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Microbial community structure and niche differentiation in healthy and infected samples of Kappaphycus alvarezii in the commercial farming sites of Tamil Nadu coastal, India

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Abstract

Seaweed cultivation, particularly of *Kappaphycus alvarezii*, plays a significant role in the coastal economy of Tamil Nadu, India. *K. alvarezii* is frequently affected by ice-ice disease caused by microbial imbalances. This disease leads to tissue bleaching, reduced biomass, and lower carrageenan yield, making the study of seaweed-associated bacterial communities crucial for sustainable cultivation. In this study, healthy and diseased samples of *K. alvarezii* were collected from five commercial farming sites. High-throughput Illumina sequencing of the V3–V4 region of the 16S rRNA gene was performed to analyze microbial diversity. Bioinformatics tools such as OTU clustering, alpha diversity indices, heat tree visualization, and functional prediction were applied to characterize differences between healthy and diseased samples across locations. The results revealed Proteobacteria as the dominant phylum, with Gammaproteobacteria being prevalent in all samples. Diseased seaweed samples exhibited greater microbial diversity, with genera including *Pseudoalteromonas* and *Vibrio* frequently associated with disease, while *Cobetia* was more abundant in healthy samples. Variations in microbial community structure were closely linked to environmental factors, including temperature, salinity, dissolved oxygen, and nutrient levels. Functional prediction showed that diseased samples had elevated pathways related to fermentation, nitrate reduction, and nitrogen respiration. Whereas healthy samples were enriched in aerobic chemoheterotrophy and hydrocarbon degradation. The study demonstrates that site-specific environmental conditions significantly influence microbial dynamics and disease progression in seaweed farms. Identifying potential pathogens and beneficial bacteria provides a foundation for developing targeted probiotics and disease management strategies that can support sustainable cultivation and improve seaweed health.

Introduction

Seaweeds, aka macroalgae, the cultivation of multiple seaweed species constitutes more than half of the cumulative global marine aquaculture production. This indicates the enormous potential of seaweed aquaculture as a sustainable and lucrative industry. The utilization of seaweed cultivation offers many benefits, ranging from mitigating environmental issues to promoting socio-economic development. Hence, it is imperative to recognize and harness the potential of seaweed aquaculture to ensure a sustainable future (FAO 2020; Wang et al., 2022). The demand for wet seaweeds has skyrocketed in recent years, with over 35 million tons produced worldwide in 2022 (FAO 2022). This versatile seaweed has proven to be a valuable resource, providing various benefits for industries such as food, medicine, and fuel. As a result, the global revenue generated by wet macroalgae production has surged to nearly $1.9 billion (FAO 2022). Investing in cultivating and harvesting this valuable resource is wise for businesses looking to capitalize on a growing market (Montúfar et al., 2023). Cultivating seaweed for ecosystem services provides a sustainable solution to ocean acidification and contributes towards carbon fixation, making it a constructive approach to protecting and preserving our oceans (Campbell et al., 2019; Montúfar et al., 2023).

K. alvarezii (Doty) is a tropical seaweed that produces a commercially valuable and significant product, carrageenan (Mamat et al., 2024; Munisamy et al., 2024; Mantri et al., 2025). Ice-ice disease is a major concern in the world of *K. alvarezii* farming. This disease leads to a substantial decline in *K. alvarezii* biomass yield and reduces the amount of carrageenan relative to the significant percentage of healthy seaweed (Rantetondok et al., 2017; Tahiluddin et al., 2022). The disease is characterized by “bleaching or whitening” indications on the seaweed thallus tissue and can be caused by various opportunistic pathogenic bacteria (Ward et al., 2022; Achmad et al., 2017; Riyaz et al., 2021). It is non-infectious and can arise from adverse environmental factors, including extreme temperature, pH, and salinity, as well as opportunistic bacterial pathogens such as *Vibrio* sp. (Arasamuthu & Patterson Edward., 2018).

The microbial communities associated with seaweeds are highly diverse, yet we have not fully understood their complexities or how they differ from those found in the open sea (Xu et al., 2022). It is vital to examine the epiphytic bacterial microbiota that inhabits seaweed since they are instrumental in influencing algal morphological development, metabolism, growth, and protection against fouling organisms (Nahor et al., 2024). By further exploring this field of study, we can uncover valuable knowledge about the captivating realm of seaweed and its associated microbial communities.

The 16S rRNA gene sequencing is the latest approach used for studies of bacterial phylogeny and taxonomy. The major reason for the use of the 16S rRNA gene sequencing is that steady function has not changed over time because it occurs in almost every bacterium, indicating that random sequence change is more accurate and the 16S rRNA gene (1500 bp) is large enough for information purposes (Janda & Abbott., 2007; Clarridge., 2004; Kembel et al., 2012). Experiments show that *Ulva* species, a type of marine green macroalga, have complex microbiomes essential for their growth. These species associate with specific bacterial communities, and studies using 16S rRNA gene sequencing have explored bacteria-driven growth patterns and the potential of using microbes in algal aquaculture (Wichard., 2023). Red seaweeds have been extensively studied with specific emphasis on *Gracilariopsis* and *Gracilaria* species. An integrative analysis of the red macroalga *Gracilariopsis lemaneiformis* through high-throughput sequencing showed that environmental factors, notably nitrogen and phosphorus levels, profoundly influence the epiphytic bacterial community structure of varying geographic locations. The study illustrated location-specific differences in bacterial community structures and their association with nutrient levels (Pei et al., 2021). Sophisticated multi-omics tools have been used in brown seaweeds. *Fucus vesiculosus* studies have integrated amplicon sequencing with metabolomics and imaging methods, and it was identified that *Alphaproteobacteria* are the dominant epibiotic bacteria, whereas fungi of the class Eurotiomycetes were imaged for the first time on an algal surface. This study showed the combination of 16S rRNA sequencing with metabolomics to clarify the surface microbiome-metabolome interactions (Parrot et al., 2019). Phylogenetic and functional characterization of microbiomes of *Sargassum* seaweed waste determined leading bacterial genera, such as *Muricauda, Aminobacterium, Mesorhizobium, Marinobacter, Reichenbachiella, Hyphomonas, Simiduia, Aquamicrobium, Oceanicola, and Alcanivorax*, which reflect bacterial diversity within brown seaweed detritus (Mohapatra., 2023). Studies employing 16S rRNA gene sequencing have described bacterial communities related to several species of seaweeds in various marine

ecosystems. These researchers have identified that bacteria associated with seaweed are dominated by phyla including Proteobacteria, Bacteroidetes, Firmicutes, Cyanobacteria, Planctomycetes, Actinobacteria, and Verrucomicrobia (Selvarajan et al., 2019; Nahor et al., 2024).

In this study, metagenomics was used to analyze the microbial diversity on the surface of diseased and healthy *K. alvarezii* among five seaweed cultivation sites. This study effectively extends our understanding of the diversity of seaweed thallus-bound microorganisms on both diseased and healthy *K. alvarezii*. This work unlocks new opportunities for the exploitation and use of seaweed resources in the future, as well as the exploration of valuable microbiomes from active seaweed cultivation sites.

Materials and methods

Sample collection

The source of the biological material was from commercial-scale seaweed farming sites in Tamil Nadu, India. In the present study, *K. alvarezii* (red seaweed) well-matured, healthy, and infected samples were collected from 5 different commercial farming sites i.e., Mangadu (9.327695"N 78°59'35.8"E), Soliyakkudi (9°42'29.6"N 78°59'35.8"E), Jegathapattinam (9°57'55.2"N 79°11'19.5"E), Nambuthalai (9°43'34.8"N 79°00'15.1"E) and Odavimadam (9°59'21.6"N 79°12'31.3"E) in the Palk Bay region, Tamil Nadu, India (Fig. 1) Table 1. The samples were immediately placed in a cool pack and transported from the farming sites to the laboratory, and were used to extract the total genomic DNA. All the sites are situated between the villages and in close proximity to shrimp culture ponds. As a result, they are frequently disturbed by heavy anthropogenic pressure from the seaweed farmers and fisher communities that enter the water. The Mangadu site, on the other hand, is located far from the fishers' village and has limited public access, making it an ideal site for seaweed farming with low anthropogenic pressure. Seawater samples were collected from all sites to investigate the relationship between seawater physicochemical parameters and both diseased and healthy seaweed.

Table. 1. Collection sites name and their code word

Site name	Sample	Code
Mangadu	Diseased	KaMD
	Healthy	KaMH
Soliyakudi	Diseased	KaSD
	Healthy	KaSH
Nambuthalai	Diseased	KaND
	Healthy	KaNH
Jegadhapattinam	Diseased	KaJD
	Healthy	KaJH
Odavimadam	Diseased	KaOD
	Healthy	KaOH

Collection sites, sample types, and their corresponding code names. “Ka” represents *Kappaphycus alvarezii*, followed by the initials of the site name and sample condition (D – Diseased, H – Healthy).

Genomic DNA extraction

Genomic DNA was extracted from disease-infected and healthy samples of *K. alvarezii*. DNA was extracted using the modified method by ONEOMICS PRIVATE LIMITED, Tiruchirappalli, Tamil Nadu (In-house Protocol). Briefly, for around 50 mg of seaweed samples (samples were chopped into pieces in a tube), 200 µL of high salt lysis buffer was added, and then crushed using a sterile micro pestle. To this, 20 µL of proteinase K (20 mg/mL) was added, and the tube was vortexed vigorously and then incubated at 55°C for 10 min. To this, an equal volume of phenol chloroform, iso amyl alcohol was added, followed by centrifugation at 14,000 RPM for 10 min. The supernatant was pipetted out and then two volumes of ice-cold Isopropanol were added and subjected to DNA precipitation at -20°C. Finally, the samples were centrifuged at 14,000 RPM for 20 min, and then the pellet was washed with 70% ethanol. Following isolation, the DNA concentrations were verified using a Qubit 4 Fluorometer (Thermo Fisher Scientific, USA), and DNA quality was determined by Nanodrop (Thermo Scientific, San Diego, CA, USA) and Agarose gel electrophoresis.

Next-Generation Amplicon Sequencing

During the first PCR, fragments of the V3–V4 regions of the 16S rRNA gene were amplified using the primers 341 F – CCTAYGGGRBGCASCAG and 806 R –GGACTACNNGGGTATCTAAT. The PCR reactions were carried out with Phusion High-Fidelity PCR Master Mix (New England Biolabs), 2 µM of forward (341 F) and reverse (806 R) primers, and about 10 ng template DNA. Thermal cycling consisted of initial denaturation at 98℃ for 1 min, followed by 30 cycles of denaturation at 98℃ for 10 s, annealing at 50℃ for 30 s, and elongation at 72℃ for 30 s, and final extension at 72℃ for 5 min. The reaction was performed on a PTC-0200 G thermocycler (Bio-Rad Laboratories, Inc., USA). Besides the PCR clean-up done to purify the PCR product in this study, it is important to state that a negative control was included in the PCR amplification to ensure there was no contamination of

reagents. For PCR product quantification and qualification, the PCR product was mixed with an equal volume of 1X loading buffer (containing SYBR green), and electrophoresis was performed on a 2% agarose gel for detection. Agilent 5400 Fragment Analyzer (Agilent 2100, USA) was used to verify the size of the PCR product. Samples with sharp peaks between 450-480 bp were chosen for further experiments. We purified the amplicon product to remove free primers and primer dimers using AMPure XP beads. Next, we attached dual indices and Illumina sequencing adapters using the Nextera XT DNA Library Preparation Kit (Illumina, USA). The amplicon was then purified again with AMPure XP beads. The libraries generated were quantified using a Qubit 4 Fluorometer (Thermo Fisher Scientific, USA). The amplicons from each reaction mixture were combined in equimolar concentrations and sequencing was carried out using the Illumina NovaSeq 6000 system (Illumina, USA) in a 2 × 250 bp paired-end run following the manufacturer's guidelines.

Bioinformatics analyses

The bioinformatics analyses were carried out by ONEOMICS PRIVATE LIMITED (Tiruchirappalli, India). Initially, the raw reads underwent demultiplexing and quality filtering using the deblur plugin in QIIME2 software (https://qiime2.org/). To provide an overview, reads with a length of around 250 base pairs were shortened at positions with an average quality score below 20 over a 50-base-pair sliding window. Reads shorter than 50 base pairs and those containing ambiguous characters were omitted from the subsequent analysis.

The operational taxonomic units (OTUs) were generated by clustering using the same deblur plugin. Non-repetitive sequences were extracted to streamline the analysis, and single non-repetitive sequences were removed. The non-repetitive sequences, excluding single sequences, were then clustered based on the OTUs using a similarity threshold of 97%, and chimeras were removed during clustering to obtain the representative OTU sequences. The OTUs obtained were matched against the SILVA database (version 138) using a confidence threshold of 70% using the feature-classifier plugin in QIIME2.

Diversity Analyses

Variation analysis of alpha diversity indices between groups

The statistical analysis of the data from QIIME2 was performed using various R packages. The QIIME2 artifacts were imported and analyzed using the R package qiime2R v0.99.6. Alpha diversity is designed to measure the diversity within a single community, i.e., the bacterial community of *K. alvarezii* samples. Boxplots were formed to analyze the difference in Alpha diversity indices between groups using the R package Vegan v2.6.6. The alpha diversity index and the Shannon index were used to determine the diversity in each sample. The abundance plots, used to determine the variation in the presence of different taxonomies between the groups, were generated using the R package Phyloseq v1.48.0. The rarefaction curve to determine the adequacy of sequencing depth was also generated using the Vegan R package. The PCA analysis was done using the QIIME2 software and visualized with the qiime2R package. The R package Metacoder R v0.3.8 was used to parse the taxonomy data and visualize the heat tree.

Heat Tree Visualization

The Heat Tree Analysis utilizes the hierarchical structure of taxonomic classifications to quantitatively (using the median abundance) and statistically depict taxonomic differences between microbial communities. Heat tree visualizations were built from the metacoder package in R v0.3.8 to represent hierarchical taxonomic hierarchies in *K. alvarezii* samples from five sampling sites (Mangadu, Soliyakudi, Nambuthalai, Jagadhapattinam, and Odavimadam).

Functional prediction

The FAPROTAX (Functional Annotation of Prokaryotic Taxa) tool was used to predict potential metabolic functions of important pathways within bacterial communities identified from healthy and diseased samples collected from five seaweed cultivation sites (Table 1).

Data availability

The metagenomic sequencing data from diseased *Kappaphycus alvarezii* have been submitted to the NCBI (https://www.ncbi.nlm.nih.gov/sra/PRJNA1248676) under the BioProject ID PRJNA1248676. The associated Sequence Read Archive (SRA) accession numbers are SRR33066784, SRR33066785, SRR33066786, SRR33066787, SRR33066788, SRR33066789, SRR33066790, SRR33066791, SRR33066792, and SRR33066793.

RESULTS

Physicochemical characterization of seawater of diseased sample collection sites

The average of all physicochemical analyses at each site is presented in (Table 2). Based on the physicochemical parameter results provided for the five cultivation sites, notable differences occurred across the five locations. Air temperatures ranged from 28.6°C to 30.6°C, with Mangadu having the highest temperature (30.6°C). Salinity was relatively consistent across most sites (33.6–36.6 PSU), except for Mangadu (36.6 PSU). The pH ranged from 8.16 to 8.41 in all sites. Nutrient levels varied, with Jegathapattinam and Odavimadam generally showing higher concentrations of nitrites, nitrates, and phosphates. Dissolved oxygen levels varied significantly, from a low of 4.8 mg/L in Odavimadam to a high of 8.4 mg/L in Mangadu.

Table 2
Mean values and standard deviation of physicochemical properties of seawater samples collected from infected seaweed sample collection sites and their dominant microbial phylum level.

Cultivation site	Soliyakudi	Jegathapattinam	Nambuthalai	Odavimadam	Mangadu
Physical parameter					
Air tem. (℃)	28.6 ± 0.5	28.3 ± 0.5	29 ± 0.0	28.3 ± 0.5	30.6 ± 0.5
Water tem. (℃)	27 ± 0.0	26 ± 0.0	26 ± 0.0	25 ± 0.0	28 ± 0.0
Salinity (psu)	33.6 ± 0.5	34 ± 0.0	34 ± 0.8	33.6 ± 1.03	36.6 ± 0.5
pH	8.29 ± 0.03	8.35 ± 0.02	8.41 ± 0.01	8.3 ± 0.01	8.16 ± 0.06
Water current (M/min)	4 ± 0.0	6.5 ± 0.0	3.4 ± 0.0	6 ± 0.0	5 ± 0.0
Chemical parameter					
No_2^-N (mg/l)	0.003 ± 0.0005	0.009 ± 0.0018	0.004 ± 0.001	0.014 ± 0.002	0.004 ± 0.004
No_3^-N (mg/l)	0.027 ± 0.0005	0.087 ± 0.0019	0.09 ± 0.02	0.044 ± 0.005	0.035 ± 0.003
Po_4^- N (mg/l)	0.038 ± 0.002	0.091 ± 0.001	0.052 ± 0.02	0.044 ± 0.004	0.048 ± 0.047
NH_3 (mg/l)	0.004 ± 0.001	0.007 ± 0.0008	0.005 ± 0.02	0.008 ± 0.0008	0.004 ± 0.001
SiO_2 (mg/l)	0.045 ± 0.0004	0.055 ± 0.005	0.053 ± 0.005	0.105 ± 0.001	0.074 ± 0.001
D.O (mg/l)	5.6 ± 0.1	5.8 ± 0.02	6.3 ± 0.4	4.8 ± 0.7	8.4 ± 0.3
Microbial Diversity (Phylum level)					
Diseased dominant bacteria	Gammaproteobacteria and Alphaproteobacteria	Gammaproteobacteria and Clostridia	Gammaproteobacteriaand Clostridia	Gammaproteobacteria, Clostridia and Alphaproteobacteria	Gammaproteobacteria and Alphaproteobacteria
Healthy dominant bacteria	Gammaproteobacteria	Gammaproteobacteria and Clostridia	Gammaproteobacteriaand Clostridia	Gammaproteobacteriaand Clostridia	Gammaproteobacteria

Physicochemical properties of seawater from infected seaweed cultivation sites. Values are presented as mean ± standard deviation. The table also shows the dominant microbial phylum present in diseased and healthy samples at each site.

Microbial Diversity and Community Composition

The bacterial community structure associated with *K. alvarezii* shows significant variation between diseased and healthy samples from different sites (Fig. 2). This study examines bacteria's phylum and genus-level diversity, revealing distinct patterns of abundance and composition that offer insights into the complex relationship between *K. alvarezii* and its associated bacterial communities.

At all the site, Proteobacteria phylum was found to be dominant, among them, Gammaproteobacteria was consistently prevalent. At the phylum level, the diseased samples from Mangadu and Soliyakudi showed a high abundance of both Gammaproteobacteria and Alphaproteobacteria. In contrast, their healthy counterparts showed a low abundance primarily of Gammaproteobacteria. The Nambuthalai and Jegadhapattinam sites displayed a high abundance of Gammaproteobacteria and *Clostridia* in diseased samples, with low to moderate abundance in healthy samples. Odavimadam uniquely showed an equal abundance in both diseased and healthy samples, though with slightly different compositions.

At the genus level (Fig. 3), in the Mangadu diseased sample, high abundance of Others, Cobatia, and Halomonas was observed, while its healthy sample contained low abundance of only *Cobatia* and *Halomonas*. In Soliyakudidiseased sample was dominated by *Pseudoalteromonas* and *Cobatia*, while Nambuthalai's diseased sample showed high abundance of unclassified genera and *Pseudoalteromonas*. In Jegadhapattinam, the diseased sample was characterized by Pseudoalteromonas and Psychrobacter, and in Odavimadam, the diseased sample contained high levels of unclassified genera and *Oceanisphaera*.

Diversity analysis

While conducting ecological analysis and studying ecological communities, it is essential to measure the community diversity. In this study, a total of 2,071,476 high-quality reads were obtained for the bacterial species, among which 80,977 were unique reads with an average length of 250 bp. It was clear that the diseased samples had more unique raw reads as compared to the healthy samples. Overall, among the five sample sites, the raw reads were comparatively fewer (1,145,158) in diseased *K. alvarzii* samples than in the healthy ones (1,219,912). The bacterial communities from the five closely located sites were compared between the healthy and diseased samples. From Jegathapattinam, Mangadu, Nambuthalai and Odavimadam sites, the healthy (243,634; 268,598; 260,420; 226,420) samples had more raw reads as compared to diseased samples (234,020; 200,594; 260,142; 203,752). Although in the Soliyakkudi site, the healthy sample (220,546) had fewer raw reads as compared to the diseased ones (246,650).

A total of 2,020 bacterial OTUs were identified across all 5 seaweed samples, i.e., 331 from Jegathapattinam, 345 from Mangadu, 504 from Nambuthalai, 581 from Odavimadam, and 259 from Soliyakkudi (Supplementary Table 1). This clearly shows that the bacterial diversity is higher in samples from Odavimadam and lower in samples from Soliyakkudi. This difference was statistically studied with the help of rarefaction curves, Shannon index, relative abundance plots, etc.

The rarefaction curve is generally used to deduce that the volume of the sequenced reads is reasonable by assessing the abundance of the bacterial OTUs, which are close to saturation. The rarefaction curves revealed that the alpha diversity of the bacterial lineages for the seaweed samples from Nambuthalai was higher among the diseased samples (OTU > 450; Raw Reads > 45,000) and Odavimadam was higher among the healthy samples (OTU > 400; Raw Reads > 50,000) compared to the other 4 sites' samples. (Fig. 4).

The Shannon index depicts the statistical results of the community diversity in each sample. The higher Shannon index value suggests that the diversity of organisms is rich. The 10 different samples (diseased and healthy each) from 5 different sites illustrate that, between the diseased and healthy samples, the former has more microbial diversity compared to the latter. Overall, the Odavimadam site has a higher Shannon index (diseased > 3.7 and healthy > 3), which portrays that there are more diversified microbes in this site compared to the other 4 sites (Fig. 5).

Relative Abundance across the sampling sites

A total of 22 bacterial phyla were detected in the *K. alvarezii* healthy seaweed samples, and about 22 bacterial phyla were detected in the *K. alvarezii* diseased seaweed samples. Proteobacteria (41.16%) and Actinobacteria (38.33%) were the most abundant phyla in each of the seaweed niches. The major phyla in healthy and diseased *K. alvarezii* seaweed samples were similar, with Proteobacteria (83.4%), Firmicutes (10.2%), and Actinobacteria (4.5%) being the most abundant phyla. The frequency of bacterial phyla in the healthy and diseased *K. alvarezii* seaweed samples varied greatly. The abundance of Proteobacteria was higher in healthy *K. alvarezii* seaweed samples (87.4%) compared to diseased *K. alvarezii* seaweed samples (75.8%), which was the most abundant phylum in all the samples, followed by Firmicutes and Actinobacteria. Of the 167 bacterial classes, Gammaproteobateria, Alphaproteobacteria, Clostridiales, Veillionellaceae had a higher abundance, in diseased *K. alvarezii* seaweed samples whereas, Gammaproteobateria, Alphaproteobacteria and Clostridiales had a higher abundance, in healthy *K. alvarezii* seaweed samples (Fig. 6). Among the multiple genus identified, *Pseudohalomonas*, *Halomonas*, *Cobetia*, *Vibrio* and *Agathobacter* were the most abundant in the diseased *K. alvarezii* samples however, *Pseudohalomonas*, *Halomonas*, *Cobetia*, and *Agathobacter* were the most abundant in the healthy *K. alvarezii* samples (Fig. 6).

In the Mangadu site's diseased sample, the major bacterial phyla were Proteobacteria (76%), Firmicutes (13%), Actinobacteria (4%) and Acidobacteria (3%). The major bacterial class were, Gammaproteobacteria (57%), Alphaproteobacteria (18%) and Clostridiales (10%) and the major bacterial genus were *Cobetia* (37%), *Halomonas* (11%), *Azospirillum* (2%), *Sphingomonas* (4%), *Agathobacter* (4%), *Vibrio* (2%), and *Pseudohalomonas* (2%). In the healthy ones the major bacterial phyla were Proteobacteria (95%), Firmicutes (3%), the major bacterial class were Gammaproteobacteria (95%), and Clostridiales (3%), the major bacterial genera were *Cobetia* (85%), *Halomonas* (9%) and *Agatobacter* (1%).

In the Soliyakkudi site, the major bacterial phyla discovered in the diseased sample were Proteobacteria (81%), and Firmicutes (11%), the major bacterial class were Gammaproteobacteria (77%), Clostridiales (8%), Alphaproteobacteria (3%), Veillonellaceae (3%) Gammaproteobacteria (2%), and finally, the major bacterial genus were *Paeudohalomonas* (38%), *Cobetia* (23%), Vibrio (10%), *Agathobacter* (4) and *Halomonas* (3%), whereas in the healthy ones the major bacterial phyla were Proteobacteria (95%), Firmicutes (4%), the major bacterial class were Gammaproteobacteria (95%), Clostridiales (3%), the major bacterial genus were *Cobetia* (66%), *Paeudohalomonas* (23%), *Halomonas* (6%), *Agathobacter* (2%).

In the Nambuthalai site, the major bacterial phyla identified in the diseased sample were Proteobacteria (68%), Firmicutes (16%), Actinobacteria (9%). The major bacterial class were Gammaproteobacteria (64%), Clostridiales (12%), Actibnobacteria (3%). Majorly bacterial genus such as *Cobetia* (29%), *Pseudohalomonas* (22%), Vibrio (6%), *Agathobacter* (4%), *Halomonas* (4%), *Alphaproteobateria* (3%) were observed. In the healthy ones the major bacterial phyla were Proteobacteria (95%), Firmicutes (7%). Gammaproteobacteria (92%), Clostridiales (4%) and Bacilli (2%) were observed as the major bacterial class. The major bacterial genus were *Cobetia* (66%), *Pseudohalomonas* (11%), *Halomonas* (14%), *Agathobacter* (2%), *Lactobacillus* (1%).

In the Jegathapattinam site, the major bacterial phyla spotted in the diseased sample were Proteobacteria (75%), Firmicutes (19%), Actinobacteria (3%) the major bacterial class were Gammaproteobacteria (74%), Clostridiales (15%), Veillonellaceae (3), Actinobacteria (3%) and Alphaproteobacteria (1%) and the major bacterial genus were *Pseudoalteriomonas* (28%), *Psychobacter* (17%), *Cobetia* (10%), *Vibrio* (7%), *Agathobacter* (6%), *Shewanella* (4%) and *Dialister* (2%) whereas in the healthy ones the major bacterial phyla were Proteobacteria (78%), Firmicutes (21%), the major bacterial class were Gammaproteobacteria (78%), Clostridiales (17%), and the major bacterial genus were *Pseudoalteriomonas* (43%), *Cobetia* (20%), *Halomonas* (11%), *Agathobacer* (4%), *Clostridiun sensu strico 1* (3%), *Pseudostreptococcaceae* (3%), *Shewanella* (2%), and *Vibrio* (1%).

In the Odavimadam site, the major bacterial phyla discovered in the diseased sample were Proteobacteria (79%), Firmicutes (13%), and Actinobacteria (4%), the major bacterial class were Gammaproteobacteria (67%), Alphaproteobacteria (9%), Clostridiales (9%), Deltaproteobacteria (5%), Veillonellaceae (3%), Actinobacteria (2%) and the major bacterial genus were *Oceanisephaera* (21%), *Pseudoateriomonas* (12%), *Cobetia* (11%), *Vibrio* (10%), *Psycrobacter* (7%), *Agathobacter* (4%), *Shewanella* (2%), *Dialister* (2%) and *Halomonas* (1%) whereas in the healthy ones the major bacterial phyla were Proteobacteria (77%), Firmicutes (14%) and Actinobacteria (5%) the major bacterial class were Gammaproteobacteria (75%), Clostridiales (9%), Alphaproteobacteria (9%), Veillonellaceae (3%), Actinobacteria (2%) and the major bacterial genus were *Cobetia* (50%), *Halomonas* (13%), *Pseudoalteriomonas* (10%), *Agathobacter* (4%), *Alphaproteobacteria* (2%) (Supplementary Fig. 1).

Heat Tree Analysis of Bacterial Communities

Heat tree analysis emphasized the taxonomic structure and abundance structures of bacterial communities linked to *K. alvarezii* across five sampling sites, each with peculiar compositions. Mangadu site shows a complex structure and balanced abundance among diverse bacterial lineages. Moderate complexity with clustered taxa and specific abundance patterns was observed at Soliyakudi site. Whereas, a simplified structure with lower diversity and dominance of certain groups was noted at the Nambuthalai site. The Jagadhapattinam site was observed with intermediate complexity and was characterized by unique patterns. The Odavimadam site revealed the highest complexity and diversity, with even abundance distribution across taxonomic levels. The heat trees effectively illustrated the quantitative differences in bacterial abundance, emphasizing the taxonomic hierarchy within each site microbiome (Fig. 7a-e).

Functional prediction of bacteria associated with diseased and healthy

The functional prediction analysis revealed that the functional groups of the bacterial microbiota associated with seaweed were similar (Fig. 8). Although the microbial composition associated with both healthy and diseased varied across collection sites. In Mangadu, the diseased state (KaMD) is characterized by high expression of fermentation, nitrate reduction, nitrate respiration, ureolysis, methylotrophy, nitrogen fixation, and methanol oxidation pathways. In contrast, the healthy state (KaMDH) shows upregulation of chemoheterotrophy, aerobic chemoheterotrophy, and hydrocarbon degradation. Soliyakudi shows a diseased condition (KaSD) with enrichment in fermentation, chloroplast activity, sulfate respiration, and nitrogen respiration. The diseased state in Soliyakudi (KaSH) exhibits a high expression of aerobic chemoheterotrophy, chemoheterotrophy, and hydrocarbon degradation. In Namuthalai, the diseased state (KaND) demonstrates elevated activity in fermentation, nitrification, aerobic ammonia oxidation, nitrate reduction, nitrogen respiration, and nitrate respiration. This contrasts with the healthy state (KaNH), which shows strong activity in aerobic chemotrophy, chemoheterotrophy, and hydrocarbon degradation. Jagathapattinam exhibits a diseased condition (KaJD) with upregulation of aerobic chemotrophy, chemoheterotrophy, and hydrocarbon degradation pathways. The healthy state (KaJH) shows strong expression of nitrification, nitrogen respiration, fermentation, nitrate reduction, and sulfate respiration pathways. Finally, Odavimadam reveals a diseased state (KaOD) with enrichment in nitrate reduction, nitrogen respiration, sulfate respiration, respiration of sulfate compounds, and fermentation. The healthy state (KaOH) exhibits high activity in nitrification, hydrocarbon degradation, aerobic chemoheterotrophy, and chemoheterotrophy (Fig. 8).

Discussion

The present study evaluated the microbial community structure of healthy and diseased *K. alvarezii* collected from five different seaweed cultivation sites in southern coast of Tamil Nadu, India (Fig. 1). In the year of 2013, severe impact was observed on cultivation of *K. alvarezii* according to the reported findings of Mantri et al. (2022), that have an influential role on seaweed cultivators to abandon their practices because of the seaweed diseases which remained unidentified (Mantri et al., 2024). In the beginning, the change of climate was considered to be the cause, however, later it was discovered that an opportunistic pathogen was found to be responsible for disease (Largo et al., 2017). This metagenomic analysis of healthy and diseased *K. alvarezii* were were collected from five different active cultivation sites helped to decipher the root cause behind seaweed disease in India.

Physicochemical parameters at five cultivation sites significantly influence microbial communities associated with seaweed in both healthy and infected samples (Table 2). Higher temperatures and salinity at Mangadu (30.6°C, 36.6 PSU) favor specific microbial taxa, altering diversity and metabolic functions. Dissolved oxygen levels (4.8 mg/L in Odavimadam to 8.4 mg/L in Mangadu) influence the abundance of aerobic and facultative anaerobic microorganisms, affecting community composition. Elevated nitrite, nitrate, and phosphate levels in Jegathapattinam and Odavimadam promote unique nutrient-cycling groups that may alter seaweed-microbe interactions. Slight variations in pH (8.16–8.41) can affect enzyme activity and overall metabolism within the seaweed microbiome, influencing responses to environmental factors (Menaa et al., 2020; Minich et al., 2018).

The analysis of microbial diversity revealed that the microbiome associated with both healthy and diseased seaweed varies at each collection site (Figs. 2 and 3) and may be influenced by environmental factors (Ndawala et al., 2022; Faisan et al., 2024). Abiotic stressors impact the structure of the bacterial community, even during a single intertidal period, due to unstable of levels of desiccation, temperature, light, and humidity (Vigil et al., 2024; Centurion et al., 2021). Consequently, the microbiomes at different sites varied significantly from one another (Vohsen et al., 2024). Furthermore, there is a phenomenon known as the host effect, which refers to the ability of seaweed to produce metabolic substances that selectively influence microbial adhesion and settlement in their environment (Mesquita et al., 2019; Singh et al., 2014). This process contributes to the development of specific microbial communities. It also activates genes in the bacterial community that are essential for metabolizing substrates or producing metabolites, many of which are beneficial to the host (Lu et al., 2025).

The identified microbiome exhibited differences from those found in both healthy and diseased individuals at various sites (Fig. 2). In the present study, Proteobacteria emerged as the predominant phylum across all sampling sites, with Gammaproteobacteria consistently detected as a major class. The

dominance of Proteobacteria in seawater samples from all five locations suggests their ecological versatility and potential role in shaping microbial community structure, as also highlighted in previous reports (Gu et al., 2023). The bacterial phylum distribution across the five sampling sites reveals important patterns in the comparison of diseased and healthy *K. alvarezii* samples. The dominance of Gammaproteobacteria and Alphaproteobacteria in diseased samples from Mangadu and Soliyakudi suggests these groups may be crucial to disease processes. The proliferation of opportunistic microorganisms in response to seaweed indicates ecological succession (Singh et al., 2018; Egan et al., 2013). The higher abundance of Gammaproteobacteria and Clostridia in diseased samples from Nambuthalai and Jegadhapattinam, compared to healthy ones, suggests a link between these bacteria and disease. Clostridia, being anaerobic, are well-suited to proliferate in environments where tissue degradation leads to diminished oxygen levels and the accumulation of organic matter, thereby creating conditions that support their growth and metabolic activity (Facimoto et al., 2024). In Odavimadam, similar bacterial abundance in both diseased and healthy samples hints at site-specific factors affecting host-microbe interactions, possibly indicating varied disease etiology or stable environmental conditions. These variations at the phylum level highlight the complexity and site-specific nature of *K. alvarezii* disease, showing that local conditions significantly influence microbial dynamics in seaweed cultivation.

The comparison at the genus level of bacterial communities from five sampling locations indicates different patterns associated with *K. alvarezii* disease and healthy samples (Fig. 3). Diseased samples in Mangadu showed high proportions of unclassified genera, *Cobetia*, and *Halomonas*, indicating greater taxonomic diversity with disease development. Existing literature demonstrates that bacterial densities on and inside ice-iced shoots of *K. alvarezii* are 10–100 fold larger than healthy tissue, with microbiome changes playing crucial role a role in the causing ice-ice disease (Riyaz et al., 2020; Largo et al., 1995).

In Soliyakudi site, diseased samples showed enrichment of *Pseudoalteromonas*, a genus reported to secrete bioactive metabolites with the potential to degrade or damage algal tissues, thereby contributing to disease progression. *Pseudoalteromonas* organisms are linked with larger organisms and possessing extracellular biologically active agents, and these algal cell wall degrading bacteria are capable of damaging algal tissues and facilitate an entry for opportunistic bacteria (Hollants et al., 2013). The adoptive evolution of algal polysaccharide degradation in *P. carrageenovora* shows them to be specialized and adapted to seaweed habitats, with an estimated half of seaweed biomass made up of polysaccharides that these bacteria are capable of breaking down (Gobet et al., 2018).

Nambuthalai site samples also contained high levels of *Pseudoalteromonas* and unclassified bacteria, suggesting polymicrobial disease communities of high complexity. At Jegadhapattinam, the presence of *Pseudoalteromonas* and *Psychrobacter* suggests distinct disease mechanisms, while high occurrences of Oceanisphaera in Odavimadam diseased samples indicate potential new pathogenic associations. The Formation of ice-ice disease in *K. alvarezii* is related to microbiome changes influencing carrageenan production and seaweed structural integrity (Riyaz et al., 2021)

This study generally demonstrated higher bacterial diversity in diseased *K. alvarezii* samples compared to healthy samples, consistent with the findings of Zozaya et al. (2015), as evidenced by Shannon diversity indices (Figs. 4 and 5). The Odavimadam site exhibited the highest overall microbial diversity. This aligns with previous studies suggesting that increased diversity may result from opportunistic colonization when host defense mechanisms are disrupted (Caballero et al., 2023). There are significant differences in bacterial population across sites, with Odavimadam presenting 581 OTUs and Soliyakkudi only 259 (Fig. 4). These discrepancies are likely due to the distinct environmental conditions at each site (Gianoulis et al., 2009). At the phylum level, Proteobacteria were the most abundant in both healthy (87.4%) and diseased samples (75.8%). Interestingly, *Vibrio* sp. was mainly detected in diseased samples, highlighting its possible role as a pathogen (Ward et al., 2022). Overall, it is clearly noticeable that microbial population was strong similar among the Mangadu, Soliyakudi, Nanbuthalai, sites. Alike the Jegadhapattinam and Odavimadam sites showed extreme similarity in microbial community.

The heat tree visualization effectively emphasized taxonomic hierarchies, revealing notable site specific variations in the diversity and abundance of *K. alvarezii* microbiomes across five sampling sites (Fig. 7). The complex relationships within microbial communities, showing that different locations host distinct bacterial assemblages with varying taxonomic complexity (Yan et al., 2024; Juhmani et al., 2020; Xie et al., 2024). The architecture of heat trees, from simple structures to complex communities, reflects local environmental and ecological influences on bacterial community assembly (Xu et al., 2022; Egan et al., 2013).

Despite the differences between groups, some genera, such as *Cobetia*, *Pseudohalomonas*, and *Halomonas*, were consistently present. *Cobetia* was more abundant in healthy samples compared to the diseased ones, suggesting its beneficial role. The association of *Vibrio* with disease highlights its potential as an early microbial indicator (Largo et al., 1998), while beneficial bacteria may provide avenues for developing probiotics to enhance seaweed health (Mahajan et al., 2025).

The functional prediction analysis indicated metabolic changes associated with both healthy and diseased samples (Fig. 8). In contrast, diseased samples displayed enrichment in fermentation, nitrate reduction, nitrogen respiration, and sulfate metabolism pathways at various sites. Meanwhile, healthy samples consistently showed enhanced expression of aerobic chemoheterotrophy and hydrocarbon degradation functions. These results reveal, discrepancies in physicochemical characteristics influenced the microbial community structures, with Odavimadam showing the lowest DO at 4.8 mg/L and Mangadu showing the highest at 8.4 mg/L. These findings clearly show that environmental determinants and disease status significantly impact the composition and functional potential of the bacterial communities associated with *K. alvarezii*.

Conclusion

This study reveals variance in microbial community structures between healthy and diseased *K. alvarezii* across five cultivation sites in Tamil Nadu, India. Proteobacteria were observed to be the dominant class across all sites, in which Gammaproteobacteria were consistently present. Diseased samples show higher bacterial diversity compared to healthy samples, indicating that opportunistic colonization occurs following disruptions in host defenses. *Pseudoalteromonas* and *Vibrio* were primarily identified in diseased samples, indicating their potential role in disease pathology. *Cobetia* was more abundant in healthy samples, indicating the beneficial association with the host. Divergences in microbial communities were site-specific, with differences in physicochemical parameters such as temperature, salinity, dissolved oxygen, and nutrient levels.

Functional prediction analysis revealed that diseased samples had elevated pathways relevant to fermentation, nitrate reduction, and sulfate metabolism. In contrast, healthy samples showed enhanced functions related to aerobic chemoheterotrophy and hydrocarbon degradation. These determinations provide valuable insights into the microbial factors contributing to *K. alvarezii* diseases in Indian cultivation sites since 2013. Identifying potential pathogens and beneficial bacteria paves the way for developing targeted disease management strategies and probiotics to improve the health of seaweed, thereby supporting the sustainable cultivation of this economically vital resource.

Declarations

Ethical approval not applicable.

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Author contribution

N. Dhanya is conducting experiments, writing, and analyses; B. Prakriti, writing, and analyses; Arup Ghosh, monitoring the project. Lakkakula Satish, conceptualization; resources; writing, original draft; writing, review, and editing.

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Data availability

Research data are not shared

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Figures



Figure 1

Infected *Kappaphycus alvarezii* seaweed sample collected from commercial farming sites in Tamil Nadu, India.

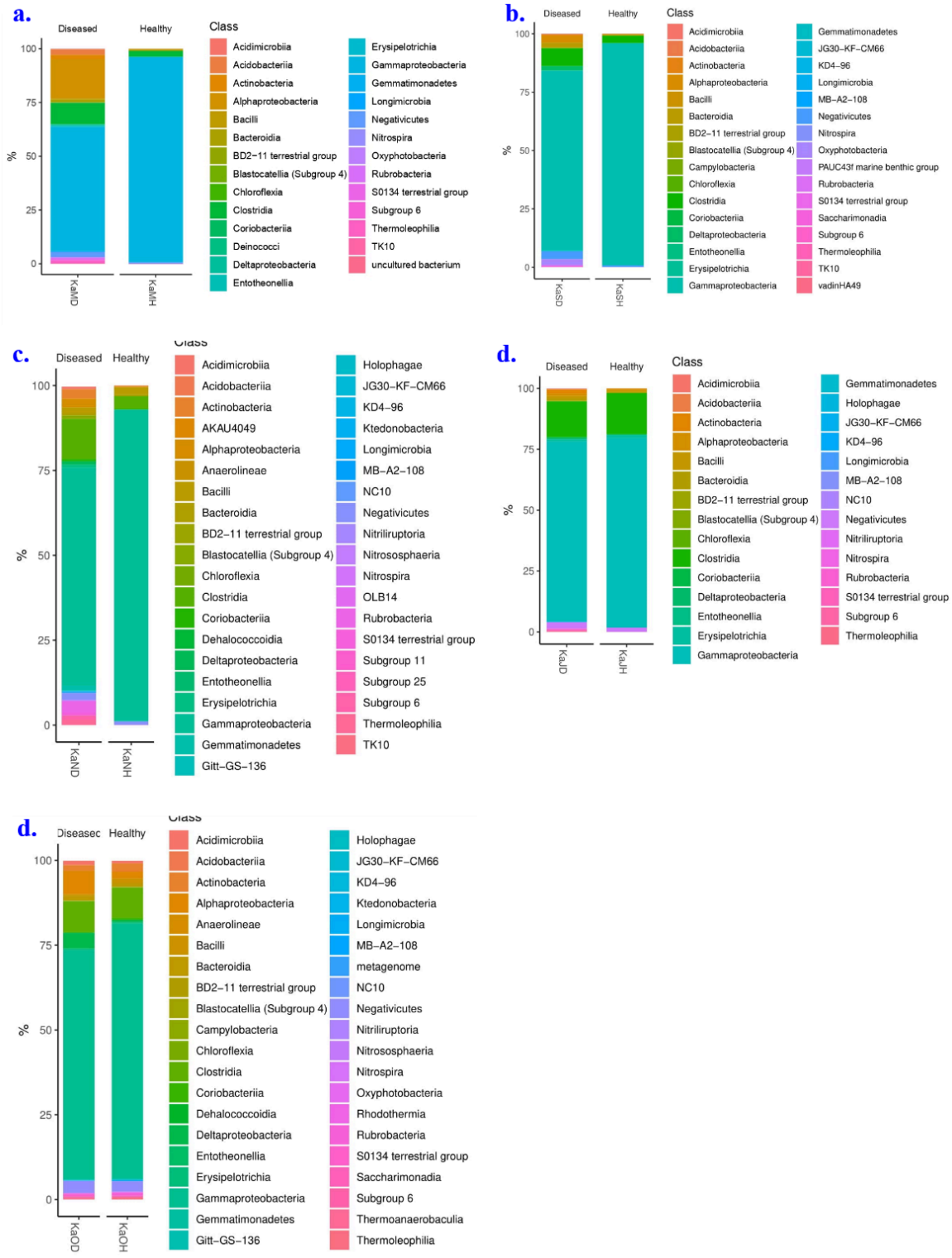


Figure 2

Taxonomic composition of the microbiota associated with healthy and diseased *K. alvarezii*: Relative abundance at the phylum level at different collection sites: a. Mangadu, b. Soliyakudi, c. Nambuthalai, d. Jegathapattinam, and e. Odavimadam.

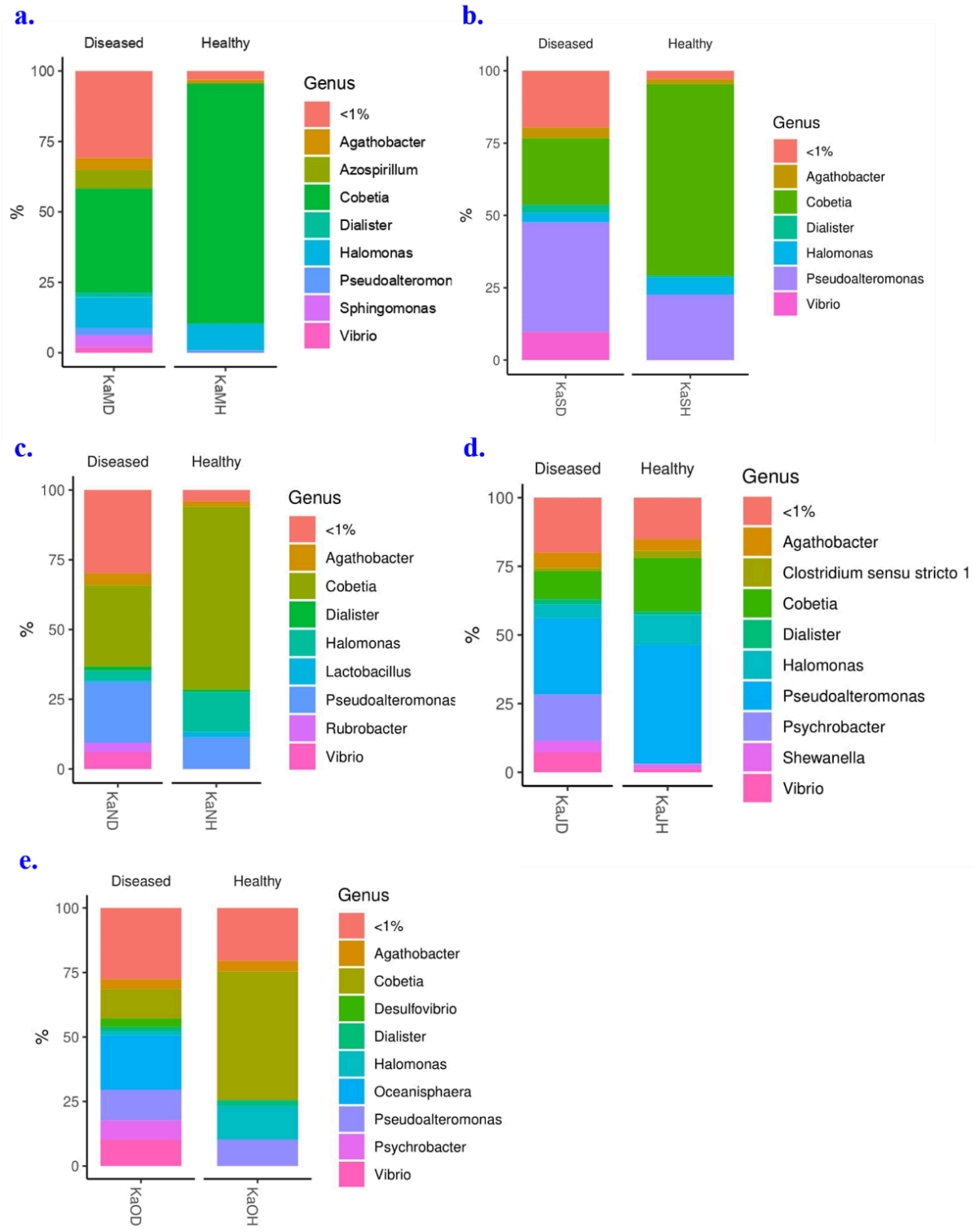


Figure 3

Taxonomic composition of the microbiota associated with healthy and diseased *K. alvarezii*: Relative abundance at genus levels at in the healthy and diseased *K. alvarezii* from 5 different cultivation sites. a. Mangadu, b. Soliyakudi, c. Nambuthalai, d. Jegathapattinam and e. Odavimadam

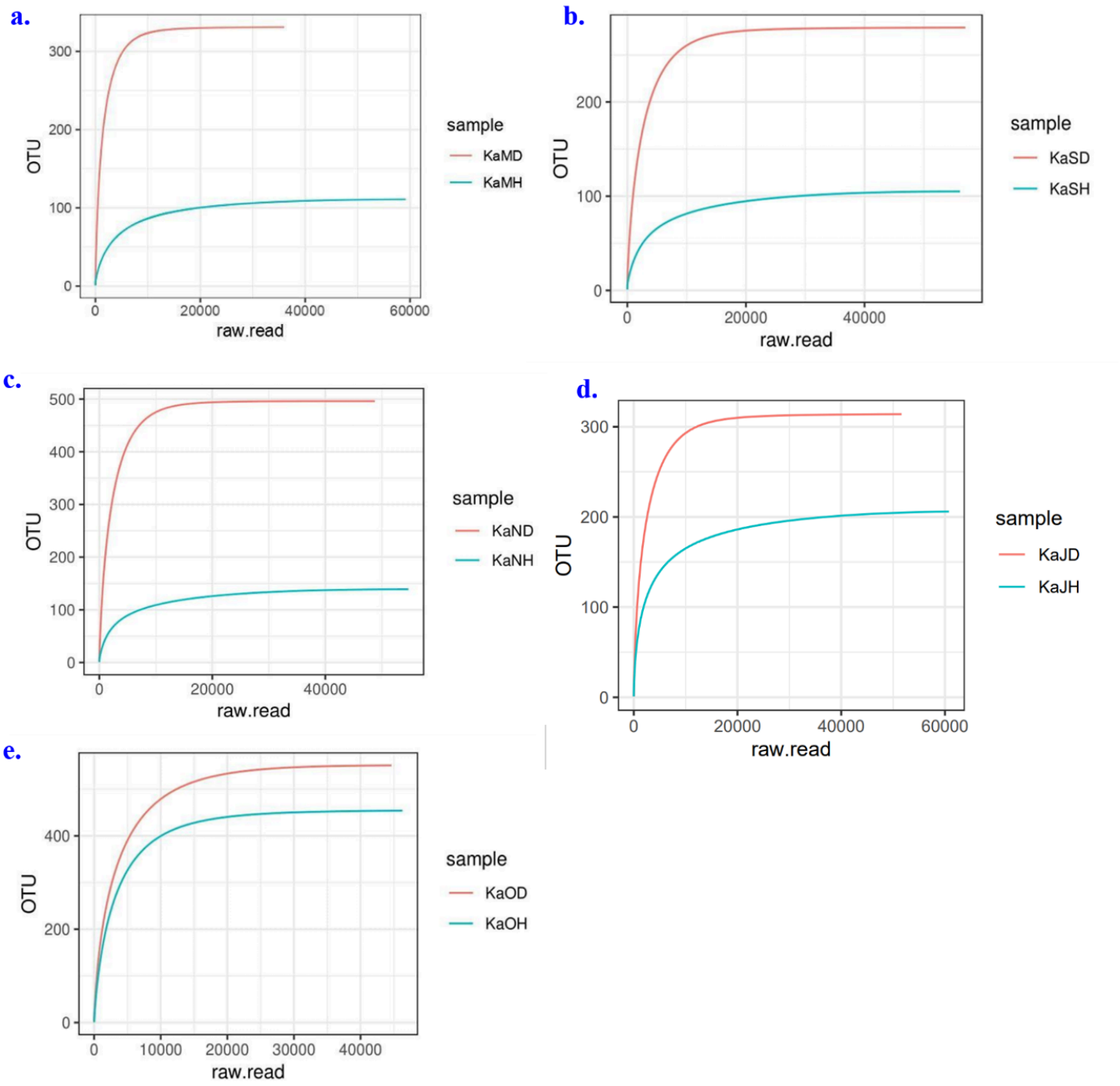


Figure 4

Refraction curve showing the comparison between the operational taxonomic units (OTU) in the healthy and diseased *K. alvarezii* from 5 different cultivation sites a. Mangadu, b. Soliyakudi, c. Nambuthalai, d. Jegathapattinam and e. Odavimadam. The Y-axis depicts the number of OTUs and the X-axis depicts the raw reads. More number of OTUs shows that there are more diverse organisms in the sample.

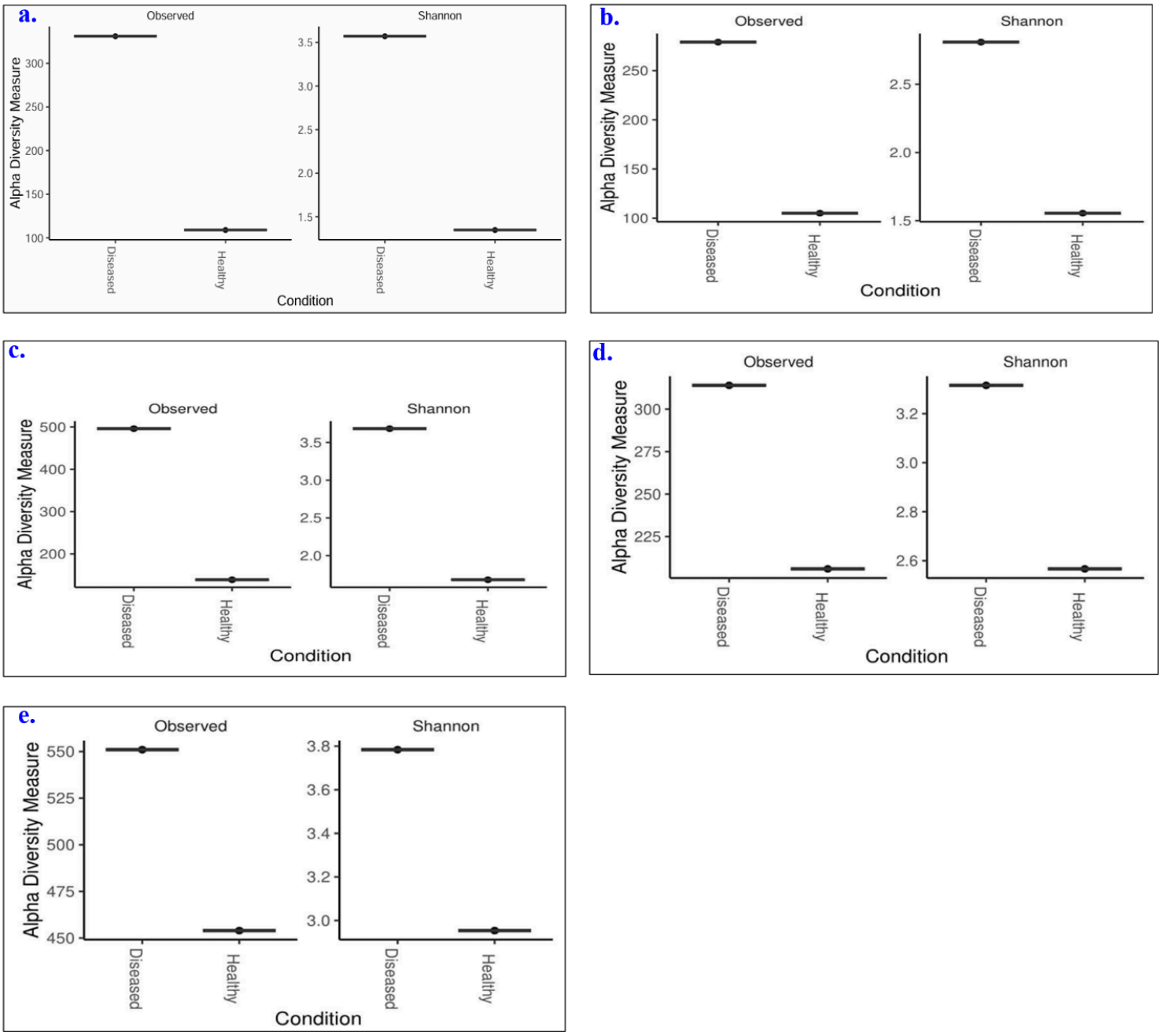


Figure 5

Shannon Index. Stastical report of the diverse communities in the healthy and diseased *K. alvarezii*. a. Mangadu, b. Soliyakudi, c. Nambuthalai, d. Jegathapattinam and e. Odavimadam

Diseased samples have more Shannon index as compared to the healthy ones, depicting that they have more diverse microbial communities. The left graph on each figure depicts the raw data and the right side is the statistically analyzed data showing the Shannon index

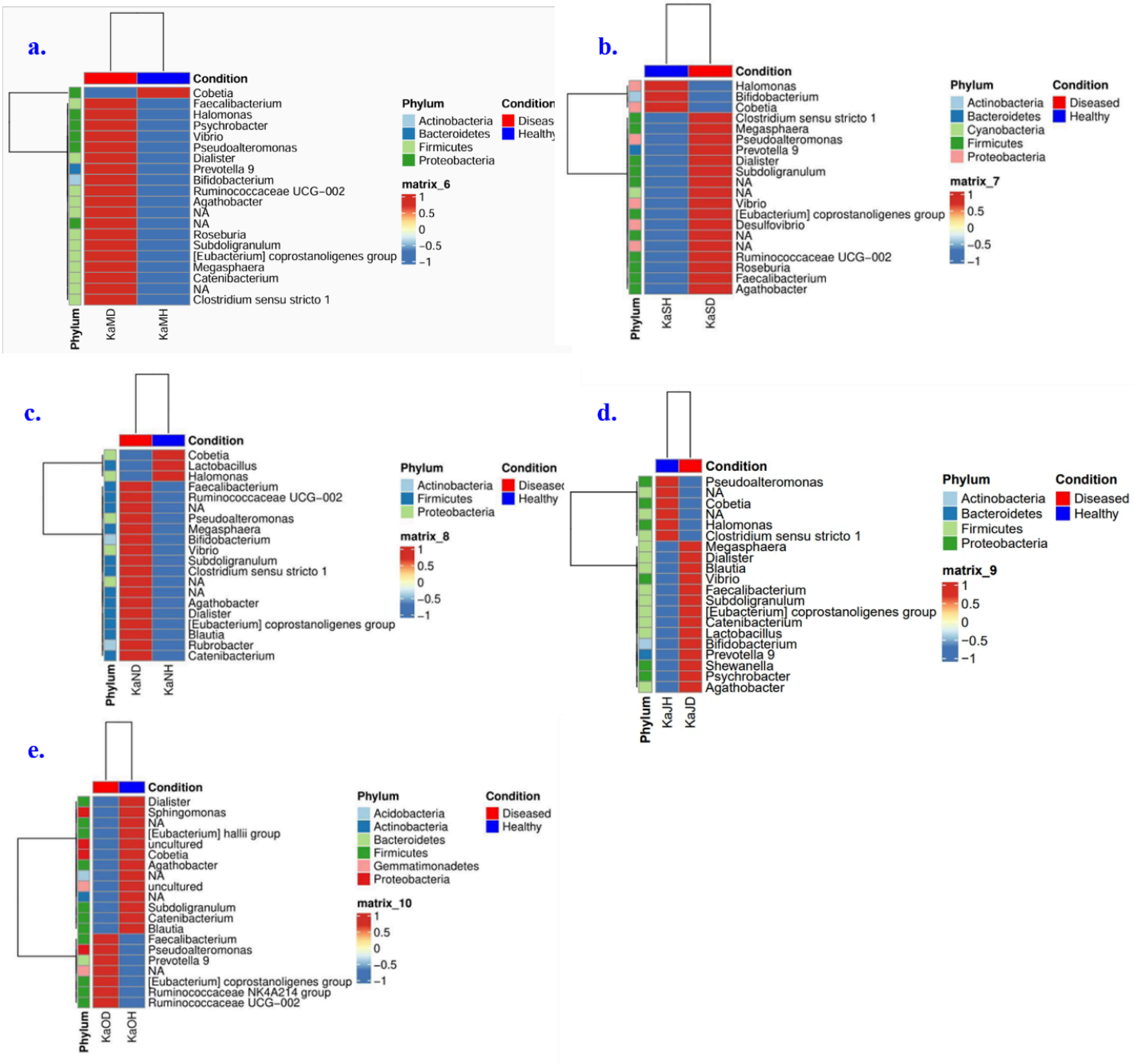


Figure 6

Complex Heat Map. Detailed analysis of the major phylum and genus present in the diseased and healthy *K. alvarezii* samples from the 5 different sites a. Mangadu, b. Soliyakudi, c. Nambuthalai, d. Jegathapattinam and e. Odavimadam. The first column shows phylum abundance, the second and third column shows the genus abundance of diseased and healthy samples. In the genus abundance, the 1 shows highest abundance and -1 depicts the lowest abundance.

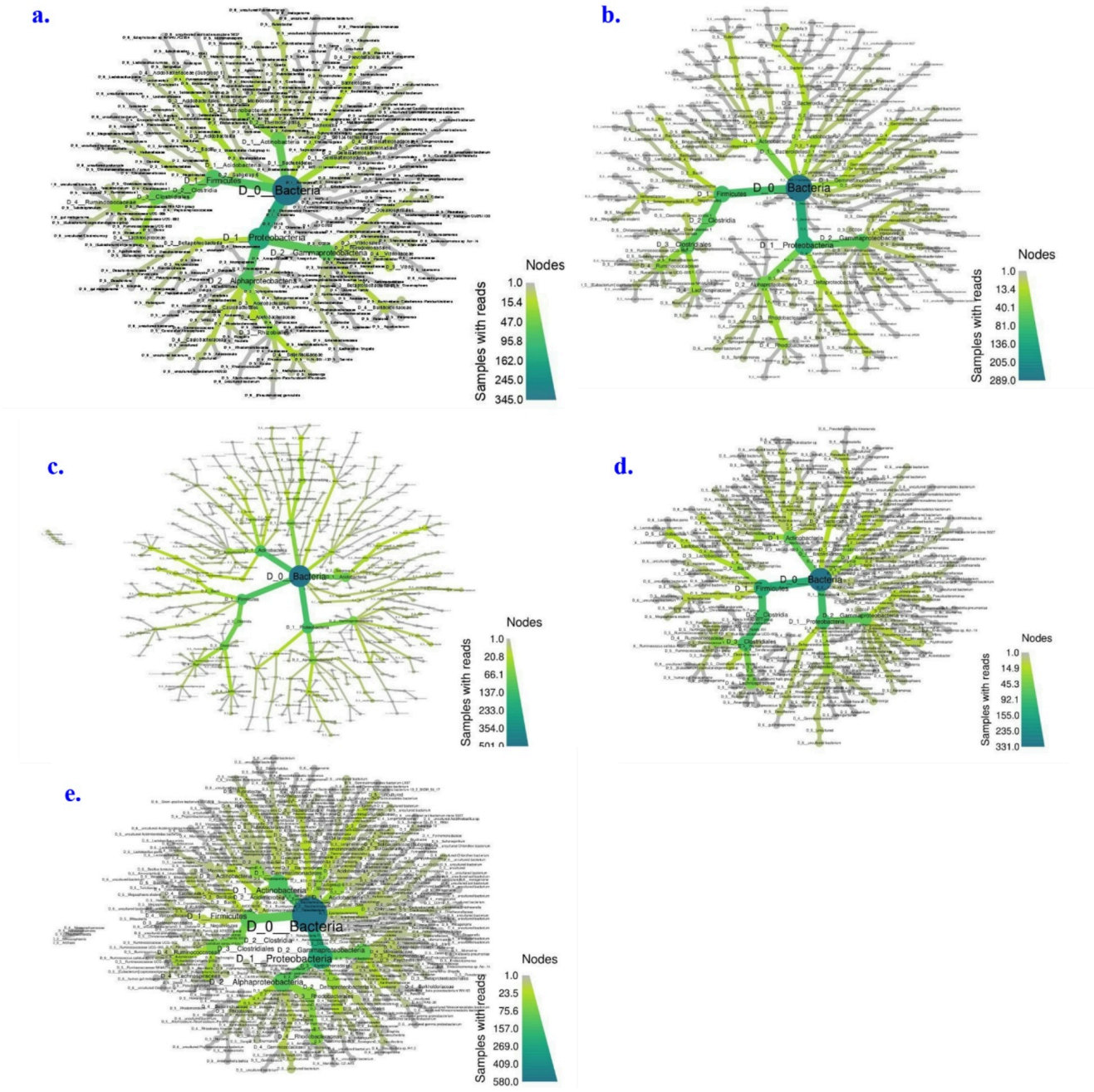


Figure 7

Heat tree of microbiome community structure found in the diseased and healthy *K. alvarezii* samples from the 5 different sites a. Mangadu, b. Soliyakudi, c. Nambuthalai, d. Jegathapattinam and e. Odavimadam. Size and color of nodes and edges show their abundance in the microbial community.



Figure 8

Predicted pathways of the microbiota associated with diseased and healthy *K. alvarezii* for biological functions. a. Mangadu, b. Soliyakudi, c. Nambuthalai, d. Jegathapattinam and e. Odavimadam.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SeaweedMetagenomeSupplementary.docx
- SeaweedMetagenomeSupplementaryExcel.xlsx