Coursework Declaration and Feedback Form

The Student should complete and sign this part

Student Number: 2721753Z	Student Name: Weichen Zhao
Programme of Study (e.g. MSc in Electronics and Electrical Engineering): MSc in Computer System Engineering	
Course Code: ENG5059P	Course Name: MSc Project
Name of **First** Supervisor: Umer Zeeshan Ijaz	Name of **Second** Supervisor: Stephen Thoms
Title of Project: Comparing effects of natural and chemical biosurfactants on microbiome to explore crude oil degradation	

Declaration of Originality and Submission Information

I affirm that this submission is all my own work in accordance with the University of Glasgow Regulations and the School of Engineering requirements Signed (Student) : Weichen Zhao	E N G 5 0 5 9 P
Date of Submission : 15/08/2023	

Feedback from Lecturer to Student – to be completed by Lecturer or Demonstrator
Grade Awarded: Feedback (as appropriate to the coursework which was assessed):

Lecturer/Demonstrator:	Date returned to the Teaching Office:

Comparing effects of natural and chemical biosurfactants on microbiome to explore crude oil degradation

Weichen Zhao

2721753Z

Supervised by Dr Umer Zeeshan Ijaz & Dr Stephen Thoms

MSc Computer System Engineering

School of Engineering

Abstract

Crude oil spills at sea can have a significant impact on marine ecosystems, trapping seabirds and small and medium-sized marine creatures, immobilizing them and eventually killing them as they run out of strength. Toxic compounds in crude oil can also enter the entire marine food chain, causing toxins to pass through all marine species within a certain range, and these toxins can eventually be ingested by us, resulting in incalculable damage. If the spilled crude oil is not cleaned up quickly, then surviving marine organisms may also pass on their toxin-induced mutations, causing far-reaching effects on the entire planet's ecosystem.

In the early days, chemical dispersants were used to degrade spilled crude oil, with Span, Tween and Finasol among the main ingredients, but there are also natural microorganisms in the ocean (such as hydrocarbon-degrading bacteria) that produce surfactants to degrade crude oil. Although these chemicals are food-grade, they may still affect marine life and potentially diminish the ability of other microorganisms to degrade crude oil. Therefore, more environmentally friendly biosurfactants, such as rhamnolipid, trehalose, and sophorolipid, are being considered.

In this project, the effects of Finasol and rhamnolipid on marine microbial communities, especially those that release biosurfactants, will be compared. The efficiency and negative effects of the two different degradation chemicals will be investigated in group experiments with different control variables. This study will use the method of 16S rRNA amplicon sequencing, combined with machine learning algorithms for data analysis and diversity study, to discover the changes in microbial communities and dominant species under different conditions.

Key words：Rhamnolipid, Finasol, Phi Network, Zeta Diversity, Biome

Acknowledgements

Many thanks to my mentor Umer Zeeshan Ijaz and his PhD students, Uzma, Kelly J Stewart and Aqsa Ameer among others. Our tutor, Umer, gave us weekly tutorials on Thursdays, checking on our progress, answering our questions and encouraging us to march forward. Even if he himself is unable to give us a tutorial due to his busy schedule, he will record a video of what he wants to talk about and send it to us for us to study and refer to at any time. Several of his students took time out of their busy schedules to help us with our projects, giving us advice and correcting our problems. Teacher and seniors were very kind and enthusiastic and provided us with tons of help. I will never forget the experience of doing the project with you all because I learned so much valuable knowledge, not only in terms of academics, but also in terms of being a man. So I would like to thank from the bottom of my heart to all the teachers and seniors who helped me, because of you, I can take another big step forward in my life.

1. Introduction

1.1 Background

Crude oil, a double-edged sword driving rapid industrial progress, has ushered in prosperity while simultaneously igniting significant environmental concerns. Its pivotal role in shaping industries is undeniable, yet the profound ecological ramifications, particularly in the aftermath of offshore oil spills, underscore the urgency for innovative solutions. The cataclysmic Gulf of Mexico oil spill in 2010, a watershed moment, disgorged approximately 700,000 tonnes of Louisiana light crude oil into the marine ecosystem, laying bare the devastating and enduring repercussions (Fredy et al., 2018). The repercussions of oil pollution ripple across avian, aquatic, and terrestrial habitats, leaving a trail of disruption, morbidity, and mortality (Sargent et al., 2011). A historic perspective reveals the devastating impact of oil pollution, with historical records documenting the demise of seabird populations attributed to oil pollution in the North Atlantic and North Sea regions between 1952 and 1962 (Sargent et al., 2011). Notably, the noxious elements in crude oil, particularly aromatic hydrocarbons, inflict dire consequences on aquatic life, leading to elevated mortality rates of marine organisms, including shrimp larvae in the aftermath of the Venezuelan oil spill (Crude oil spills, 2016).

Aiding in oil spill mitigation, microbial approaches have gained traction as effective interventions. Microorganisms specialized in hydrocarbon degradation facilitate the conversion of pollutants into benign byproducts like water and carbon dioxide. Chemical dispersants, recognized as the primary agents for expediting petroleum degradation, have undergone an evolution since the 1970s, with hundreds of formulations deployed worldwide (Kleindienst et al., 2015). The predominance of aromatic hydrocarbons in early oil spills, notorious for their recalcitrance, led to their replacement with natural surfactants, such as glycolipids derived from vegetable oil, yielding improved ecological compatibility (Crude oil spills, 2016).

This study explores the intricate interactions between the chemical dispersant Finasol, the biosurfactant rhamnolipid, and microbial communities. Using 16S rRNA sequencing, the dynamics of microbial communities under the influence of these substances and the dominant strains in different environments were elucidated, thus revealing the underlying mechanisms driving their dynamics. Of particular interest is the investigation of taxonomic variation within the microbial community of the ecologically important Faroe-Shetland Channel, an area that is emblematic of a diverse marine ecosystem with a history of oil exploration (Crude Oil Spill, 2016).

Furthermore, the study capitalizes on recent advances in ecological modeling, notably the application of Phi Network and Zeta Diversity, to dissect microbial community assembly processes. These models elucidate the delicate balance between stochastic and deterministic mechanisms, providing insights into how chance colonization, ecological drift, and species-specific traits shape community composition. Of significant import is the evaluation of how chemical dispersants and biosurfactants impact microbial diversity, community assembly, and the intricate relationships between taxa and their functional roles, bridging the gap between taxonomic composition and ecological function (Crude oil spills, 2016).

The emergence of biosurfactants as a natural-based solution to mitigate the ecological toll of

oil spill responses reinforces the necessity for empirical validation. While earlier studies hint at distinct microbial responses to chemical dispersants and biosurfactants, this study presents a pioneering attempt to synergize ecological modeling and microbial community analysis (Nikolova et al., 2021; Thomas et al., 2021). By unraveling the intricate nexus between oil spill response agents and microbial dynamics, this study contributes a crucial dimension to environmental management and preservation.

1.2 Case study

The Faroe-Shetland Channel (FSC), a marine nature reserve spanning the North Atlantic between the Shetland Islands and the Faroe Islands, stands as a biodiverse hotspot and a critical fishing zone. With an expansive area of 5278 km2, the FSC boasts a unique ecological mix, where relatively warm North Atlantic seawater intermingles with subzero deep water from the Norwegian Sea, nurturing a complex and thriving ecosystem (Logan et al., 2018; Corvec, 2018). Given the FSC's rich biodiversity, history of oil exploitation, and sensitive ecological equilibrium, the potential occurrence of oil spill accidents in this region poses a substantial threat. The intricate interplay between warm and cold waters, combined with the region's harsh and cold weather conditions, further compounds the challenges of managing and mitigating oil spills. Notably, an oil spill in the FSC has the potential to disrupt both the ecological community and the flourishing fishing industry, necessitating effective and sustainable oil spill response strategies.

To address this pressing concern, our case study focuses on investigating the impact of two distinct oil spill response tools, namely the synthetic dispersant Finasol and the microbial biosurfactant rhamnolipid, on the microbial community dynamics within the FSC. Building upon the pioneering work in the field, particularly in the context of the FSC (Crude oil spills, 2016), we aim to unveil the potential shifts in microbial community composition and assembly processes caused by these response tools.

Utilizing the power of Phi network and Zeta Diversity, we seek to simulate the intricate interplay between the FSC's ecological community and the application of Finasol and rhamnolipid. By employing cutting-edge ecological modeling techniques, we aim to elucidate the key taxa that drive changes in microbial community structure in response to these oil spill response tools. Additionally, we intend to discern the underlying ecological mechanisms, be they stochastic or deterministic, that govern the observed shifts in microbial composition.

Our study site, the FSC, presents a unique opportunity to explore these dynamics. With its history of oil exploration, dynamic physical circulation, and intricate mix of water masses, the FSC offers a microcosm through which we can analyze the intricate ecological repercussions of oil spill response tools (Faroe-Shetland Channel study, 2022). By discerning the taxonomic shifts induced by Finasol and rhamnolipid, we contribute to a deeper understanding of their respective ecological impacts and their potential role in shaping the microbial response during oil biodegradation.

Ultimately, this case study endeavors to contribute valuable insights into the selection and application of oil spill response tools in sensitive marine ecosystems. By uncovering the ecological consequences of these tools and their effects on microbial community dynamics, we aim to provide a foundation for informed decision-making in future oil spill response strategies,

ensuring the preservation of delicate marine ecosystems and the industries that rely upon them.

1.3 Aims and Objectives

This study aims to conduct a comprehensive analysis of the effects of chemical dispersants (Finasol) and biosurfactants (rhamnolipid) on the microbial community within the Faroe Shetland Channel. The primary objectives include:

1. Comparing the response of microorganisms to Finasol and rhamnolipid treatments, and assessing their impact on community diversity.
2. Investigating the dynamic shifts in microbial community composition under different treatment conditions.
3. Identifying and characterizing the dominant bacterial taxa prevalent in samples subjected to dispersant treatments.
4. Unveiling the intricate interplay between microorganisms and their environment within distinct treatment contexts.

2. Methodology

2.1 Sample Acquisition and Preparation

The sample collection approach involves the preparation of water accommodated fractions (WAFs) to study the effects of different dispersants on microbial communities in the Faroe Shetland Channel (FSC). Surface seawater from FSC is collected and used as the basis for creating distinct treatment groups. The preparation process includes the addition of different components such as crude oil, chemical dispersants, and biosurfactants to assess the influence on microbial communities over a specified time frame.

The sample collection procedure encompasses the creation of WAFs through a systematic process that examines the response of microbial communities to varying dispersant treatments. Initially, FSC surface seawater is gathered to serve as the foundational medium. This seawater is utilized to establish different experimental conditions. The process begins with the formulation of three key WAFs: WAF, CEWAF, and BEWAF. These fractions are developed by combining predetermined quantities of seawater, crude oil, and either biological or chemical surfactants. Importantly, the ratios of components are controlled to ensure consistency, with seawater and crude oil maintaining a uniform volume (seawater: 1500ml, crude oil: 120ml).

The composition of the WAFs is strategically adjusted to create distinct treatment groups. WAF consists of seawater and crude oil, while CEWAF incorporates the chemical dispersant Finasol, seawater, and crude oil. The ratio of chemical surfactant and crude oil is carefully maintained at 1:20. In contrast, BEWAF is designed with a biological surfactant – rhamnolipid – in addition to seawater and crude oil. To assess the impact of dispersants alone, control groups are established: SWD containing seawater and Finasol, and SWBS containing seawater and biosurfactant rhamnolipid.

The prepared treatment groups, including the six treatment samples (WAF, CEWAF, BEWAF, SWD, SWBS, SW), are mixed for a minimum of 48 hours. This duration ensures the complete dispersion of oil and the establishment of a representative microenvironment. A crucial control sample containing only seawater serves as a baseline comparison. The samples are collected on specified days (3, 7, 14, and 28) to evaluate the changes in hydrocarbon composition due to biodegradation. This systematic approach provides valuable insights into the dynamics of microbial communities under the influence of different dispersants in the FSC.

2.2 Amplicon Sequence Variants

Amplicon Sequence Variants (ASVs) play a pivotal role in marker gene sequencing, such as the 16S rRNA gene in bacteria, offering insights into the microbial genome and corresponding species information (Schlomann et al., 2019). However, the challenges arise when dealing with the massive volume of sequencing data, often yielding tens of thousands of sequences per sample. This creates a formidable workload and computational burden. Moreover, inherent in the process of amplification and sequencing of marker genes, there exists a slight probability of sequencing errors, which can undermine the accuracy of subsequent analyses.

To address these issues effectively, Operational Taxonomic Units (OTUs) have been introduced in diversity analysis. Initially, during OTU clustering, the UPARSE algorithm is employed to

extract unique sequences, thereby diminishing redundant computational demands (Booth et al., 2020). Sequences are categorized based on distinct levels of similarity within the OTU framework, subsequently forming clusters based on sequence similarity.

In recent years, the trend has shifted towards adopting Amplicon Sequence Variants (ASVs) to mitigate the impact of sequencing errors and supplant OTUs. The default OTU clustering step, which utilizes a 97% sequence similarity threshold in the UPARSE algorithm, tends to obscure sequences containing errors, consequently leading to inaccurate abundance estimates for certain OTUs (Alisa et al., 2022). Additionally, the true variation within sequences can be masked by a relatively broad similarity threshold. To enhance accuracy, the DADA2 algorithm has emerged, amalgamating sequencing precision and employing a divisive partitioning algorithm for final clustering, accompanied by p-value calculations. This refined clustering outcome, termed ASV, mirrors the concept of OTU clustering with 100% similarity, thereby elevating the precision of clustering (Guckenheimer et al., 2013).

In the context of our project, millions of sequences procured through 16S rRNA sequencing have undergone refinement and filtration through the DADA2 algorithm. The resultant ASV samples have been subjected to diverse analyses, including diversity assessment, differential analysis, and regression analysis, facilitated by the R-Studio statistical software (Guckenheimer et al., 2013).

In essence, Amplicon Sequence Variants (ASVs) have become an indispensable tool in the precise analysis of microbial communities, addressing challenges arising from sequencing errors and paving the way for comprehensive exploration of microbial diversity and functionality (Schlomann et al., 2019; Guckenheimer et al., 2013).

2.3 High-Throughput Sequencing

The DNA extracts collected from the samples underwent sequencing using second-generation sequencing technology from Illumina. The two-step amplification procedure was employed to amplify the 16S rRNA sequence. High-throughput sequencing was carried out on the microbial population, enabling comprehensive analysis of gene composition and diversity within the environmental microbiome.

2.4 DADA2 algorithm with the bioinformatics pipeline QIIME2

In the pursuit of intricate biological insights into the microbial community species, the 16S rRNA gene sequences obtained through sequencing were subjected to thorough analysis within the QIIME2 bioinformatics pipeline. A pivotal role was played by the DADA2 algorithm, harnessed as a powerful plugin module, meticulously addressing the intricacies of sequence duplication and errors (Schlomann et al., 2019). The Divisive Amplicon Denoising Algorithm 2, an R package renowned for its adeptness in modeling and rectifying amplicon sequencing errors across a multitude of sequencing platforms, was meticulously incorporated (Phadnis et al., 2018). This algorithm, integral to amplicon analysis, possesses the remarkable capability to accurately discern sample sequences, delving into the minutiae of single nucleotide disparities. The core functionality of DADA2 revolves around the construction of an error rate model that speculates the origin of an amplicon sequence from its template. It seamlessly factors in the

inherent error model of the data at hand, thereby yielding the probabilities of various transpositions. Embarking on the sequence preprocessing journey, each sequence is adeptly truncated to meet specified length criteria, as determined by approximate sequence length and minimum quality score benchmarks. Subsequently, sequences falling below the prescribed length threshold are prudently excised from the dataset. Duplication of sequences across all samples is diligently eliminated, affording the preservation of a unique sequence set. Herein, the algorithm delves into calculating the average quality score of each nucleotide base and the prevalence of each distinct sequence.

Elevating its prowess, DADA2 deftly navigates the intricate landscape of sequencing errors, whereby an erroneous OTU sequence may inadvertently encompass a myriad of sequences, encompassing both the accurate sequence with considerable abundance and erroneous sequences with scant representation (Dizay et al., 2017). Employing a multifaceted approach, DADA2 capitalizes on the interplay between sequence abundance, quality scores, and sequence relationships, effectively rectifying erroneous base calls and unraveling the veritable sequence identity. Notably, the algorithm further administers the crucial task of identifying and removing chimeric sequences, expertly discerning sequences with modest abundance that mirror multiple distinct sequences (Aubin et al., 2020). The culmination of these intricate steps culminates in the construction of an Amplicon Sequence Variant (ASV) table, facilitated by splicing sequences with a 20-base pair overlap.

2.5 SparCC and Spec-easi

Microbiomes embody intricate microbial communities where both their structure and function are profoundly governed by an intricate interplay of microbe–microbe and microbe–host interactions. These interactions span a gamut of mechanisms, encompassing direct cell-to-cell communication and interspecies signaling to more nuanced metabolite sensing. These intricate interactions play a pivotal role in shaping disease progression and clinical outcomes (Magalhaes et al., 2016). A striking illustration of intricate microbial dynamics exacerbating diseases is the phenomenon of polymicrobial synergism, wherein infections involving multiple interdependent bacterial species result in greater severity than single-agent infections. Polymicrobial synergism is associated with heightened antibiotic resistance, biofilm formation, tissue damage, and adaptation to the environment (Dalton et al., 2011; Murray et al., 2014). Hence, comprehending the microbiome in its entirety, including the intricate choreography between microbial taxa and their interactions with host organisms, is paramount in understanding the diverse roles microbiomes play in host health, development, dysbiosis, and the intricate realm of polymicrobial infections.

Despite the profound expansion in microbiome studies catalyzed by next-generation sequencing technologies, the methodological panorama for unraveling microbe–microbe and host–microbe interactions remains surprisingly limited (Legendre et al., 2012). Enter network theory, especially manifested through system-oriented, graph-theoretical approaches. This framework offers a promising avenue for holistic microbiome analysis, facilitating a deeper understanding of the intricate ecological and evolutionary dynamics at play. Network theory empowers the modeling and analysis of a microbiome and its intricate interactions within an integrated network. Importantly, the architectural features of networks seem to exhibit

universality across a spectrum of complex systems, transcending domains from microbiomes and molecular interaction networks to computer networks, microcircuits, and social networks (Barabasi et al., 2004). This universality provides a conduit for harnessing insights garnered from well-studied non-biological systems to untangle the interwoven relationships shaping microbial interactions.

An array of techniques, varying in efficacy and accuracy, have been harnessed to construct networks grounded in microbiome data. The simplest techniques draw upon (dis)similarity- or distance-based methodologies. However, the preponderant methods are correlation-based techniques, wherein meaningful pairwise associations among operational taxonomic units (OTUs) are unveiled using correlation coefficients such as Pearson's or Spearman's coefficients. Nonetheless, reliance on correlation coefficients for uncovering dependencies among members of a microbiome is fraught with inherent limitations. These encompass the potential to detect spurious correlations due to compositional bias and the challenge of statistical underpowering due to modest sample sizes.

Amid burgeoning concerns surrounding correlation-based analyses, a cohort of methodologies resilient to compositional bias has emerged. A prime illustration is SparCC (Sparse Correlations for Compositional data). This technique leverages linear Pearson's correlations between log-transformed components to discern associations within compositional data (Friedman et al., 2012). Another intriguing approach is SPIEC-EASI (SParse InversE Covariance Estimation for Ecological Association Inference), a statistical methodology marrying data transformations tailored for compositional data analysis with a graphical model inference framework, predicated on the notion of a sparse underlying ecological association network (Kurtz et al., 2015).

SparCC networks crystallize by introducing the OTU table—comprising absolute abundance values—to the sparcc function nestled within the SpiecEasi package. Subsequently, the correlation matrix metamorphoses into an adjacency matrix through an aptly chosen threshold. Likewise, SPIEC-EASI networks spring to life through the utilization of the spiec.easi function inherent in the SpiecEasi package. The resultant entity encapsulates a matrix christened "refit," akin to a sparse adjacency matrix that seamlessly facilitates the construction of the microbiome network.

2.6 Statist

For the analytical phase, statistical computations and data processing were executed using the R-Studio software version 4.3.0. To explore the genetic landscape within the collected samples, the DADA2 algorithm was harnessed, allowing for a meticulous dissection of gene sequences. This endeavor encompassed a comprehensive assessment, addressing factors of diversity, environmental context, and temporal dynamics. To facilitate these intricate analyses, the microbiome package was employed, acting as the conduit for multifaceted tasks, including diversity quantification, subset examination, and core microbiome analysis.

2.6.1 Phi Network and IVI

Unlocking the intricate tapestry of network connections holds significant potential for effectively managing intricate systems. An overarching challenge in this endeavor is the

identification of pivotal nodes capable of exerting the most substantial influence on the entire network. This pursuit is instrumental in enhancing network efficiency and mitigating costs. This article introduces a groundbreaking algorithm termed the Integrated Value of Influence (IVI), which amalgamates key topological attributes of the network to pinpoint its central nodes. The IVI algorithm is a versatile tool applicable across diverse domains, including sociology, economics, transportation, biology, and medicine. For instance, within biomedical research, accurately discerning influential nodes within a disease-associated network could catalyze the revelation of novel biomarkers and therapeutic targets, thereby significantly impacting society. The realm of computational complex systems theory aims to furnish a panoramic, macroscopic view of network interactions, unveiling critical properties often elusive to reductionist methodologies. Network science has permeated numerous scientific spheres, encompassing social networks, traffic systems, telecommunications, cartography, chemistry, biochemistry, and biology at large (Frainay et al., 2017; Hochberg et al., 2018) . As we navigate the era of high-throughput biological assays, systems biology techniques have gained momentum in analyzing diverse biological networks, including gene regulatory networks, protein-protein interactions (PPIs), and neural transmissions (Tieri et al., 2019). These approaches hinge on network topology analysis and computation of centrality metrics, thereby elucidating deeper biological significance and spotlighting pivotal regulatory molecules. While hub nodes boast numerous connections, the role of spreader nodes in fostering information dissemination throughout the network is crucial (Kitsak., 2010). Notably, both these aspects, hubness and spreading potential, are often employed independently to identify influential nodes. Remarkably, nodes possessing simultaneously high connection frequency and expansive spreading potential emerge as the most pivotal constituents within a network.

The concurrent integration of an array of centrality metrics has been employed as a strategy to pinpoint the most influential nodes within a network. For instance, researchers (del Rio et al., 2009) demonstrated that while individual centrality metrics might not robustly predict network vital nodes, combining two metrics that encompass local and global network features yield more accurate prognostications. In the realm of systems biology, nodes exhibiting high degree and betweenness centrality are often recognized as influential nodes, encapsulating both local significance and global network flow. However, newer algorithms for identifying influential nodes, such as collective influence, local H index, and ClusterRank, have surfaced but are yet to be widely embraced, especially within biological contexts. Furthermore, no existing algorithm harmoniously integrates these centrality metrics to synergistically harness their efficacy. Moreover, in several networks, certain nodes occupy central positions and exhibit high degree centrality, yet possess low betweenness centrality due to limited connections beyond the main module (Oldham et la., 2019). Consequently, these nodes might boast high local centrality but subdued global centrality or vice versa, contingent on their network position (Guimera et al., 2005). The measurement of betweenness centrality is consequently influenced by a node's network position and warrants cautious application in identifying network spreaders or developing innovative influential node identification algorithms. Intriguingly, addressing the positional bias of betweenness centrality has remained largely uncharted territory, and no computational solution has been devised to rectify this bias.

To surmount these challenges, researchers devised the Integrated Value of Influence (IVI) formula, a pioneering approach that harmonizes key network centrality metrics to concurrently

neutralize their biases and discern pivotal regulatory molecules within the network. The IVI algorithm represents a revolutionary advancement, seamlessly amalgamating six pivotal network centrality metrics. To counterbalance the positional bias inherent in betweenness centrality, we harnessed an alternative metric called neighborhood connectivity. Rigorous evaluation of the interplay between each pair of selected centrality metrics facilitated the formulation of appropriate integration functions. After meticulous scrutiny of the centrality metrics presented in the literature, six pivotal metrics—degree centrality, ClusterRank, neighborhood connectivity, local H index, betweenness centrality, and collective influence—were identified as paramount for discerning a network's influential nodes. Each of these centrality metrics captures distinct topological dimensions of the graph, encompassing local, semi-local, and global topology. An additional advantage of these metrics is their independence from the requirement for a fully connected graph or module for calculation.

The Integrated Value of Influence (IVI) algorithm epitomizes a remarkable breakthrough in understanding and harnessing the dynamics of complex networks. By synergizing various centrality metrics and addressing the limitations inherent in traditional approaches, the IVI algorithm enhances our capacity to identify pivotal nodes in intricate networks across diverse domains. This innovation promises transformative implications for disciplines ranging from sociology to medicine, empowering us to navigate the intricate web of interconnected nodes with unprecedented precision and efficacy.

Quantifying the influence of individual nodes in complex networks is a fundamental pursuit with applications spanning diverse domains, from social networks to biological systems. In this study, we introduce the Integrated Vertex Influence (IVI) metric, which seamlessly integrates the Spreading Score and Hubness Score to provide a comprehensive assessment of node importance, considering both information dissemination and local prominence.

The Spreading Score, a measure of a node's potential to propagate information within a network, is tailored to address the challenge of varying scales across centrality metrics. By applying the Min-Max feature scaling method, we normalize centrality measures, preserving their relative weight ratios (Han et al., 2011). Building on the basis of four crucial measurements - normalized neighborhood connectivity (NC), ClusterRank (CR), betweenness centrality (BC), and collective influence (CI) - we introduce the Spreading Score as the amalgamation of these metrics. This composite score aptly captures the nodes' capacity for information diffusion.

$$\text{Spreading}_{\text{score}_i} = \left(\text{NC}_i' + \text{CR}_i'\right)\left(\text{BC}_i' + \text{CI}_i'\right),$$

Formula 1: Spreading Score formula

Likewise, the Hubness Score illuminates nodes' local prominence within their immediate surroundings. By combining local H index (LH index) and degree centrality (DC), this score encapsulates nodes' dominance in local territories. The Hubness Score (HS) for node i is computed as:

$$\text{Hubness}_{\text{score}_i} = DC'_i + LH'_{\text{index}_i},$$

Formula 2: Hubness Score formula

The IVI metric emerges as a pivotal contribution by synergizing Spreading and Hubness Scores to offer a comprehensive estimation of node influence. Leveraging the Multiplication function, we integrate these scores, considering their multiplicatively enhanced impact. IVI encapsulates the most vital aspects of local, semi-local, and global centrality measures - degree centrality, ClusterRank, neighborhood connectivity, local H index, betweenness centrality, and collective influence. Crucially, IVI simultaneously addresses positional biases inherent in network analysis.

$$IVI_i = \left(\text{Hubness}_{\text{score}_i}\right)\left(\text{Spreading}_{\text{score}_i}\right),$$

Formula 3: IVI Score formula

The IVI metric stands as a testament to the evolving landscape of network analysis, fostering a holistic understanding of node influence. By integrating Spreading and Hubness Scores, IVI provides a nuanced appraisal of nodes' roles in information dissemination and local prominence. This novel approach advances our capacity to comprehend the intricate dynamics of complex networks across various disciplines, significantly enhancing our ability to discern key players and their impact.

2.6.2 Zeta Diversity

In the realm of biodiversity research, unraveling the intricate tapestry of patterns and drivers is a fundamental pursuit. Researchers have embarked on this journey, proposing a groundbreaking concept known as zeta (z) diversity—a metric designed not only to harmonize incidence-based diversity measures but also to unveil the underlying intricacies within patterns and relationships. Unlike conventional methods focused on species compositional turnover, zeta diversity introduces a comprehensive framework by quantifying the entire spectrum of diversity components across multiple assemblages, thereby providing a panoramic view of the spatial arrangements governing multispecies distributions.

Researchers unveiled the versatility and ecological significance of zeta diversity through its application across various contexts. Scaling with sample size, spatial granularity, and geographical extent, zeta diversity emerged as a versatile tool that reconciled diverse biodiversity patterns. It masterfully elucidated species accumulation curves, the species-area relationship, multispecies occupancy patterns, and the influence of species endemism—offering profound insights that reverberate across the ecological landscape.

Distinct forms of zeta diversity, such as the exponential and power-law relationships, unearthed invaluable insights into the assembly processes. By distinguishing between stochastic and niche assembly processes, zeta diversity facilitated a deeper understanding of the intricate

mechanisms steering biodiversity patterns. This understanding transcended the realms of species composition, turnover dynamics, co-occurrence patterns, community assembly processes, and the subsequent repercussions of environmental change on biodiversity.

The significance of zeta diversity was further underscored by its inherent versatility in addressing multiple facets of species incidence and compositional turnover. Serving as a bridge between these descriptors, zeta diversity illuminated the dynamics of species diversity across diverse scenarios, encompassing a multitude of biodiversity patterns. By orchestrating a synthesis of these facets, zeta diversity emerged as a unifying force that provided a holistic comprehension of ecological relationships, coexistence dynamics, and the broader ecological tapestry.

In summation, the pioneering concept of zeta diversity, championed by researchers, marked a significant stride in the area of biodiversity pattern elucidation. Its holistic framework transcended traditional measures, offering a nuanced understanding of the intricate drivers shaping biodiversity and their intricate interplay with changing environmental dynamics.

Zeta diversity, a concept pivotal to the study of biodiversity, encompasses several interconnected aspects that shed light on species distribution patterns and their underlying dynamics. Researchers have identified crucial concepts within the field of zeta diversity, each serving a distinct analytical or ecological purpose.

Zeta Diversity Decline: Researchers employed the Min-Max feature scaling technique to normalize various centrality measures, while simultaneously preserving their relative weight ratios. The essence of this concept encompasses the alteration in the number of shared species as the count of included sites in the comparison, or the zeta order, increases. Ecologically, it encompasses the contribution of species with varying ranges, spanning from narrow to wide, to compositional changes. This contribution operates implicitly over spatial, temporal, or even categorical aspects such as samples, sites, or hosts. It's imperative to recognize that Zeta diversity is rooted in incidence-based calculations, capturing the rarity and commonness of species, as reflected by their range, occurrence, or area of occupancy.

Zeta Ratio: In the analytical sphere, the Zeta ratio emerges as a vital metric for understanding the retention of species within additional cases. Specifically, the Zeta ratio outlines the probability of preserving, or rediscovering, a species belonging to the same order of commonness as the zeta order. As for its application, this metric proves valuable in constructing the species retention rate, offering insights into the stability of species presence across different cases.

Retention Rate (Based on Zeta Ratio): The Retention rate, a product of the Zeta ratio, captures the degree to which common species exhibit a higher likelihood of persisting across sites compared to their rare counterparts, as the zeta order increases. This ecological concept embodies the pace at which species endure in a community over various sites or landscapes, reflecting the tenacity of widespread or commonly occurring species. The Retention rate holds multifaceted applications: it is instrumental in visually depicting turnover at high orders where absolute changes might appear minimal. Additionally, it serves to evaluate the spatial extent of a community or metacommunity concerning the extent of sampling. Furthermore, the Retention rate serves as a tool to discern differences in species retention rates across taxonomic groups, habitats, conditions of interest, or in contrast to the null expectation. It is also adept at unveiling ecotones or abrupt shifts in composition.

Zeta Diversity Decay (Linked to Distance Decay): The concept of Zeta diversity decay encapsulates the fluctuation in shared species concerning the increasing distance between sites or temporal gaps between surveys, across different zeta orders. This phenomenon intertwines spatial and temporal aspects. From a spatial perspective, it unveils the transformation in compositional similarity between communities with expanding distances. Temporally, it unveils the variation in compositional similarity over different periods. This concept's implications encompass selecting appropriate spatial and temporal dimensions when designing survey and monitoring schemes. It also quantifies the distances over which community compositions alter, enabling comparisons between the turnover rates of rare and common components across various conditions and circumstances.

The elucidation of these concepts within Zeta diversity provides an enriched framework for unraveling species distribution patterns and their dynamic variations across space and time. These interconnected concepts, addressing aspects like commonness, persistence, turnover, and distance, offer a nuanced comprehension of ecosystem dynamics and the factors influencing biodiversity patterns.

3. Results

3.1 Network

In the domain of Network studies, the Hubness score signifies the potency of individual vertices within their respective contexts, while the Spreading score offers insights into their capacity for information propagation. When combined, these scores yield the IVI score, providing a comprehensive indicator of a vertex's impact across the entire Network. If the IVI score is higher, it signifies a greater impact of the respective vertex on the entire Network, and it also means that the microbial species corresponding to the vertex is the dominant species in this environment. Thus, the objective of this experiment is to generate the IVI Network graph for each sample along with its corresponding IVI score table. In the IVI Network graph, the darker the color of a node means that it has a higher IVI score, and the lighter the color, the lower the IVI score. Subsequently, the objective is to extract the top microbial species with elevated IVI scores for further analysis.

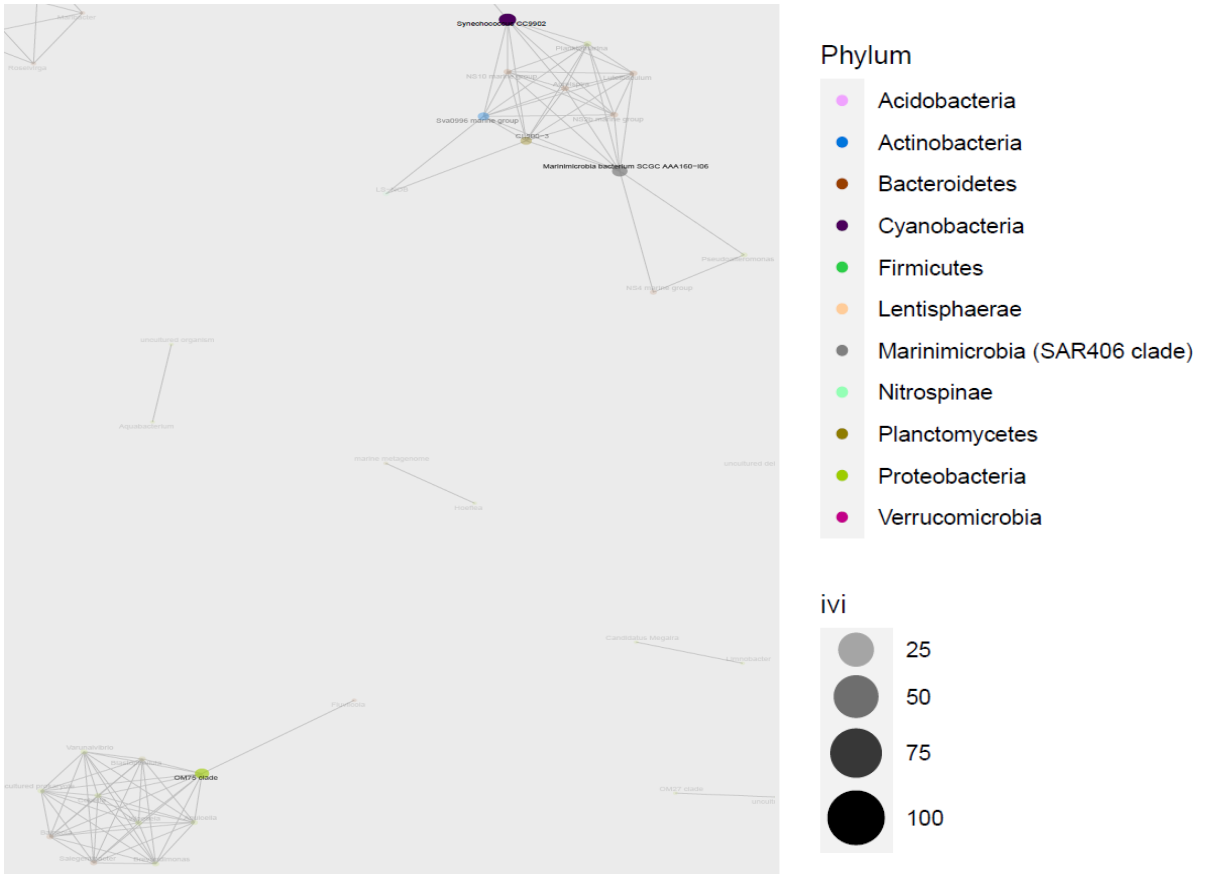
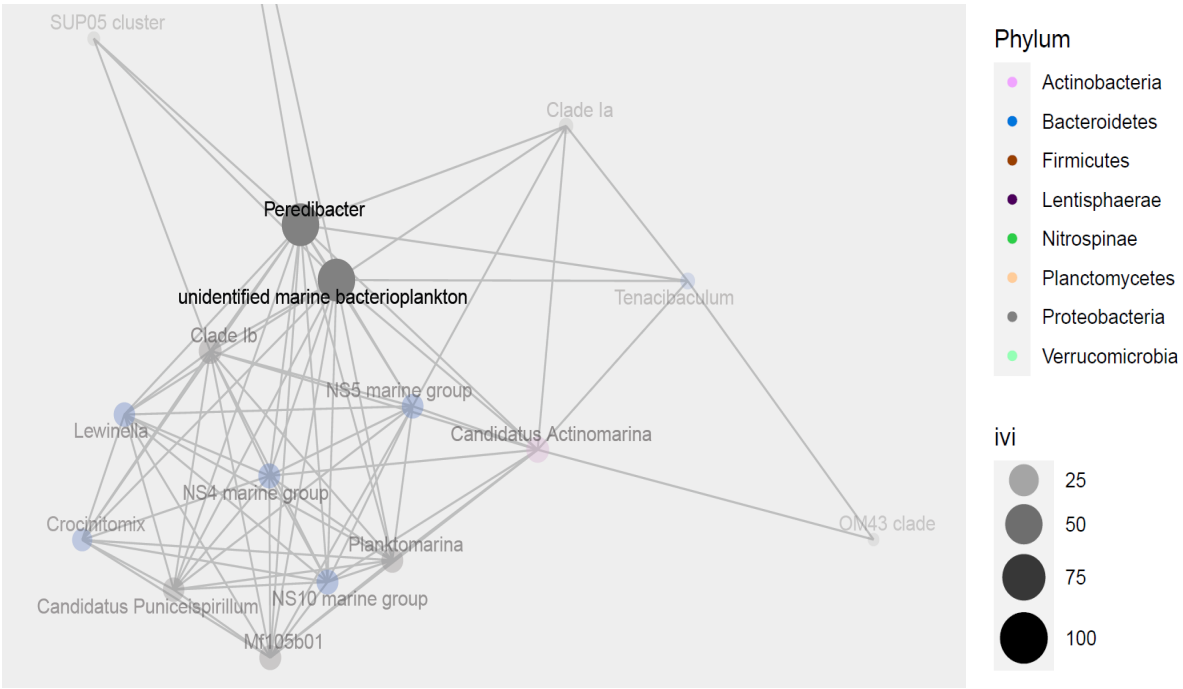


Figure 1

spreading_score	hubness_score	ivi	Phylum	Genus
100	73.98314607	100	Cyanobacteria	Synechococcus CC9902
64.28739271	81.15168539	70.4307071	Marinimicrobia (SAR406 clade)	Marinimicrobia bacterium SCGC AAA160-I06
42.54558493	100	57.1776015	Proteobacteria	OM75 clade

Table 1

Figure 1 and Table 1 show the IVI Network and IVI score for the WAF group. The three microorganisms shown in the table, Synechococcus CC9902, Marinimicrobia bacterium SCGC AAA160-I06 and OM75 clade are exactly the three dominant microorganisms in the



WAF environment.

Figure 2

spreading_score	hubness_score	ivi	Phylum	Genus
98.57772901	65.23137255	100	Proteobacteria	unidentified marine bacterioplankton
98.57772901	65.23137255	100	Proteobacteria	Peredibacter

Table 2

Figure 2 and Table 2 show the IVI Network and IVI score for the CEWAF group. The two microorganisms shown in the table, unidentified bacterioplankton and Peredibacter are exactly the two dominant microorganisms in the CEWAF environment.

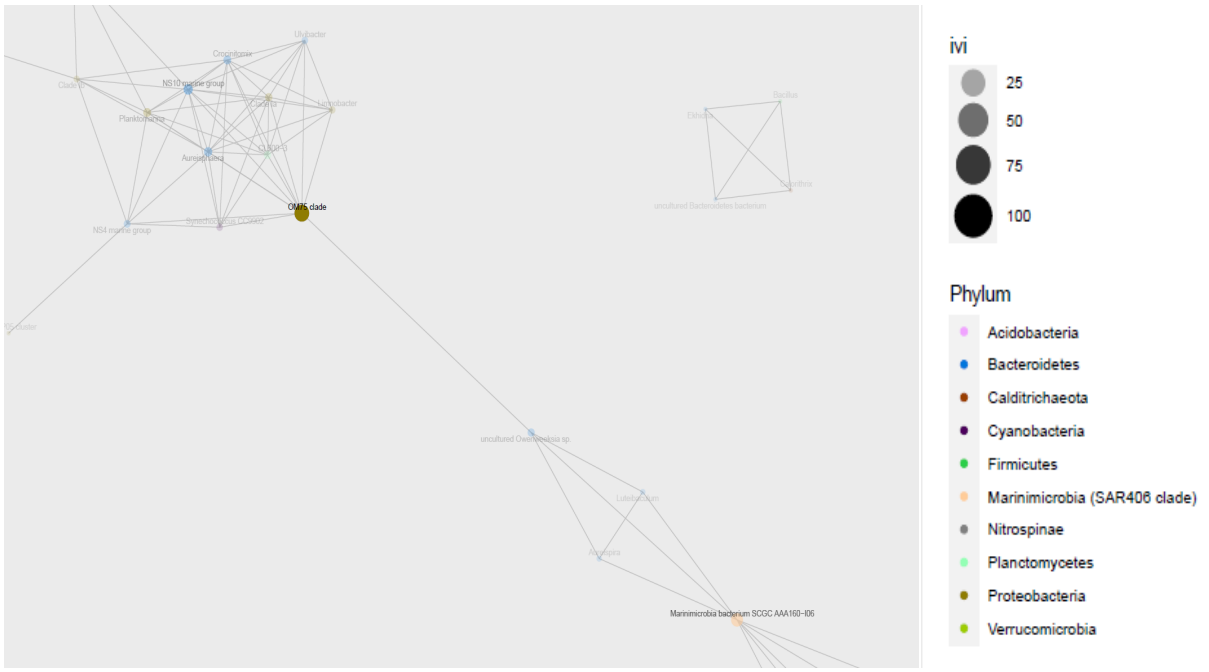


Figure 3

spreading_score	hubness_score	ivi	Phylum	Genus
100	90.62158055	100	Proteobacteria	OM75 clade
73.64882216	65.44528875	53.479099	Marinimicrobia (SAR406 clade)	Marinimicrobia bacterium SCGC AAA160-I06

Table 3

Figure 3 and Table 3 show the IVI Network and IVI score for the BEWAF group. The two microorganisms shown in the table, OM75 clade and Marinimicrobia bacterium SCGC AAA160-I06 are exactly the two dominant microorganisms in the BEWAF environment.

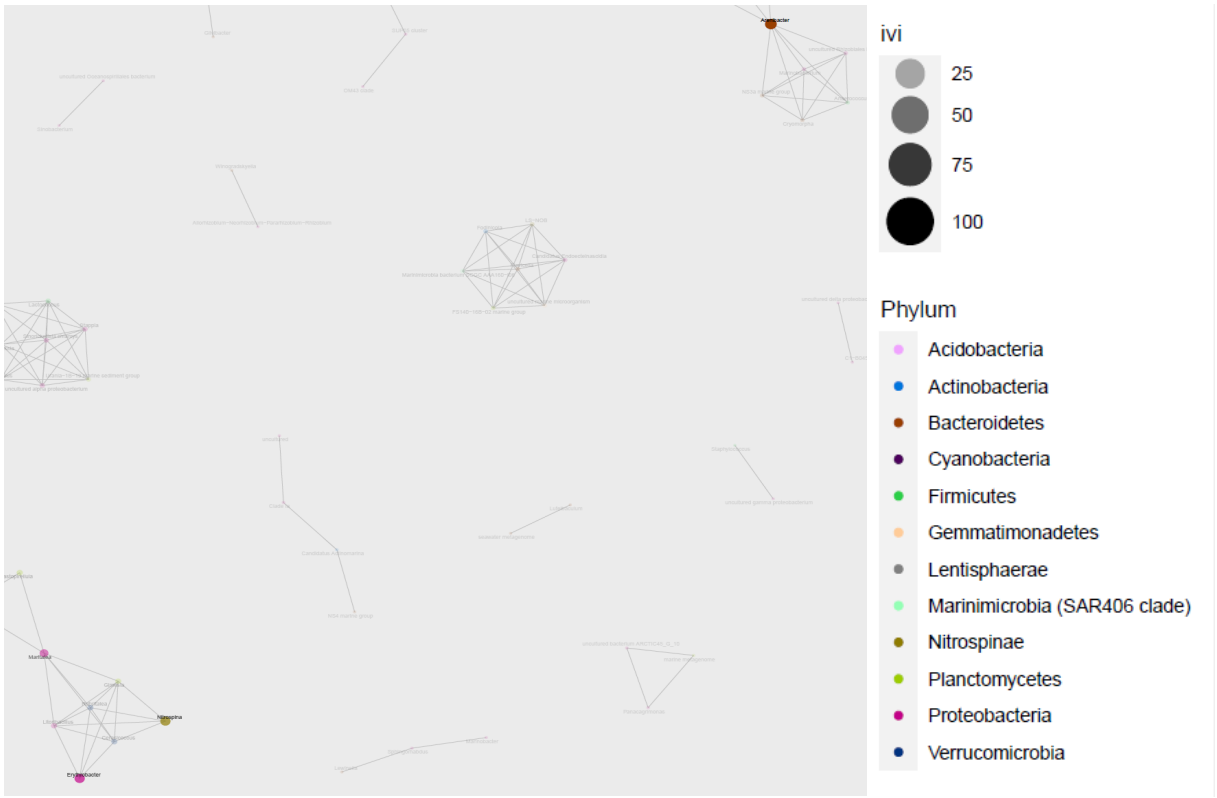


Figure 4

spreading_score	hubness_score	ivi	Phylum	Genus
69.71622109	65.125	100	Bacteroidetes	Arenibacter
100	29.6875	66.006211	Proteobacteria	Erythrobacter
100	29.6875	66.006211	Nitrospinae	Nitrospina

Table 4

Figure 4 and Table 4 show the IVI Network and IVI score for the SW group. The three microorganisms shown in the table, Arenibacter, Erythrobacter and Nitrospina are exactly the three dominant microorganisms in the SW environment.

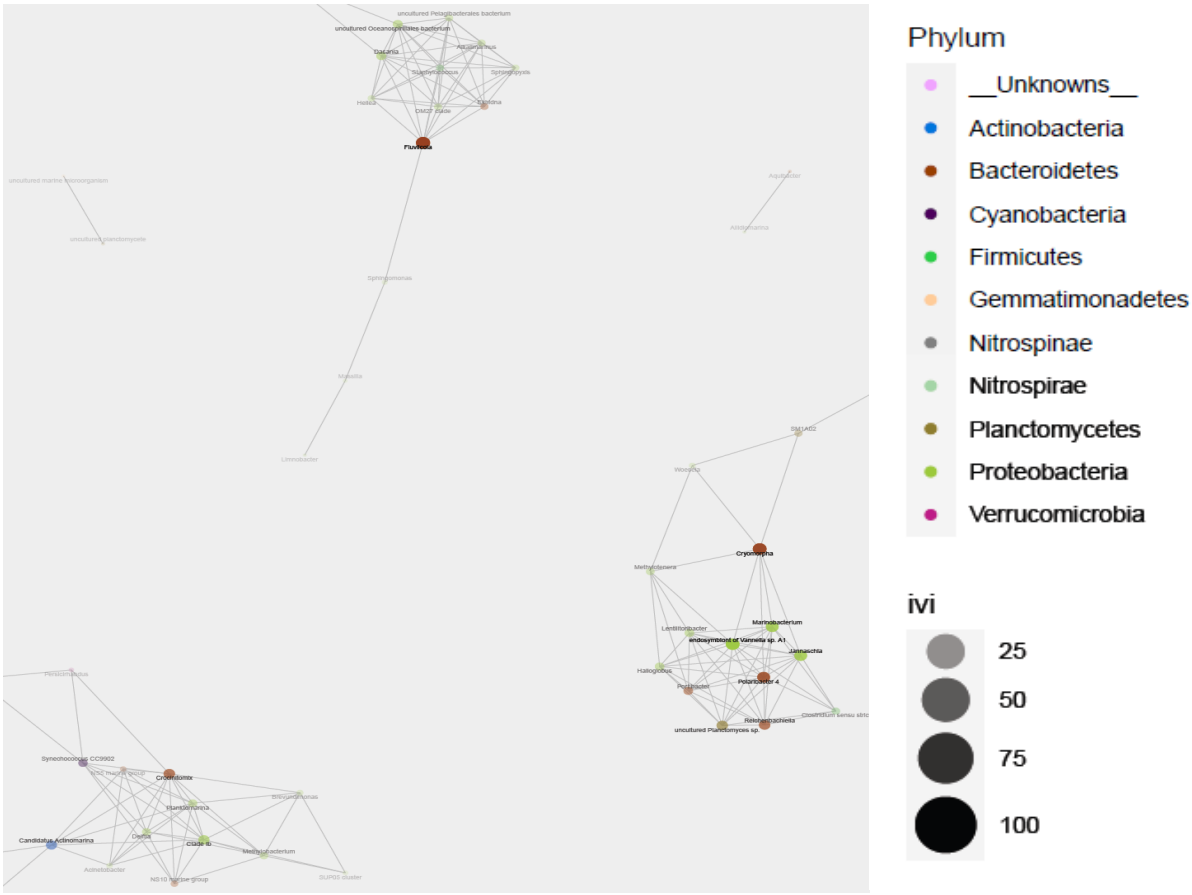


Figure 5

spreading_score	hubness_score	ivi	Phylum	Genus
99.20793425	84.32758621	100	Proteobacteria	SAR92 clade
62.64039539	85.49137931	64.0073853	Bacteroidetes	NS5 marine group

Table 5

Figure 5 and Table 5 show the IVI Network and IVI score for the SWD group. The two microorganisms shown in the table, SAR92 clade and NS5 marine group are exactly the two dominant microorganisms in the SWD environment.

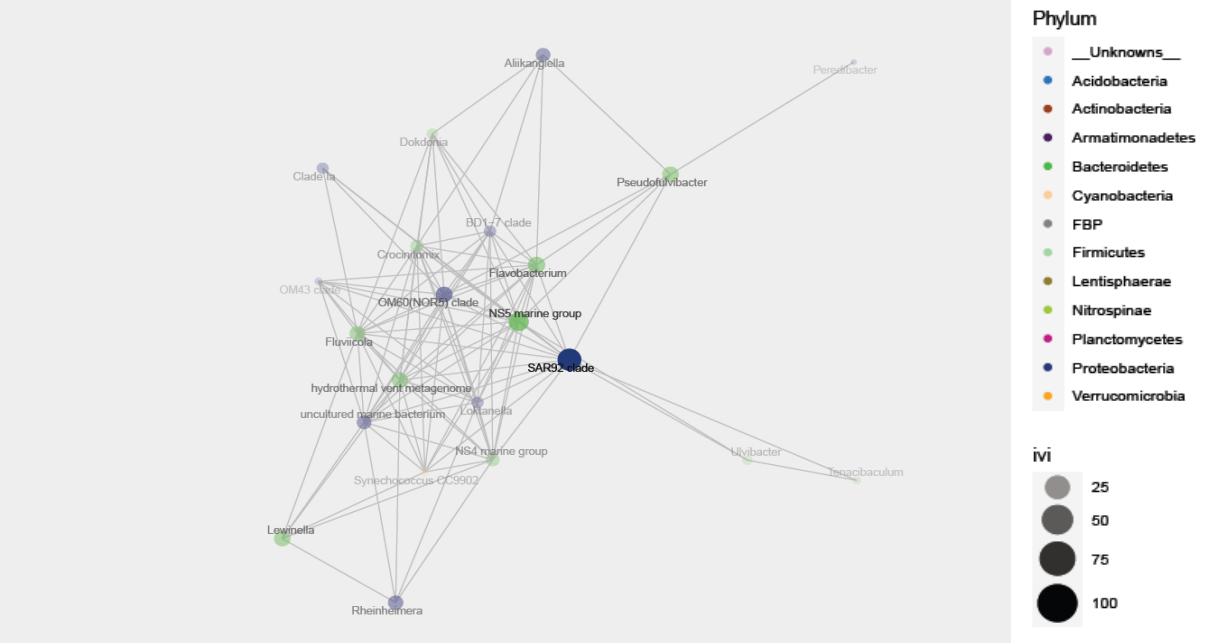


Figure 6

spreading_score	hubness_score	ivi	Phylum	Genus
68.12658145	91.59651163	100	Bacteroidetes	Fluviicola
58.26939553	100	93.2183324	Proteobacteria	endosymbiont of Vannella sp. A1
100	57.17674419	92.1285086	Bacteroidetes	Cryomorpha

Table 6

Figure 4 and Table 4 show the IVI Network and IVI score for the SWBS group. The three microorganisms shown in the table, Fluviicola, endosymbiont of Vannella sp. A1 and Cryomorpha are exactly the three dominant microorganisms in the SWBS environment.

3.2 Zeta Diversity

This study focuses on four metrics when discussing Zeta Diversity, which are Zeta Diversity Decline, Ratio of Zeta Diversity Decline, Exponential Regression and Power Law Regression. When we move from examining species shared between two communities (ζ2) and gradually increase the number of communities under consideration, we typically find that the number of shared species declines. This decline is termed the Zeta Diversity Decline. It provides a deeper understanding of species sharedness among biological communities. The Ratio of Zeta Diversity Decline is another representation of the Zeta Diversity Decline, typically calculated between successive ζ values. Specifically, it's the ratio between ζn and ζ(n+1), which is ζ(n+1)/ ζn. In other words, this is the probability that an already discovered species remains after adding samples or sites. This ratio offers a quantitative insight into the rate of decline in species sharedness when adding a new community to the mix. Exponential regression and power law regression are two regression methods in statistics used to fit data points to an exponential or power function. Using regression can help us better understand and predict the pattern of Zeta Diversity Decline as the number of biomes increases. When the decline is

more random it will fit an exponential regression, and conversely when the decline is deterministic it will fit a power law regression. There are also two metrics, Zeta Diversity, which indicates the number of species that are common to multiple biomes, and Zeta Order, which indicates the number of biomes examined.

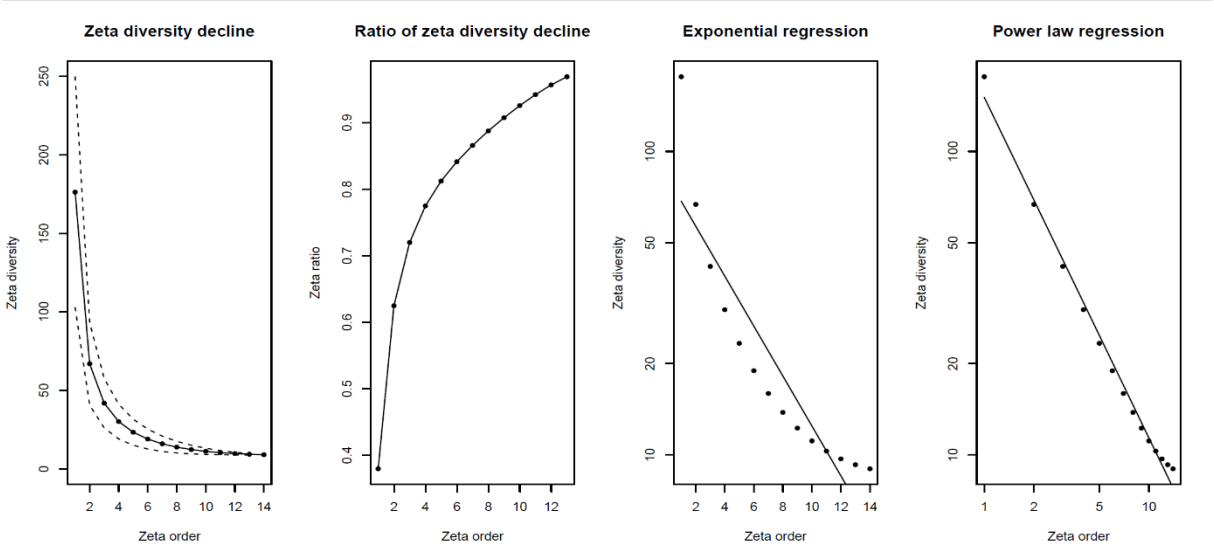


Figure 7: Four graphs related to zeta diversity from the WAF group

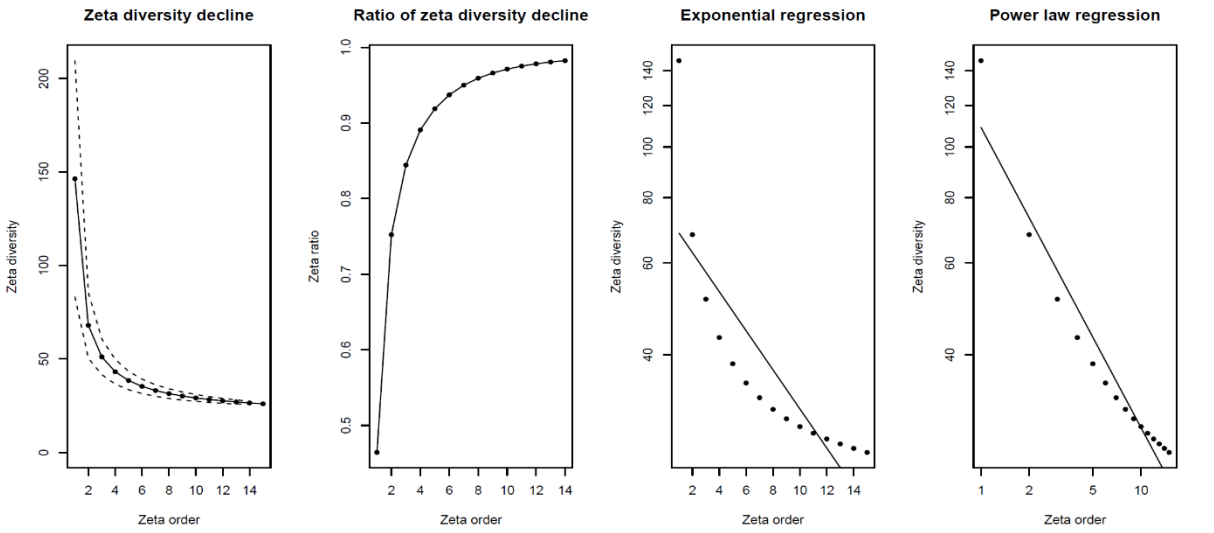


Figure 8: Four graphs related to zeta diversity from the CEWAF group

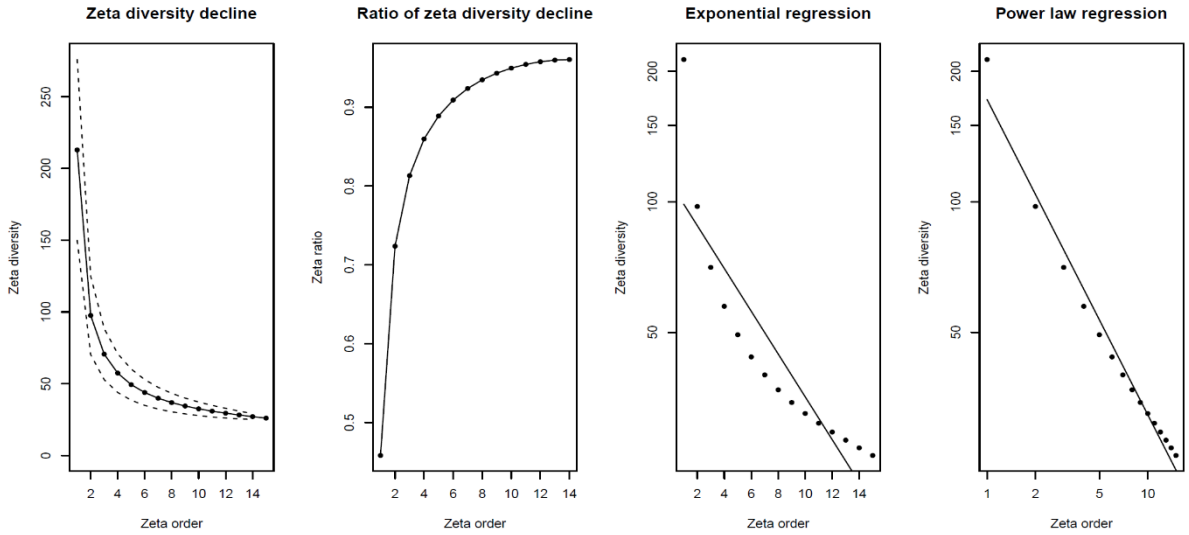


Figure 9: Four graphs related to zeta diversity from the BEWAF group

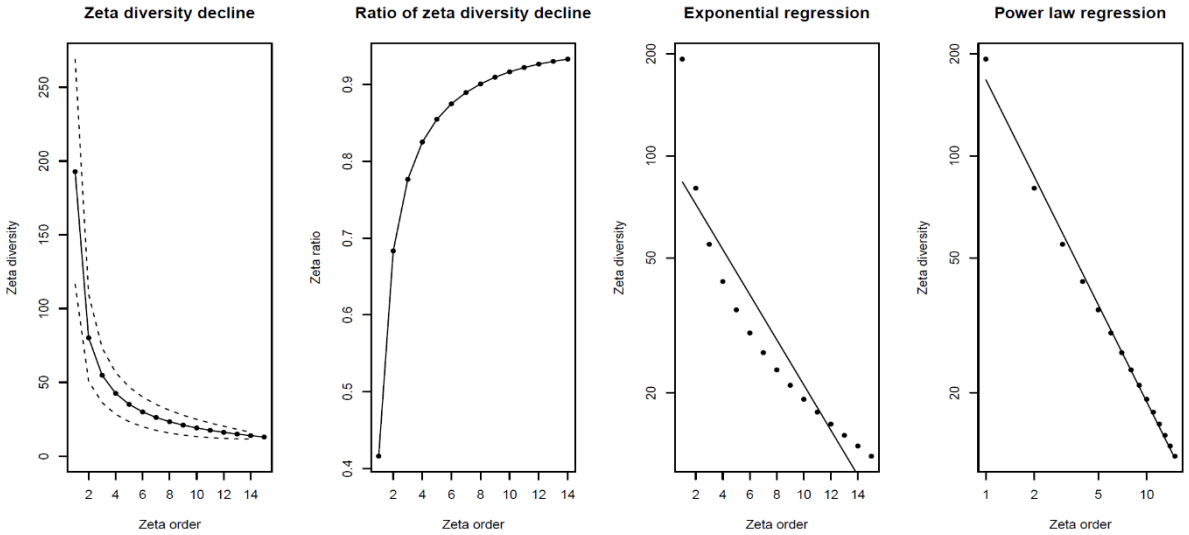


Figure 10: Four graphs related to zeta diversity from the SW group

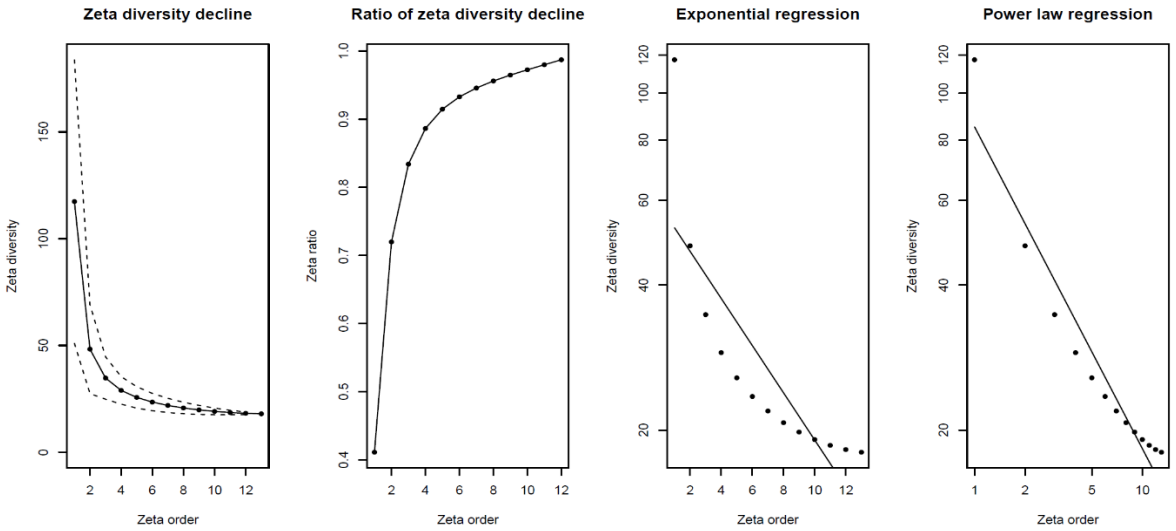


Figure 11: Four graphs related to zeta diversity from the SWD group

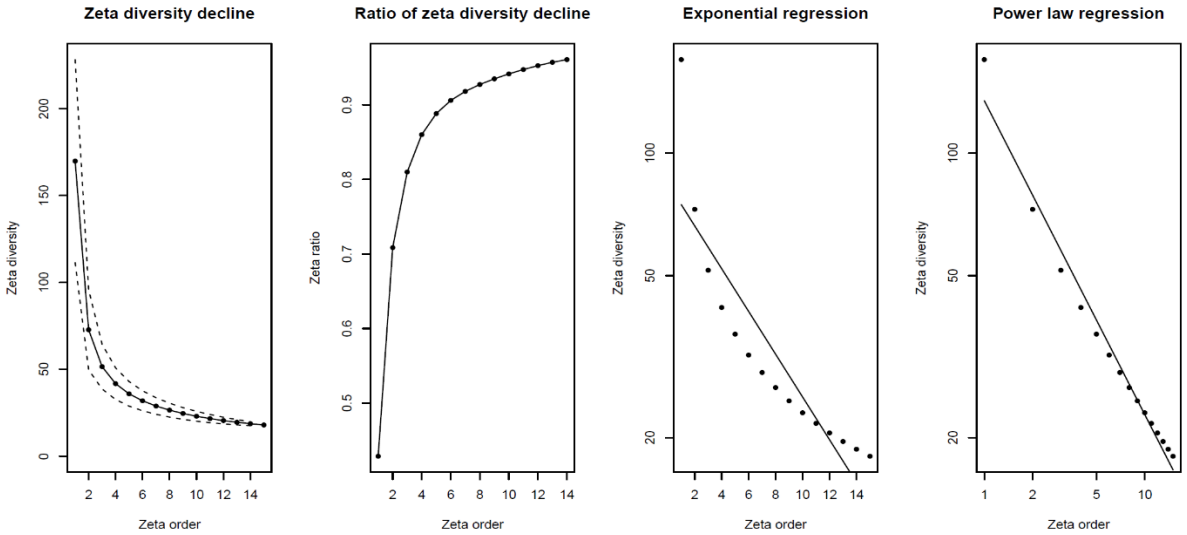


Figure 12: Four graphs related to zeta diversity from the SWBS group

4. Discussion

4.1 Network

4.1.1 Comparison of WAF, CEWAF and BEWAF results in Network

Based on the results of the charts in the previous chapter, it is clear that in the three groups of samples, WAF and BEWAF have extremely similar dominant species, and the dominant species even have the same genus. This suggests that the use of the biodispersant rhamnolipid has very little effect on the overall species structure of the microorganisms in the case of an oil spill. In contrast, all dominant species were altered in the CEWAF group using the chemical dispersant finasol. Colwellia was the dominant species in all three groups in the results of previous similar experiments, but this result was not observed in the present study, possibly because Colwellia was one of the dominant species in the original community in the FSC waters, leading to its higher initial abundance measured in previous similar experiments.

4.1.2 Comparison of SW, SWD and SWBS results in Network

The results for the three control groups were slightly more unexpected, with all three samples having different genus of dominant species, suggesting that when the oil is fully degraded, the residual finasol or rhamnolipid dispersants will still have an impact on the microbial community in the water. But this does not necessarily mean that the impact is negative; after all, the ecological functions of these microorganisms, the ecological balance of the environment and the food chain are not necessarily seriously affected. Therefore, the comparison of the three control groups with each other does not lead to a large number of meaningful conclusions.

4.1.3 WAF and BEWAF dominant species, OM75 clade and Marinimicrobia bacterium SCGC AAA160-I06

In the WAF and BEWAF samples, two dominant microbial taxa emerged prominently: members of the order Thalassobaculales (with special emphasis on the OM75 clade) and the relatively newly identified Marinimicrobia bacterium SCGC AAA160-I06.

The Thalassobaculales, notably the OM75 clade, are part of the Alphaproteobacteria class, which has been shown to have versatility in hydrocarbon degradation, especially in marine environments. The significant dominance of OM75 clade in the WAF and BEWAF samples suggests their potential role in processing petroleum or its derivatives, possibly indicating their capability to adapt and thrive in oil-contaminated environments.

On the other hand, Marinimicrobia bacterium SCGC AAA160-I06 represents a more mysterious aspect of marine microbial ecology. Being relatively new to scientific literature, its exact ecological role remains to be elucidated. However, its dominance in both WAF and BEWAF samples emphasizes the need to understand its potential interactions with hydrocarbons and the broader implications for marine ecosystems.

4.1.4 The predominant taxa in both SW and SWD: Flavobacteriaceae

The family Flavobacteriaceae consistently featured as a dominant player in both the SW and SWD treatments. Originating from the phylum Bacteroidetes, this family is ubiquitously

found in diverse environments, especially in aquatic habitats. Many of its members have been recognized for their adeptness at degrading organic compounds, from complex molecules to polysaccharides and proteins. Notably, while the addition of Finasol in the SWD treatment did not significantly alter the dominance of Flavobacteriaceae, suggesting a level of resilience or compatibility with this dispersant, it's vital to consider the broader ecological impacts of dispersants beyond just microbial community structures.

4.1.5 Pervasive Dominance of Thalassobaculales in Various Treatments

A remarkable consistency was observed in our experiments: members of the Thalassobaculales order were dominant in five out of the six treatments, with only CEWAF being the exception. This prevalent dominance of Thalassobaculales, irrespective of variations in environmental conditions brought about by the presence of oil and two distinct dispersants, speaks to their robustness and adaptability in marine ecosystems.

Several aspects might shed light on the ubiquitous presence of Thalassobaculales:

Environmental Fitness: Thalassobaculales could have metabolic pathways that favor them in oil-affected habitats, potentially utilizing hydrocarbon components for energy.

Competitive Edge: The dominance of this order might suggest they have a competitive advantage in accessing resources under the given experimental conditions.

Generalist Nature: Being generalists, they might be equipped to thrive in diverse conditions, explaining their consistent presence across treatments.

The resilience of Thalassobaculales underscores their potential significance in marine environments, especially when subjected to variations in conditions.

4.1.6 Difference in Core Taxa in WAF Treatments: Alcanivorax

Historically, Alcanivorax is characterized as a crucial marine bacterium for oil degradation, often found in low concentrations in uncontaminated upper ocean areas (Emmanuel et al., 2016). Remarkably, in certain saline conditions, it's believed that Alcanivorax can represent up to 80% of oil-degrading microorganisms. While this bacterium primarily depends on alkanes and possesses strong degradation capabilities, its presence was not detected in any of our experimental groups. Generally, populations of this microbe surge upon the introduction of oil to the environment. The stark difference in our observations may suggest unique environmental or experimental factors that prevented its proliferation. The colder conditions of the FSC in the North Atlantic could potentially play a role in hindering the growth of Alcanivorax.

4.1.7 Divergence from Previous Studies on Dominant Taxa in Finasol Environments

In prior investigations, members of Rhodobacteracaea emerged as a significant presence in Finasol-treated environments. Vibrionaceae was also notably observed predominantly in environments treated with Finasol, with a distinct enrichment in SWD.

However, our current findings present a contrast. Neither Rhodobacteracaea nor Vibrionaceae were dominant in our study's primary taxa. This discrepancy might arise from multiple factors. Environmental variations, for instance, can influence microbial responses, as changes in experimental conditions, such as temperature, salinity, or nutrient availability, may yield different results. The source and historical background of the microbial communities studied might also play a role in their varied reactions. Moreover, the precise composition or batch of Finasol used in different studies could be another determinant, potentially influencing the

microbial responses. Additionally, microbial communities are known for their evolutionary adaptability, which could reflect in the different dominant taxa in separate studies.
While Rhodobacteracaea and Vibrionaceae have demonstrated capabilities to metabolize hydrocarbons and surfactant by-products, other microbial taxa may have taken on this role under our study's specific conditions. A more in-depth exploration is necessary to truly understand these microbial shifts and their implications in Finasol-treated marine environments.

4.1.8 Variation in the Core Taxa of BEWAF: Cycloclasticus

In past research, Cycloclasticus presented as a prominently enriched taxa in early experimental stages, particularly in treatments with rhamnolipid and pure oil. These observations revealed that Cycloclasticus levels surged within the initial week and remained consistent throughout the experimental period. However, my investigations did not note the presence of Cycloclasticus in any Finasol treatments. This absence is quite significant. Within the context of oil spill research concerning the Gulf of Mexico, Cycloclasticus has been identified as a pivotal bacterium involved in the chemical processing of polycyclic aromatic hydrocarbons in such environments(Xuemei et al., 2016). The lack of Cycloclasticus in this study's chemical treatments suggests that Finasol might exert a detrimental effect on this taxa. Given Cycloclasticus's critical role in degrading aromatic hydrocarbons, its absence could potentially impact the biodegradation rate of aromatics during chemical treatment. This finding underscores the need to reconsider the composition of Finasol for future applications.

4.2 Zeta Diversity

4.2.1 Summary of the six sample groups in terms of zeta diversity decline

All six groups showed very similar trends in zeta diversity decline curves. This suggests that there is consistency between the groups in the pattern of change in the species composition of the samples. Specifically, if the trends in the curves for zeta diversity decline are the same, this indicates that species composition is changing or being lost in a similar manner across the groups. This situation may result from the presence of some mechanism, which may be related to ecological processes such as microbial interactions, resource competition, and species substitution inherent in the environment. The similarity in trends could offer valuable insights into how microbial communities respond to environmental changes or perturbations. However, it might also limit understanding the subtle differences in microbial responses between different treatments.
But there is also a very clear difference in these six curves. When $\zeta = 1$, the two groups with Finasol added (SWD and CEWAF) have significantly lower values of Zeta Diversity compared to the other four groups, which means that the number of species in the samples is significantly reduced after the addition of Finasol. This means that Finasol is unfavorable to the survival of certain species, but rhamnolipid does not have this problem, suggesting that rhamnolipid is better than Finasol in maintaining the diversity of marine microorganisms. Although the different sample treatments may have affected the absolute number of species or their co-occurrence, their effects on the overall pattern of change in species composition were similar, meaning that no significant treatment effects were captured here.

4.2.2 Stochastic and deterministic situations of samples obtained from regression models

Unexpectedly, all six groups followed the power law regression, even for the SW blank control group. We can therefore infer that all treatment and control groups seem to have a similar way of responding to environmental changes or perturbations. This might suggest that these microbial communities have an inherent and relatively stable pattern of response, regardless of their initial conditions or specific treatments. This could be dictated by specific environmental conditions of that region, niche competition, predator-prey dynamics, etc.

This result may imply that the microbial community in this marine region has a strong robustness and resilience, capable of resisting external disturbances and striving to maintain its original ecological dynamics and structure. Or it may be that although the external factors introduce new substances and pressures, they might not be strong enough to entirely change or reshape the basic dynamics of this ecological system. This could explain why all the groups, including those treated, follow the same power law pattern.

The microbial community decline in this marine region seems to be governed by an inherent, non-random ecological mechanism, which demonstrates significant stability and robustness across different disturbances.

5. Conclusion

The intricate web of interactions that form the basis of microbial community structures remains a subject of immense fascination and importance, particularly within the context of marine environments. Through a comprehensive analysis of the effects of the biodispersant rhamnolipid and the chemical dispersant Finasol on microbial communities during oil spills, this research delves deep into the ecological ramifications of using these compounds.

The SWBS and SWD groups were not only used as control groups for BEWAF and CEWAF, but also as experimental groups for SW to simulate the effects of the residual dispersant on the biotic community after all the crude oil has been degraded. Although this part of the Network study did not produce valid results, the Zeta Diversity study concluded that rhamnolipid had a lower impact on marine microorganisms than Finasol in a situation where there is no oil to be degraded.

While both the BEWAF and CEWAF groups obtained similar results in both the Network study and the Zeta Diversity study, rhamnolipid does not cause more changes in the original community structure, which means that the rhamnolipid environments are closer to the natural circumstances compared to the Finasol environments.

By comparing the regression models of the six samples, we have another surprise gain, which is that the resilience and robust nature of marine microbial communities in the Faroe-Shetland Channels (FSC) have been made evident. Our research shows that while external disturbances can temporarily affect the marine microbiome, its inherent stability allows it to resist drastic changes.

Generally speaking, rhamnolipid, as a biodispersant, has been observed not to significantly alter dominant species within microbial communities, preserving essential species and promoting biodegradation, presenting it as a potentially effective alternative in addressing oil spills. In contrast, Finasol, a chemical dispersant, can introduce considerable selective pressures, leading to significant disruptions in microbial diversity.

This study underscores the pivotal role marine microbial communities play in responding to oil spills. Our findings advocate for further research into sustainable and efficient measures like rhamnolipid to combat the challenges of oil spills.

6. Future work

Oil spills present grave environmental challenges, necessitating the investigation into microbial species that can proficiently degrade oil. In our research, we meticulously sourced microbial specimens from the Faroe-Shetland Channel, aiming to identify bacteria that demonstrate heightened adaptability when exposed to two specific dispersants. This model of simulation is versatile and can be adapted to other regions susceptible to oil spills, allowing for proactive identification and deployment of appropriate dispersants tailored to the local microbial populace. As we advance, there are still areas in our study that require refinement and emerging challenges we anticipate confronting in the future. Here are my three summarized points of greatest need for improvement, or possible future difficulties:

1. The samples contain a large number of microorganisms that have not yet been fully investigated, and some of the newly discovered microorganisms are in a completely new field, even from phylum level. More problematically, these newly discovered microorganisms are often the dominant species in the samples, which makes it difficult to analyze the dominant species once we have found it. And we can't conclude why it is the dominant species, but we can only make simple comparisons between different samples. While this isn't too much of an obstacle to drawing the conclusions we want, it does prevent us from digging deeper for more valuable information.

2. The zeta diversity and regression models for all six sets of samples have almost identical trends, with only the minor difference of initial Zeta Diversity values. The almost no difference in the results makes it difficult to find more profound conclusions, possibly because Zeta Diversity itself is not suitable for this kind of research, or more likely, the sample quality is not stable due to too many interfering factors in the sampling. This is also reflected in the Network analysis, where the IVI Networks for the SW, SWD and SWBS groups are almost worthless, and although they are not the primary subjects of the study, it is still expected that all the data I have processed will provide useful results.

3. Since the dangers of crude oil spills are extremely high, modern protective measures have tried to minimize the possibility of a spill, making dispersants the last line of defense that is better left unused. This is coupled with the fact that Finasol is cheaper to produce, is available in larger quantities, has a more complete product chain, and is itself food-grade. Therefore, there is still a long way to go before Finasol is replaced by Rhamnolipid. After all, it is always the economics of use that take precedence, and in this case the advantages of Rhamnolipid cannot be fully realized.

Reference

Alvarado, F., Williams, D. R., Arroyo-Rodríguez, V., & Escobar, F. (2018). Commentary: Forest Cover Is Critical for Biodiversity Conservation in Tropical Livestock-Dominated Landscapes. Tropical Conservation Science, 11, 194008291878316. https://doi.org/10.1177/1940082918783160

Aubin, G. G., Haloun, A., Treilhaud, M., Reynaud, A., & Corvec, S. (2013). Gallibacterium anatis Bacteremia in a Human. Journal of Clinical Microbiology, 51(11), 3897–3899. https://doi.org/10.1128/jcm.01638-13

Balaban, A. T. (1985). Applications of graph theory in chemistry. Journal of Chemical Information and Modeling, 25(3), 334–343. https://doi.org/10.1021/ci00047a033

Booth, S. C., & Smith, W. P. J. (2019). Light sheets unveil host–microorganism interactions. Nature Reviews Microbiology, 18(2), 65–65. https://doi.org/10.1038/s41579-019-0318-y

Corvec, S. (2018). Clinical and Biological Features of Cutibacterium (Formerly Propionibacterium) avidum, an Underrecognized Microorganism. Clinical Microbiology Reviews, 31(3). https://doi.org/10.1128/cmr.00064-17

Dalton, T., Dowd, S. E., Wolcott, R. D., Sun, Y., Watters, C., Griswold, J. A., & Rumbaugh, K. P. (2011). An In Vivo Polymicrobial Biofilm Wound Infection Model to Study Interspecies Interactions. PLoS ONE, 6(11), e27317. https://doi.org/10.1371/journal.pone.0027317

del Rio, G., Koschützki, D., & Coello, G. (2009). How to identify essential genes from molecular networks? BMC Systems Biology, 3(1). https://doi.org/10.1186/1752-0509-3-102

Dizay, H. H., Lau, D. G., & Nottage, W. M. (2017). Benzoyl peroxide and clindamycin topical skin preparation decreases Propionibacterium acnes colonization in shoulder arthroscopy. Journal of Shoulder and Elbow Surgery, 26(7), 1190–1195. https://doi.org/10.1016/j.jse.2017.03.003

Frainay, C., & Jourdan, F. (2017). Computational methods to identify metabolic sub-networks based on metabolomic profiles. Briefings in Bioinformatics, 18(1), 43–56. https://doi.org/10.1093/bib/bbv115

Friedman, J., & Alm, E. J. (2012). Inferring Correlation Networks from Genomic Survey Data. PLoS Computational Biology, 8(9), e1002687. https://doi.org/10.1371/journal.pcbi.1002687

Guimerà, R., & Nunes Amaral, L. A. (2005). Functional cartography of complex metabolic networks. Nature, 433(7028), 895–900. https://doi.org/10.1038/nature03288

Herbst, A. C., Johnson, M. G., Gammons, H., Reedy, S. E., Urschel, K. L., Harris, P. A., & Adams, A. A. (2022). Development and Evaluation of a Muscle Atrophy Scoring System (MASS) for Horses. Journal of Equine Veterinary Science, 110, 103771. https://doi.org/10.1016/j.jevs.2021.103771

Hochberg, D., & Ribó, J. M. (2018). Stoichiometric network analysis of entropy production in chemical reactions. Physical Chemistry Chemical Physics, 20(36), 23726–23739. https://doi.org/10.1039/c8cp04398a

Kitsak, M., Gallos, L. K., Havlin, S., Liljeros, F., Muchnik, L., Stanley, H. E., & Makse, H. A. (2010). Identification of influential spreaders in complex networks. Nature Physics, 6(11), 888–893. https://doi.org/10.1038/nphys1746

Kleindienst, S., Seidel, M., Ziervogel, K., Grim, S., Loftis, K., Harrison, S., Malkin, S. Y., Perkins, M. J., Field, J., Sogin, M. L., Dittmar, T., Passow, U., Medeiros, P. M., & Joye, S. B. (2015). Chemical dispersants can suppress the activity of natural oil-degrading microorganisms. Proceedings of the National Academy of Sciences, 112(48), 14900–14905. https://doi.org/10.1073/pnas.1507380112

Kurtz, Z. D., Müller, C. L., Miraldi, E. R., Littman, D. R., Blaser, M. J., & Bonneau, R. A. (2015). Sparse and Compositionally Robust Inference of Microbial Ecological Networks. PLOS Computational Biology, 11(5), e1004226. https://doi.org/10.1371/journal.pcbi.1004226

Magalhães, A. P., Azevedo, N. F., Pereira, M. O., & Lopes, S. P. (2015). The cystic fibrosis microbiome in an ecological perspective and its impact in antibiotic therapy. Applied Microbiology and Biotechnology, 100(3), 1163–1181. https://doi.org/10.1007/s00253-015-7177-x

Murray, J. L., Connell, J. L., Stacy, A., Turner, K. H., & Whiteley, M. (2014). Mechanisms of synergy in polymicrobial infections. Journal of Microbiology, 52(3), 188–199. https://doi.org/10.1007/s12275-014-4067-3

Nikolova, C. N., Ijaz, U. Z., Magill, C., Kleindienst, S., Joye, S. B., & Gutierrez, T. (2021). Response and oil degradation activities of a northeast Atlantic bacterial community to biogenic and synthetic surfactants. Microbiome, 9(1). https://doi.org/10.1186/s40168-021-01143-5

Nyankson, E., Demir, M., Gonen, M., & Gupta, R. B. (2016). Interfacially Active Hydroxylated Soybean Lecithin Dispersant for Crude Oil Spill Remediation. ACS

Sustainable Chemistry & Engineering, 4(4), 2056–2067. https://doi.org/10.1021/acssuschemeng.5b01403

Oldham, S., Fulcher, B., Parkes, L., ArnatkevičiūtėA., Suo, C., & Fornito, A. (2019). Consistency and differences between centrality measures across distinct classes of networks. PLOS ONE, 14(7), e0220061. https://doi.org/10.1371/journal.pone.0220061

Phadnis, J., Gordon, D., Krishnan, J., & Bain, G. I. (2016). Frequent isolation of Propionibacterium acnes from the shoulder dermis despite skin preparation and prophylactic antibiotics. Journal of Shoulder and Elbow Surgery, 25(2), 304–310. https://doi.org/10.1016/j.jse.2015.08.002

Sandefur, C. I., Mincheva, M., & Schnell, S. (2013). Network representations and methods for the analysis of chemical and biochemical pathways. Molecular BioSystems, 9(9), 2189–2189. https://doi.org/10.1039/c3mb70052f

Sargent, R. D., Kembel, S. W., Emery, N. C., Forrestel, E. J., & Ackerly, D. D. (2011). Effect of local community phylogenetic structure on pollen limitation in an obligately insect-pollinated plant. American Journal of Botany, 98(2), 283–289. https://doi.org/10.3732/ajb.1000329

Schlomann, B. H., Wiles, T. J., Wall, E. S., Guillemin, K., & Parthasarathy, R. (2019). Sublethal antibiotics collapse gut bacterial populations by enhancing aggregation and expulsion. Proceedings of the National Academy of Sciences of the United States of America, 116(43), 21392–21400. https://doi.org/10.1073/pnas.1907567116

Thomas, G. E., Brant, J. L., Campo, P., Clark, D. R., Coulon, F., Gregson, B. H., McGenity, T. J., & McKew, B. A. (2021). Effects of Dispersants and Biosurfactants on Crude-Oil Biodegradation and Bacterial Community Succession. Microorganisms, 9(6). https://doi.org/10.3390/microorganisms9061200

Virgin, L. N. (2006). A Reflection on Nonlinear Oscillations, Dynamical Systems, and Bifurcations of Vector Fields by J. Guckenheimer and P. J. Holmes. Journal of Computational and Nonlinear Dynamics, 1(4), 277–278. https://doi.org/10.1115/1.2338659