Dead Ecosystems Tell No Tales: How Mangrove Mortality Silences Polyextremophilic Enzymes Screening

POLYEXTREMOPHILIC ENZYMES BY MANGROVE MICROBIOME

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Abstract:

The reliance on fossil energy is a significant issue that poses serious environmental and economic problems, fueling the search to find alternative sources of energy that are sustainable. The present study harnesses combined metagenomic-culturomics research to realise the bio-technological capabilities of mangrove microbiomes, screening bacterial isolates of alive and dead ecosystems for poly-extremophilic lignocellulolytic enzymes to solve the fossil energy crisis. The metagenomic profiling showed that changes in the microbial community composition and predicted functional potential differences were significant between alive and dead mangrove ecosystems. Mangrove metagenomic bacterial communities were more prosperous regarding metabolic diversity and the abundance of genes encoding biosynthetic enzymes. Likewise, PICRUSt2 assisted in comprehending that dead mangrove sites were enriched in necrotrophic and stress-related enzymes, including cytochrome c oxidase and N-acetylmuramoyl-L-alanine amidase. A significant percentage of isolates exhibited acidothermolytic capabilities. Interestingly, the enzyme versatility was more widely observed in Alive mangrove locations, whereas some dead mangrove locations contained poly-extremophilic lignocellulolytic capacity. Notably, *Vibrio* abundance became a relative measure of ecosystem vitality, directly portraying lignocellulolytic enzyme biotropy. This study overturns the conventional divide between ecology and biotechnology, showing that mangrove microbiomes are living libraries of industrial biocatalysts--in cases where degraded ecosystems constitute irreversible biotechnological losses.

Keywords: Ecosystem biomarkers, extremozymes, lignocellulosic biofuels, mangrove microbiome, poly extremozymes, sustainable biotechnology

1. Introduction

The dependence on traditional fossil fuels presents notable environmental, economic, and energy challenges, sparking an exciting quest for sustainable alternatives. A worsening climatic emergency and increased environmental demands have intensified the search for sustainable alternatives to using fossils, with transport representing 14 % of greenhouse-gas emissions in the energy sector (Lamb et al., 2021). Of the renewable energy options, lignocellulosic biofuels are the most promising, with 86-108 % greenhouse-gas reduction, compared with the petroleum-based fuels, and they do not compete with agriculture (Wang et al., 2012).

Lignocellulosic biomass is the largest renewable carbon source on earth, as it is estimated to provide about 200 billion tonnes annually in the form of agricultural wastes, forestry wastes, and energy crops (*FAO Knowledge Repository*, n.d.). In relation, the lignin matrix inhibits the accessibility of cellulose and hemicellulose for further processing (H. Chen et al., 2016). Efficient bioconversion will thus require microbial enzymes such as laccase, cellulase, and xylanase with the ability to break down these complicated polysaccharides into sugar substrates (Joshi et al., 2022; Obeng et al., 2017; Wen et al., 2009). Xylose isomerase (XI) plays a crucial role by converting xylose, the second most abundant lignocellulosic sugar, into xylulose for microbial fermentation, enhancing biofuel yield and economic viability (Hahn-Hägerdal et al., 2007; Zhang et al., 2007; Kim & Dale, 2005).

The traditional degradation of lignocellulose is based on the use of energy-intensive physical methods in the form of milling, grinding, and even irradiation, which often result in the incomplete degradation of the compounds and the production of highly undesirable by-products (Marzo-Gago et al., 2023; Rabemanolontsoa & Saka, 2016). Extremozymes offer superior alternatives, demonstrating remarkable catalytic activity and stability under extreme industrial settings (Chettri et al., 2021; Espina et al., 2021; Rotter et al., 2021).

Enzymes in the mangrove microbiome represent an untapped industrial resource. Mangrove-derived mesophilic bacteria produce remarkably stable enzymes that challenge conventional assumptions about growth-activity relationships (Ravi et al., 2025). Furthermore, mangrove bacterial species secrete xylanase exhibiting exceptional elevated temperatures, optimum activity at 60°C (P. D. Parab & Khandeparker, 2025). This disconnect between organismal growth optima and enzyme functionality suggests environmental pressures select for intrinsically robust enzyme architectures regardless of host classification (Yu et al., 2023).

Despite recognition of mangrove biotechnological potential, systematic screening for polyextremophilic lignocellulases remains limited. Previous studies focused on enzyme activities under single-extremophilic conditions, overlooking poly-extremophilic functionality (Pérez-Llano et al., 2020; Rodríguez et al., 2024). Additionally, the

relationship between ecosystem health and microbial enzyme diversity is poorly understood, though degraded mangroves show an 80% reduction in functional enzyme diversity (T. Chen, 2024; Williams et al., 2025).

Indian mangroves cover 4,991.68 km² across nine coastal states, with Goa's mangroves experiencing significant anthropogenic pressure (*India State of Forest Report 2023*, n.d., p. 85; A. Parab, 2025). The distinction between alive (AM) and dead (DM) mangrove sites provides unique opportunities to investigate how ecosystem deterioration influences microbial community structure and biotechnological potential (Kutty et al., 2021).

This study addresses critical knowledge gaps through a comprehensive screening of bacterial isolates for lignocellulolytic activities under multiple extreme conditions. Our approach combines: (1) systematic sampling from both alive and dead mangrove sites; (2) screening for four key enzymes under normal, acidic (pH 5), thermophilic (50°C), and acido-thermophilic conditions; (3) integrated metagenomic and culture-based approaches; and (4) correlation of enzyme activities with environmental parameters.

We propose that mangrove ecosystems represent untapped reservoirs of industrially relevant poly-extremozymes, establishing a framework for sustainable bioprospecting that aligns conservation objectives with industrial innovation while providing breakthrough solutions for cost-effective lignocellulosic biofuel production.

2. Materials and methods

2.1. Sampling Locations

For sampling 13 mangrove locations along the west coast of Goa, and categorising them regarding vegetation health and historical mortality as 10 living mangroves (AM; M1-M10) and 3 dead mangroves (DM; D1-D3)(Figure 1-2, Supplementary Material 1; Table S1). The water, sediment and root samples were gathered during low tide, transported at 4°C and processed within 6 h.

TABLE 1: The table shows the pH, temperature, salinity, location and number of isolates from different sampling sites.

FIGURE 1: Study sites showcasing the vast difference between AM and DM

FIGURE 2: Map of the Mangrove Sampling Sites Across Goa. Sites M1-M10 are denoted for AM sites. Sites D1-D3 are denoted for DM sites.

2.2. Physico-chemical Analysis

Calibrated probes were used to monitor in situ pH, temperature and salinity. On a SKALAR autoanalyser, using standard procedures, dissolved nutrients (NO_3^-, NO_2^-, PO_4^{3-}, NH_4^+, SiO_4^{4-}) were determined (Grasshoff et al., 2009).

2.3. DNA Extraction and Metagenomic Sequencing

The DNA was purified with the NucleoSpin workflow: optimised to extract DNA in 350 mg sediment, pre-wash in 8.5% NaCl, chemical lysis in SL1/SL2/SX and bead-beating (2 × 7 min). Full-length 16S rRNA amplicons were produced with the ONT 16S barcoding kit 24 V14 (SQK-16S114.24) in the presence of LongAmp polymerase (200 nM primers; 12.5 µL mix; 5 µL template; 25 µL total). Cycling (adapted from Matsuo, 2023) was: 95 °C 3 min; 5 cycles of 95 °C 15 s, 55 °C 15 s, 65 °C 90 s; 25 cycles of 95 °C 15 s, 62 °C 15 s, 65 °C 90 s; final extension 65 °C 5 min (Matsuo, 2023). The library was sequenced using flow cell R10 (FLO-MIN114), and base calling was performed on MinION Mk1B using an accurate base calling model and read filtering of min_score = 9. Epi2me was used to demultiplex the samples, filter reads retaining a size range of 1.0–1.8 kb, and assign the reads to its taxonomic group with default parameters using Kraken2 and SILVA 16S (Supplementary Material 1; Figure S1). Functional prediction was carried out using PICRUSt2 (v2.5.3) with NSTI cutoff 2.0.

2.4. Collection of the culturable bacterial diversity

Site-specific water was used to recover culturable bacteria in modified nutrient agar plates. Serial diluents (10^{-1} to 10^{-2}) were inoculated and incubated at 37 °C for 24 hours. Sequencing 16S rDNA (primers 27F/1492R; Eurofins) and NCBI BLAST provided evidence of the taxonomy: The sequences were deposited as accession numbers PV789845-PV790020 (Data in Supplementary Material 2).

2.5. Qualitative Screening of Lignocellulolytic Activity

The lignocellulolytic potential of 212 isolates was screened on a basal salt solution (BSS; per litre NaCl 25 g, MgSO4 0.75 g, NH4Cl 0.5 g, CaCl2 0.1 g, K2HPO4 (10%) 7 ml, KH2PO4 (10%) 3 ml; agar 3%) at pH 7/5 and 37/50 °C. A pH 5 was established for the growth medium by making a BSS solution in citrate buffer with double-standard agar. For cellulase screening, the Gram's iodine method was used; briefly, the isolates were grown in carboxymethyl cellulose (CMC) (0.5%), peptone B (0.2%), and yeast extract (0.2%) (Kasana et al., 2008). For Laccase screening 2, 6-dimethoxy phenol (DMP) 0.65 mM was added to Luria Bertani (LB) plates (Ambika et al., 2022). Further, for xylanase, xylan (0.5%), peptone B (0.2%), and yeast extract (0.2%) were added to the media, and Congo red was used for screening (Khandeparker et al., 2017). TTC (2,3,5-triphenyl tetrazolium

chloride) medium was used to prepare xylose plates for screening the XI enzyme. TTC medium is composed of NaCl, 25.00 g; NH_4Cl, 0.5g; K_2HPO_4 (10%), 7.00 mL; KH_2PO_4(10%), 3.00 mL; $MgSO_4$, 0.75 g; 2,3,5-triphenyl tetrazolium chloride (TTC), 30.00 mg; xylose 10.00 g; agar, 3% in 1000 ml distilled water (modified method from (Dien et al., 2000)). For acidic TTC medium, pH is adjusted to 5 using 1 N HCl, and the amount of agar added is increased to double (6%). All the inoculated plates were kept for incubation for 24 hours and observed.

3. Results

3.1. Physicochemical characterisation reveals distinct environmental signatures

The concentrations of nutrients in water samples were dissimilar across the research sites (Table 1). According to t-tests, there were no significant differences between the parameters of individuals within the AM and DM groups. However, multivariate analysis through MANOVA showed that the overall environmental variables had significant differences between the two groups (Pillai Trace = 0.769, p = 0.034; Wilks Lambda = 0.231, p = 0.034).

Table 1: The Table shows the nitrate, nitrite, phosphate, ammonia, and silicate from different sampling sites.

3.2. Metagenomic analysis reveals distinct microbial community structures

Oxford Nanopore sequencing of mangrove soils determined Bacillota and Pseudomonadota as the abundant phyla in all sampling sites (Fig. 3) (Data in Supplementary Material 3). The raw metagenomic sequence data generated in this study have been deposited in the NCBI Sequence Read Archive (SRA) under the number SUB15472690 (Accession number SAMN50082737-SAMN50082750). The taxonomic dissection at the order and family level is presented in Supplementary Material 1; Figure S2, in detail. The phylogenetic analysis has revealed site-specific bacterial signals; Caldisericia was specifically present on D3, and Clostridia were ubiquitous. Alpha-diversity (Supplementary Material 1; Figure S3) established that all sites classified as AM were enriched with more evenly distributed species compared to DM, where a few but more abundant species constituted the community assemblage. The dissimilarity of compositions of experimental groups was additionally highlighted by the beta diversity analysis using PCoA (Supplementary Material 1; Figure S4), corroborating the assumption that separate microbial assemblages existed in the “Alive” and the “Dead” environments.

FIGURE 3: Phylogenetic tree representing microbiome data (abundance and location).

The innermost circle shows a dendrogram of the phylum and class. The subsequent rings indicate phyla and classes present in the metagenomic study. The 13 outer rings display heatmaps representing the abundance of taxon-specific organisms from M1–M10 and D1–D3 sampling sites. Colour intensity in each segment reflects taxon abundance. For example, the Caldisericia class was detected only at site D3, while Clostridia were abundant across all sites.

3.3. Correlation between environmental parameters and metagenomics

To better understand the correlation between environmental parameters and metagenomics, PCoA was used (999 permutations). PCoA analysis was based on the Jensen-Shannon distance matrix for the predicted enzymatic presence (Data in Supplementary Material 4) and the Euclidean matrix on environmental parameters with z-log normalisation (Figure 4). The research at hand has revealed the high functional dissimilarity between bacterial communities of AM and DM sites (PERMANOVA: $F = 2.51$, $R^2 = 0.186$, $p = 0.047$), and the Betadisper test supported the homogeneity of group dispersion ($F = 2.81$, $p = 0.122$). Overwhelming differences were observed in the glutathione transferase (EC 2.5.1.18), which was 2.2-fold more active in the AM sites than in the DM. On the other hand, cytochrome c oxidase (EC 1.10.3.12) and N-acetylmuramoyl-L-alanine amidase (EC 3.5.1.28) had a relative activity of 2.8-fold and 1.3-fold, respectively, higher in DM sites. Enzymes such as glutamine synthetase (EC 6.3.1.2) and ligase activities (EC 6.6.1.2) were vastly enriched in AM communities. Similar differences were seen with KO data (Supplementary Material 1; Figure S5).

FIGURE 4: Principal Coordinates Analysis (PCoA) of metagenomic bacterial communities and PICRUSt2-predicted enzyme commission (EC).

3.4. Environmental parameters drive genus-specific culturable bacterial distributions

Now, to better understand the culturomics, 16S rRNA sequencing was performed, in which it was found that among all genera, *Bacillus* was dominant in all locations, highlighting their ecological significance in mangrove environments (Supplementary Material 1; Figure S6). The alpha diversity of the culturable bacteria displayed no significant differences between the sites, but the PCoA carried out on Bray-Curtis dissimilarity (Supplementary Material 1; Figure S7) demonstrated the significant structural differences between AM and DM populations, which PERMANOVA ($F = 2.33$, $p = 0.05$) confirmed and explained 52.7 % of the total variance. Notably, no large-scale disparity in beta-dispersion was observed, which means that such between-groups patterns are a real community-level change instead of dispersion effects (Supplementary Material 1; Figure S8).

3.5. Correlation between environmental parameters and culturable genera

To further explain the variety of the lignocellulase producing microbes, the isolates were grown under various growth conditions in a defined medium and screening was performed (Supplementary Material 1; Figure S9). There was no homogeneity of enzymatic activity between the different sampling sites in comparative assays (Supplementary Material 1; Figure S10). It was worth noting that the percentage of culturable bacteria with cellulase activity was high in the isolates grown under normal (37°C and 5pH). On the other hand, laccase activity was mainly absent in thermolytic conditions. Xylanase possessed significant enzymatic activity solely in conditions of low pH, namely in M2, to the greatest extent. XI was highly active regardless of substrate or growth substrate, possibly showing its adaptive characteristic or some other ecological significance. The microbial communities found in M7 were characterised by an expanded enzymatic activity on a wide range of conditions, making this zone a potential hot zone to use in biotechnology (Data in Supplementary Material 5).

To better demonstrate the correlation between environmental parameters and culturable genera, a heatmap was plotted (Figure 5). Hellinger-transformed, bacterial community composition data were normalised to make relative abundances and avoid the effects of low-abundance taxa and environmental parameters were normalised with z-score normalisation. Ordination axes were then plotted against environmental variables using envfit, and the strength of the correlation was given as strong |r| 0.70, moderate |r| = 0.50-0.69, and weak |r| = 0.30-0.49, with the significance being $p < 0.05$. Under normal and thermolytic conditions, significant correlations were found only with silicate ($p = 0.042$ and $p = 0.039$, respectively), and all 23 genera were employed, and the correlation patterns between the treatments were identical. The highest positive correlation was with *Bacillus* and silicate ($r = 0.73$, $p < 0.05$), as well as the lowest negative correlation with *Vibrio* ($r = -0.54$, $p < 0.05$).

FIGURE 5: Heatmap showing the correlation between environmental parameters and bacterial genera exhibiting enzymatic activities (cellulase, laccase, xylanase, and XI) under different conditions (pH 7/5 and 37/50°C) across various sampling sites.

Brevibacterium, *Enterobacter*, *Exiguobacterium* and *Staphylococcus* showed moderate positive correlations with silicate ($r = 0.40$-0.47, $p < 0.05$). On the contrary, acid and acidothermolytic had no significant correlations over all the environmental parameters ($p > 0.05$ in all the tests). However, there was a strong underlying correlation, such as $r = 0.98$, between *Brevibacterium*, *Enterobacter*, and *Exiguobacterium* with nitrate, and between

Marinomonas and *Pontibacillus* with ammonia.

The results presented above raise the question of how different enzymes and the isolates' conditions were correlated (Data in Supplementary Material 6). Principal coordinate analysis (PCoA) was performed on combined distance matrices incorporating weighted UniFrac phylogenetic distances and standardised environmental parameters. Bacterial community data were Hellinger-transformed, while environmental parameters were z-score normalised. Distance matrices were scaled to a unit range and combined additively before ordination. Permutational multivariate analysis of variance (PERMANOVA) was conducted on centroid coordinates with 999 permutations to test for significant differences between viability groups (Figure 6).

Fig. 6: Principal Coordinates Analysis (PCoA) of environmental parameters and culturable bacterial genera. Enzymatic activities are indicated as follows: cellulase (triangle), laccase (square), xylanase (plus), and XI (circle) under all tested conditions from various sampling sites. AM sites are shown in blue, DM sites in red, with overall centroids indicated by diamond shapes.

With PCoA ordination, 42.04% and 18.27% of the overall variation were accounted for on axes 1 and 2, respectively. The statistically significant separation between viable and nonviable bacterial communities using comparison of condition-group centroids was 15.44 (df = 1, 6) at $p = 0.035$ and $R^2 = 0.72$. AM and DM communities were always at opposite PCoA2 space in the enzyme treatments. The highest centroid separation value was found in the xylanase treatment (PCoA1 difference = 0.838), followed by XI (0.469), laccase (0.268), and cellulase (0.223). The findings can be considered a definite bifurcation where the world of AM communities resides at (0.106, -0.054) and DM communities at (-0.345, 0.177). Homozygosity of within-group variance was supported by Beta-dispersion analysis of six groupings of the conditions ($F_{1,6} = 3.34$, $p = 0.117$), so the effect was likely due to actual structural differences and not dispersion. Similar results were observed in nMDS (Supplementary Material 1; Figure S11).

4. Discussion

4.1. Metagenomic Communities: Taxonomic and Functional Restructuring in Dead and Alive Mangroves

The metagenomic comparison deepens the taxonomic and functional rearrangements identified between AM and DM sites, including the fundamental shifts in communities and metabolic capabilities. Although Pseudomonadota and Bacillota dominated in all the sites,

40.5% and 28% of total abundance, respectively, when the ecosystem state was compared, there was a significant change in their relative abundance. The abundance ratio of 11.7:1 in favour of AM sites indicates the loss of microbial biomass during DM, which aligns with the findings by mangrove-degraded systems around the globe (Alsharif et al., 2024; He et al., 2025; Costa et al., 2023).

The appearance of Caldisericota can also be compared to the geochemical changes observed in the Brazilian and Australian systems, where the demise of mangroves generates reducing conditions with active CO_2 efflux and decreased pH (Queiroz et al., 2018; Sippo et al., 2019). A striking positional skew was observed in the proportion of DM site abundance in Bacillota (28% of the total abundance of Bacillota), with the appearance of a higher abundance in Clostridia (270 in DM vs 1477 in AM sites). Such a trend indicates the switch in the metabolism toward anaerobic fermentation in alive mangroves (Nóbrega et al., 2022; Wainwright et al., 2023). The Clostridia phylum is present at all sites, with rising relative abundance in AM areas. In AM sites, the consistent input of organic material by roots and litter led to diverse bacteria, even anaerobic bacteria such as Clostridia and Bacteroidia. Conversely, stagnant waters of DM sites do not have fresh organic matter, resulting in a general decrease in microbial biomass (including anaerobes). The water has low nutrient cycling, and the broken redox gradients make these sediments less favourable to strict anaerobic communities.

The alpha diversity analysis indicated a precipitous decline in species richness, ranging between 477.2 species per AM and 212.0 per DM site, which translates to a diversity loss of 55.6 and is associated with ecosystem degradation. This loss of diversity surpasses the losses seen in other highly-stressed coastal marine environments and suggests that mangrove deletion leads to a great deal of community simplification via environmental filtering (W. Chen & Wen, 2021; Zhuang et al., 2020). This overall trend in all the dead sites indicates deterministic historical assembly processes and not stochastic processes (W. Chen & Wen, 2021).

The integrated analysis of PICRUSt2-predicted EC and KO abundances reveals fundamental metabolic potential divergence between AM and DM bacterial communities, with enzymatic and genetic predictions supporting a shift from aerobic to anaerobic metabolic pathways following mangrove mortality. The 2.8-fold enrichment of predicted cytochrome c oxidase abundance in DM sites, coupled with reduced ABC transporter gene predictions, indicates compromised respiratory potential and membrane integrity maintenance capacity (Luo et al., 2017; Srikanth et al., 2016). This predicted metabolic shift is further evidenced by the dramatic reduction in biosynthetic enzyme predictions (EC 6.3.1.2, EC 6.6.1.2) and corresponding decreases in DNA repair machinery predictions (K01999, K02004), suggesting that bacterial communities in DM sites possess limited

anabolic and maintenance metabolic potential (Atwood et al., 2017; Luo & Gu, 2018). The persistence of cell wall degradation enzyme predictions (EC 3.5.1.28) and formaldehyde dehydrogenase (EC 1.2.7.3) in DM communities reflects the predicted transition to necrotrophic metabolic capacity, where bacterial communities increasingly rely on decomposition-related functions rather than primary metabolic processes (Holguin et al., 2001). These findings align with previous studies showing that mangrove degradation leads to fundamental shifts in predicted microbial biogeochemical cycling capacity, with implications for carbon sequestration and nutrient availability (Donato et al., 2011).

4.2. Culturable Bacterial Communities: Physiological Adaptation and Environmental Filtering in Dead and Alive Mangroves

Taxonomic stability at the genus level, such as that of *Bacillus*, was present in each site despite the dramatic metagenomic differences. The rigorous separation in ordination (PERMANOVA: $F = 2.33$, $p = 0.05$, $R^2 = 17.5\%$) shows that the ecosystem status has fundamental reorganisation of community functionality based on selective pressure on the enzymatic potential, unlike the complete replacement of taxa (Gong et al., 2019; Ramette, 2007). The result underlines past research on the Goan mangrove bacterial community to provide the hidden physiological diversity underneath well-documented taxonomic backbones (Haldar & Nazareth, 2018; P. D. Parab et al., 2020).

The combination of cellulase, xylanase, laccase, and xylose isomerase is a minimal set of biochemical tools that makes up lignocellulose degradation to allow the use of pentose sugar. The AM-DM site differential expression of enzymes is noticeable. This variation has led to diversity within the enzymes for industrial applications. Overlap between ecosystem adaptation and industrial needs makes these enzymes dual-purpose biomarkers, which provide information both on ecosystem health and on biotechnological potential.

The enzyme screening outcomes shed light on the adaptive machineries that sustain the community through the transitions among these ecosystem states. Not only is the activity of extremophilic enzymes predominant in the dead sites, but 42 isolates showed cellulase activity at pH 5 with 50 °C, with acidothermolytic bacteria of *Bacillus* and *Vibrio* (Supplementary Material 1; Fig. 9). These findings significantly expand the known diversity of lignocellulolytic bacteria in mangrove systems, which have been predominantly studied under normal conditions, revealing a previously uncharacterized reservoir of extremophilic enzymatic potential (Arfi et al., 2013).

The fundamental changes in the community-environment relationship caused by stress gradients were identified through the analysis of environmental correlation. In both normal and thermolytic situations, silicate was the only meaningful correlate ($p = 0.042$ and $p = 0.039$, respectively), and *Bacillus* had the strongest positive correlation ($r = 0.73$, $p < 0.05$).

Such dependence on *Bacillus* indicates that this bacterium is a silicate bioindicator, possibly due to its role in biogeochemical cycling processes and the processes of cell wall synthesis (Hui et al., 2024; Jadtanim et al., 2024). That the apparent statistical significances are lost in acidic conditions, yet the population correlation coefficients remain substantial, suggests that the acidic condition itself irreversibly disorients the canonical natural bacterium-geochemical relationship owing to effects on membrane permeability and enzyme reaction rate (Bonugli-Santos et al., 2010).

Environmental correlation analysis revealed fundamental shifts in community-environment relationships across stress gradients. Under normal and thermolytic conditions, silicate emerged as the sole significant correlate ($p = 0.042$ and $p = 0.039$, respectively), with *Bacillus* showing the strongest positive correlation ($r = 0.73$, $p < 0.05$). This relationship suggests *Bacillus* serves as a bioindicator of silicate availability, potentially reflecting its role in biogeochemical cycling and cell wall synthesis processes (Afridi et al., 2024; Alongi et al., 1993). The loss of statistical significance under acidic conditions, despite maintaining strong correlation coefficients, indicates that acid stress fundamentally disrupts traditional bacterial-geochemical relationships through altered membrane permeability and enzyme kinetics (Nimnoi & Pongsilp, 2022).

The ecological implication does not stick merely to enzymatic documentation, but goes so far as to predict the functional level within an ecosystem. The abundance of *Vibrio* in AM sites and its depletion in DM sites is a crucial loss of chitinase activity and organic matter processing that is necessary to mangrove nutrient cycling (Zhou et al., 2017). With specific adaptations of *Vibrio* in the environment of variable salinity and high organic matter due to specialised enzyme systems, and considering the reduction in *Vibrio*, concerning ecosystem degradation, it plays a sensitive microbial indicator to the degradation of the ecosystem, similar to the indicators presented by the vegetation(Brumfield et al., 2020; Muwawa et al., 2021). Disruption of the *Vibrio*-mediated nutrient cycling processes could speed the mangrove decline due to positive feedback between simplification of microbial communities and collapse of the ecosystems, meaning that the decline process is interconnected between the aboveground and belowground ecosystem parts (De Santana et al., 2021; Erazo & Bowman, 2021).

The current research is one of the early steps to systematically explore the lignocellulolytic bacterial communities of the AM and DM ecosystems. We combined the genome-wide snapshot approach to metagenomic profiling with culture-based screening along environmental parameters. The results revealed how not just taxonomic structure, but also the functional diversity of the microbial community, responds to an ecosystem in deteriorated condition. Although genera like *Bacillus* are maintained in both ecosystem conditions, we found considerable reshaping in the community functions. The result

dictated divergence between aerobic, anaerobic and biosynthetic pathways in AM sites and the necrotrophic and stress-resistant metabolic strategy in the DM environment. In particular, the ability of extremophilic and polyextremophilic lignocellulolytic enzymes, especially in stressed DM bacteria, points to the enormous but underused biotechnological potential of mangrove microbiomes. Not only do enzymes like cellulase, xylanase, laccase and xylose isomerase indicate the health of ecosystems, but they also present sustainability in biofuel production using lignocellulose. Finally, the study underlines both aspects of mangrove ecosystems as reservoirs of biodiversity and storage of industrial enzymes. Not only should protecting such fragile coastal ecosystems be a priority, but it should also be a critical strategic activity in tapping the unique, environmentally friendly mode of addressing worldwide problems, namely energy conundrums. Their ability to support life in functional versatility, as seen in the case of mangrove microbial communities, can usher in the innovation of green technology, besides acting as sensitive leading signatures of environmental change.

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7. Data Availability Statement

The data for this work are accessible in NCBI, with accession numbers for genomics sample SAMN50082737-SAMN50082750 and Sanger sequencing PV789845- PV790020.

8. Author Contributions

Rishi Gandhi (Conceptualization, Investigation, Writing - original draft, Methodology, Visualization, Software, Formal analysis, Data curation), Rakhee khandeparker (Conceptualization, Methodology, Data curation, Supervision, Resources, Project administration, Visualization, Writing - review & editing), Nikhita P. (Conceptualization, Investigation, Methodology, Writing – orig18inal draft, Visualization, Data curation, Formal analysis), Mandar Paingankar (Formal analysis, Conceptualization, Investigation, Writing - review & editing, Software, Methodology, Resources, Data curation), Khanjan Chudasama (Conceptualization, Investigation, Writing - review & editing, Methodology, Formal analysis, Data curation)

9. Tables and Figures

TABLE 1: *Table shows the nitrate, nitrite, phosphate, ammonia, and silicate from different sampling sites.*

Sampling sites	Nitrate µM/L	Nitrite µM/L	Ortho-Phosphate µM/L	Ammonia µM/L	Silicate µM/L
M1	14.57	36.7	5.43	18.04	95.28
M2	1.75	2.79	3.23	1.23	42.81
M3	7.1	3.02	11.24	39.48	350.47
M4	1.58	2.04	0.73	1.28	34.88
M5	2.19	2.53	5.54	9.08	47.05
M6	2.07	2.17	0.1	6.05	55.52
M7	1.3	1.96	0.15	4.6	112.5
M8	1.52	2.17	1.68	4.13	71.71
M9	1.95	2.98	1.37	10.75	21.36
M10	4.34	4	2.07	30.32	20.26
D1	92.26	3.43	3.71	1.69	246.61
D2	5.21	4.5	0.81	190.9	73.94
D3	14.57	36.7	5.43	18.04	95.28

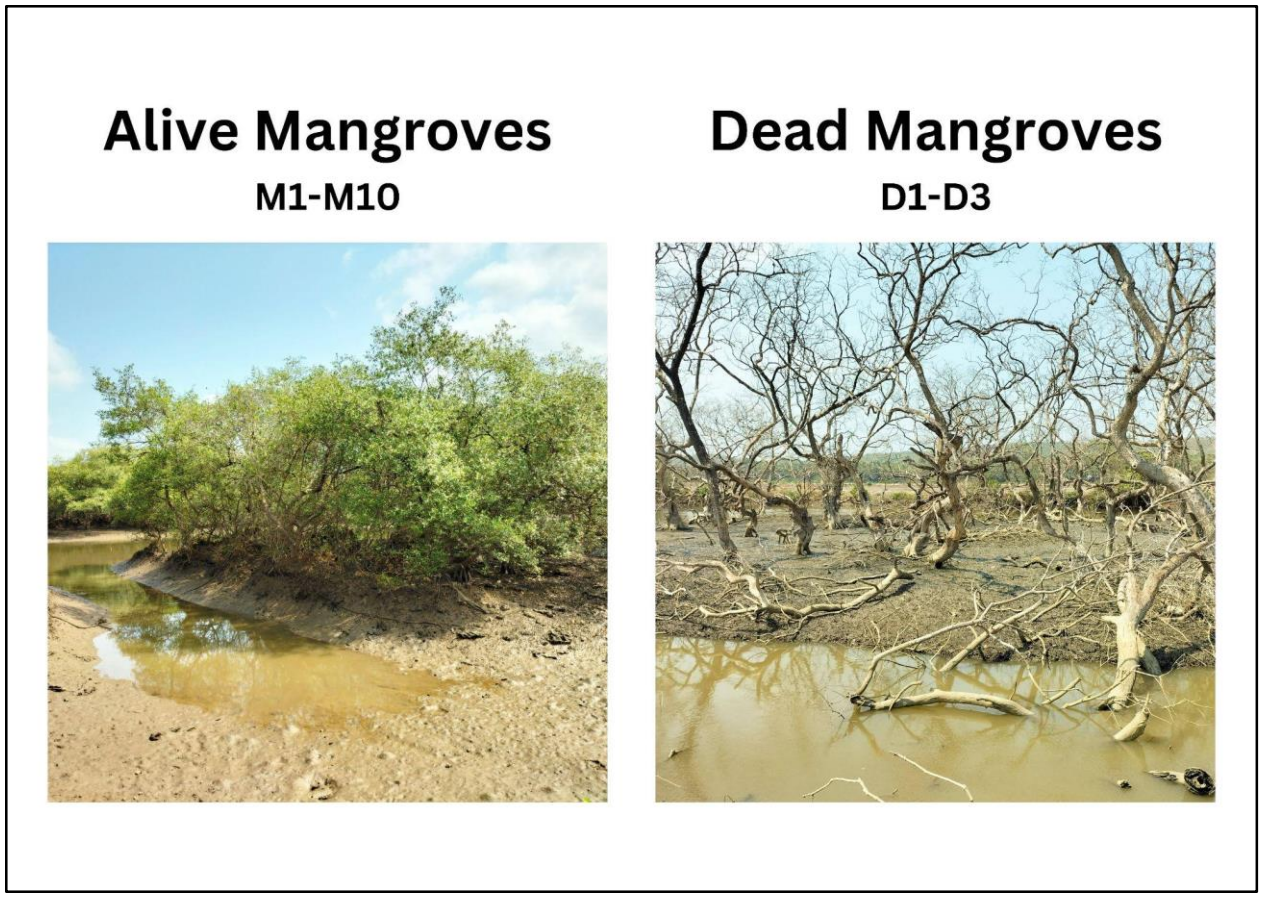

FIGURE 1: Study sites showcasing the vast difference between AM and DM

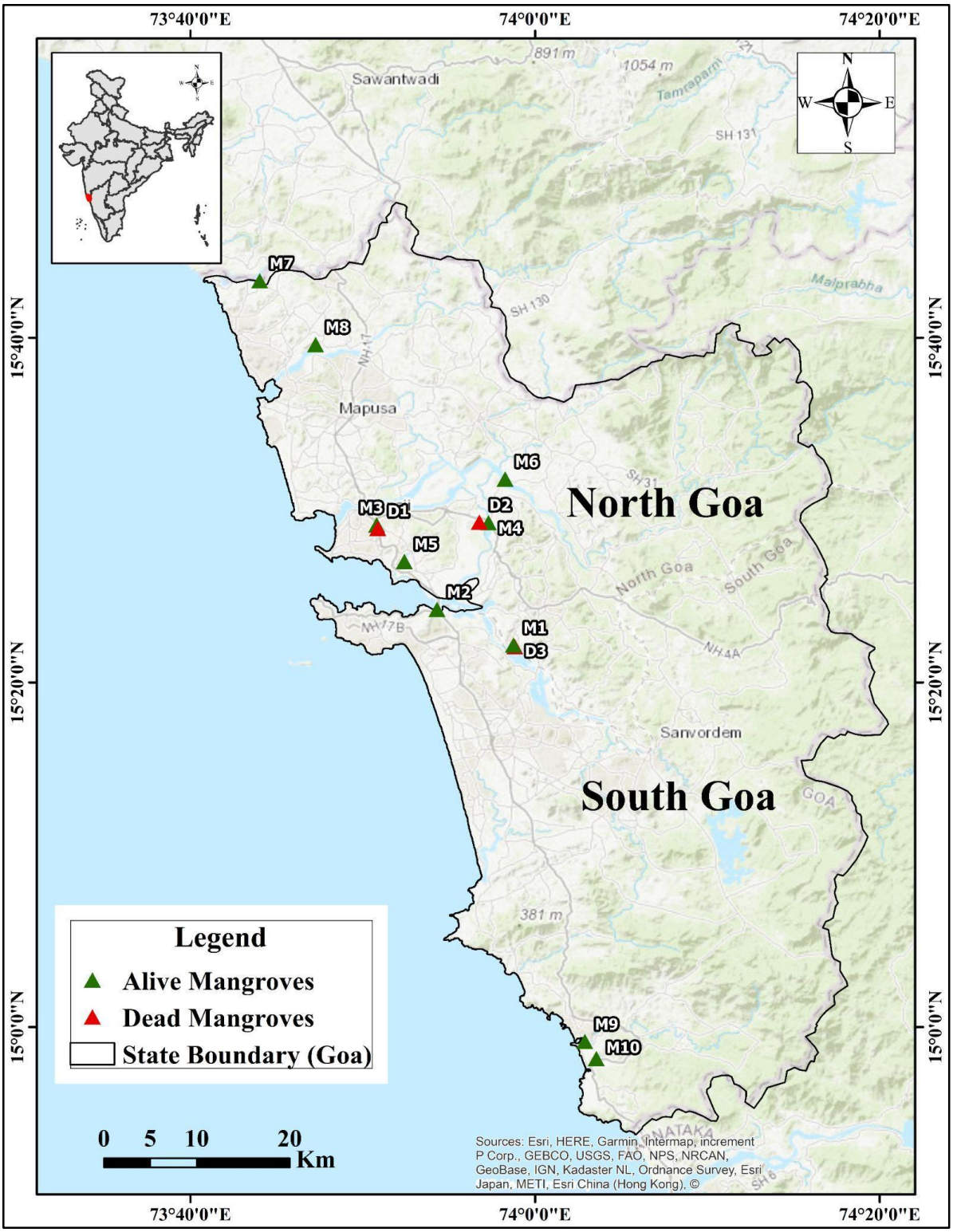

FIGURE 2: Map of the Mangrove Sampling Sites Across Goa. Sites M1-M10 are denoted for AM sites. Sites D1-D3 are denoted for DM sites.

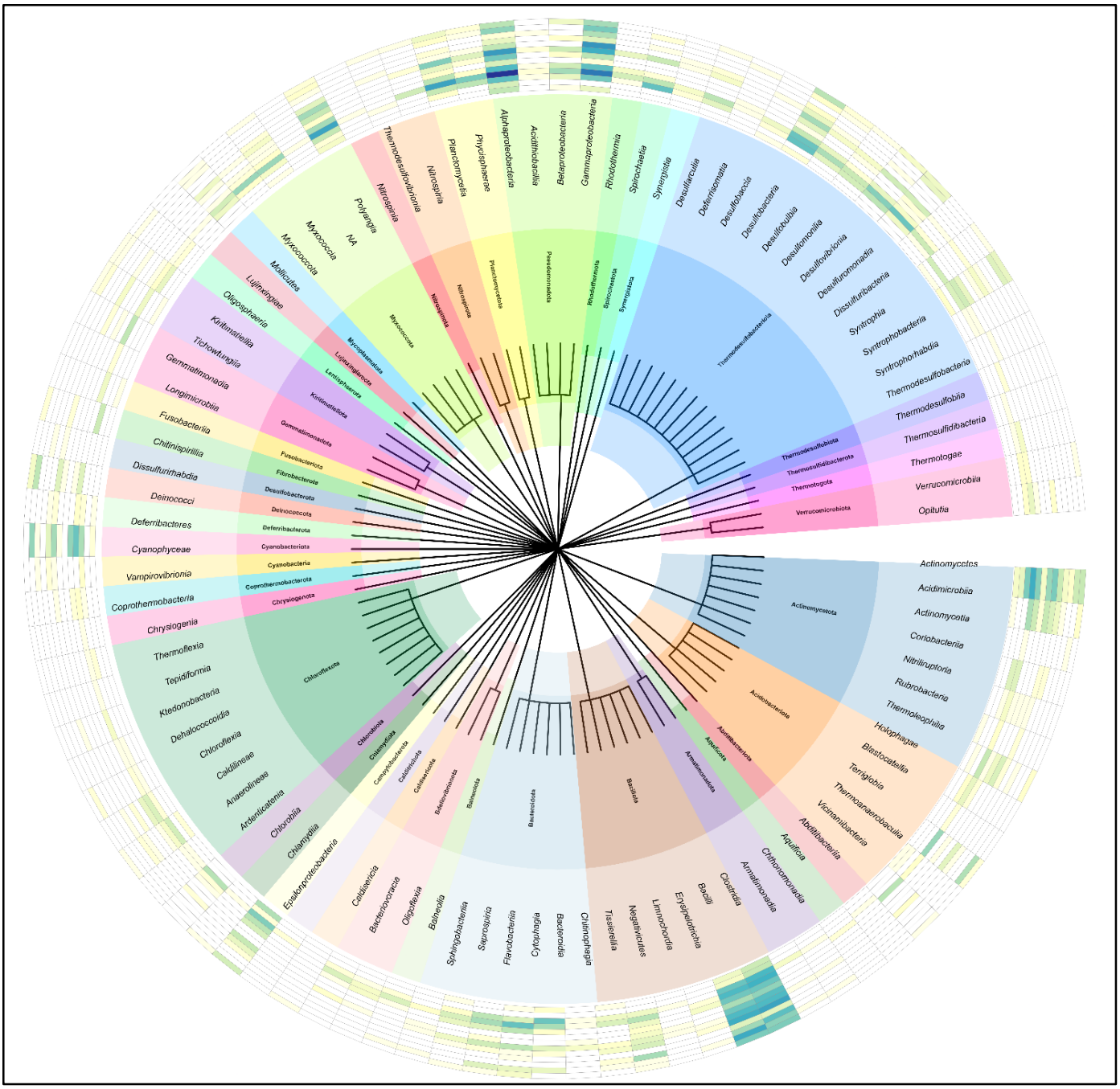

FIGURE 3: Phylogenetic tree representing microbiome data (abundance and location). The innermost circle shows a dendrogram of the phylum and class. The subsequent rings indicate phyla and classes present in the metagenomic study. The 13 outer rings display heatmaps representing the abundance of taxon-specific organisms from M1–M10 and D1–D3 sampling sites. Colour intensity in each segment reflects taxon abundance. For example, the Caldisericia class was detected only at site D3, while Clostridia were abundant across all sites.

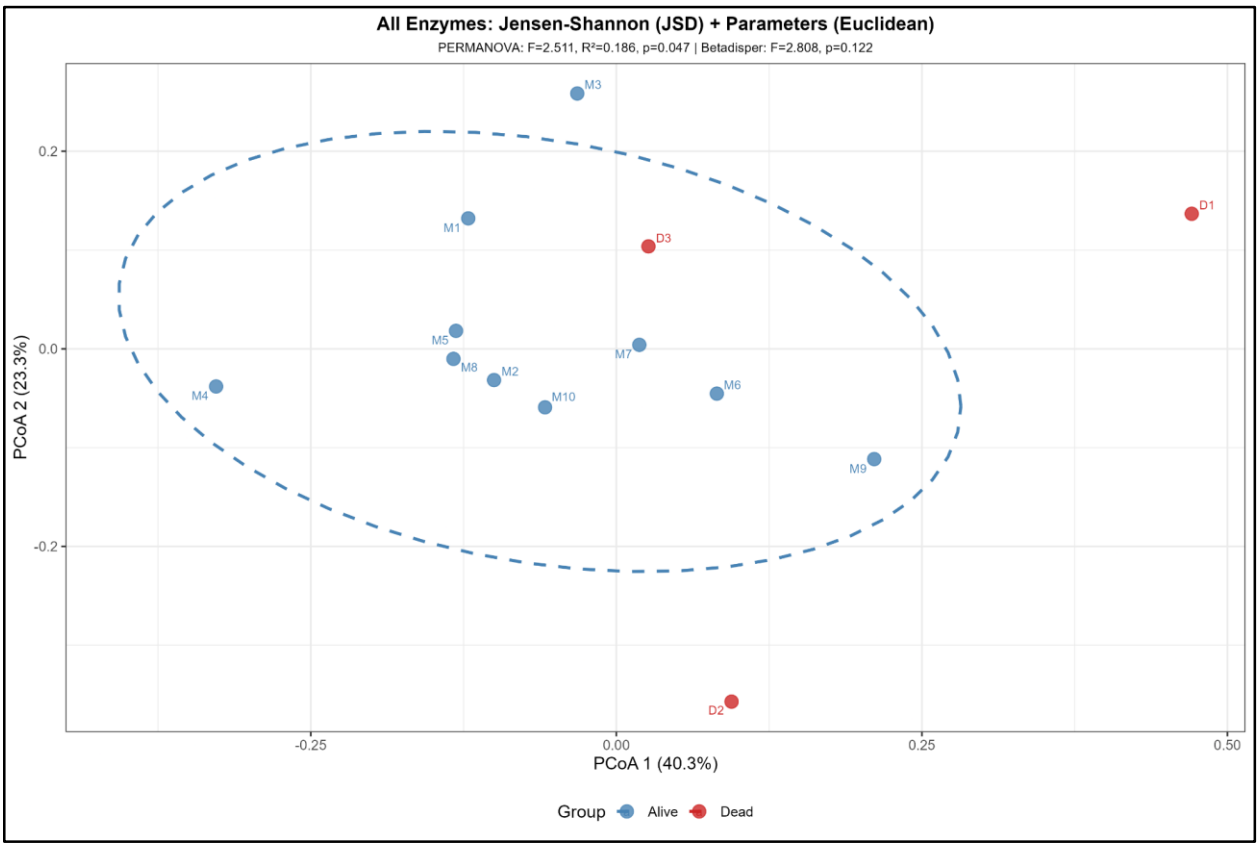

FIGURE 4: Principal Coordinates Analysis (PCoA) of metagenomic bacterial communities and PICRUSt2-predicted enzyme commission (EC).

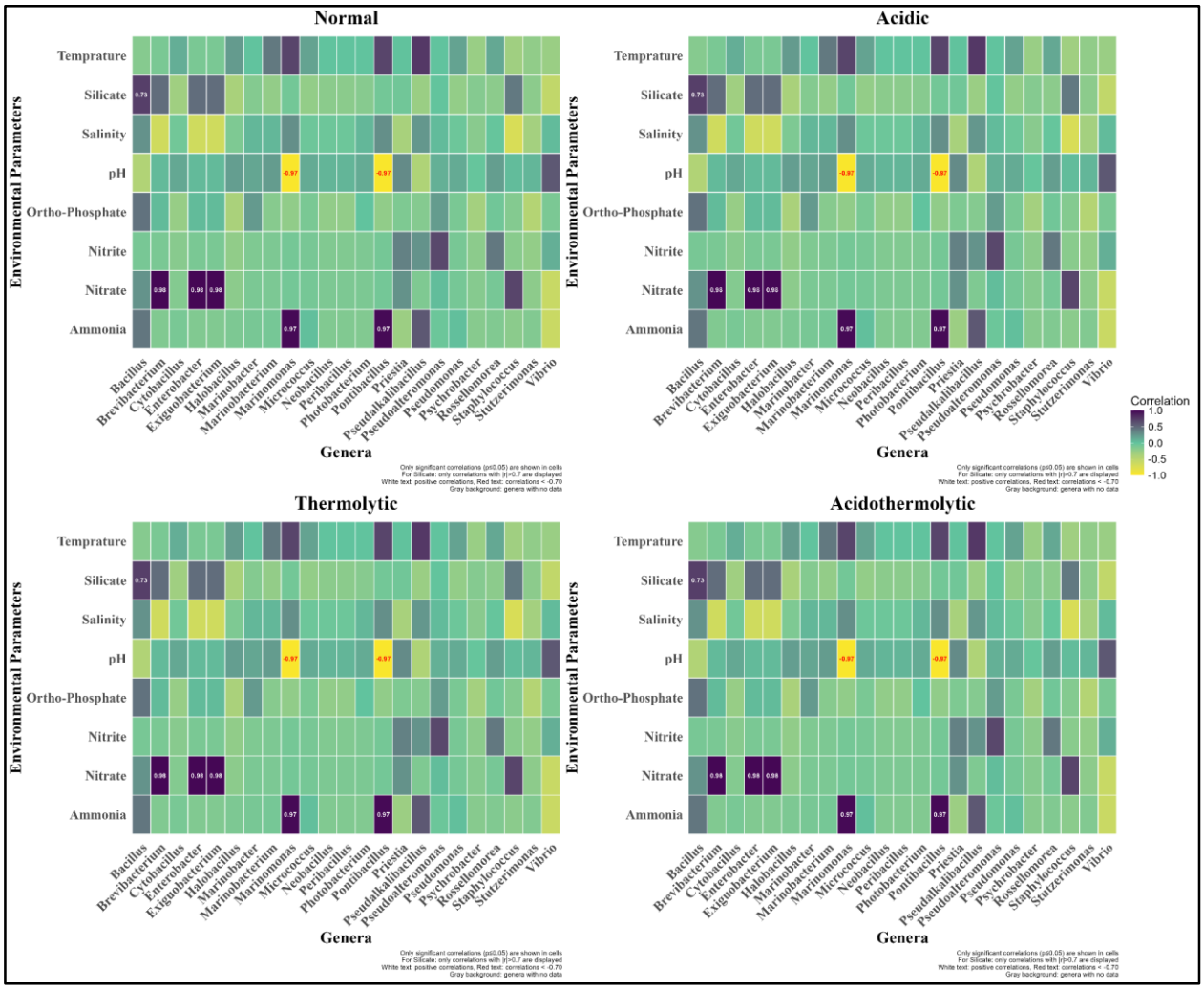

FIGURE 5: Heatmap showing the correlation between environmental parameters and bacterial genera exhibiting enzymatic activities (cellulase, laccase, xylanase, and XI) under different conditions (pH 7/5 and 37/50°C) across various sampling sites.

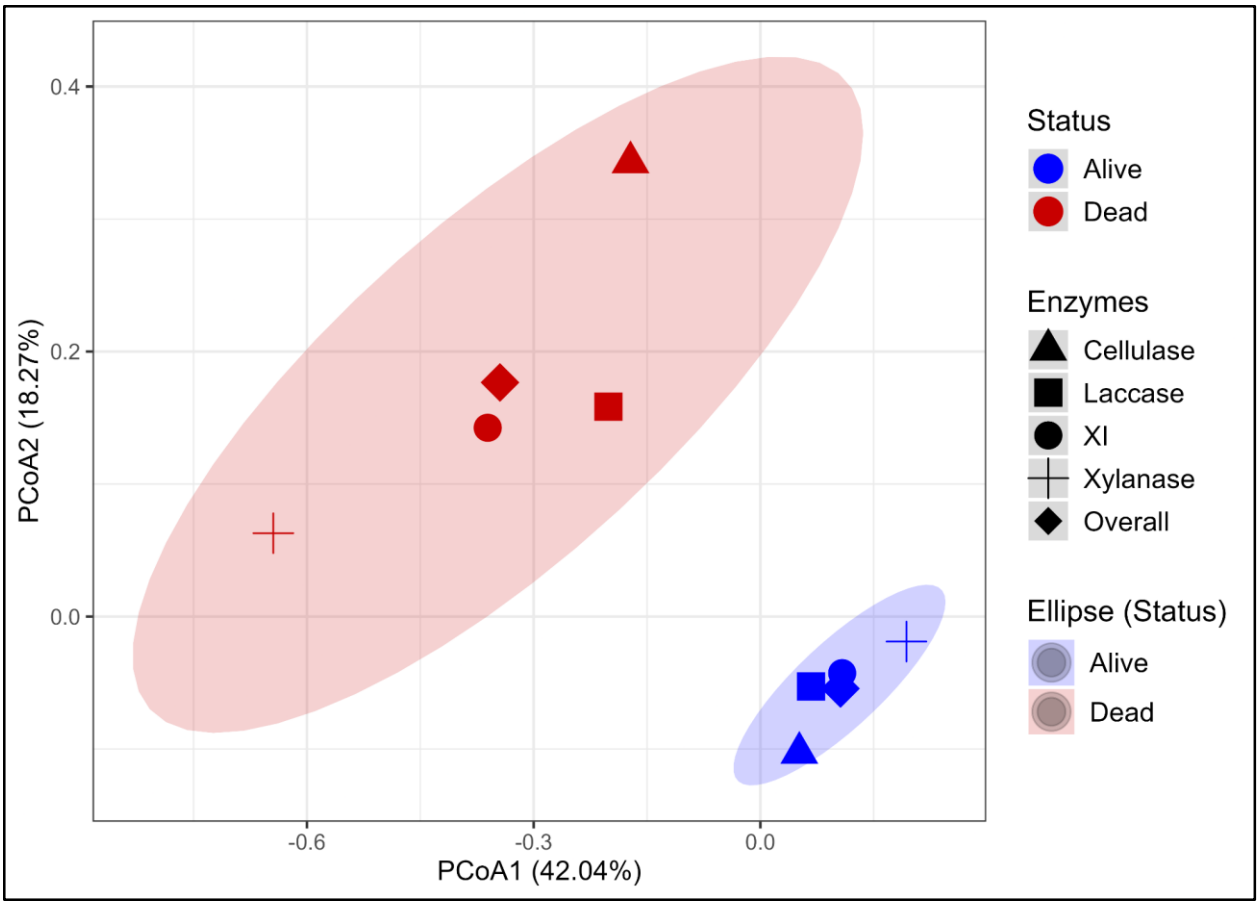

Fig. 6: Principal Coordinates Analysis (PCoA) of environmental parameters and culturable bacterial genera. Enzymatic activities are indicated as follows: cellulase (triangle), laccase (square), xylanase (plus), and XI (circle) under all tested conditions from various sampling sites. AM sites are shown in blue, DM sites in red, with overall centroids indicated by diamond shapes.