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An Integrated Culture and Metagenomic Based Study of Microbial Diversity and Role of Potentially Opportunistic Microbial Flora in a Carcass Dumping Site in Keru, Jodhpur

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Abstract

Vultures are natural scavengers of the environment which can remove animal carcasses and help in retarding the growth of microorganisms associated with decomposition. In the present study, carcass dumping sites of Keru, Jodhpur (Rajasthan, India) were used to study microbial diversity, community structure and functional potential. 16S rRNA gene sequencing amplicon and shotgun metagenomic analysis confirmed the various forms of microbes are associated with the carcass. Studies confirmed that the dominated microflora of sites are Proteobacteria, Firmicutes, Actinobacteria and Bacteroidetes, which are prominently associated with organic matter degradation. Samples DL, Dg, and Tg represented higher PD values, representing higher phylogenetic relationships with CL-2 and TL which showed comparatively lesser PD values, indicating more phylogenetically related communities. A significant value of variation was described by the ordination axes (PC1: 24.1%, PC2: 18.0%, and PC3: 14.9%), showing that significant community-level differences were observed within low-dimensional space. The spatial heterogeneity of carcass waste prominent habitats confirmed that where organic inputs and microhabitat conditions vary between sites, data showed OTU richness and ranged from 1,089 to 1,721 OTUs per sample, with the highest richness in Tg and the lowest in CL-2 samples. The current work demonstrated that diverse, functionally adaptive microbial communities are interlinked environmental compartments supported by extended corpse persistence in the absence of effective scavenger-mediated clearance.

Key words: Vultures, Carcass, Scavengers, Diversity, Metagenomics, Scavenger decline,

Introduction

Vultures are scavengers that remove carcasses through an ecological process. The death rate of vulture populations is very high in South Asia which alters the dynamics of carcass removal and has high health risks. Vulture populations have experienced a striking depletion in recent decades, due to the dumping of waste, and changes in carcass management practices. Carcass disposal at dumping sites is highly required because it has critical ecological and public health concerns and plays a combined role in waste management (Grootaers et al., 2025; Green et al., 2004; Thakur, 2014). In natural ecosystems, vertebrate scavengers (vultures, hyenas, and crows etc.) play a key role in expeditiously removing animal remains, that confer the removal and management of pathogenic microorganisms. The importance of scavengers is to take part in nutrient recycling, carcass removal, and maintaining hygiene (Ogada et al., 2012; Lobello et al., 2025).



By consuming carcasses, vultures diminish the spread of microbial proliferation across soil, water, and air (DeVault et al., 2003; Santangeli et al., 2025). The depletion of these scavengers can therefore perturb ecosystem-level regulation and management of disease (Beasley et al., 2019). Scavengers are capable of reducing water contamination by consuming carrion. If carcasses are thrown into water bodies, they deplete dissolved oxygen and increase fecal coliforms (Cabral, 2010; Sonawane et al., 2025).

Carcass dumping ecosystems in semi-arid and arid regions are unexplored, so it needs to be explored microbial community composition (Adnani et al., 2018; Stewart, 2012). The recent development techniques have changed the knowledge of microbial diversity by using sequencing technologies (Thompson et al., 2017; Knight et al., 2018). Gene sequencing the variable regions (V3–V4) has been regularly employed as a standard technique for knowing microbial diversity (Johnson et al., 2019; Caporaso et al., 2011; Nearing et al., 2018). By utilizing recent approaches and decomposition-related environments, researchers have identified a variety of microbial associations (Khan et al., 2021; Tomassini et al., 2025; Ogunyemi et al., 2025; Yang et al., 2024). Decomposed animal remnants harbor a huge load of bacterial genera that include *Salmonella*, *Campylobacter*, *Clostridium*, *Pseudomonas* and *Escherichia* (Jones et al., 2008; Batista-Barwinski et al., 2024; Schmeller et al., 2020). These pathogens can spread several diseases if they mix with water and air. In arid regions, wind is responsible for the dispersal of bioaerosols from carcass and waste decomposition sites, which further extends microbial influence in dumping areas (Larsson and Flach, 2022; Allen et al., 2010; Martínez, 2008).

This study describes integrated microbial assessments by the molecular approach in a carcass dumping ecosystem. By using different microbiological approaches viz., combining cultivation, amplicon sequencing, and shotgun metagenomics, studies showed different microbial community structures and functional potential across soil and water. The work highlights that scavengers played an important role in carcass removal along with the microbial community. These findings paved the way for the role of vultures in ecosystem regulation and also played a role in decomposition-associated microbiomes in carcass management.

Materials and Methods

Study area and sample collections

This study was performed around the carcass and waste dumping areas of Keru, Jodhpur, Rajasthan, India. Soil samples were collected from carcass dumping sites using sterile polybags. Soil samples were transported on ice packs and processed within 24 h for further microbiological studies. Vulture droppings were collected from Keru, Jodhpur, Rajasthan, selected feeding areas using sterile spatulas. Dropping samples were placed and stored in sterile containers and processed immediately. All sampling was conducted in accordance with ethical guidelines of wildlife research. Water samples were obtained from Badli Pond, located near the dumping site and potentially influenced by runoff. Water samples were collected in sterile bottles from below the water surface to avoid contamination from surface debris. Water samples were transported under cooled conditions and processed for further study. The settle plate method was used for enumeration of airborne microorganisms from the dumping site (Pasquarella et al., 2000).

Culture-Based Isolation and Phenotypic Characterization

Serial dilution techniques were employed to study viable bacteria from collected soil, water, air, and vulture droppings using the standard method (Sanders, 2012). Appropriate diluted samples were plated on nutrient agar medium and incubated. Colonies appearing on a solid medium were identified and their morphological features (size, shape, color and texture) were noted and colonies were purified. Pure isolates were further studied to know the Gram reaction and biochemical and molecular level identification (Brown and Smith, 2015).

DNA Extraction and Identification of Bacterial Isolates

Newly isolated bacterial culture genomic DNA was analyzed using standard extraction methods. DNA concentration studies were assessed before amplification. 16S rRNA gene sequencing was performed for molecular identification. For this identification, universal amplification bacterial primers showing variable domains were employed for PCR studies. Agarose gel electrophoresis was used for amplicon purification and sequencing studies. For taxonomic relationship nucleotide databases, assignments were used for comparison taxonomic resolution.

Ecological Context and Vulture Identification

Vulture species present in the Keru, Jodhpur, Rajasthan region were analyzed using field observations, standard taxonomic keys and previously published ecological data. Species of vulture identification was done by analyzing their morphological characteristics and known distribution patterns in this region, without direct handling. These types of observational studies provide ecological context to know microbial findings compared to their direct relationships.

16S rRNA Gene Amplicon Sequencing and Processing

The 16S rRNA gene sequencing of the V₃–V₄ region of microbial communities was performed by the culture-independent analysis method. FastQC was confirmed by assessing using raw paired-end reads. Ambiguous and low-quality reads were separated, and reads with above 30 Phred quality scores were used for further study. The QIIME pipeline method was used to assess processed sequences (Caporaso et al., 2010). Operational taxonomic units (OTUs) were brought out by employing a closed-reference clustering approach to contrast the SILVA database (Quast et al., 2013). To evaluate the credibility of downstream functional predictions, Nearest Sequenced Taxon Index (NSTI) data scores were analyzed (Langille et al., 2013).

Diversity and Statistical Analyses

Shannon, Simpson, and Chao1 indices were used to assess alpha diversity within-sample richness and evenness. To analyze the differences in community composition in various samples, were examined to study beta diversity analyses, together with principal coordinate analysis (PCoA) and principal component analysis (PCA). Rarefaction analyses were used to study sequencing depth in dominant taxa and rare taxa. G-tests and Kruskal–Wallis tests were accustomed to notice statistical comparisons of OTU abundances and to study analysis of variance (ANOVA). Krona charts were noticed to evaluate taxonomic profiles of dominant genera (Ondov et al., 2011). Statistical analysis was employed at the value of $p < 0.05$.

Shotgun Metagenomic Sequencing and Analysis

The shotgun metagenomics method was employed to know a dominant taxon by independent and higher-resolution confirmation. This method is used to validate shotgun data, functional gene annotation and dominant metabolic pathways associated with carcass decomposition. Prodigal in metagenomic frames of mode were predicted using open reading frames. CDD, COG, KOG, Pfam, SMART, TIGRFAM, and NCBIfam were used to perform annotation.

Meteorological and Ecological Observations

Meteorological records were recorded regularly during each sampling and rainfall to study microbial community. Vulture activity was analyzed with the help of visual head counts and photographic documentation during daylight hours.

Results and Discussion

Study area and sample analysis

Environmental samples were collected from soil, water, air, and the vulture-associated region at the Keru carcass dumping site. Dumping-site soils showed dense and morphologically diverse bacterial populations of persistent heterotrophic communities. The detection of culturable airborne and waterborne bacteria highlights active bioaerosol and water dwelling potential microbial dispersal from the dumping site, respectively (Pasquarella et al., 2000; Hyvärinen et al., 2008; Drury et al., 2013; Fierer et al., 2012; Metcalf et al., 2016). In addition, bacterial isolates from vulture droppings confirmed the presence of various gut-associated microbiota, and ecological linkages between scavengers and carcass-derived microbial communities (Roggenbuck et al., 2014). Collectively, these findings suggest that longer carcass disposal promotes stable microbial communities across soil, water, and air in the Keru dumping site of Jodhpur.

Isolation of bacterial DNA from pure isolates

Genomic DNA was extracted from selective bacterial isolates. 16S rRNA gene-based bacterial identification confirmed viable and taxonomically diverse microbial strains in carcass environments. The molecular level identification study at the genus and species level confirmed clear data for genotypic and revealing diversity of isolates (Stewart, 2012). Although causality was not observed, the co-occurrence of various bacterial taxa and presence of scavengers confirmed ecological connectivity between carcass-derived microbial communities and scavenger present in dumping sites.

16S rRNA (V₃–V₄) Amplicon Sequencing

16S rRNA sequencing of the V₃–V₄ region confirmed that the generated ~0.7–1.0 million paired-end reads per sample, with consistent GC content (~54–55%), indicate a uniform composition of base. The initial quality assessment study revealed low-quality bases toward read termini, which is a common feature of amplicon datasets that can be effectively removed via stringent quality filtering (Table 1). The V₃–V₄ region depicts taxonomic resolution of soil, water, and waste impacted environments; this suggests the suitability for the present study (Klindworth et al., 2013; Johnson et al., 2019; Caporaso et al., 2012; Knight et al., 2018). Retention of reads with >30 scores Phred confirmed high confidence data for OTU clustering downstream and assignment of the taxonomic study.

Table 1. Amplicon sequencing data of 16S rRNA (V3–V4) from Keru dumping site samples

Sample ID	Forward Reads (R1)	GC Content (%)	Reverse Reads (R2)	GC Content (%)
CL_240315_1_S156	750,569	54	750,569	54
CL_240315_2_S157	852,945	55	852,945	55
Dg_240315_S158	951,101	55	951,101	55
DL_240315_S159	700,387	55	700,387	55
Pg_240315_S161	691,726	55	691,726	55
PL_240315_S160	757,272	55	757,272	55
Tg_240315_S163	1,001,476	55	1,001,476	54
TL_240315_S162	966,004	55	966,004	55

OTU Picking and Microbial Community Structure

By using the QIIME pipeline for related-reference OTU clustering, which used the SILVA database, this confirmed 4,143 OTUs in eight samples. This finding represents high microbial richness in the dumping ecosystem of Keru (Caporaso et al., 2010; Quast et al., 2013). After rigorous study, ~4.64 million high-quality reads were owned, with similar uniform sequencing data across samples (4.36×10^5 – 7.36×10^5 reads per sample) (Bokulich et al., 2013; Knight et al., 2018). Data obtained from OTU richness varied from 1,089 to 1,721 per sample (Table 2). The highest abundance in Tg and lowest in CL-2, represent contiguous heterogeneity in waste- influenced environments (Schloss et al., 2009; Knight et al., 2018).

Observed OTU studies showed rarefaction analysis based on an increase in richness with various depth sequencing without abrupt saturation of curves in all samples (Fig. 1). Higher OTU (Tg and DL) counts represented steeper accumulation patterns in samples while lower richness (CL-2 and TL) was reported in various trajectories (Table 3). These shifts demonstrate organic-rich ecosystems where dominant taxa were well shown whereas rare taxa continued to be detected (Fierer et al., 2012; Lynch and Neufeld, 2015). The medium value of OTU density (0.331), with rarefaction patterns, confirms the site-specific taxa and core microbiome in the carcass site.

Table 2. Statistics analysis of OTU picking and sequencing of depth across samples

Metric	Value
Number of samples	8
Total OTUs (observations)	4,143
Total reads retained	4,638,957
OTU table density	0.331
Reads per sample (min–max)	436,391 – 735,962
Reads per sample (mean $\pm$ SD)	579,869 $\pm$ 89,057
OTUs per sample (min–max)	1,089 – 1,721
OTUs per sample (mean $\pm$ SD)	1,373 $\pm$ 200

Table 3. OTU picking results generated using QIIME using SILVA database

Sample ID	Reads retained	OTUs observed
Pg	436,391	1,194
DL	511,186	1,358
CL-1	519,733	1,524
PL	562,965	1,382
CL-2	588,636	1,089
Dg	609,866	1,535
TL	674,218	1,181
Tg	735,962	1,721

Nearest Sequenced Taxon Index (NSTI) score analysis

NSTI of all samples varied from 0.033 to 0.055 (Table 4) confirming close phylogenetic relatedness in current databases of detected taxa and sequenced reference genomes analysis. This low NSTI data supports the precision of functional predictions obtained from 16S rRNA gene data and provides evidence the reliable taxonomic data study. Obtained NSTI values were closely related to the threshold of ~0.10 or predictive data of environmental microbiome studies in waste and soil-impacted systems (Langille et al., 2013; Douglas et al., 2020). Previous studies of relative agreement of NSTI distributions have been reported by other sites (Knight et al., 2018). The NSTI analysis suggests the suitability of the Keru data information is very suitable to predict and comparing ecological interpretations.

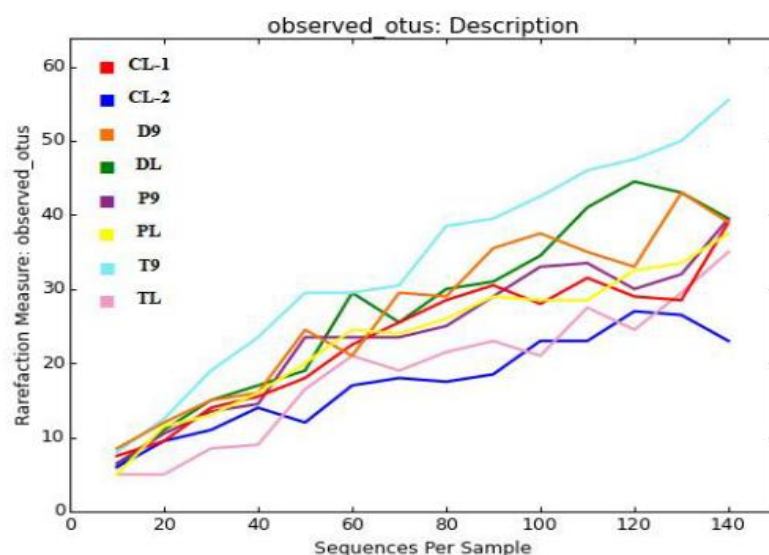


Fig. 1. Rarefaction analysis of observed OTUs across samples from the Keru carcass dumping ecosystem

Table 4. 16S rRNA V3–V4 datasets of nearest sequenced taxon index (NSTI) scores. Lower values of NSTI suggest closer phylogenetic relatedness of observed taxa and sequenced genomes

Sample ID	NSTI metric	Weighted NSTI value
TL	Weighted NSTI	0.041161
CL-1	Weighted NSTI	0.039955
CL-2	Weighted NSTI	0.033181
D9	Weighted NSTI	0.046911
T9	Weighted NSTI	0.046462
DL	Weighted NSTI	0.044762
P9	Weighted NSTI	0.049688
PL	Weighted NSTI	0.054663

Taxonomic Composition of Microbial Communities

16S rRNA gene sequence studies revealed varied taxonomic profiling of microbial communities of sample analysis of the Keru dumping ecosystem. The most dominant phyla were Proteobacteria, Firmicutes, Actinobacteria, and Bacteroidetes associated with the dumping site of carcass disposal (Fierer et al., 2012; Delgado-Baquerizo et al., 2018). Most prominently reported taxa, stress-tolerant and ammonia-oxidizing microorganisms suggest dominance of specific related genera (Prosser and Nicol, 2012).

Observed analysis suggests richness of Alpha proteobacteria and Gamma proteobacteria was higher in waste-impacted and carcass samples, which is very consistent (Newton et al., 2011). Presence of Clostridia and other anaerobes indicates the development of anaerobic conditions during carcass decomposition (Hyde et al., 2013; Metcalf et al., 2016). The presence of Actinobacteria selectively suggests their well-known effect in the degradation of complex organic matter and high stress tolerance (Barka et al., 2016).

Stacked bar plots (Fig. 2) and Krona diagrams (Fig. 3) analysis of area charts disclosed profound shifts in site-specific dominant taxa and uniform successional patterns. These taxonomic studies revealed concordant β -diversity and coexistence of a core microbiome in local complex terrestrial environmental conditions (Ramette, 2007; Knight et al., 2018). Collectively, present findings represent that reduced scavenger arbitrate carcass removal suggests the establishment of complex taxonomical microbial communities alters prolonged enrichment and underlines the importance of scavengers in regulating microbial structure and environmental fitness (Markandya et al., 2008).

Principal Component Analysis (PCA) of Microbial Communities

PCA data confirmed clear differentiation in microbial communities in eight samples, suggesting valuable variation in microbial community composition within the Keru carcass dumping ecosystem (Fig. 4). Samples CL-1 and D9 were second principal component separated using secondary gradients in the community that show differences in oxygen availability, disturbance intensity, and moisture. In contrast, CL-2, P9, DL and TL clustered more closely, indicating incomplete overlap in community composition and the sharing of shared core taxa. A similar type of pattern has been reported in decomposition-driven environments and waste-impacted areas, by various coauthors (Lauber et al., 2009; Fierer et al., 2012).

Alpha Diversity Patterns across Samples

Shannon, Simpson, and Chao1 indices revealed the alpha diversity of differences in microbial diversity of a sample of the Keru dumping ecosystem (Fig. 5). Tg represents the highest Shannon diversity showing rich taxonomy and even community, whereas TL and CL-2 exhibited lower values in data. Simpson index data showed a similar type of pattern, with greater predominance in CL-2 and TL and higher consistency in Tg and DL. Such diversity inclination is critical of waste affected environments where restricted differences in oxygen availability, disturbance intensity, and nutrients positively influence microbial populations (Lauber et al., 2009; Fierer et al., 2012). A similar pattern of variability in alpha diversity has been described for waste and soil related microbiomes shaped by nutrient gradients and disturbance analysis (Schloss et al., 2009; Knight et al., 2018).

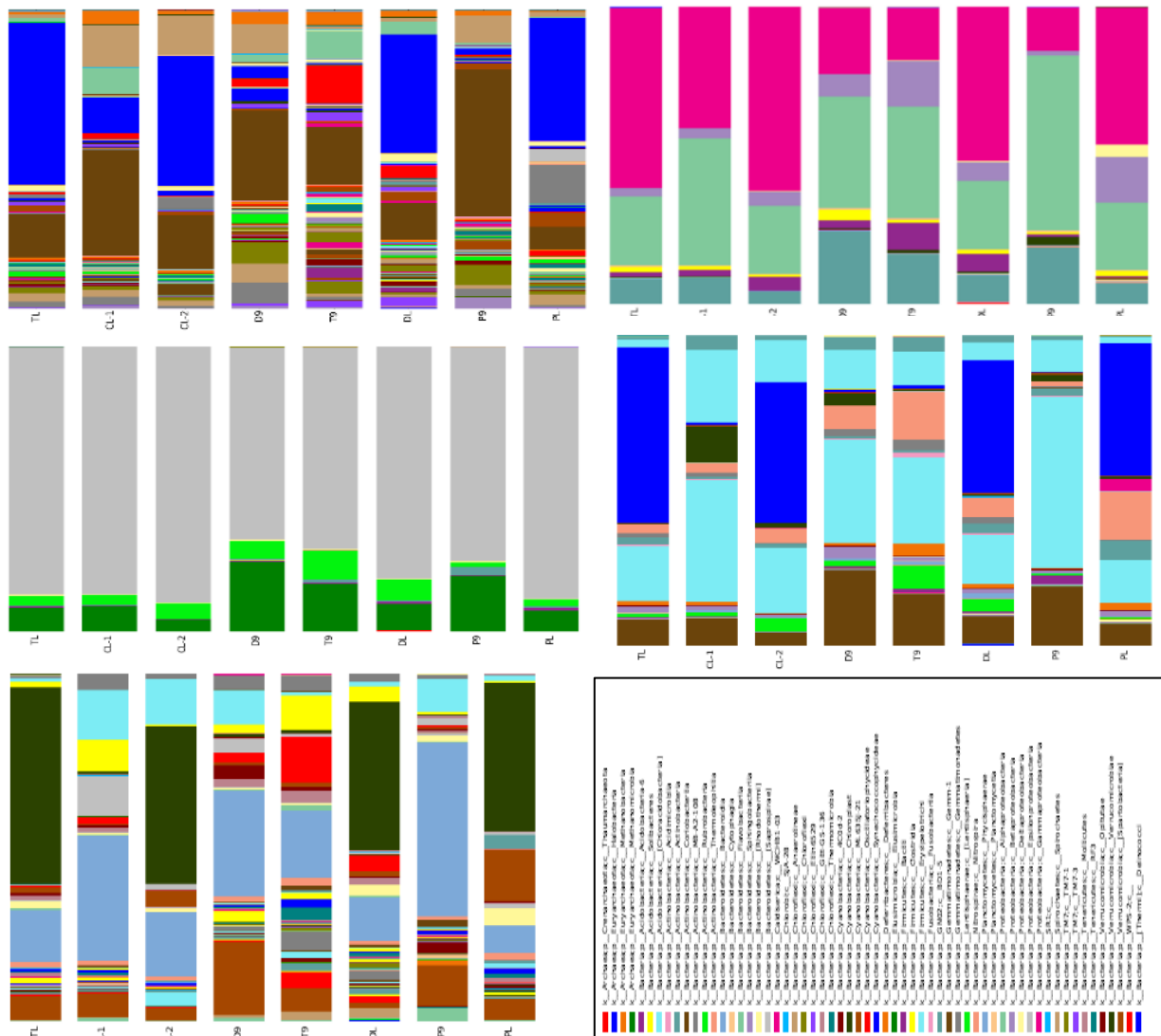


Fig. 2. Stacked bar plots with all taxa

Phylogenetic Diversity Rarefaction Analysis

Rarefaction analysis was performed by using Faith's phylogenetic diversity showed a steady relatedness in effective recovery of evolutionary lineages of microbial communities obtained from Keru sites of Jodhpur (Fig. 6). Samples D9, T9 and DL, revealed higher PD values, showing greater phylogenetic broadness, whereas TL and CL-2 represented comparatively lower PD, recommending more phylogenetic relatedness of communities (Fierer et al., 2012).

The absence of a noticeable elevated pattern further suggests the endurance of a phylogenetically diverse biosphere, which suggests complex environmental microbiomes. The close relatedness between PD rarefaction trends, alpha diversity metrics, and OTU accumulation curves suggests that observed variations among varied samples show genuine ecological variation instead limitations in sequencing depth. A similar type of observation has been reported in soil and organic waste studies in previous reports (Knight et al., 2018). Overall, tree analysis of PD suggests that the Keru carcass dumping ecosystem effectively supports phylogenetically varied microbial populations.

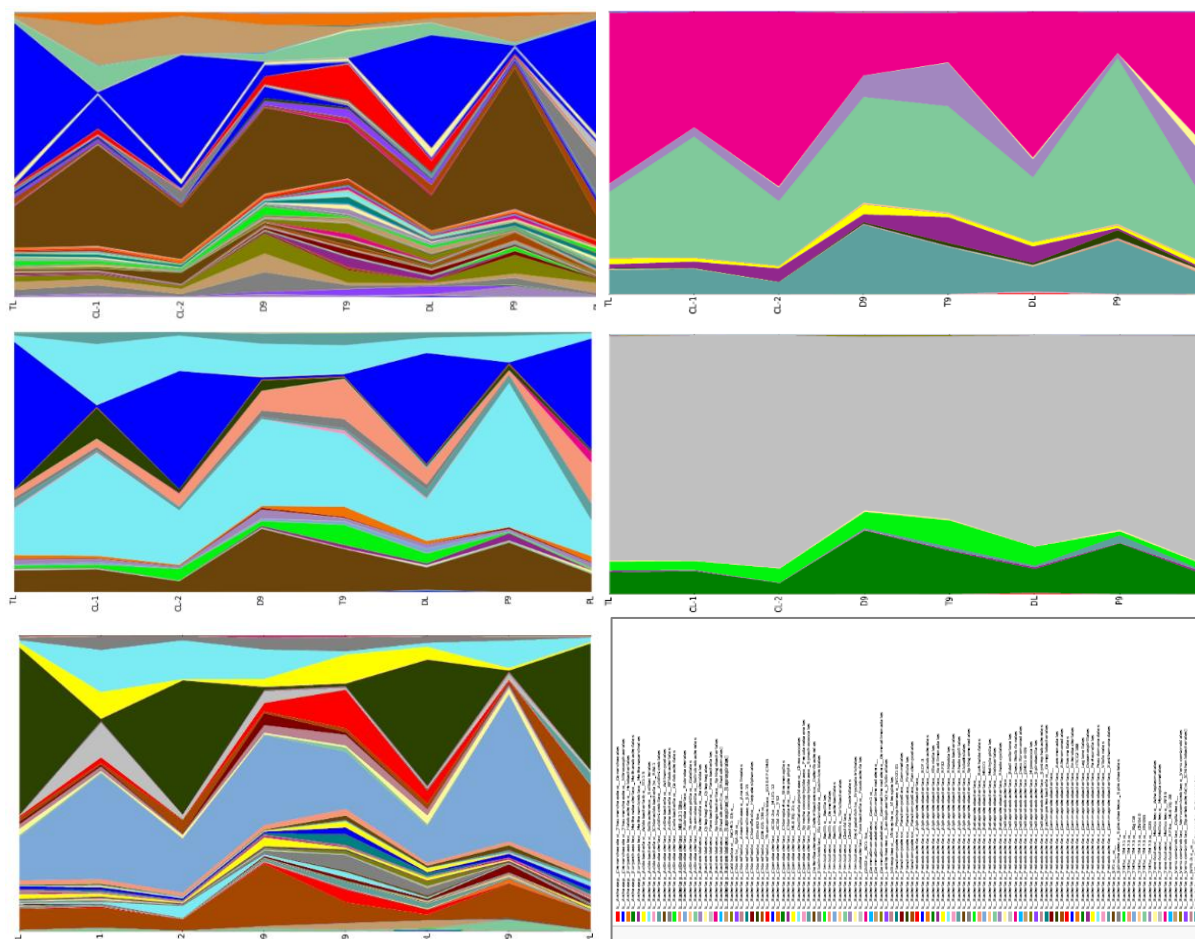


Fig. 3. Individual Krona plots (one per sample)

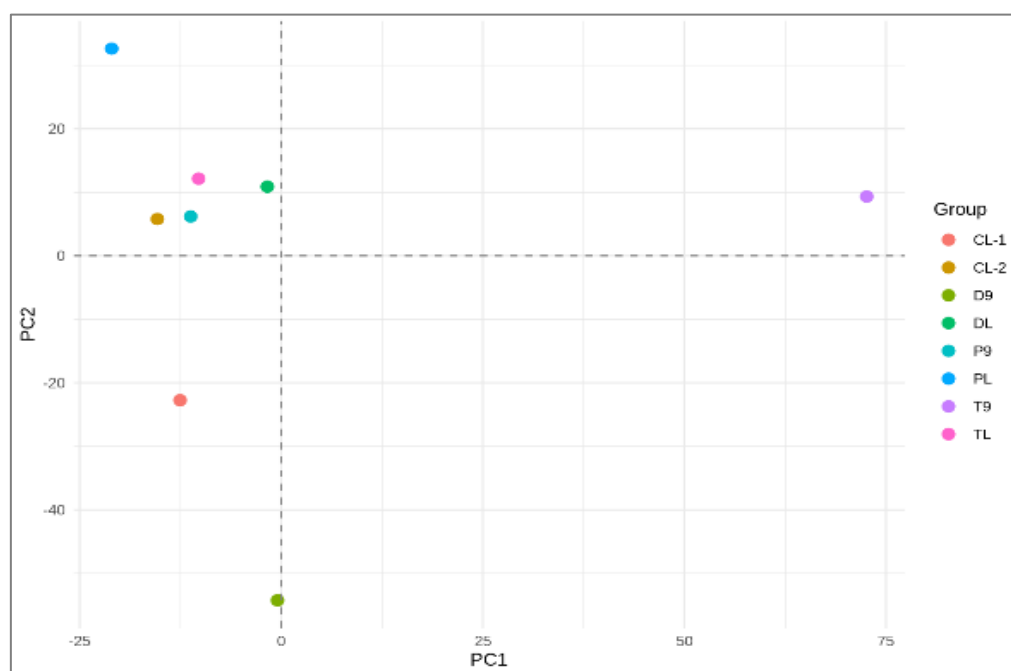


Fig. 4. Principal component analysis (PCA) of microbial community data suggests 16S rRNA gene OTU abundance. Each point PC1 and PC2 reflects differences in community and separation of structure and sampling locations.

Chao1-Based Rarefaction Analysis

Chao1 richness rarefaction analysis was performed using an estimator with a consistent increase in evaluated species richness with the help of the sequencing depth of all samples that indicates a successful recovery of taxa of the Keru carcass dumping ecosystem (Fig. 7). CL-1, DL, and T9 samples showed higher Chao1 values, representing higher richness and a greater endowment of rare taxa, whereas CL-2 and TL represented relatively lower estimates, showing more awkward richness. The absence of untimely plateaus suggests continued observation of rare OTUs

with increased sequencing effort influenced by sustained decomposition. Site-specific heterogeneity of waste-impacted ecosystems, represents localized inputs of organic waste spawning diversely distributed assemblages of microbial species (Lynch and Neufeld, 2015).

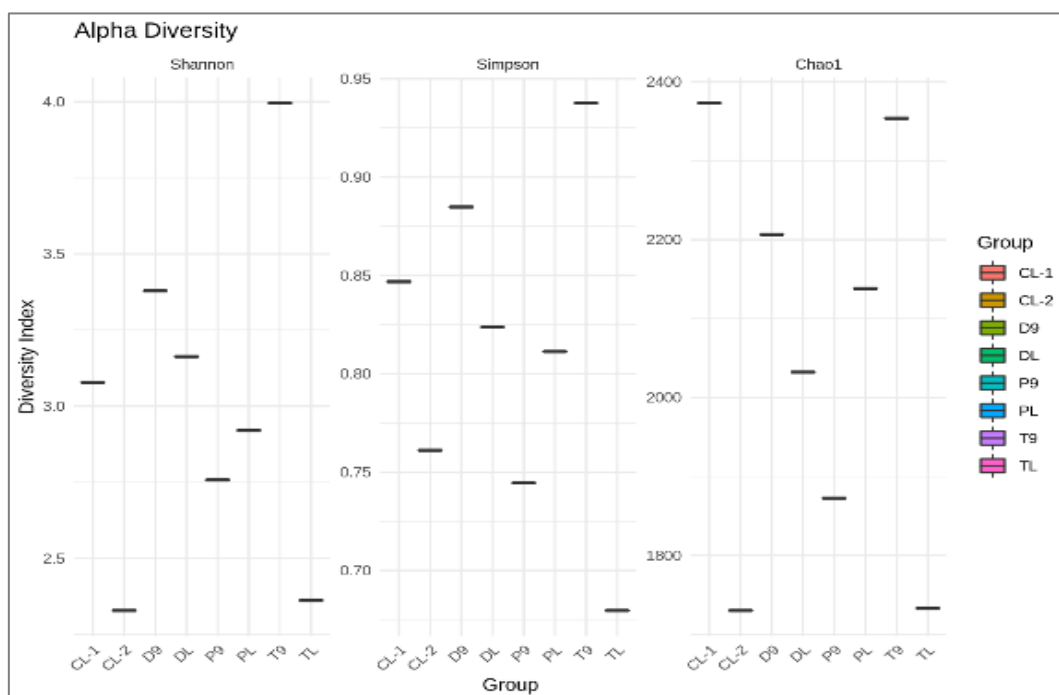


Fig. 5. Shannon, Simpson, and Chao1 based study confirmed alpha diversity of microbial communities of calculated 16S rRNA gene OTU data. Each point of graph represents an individual sample, representing variation in community richness and evenness among locations of sampling.

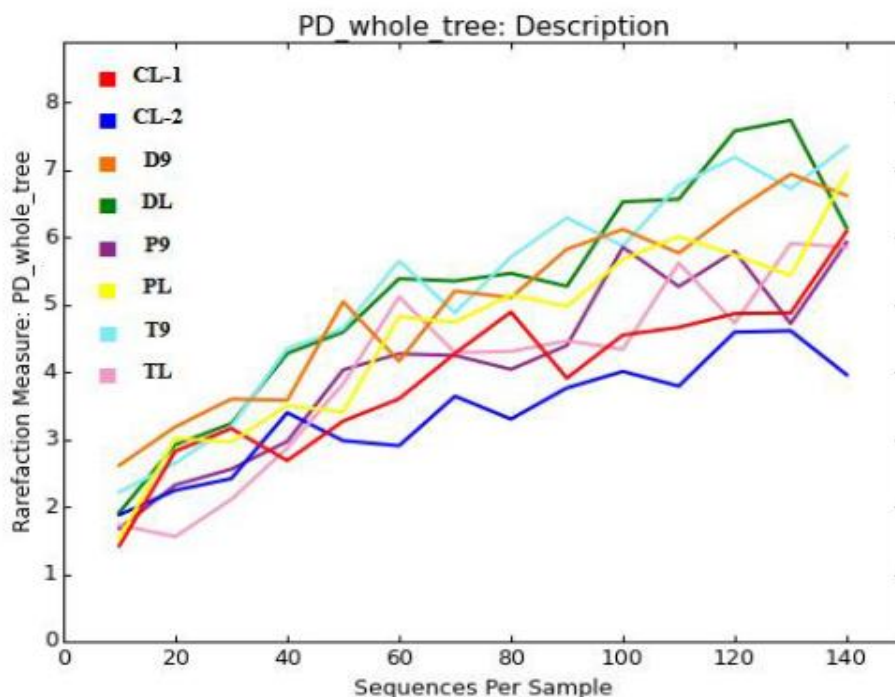


Fig. 6. Faith's phylogenetic diversity (PD whole tree) for analysis of rarefaction curves based on calculated from 16S rRNA gene OTU data. Curves of graph show the accumulation of phylogenetic diversity with increasing sequencing depth of samples, showing variation in evolutionary diversity among sites.

Rarefaction Analysis Based on Observed OTUs

Rarefaction analysis derived from observed OTUs represented a richness of species with sequencing depth in all samples, showing the 16S rRNA gene sequencing analysis of the Keru carcass dumping ecosystem (Fig. 8). Although no data of curves are shown, there is a complete plateau and a gradual tapering of OTU accumulation at the higher depths of sequencing, suggesting a sufficient assemblage of dominant taxa with rare OTUs. Samples DL and T9

showed higher richness, whereas TL and CL-2 detected relatively lower OTU counts, showing strong heterogeneity in environmental conditions of the sampling site. Such analyses are the good agreement of the previous studies of decomposition-driven ecosystems, and waste-impacted studies showed good microbial assemblage. Previous studies suggested that observed OTU-based rarefaction furnish a strong first-order agreement of sampling adequacy and within-sample diversity in environmental microbiome analyses (Schloss et al., 2009; Knight et al., 2018).

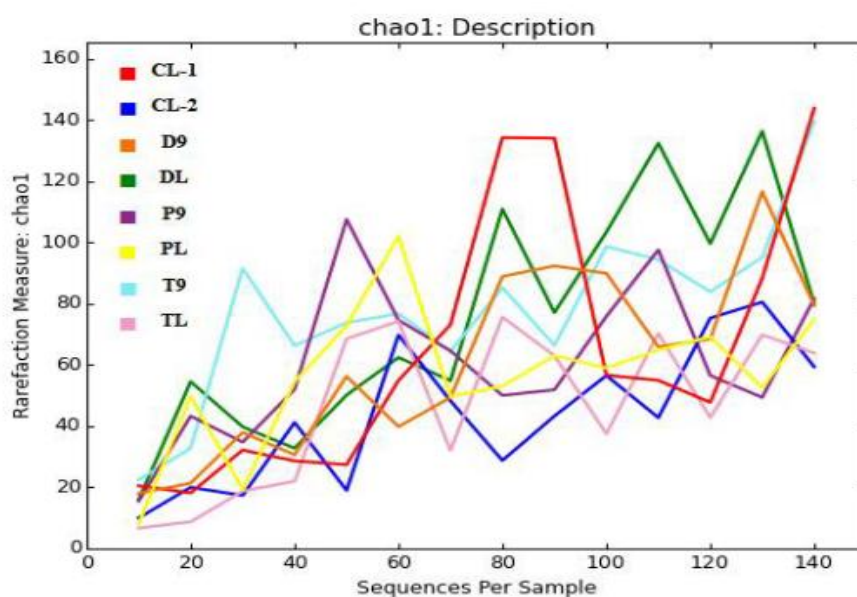


Fig. 7. 16S rRNA gene OTU data were used to calculate rarefaction curves based on the Chao1 richness estimator. Curves represent the accumulation of estimated species richness with increasing sequencing depth across samples, showing differences in rare taxon representation between sites.

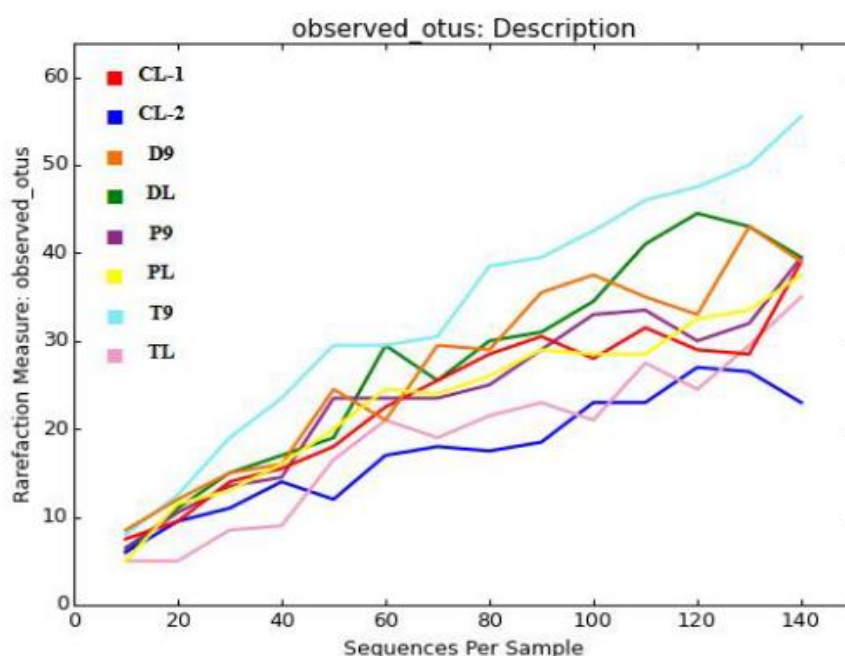


Fig. 8. OTUs calculated from 16S rRNA gene sequencing data applied to make rarefaction curves. Curves represent the accumulation of analyzed operational taxonomic units with increasing sequencing depth across samples, showing variation in microbial richness.

Overall, these results approve sufficient effort of sequencing for comparative analysis of diversity and verify the complexity of microbial communities related to long-term carcass disposal.

Shannon Diversity-Based Rarefaction Analysis

Rarefaction-based analysis of the Shannon diversity index confirmed a successive increase in diversity with the depth of sequencing across all samples, confirming enhanced resolution of both richness and evenness between microbial communities as effort increased in sampling (Fig. 9). T9 effectively confirmed the highest Shannon

diversity, showing a more even distribution of taxa, whereas TL and CL-2 represented lower values of taxa. Such a type of variability is distinctive decomposition-driven ecosystems and the waste-impacted influence of microbial evenness (Fierer et al., 2012). A similar type of Shannon rarefaction trends was reported in previous studies of soil and organic waste microbiomes of related areas (Knight et al., 2018).

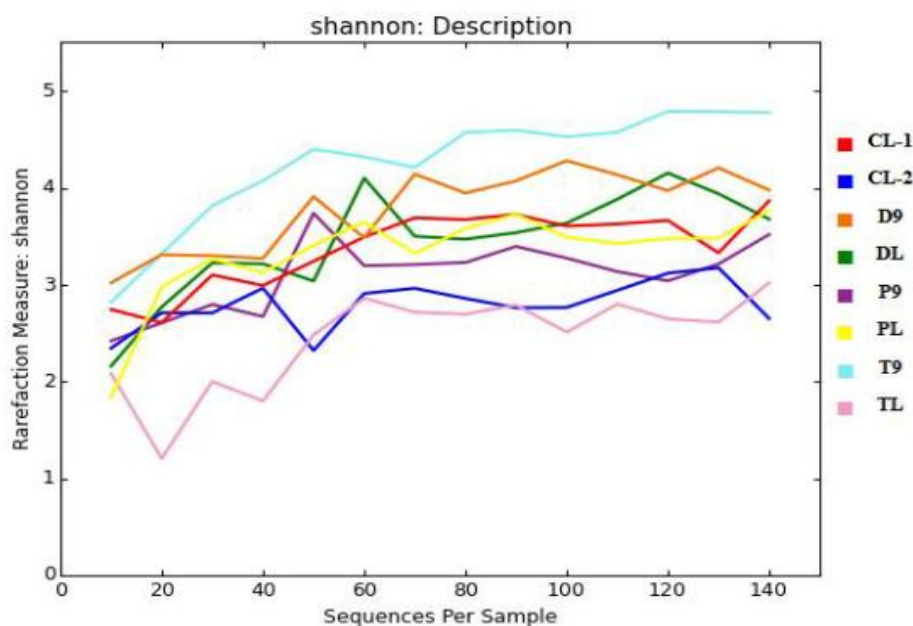
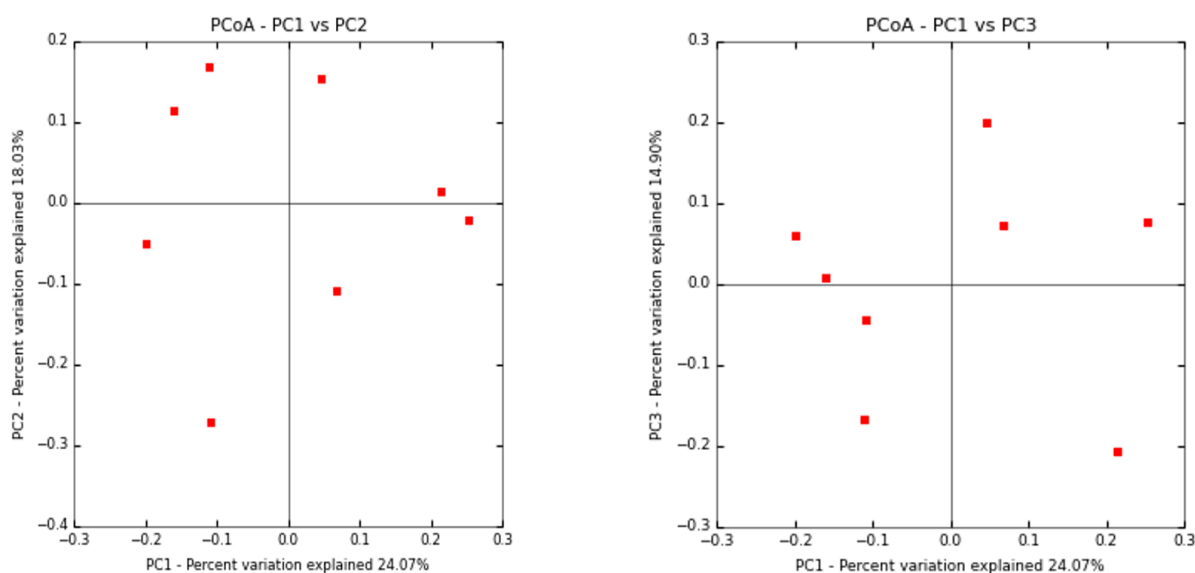


Fig. 9. Shannon diversity index was used to calculate 16S rRNA gene sequencing data to form rarefaction curves.

Beta Diversity and Ordination Analysis

Analysis of beta diversity revealed noticeable differences in composition of the microbial community among various samples collected from the Keru carcass dumping ecosystem, as analyzed by principal coordinates analysis (PCoA) in two- and three-dimensional analysis (Fig. 10). PCoA plots confirmed partial clustering of samples showed clear separation of coexistence of shared core taxa and site-specific community features. The first three ordination axes explained a reasonable percentage of the total variation (PC1: 24.1%, PC2: 18.0%, PC3: 14.9%), representing higher-level community variations that were described within low-dimensional space. Fair separation of reported samples along with axes demonstrates the assemblage of microbial sampling of a homogeneous community. Comprehensive analysis of beta diversity confirms that microbial communities of the Keru dumping ecosystem of Jodhpur strongly suggest controlled localized environmental variations of microbial populations. The present study describes a consolidated culture-based and metagenomic technique to characterize diversity and microbial community, and envisage functional capacity between various environmental matrices at the site of a carcass dumping ecosystem in the Keru region of Jodhpur.



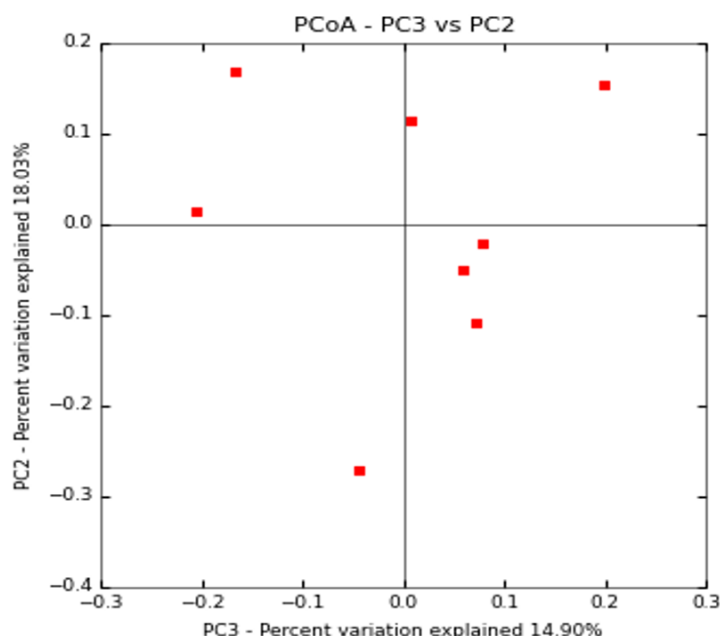


Fig. 10. Beta diversity analysis using PCoA showed clear segregation among samples, with PC₁, PC₂, and PC₃ showing 24.1%, 18.0%, and 14.9% of the total variation, respectively, representing higher spatial structuring of microbial communities

Conclusion

This study suggests culture and metagenomic based analysis are highly suitable methods to understand microbial communities in a vulture depleting and carcass dumping ecosystem in Keru, Jodhpur. Present data also described as the assemblage of microbial composition across soil, water, air and vulture associated data were highly diverse and phylogenetically broad. Amplicon sequencing based data suggests heterogeneity of microbial taxa and site-specific microflora adapted to sustained organic enrichment and sustainability. Predictive functional profiling revealed the metabolic potential of decomposition, stress tolerance and, nutrient cycling with ecological consequences by scavenger mediated carcass removal. The Keru region near Jodhpur, Rajasthan, showed that this site receives animal carcasses from nearby areas and is effectively influenced by nearby waste disposal practices, which is making this site an organic rich microhabitat.

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