of BGCs	Most similar known BGCs														Most likely type
			NRPS	PKS I	PKS II	PKS III	Prodigiosin	Hydrogen cyanide	Cyanobactin	RiPP-like	Terpene-precursor	betalactone	Redox cofactor	Arylopolyene	PUFA	Ectoine	
S1	*Vibrio hispanicus*	24	14	3		1	1	1	1	1	1					1	Andrimid
S2	*Phycrobacter pulmonis*	3									1	1	1				Ectoine
S5	*Pseudoalteromonas spiralis*	3	1							1	1						Pentabromopseudilin
S6	*Lysobacter luteus*	3								1	1			1			Heat-stable antifungal
S7	*Lysobacter luteus*	3								1	1			1			Heat-stable antifungal
S9	*Chrysobacterium indolthethicum*	2	1					1				1					Flexiruben pigments
S10	*Variovorax paradoxus*	3								1	1				1		Carotenoid like
S11	*Psychromonas arctica*	2								1					1		Polyunsaturated fatty

Isolated fungi from seagrass habitat

To expand upon the scope of the project, fungal species were identified from the Llanelli seagrass meadow and the seagrass aquarium in Swansea University. The species shown in Figure 8 shows strains isolated from the blades and roots from the mudflat whereas Figure 10 shows the strains isolated from root cuttings from the seagrass aquarium. From this, two of the endophytic strains from the Llanelli mudflats that showed to grow from inside the seagrass blade and two strains from the aquarium root cuttings were sequenced with the ITS one and four primers for species identification along with microscopy of the mycelium and spores (Figure 9). The analysis identified that *Pyrenochaetopsis leptospora and Pseudeurotium zonatum* were slow growing species with different morphologies, isolated from inside the seagrass blades. *Penicillium commune & Pyrenochaetopsis leptospora* were species isolated from the root cuttings of *Z. marina* from the aquarium, which showed rapid growth rates and large colony sizes (Figure 9). The fungi cultures isolated from Llanelli seagrass meadow revealed a high diversity of species for identification. Figure 8 indicates that the fungi collected from the seagrass meadow have a wide range of morphologies and growth rates. For this reason, DNA extractions, inclusion in co-cultures and mono-cultures along with bacteria proved by trial experiments to be a logistical challenge. Therefore instead, select isolates were analysed and examined under a microscope (Figure 9). For future work the strains were stored in cryotubes at –70 °C for analysis for potential bioactive compounds.

Figure 8: Overview of fungi growths isolated from Llanelli seagrass meadows

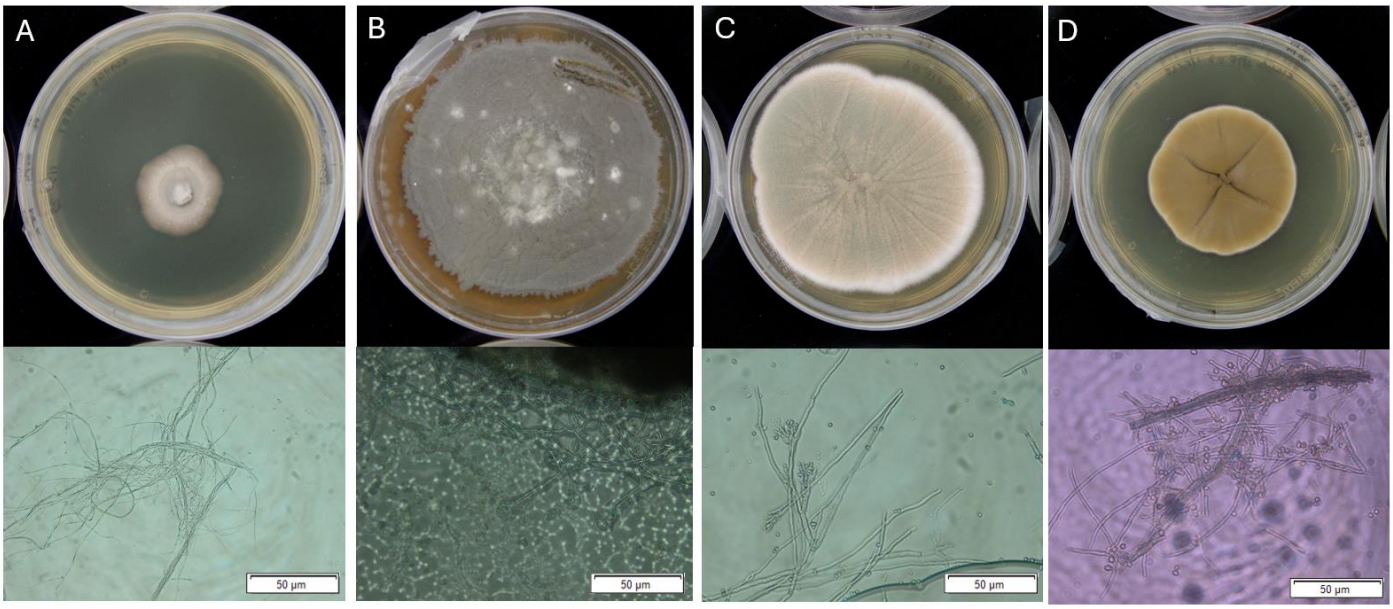


Figure 9: Identified fungi species (Grown on MEAA for one week) from Llanelli seagrass meadow with the mycelium and spores pictured with an optical microscope below the colony; *Pyrenochaetopsis leptospora* (A), *Pseudeurotium zonatum* (B), *Penicillium commune* (C), *Cladosporium halotolerans* (D). *Pyrenochaetopsis leptospora* and *Pseudeurotium zonatum* were endophytic strains isolated from inside the seagrass plant whereas *Penicillium commune* & *Pyrenochaetopsis leptospora* are epiphytic fungi that grew on the outside of the roots of the seagrass plant.

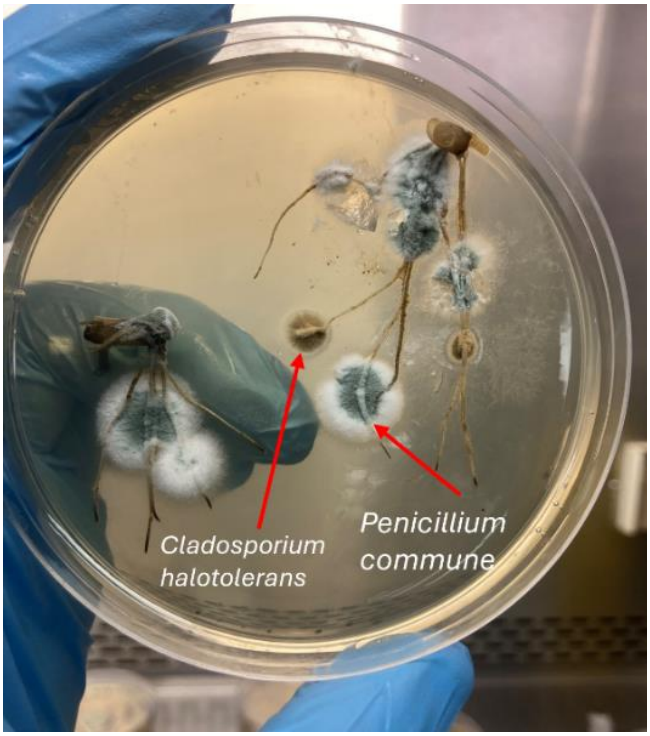


Figure 10: Labelled photo of isolated fungi strains from *Z. notlii* roots planted on MEAA

Discussion

To map out the pairwise interaction of the seagrass microbiome, we herein established a workflow using high throughput lab automation to monitor the growth curves in varied nutrient media of the twelve strains of bacteria; *Vibrio hispanicus* S1, *Phycrobacter pulmonis*, S2, *Rhodococcus cerasti* S3, *Pseudoalteromonas fulgina*, S4, *Pseudoalteromonas spiralis* S5, *Lysobacter leutus* S6, *Lysobacter leutus* S7, *Marinomonas primoryensis* S8, *Chrysobacerium indolthethicum* S9, *Variovorax paradoxus* S10, *Psychromonas arctica* S11, *Rhodococcus yunnanensis* S12. With the growth data measured, the extraction of AUC values for all conditions and conversion of this data into interaction plots allowed detailed analysis into the effect nutrient media can have on co-cultures of bacteria. Seagrass habitats are known to have higher nutrient concentrations from inputs such as sewage pollution (Zhang et al., 2023). Common pollutants such as nitrates, phosphate, iron and copper can increase the toxicity of the microbiome habitat. To understand in greater detail the extent to which these nutrients may affect the microbiome functioning, medium was prepared based on environmentally representative level of nutrients. This allows examination of how the seagrass microbiome can be sensitive to nutrient loading and how it may change the interactions between co-cultures.

The growth behaviours of the twelve bacterial strains were distinct in mono-culture demonstrating their metabolic diversity, with *L. luteus* S6 reaching the largest and *Py. arctica* S11 consistently showing the smallest colony sizes. The growth curves also revealed strains that showed immediate growth to twice their original starting size peaking after two days, whereas many of the other strains showed steady growth peaking after 7 days. Strains that were slow growing may be more useful to the seagrass plant, such as *V. paradoxus,* a pioneering species *which* has a large genome able to metabolise a wide range of carbon compounds allowing it to engineer the microhabitat to be more hospitable to other useful strains (Kaufmann et al., 2025). The slower growing species may be associated with services provided to seagrass meadows, such as disease protection, sulphide detoxification or nitrogen fixation (Tarquinio et al., 2021). For example, *Py. arctica* S11 has been described to play a role in nutrient cycling in seagrass

meadows (Walker et al., 2024). Other slower growing species such as *M. primoryensis* S8 *have* been linked to biofilm formation and polysaccharide degradation in the phyllosphere, suggesting their importance continues from germination seed into mature plant (Rabbani et al., 2021). Another slow growing bacterium was *C. indolthethicum* S9 which is often found in seagrass root sediments and have shown evidence in their role in disease prevention and production of anti-microbial compounds (Mohapatra et al., 2022).

The growth of bacterial co-cultures in control F2 media provided a baseline for understanding how seagrass-associated bacteria interact in facilitative, neutral and inhibitory ways, and confirmed that high throughput technologies can be used for detailed analysis of microbial interactions. The Rotor+ pinning robot, in combination with the Reshape machine image analysis technology can provide high-resolution data on bacterial growth in controllable conditions. This workflow advances previous methods applied to co-cultivation, such as microbial pinning onto a lawn of the microbial partner using Rotor+ pinning in unison with advanced metabolomic analysis into metabolite production (Blasche et al., 2021). The novel use of the Reshape machine in this project proves there is an alternative, efficient approach to analysing bacterial growth rates and interactions. The droplet co-cultivation is another high throughput way co-cultures can be examined and may be more suitable at isolating a co-culture from other co-cultures as well as detecting metabolite exchanges (Tan et al., 2020). However, use of the Reshape machine allows for the creation of growth curves as well as observing behaviours such as swarming which would not have been possible in droplet co-cultivation. It can also be advanced by combination with bioassays, such as polymer degradation and signal molecule production in the underlying agar. What must be acknowledged is that the use of the Reshape machine to build interaction networks is a new process, not found in literature and is a protocol which can be refined further. However, these methods can now be used for large-scale microbial analysis for multiple purposes, such as discovering bioactive molecules produced in co-culture.

Across the different nutrient additions (nitrate, phosphate, iron, copper), medium nitrate addition caused significant increase in growth in some strains, namely *C. indolthethicum*

S9, *V. paradoxus* S10, *R.* yunnanensis S12, while three strains, namely *R. cerasti* S3, *Ps. fulgina* S4, *L. luteus* S6, did not show significantly higher growth in medium concentration and were significantly inhibited under high nitrate. This mirrors patterns in the marine environment where nitrate enrichment can polarise and restructure communities with some strains better adapted for uptake which can often result in reduced biodiversity (Xu et al., 2022). It is difficult to predict exactly the effect this has on seagrass plants. However, a possible explanation is that the shift in microbial diversity will affect the resilience seagrass ecosystems can have to factors such as diseases or sulphide toxicity (Martin et al., 2019; Mohapatra et al., 2022). The phosphate condition caused minor changes to the monocultures with no strong improvements in growth rates. The only significant improvements were with *Pb. pulmonis* S2 and *Ps. fulgina* S4. Then in the high phosphate condition, there was a shift to only three surviving strains, namely *R. cerasti* S3, *Ps. spiralis* S5 and *R. yunnanensis* S12, which demonstrates a significant shift in microbial diversity. The reduced bacterial diversity in excess phosphate could lead to rapid growth of microalgae or other detrimental species that can readily grow and alter the water and sediment chemistry, possibly causing negative impacts such as anoxia of sulphide accumulation in the benthos causing significant harm to seagrass plants (López et al., 1998). In mono-cultures of *V. paradoxus* S10, growth rates were highest upon iron addition, a nutrient essential for sulphide detoxification in seagrass sediments (Haviland et al., 2024). Recording *V. paradoxus* S10 successful growth is important as the bacteria is known to be associated with nutrient cycling and degradation of organic compounds (Franzetti et al., 2012) and has been shown to positively affect cotyledon length in seagrass germination experiments (Jones, 2025). This suggests it could be more useful in mono-culture than other co-cultures as a useful additive for seagrass nursery work due to its nutrient resilience, to improve seed establishment rates. The medium copper concentration is one of the more extreme conditions to be exposed to, as no other condition caused the bacteria to swarm, which is a clear sign that the strains are actively moving to escape to a less toxic area. Normal concentration to cause bacterial swarming is anything within the micromolar range (Gautam et al., 2023). Growth rates were generally lower in copper mono-culture in comparison to co-culture, potentially indicating that the microbiome can respond to elevated copper concentrations with significantly reduced alpha diversity in areas

suffering from coastal sewage pollution (Signorini et al., 2022). Another explanation of the mono-cultures' underperformance is that in stressful environments, co-cultures switch to facilitative relationships, sharing protective metabolites and extracellular molecules to neutralise the copper toxicity (Martino et al., 2024).

In control media, 45% of co-cultures were classified as neutral interactions, 39% as negative and only 23% were positive. This follows currently understood patterns of microbial interactions where resource competition can cause antagonistic behaviours between strains (Qiao et al., 2023) and neutral interactions are a symptom of sufficient resource availability (Solowiej-Wedderburn et al., 2025). An example of this can be seen in the medium phosphate condition where, there was no increase in AUC values, however 68% of interactions were neutral, a 23% increase from control media. Previous studies have trialled planting *Z. marina* seeds along with enhanced nitrate and phosphate resulting in increased shoot length, indicating success in early growth (Unsworth et al., 2022). As the seagrass microbiome has been linked as being of vital importance to the health of the plant (Martin et al., 2019), nutrient additions to seagrass should also consider how the microbiome responds to nutrients such as nitrates and phosphates. As this study was performed excluding the role of seagrass in the bacterial interactions, further work should examine the difference seagrass can have on the co-culture growths as seagrass plants are known to provide nutrients to its microbiome in a mutualistic relationship (Li et al., 2025). An example in seagrass is where the root exudates can release carbon and nitrogen sources to shape bacterial communities and interactions to benefit the meadow (Ettinger, 2020). Seagrass do this as growth-promoting bacteria can increase seagrass fragment survivorship by up to 90% (Marletta et al., 2025). Therefore, reviewing co-cultures that proved to work in additional nutrients to benefit seagrass germination, could make a huge ecological difference as current field germination and emergence statistics are <5% (Orth et al., 2003), whereas lab germination can amount to as high as 60% (Balestri & Lardicci, 2012). The difference in germination success is often attributed to abiotic factors such as nutrient levels, sediment type, sulphides and pathogens, all creating stress that affects the germinating seed (Govers et al., 2017, Xu et al., 2021, Probert & Brenchley, 1999). Given that certain co-cultures can successfully grow in these conditions (examples such as *Ps. spiralis and*

R. cerasti S5-S3 in phosphate or *Py. arctica* and *R. yunnanensis* S11-S12 in copper), this symbiosis could be potential use as probiotics combined with controlled nutrient additions addressing the abiotic bottlenecks that limit germination.

Seagrass requires nitrates as a vital source for growth through ammonification and nitrification before assimilation (Sun et al., 2024). Multiple studies are trialling nutrient enrichment to improve seagrass health. A study in the Maldives used nitrogen fertilizers in a seagrass meadow to improve sediment stabilisation to the surrounding coral communities (Arnull et al., 2024). While the study did show improved seagrass growth, the increased nitrogen was also traced to cause a bloom of cyanobacteria and filamentous green-algal blooms (Arnull et al., 2024). This is evidence to a shift in restoration projects aiming to improve the health and resistance of ecosystems through a bottom-up approach of providing nutrients required by plants to improve growth. However, before such schemes become widely used, what should be factored into these methods is the response of the microbiome to the nutrient enhancement. Understanding how surplus nutrients intended or as sewage affects the functioning of microbial communities in environments are becoming increasingly important (Zhang et al., 2023). Addition of nitrate (concentration of 5.00×10^{-3} mmol/L) generally caused an increase in growth rates in the co-cultures in comparison to control media as well as mono-cultures. However, when nitrate levels became too high (concentration of 1×10^{-2} mmol/L), some seagrass bacteria show less growth than in the control. Only 16% of co-cultures, including strains *C. indolthethicum* S9, *Py. arctica* S11 & *R. yunnanensis* S12, improved in the high nitrate condition. This shows that while strains can show resilience to increased nitrate too much has negative effects on bacterial growth. Genomic data in combination with literature suggests these strains contain nitrogen detoxification pathways and mechanisms to manage oxidative stress (Damas et al., 2022) which could be why certain strains were able to grow in the high nitrate condition. This research suggests high nitrate concentrations reduce growth rates as well as shifting the microbial interactions to have less inhibitory behaviour with 13% negative and 71% neutral interactions likely due to the nutrient stress. Overall, increased nitrate additions to seagrass meadows may be a suitable addition to improve the growth of the microbial

communities if levels remain around 5×10^{-3} mmol/L, as exceeding this could have be damaging to the diversity of the microbiome shown by the reduced abundances of growths. Further studies also suggest that nitrate pulses can temporarily increase microbial productivity, however, they risk long term destabilisation through knock on effects causing blooms in pathogens and invasive species (Gomez Isaza et al., 2020). Over exposure to nitrate additions from sewage could risk long term damage to seagrass communities as the hysteresis effect suggests it would be energetically expensive to recover the ecosystem from such environmental shifts. However as shown in this research, pairwise co-cultures under medium nitrate can improve growth rates. As the higher nitrate condition is also designed to simulate unnatural conditions more associated with sewage pollution, evidence suggests these levels cause dramatically lower diversity, growth rates and reduced facilitative interaction between co-cultures as strains do not have the capabilities to tolerate such high levels of nitrates. Persistent nitrate enrichment has been known to inhibit the growth of sulphate reducing species (He et al., 2010). This could suggest that while nitrates may promote certain cultures of bacteria, there is the possibility that the impaired function of the sulphate reducing community that is present in the seagrass microbiome could suffer significant inhibition resulting in reduced health of seagrass plants (Cifuentes et al., 2000). The anthropogenic introduction of nitrates rarely occurs alone in marine environments and is often associated with other frequently seen pollutants such as phosphate.

The herein presented data also provides useful insights into the implications of elevated phosphate in understanding the implications of enhanced nutrient levels on seagrass bacteria. Enhancing phosphate levels can lower the growth rates of many seagrass bacteria, with up to a concentration of 1×10^{-3} mmol/L (levels not naturally achieved according to Copernicus databases) causing complete inhibition of bacterial growth. The strains that did grow, *Ps. spiralis S5* and *R. cerasti S3* showed mutualistic behaviour to each other that improved their individual AUC values, showing a symbiotic relationship to improve phosphate resilience. *Pseudoalteromonas* is known to have several roles in microbial defence in marine systems with studies reviewing its potential as an antifoulant or to produce antimicrobial compounds to defend corals and sponges along with inhibiting algal spore germinations (Hentschel, 2001; Pernthaler et al., 2001;Egan et

al., 2001). Which highlights its importance in controlling the damaging growths associated with eutrophication. Therefore, phosphate nutrient additions to seagrass meadows should be used conservatively as its toxicity to the microbiome will shift the communities to be dominated by strains whose main role may be controlling the high phosphate to limit microalgal growth. The bioactive potential of *Ps. spiralis S5* as identified through a combination of genome analysis and literature searching is extensive as it contains reservoirs of metabolites that could be explored for commercial use in addition to the strain's usefulness for conservation (Paulsen et al., 2019). *Ps. spiralis'* growth with *R. cerasti* may also be significant as *R. cerasti* is known for its ability in maintaining a homeostatic environment in the benthos (Nazari et al., 2022). The mechanisms for these growth behaviours are uncertain as not often evidenced in literature. A possibility is that *Pseudoalteromonas* secretes extracellular metabolites to control the excess phosphate along with *R. cerasti* biochemical defences, to maintain ideal concentration gradients while benefiting from the extracellular proteins secreted by *Pseudoalteromonas* (Ivshina et al., 2021). Analysis into the BGC's of *Ps. Spiralis* also suggest the presence of brominated compounds and polyketides. This could explain the inhibitory interactions seen in as *Ps. spiralis* S5 had within the experiments (Offret et al., 2016). However, when co-cultured with *R. cerasti* S3 it showed clear high growth rates when phosphate levels were elevated. Studies have shown *R. cerasti* S3 abilities to detoxify anthropogenic hydrocarbons such as petroleum or plastics (Vaksmaa et al., 2021). Its ability to behave as a bioremediation agent, along with its evidence to build biofilms intended to protect from toxins while also promoting efficient metabolite exchange, highlight its importance as a regenerative species to seagrass is stressed environments (Nazari et al., 2022). The evidence of the mutualistic relationship in phosphate between the two species could suggest its use to meadows under stress could stabilise the conditions surrounding the microbiome.

The iron conditions produced the highest abundance of growth across the strains. Marine open ocean systems are typically growth limited due to minimal iron, however coastal and intertidal systems are known to have significantly higher levels due to terrestrial runoff, upwelling and other anthropogenic sources (Sunda & Huntsman, 1995). This may explain how none of the strains failed to grow as intertidal bacteria are

better adapted to the dynamic environment where iron levels can significantly fluctuate (Zhang et al., 2013). There were varied responses from the same species, *L. luteus* S6 & S7, unlike S6, the growths in S7 were supressed whenever in co-culture, highlighting that different strains of the same species can show variability in growth rates and interactions to other bacteria. Despite high genetic similarity they still showed differences in responses to co-cultures which highlights their metabolic versatility. Marine bacteria commonly cope with high iron stress through siderophore productions or the secretion of biofilms to cope with the toxicity (Chandrangsu et al., 2017). The Lysobacter genus is known to contain ferric uptake regulators which assist the bacteria in avoiding the toxicity which may explain how these strains were able to grow (Tang et al., 2023). In the medium iron condition, multiplcocultures had inhibitory interactions with their partners with only three interactions being positive, *M. primoryensis* and *C. indolthethicum* S8-S9, *V. hispanicus* and *C. indolthethicum* S1-S9 and *L. luteus* and *C. indolthethicum* S6-S9. *C. indolthethicum* S9 showed a common facilitative relationship on its partner which suggests iron supports this strain in cooperating with other species, however all bacterial relationships degraded from positive to negative in the higher iron condition. This is likely due to the extreme oxidative stress damaging and impairing growth (Coimbra et al., 2024). Iron polluted environments may cause the seagrass microbiome to have reduced symbiosis through this oxidative stress which likely impairs functional roles to the seagrass plant. The *V. paradoxus* S10 monoculture showed higher growth response to medium iron than it did in co-cultures. Analysis into the BGC's identified a broad catabolic versatility containing genes for xenobiotic degradation. This suggests that *V. paradoxus* S10 was able to withstand the medium iron condition. However, these mechanisms were supressed when in co-culture represented through AUC values. The only positive co-culture interaction in high iron was the S7-S11 condition. The *Py. arctica* S11 showed slow growth across all conditions fitting with current trends known for this species of psychrophile (Dasauni & Nailwal, 2020). The positive interaction in the networks could be because *Py. arctica* S11 had minimal overlap in resource with *L. luteus* S7 therefore had minimal competition for resources and is less likely attributed to a facilitative relationship. As *L. luteus* S7 showed reduced growth in co-cultures, the minimal overlap in resources when in its co-culture with *Py. arctica* S11 is likely what caused this co-culture to show more growth.

Copper is required by marine bacteria in trace amounts to provide structure for catalytic enzymes involved in electron transport (Gautam et al., 2023). However, the concentrations used in this research exceed these requirements. When testing the bacteria strains in the medium copper condition, it produced more beneficial interactions than the other tested conditions, potentially due to the toxicity forcing cooperations for survival (Ly-Sauerbrey et al., 2025). Similar studies show that strains exposed to high concentrations of copper produce biofilms to mitigate environmental harm along with sharing the use of metabolites excreted by strains to assist resilience in neighbouring strains (Amarnath et al., 2023). The observed bacterial swarming is known to facilitate biofilms and indicates that the bacteria are actively attempting to relocate to regions lower in copper (Gautam et al., 2023; Iago Grobas et al., 2021). This highlights the sensitivity of the seagrass microbiome to copper concentrations as the bacteria showed evidence of multiple methods to maintain resilience to the medium concentration of copper. This could affect the seagrass microbiome significantly as stress to the microbiome could weaken the plants resilience to photosynthesise, potentially causing fatal effects on their longevity (De los Santos et al., 2019). *Py. arctica* and *R. yunnanensis* S11-S12 was the most resilient co-culture to copper toxicity. Research indicates that *R. yunnanensis* S12 has a well-studied ability to detoxify heavy metals through various mechanisms such as efflux pumps and mycolic acid-rich cell walls designed to reduce the physiological stress of copper (Presentato et al., 2019). The partner *Py. arctica* S11 grew poorly in mono-culture but showed higher growth in the co-culture, possibly due to sharing the micro-environment with *R. yunnanensis* S12. The persistence of *Py. arctica* S11 to grow in co-cultures in high concentration conditions which inhibited many of the other strains, suggests it is a highly resilient bacteria with a diverse genome to accommodate stressful environments. This suggests that *Py. arctica* S11 can produce important biosynthetic proteins to tolerate the high nutrient concentrations. . This is evidenced as *Py. arctica* S11 is often seen in co-cultures where its partner strain showed increased growth than it did apart in its own mono-culture. Reviewing the literature in combination with the BGC's of the strain revealed the presence of carbohydrate metabolism, nitrate transporters and TMAO-reducing gene cluster composed of torD, torA and torT which support adaption to nutrient rich marine environments (Zhang et al., 2018). This could suggest an explanation as to why the

partner strain in co-cultures showed promotional growth when paired with *Py. arctica* S11. It could be possible that *Py. arctica* S11 has functions to assist growth in its partner strain in ecologically challenging environment. A possible explanation of why the co-culture *R. yunnanensis* and *Py. arctica* S12-S11 outperformed the *R. yunnanensis* S12 mono-culture could be that the strains shared use of copper binding proteins present in *R. yunnanensis* S12 that have cupredoxin like folds that help the strains bind copper to enzymes such as laccases and mitigate the toxicity (Santo et al., 2013). All other strains showed significant inhibition compared to the control growths which suggests that 2.93×10^{-2} mmol/L of copper creates an extremely toxic environment through which the seagrass microbiome cannot survive and should be factored into reforesting efforts as estuaries with high levels of copper should be avoided to maximise seedling establishment.

The isolation methodology extracting fungi from the Llanelli seagrass mudflat was successful and 10 morphologically different fungal strains were isolated. From these strains, four were further investigated because of the location that they were isolated from as *Pyrenochaetopsis leptospora*, *Pseudeurotium zonatum* appeared to grow from the inside of the seagrass blade and *Penicillium commune* and *Cladosporium halotolerans* were isolated from the roots. The *P*c. *leptospora* and *P*e. *zonatum* strains are believed to play useful roles in plant disease protection through novel metabolites (Rajakaruna et al., 2024). *P*c. *leptospora* & *Pe. zonatum* are a common seagrass fungi associated with decomposition and controlling disease levels amongst plants (Liu & Howell, 2020). They have both been identified in terrestrial soil systems to be an important species contributing towards decomposition and nutrient cycling (Landinez-Torres et al., 2019; Dasauni & Nailwal, 2020; Lior Granit et al., 2025). Therefore, their presence inside the seagrass blades may be due to migration from the water column to inside the plant. Therefore, its role in seagrass blades could be a supporting role in recycling nutrients or a protective role using secretion of metabolites to reduce epiphytic algae growing on the surface of the *Z. notlii* blades. Both *Pen. commune* and *Cs. halotolerans* were isolated from the roots of *Z. notlii* which emphasise that these species grow on the surface of the seagrass roots and could have ecologically important roles in supporting the health and function of seagrass. *Penicillium* species are common around

seagrass meadows and are known to produce anti-microbial compounds which could assist towards disease prevention (Petersen et al., 2019). Furthermore, they are known to produce bioactive compounds with strong anti-quorum sensing properties often intended to disrupt biofilms and bacterial growth (Petersen et al., 2019). There is also evidence suggesting they can exhibit cellulase and protease activity which improve nutrient uptake from seagrass roots (Park et al., 2019). *Cs. halotolerans* is also an important fungal species commonly found in marine habitats. They have protective effects on other epipelagic habitats such as coral health under thermal stress, and improve efficiency of algae symbionts (Lior Granit et al., 2025). They are also useful for root-based processes as they can decompose lignin-cellulostic materials into simpler molecules for nutrient uptake and mitigating pollutants through production of metabolites capable of biodegrading polyester polyurethane (Zhang et al., 2022). Their highly diverse assemblage of antimicrobial, antifouling, antiviral and anticancer compounds, is vitally important species guarding the root sediments of seagrass meadows (Mohamed & Ibrahim, 2021).

Conclusion

This study successfully adopted high throughput techniques and revealed bacterial co-cultures are sensitive to the environment and nutrient variability. In control media, there were 29 neutral interactions, 25 negative and 15 positive interactions across the co-cultures produced. These interactions were further successfully repeated in a range of media to show how bacterial communities can shift depending on the concentration of nutrient enhancement they are exposed to. Identification of nutrient resilient strains highlights co-cultures that can be resilient to specific nutrient stressors as well as adaptations through metabolite production. This was then further represented in the construction of interaction networks and backed up with evidence when appropriate from bioinformatics performed on sequenced genomes. Symbiotic relationships were discovered in multiple strains; examples include *Ps. spiralis* & *R.cerasti* S5-S3 in phosphate or *R. yunnanensis & C. indolthethicum* S12-S9 in nitrate. These could be investigated further as a potential probiotic to be delivered to seagrass in specific nutrient stressed habitats. The genomic preliminary data indicates the importance that seagrass bacteria and fungi could have in the discovery of novel biosynthetic compounds which should be pursued in further studies. Future work could also include looking at more complex co-cultures of bacteria strains and testing differences in growth when co-cultures are planted next to mature seagrass seeds to investigate if certain combinations of strains have positive effects on seagrass seed germination and early development. This could be done with and without nutrient additions as early trials to a probiotic approach to assist seagrass growth. By simulating nutrient conditions above naturally occurring levels, we can predict strains that have higher tolerances to nutrient pollution. Continuations of this study could test responses with co-cultures of greater complexity in media containing multiple nutrient additions to build a more complex understanding of the functioning of the microbiome. Also, investigation into the metabolites produced by both the bacterial and fungal strains analysed in this project could be a promising area for the discovery of novel biosynthetic compounds with commercial uses. However, the outcomes of this research prove how novel high throughput technologies can be adapted into a pipeline to create interaction networks of seagrass associated bacteria. These networks are vital for understanding the mechanisms underpinning plant- microbe and

microbe-microbe interactions as well as studying the potential bacteria have for producing metabolites that can be important in overall ecosystem functioning.

References

Amarnath, K., Narla, A.V., Pontrelli, S., Dong, J., Reddan, J., Taylor, B.R., Caglar, T., Schwartzman, J., Sauer, U., Cordero, O.X. & Hwa, T. (2023) 'Stress-induced metabolic exchanges between complementary bacterial types underly a dynamic mechanism of inter-species stress resistance', *Nature Communications*, 14(1), p. 3165.

AntiSMASH (2025) *antiSMASH bacterial version*. [Online] [online]. Available from: https://antismash.secondarymetabolites.org/#.

Arnull, J., Hashim, A.S., Ganeshram, R.S., Moosa, H., Wilson, A.M.W. & Tudhope, A.W. (2024) 'Seagrass is an early responder to nitrogen enrichment in oligotrophic oceanic coral atoll environments', *Marine Pollution Bulletin*, 209p. 117224.

AutoMLST (2025) *autoMLST: Automated Multi-Locus Species Tree*. [Online] [online]. Available from: https://automlst.ziemertlab.com/results/1ec1f9f9-1352-4e05-a0ee-84dd19c34028/report/# (Accessed 10 September 2025).

Balestri, E. & Lardicci, C. (2012) 'Nursery-propagated plants from seed: a novel tool to improve the effectiveness and sustainability of seagrass restoration' C. Frid (ed.), *Journal of Applied Ecology*, 49(6), pp. 1426–1435.

Blasche, S., Kim, Y., Mars, R.A.T., Machado, D., Maansson, M., Kafkia, E., Milanese, A., Zeller, G., Teusink, B., Nielsen, J., Benes, V., Neves, R., Sauer, U. & Patil, K.R. (2021a) 'Metabolic cooperation and spatiotemporal niche partitioning in a kefir microbial community', *Nature Microbiology*, 6(2), pp. 196–208.

Chandrangsu, P., Rensing, C. & Helmann, J.D. (2017) 'Metal homeostasis and resistance in bacteria', *Nature Reviews Microbiology*, 15(6), pp. 338–350.

Cifuentes, A., Anton, J., de Wit, R. & Rodriguez-Valera, F. (2003) 'Diversity of Bacteria and Archaea in sulphate-reducing enrichment cultures inoculated from serial dilution of Zostera noltii rhizosphere samples', *Environmental Microbiology*, 5(9), pp. 754–764.

Cifuentes, A., AntónJ., Benlloch, S., Donnelly, A., Herbert, R.A. & Rodríguez-ValeraF. (2000) 'Prokaryotic Diversity in *Zostera noltii* -Colonized Marine Sediments', *Applied and environmental microbiology*, 66(4), pp. 1715–1719.

Coimbra, C., Morais, P.V. & Branco, R. (2024) 'Iron homeostasis as a cell detoxification

mechanism in Mesorhizobium qingshengii J19 under yttrium exposure', *Frontiers in Microbiology*, 15.

Conte, C., Rotini, A., Manfra, L., D'Andrea, M.M., Winters, G. & Migliore, L. (2021) 'The Seagrass Holobiont: What We Know and What We Still Need to Disclose for Its Possible Use as an Ecological Indicator', *Water*, 13(4), p. 406.

Copernicus (2020) *Homepage | Copernicus*. [Online] [online]. Available from: https://www.copernicus.eu/en.

Crump, B.C., Wojahn, J.M., Tomas, F. & Mueller, R.S. (2018) 'Metatranscriptomics and Amplicon Sequencing Reveal Mutualisms in Seagrass Microbiomes', *Frontiers in Microbiology*, 9.

Cúcio, C., Engelen, A.H., Costa, R. & Muyzer, G. (2016) 'Rhizosphere Microbiomes of European + Seagrasses Are Selected by the Plant, But Are Not Species Specific', *Frontiers in Microbiology*, 7.

Damas, M.S.F., Ferreira, R.L., Campanini, E.B., Soares, G.G., Campos, L.C., Laprega, P.M., Soares da Costa, A., Freire, C.C. de M., Pitondo-Silva, A., Cerdeira, L.T., Cunha, A.F. da & Pranchevicius, M.-C. da S. (2022) 'Whole genome sequencing of the multidrug-resistant Chryseobacterium indologenes isolated from a patient in Brazil', *Frontiers in Medicine*, 9.

Dasauni, K. & Nailwal, T.K. (2020) 'Biodiversity of microbial life: Indian Himalayan region', in *Microbiology*. [Online]. Academic Press. pp. 1–17.

de los Santos, C.B., Arenas, F., Neuparth, T. & Santos, M.M. (2019) 'Interaction of short-term copper pollution and ocean acidification in seagrass ecosystems: Toxicity, bioconcentration and dietary transfer', *Marine Pollution Bulletin*, 142pp. 155–163.

do Amaral Camara Lima, M., Bergamo, T.F., Ward, R.D. & Joyce, C.B. (2023) 'A review of seagrass ecosystem services: providing nature-based solutions for a changing world', *Hydrobiologia*, 850(12-13), pp. 2655–2670.

Egan, S., Harder, T., Burke, C., Steinberg, P., Kjelleberg, S. & Thomas, T. (2013) 'The seaweed holobiont: understanding seaweed–bacteria interactions', *FEMS Microbiology Reviews*, 37(3), pp. 462–476.

Egan, S., James, S., HolmstrÃ¶m, C. & Kjelleberg, S. (2001) 'Inhibition of algal spore germination

by the marine bacterium Pseudoalteromonas tunicata', *FEMS Microbiology Ecology*, 35(1), pp. 67–73.

Ettinger, C.L. (2020) *Taxonomic Diversity of the Bacterial and Fungal Communities Associated with the Seagrass, Zostera marina*. [Online] [online]. Available from: https://www.proquest.com/dissertations-theses/taxonomic-diversity-bacterial-fungal-communities/docview/2460090049/se-2?accountid=14680 (Accessed 24 April 2025).

Fahimipour, A.K., Kardish, M.R., Lang, J.M., Green, J.L., Eisen, J.A. & Stachowicz, J.J. (2017) 'Global-Scale Structure of the Eelgrass Microbiome' Harold L. Drake (ed.), *Applied and Environmental Microbiology*, 83(12),.

Franzetti, A., Gandolfi, I., Raimondi, C., Bestetti, G., Banat, I.M., Smyth, T.J., Papacchini, M., Cavallo, M. & Fracchia, L. (2012) 'Environmental fate, toxicity, characteristics and potential applications of novel bioemulsifiers produced by Variovorax paradoxus 7bCT5', *Bioresource Technology*, 108pp. 245–251.

Gautam, P., Erill, I. & Cusick, K.D. (2023) 'Linking Copper-Associated Signal Transduction Systems with Their Environment in Marine Bacteria', *Microorganisms*, 11(4), pp. 1012–1012.

Gomez Isaza, D.F., Cramp, R.L. & Franklin, C.E. (2020) 'Living in polluted waters: A meta-analysis of the effects of nitrate and interactions with other environmental stressors on freshwater taxa', *Environmental Pollution*, 261(0269-7491), p. 114091.

Govers, L.L., van der Zee, E.M., Meffert, J.P., van Rijswick, P.C.J., Man in 't Veld, W.A., Heusinkveld, J.H.T. & van der Heide, T. (2017) 'Copper treatment during storage reduces Phytophthora and Halophytophthora infection of Zostera marina seeds used for restoration', *Scientific Reports*, 7p. 43172.

Haviland, K.A., Howarth, R.W., Giblin, A.E. & Marino, R. (2024) 'The potential role of sediment iron and sulfur speciation in seagrass meadow loss and recovery', *Ocean-Land-Atmosphere Research*, 3.

He, Q., He, Z., Joyner, D.C., Marcin Joachimiak, Price, M.N., Yang, Z.K., Yen, H.-C.B., Hemme, C.L., Chen, W., Fields, M.M., Stahl, D.A., Keasling, J.D., Keller, M., Arkin, A.P., Hazen, T.C., Wall, J.D. & Zhou, J. (2010) 'Impact of elevated nitrate on sulfate-reducing bacteria: a comparative Study of *Desulfovibrio vulgaris*', *The ISME Journal*, 4(11), pp. 1386–1397.

Hentschel, U. (2001) ‘Isolation and phylogenetic analysis of bacteria with antimicrobial activities from the Mediterranean sponges Aplysina aerophoba and Aplysina cavernicola’, *FEMS Microbiology Ecology*, 35(3), pp. 305–312.

Hurtado-McCormick, V., Kahlke, T., Petrou, K., Jeffries, T., Ralph, P.J. & Seymour, J.R. (2021) ‘Corrigendum: Regional and Microenvironmental Scale Characterization of the Zostera muelleri Seagrass Microbiome’, *Frontiers in Microbiology*, 12.

Iago Grobas, Polin, M. & Munehiro Asally (2021) ‘Swarming bacteria undergo localized dynamic phase transition to form stress-induced biofilms’, *eLife*, 10.

Ivshina, I.B., Kuyukina, M.S., Krivoruchko, A.V. & Tyumina, E.A. (2021) ‘Responses to Ecopollutants and Pathogenization Risks of Saprotrophic Rhodococcus Species’, *Pathogens*, 10(8), p. 974.

Jones, J. & Sonnenchein, E. (2024) *Deciphering the seagrass microbiome for advanced ecosystem restoration*. [Online]

Kaufmann, H., Salvador, C., Salazar, V.W., Cruz, N., Dias, G.M., Diogo Tschoeke, Campos, L., Tomoo Sawabe, Miyazaki, M., Maruyama, F., Thompson, F. & Thompson, C. (2025) ‘Genomic Repertoire of Twenty-Two Novel Vibrionaceae Species Isolated from Marine Sediments’, *Microbial Ecology*, 88(1),.

Kirchman, D., Mazzella, L., Alberte, R. & Mitchell, R. (1984) ‘Epiphytic bacterial production on Zostera marina’, *Marine Ecology Progress Series*, 15pp. 117–123.

Landinez-Torres, A., Panelli, S., Picco, A.M., Comandatore, F., Tosi, S. & Capelli, E. (2019) ‘A meta-barcoding analysis of soil mycobiota of the upper Andean Colombian agro-environment’, *Scientific Reports*, 9(1),.

Li, X., Wang, H., Zang, Y., Xue, S., Xin, J., Liu, L., Tang, X. & Chen, J. (2025) ‘Exploring the structure and assembly of seagrass microbial communities in rhizosphere and phyllosphere’ Gladys Alexandre (ed.), *Applied and Environmental Microbiology*, 91(3),.

Lior Granit, Levi, R., Lifshitz, N., Guilhem Banc-Prandi, Zelinger, E., Ronen, B., Kraut-Cohen, J., Ankur Naqib, Green, S.J., Fine, M. & Oded Yarden (2025) ‘Beneficial and detrimental fungi within the culturable mycobiome of the Red Sea coral *Stylophora pistilatta*’, *The ISME Journal*,

Liu, D. & Howell, K. (2020) ‘Community succession of the grapevine fungal microbiome in the annual growth cycle’, *Environmental Microbiology*, 23(4), pp. 1842–1857.

Liu, S., Jiang, Z., Deng, Y., Wu, Y., Zhang, J., Zhao, C., Huang, D., Huang, X. & Trevathan-Tackett, S.M. (2018) ‘Effects of nutrient loading on sediment bacterial and pathogen communities within seagrass meadows’, *MicrobiologyOpen*, 7(5), p. e00600.

Llagostera, I., Cervantes, D., Sanmartí, N., Romero, J. & Pérez, M. (2016) ‘Effects of Copper Exposure on Photosynthesis and Growth of the Seagrass Cymodocea nodosa: An Experimental Assessment’, *Bulletin of Environmental Contamination and Toxicology*, 97(3), pp. 374–379.

López, N.I., Duarte, C.M., Ferrán Vallespinós, Romero, J. & Alcoverro, T. (1998) ‘The effect of nutrient additions on bacterial activity in seagrass (Posidonia oceanica) sediments’, *Journal of Experimental Marine Biology and Ecology*, 224(2), pp. 155–166.

Ludwig, W. (2007) ‘Nucleic acid techniques in bacterial systematics and identification’, *International Journal of Food Microbiology*, 120(3), pp. 225–236.

Ly-Sauerbrey, Y., Anton, R., Kopruch, L., Krämer, C.L., Boschert, A.L., Neidhöfer, C., Schwengers, O., Zander, D. & Leuko, S. (2025) ‘Comparison of single bacteria and a bacterial reference community in a test against coated surfaces of varying copper content’, *Frontiers in Microbiology*, 16.

Macherey-Nagel (2024) *NucleoSpin TriPrep, Mini kit for RNA, DNA, and protein purification*. [Online] [online]. Available from: https://www.mn-net.com/nucleospin-triprep-mini-kit-for-rna-dna-and-protein-purification-740966.50 (Accessed 1 August 2025).

Marletta, G., Sacco, D., Danovaro, R. & Bianchelli, S. (2025) ‘Effectiveness of growth promoters for the seagrass (Cymodocea nodosa) restoration’, *Frontiers in Plant Science*, 16.

Martin, B.C., Alarcon, M.S., Gleeson, D., Middleton, J.A., Fraser, M.W., Ryan, M.H., Holmer, M., Kendrick, G.A. & Kilminster, K. (2019) ‘Root microbiomes as indicators of seagrass health’, *FEMS Microbiology Ecology*, 96(2),.

Martin, B.C., Bougoure, J., Ryan, M.H., Bennett, W.W., Colmer, T.D., Joyce, N.K., Olsen, Y.S. & Kendrick, G.A. (2019) ‘Oxygen loss from seagrass roots coincides with colonisation of sulphide-oxidising cable bacteria and reduces sulphide stress’, *The ISME Journal*, 13(3),

pp. 707–719.

Martino, R.D., Picot, A. & Mitri, S. (2024) ‘Oxidative stress changes interactions between 2 bacterial species from competitive to facilitative’, *PLoS Biology*, 22(2), pp. e3002482–e3002482.

Matsapume Detcharoen, Ekkalak Rattanachot & Anchana Prathep (2024) ‘Metagenomics insights into microbial diversity shifts among seagrass sediments’, *bioRxiv (Cold Spring Harbor Laboratory)*,

MCROY, C.P. & GOERING, J.J. (1974) ‘Nutrient transfer between the seagrass Zostera marina and its epiphytes’, *Nature*, 248(5444), pp. 173–174.

Mejia, A.Y., Rotini, A., Lacasella, F., Bookman, R., Thaller, M.C., Shem-Tov, R., Winters, G. & Migliore, L. (2016) ‘Assessing the ecological status of seagrasses using morphology, biochemical descriptors and microbial community analyses. A study in Halophila stipulacea (Forsk.) Aschers meadows in the northern Red Sea’, *Ecological Indicators*, 60pp. 1150–1163.

Millan, C. (2022) ‘The effect of seagrass components on the associated bacterial diversity and antimicrobial activity in Zostera marina’, *PEARL*,

Mohamed, G.A. & Ibrahim, S.R.M. (2021) ‘Untapped Potential of Marine-Associated Cladosporium Species: An Overview on Secondary Metabolites, Biotechnological Relevance, and Biological Activities’, *Marine Drugs*, 19(11), p. 645.

Mohapatra, M., Manu, S., Dash, S.P. & Rastogi, G. (2022) ‘Seagrasses and local environment control the bacterial community structure and carbon substrate utilization in brackish sediments’, *Journal of Environmental Management*, 314p. 115013.

Molecular Evolutionary Genetics Analysis (2019) *Home*. [Online] [online]. Available from: https://www.megasoftware.net/.

Nazari, M.T., Simon, V., Machado, B.S., Crestani, L., Marchezi, G., Concolato, G., Ferrari, V., Colla, L.M. & Piccin, J.S. (2022) ‘Rhodococcus: A promising genus of actinomycetes for the bioremediation of organic and inorganic contaminants’, *Journal of Environmental Management*, 323p. 116220.

Offret, C., Desriac, F., Le Chevalier, P., Mounier, J., Jégou, C. & Fleury, Y. (2016) ‘Spotlight on

Antimicrobial Metabolites from the Marine Bacteria Pseudoalteromonas: Chemodiversity and Ecological Significance', *Marine Drugs*, 14(7), p. 129.

Orth, R., Fishman, J., Harwell, M. & Marion, S. (2003) 'Seed-density effects on germination and initial seedling establishment in eelgrass Zostera marina in the Chesapeake Bay region', *Marine Ecology Progress Series*, 250pp. 71–79.

Orth, R.J., Harwell, M.C. & Inglis, G.J. (2006) 'Ecology of Seagrass Seeds and Seagrass Dispersal Processes', *SEAGRASSES: BIOLOGY, ECOLOGYAND CONSERVATION*, pp. 111–133.

Oxford Nanopore Technologies (2021) *How Nanopore Sequencing Works*. [Online] [online]. Available from: https://nanoporetech.com/platform/technology.

Park, M.S., Oh, S.-Y., Fong, J.J., Houbraken, J. & Lim, Y.W. (2019) 'The diversity and ecological roles of Penicillium in intertidal zones', *Scientific Reports*, 9(1),.

Paulsen, S.S., Strube, M.L., Bech, P.K., Gram, L. & Sonnenschein, E.C. (2019) 'Marine Chitinolytic Pseudoalteromonas Represents an Untapped Reservoir of Bioactive Potential' Steven J. Hallam (ed.), *mSystems*, 4(4),.

Pedersen, T.L. (2024) 'A Tidy API for Graph Manipulation [R package tidygraph version 1.3.1]', *R-project.org*,

Pedersen, T.L. & RStudio (2022) *ggraph: An Implementation of Grammar of Graphics for Graphs and Networks*. [Online] [online]. Available from: https://cran.r-project.org/web/packages/ggraph/index.html.

Pernthaler, A., Pernthaler, J., Eilers, H. & Amann, R. (2001) 'Growth Patterns of Two Marine Isolates: Adaptations to Substrate Patchiness?', *Applied and Environmental Microbiology*, 67(9), pp. 4077–4083.

Petersen, L.-E., Marner, M., Labes, A. & Tasdemir, D. (2019) 'Rapid Metabolome and Bioactivity Profiling of Fungi Associated with the Leaf and Rhizosphere of the Baltic Seagrass Zostera marina', *Marine Drugs*, 17(7), p. 419.

Pita, P., Fernández-Márquez, D. & Freire, J. (2018) 'Spatiotemporal variation in the structure of reef fish and macroalgal assemblages in a north-east Atlantic kelp forest ecosystem: implications for the management of temperate rocky reefs', *Marine and Freshwater Research*, 69(4), p. 525.

Presentato, A., Piacenza, E., Cappelletti, M. & Turner, R.J. (2019) 'Interaction of Rhodococcus with Metals and Biotechnological Applications', *Microbiology Monographs*, pp. 333–357.

Probert, R.J. & Brenchley, J.L. (1999) 'The effect of environmental factors on field and laboratory germination in a population of Zostera marina L. from southern England', *Seed Science Research*, 9(4), pp. 331–339.

Project seagrass (n.d.) *Project Seagrass | Advancing the conservation of seagrass through education, influence, research and action*. [Online] [online]. Available from: https://www.projectseagrass.org/.

Qiao, Y., Huang, Q., Guo, H., Qi, M., Zhang, H., Xu, Q., Shen, Q. & Ling, N. (2023) 'Nutrient status changes bacterial interactions in a synthetic community', *Applied and Environmental Microbiology*,

Rabbani, G., Yan, B.C., Lee, N.L.Y., Ooi, J.L.S., Lee, J.N., Huang, D. & Wainwright, B.J. (2021) 'Spatial and Structural Factors Shape Seagrass-Associated Bacterial Communities in Singapore and Peninsular Malaysia', *Frontiers in Marine Science*, 8.

Rajakaruna, O., Wijayawardene, N.N., Udagedara, S., Jayasinghe, P.K., Gunasekara, S.S., Boonyuen, N., Bamunuarachchige, T.C. & Ariyawansa, K.G.S.U. (2024) 'Exploring Fungal Diversity in Seagrass Ecosystems for Pharmaceutical and Ecological Insights', *Journal of Fungi*, 10(9), p. 627.

Ransome, E. (2024) *2024_54_DoLS_ER: Enhancing seagrass growth and resilience for restoration purposes with microbial manipulations*.

Rodrigues, C.J.C. & de Carvalho, C.C.C.R. (2022) 'Cultivating marine bacteria under laboratory conditions: Overcoming the "unculturable" dogma', *Frontiers in Bioengineering and Biotechnology*, 10.

Santo, M., Weitsman, R. & Sivan, A. (2013) 'The role of the copper-binding enzyme – laccase – in the biodegradation of polyethylene by the actinomycete Rhodococcus ruber', *International Biodeterioration & Biodegradation*, 84pp. 204–210.

Shakya, M., Lo, C.-C. & Chain, P.S.G. (2019) 'Advances and Challenges in Metatranscriptomic Analysis', *Frontiers in Genetics*, 10.

Signorini, M., Midolo, G., Cesco, S., Mimmo, T. & Borruso, L. (2022) 'A Matter of Metals: Copper

but Not Cadmium Affects the Microbial Alpha-Diversity of Soils and Sediments — a Meta-analysis', *Microbial Ecology*,

Solowiej-Wedderburn, J., Pentz, J.T., Lizana, L., Schroeder, B.O., Lind, P.A. & Libby, E. (2025) 'Competition and cooperation: The plasticity of bacterial interactions across environments', *PLoS Computational Biology*, 21(7), pp. e1013213–e1013213.

Sun, H., Liu, X., Wang, T., Liu, S., Zhang, R., Guo, X., Yu, Z., Zhao, Y., Shen, P. & Zhang, Y. (2024) 'Rhizosphere microbiomes are closely linked to seagrass species: a comparative study of three coastal seagrasses', *Applied and Environmental Microbiology*,

Sunda, W.G. & Huntsman, S.A. (1995) 'Iron uptake and growth limitation in oceanic and coastal phytoplankton', *Marine Chemistry*, 50(1-4), pp. 189–206.

Tan, J.Y., Wang, S., Dick, G.J., Young, V.B., Sherman, D.H., Burns, M.A. & Lin, X.N. (2020) 'Co-cultivation of microbial sub-communities in microfluidic droplets facilitates high-resolution genomic dissection of microbial "dark matter"', *Integrative Biology*, 12(11), pp. 263–274.

Tang, B., Wang, B., Xu, Z., Hou, R., Zhang, M., Chen, X., Liu, Y. & Liu, F. (2023) 'Iron ions regulate antifungal HSAF biosynthesis in *Lysobacter enzymogenes* by manipulating the DNA-binding affinity of the ferric uptake regulator (Fur)', *Microbiology Spectrum*, 11(5),.

Tarquinio, F., Attlan, O., Vanderklift, M.A., Berry, O. & Bissett, A. (2021b) 'Distinct Endophytic Bacterial Communities Inhabiting Seagrass Seeds', *Frontiers in Microbiology*, 12.

Tarquinio, F., Hyndes, G.A., Laverock, B., Koenders, A. & Säwström, C. (2019) 'The seagrass holobiont: understanding seagrass-bacteria interactions and their role in seagrass ecosystem functioning', *FEMS Microbiology Letters*, 366(6),.

Trivedi, P., Leach, J.E., Tringe, S.G., Sa, T. & Singh, B.K. (2020) 'Plant-microbiome interactions: from community assembly to plant health', *Nature Reviews. Microbiology*, 18(11), pp. 607–621.

Ugarelli, K., Chakrabarti, S., Laas, P. & Stingl, U. (2017) 'The Seagrass Holobiont and Its Microbiome', *Microorganisms*, 5(4), p. 81.

Unsworth, R.K.F., Nordlund, L.M. & Cullen-Unsworth, L.C. (2018) 'Seagrass meadows support global fisheries production', *Conservation Letters*, 12(1), p. e12566.

Unsworth, R.K.F., Rees, S.C., Bertelli, C.M., Esteban, N.E., Furness, E.J. & Walter, B. (2022) *Nutrient additions to seagrass seed planting improve seedling emergence and growth*, 13.

Vaksmaa, A., Hernando-Morales, V., Zeghal, E. & Niemann, H. (2021) 'Microbial Degradation of Marine Plastics: Current State and Future Prospects', *Biotechnology for Sustainable Environment*, pp. 111–154.

Valdez, S.R., Zhang, Y.S., van der Heide, T., Vanderklift, M.A., Tarquinio, F., Orth, R.J. & Silliman, B.R. (2020) 'Positive Ecological Interactions and the Success of Seagrass Restoration', *Frontiers in Marine Science*, 7.

Vasco, Lobo-Arteaga, J., Santos, R., Leitão-Silva, D., Veronez, A.C., Neves, J.M., Nogueira, M., Creed, J.C., Bertelli, C.M., Jimena Samper-Villarreal & Pettersen, S. (2022) *Seagrasses benefit from mild anthropogenic nutrient additions*, 9.

Walker, L.D., Gribben, P.E., Glasby, T.M., Marzinelli, E.M., Varkey, D.R. & Dafforn, K.A. (2024) 'Above and below-ground bacterial communities shift in seagrass beds with warmer temperatures', *Frontiers in Marine Science*, 11.

Waycott, M., Duarte, C.M., Carruthers, T.J.B., Orth, R.J., Dennison, W.C., Olyarnik, S., Calladine, A., Fourqurean, J.W., Heck, K.L., Hughes, A.R., Kendrick, G.A., Kenworthy, W.J., Short, F.T. & Williams, S.L. (2009) 'Accelerating Loss of Seagrasses across the Globe Threatens Coastal Ecosystems', *Proceedings of the National Academy of Sciences*, 106(30), pp. 12377–12381.

Wickham, H. (2019a) 'Create Elegant Data Visualisations Using the Grammar of Graphics [R package ggplot2 version 3.2.1]', *R-project.org*,

Wickham, H. (2019b) 'randomcoloR: Generate Attractive Random Colors', *R-project.org*,

Wickham, H. (2020) *tidyverse/tidyverse*. [Online] [online]. Available from: https://github.com/tidyverse/tidyverse.

Wickham, H., François, R., Henry, L., Müller, K. & RStudio (2020) *dplyr: A Grammar of Data Manipulation*. [Online] [online].

Wickham, H. & Henry, L. (2020) *tidyr: Tidy Messy Data*. Available from: https://cran.r-project.org/web/packages/tidyr/index.html.

Wickham, H. & RStudio (2023) *forcats: Tools for Working with Categorical Variables (Factors)*. [Online] [online]. Available from: https://cran.r-project.org/web/packages/forcats/index.html.

Wickham, H. & RStudio (2019) *stringr: Simple, Consistent Wrappers for Common String Operations*. [Online] [online]. Available from: https://cran.r-project.org/web/packages/stringr/index.html.

Xu, S., Wang, P., Wang, F., Zhang, X., Song, X. & Zhou, Y. (2021) 'Responses of eelgrass seed germination and seedling establishment to water depth, sediment type, and burial depth: implications for restoration', *Marine Ecology Progress Series*, 678pp. 51–61.

Xu, Y.-F., Dong, X.-M., Luo, C., Ma, S.-N., Xu, J.-L. & Cui, Y.-D. (2022) 'Nitrogen Enrichment Reduces the Diversity of Bacteria and Alters Their Nutrient Strategies in Intertidal Zones', *Frontiers in Marine Science*, 9.

Yan, B., Rabbani, G., Lee, N., Ooi, J., Lee, J., Huang, D. & Wainwright, B. (2021) 'The microbiome of the seagrass Halophila ovalis: community structuring from plant parts to regional scales', *Aquatic Microbial Ecology*, 87pp. 139–150.

Yogev Yonatan, Kahn, S. & Bashan, A. (2024) 'Interactions-based classification of a single microbial sample', *Cell Reports Methods*, 4(5), pp. 100775–100775.

Zhang, K., Hu, J., Yang, S., Xu, W., Wang, Z., Zhuang, P., Grossart, H.-P. & Luo, Z. (2022) 'Biodegradation of polyester polyurethane by the marine fungus Cladosporium halotolerans 6UPA1', *Journal of Hazardous Materials*, 437p. 129406.

Zhang, M., Yu, X., Chen, Z., Wang, Q., Zuo, J., Shi, Y. & Guo, R. (2023) 'A Mini-Review of Seagrass Bed Pollution', *Water*, 15(21), pp. 3754–3754.

Zhang, W., Tian, R.-M., Sun, J., Salim Bougouffa, Ding, W., Cai, L., Lan, Y., Tong, H., Li, Y., Jamieson, A.J., Bajic, V.B., Drazen, J.C., Bartlett, D. & Qian, P.-Y. (2018) 'Genome Reduction in *Psychromonas* Species within the Gut of an Amphipod from the Ocean's Deepest Point', *mSystems*, 3(3),.

Zhang, W., Wang, Y., Lee, O.O., Tian, R., Cao, H., Gao, Z., Li, Y., Yu, L., Xu, Y. & Qian, P.-Y. (2013) 'Adaptation of intertidal biofilm communities is driven by metal ion and oxidative stresses', *Scientific Reports*, 3(1),.

Appendix 1

Plating tables for bacterial pinning

Appendix Table A1: Plating tables demonstrating the organisation of a 96 well plate before being used in the Rotor + pinning machine for co-culture creation. Number in plating table corresponds to species number eg: 1 is S1, 2 is S2

Species 1-6 plate A	1	2	3	4	5	6	7	8	9	10	11	12
A	1	2	3	4	5	6	1	2	3	4	5	6
B	1	2	3	4	5	6	1	2	3	4	5	6
C	1	2	3	4	5	6	1	2	3	4	5	6
D	1	2	3	4	5	6	1	2	3	4	5	6
E	1	2	3	4	5	6	1	2	3	4	5	6
F	1	2	3	4	5	6	1	2	3	4	5	6
G	1	2	3	4	5	6	1	2	3	4	5	6
H	1	2	3	4	5	6	1	2	3	4	5	6

Species 1-6 Plate B	1	2	3	4	5	6	7	8	9	10	11	12
A	1	2	3	4	5	6	1	2	3	4	5	6
B	6	1	2	3	4	5	6	1	2	3	4	5
C	5	6	1	2	3	4	5	6	1	2	3	4
D	4	5	6	1	2	3	4	5	6	1	2	3
E	3	4	5	6	1	2	3	4	5	6	1	2
F	2	3	4	5	6	1	2	3	4	5	6	1
G	1	2	3	4	5	6	1	2	3	4	5	6
H	1	1	2	3	4	5	6	1	2	3	4	5
Species 7-12 Plate A	7	8	9	10	11	12	7	8	9	10	11	12
A	7	8	9	10	11	12	7	8	9	10	11	12
B	7	8	9	10	11	12	7	8	9	10	11	12

C	7	8	9	10	11	12	7	8	9	10	11	12
D	7	8	9	10	11	12	7	8	9	10	11	12
E	7	8	9	10	11	12	7	8	9	10	11	12
F	7	8	9	10	11	12	7	8	9	10	11	12
G	7	8	9	10	11	12	7	8	9	10	11	12
H	7	8	9	10	11	12	7	8	9	10	11	12
Species 7-12 Plate B	1	2	3	4	5	6	7	8	9	10	11	12
A	7	8	9	10	11	12	7	8	9	10	11	12
B	12	7	8	9	10	11	12	7	8	9	10	11
C	11	12	7	8	9	10	11	12	7	8	9	10
D	10	11	12	7	8	9	10	11	12	7	8	9
E	9	10	11	12	7	8	9	10	11	12	7	8

F	8	9	10	11	12	7	8	9	10	11	12	7
G	7	8	9	10	11	12	7	8	9	10	11	12
H	12	7	8	9	10	11	12	7	8	9	10	11
Species 1-6 plate A	1	2	3	4	5	6	7	8	9	10	11	12
A	1	2	3	4	5	6	1	2	3	4	5	6
B	1	2	3	4	5	6	1	2	3	4	5	6
C	1	2	3	4	5	6	1	2	3	4	5	6
D	1	2	3	4	5	6	1	2	3	4	5	6
E	1	2	3	4	5	6	1	2	3	4	5	6
F	1	2	3	4	5	6	1	2	3	4	5	6
G	1	2	3	4	5	6	1	2	3	4	5	6
H	1	2	3	4	5	6	1	2	3	4	5	6

Species 1-12 Plate B	1	2	3	4	5	6	7	8	9	10	11	12
A	7	8	9	10	11	12	7	8	9	10	11	12
B	12	7	8	9	10	11	12	7	8	9	10	11
C	11	12	7	8	9	10	11	12	7	8	9	10
D	10	11	12	7	8	9	10	11	12	7	8	9
E	9	10	11	12	7	8	9	10	11	12	7	8
F	8	9	10	11	12	7	8	9	10	11	12	7
G	7	8	9	10	11	12	7	8	9	10	11	12
H	12	7	8	9	10	11	12	7	8	9	10	11

Appendix 2

T-test comparing AUC of co-cultures across media

Appendix Table A2 : Summary of T tests comparing AUC of co-cultures in nutrient additions to the AUC of the same co-culture in control media.

Co-culture	Condition compared to	Condition compared	Estimate difference	P value
S5-S5	Control	Medium phosphate	1104.046713	2.32E-14
S5-S5	Control	High phosphate	-715.2269815	6.32E-10
S3-S3	Control	High iron	-356.042406	1.21E-07
S7-S7	Control	High iron	125.2693421	9.16E-07
S5-S5	Control	High nitrate	-384.125311	1.23E-05
S5-S5	Control	Medium copper	-465.3574904	1.35E-05
S5-S5	Control	High iron	-375.635311	1.65E-05
S2-S2	Control	High phosphate	-488.6383929	1.80E-05
S3-S3	Control	Medium copper	-351.4035429	2.68E-05
S6-S6	Control	High phosphate	-1204.657338	2.72E-05
S12-S11	Control	High iron	162.55	3.60E-05
S11-S11	Control	High iron	114.2410256	4.89E-05
S12-S11	Control	Medium phosphate	179.5966667	8.48E-05
S12-S11	Control	Medium copper	126.2167662	1.26E-04
S5-S4	Control	Medium phosphate	827.9	7.75E-05
S4-S9	Control	High iron	-449.59625	1.01E-04
S12-S12	Control	Medium iron	193.8664402	1.04E-04
S10-S10	Control	Medium iron	524.6816667	2.08E-04
S3-S3	Control	High nitrate	-219.2241429	5.84E-04
S12-S11	Control	Medium iron	233.6775	9.09E-04
S12-S11	Control	Medium nitrate	117.845	0.001754825
S7-S7	Control	Medium iron	282.4151667	9.28E-04
S6-S6	Control	Medium phosphate	-761.4445455	0.001386674
S10-S9	Control	High iron	100.2125	0.00117012
S5-S5	Control	Medium nitrate	-288.4888636	0.003202976
S6-S6	Control	Medium iron	-674.8645455	0.003026093
S6-S6	Control	Medium nitrate	-532.0045455	0.010543114
S10-S10	Control	Medium nitrate	191.05875	0.008207004

S11-S11	Control	Medium copper	67.53823418	0.007466583
S4-S9	Control	Medium iron	-318.3635714	0.010216058
S6-S6	Control	Medium copper	-550.5405455	0.026100626
S11-S11	Control	Medium iron	292.7096923	0.016192474
S10-S11	Control	Medium nitrate	77.4525	0.009484398
S4-S9	Control	Medium nitrate	-288.791	0.021811318
S3-S3	Control	Medium nitrate	-213.4821429	0.016955311
S7-S12	Control	High iron	76.95696429	0.006348492
S12-S9	Control	Medium iron	260.9333333	0.009896437
S12-S9	Control	High iron	145.1	0.010718527
S10-S10	Control	High iron	82.57333333	0.026159561
S7-S7	Control	Medium nitrate	166.8278846	0.021798118
S4-S7	Control	High iron	-301.295	0.018236534
S12-S9	Control	Medium nitrate	133.935	0.024354338
S5-S12	Control	High phosphate	-600.2894141	0.009943377
S1-S1	Control	High phosphate	-702.2806461	0.008398562
S6-S11	Control	Medium copper	-758.6845	0.026662303
S12-S9	Control	High nitrate	180.675	0.042932538
S7-S12	Control	High nitrate	-33.75428571	0.02737314
S10-S11	Control	Medium iron	138.165	0.047285412
S5-S3	Control	High phosphate	644.9508333	0.019838923
S5-S3	Control	Medium iron	391.545	0.024586122
S12-S12	Control	Medium phosphate	50.53289855	0.033984105
S12-S12	Control	High iron	51.42693364	0.042252847
S1-S1	Control	High iron	-378.4941667	0.038419838
S3-S12	Control	High phosphate	-540.1302778	0.032880723
S5-S11	Control	High iron	-558.52	0.038673388
S9-S9	Control	High copper	-234.16	0.027122866

Appendix 3

T-test comparing AUC of co-cultures to mono-culture in same media

Appendix Table A3: Summary statistics of T test comparing the Area Under Curve (AUC) of co-cultures in different media to the mono-culture in the same media to test which co-culture growths significantly alter each other. The starter represents the mono-culture that the co-culture is compared against.

Condition	Starter	Co-culture being compared against starter mono-culture	AUC of co-culture	AUC of mono-culture	P value
0.0000135 mmol/L FeSO4,7H20	S1	S1-S11	527.044	415.915	0.565848
0.0000135 mmol/L FeSO4,7H20	S1	S1-S9	581.6333	415.915	0.284947
0.0000135 mmol/L FeSO4,7H20	S10	S10-S11	331.35	705.4617	0.00097
0.0000135 mmol/L FeSO4,7H20	S10	S10-S9	274.13	705.4617	0.00379
0.0000135 mmol/L FeSO4,7H20	S11	S11-S10	391.9	474.692	0.355499
0.0000135 mmol/L FeSO4,7H20	S11	S11-S12	373.54	474.692	0.299024
0.0000135 mmol/L FeSO4,7H20	S11	S11-S8	360.265	474.692	0.466924
0.0000135 mmol/L FeSO4,7H20	S11	S11-S9	362.29	474.692	0.635703
0.0000135 mmol/L FeSO4,7H20	S12	S12-S11	385.0875	474.4369	0.097437

0.0000135 mmol/L FeSO4,7H20	S12	S12-S8	326.9033	474.4369	0.002129
0.0000135 mmol/L FeSO4,7H20	S12	S12-S9	397.0133	474.4369	0.133251
0.0000135 mmol/L FeSO4,7H20	S3	S3-S11	603.61	734.4276	0.741768
0.0000135 mmol/L FeSO4,7H20	S3	S3-S2	692.125	734.4276	0.027262
0.0000135 mmol/L FeSO4,7H20	S3	S3-S4	647.795	734.4276	0.000198
0.0000135 mmol/L FeSO4,7H20	S3	S3-S5	690.34	734.4276	0.587618
0.0000135 mmol/L FeSO4,7H20	S3	S3-S9	577.598	734.4276	0.120997
0.0000135 mmol/L FeSO4,7H20	S4	S4-S11	472.1583	575.5183	0.463139
0.0000135 mmol/L FeSO4,7H20	S4	S4-S2	652.265	575.5183	0.315175
0.0000135 mmol/L FeSO4,7H20	S4	S4-S3	599.4	575.5183	0.669795
0.0000135 mmol/L FeSO4,7H20	S4	S4-S5	672.915	575.5183	0.252332
0.0000135 mmol/L FeSO4,7H20	S4	S4-S9	408.4214	575.5183	0.126959

0.0000135 mmol/L FeSO4,7H20	S5	S5-S11	621.4883	678.5875	0.716433
0.0000135 mmol/L FeSO4,7H20	S5	S5-S3	721.4625	678.5875	0.728163
0.0000135 mmol/L FeSO4,7H20	S5	S5-S4	664.1088	678.5875	0.868505
0.0000135 mmol/L FeSO4,7H20	S5	S5-S7	512.42	678.5875	0.48628
0.0000135 mmol/L FeSO4,7H20	S5	S5-S9	300.3783	678.5875	0.008119
0.0000135 mmol/L FeSO4,7H20	S6	S6-S11	556.066	542.49	0.90273
0.0000135 mmol/L FeSO4,7H20	S6	S6-S9	505.075	542.49	0.794625
0.0000135 mmol/L FeSO4,7H20	S7	S7-S11	361.6767	553.4927	0.022572
0.0000135 mmol/L FeSO4,7H20	S7	S7-S12	539.1683	553.4927	0.928086
0.0000135 mmol/L FeSO4,7H20	S8	S8-S11	389.985	383.5106	0.905312
0.0000135 mmol/L FeSO4,7H20	S8	S8-S12	364.894	383.5106	0.660973
0.0000135 mmol/L FeSO4,7H20	S8	S8-S7	437.28	383.5106	0.580696

0.0000135 mmol/L FeSO4,7H20	S8	S8-S9	231.64	383.5106	0.56656
0.0000135 mmol/L FeSO4,7H20	S9	S9-S12	402.4982	336.8733	0.455018
0.0000135 mmol/L FeSO4,7H20	S9	S9-S7	385.7933	336.8733	0.556611
0.0000135 mmol/L FeSO4,7H20	S9	S9-S8	472.9867	336.8733	0.157868
0.000027 mmol/L FeSO4,7H20	S10	S10-S11	252.645	263.3533	0.850814
0.000027 mmol/L FeSO4,7H20	S10	S10-S12	265.805	263.3533	0.966928
0.000027 mmol/L FeSO4,7H20	S10	S10-S7	290.26	263.3533	0.667244
0.000027 mmol/L FeSO4,7H20	S10	S10-S8	294.865	263.3533	0.377151
0.000027 mmol/L FeSO4,7H20	S10	S10-S9	256.235	263.3533	0.838603
0.000027 mmol/L FeSO4,7H20	S11	S11-S10	293.9867	296.2233	0.912914
0.000027 mmol/L FeSO4,7H20	S11	S11-S12	317.4767	296.2233	0.361378
0.000027 mmol/L FeSO4,7H20	S11	S11-S7	393.6633	296.2233	0.27024

0.000027 mmol/L FeSO4,7H20	S11	S11-S8	269.355	296.2233	0.36689
0.000027 mmol/L FeSO4,7H20	S11	S11-S9	274.8125	296.2233	0.361105
0.000027 mmol/L FeSO4,7H20	S12	S12-S11	313.96	331.9974	0.339037
0.000027 mmol/L FeSO4,7H20	S12	S12-S7	319.65	331.9974	0.860565
0.000027 mmol/L FeSO4,7H20	S12	S12-S8	328.7225	331.9974	0.882381
0.000027 mmol/L FeSO4,7H20	S12	S12-S9	281.18	331.9974	0.146646
0.000027 mmol/L FeSO4,7H20	S3	S3-S10	191.98	424.9047	0.399666
0.000027 mmol/L FeSO4,7H20	S3	S3-S11	341.1475	424.9047	0.240555
0.000027 mmol/L FeSO4,7H20	S3	S3-S12	326.7275	424.9047	0.03846
0.000027 mmol/L FeSO4,7H20	S3	S3-S4	391.72	424.9047	0.092495
0.000027 mmol/L FeSO4,7H20	S3	S3-S5	362.57	424.9047	0.077953
0.000027 mmol/L FeSO4,7H20	S3	S3-S8	289.33	424.9047	0.015387

0.000027 mmol/L FeSO4,7H20	S3	S3-S9	315.25	424.9047	
0.000027 mmol/L FeSO4,7H20	S4	S4-S10	272.8975	416.3168	0.220143
0.000027 mmol/L FeSO4,7H20	S4	S4-S11	299.605	416.3168	0.30146
0.000027 mmol/L FeSO4,7H20	S4	S4-S12	227.49	416.3168	0.183657
0.000027 mmol/L FeSO4,7H20	S4	S4-S3	396.5238	416.3168	0.368355
0.000027 mmol/L FeSO4,7H20	S4	S4-S5	376.2325	416.3168	0.513048
0.000027 mmol/L FeSO4,7H20	S4	S4-S7	309.275	416.3168	0.161845
0.000027 mmol/L FeSO4,7H20	S4	S4-S8	292.905	416.3168	0.004416
0.000027 mmol/L FeSO4,7H20	S4	S4-S9	277.1888	416.3168	0.008701
0.000027 mmol/L FeSO4,7H20	S5	S5-S11	276.385	369.3411	0.013559
0.000027 mmol/L FeSO4,7H20	S5	S5-S12	298.77	369.3411	0.000382
0.000027 mmol/L FeSO4,7H20	S5	S5-S3	364.9175	369.3411	0.789389

0.000027 mmol/L FeSO4,7H20	S5	S5-S4	372.83	369.3411	0.801179
0.000027 mmol/L FeSO4,7H20	S5	S5-S7	323.385	369.3411	0.132728
0.000027 mmol/L FeSO4,7H20	S5	S5-S8	317.11	369.3411	0.036183
0.000027 mmol/L FeSO4,7H20	S5	S5-S9	308.56	369.3411	0.325961
0.000027 mmol/L FeSO4,7H20	S7	S7-S11	324.515	396.3468	0.084248
0.000027 mmol/L FeSO4,7H20	S7	S7-S12	363.7313	396.3468	0.204837
0.000027 mmol/L FeSO4,7H20	S7	S7-S9	273.745	396.3468	0.07963
0.000027 mmol/L FeSO4,7H20	S8	S8-S10	269.1725	303.0522	0.355375
0.000027 mmol/L FeSO4,7H20	S8	S8-S11	298.5825	303.0522	0.73445
0.000027 mmol/L FeSO4,7H20	S8	S8-S12	303.0525	303.0522	0.999986
0.000027 mmol/L FeSO4,7H20	S8	S8-S7	376.0738	303.0522	0.04001
0.000027 mmol/L FeSO4,7H20	S8	S8-S9	269.3775	303.0522	0.120858

0.000027 mmol/L FeSO4,7H20	S9	S9-S10	261.1225	295.7393	0.09242
0.000027 mmol/L FeSO4,7H20	S9	S9-S11	266.905	295.7393	0.261929
0.000027 mmol/L FeSO4,7H20	S9	S9-S12	289.0025	295.7393	0.773357
0.000027 mmol/L FeSO4,7H20	S9	S9-S7	335.015	295.7393	0.579677
0.000027 mmol/L FeSO4,7H20	S9	S9-S8	302.2213	295.7393	0.822114
0.0005 mmol/L PO4	S12	S12-S11	331.0067	331.1033	0.993918
0.0005 mmol/L PO4	S12	S12-S8	321.62	331.1033	0.901385
0.0005 mmol/L PO4	S12	S12-S9	323.57	331.1033	0.800477
0.0005 mmol/L PO4	S3	S3-S12	426.93	744.5064	0.109183
0.0005 mmol/L PO4	S3	S3-S2	574.592	744.5064	0.032358
0.0005 mmol/L PO4	S5	S5-S12	1189.848	1849.023	0.330615
0.0005 mmol/L PO4	S5	S5-S3	1075.723	1849.023	0.132737
0.0005 mmol/L PO4	S5	S5-S4	1360.575	1849.023	6.19E-05
0.0005 mmol/L PO4	S7	S7-S12	179.01	219.9	0.722947
0.001 mmol/L PO4	S3	S3-S12	8.157222	649.9957	6.70E-07

0.001 mmol/L PO4	S3	S3-S5	984.89	649.9957	0.427634
0.001 mmol/L PO4	S5	S5-S12	3.730586	29.74938	0.000772
0.001 mmol/L PO4	S5	S5-S3	974.8683	29.74938	0.004472
0.005mmol/L NO3	S10	S10-S11	270.6375	371.8388	0.102967
0.005mmol/L NO3	S10	S10-S12	284.7075	371.8388	0.150843
0.005mmol/L NO3	S10	S10-S9	144.2767	371.8388	0.004994
0.005mmol/L NO3	S11	S11-S10	266.88	181.9445	0.109577
0.005mmol/L NO3	S12	S12-S11	269.255	302.3321	0.182498
0.005mmol/L NO3	S12	S12-S7	300.105	302.3321	0.911269
0.005mmol/L NO3	S12	S12-S9	270.015	302.3321	0.050112
0.005mmol/L NO3	S2	S2-S12	428.16	598.1182	0.146054
0.005mmol/L NO3	S4	S4-S12	392.055	530.31	0.467804
0.005mmol/L NO3	S4	S4-S9	437.994	530.31	0.352349
0.005mmol/L NO3	S5	S5-S11	129.9	456.4875	0.194637
0.005mmol/L NO3	S5	S5-S12	236.18	456.4875	0.131829
0.005mmol/L NO3	S5	S5-S9	275.6	456.4875	0.013623
0.005mmol/L NO3	S7	S7-S10	142.995	437.9054	0.156775

0.005mmol/L NO3	S7	S7-S11	249.04	437.9054	0.071049
0.005mmol/L NO3	S7	S7-S12	301.3629	437.9054	0.05156
0.005mmol/L NO3	S7	S7-S9	301.2825	437.9054	0.079194
0.01mmol/L NO3	S10	S10-S12	182.73	123.54	0.638927
0.01mmol/L NO3	S11	S11-S12	190.08	147.618	0.573248
0.01mmol/L NO3	S11	S11-S9	144.9225	147.618	0.966798
0.01mmol/L NO3	S12	S12-S11	171.765	274.1824	0.00348
0.01mmol/L NO3	S12	S12-S9	316.755	274.1824	0.105523
0.01mmol/L NO3	S2	S2-S1	568.128	593.9636	0.342799
0.01mmol/L NO3	S2	S2-S12	447.315	593.9636	0.518922
0.01mmol/L NO3	S2	S2-S7	451.68	593.9636	0.558729
0.01mmol/L NO3	S3	S3-S2	556.08	561.723	0.937129
0.01mmol/L NO3	S4	S4-S1	544.5	471.9833	0.292555
0.01mmol/L NO3	S4	S4-S12	342.4725	471.9833	0.363041
0.01mmol/L NO3	S4	S4-S2	563.445	471.9833	0.737732
0.01mmol/L NO3	S4	S4-S3	454.6843	471.9833	0.511805
0.01mmol/L NO3	S5	S5-S12	293.51	360.8511	0.022484

0.01mmol/L NO3	S5	S5-S2	505.08	360.8511	0.646602
0.01mmol/L NO3	S5	S5-S3	423.4425	360.8511	0.32729
0.01mmol/L NO3	S5	S5-S4	358.962	360.8511	0.916917
0.01mmol/L NO3	S7	S7-S11	209.805	274.2533	0.271072
0.01mmol/L NO3	S7	S7-S12	253.02	274.2533	0.30357
0.01mmol/L NO3	S7	S7-S9	291.435	274.2533	0.69465
0.01mmol/L NO3	S9	S9-S10	144.945	242.0267	0.089799
0.01mmol/L NO3	S9	S9-S11	257.64	242.0267	0.597521
0.01mmol/L NO3	S9	S9-S8	211.2975	242.0267	0.446237
Control	S1	S1-S2	568.53	712.7475	0.299727
Control	S1	S1-S5	840.39	712.7475	0.412828
Control	S10	S10-S11	193.185	180.78	0.44161
Control	S10	S10-S12	200.6775	180.78	0.730744
Control	S10	S10-S9	156.0225	180.78	0.244361
Control	S11	S11-S12	255.7575	181.9823	0.011707
Control	S11	S11-S3	543.575	181.9823	0.299593
Control	S11	S11-S4	301.08	181.9823	0.093668
Control	S11	S11-S5	495.67	181.9823	0.02801
Control	S11	S11-S6	368.835	181.9823	3.46E-07
Control	S12	S12-S11	151.41	280.5704	0.000153
Control	S12	S12-S3	497.15	280.5704	0.368177
Control	S12	S12-S5	520.23	280.5704	0.208331

Control	S12	S12-S6	486.515	280.5704	0.109362
Control	S12	S12-S7	279.11	280.5704	0.957189
Control	S12	S12-S9	136.08	280.5704	1.43E-07
Control	S2	S2-S1	640.77	499.1813	0.053356
Control	S2	S2-S12	382.6125	499.1813	0.08696
Control	S2	S2-S3	322.77	499.1813	0.600942
Control	S2	S2-S4	610.215	499.1813	0.728866
Control	S2	S2-S7	291.675	499.1813	0.014703
Control	S2	S2-S8	250.32	499.1813	0.356964
Control	S2	S2-S9	288.345	499.1813	0.44925
Control	S3	S3-S12	548.2875	780.9471	0.204203
Control	S3	S3-S8	429.495	780.9471	0.558222
Control	S3	S3-S9	414.6825	780.9471	0.194737
Control	S4	S4-S10	513.605	471.7275	0.818209
Control	S4	S4-S11	751.525	471.7275	0.10353
Control	S4	S4-S3	407.93	471.7275	0.55963
Control	S4	S4-S5	299.68	471.7275	0.633912
Control	S4	S4-S7	610.57	471.7275	0.187399
Control	S4	S4-S9	726.785	471.7275	0.026353
Control	S5	S5-S1	614.64	744.9764	0.693542
Control	S5	S5-S11	834.905	744.9764	0.377016
Control	S5	S5-S12	604.02	744.9764	0.371856
Control	S5	S5-S3	329.9175	744.9764	0.009546
Control	S5	S5-S4	532.675	744.9764	0.150936
Control	S5	S5-S6	785.1275	744.9764	0.582022
Control	S5	S5-S7	522.5	744.9764	0.011072
Control	S6	S6-S11	945.4825	1217.355	0.341116
Control	S6	S6-S12	766.9875	1217.355	0.103975

Control	S6	S6-S3	79.185	1217.355	4.78E-05
Control	S6	S6-S5	571.102	1217.355	0.029759
Control	S7	S7-S11	246.6375	271.0775	0.498716
Control	S7	S7-S12	286.7743	271.0775	0.347235
Control	S7	S7-S3	529.325	271.0775	0.299257
Control	S7	S7-S9	286.5675	271.0775	0.703942
Control	S8	S8-S2	217.41	383.322	0.453256
Control	S8	S8-S3	492.745	383.322	0.348778
Control	S9	S9-S3	442.21	272.2	0.216696
Control	S9	S9-S4	348.295	272.2	0.429439

Appendix 4

Fungal ITS4 sequences

ITS 4 regions for the four fungal strains studied in this thesis, *Pyrenochaetopsis leptospora (*SPIE01), *Pseudeurotium zonatum* (SPIE02), *Penicillium commune* (SPIE04), *Cladosporium halotolerans* (SPIE07).

>*SPIE01_ITS4 Pyrenochaetopsis leptospora*

AACGGGTCCTACCTGATCCGAGGTCAACGTTTGAATAAGGCTTCATGGACGCCGACGTAGAAGGGAGAGAC
GCAAATTGTGCTGCGCTCTACCACCAGTACGTTGGCCGCCAATCGCTTTCAGGCGAGTCCTTTGCAGGACAA
ACGCCCAACACCAAGCAGTGCTTGAGGGTACAAAATGACGCTCGAACAGGCATGCCCCATGGAATACCAA
GGGGCGCAATGTGCGTTCAAAGATTCGATGATTCACTGAATTCTGCAATTCACACTACTTTTCGCATTTCGCTG
CGTTCTTCATCGATGCCAGAACCAAGAGATCCGTTGTTGAAAGTTGTAATTATTATTTGTTTTCTGACGCTGATTG
CAACTACAAAAAGGTTTGATATTGTCCAACCGGCAGGCAAGCCCGCCGAGGAAACATACGGTACGCAAAAG
ACAAGGGTGCAGCAAGGACCGAAGTCCGCTGTTTTGATAATGATCCTTCCGCAGGTTCACCCTACGGAAGGA

>SPIE02_ITS4 *Pseudeurotium zonatum*

GGGGGTCCTACCTGATCCGAGGTCAACCTGAAAAATTGGGGGTTGCTGGCAAGCACGCACCCGGGTCTCC
AAAGCGAGAAGAATTACTACGCTTGAAGCCGAATGGCACCGCCACTGATTTTAAGGCCTGCCGGGACCGGC
AGAGCCCAATACCAAGCAGAGCTTGAGGGTTGTAATGACGCTCGAACAGGCATGCCCCCCGGAATACCAG
GGGGCGCAATGTGCGTTCAAAGATTCGATGATTCACTGAATTCTGCAATTCACATTACTTATCGCATTTCGCTG
CGTTCTTCATCGATGCCAGAACCAAGAGATCCGTTGTTGAAAGTTTTAACTATTATATAGTACTCAGACAATATGA
ACAAACAGAGTTTTAGGTCCTCTGGCGGGCACTGACCAGCCGAAGCCGGTGGTCCGAGGACGGGCCCGC
CAAAGCAACAAAGGTATAATAAACAAAGGGTGGGAGGTATACCCCGAAGGGCAACGTCTCTTTAATGATCCTT
CCGCAGGTCACCCTA

>SPIE04_ITS4 *Penicillium commune*

GGGGTCCTACCTGATCCGAGGTCAACCTGGGATAAAATTTGGGTTGATCGGCAAGCGCCGGCCGGGCCTAC
AGAGCGGGTGACAAAGCCCCATACGCTCGAGGACCGGACGCGGTGCCGCCGCTGCCTTTCGGGCCCGTC
CCCCGGAGATCGGAGGACGGGGCCCAACACACAAGCCGGGCTTGAGGGCAGCAATGACGCTCGGACAG
GCATGCCCCCCGGAATACCAGGGGGCGCAATGTGCGTTCAAAGACTCGATGATTCACTGAATTTGCAATTCA
CATTACGTATCGCATTTCGCTGCGTTCTTCATCGATGCCGGAACCAAGAGATCCGTTGTTGAAAGTTTTAAATAA
TTTATATTTTCACTCAGACTTCAATCTTCAGACAGAGTTCGAGGGTGTCTTCGGCGGGCGCGGGCCCGGGGG
CGTGAGCCCCCCGGCGGCCAGTTAAGGCGGGCCCGCCGAAGCAACAAGGTAAAATAAACACGGGTGGGA
GGTTGGACCCAGAGGGCCCTCACTCGGTAATGATCCTTCCGCAGGTCACCCTTACGGAAGGTT

>SPIE07_ITS4 *Pyrenochaetopsis leptospora*

GGGTCCTACCTGATCCGAGGTCAACCTTAGAAATGGGGTTGTTTTACGGCGTGGCCTCCCGCGGCACCCTTT
AGCGAATAGTTTCCACAACGCTGAGGGGACGAAAGACCCAGCCGGTCGATTTGAGGCGCGCGGCGGACC
GCGTCGCCCAATACCAAGCGAGGCTTGAGTGGTGAAATGACGCTCGAACAGGCATGCCCCCCGGAATACC
AGGGGGCGCAATGTGCGTTCAAAGATTCGATGATTCACTGAATTCTGCAATTCACATTACTTATCGCATTTCGCT
GCGTTCTTCATCGATGCCAGAACCAAGAGATCCGTTGTTGAAAGTTTTAATTTATTAATTAAATTTACTCAGACTG
CAAAGTTACGCAAGAGTTTGAAATGTCCACCCGGGGCCCCCGCCCGGAGGCAGGGTCGCCCCGGAGGCA
ACAGAGTCGGACAACAAAGGGTTGTGAACATCCCGGCCCGAGGGCCGGGGTCAACTTGTAATGATCCCTCC
GCAGGTCACCC

Appendix 5-

F/2 Medium Recipe

f/2 Medium

Stocks		**per 200 ml**
(1)	$NaNO_3$	15 g
(2)	$NaH_2PO_4.2H_2O$	1.13 g
(3)	Trace elements (x10 concentration):	**per 200 ml**
	Na_2EDTA	8.32 g
	$FeCl_3.6H_2O$	6.30 g
	$CuSO_4.5H_2O$	0.02 g
	$ZnSO_4.7H_2O$	0.044 g
	$CoCl_2.6H_2O$	0.02 g
	$MnCl_2.4H_2O$	0.36 g
	$Na_2MoO_4.2H_2O$	0.012 g
(4)	Vitamin mix:	
		per litre
	Cyanocobalamin (Vitamin B_{12})	0.0005 g
	Biotin	0.0005 g
	Thiamine HCl (Vitamin B_1)	0.1g

Appendix 6

Ethics form

Swansea University
Prifysgol Abertawe

Approval Date: 23/09/2025

Research Ethics Approval Number: 1 2025 14322 14075

Thank you for completing a research ethics application for ethical approval and submitting the required documentation via the online platform.

Project Title	The focus is on deciphering the interactions between various bacteria species within the seagrass environment. Methodology involves developing imaging technology to analyze bacterial growth patterns.
Applicant name	PHILIP ELLWOOD
Submitted by	PHILIP ELLWOOD /
Full application form link	https://swansea.forms.ethicalreviewmanager.com/Project/Index/16930

The Science and Engineering ethics committee has approved the ethics application, subject to the conditions outlined below:

Approval conditions

1. The approval is based on the information given within the application and the work will be conducted in line with this. It is the responsibility of the applicant to ensure all relevant external and internal regulations, policies, and legislations are met.
2. This project may be subject to periodic review by the committee. The approval may be suspended or revoked at any time if there has been a breach of conditions.
3. Any substantial amendments to the approved proposal will be submitted to the ethics committee prior to implementing any such changes.

Specific conditions in respect of this application:

The application has been classified as Low Risk to the University.

Conditions as per Declaration form.

Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees. It complies with the guidelines of UKRI and the concordat to support Research Integrity.

Science and Engineering Research and Ethics Chair

Swansea University.

If you have any queries regarding this notification, then please contact your research ethics administrator for the faculty.

- For Science and Engineering contact FSE-Ethics@swansea.ac.uk
- For Medicine, Health and Life Science contact FMHLS-Ethics@swansea.ac.uk
- For Humanities and Social Sciences contact FHSS-Ethics@swansea.ac.uk

Dyddiad Cymeradwyo: 23/09/2025

Rhif Cymeradwyo Moeseg Ymchwil:

Diolch am gwblhau cais moeseg ymchwil am gymeradwyaeth foesegol ac am gyflwyno'r ddogfennaeth ofynnol drwy'r platfform ar-lein.

Appendix 7

Expenditure

Item	Supplier	Specs	Price (£)
Guillards (F2) Marine water enrichment solution	Sigma Aldrich	500 ml	63.2
Wizard® HMW DNA Extraction Kit, 50 preps	Promega		223
Gloves			49
Pipette tips			50
Dreamtaq (200 reactions)		X8	94.65
Rotor+ Singer instrument bacterial pinning pads	Rotor+		40
Sodium nitrate	(Thermo scientific)		64
Sodium phosphate	(Thermo scientific)		35
Copper chloride	(Acros organics)		44
Ferrous sulphate	(Thermo scientific)		27
Bacterial genome sequencing			300
Total			989.85

Appendix 8

Statement of contributions

Contributor Role	Persons involved
Conceptualization	PE, ECS, CG
Data curation	PE
Formal Analysis	PE (HW help with genome assembly)
Funding acquisition	N/A
Investigation	PE
Methodology	PE, ECS, CG
Project administration	PE, ECS, CG
Resources	PE, ECS, CG
Software	PE
Supervision	ECS, CG
Validation	N/A
Writing original draft	PE
Writing review and editing	PE, ECS, CG

Appendix 9

Risk assessment

Swansea University Prifysgol Abertawe | HEALTH & SAFETY IECHYD A DIOGELWCH

Risk Assessment of An Activity Involving Deliberate Work with Pathogenic Microorganisms or Samples with Potential to Harbour Pathogenic Microorganisms

This risk assessment form should be used to assist in the assessment of risks from an activity involving deliberate work with an infectious of harmful biological agent. The aim of the assessment is to identify those at risk from infection or other harm and the measures required to eliminate or control the risks to human health and the environment to an acceptable level.

Section 1: General Information	
1.1 Principal Investigator/ Academic Supervisor:	
Name	Faculty
Eva Sonnenschein	FSE
Email	Date
[redacted]	06/02/25

1.2 Premises where this work will be carried out:		
Building	Laboratories	Containment level
Wallace	123, 124	1

Section 2: Project Information	
2.1 Person undertaking this risk assessment (if different from above):	
Name	Faculty
Philip Ellwood	Science and Engineering
2.2 Project title:	
Unravelling microbial interactions in the seagrass microbiome	
2.3 Project Reference Number: Suggested format YYMMDD, PI initials, BIO (number if more than one in a month)	

250206_MME_ECS

2.3 Is this proposal an extension of a previously approved project?	
Previously approved If yes, please complete the form emphasising the connection between the original project and this application.	☐ Yes ☒ No
Previous reference number	

2.4 Brief overview of the work (in layman's terms)**:**
Please describe the project, detailing aims and objectives, significance, and outcomes, indicating how the biological agent will help to achieve the objectives of the project. This description should contain enough detail to help a non-specialist to understand the project.

This research seeks to investigate the bacteria inhabiting seagrass ecosystems, which are vital for supporting marine life. The primary focus is on deciphering the interactions between various bacterial species within the seagrass environment. These interactions can range from beneficial to detrimental or neutral, potentially influencing seagrass health, which in turn affects marine biodiversity and water quality. Our methodology involves developing an advanced technique to assess how different microbial strains interact. We'll utilize an automated system to cultivate pairs of microbes on specialised nutrient-rich surfaces (agar plates), allowing for simultaneous testing of numerous combinations. We'll monitor these microbial pairs over time, employing imaging technology to analyse their growth patterns. This will enable us to categorize their interactions as positive, negative, or neutral. By classifying these relationships, we aim to construct a comprehensive network illustrating the interconnections among diverse microorganisms in seagrass habitats.

2.5 Is this biological agent going to form part of an undergraduate practical class?	☐ Yes ☒ No
2.6 Faculty Contacts:	
Health, Safety and Resilience Advisor	Lorraine Wild and Will Finn-Lewis
Biological Safety Officer	Dr Tamsyn Uren Webster

Section 3: Identification of Biological Hazards					
3.1 List microorganisms deliberately used/ or pathogenic microorganisms that potentially can be present in the sample(s):					

Name of microorganism	*Chryseobacterium indolthetic um*	*Psychromonas arctica*	*Vibrio hispanicus*	*Colwellia echini*	*Psychromonas marina*	*Marinomonas primoryensis*	*Pseudoalteromonas fuliginea*	*Variovorax paradoxus*
Identified as human pathogen on ACDP list[1]	☐ Yes ☒ No	☐ Yes ☒ No	☐ Yes ☒ No	☐ Yes ☒ No	☐ Yes ☒ No	☐ Yes ☒ No	☐ Yes ☒ No	☐ Yes ☒ No
If yes, please state hazard group	☐ 2 ☐ 3 ☐ 4	☐ 2 ☐ 3 ☐ 4	☐ 2 ☐ 3 ☐ 4	☐ 2 ☐ 3 ☐ 4	☐ 2 ☐ 3 ☐ 4	☐ 2 ☐ 3 ☐ 4	☐ 2 ☐ 3 ☐ 4	☐ 2 ☐ 3 ☐ 4
If not on ACDP list, is there any evidence to support the microorganism may present a risk to human health	NA	Some studies suggest it could show mild haemolytic activity	Within the family of Vibrionaceae are typically associated with risks to human health.	Its ability to degrade agar and carrageenan	NA	NA	NA	NA
Normal routes of human infection	☐ Inhalation ☐ Oral/ ingestion ☐ Mucocutaneous ☐ Percutaneous ☐ Via vector (e.g., insect) ☐ Allergen	☐ Inhalation ☐ Oral/ ingestion ☐ Mucocutaneous ☐ Percutaneous ☐ Via vector (e.g., insect) ☐ Allergen	☐ Inhalation ☒ Oral/ ingestion ☐ Mucocutaneous ☐ Percutaneous ☒ Via vector (e.g., insect)	☐ Inhalation ☐ Oral/ ingestion ☐ Mucocutaneous ☐ Percutaneous ☐ Via vector (e.g., insect) ☐ Allergen	☐ Inhalation ☐ Oral/ ingestion ☐ Mucocutaneous ☐ Percutaneous ☐ Via vector (e.g., insect) ☐ Allergen	☐ Inhalation ☐ Oral/ ingestion ☐ Mucocutaneous ☐ Percutaneous ☐ Via vector (e.g., insect) ☐ Allergen	☐ Inhalation ☐ Oral/ ingestion ☐ Mucocutaneous ☐ Percutaneous ☐ Via vector (e.g., insect) ☐ Allergen	☐ Inhalation ☐ Oral/ ingestion ☐ Mucocutaneous ☐ Percutaneous ☐ Via vector (e.g., insect) ☐ Allergen

			☐ Allergen					
Multiplicity of infection if known (i.e., number of organisms required to establish an infection)	Not known as human pathogen	Not known as human pathogen	pathogenic to fish and crustaceans	Not known as human pathogen	Not known as human pathogen	Not known as human pathogen	Not known as human pathogen	Not known as human pathogen
Consequence of infection to humans	Medium	Low	Low-potential gastrointestinal issues	NK	NK	NK	NK	NA
Is the microorganism a specified animal pathogen (SAPO[2])	☐ Yes ☒ No	☐ Yes ☒ No	☐ Yes ☒ No	☐ Yes ☒ No	☐ Yes ☒ No	☐ Yes ☒ No	☐ Yes ☒ No	☐ Yes ☒ No
If yes, please state SAPO hazard group	☐ 2 ☐ 3 ☐ 4	☐ 2 ☐ 3 ☐ 4	☐ 2 ☐ 3 ☐ 4	☐ 2 ☐ 3 ☐ 4	☐ 2 ☐ 3 ☐ 4	☐ 2 ☐ 3 ☐ 4	☐ 2 ☐ 3 ☐ 4	☐ 2 ☐ 3 ☐ 4
Detail of any other harm the microorganism may pose to the environment? e.g., harmful to plants,	Can kill limpets, muscles and urchins in high concentrations	NA	NA	Extensive spread could pose damage to local macro algae	NA	NA	NA	NA

insects, etc.								
Consequence of spread in environment	Water source contamination	NA	NK	NA	NA	NA	NA	NA
Route of transmission for environmental pathogens (including animals)	Water borne	NA	NA	NA	NA	NA	NA	NA
Any additional risk to health/ environment e.g. Hyper virulence, multiple antibiotic resistance	NA	NA	NA	NA	NA	NA	NA	NA
Listed on Schedule 5[3]	☐ Yes ☒ No	☐ Yes ☒ No	☐ Yes ☒ No	☐ Yes ☒ No	☐ Yes ☒ No	☐ Yes ☒ No	☐ Yes ☒ No	☐ Yes ☒ No
How likely is that Cat 3 and Cat 4 biological agents will be present in the material / sample to be	☐ Definitely present ☐ Likely present ☒ Unlikely present	☐ Definitely present ☐ Likely present	☐ Definitely present ☐ Likely present	☐ Definitely present ☐ Likely present ☒ Unlikel	☐ Definitely present ☐ Likely present ☒ Unlikel	☐ Definitely present ☐ Likely present ☒ Unlikel	☐ Definitely present ☐ Likely present ☒ Unlikely present	☐ Definitely present ☐ Likely present ☒ Unlikel

handled?		☒ Unlikely present	☒ Unlikely present	y present	y present	y present		y present
Will you intentionally isolate, propagate or otherwise increase the risk of the above mentioned (if any) CAT3 and CAT4 biological agents?	NA	NA	NA	NA	NA	NA	NA	NA
[1] ACDP Approved List of (Human) Pathogens [2] *SAPO Pathogens* [3] Schedule 5 Pathogens on the Anti-terrorism & Security								

Section 3: Identification of Biological Hazards									
3.1 List microorganisms deliberately used/ or pathogenic microorganisms that potentially can be present in the sample(s):									
Name of microorganism	*Pseudoalteromonas spiralis*	*Lysobacter luteus*	*Variovorax robiniae*	*Alkalicoccobacillus plakortidis*	*Celeribacter baekdonensis*	*Rhodococcus cerastii*	*Rhodococcus yunnanensis*	*Vreelandella titanicae*	
Identified	☐ Yes ☒ No	☐ Yes ☒ No	☐ Yes ☒ No	☐ Yes ☒ No	☐ Yes ☒ No	☐ Yes ☒ No	☐ Yes ☒ No	☐ Yes ☒ No	☐ Yes ☒ No

as human pathogen on ACDP list[1]									
If yes, please state hazard group	☐ 2 ☐ 3 ☐ 4	☐ 2 ☐ 3 ☐ 4	☐ 2 ☐ 3 ☐ 4	☐ 2 ☐ 3 ☐ 4	☐ 2 ☐ 3 ☐ 4	☐ 2 ☐ 3 ☐ 4	☐ 2 ☐ 3 ☐ 4	☐ 2 ☐ 3 ☐ 4	☐ 2 ☐ 3 ☐ 4
If not on ACDP list, is there any evidence to support the microorganism may present a risk to human health	NA	NA	NA	NA	NA	Rhodococcus are opportunistic pathogens some species are known to cause infections in immunocompromised individuals	Rhodococcus are opportunistic pathogens some species are known to cause infections in immunocompromised individuals	NA	
Normal routes of human infection	☐ Inhalation ☐ Oral/ ingestion ☐ Mucocutaneous	☐ Inhalation ☐ Oral/ ingestion ☐ Mucoc	☐ Inhalation ☐ Oral/ ingestion ☐ Mucoc	☐ Inhalation ☐ Oral/ ingestion	☐ Inhalation ☐ Oral/ ingestion ☐ Mucocutaneous	☐ Inhalation ☒ Oral/ ingestion ☐ Mucocutaneous	☐ Inhalation ☒ Oral/ ingestion ☐ Mucocutaneous	☐ Inhalation ☐ Oral/ ingestion ☐ Mucoc	☐ Inhalation ☐ Oral/ ingestion ☐ Mucocutaneous

	☐ Percutaneous ☐ Via vector (e.g., insect) ☐ Allergen	utaneous ☐ Percutaneous ☐ Via vector (e.g., insect) ☐ Allergen	utaneous ☐ Percutaneous ☐ Via vector (e.g., insect) ☐ Allergen	☐ Mucocutaneous ☐ Percutaneous ☐ Via vector (e.g., insect) ☐ Allergen	☐ Percutaneous ☐ Via vector (e.g., insect) ☐ Allergen	☐ Percutaneous ☐ Via vector (e.g., insect) ☐ Allergen	☐ Percutaneous ☐ Via vector (e.g., insect) ☐ Allergen	utaneous ☐ Percutaneous ☐ Via vector (e.g., insect) ☐ Allergen	☐ Percutaneous ☐ Via vector (e.g., insect) ☐ Allergen
Multiplicity of infection if known (i.e., number of organisms required to establish an infection)	Not known as human pathogen	Not known as human pathogen	Not known as human pathogen	Not known as human pathogen	Not known as human pathogen	Not known as human pathogen	Not known as human pathogen	Not known as human pathogen	
Consequence of infection to humans	NA	NA	NA	NA	NA	NA	NA	NA	
Is the microorganism a specified animal	☐ Yes ☒ No	☐ Yes ☒ No	☐ Yes ☒ No	☐ Yes ☒ No	☐ Yes ☒ No	☐ Yes ☒ No	☐ Yes ☒ No	☐ Yes ☒ No	☐ Yes ☐ No

pathogen (SAPO[2])									
If yes, please state SAPO hazard group	☐ 2 ☐ 3 ☐ 4	☐ 2 ☐ 3 ☐ 4	☐ 2 ☐ 3 ☐ 4	☐ 2 ☐ 3 ☐ 4	☐ 2 ☐ 3 ☐ 4	☐ 2 ☐ 3 ☐ 4	☐ 2 ☐ 3 ☐ 4	☐ 2 ☐ 3 ☐ 4	☐ 2 ☐ 3 ☐ 4
Detail of any other harm the microorganism may pose to the environment? e.g., harmful to plants, insects, etc.	NA	NA	NA	NA	NA	NA	NA	NA	
Consequence of spread in environment	NA	NA	NA	NA	NA	NA	NA	NA	
Route of transmission	NA	NA	NA	NA	NA	NA	NA	NA	

for environmental pathogens (including animals)									
Any additional risk to health/ environment e.g. Hyper virulence, multiple antibiotic resistance	NA	NA	NA	NA	NA	NA	NA	NA	
Listed on Schedule 5[3]	☐ Yes ☒ No	☐ Yes ☒ No	☐ Yes ☒ No	☐ Yes ☒ No	☐ Yes ☒ No	☐ Yes ☒ No	☐ Yes ☒ No	☐ Yes ☒ No	☐ Yes ☒ No
How likely is that Cat 3 and Cat 4 biological agents will be present in the	☐ Definitely present ☐ Likely present ☒ Unlik	☐ Definitely present ☐ Li	☐ Definitely present ☐ Li	☐ Definitely present ☐ Likely present	☐ Definitely present ☐ Likely present ☒ Unlikely	☐ Definitely present ☐ Likely present ☒ Unlikely pre	☐ Definitely present ☐ Likely present ☒ Unlikely present	☐ Definitely present ☐ Li	☐ Definitely present ☐ Likely present ☐ Unlikely

material/ sample to be handled?	ely present	kely present ☒ Unlikely present	kely present ☒ Unlikely present	☒ Unlikely present	present	sent		kely present ☒ Unlikely present	present
Will you intentionally isolate, propagate or otherwise increase the risk of the above mentioned (if any) CAT3 and CAT4 biological agents?	NA	NA	NA	NA	NA	NA	NA	NA	

Section 3: Identification of Biological Hazards				
3.1 List microorganisms deliberately used/ or pathogenic microorganisms that potentially can be present in the sample(s):				
Name of microorganism	Pyrenochaetopsis leptospora	Pseudeurotium zonatum	Penicillium commune	Cladosporium halotolerans
Identified as human pathogen on ACDP list[1]	☐ Yes ☒ No	☐ Yes ☒ No	☐ Yes ☒ No	☐ Yes ☒ No
If yes, please state hazard group	☐ 2 ☐ 3 ☐ 4	☐ 2 ☐ 3 ☐ 4	☐ 2 ☐ 3 ☐ 4	☐ 2 ☐ 3 ☐ 4
If not on ACDP list, is there any evidence to support the microorganism may present a risk to human health	NA	NA	NA	NA
Normal routes of human infection	☐ Inhalation ☐ Oral/ ingestion ☐ Mucocutaneous ☐ Percutaneous ☐ Via vector (e.g., insect)	☐ Inhalation ☐ Oral/ ingestion ☐ Mucocutaneous ☐ Percutaneous ☐ Via vector (e.g., insect) ☐ Allergen	☐ Inhalation ☒ Oral/ ingestion ☐ Mucocutaneous ☐ Percutaneous ☒ Via vector (e.g., insect) ☐ Allergen	☐ Inhalation ☐ Oral/ ingestion ☐ Mucocutaneous ☐ Percutaneous ☐ Via vector (e.g., insect) ☐ Allergen

	☐ Allergen			
Multiplicity of infection if known (i.e., number of organisms required to establish an infection)	Not known as human pathogen	Not known as human pathogen	pathogenic to fish and crustaceans	Not known as human pathogen
Consequence of infection to humans	Medium	Low	Low	NK
Is the microorganism a specified animal pathogen (SAPO[2])	☐ Yes ☒ No	☐ Yes ☒ No	☐ Yes ☒ No	☐ Yes ☒ No
If yes, please state SAPO hazard group	☐ 2 ☐ 3 ☐ 4	☐ 2 ☐ 3 ☐ 4	☐ 2 ☐ 3 ☐ 4	☐ 2 ☐ 3 ☐ 4
Detail of any other harm the microorganism may pose to the environment?	NA	NA	NA	NA

e.g., harmful to plants, insects, etc.				
Consequence of spread in environment	NA	NA	NK	NA
Route of transmission for environmental pathogens (including animals)	NA	NA	NA	NA
Any additional risk to health/ environment e.g. Hyper virulence, multiple antibiotic resistance	NA	NA	NA	NA
Listed on Schedule 5[3]	☐ Yes ☒ No	☐ Yes ☒ No	☐ Yes ☒ No	☐ Yes ☒ No
How likely is that Cat 3	☐ Definitely present	☐ Definitely present ☐ Likely present	☐ Definitely present ☐ Likely present	☐ Definitely present ☐ Likely present

and Cat 4 biological agents will be present in the material/ sample to be handled?	☐ Likely present ☒ Unlikely present	☒ Unlikely present	☒ Unlikely present	☒ Unlikely present
Will you intentionally isolate, propagate or otherwise increase the risk of the above mentioned (if any) CAT3 and CAT4 biological agents?	NA	NA	NA	NA
[1] ACDP Approved List of (Human) Pathogens [2] *SAPO Pathogens* [3] Schedule 5 Pathogens on the Anti-terrorism & Security				

Section 4: Experimental Procedures

4.1 Description of experimental procedures:

Co-cultivation of bacteria isolated from seagrass to determine how microbial strains interact with each other. The strains will be cultured on agar and potentially liquid broth and incubated at 25 °C.

4.2 Quantities used and frequency of use:			
This information will enable you to determine the likelihood of exposure and therefore the risks from this particular activity. Please indicate maximum culture volumes at any time shown as multiples of flask volumes to give an idea of scale.			
Max. volume per culture/ sample	500ml	Max. volume per experiment	500ml
Frequency of experiments		weekly	

Section 5: Measures to Prevent or Control Exposure

5.1 Preventing exposure

a) Could a less hazardous substance (or form of the substance) be used instead? If it can, then it should be used, or justification be given here why it is not being used. COSHH requires substitution with less hazardous materials wherever possible, but there may be good reasons for not using them.

N/A

5.2 Controlling Exposure

b) Containment Level - what containment level is required for this work with regard to COSHH/ SAPO?

☒ 1	☐ 2	☐ 3

c) CL3 only – application for derogation from the following controls (list if relevant and justify):	
d) Will the work be segregated from others not involved in the work and if not, how will they be informed of the hazards?	

5.3 Engineering Controls (Containment & Ventilation)

a) Is a microbial safety cabinet (or isolator for in vivo work) required? These must be	☐ Yes ☒ No

used for activities generating potentially infectious aerosols or splashes.	
If yes, please state which class and describe underneath what processes will use it:	Class: ☐ I ☐ II ☐ III
If required, what processes require its use?	
b) Specify other local ventilation control measures considered appropriate (e.g., downdraft table, isolator):	NA
c) Will centrifugation be used?	☐ Yes ☒ No
If yes, will buckets and rotors be sealed?	☐ Yes ☒ No
If yes, where will buckets or rotors be opened?	
If yes, how will spillages in the centrifuge be dealt with?	
d) Will incubators be used?	☒ Yes ☐ No
If yes, what type (e.g., shaking)?	Shaking and temperature controlled
If yes, how will spillages in the incubator be dealt with?	Wipe up with disinfectant and blue roll
e) Will sharps be used:	☐ Yes ☒ No
If yes, list and justify their use:	
f) Will animals be deliberately infected with these biological agents?	☐ Yes ☒ No
g) Do you require a licence/ permit? https://staff.swansea.ac.uk/media/tgn002-abps-and-import-permits.pdf	☐ Yes ☒ No
h) If yes, describe the procedure, control measures and whether shedding of infectious agents by animals is expected?	

5.4 Personal Protective Equipment (PPE):			
Lab coat	Gloves	Eye or face (specify)	Other
☒ Yes	☒ Yes ☐ No	☐ Yes ☒ No	☐ Yes ☒ No
Details:	Details:	Details:	Details:

5.5 Transportation	
a) How will viable material be transported within the laboratory?	In covered flasks and beakers with liquid media. On agar plates

b) How will viable material be transported locally outside the laboratory?	In covered flasks or beakers with liquid media or on agar plates in secondary container
c) Will viable material be shipped anywhere (off campus)?	☐ Yes ☒ No
If yes, what will be shipped?	
If yes, how will this be shipped (e.g., Category A, Category B, Exempt, Non-hazardous)?	

5.6 Waste Disposal Procedures:
(Disinfectants, concentrations, exposure times, autoclaving procedures, incinerator procedures, include any animal related wastes.)

Waste	Decontamination method (include details on efficacy)	Disposal route e.g., drain/ incineration/ landfill
Liquid waste	Autoclaving (121 °C for 15 mins) to kill the microorganisms	Drain
Solid waste	Autoclaving (121 °C for 15 mins) to kill the microorganisms	Autoclaved waste or Incineration
Sharp waste	In yellow bins as non- hazardous waste	Contractor for University waste disposal

5.7 Emergency Procedures

Spillages

- If covered by local rules/ standard operating procedure, please attach.
- If not covered by local rules/ standard operating procedure. Remember to take into account route of exposure

a) Inside primary containment (if relevant e.g., MSC, isolator):	Put on required PPE and stop source of spill, this may involve removing equipment away to thoroughly clean the areas. Cover the spill with paper towel, spray spillage area with 5% BioCleanse and leave to disinfect for 5 minutes. After 5 minutes wipe the areas with paper towel and discard the paper towel in autoclave bag.
b) Outside primary containment but within the laboratory (secondary containment):	Put on the required PPE equipment. Do not stand near the spill for 5 minutes to allow aerosols to settle. Move any chairs or waste bins away to thoroughly clean the area. Cover the spill with paper towel, spray spillage area with

	5% BioCleanse and leave to disinfect for 5 minutes. After 5 minutes wipe the areas with paper towel and discard the paper towel in autoclave bag.
c) Outside secondary containment (if relevant):	Only small quantities of bacterial cultures (max 500ml) will be outside the laboratory, primarily for use on microscopes or moving to incubator. Bacteria should always be placed within a leak-proof receptacle to contain any spillages during transport. Leaks within the receptacle may be cleaned with 5% BioCleanse upon return to the laboratory. Alert nearby personnel and evacuate area, if necessary, put on appropriate PPE to contain the spill using absorbent materials. Disinfect the area and dispose of contaminated materials in designated biohazard waste containers. Report incident to supervisor and laboratory technicians. Update safety protocols if required.
d) Other procedures (e.g., first aid following any accidental exposure, needle stick, etc.):	Notify someone such as supervisor or laboratory technician or a first aid officer. In the event of exposure, affected individuals should always go directly to A & E in the event of accidental exposure. If appropriate, reserve the culture to inform the health care team of the containment. The project supervisor should always be notified, and later details should be noted on the report form found at https://staff.swansa.ac.uk/healthsafety/report-it/

Section 6: Personnel and Health Issues

6.1 Vaccination

For ACDP 2 or above human pathogens Is an effective vaccination available for any of the pathogens associated with this work?	NA

6.2 Is health surveillance/ health clearance required?	
Staff and postgraduate research students	☐ Yes ☒ No
Taught students (undergraduate and MSc)	☐ Yes ☒ No (Initial health clearance only)
6.3 Identify any groups of workers who may be at increased risk from this material:	
Anyone who might have compromised resistance to disease for any reason should seek advice from the University Occupational Health Service regarding the need for additional precautions. (For example, pregnant workers, young persons under 18, disabled workers, those with pre-existing disease that increases susceptibility).	Individuals with the following risk factors may be at increased risk of infection from bacteria listed above - <18 years of age - >65 years of age - Immunocompromised individuals - Pregnancy
6.4 Information, Instruction, and Training	
Describe the training that will be given to all those affected (directly or indirectly) by the work activity.	Individuals will be given lab induction and equipment training prior to lab work. Relevant COSHH forms and risk assessments should also be signed before work.

Section 7: Declaration and Approval

7.1 Principal Investigator:

I the undersigned:

- Confirm that all information contained in this assessment is correct and up to date.
- Will ensure that suitable and sufficient instruction, information, and supervision is provided to all individuals working on the activity.
- Will ensure that no work will be carried out until this assessment has been completed and approved, and that all necessary control measures are in place.
- Will ensure that all information contained in this assessment will remain correct and up to date and re-submit for approval if any significant changes occur.
- Work will only be undertaken in appropriate facilities.

Name	Signature	Date

Eva Sonnenschein	[redacted]	06/02/25

7.2 Approval on behalf of the Faculty:

- Approval of Hazard Group 1 only.
- I support the presentation of this proposal to the Biological Hazards and Genetic Modified Organisms Sub-Committee (for ACDP Hazard Group 2-4, SAPO Hazard Group 2-4 and organisms listed on schedule 5).

The person supporting this proposal must not be involved in the project being proposed.

Faculty BSO	Name	
	Signature	
	Date	
University BSO	Name	
	Signature	
	Date	

7.3 Approval on behalf of the University:

- The risk assessment has been reviewed and approved by the Biological Hazards and GMO Sub-Committee.
- Approval of ACDP Hazard Group 2-4, SAPO Hazard Group 2-4 and organisms listed on schedule 5.

Chair of Biological Hazards and GMO Sub-Committee	Name	
	Signature	
	Date	

Section 8: List of Workers Under This Project

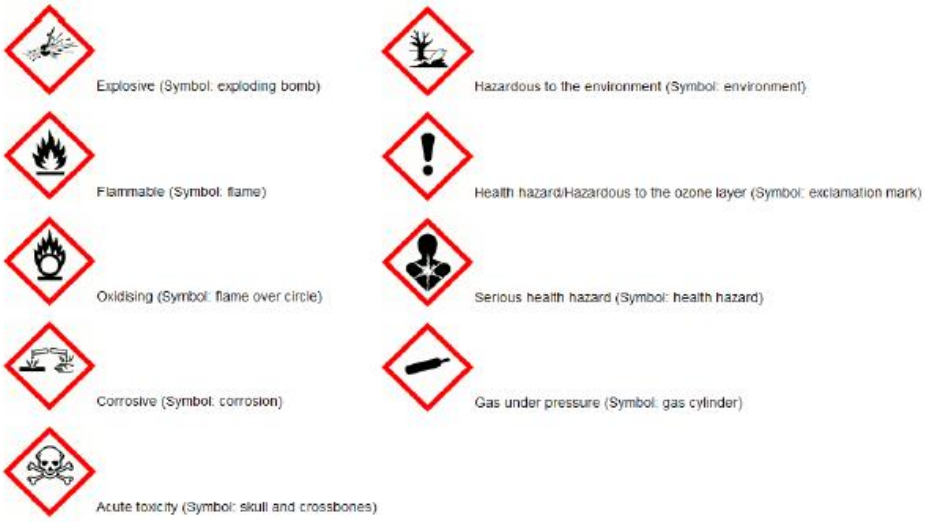
List any persons who will be working on this project. They must have access to this risk assessment and other associated risk assessments. Those listed should sign and date to confirm they have read understand the risk assessment.

Full Name	Worker Type					Signature and date
	Staff	PG Research	PG Taught	UG	Other	
Philip Ellwood	☐	☒	☐	☐	☐ Details	Philip Ellwood 31/01/25
	☐	☐	☐	☐	☐ Details	
	☐	☐	☐	☐	☐ Details	
	☐	☐	☐	☐	☐ Details	
	☐	☐	☐	☐	☐ Details	
	☐	☐	☐	☐	☐ Details	
	☐	☐	☐	☐	☐ Details	

This can also be completed manually and kept as a hard copy in the laboratory – copies must be available for review by Biological Safety Officer/ Biological Safety Advisor.

CHEMICAL RISK ASSESSMENT

GB CLP hazard pictograms



Hazard group

A	B	C	D	E
H304, H315, H319, H336 and all H-numbers not otherwise listed	H302, H312, H332, H371	H301, H311, H314, H317, H318, H331, H335, H370, H373	H300, H310, H330, H351, H360, H361, H362, H372	H334, H340, H341, H350
Least Hazardous Substances →			More Hazardous Substances	Special cases
Notes: All phrases/hazard statements now considered a risk on or via the skin.				

Hazard phrases associated with hazard groups A-E (chemicals caused harm by inhalation) and hazard groups SA-SE (chemicals causing harm via skin or eye contact). More information available here:
https://www.she.stfc.ac.uk/Pages/ChemicalRiskAssessment.pdf

Protocol Risk Assessment

Protocol Risk Assessment Form

(Expand or contract fields, or append additional sheets as required; insert NA if not applicable)

Protocol #HSEMK01	**Title: Agarose gel electrophoresis**
Associated Protocols #.....	Location (e.g. Boat, Field (give locations, Laboratory, Office, Facility). Identify here risks and control measures for work in this environment, additional to Local Rules:

	Wallace lab 131A
Description: https://benchling.com/s/prt-hJJiAOFxFOrfma9u76tJ?m=slm-IYUP7ezyqAbbWCyhRwAS	

CHEMICAL RISK – Summary sheet

A copy of each Chemical COSSH form should be readily available in the lab for use (e.g. in an emergency)

Chemical Name (& Conc.)	**GHS symbols**	**Skin/Eyes Group**	**Inhalation Group**	**Quantity**	**In use dustiness or volatility**	**Disposal**	**Primary containment & storage**	**Other comments**
Agarose		N/A	N/A	1 - Small	Low dustiness	Waste material must be disposed of in accordance with the national and local regulations. Leave chemicals in original containers. No mixing with other waste. Handle uncleaned containers like the product itself. Notice Directive on waste	Tightly closed. Dry. Storage class (TRGS 510): 11: Combustible Solids	

						2008/98/EC.		
Tris-acetate-EDTA (TAE) buffer	SC		D	2 - Medium	Choose an item.	Waste material must be disposed of in accordance with the national and local regulations. Leave chemicals in original containers. No mixing with other waste. Handle uncleaned containers like the product itself. Notice Directive on waste 2008/98/EC.	Keep containers tightly closed in a dry, cool and well-ventilated place. Storage class (TRGS 510): 12: Non-flammable liquids in non-flammable packages.	H226, H301, H310, H314, H315, H317, H318, H319, H330, H400, H410, H411
Nucleic acid stain (e.g. SYBR Safe)		N/A	N/A	1 - Small	Choose an item.	SYBR Safe DNA gel stain is not classified as hazardous waste under U.S. Federal regulations (Resource Conservat	Keep in a dry, cool and well-ventilated place. Keep in properly labelled containers. Incompatible with:	

						ion and Recovery Act (RCRA)). SYBR Safe stain meets the requirements of the U.S. Clean Water Act and the U.S. National Pollutant Discharge Elimination System (NPDES).	Strong acids. Strong oxidising agents	
GeneRuler 1 kb Plus DNA Ladder, ready-to-use		N/A	N/A	1 - Small	Choose an item.	The generation of waste should be avoided or minimized wherever possible. Empty containers or liners may retain some product residues. This material and its container must be disposed of in according to	Keep in a dry, cool and well-ventilated place. Keep in properly labelled container. Store at -20°C.	

						approved disposal technique. Disposal of this product, its solutions or of any by-products, shall comply with the requirements of all applicable local, regional or national/federal regulations.		

PROTOCOL RISK MANAGEMENT

Secondary Containment (of protocol): open bench/fume hood/special (highlight/state)

Working Practice – Good Laboratory Practice under local rules PLUS the following: (highlight/state)

Other risks & control measures e.g. pressure, temperature, electrical, mechanical, autoclave, field, boat.

Identify all specific control measures (highlight or delete) - latex/nitrile/heavy gloves; safety glasses, screens; full face mask; dust mask; protective shoes; spillage tray; ear-defenders; other (state) n.b. details for each chemical are in individual COSSH forms

Agarose: Use equipment for eye protection tested and approved under appropriate government standards such as NIOSH (US) or EN 166(EU). Safety glasses. Gloves material: Nitrile rubber Minimum layer thickness: 0.11 mm Break through time: 480 min Material tested:KCL 741 Dermatril® L. Respiratory protection required when dusts are generated, Filter type: Filter type P1.

TAE buffer: Use equipment for eye protection tested and approved under appropriate government standards such as NIOSH (US) or EN 166(EU). Safety glasses. Gloves

material: Nitrile rubber Minimum layer thickness: 0.11 mm Break through time: 480 min Material tested:KCL 741 Dermatril® L. Protective clothing. Respiratory protection required when vapours/aerosols are generated, Filter type: Filter type ABEK.

Nucleic acid stain (SYBR Safe): In case of insufficient ventilation wear respirators and components tested and approved under appropriate government standards. Wear suitable gloves Glove material: Compatible chemical-resistant gloves. Tight sealing safety goggles. Wear suitable protective clothing.

GeneRuler 1 kb Plus DNA Ladder, ready-to-use: In case of insufficient ventilation wear respirators and components tested and approved under appropriate government standards. Wear suitable gloves Glove material: Compatible chemical-resistant gloves. Tight sealing safety goggles. Wear suitable protective clothing.

Justification and controls for any work outside normal hours

Emergency procedures (e.g. first aid, spillage clearance; communication methods) n.b. full emergency plans for each chemical are detailed in individual COSSH forms

Agarose: Avoid inhalation of dusts. Evacuate the danger area, observe emergency procedures, consult an expert. Do not let product enter drains. Cover drains. Collect, bind, and pump off spills. Observe possible material restrictions (see sections 7 and 10). Take up dry. Dispose of properly. Clean up affected area. Avoid generation of dusts.

TAE buffer: Do not breathe vapors, aerosols. Avoid substance contact. Ensure adequate ventilation. Evacuate the danger area, observe emergency procedures, consult an expert. Do not let product enter drains. Cover drains. Collect, bind, and pump off spills. Observe possible material restrictions (see sections 7 and 10). Take up with liquid-absorbent material (e.g. Chemizorb®). Dispose of properly. Clean up affected area.

Nucleic acid stain (SYBR Safe): Eliminate all ignition sources (no smoking, flares, sparks or flames in immediate area) Use personal protection equipment. Soak up with inert absorbent material.

GeneRuler 1 kb Plus DNA Ladder, ready-to-use: Ensure adequate ventilation. Always wear recommended Personal Protective Equipment. Use personal protection equipment. Soak up with inert absorbent material.

Supervision/training for worker (highlight) N.B. All relevant training forms (e.g. for specific laboratories) should be attached
None required Already trained Training required Supervised always

Declaration: I declare that I have assessed the hazards and risks associated with my work and will take appropriate measures to decrease these risks, as far as possible eliminating them, and will monitor the effectiveness of these risk control measures.

Name & signature of worker Philip Ellwood

Name & counter-signature of supervisor: .....Eva Sonnenschein & Claudio Greco

Date: .18/02/2025.....

Date of first reassessment Frequency of reassessments

Protocol Risk Assessment Form

(Expand or contract fields, or append additional sheets as required; insert NA if not applicable)

Protocol #HSEMM01	Title: Media Preparation (Agar-based media)
Associated Protocols #.....	Location: Laboratory. Identify here risks and control measures for work in this environment, additional to Local Rules: Wallace lab 123
Description: Chemicals used In making the agar media for the co-cultivation experiments are irritants that need to be acknowledged in a chemical risk assessment. Preparation of marine agar and broth media for cultivation experiments.	

CHEMICAL RISK – Summary sheet

A copy of each Chemical COSSH form should be readily available in the lab for use (e.g. in an emergency)

Chemical Name (& Conc.)	**GHS symbols**	**Skin/ Eyes Group**	**Inhalation Group**	**Quantity**	**In use dustiness or volatility**	**Disposal**	**Primary containment & storage**	**Other comments**

Marine broth (powder)		N/A	N/A	1 – Small	Low dustiness	Autoclave waste, dispose as general laboratory waste	Store dry, sealed container	Nutrient source, minimal hazard
Marine agar (powder)		N/A	N/A	1 – Small	Low dustiness	Autoclave waste, dispose as general laboratory waste	Store dry, sealed container	Solidifying agent, minimal hazard
Sodium nitrate ($NaNO_3$)	Oxidiser, irritant	SE (irritant)	D	1 – Small	Medium (dust)	Collect as hazardous waste	Store cool, dry, away from organics	Oxidiser, may intensify fire
Sodium phosphate (Na_2HPO_4)	Irritant	SE (irritant)	D	1 – Small	Low dustiness	Aqueous waste can be neutralised and disposed via drain	Store dry, sealed container	Irritant to eyes and skin
Copper chloride ($CuCl_2 \cdot 2H_2O$)	Harmful, environmental hazard	SE (irritant)	D	1 – Small	Medium (dust)	Collect as hazardous chemi	Store in dry, sealed container	Toxic to aquatic life

						cal waste		
Ferrous sulphate ($FeSO_4 \cdot 7 H_2O$)	Irritant, environmental hazard	SE (irritant)	D	1 – Small	Medium (dust)	Collect as hazardous chemical waste	Store dry, sealed container	Harmful if ingested, aquatic hazard

PROTOCOL RISK MANAGEMENT

Secondary Containment (of protocol): open bench/fume hood/special (highlight/state)

Working Practice – Good Laboratory Practice under local rules PLUS the following: (highlight/state)

Other risks & control measures e.g. pressure, temperature, electrical, mechanical, autoclave, field, boat.

Identify all specific control measures (highlight or delete) - latex/nitrile/heavy gloves; safety glasses, screens; full face mask; dust mask; protective shoes; spillage tray; ear-defenders; other (state) n.b. details for each chemical are in individual COSSH forms

Justification and controls for any work outside normal hours

Emergency procedures (e.g. first aid, spillage clearance; communication methods) n.b. full emergency plans for each chemical are detailed in individual COSSH forms

Supervision/training for worker (highlight) N.B. All relevant training forms (e.g. for specific laboratories) should be attached
None required Already trained Training required Supervised always

Declaration: I declare that I have assessed the hazards and risks associated with my work and will take appropriate measures to decrease these risks, as far as possible eliminating them, and will monitor the effectiveness of these risk control measures.

Name & signature of worker Philip Ellwood

Name & counter-signature of supervisor: Eva Sonnenschein & Claudio Greco

Date: ....17/03/2025.....

Date of first reassessment Frequency of reassessments