

Unveiling Macrofungal Diversity Across Dry Deciduous Forest Protected Areas of Rajasthan, India: A Multivariate and Visual Analytical Approach

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ABSTRACT

Macrofungi provide essential ecological functions as decomposers and symbionts; yet, their diversity is little studied in dry deciduous forests. This research examines macrofungal diversity and community composition in three separate wildlife sanctuaries in Rajasthan, India: Mount Abu Wildlife Sanctuary (MAWS), Kumbhalgarh Wildlife Sanctuary (KWS), and Sitamata Wildlife Sanctuary (SWS). A total seventy-seven macrofungal species were validated by using of ITS-5.8S rRNA gene sequences. MAWS demonstrating the greatest species richness (74 species), Shannon–Wiener diversity ($H' = 4.20$), and evenness. Comparative diversity analysis indicated that more than half (53.24%) of the species were common across all three locations, signifying a shared core population with stable fungal community, while several taxa were distinct to individual sites, illustrating ecological variability. Unique species assemblages were identified at each location, shaped by microclimatic and ecological variables. Rank-abundance curves and beta diversity indicators indicated a more equitable community at MAWS and greater compositional similarities between KWS and SWS. K-means clustering and Venn diagram analysis further corroborated the uniqueness of macrofungal communities across various settings. This research offers new insights on macrofungal diversity in dry deciduous forest habitats, contrasting with earlier studies that concentrated mostly on moist tropical forests, and proposes integrated analytical approaches for assessing community dynamics. The results highlight the significance of habitat variability and microclimatic elements in maintaining fungal diversity and provide a crucial foundation for future conservation efforts and ecological research in dry deciduous forest, Rajasthan India.

Keywords: Community structure, Diversity indices, K-means clustering, Macrofungi

INTRODUCTION

Soil fungus are essential for preserving the health and sustainability of arid, semi-arid and dry deciduous forest ecosystem including those in Rajasthan, India. These places exhibit limited precipitation, inadequate organic matter, nutrient-deficient soils, and severe temperatures, rendering the role of soil fungus crucial for ecosystem resilience. From an ecological perspective, soil fungi contribute to nutrient cycling specifically, saprophytic fungus serve as essential decomposers of organic materials. In dry locations with little plant litter and sluggish decomposition rates, fungus expedite the degradation of refractory components (such as cellulose and lignin), thereby releasing nutrients like nitrogen and phosphorus into the soil (Verma and Gupta, 2022). Mycorrhizal fungi establish symbiotic relationships with plant roots, improving water and nutrient absorption,

especially phosphorus (Sharma and Adholeya, 2004). Fungal mycelia enhance plant drought resilience, maintain soil structure, and facilitate vegetation persistence in extreme climates (Mathur *et al.*, 2019). Fungi generate hyphae and adhesive proteins, which adhere soil particles and improve soil aggregation with enhances soil porosity and water retention (Rilling, 2004). Soil fungus facilitate the formation of various plant communities by promoting nutrient availability and moderating interspecies interactions. In dry regions, when resources are few, fungi alleviate plant stress and sustain productivity (Allen, 2007). Additionally, macrofungi contribute to carbon sequestration via their biomass and by stabilizing organic materials. Fungus play a role in microcarbon sinks, mitigating climatic unpredictability.

Macrofungi are adaptable to the varied climatic conditions of Rajasthan, India and contribute to the ecological equilibrium of the region. In the delicate dry deciduous forest ecosystems of Rajasthan, macrofungus serve not just as decomposers but also as ecosystem engineers. They maintain nutrient cycles, promote plant vitality, mitigate desertification, and improve soil stability. Comprehending and measuring their variety is essential for conservation, particularly in protected regions such as wildlife sanctuaries, where maintaining soil health is vital for the long-term sustainability of ecosystems.

Although research has recorded the existence of saprophytic fungi and actinomycetes in arid and semi-arid regions (Gehlot et al., 2016; Parihar et al., 2022). Gehlot et al. (2016) did a research examining soil fungus diversity in the Indian Thar Desert. The research indicated elevated alpha diversity in Jodhpur (69 species) and reduced diversity in Jaisalmer (48 species). Hyphomycetes constituted the principal fungal community, with *Aspergillus fumigatus* being the most prevalent species. The research underscores the abundant fungal species seen in the dry areas of Rajasthan. Gehlot et al., (2016) executed a survey examining the variety of Macrofungi in Rajasthan, especially in the arid and semi-arid area of Rajasthan. The research record species of 69 macrofungi. These fungi are adaptable to the varied climatic conditions of Rajasthan and contribute to the ecological equilibrium of the region. In the delicate dry and semi-arid ecosystems of Rajasthan, soil fungus serve not just as decomposers but also as ecosystem engineers. They maintain nutrient cycles, promote plant vitality, mitigate desertification, and improve soil stability. Comprehending and measuring their variety is essential for conservation, particularly in protected regions such as wildlife sanctuaries, where maintaining soil health is vital for the long-term sustainability of ecosystems.

Despite the ecological significance of soil fungus including macrofungi in dry deciduous forest habitats there is a notable lack of extensive research examining fungal community diversity in wildlife sanctuaries in Rajasthan. Most current study on soil mycobiota on the region has focused on agricultural soils with little emphasis on protected and environmentally sensitive regions. Research has also investigated on AM fungi. There is a

notable lack of extensive research examining fungal community diversity in wildlife sanctuaries in Rajasthan such as the Mount Abu Wildlife Sanctuary (MAWS), Sitamata Wildlife Sanctuary (SWS), or Kumbhalgarh Wildlife Sanctuary (KWS). These safeguarded environments may include unique and ecologically important fungal communities owing to their specific microclimates, soil compositions, and undisturbed vegetation. Furthermore, there is an absence of integration of contemporary ecological methodologies, such as K-means clustering and Venn diagram-based analysis, in delineating fungal community patterns across various protected areas. This disparity highlights the pressing necessity for focused research that integrates genomic diversity evaluations with sophisticated statistical methodologies, thereby enhancing our comprehension of fungal ecology in Rajasthan's wildlife sanctuaries and guiding conservation efforts.

This study use statistical clustering and visual analytical methods to investigate and compare macrofungal populations across three wildlife sanctuary sites. K-means clustering, a prevalent unsupervised machine learning approach, will be utilized to discern patterns and classifications within fungal community data based on species abundance and diversity parameters. This method facilitates the categorization of ecologically analogous locations and the identification of latent gradients in community composition that may not be readily observable by conventional diversity indices. K-means has been effectively utilized in microbial ecology to reveal clustering patterns in bacterial and fungal populations across environmental gradients.

Additionally, Venn diagrams will be employed to illustrate the intersection and distinctiveness of fungal species throughout the three sanctuary locations. This picture facilitates comprehension of community exclusivity and collective ecological functions. Venn diagram analysis has demonstrated its use in fungal ecology by depicting core and site-specific taxa throughout intricate ecosystems, including forests, grasslands, and polluted soils. Collectively, these analytical instruments will furnish a thorough framework for delineating and contrasting fungal biodiversity in the protected ecosystems of Rajasthan, yielding insights into habitat-specific fungal processes. This study will use K-means clustering for community

classification and Venn diagrams for inter-site analysis to offer a comprehensive knowledge of fungal diversity trends in Rajasthan's protected regions. These approaches will facilitate the identification of essential fungal species that uphold the functional integrity of ecosystems and enable the evaluation of ecological connection among sanctuary locations. This comprehensive strategy will also aid in the management and maintenance of soil fungus diversity within these distinctive animal habitats.

The major objective of this study is to evaluate and compare the diversity of soil fungal communities throughout three designated wildlife sanctuaries in Rajasthan, which exemplify different ecological environments within dry deciduous forest ecosystem. This research aims to bridge the information gap by integrating genetic identification methods with statistical and visual analysis tools to reveal trends in fungal diversity and distribution. The explicit aims are: (a) to isolate, identify, and quantify macrofungus population from three wildlife sanctuary locations in Rajasthan via molecular techniques, (b) to assess the diversity and richness of macrofungi at each location utilizing suitable diversity indices (c) to use K-means clustering for the classification and grouping of fungal communities based on similarities in species composition and environmental characteristics, (d) to create Venn diagrams for the visualization and interpretation of the common and distinct fungal species across the three research sites and (e) to establish baseline data for conservation initiatives by cataloguing the fungal species in ecologically sensitive and inadequately studied wildlife areas of Rajasthan.

MATERIALS AND METHODS

Survey and Collection of Macrofungi specimens

A repeated survey were made during the rainy season (July to October months of year 2021 to 2023) of the three sanctuaries, viz., Mount Abu Wildlife Sanctuary (24°36' N latitude and 72°42' E longitude), Kumbhalgarh Wildlife Sanctuary (25°08' N latitude and 73°35' E longitude), and Sitamata Wildlife Sanctuary (24.04' N latitude and 74.25' E longitude), located at the Aravalli Mountain range (an eroded stub of ancient mountains, origin Pre-Cambrian Period) to explore and collect the specimens of macrofungi. Macrofungi specimens carefully extracted from ground and put into small card board box to bring

to laboratory. Field notes on the specimen including habitats, size, shape, smell, colour, GPS mapping, photography and other macroscopic characteristics features were carefully recorded for identification purposes.

Identification of macrofungi specimens

Identification of macrofungi specimens was done by using a combined approach of morphology as well as molecular characteristic features. Morphological characteristic features include phenotypic parameters viz. viz. different parts of fruiting bodies like shape, size, color, texture, cap, stalk, attachment of stipe gills, volva, annulus, basidia, cystidia, basidiospores, hyphae, mycelia, etc. These phenotypic parameters were the best match with taxonomic literature provided by Singer (1986), Pegler (1997), Jordan (2004), Phillips (2006), Cannon and Krick (2007), and Mohanan (2011). Identification of the same macrofungal specimens at the molecular level was performed using ITS-5.8S gene sequences (rRNA gene) as the molecular marker method described by White et al. (1990).

Community Dynamics with Quantitative Ecological Attributes

a) Quadrature Studies

In the present study, the density, frequency, abundance and Relative Importance Values (RIV) of different species collected from three sancturies quadrade sites of a particular habitat were quantified by using following ecological formulas (Mathur *et al.*, 2019 and Uddin *et al.*, 2020). Density was calculated for all the collected species by expressing the numerical strength of each individual.

$$\text{Density of a species} = \frac{\text{Total number of individuals of a species in all the sampling areas}}{\text{Total number of area studied}}$$

Frequency (%): Frequency is the percentage of occurrence of individual species in an area. It was studied by sampling the study area at several places at random and recorded the names of the species that occurred in each sampling units. It is calculated by the following equation:

$$\text{Frequency of a species} = \frac{\text{Total number of sampling areas in which species occur}}{\text{Total number of area studied}} \times 100$$

Abundance: It is the study of the number of individuals of different species in the community per unit area. It is represented by the equation:

Abundance of a species

$$= \frac{\text{Total number of individuals of a species in all the sampling areas}}{\text{Total number of sampling areas in which the species occurs}}$$

$$\text{Relative Density of a Species} = \frac{\text{Density of a species}}{\text{Sum of all the densities}} \times 100$$

$$\text{Relative Frequency of a Species} = \frac{\text{Frequency of a species}}{\text{Sum of all the frequencies}} \times 100$$

$$\text{Relative Abundance of a Species} = \frac{\text{Abundance of a species}}{\text{Total Abundance of all the species}} \times 100$$

Relative Importance Value (RIV)

$$= \frac{\text{Relative Density} + \text{Relative Frequency} + \text{Relative Abundance}}{3}$$

Dominance Diversity (DD) Curve

The DD Curve was developed to assess the abundance patterns of various species across several zones. This is a facet of biodiversity that summarizes the prevalence or scarcity of a species in relation to other species within a certain area (Chaithaisong et al. 2022). The current investigation involved the log transformation of the RIV of several species, which was thereafter organized in decreasing order and visually represented using microsoft excel software. In a community, three fundamental forms of distribution are observed: geometric, broken-stick, and lognormal. The geometric distribution type predominates in relatively species-poor communities where a singular environmental resource, such as moisture, is crucial for species survival and is exploited in a highly hierarchical manner. In this scenario, a single dominant species monopolizes a substantial portion of the resources; the subsequent most successful species monopolizes a lesser portion of the remaining resources, and so on. The broken-stick model posits that species within a community split or consume essential resources without overlap, whereas the log-normal model is characterized by a vast species assembly with sub-equal abundance (Clark 1990). The lognormal distribution posits that the significance of species is influenced by the interplay of several factors that dictate success within the niche hierarchy (Whittaker 1977).

Diversity Analysis (λ and β)

Species diversity was evaluated at two levels: Alpha diversity (diversity within a habitat) was determined using indices such as Shannon's

diversity index (H'), Simpson's diversity index (λ), and Evenness ($E5$). The alpha diversity indexes are mathematically independent of one another. Beta diversity, assessed using the Whittaker index, will yield a comparative analysis of similarities among the tested ecosystems. The diversity indices were computed utilizing Paleontological Statistics (PAST) Version 1.92 software. Employed diverse alpha diversity indices as advocated by the research of Morris et al. (2014), which indicated that analyzing diversity through different indices can yield enhanced understanding of system interactions.

Alpha Diversity: The alpha diversity of a site is determined by the interplay of local biotic and abiotic factors and immigration from other areas (Whittaker 1960). Alpha diversity consists of two components: species richness and evenness or equitability. Species richness serves as an indication of the relative abundance of species within a community (Peet 1974) and may be quantified by the overall species count in a specific community or the species density per unit area. Species richness was analyzed using basic species enumeration and the use of Margalef and Menhinick richness indexes.

Simpson index of dominance (λ): This index basically measures the probability that two individuals selected at random from a sample will belong to same species (Sultana et al. 2014) and it ranges from 0 to 1. Simpson index was calculated by using the density of one organism divided by the total density of all organisms, and it can be expressed in following mathematical equation:

$$D = \sum P_i^2$$

Because Simpson index is actually an index of dominance, and then tend to be inversely to evenness and richness and in our study this trend was also recorded with richness.

Shannon and Weaver index: This diversity index was calculated by using following formula.

$$H = -\sum P_i \log_e P_i$$

Shannon-weaver index is based on information theory (the information content is a measure of the amount of uncertainty). It generally falls between 1.5 and 3.5, and rarely exceeds 4.5 (Mathur, 2005). Higher Shannon Weaver index values indicated that many species are represented by the same number and low value showed complete dominance of one

species. In other words high value indicates high diversity at particular zone. Simpson index emphasis on dominance species value where is Shannon index is highly influence by rare species.

Species Evenness: Evenness or equitability represents the distribution of individuals among the species. It sometimes defined as the ratio of observed diversity to maximum diversity (Margalef, 1958). The Evenness or equitability was calculated by the formula suggested by the Pielou (1965).

$$E = H/\log_{10}S$$

H= Shannon index, S= total number of species of the stand.

Beta Diversity: Inter-community or inter-habitat diversity is termed β - diversity. Routledge (1977) defined it mathematically as “the inverse of mean proportion of communities or habitats occupied by a single species”. It is also defined as extent of species replacement or species turnover along an environmental gradient (Whittaker 1972, Cody 1975 and 1993). The beta diversity was calculated by Cody (1993) formula:

$$B_n = 1 - C \frac{(S_1 + S_2)}{S_1 \times S_2}$$

Where, S_1 and S_2 are the number of species in sampling areas 1 and 2, respectively. C is the number of species that are shared by areas 1 and 2, and β_n is Cody's new measure of beta diversity.

Multivariate Analysis

K-Means Clustering Analysis

To identify patterns and groupings among macrofungal species based on their abundance across different sanctuaries, a K-means clustering analysis was conducted using OriginPro 2023 software. The abundance data of macrofungal species collected from the three study sites—Mount Abu Wildlife Sanctuary (MAWS), Kumbhalgarh Wildlife Sanctuary (KWS), and Sitamata Wildlife Sanctuary (SWS)—were initially organized in a spreadsheet, with species listed as rows and relative abundance values across sanctuaries as columns. The data were standardized to mitigate bias arising from varying scales. Quantity of Clusters (k): Established by doing trial iterations from $k = 2$ to $k = 5$ and selecting $k = 3$ based on the minimal Within-Cluster Sum of Squares (WCSS) and visual clarity.

The Euclidean distance was employed to assess similarities across species according to their abundance patterns. K-means++ was employed for centroid initialization, and the algorithm executed for a maximum of 10 iterations or until convergence was achieved. The outputs of this program evaluated the cluster membership of each species, the centroid positions for each cluster, the within-cluster variance (WCSS), and the average and maximum distances from species to their corresponding cluster centroids. Cluster membership was illustrated by 2D scatter plots (Principal Component axis) to demonstrate cluster separation, with summary tables presenting compactness metrics including WCSS, average distance, and maximum distance for each cluster.

Venn Diagram Analysis

A Venn diagram analysis was conducted using Venny 2.1 (Oliveros 2007–2015) to analyze the presence and overlap of macrofungal species across the three research sites: Mount Abu Wildlife Sanctuary, Kumbhalgarh Wildlife Sanctuary, and Sitamata Wildlife Sanctuary. For this analysis, species presence-absence data and abundance data from each sanctuary were compiled into distinct lists, and a Venn diagram incorporating both data sets was generated using the online tool Venny 2.1 (<https://bioinfogp.cnb.csic.es/tools/venny/index.html>).

RESULTS

Macrofungi species designation

The present study was established with the collection of 881 macrofungal basidiocarps from different localities of the above three sanctuaries. Out of which, 393 macrofungal basidiocarp specimens were from MAWS, 264 basidiocarp were from KWS and 224 basidiocarp specimens were collected from the SWS. On the basis of morphological resemblances, 77 basidiocarp specimens were scrutinised for species designation at the molecular level (ITS-5.8S gene sequences with the GenBank reference sequences having a 99.1% similarity threshold for the name of species validation (Borg Dahl et al., 2017). Although, some specimens have less than 99.1% similarity to their best match from the sequences of GenBank (NCBI), but incorporation of morphology characteristics features (macroscopic and microscopic features), correct species were identified. The highest richness (74) was seen at

MAWS, followed by KWS (59), with the lowest richness reported at SWS (54) (**Table 1**). Of the species, 53.24% (41) were observed at all three locations. Twenty-eight species were prevalent in two sanctuaries, of which fifteen species (*Crepidotus dentatus*, *Stropharia* sp., *Disciseda hyalothrix*, *Omphalotus olivascens*, *Amanita manicata*, *Entoloma* sp., *Clitopilus prunulus*, *Scleroderma bovista*, *Coriolopsis trogii*, *Earliella scabrosa*, *Podoscypha petalodes*, *Geastrum triplex*, *Geastrum xerophilum*, and *Stereopsis radicans*) were recorded at Mount Abu and Kumbhalgarh Wildlife Sanctuary. Likewise, 11 species (*Leucocoprinus cepistipes*, *Crepidotus indicus*, *Calvatia holothurioides*, *Marasmius hypochroides*, *Collybiopsis menehune*, *Pluteus* sp., *Entoloma luteodiscum*, *Trametes elegans*, *Lentinus* sp., *Auricularia srilankensis*, and *Fomitiporia bannaensis*) were prevalent in the Mount Abu and Sitamata Sanctuaries. *Amanita nana* and *Itajahya roseae* were prevalent in the Kumbhalgarh and Sitamata regions. *Agaricus glabriusculus*, *Agaricus* sp., *Leucocoprinus birnbaumii*, *Chlorophyllum palaeotropicum*, *Candolleomyces asiaticus*, *Gyrodontium sacchari*, *Punctularia atropurpurascens*, and *Rhodophana* sp. were

habitat-specific to the Mount Abu and Kumbhalgarh regions, respectively.

Quadrat Data Analysis

The density, frequency, abundance, and their Relative Importance Values (RIV) of the collected species at the three research locations are presented in **Table 1**. *Schizophyllum commune* (JP112) and *Ramaria* sp. (JP70) have the greatest Relative Importance Value (RIV) of 2.39, signifying their dominating ecological importance within their respective habitats. Species such as *Coprinus* sp. JP203, *Termitomyces* sp. JP97, and *Podoscypha petalodes* JP57 demonstrate elevated RIVs, distinguishing them as significant. Species with Relative Importance Values (RIVs) below 1.0 exhibit diminished ecological significance regarding dominance and frequency, although remain integral to the community. The significant variance in species presence among the locations indicates habitat-specific dispersion and niche preferences. The table elucidates the composition and ecological function of macrofungi in the region. A few species predominate in the fungal ecosystem, although several others appear intermittently, enhancing total biodiversity.

Table 1: Macro-fungi recorded at the three studied sites with their Frequency (F), Density (D) and Abundance (A) and Relative Importance Values (RIV)

S. No.	Name of Macro-fungi Species	Studied Sites			F	D	A	Relative			
		MAWS	KWS	SWS				F	D	A	RIV
1	<i>Agaricus subrufescens</i> isolate JP24	7	4	3	1.00	4.67	4.67	1.60	1.64	1.36	1.54
2	<i>Agaricus glabriusculus</i> isolate JP206	3	0	0	0.33	1.00	3.00	0.53	0.35	0.87	0.59
3	<i>Agaricus parviniveus</i> isolate JP202	1	2	2	1.00	1.67	1.67	1.60	0.59	0.49	0.89
4	<i>Agaricus</i> sp. isolate JP27	4	0	0	0.33	1.33	4.00	0.53	0.47	1.17	0.72
5	<i>Xanthagaricus epipastus</i> isolate JP42	5	2	1	1.00	2.67	2.67	1.60	0.94	0.78	1.11
6	<i>Coprinus sterquilinus</i> isolate JP103	4	5	7	1.00	5.33	5.33	1.60	1.88	1.55	1.68
7	<i>Coprinus</i> sp. JP203	9	6	8	1.00	7.67	7.67	1.60	2.70	2.24	2.18
8	<i>Leucocoprinus birnbaumii</i> isolate JP29	4	0	0	0.33	1.33	4.00	0.53	0.47	1.17	0.72

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S. No.	Name of Macro-fungi Species	Studied Sites			F	D	A	Relative			
		MAWS	KWS	SWS				F	D	A	RIV
9	<i>Leucocoprinus cepistipes</i> isolate JP-31	3	0	1	0.67	1.33	2.00	1.07	0.47	0.58	0.71
10	<i>Chlorophyllum palaeotropicum</i> isolate JP201	2	0	0	0.33	0.67	2.00	0.53	0.23	0.58	0.45
11	<i>Microspalliota sp.</i> isolate JP59	5	4	3	1.00	4.00	4.00	1.60	1.41	1.17	1.39
12	<i>Tulostoma operculatum</i> isolate JP45	3	7	6	1.00	5.33	5.33	1.60	1.88	1.55	1.68
13	<i>Podaxis pistillaris</i> isolate JP73	6	8	7	1.00	7.00	7.00	1.60	2.46	2.04	2.04
14	<i>Bolbitius sp.</i> isolate JP109	3	1	2	1.00	2.00	2.00	1.60	0.70	0.58	0.96
15	<i>Conocybe aurea</i> isolate JP88	2	2	1	1.00	1.67	1.67	1.60	0.59	0.49	0.89
16	<i>Conocybe utricystidiata</i> isolate JP38	5	4	5	1.00	4.67	4.67	1.60	1.64	1.36	1.54
17	<i>Crepidotus applanatus</i> isolate JP3	8	5	4	1.00	5.67	5.67	1.60	1.99	1.65	1.75
18	<i>Crepidotus dentatus</i> isolate JP66	8	3	0	0.67	3.67	5.50	1.07	1.29	1.60	1.32
19	<i>Crepidotus indicus</i> isolate JP81	4	0	5	0.67	3.00	4.50	1.07	1.06	1.31	1.15
20	<i>Stropharia sp.</i> isolate JP49	4	5	0	0.67	3.00	4.50	1.07	1.06	1.31	1.15
21	<i>Psathyrella rogueiana</i> isolate JP113	6	4	2	1.00	4.00	4.00	1.60	1.41	1.17	1.39
22	<i>Coprinellus aureogranulatus</i> isolate JP74	2	4	2	1.00	2.67	2.67	1.60	0.94	0.78	1.11
23	<i>Candolleomyces asiaticus</i> isolate JP98	5	0	0	0.33	1.67	5.00	0.53	0.59	1.46	0.86
24	<i>Lycoperdon pratense</i> isolate JP54	6	4	3	1.00	4.33	4.33	1.60	1.52	1.26	1.46
25	<i>Calvatia holothurioides</i> isolate JP58	3	0	1	0.67	1.33	2.00	1.07	0.47	0.58	0.71
26	<i>Disciseda hyalothrix</i> isolate JP207	3	1	0	0.67	1.33	2.00	1.07	0.47	0.58	0.71
27	<i>Marasmius brunneoaurantiacus</i> isolate JP16	4	3	2	1.00	3.00	3.00	1.60	1.06	0.87	1.18
28	<i>Marasmius hypochroides</i> isolate JP51	3	0	2	0.67	1.67	2.50	1.07	0.59	0.73	0.79
29	<i>Marasmius leveilleanus</i> isolate JP101	1	1	2	1.00	1.33	1.33	1.60	0.47	0.39	0.82
30	<i>Porothelium sp.</i> isolate JP15	8	3	5	1.00	5.33	5.33	1.60	1.88	1.55	1.68

S. No.	Name of Macro-fungi Species	Studied Sites			F	D	A	Relative			RIV
		MAWS	KWS	SWS				F	D	A	
31	<i>Gerronema keralense</i> isolate JP52	8	1	2	1.00	3.67	3.67	1.60	1.29	1.07	1.32
32	<i>Omphalotus olivascens</i> isolate JP26	6	8	0	0.67	4.67	7.00	1.07	1.64	2.04	1.58
33	<i>Collybiopsis menehune</i> isolate JP41	3	0	4	0.67	2.33	3.50	1.07	0.82	1.02	0.97
34	<i>Mycena sp.</i> isolate JP56	4	6	7	1.00	5.67	5.67	1.60	1.99	1.65	1.75
35	<i>Hymenopellis furfuracea</i> isolate JP61	2	1	0	0.67	1.00	1.50	1.07	0.35	0.44	0.62
36	<i>Pluteus sp.</i> isolate JP23	2	0	3	0.67	1.67	2.50	1.07	0.59	0.73	0.79
37	<i>Amanita manicata</i> isolate JP8	6	4	0	0.67	3.33	5.00	1.07	1.17	1.46	1.23
38	<i>Amanita nana</i> isolate JP208	0	3	2	0.67	1.67	2.50	1.07	0.59	0.73	0.79
39	<i>Pleurotus djamor</i> isolate JP82	6	8	5	1.00	6.33	6.33	1.60	2.23	1.85	1.89
40	<i>Entoloma luteodiscum</i> isolate JP102	8	0	9	0.67	5.67	8.50	1.07	1.99	2.48	1.85
41	<i>Entoloma sp.</i> isolate JP25	4	3	0	0.67	2.33	3.50	1.07	0.82	1.02	0.97
42	<i>Clitopilus prunulus</i> isolate JP33	8	6	0	0.67	4.67	7.00	1.07	1.64	2.04	1.58
43	<i>Rhodocybe brunneoaurantiaca</i> isolate JP40	3	4	2	1.00	3.00	3.00	1.60	1.06	0.87	1.18
44	<i>Rhodophana sp.</i> isolate JP139	0	1	0	0.33	0.33	1.00	0.53	0.12	0.29	0.31
45	<i>Tricholosporum sp.</i> isolate JP48	6	5	6	1.00	5.67	5.67	1.60	1.99	1.65	1.75
46	<i>Termitomyces sp.</i> isolate JP97	10	8	6	1.00	8.00	8.00	1.60	2.81	2.33	2.25
47	<i>Schizophyllum commune</i> isolate JP112	11	8	7	1.00	8.67	8.67	1.60	3.05	2.53	2.39
48	<i>Pisolithus albus</i> isolate JP30	8	4	5	1.00	5.67	5.67	1.60	1.99	1.65	1.75
49	<i>Scleroderma albidum</i> isolate JP84	11	6	1	1.00	6.00	6.00	1.60	2.11	1.75	1.82
50	<i>Scleroderma citrinum</i> isolate JP76	7	4	5	1.00	5.33	5.33	1.60	1.88	1.55	1.68
51	<i>Scleroderma bovista</i> isolate JP37	3	2	0	0.67	1.67	2.50	1.07	0.59	0.73	0.79
52	<i>Gyrodontium sacchari</i> isolate JP44	5	0	0	0.33	1.67	5.00	0.53	0.59	1.46	0.86
53	<i>Trametes ellipsospora</i> isolate JP5	6	4	3	1.00	4.33	4.33	1.60	1.52	1.26	1.46
54	<i>Trametes pavonia</i> isolate JP12	4	2	6	1.00	4.00	4.00	1.60	1.41	1.17	1.39

Unveiling Macrofungal Diversity Across Dry Deciduous Forest Protected Areas of Rajasthan, India: A Multivariate and Visual Analytical Approach

S. No.	Name of Macro-fungi Species	Studied Sites			F	D	A	Relative			RIV
		MAWS	KWS	SWS				F	D	A	
55	<i>Trametes elegans</i> isolate JP21	5	0	4	0.67	3.00	4.50	1.07	1.06	1.31	1.15
56	<i>Microporus xanthopus</i> isolate JP17	8	6	7	1.00	7.00	7.00	1.60	2.46	2.04	2.04
57	<i>Ganoderma multipileum</i> isolate JP22	6	4	5	1.00	5.00	5.00	1.60	1.76	1.46	1.61
58	<i>Coriolopsis trogii</i> isolate JP65	8	6	0	0.67	4.67	7.00	1.07	1.64	2.04	1.58
59	<i>Hexagonia tenuis</i> isolate JP80	4	6	2	1.00	4.00	4.00	1.60	1.41	1.17	1.39
60	<i>Earliella scabrosa</i> isolate JP90	3	2	0	0.67	1.67	2.50	1.07	0.59	0.73	0.79
61	<i>Lentinus sp.</i> isolate JP205	8	0	4	0.67	4.00	6.00	1.07	1.41	1.75	1.41
62	<i>Phlebiopsis lacerata</i> isolate JP47	6	3	2	1.00	3.67	3.67	1.60	1.29	1.07	1.32
63	<i>Porostereum fulvum</i> isolate JP99	3	1	2	1.00	2.00	2.00	1.60	0.70	0.58	0.96
64	<i>Podoscypha petalodes</i> isolate JP57	9	6	0	0.67	5.00	7.50	1.07	1.76	2.19	1.67
65	<i>Efibula tuberculata</i> isolate JP91	2	1	4	1.00	2.33	2.33	1.60	0.82	0.68	1.04
66	<i>Auricularia nigricans</i> isolate JP-32	7	5	5	1.00	5.67	5.67	1.60	1.99	1.65	1.75
67	<i>Auricularia srilankensis</i> JP204	2	0	2	0.67	1.33	2.00	1.07	0.47	0.58	0.71
68	<i>Fomitiporia bannaensis</i> isolate JP62	7	0	4	0.67	3.67	5.50	1.07	1.29	1.60	1.32
69	<i>Fuscoporia senex</i> isolate JP77	6	3	2	1.00	3.67	3.67	1.60	1.29	1.07	1.32
70	<i>Fuscoporia gilva</i> isolate JP107	9	5	8	1.00	7.33	7.33	1.60	2.58	2.14	2.11
71	<i>Phylloporia sp.</i> Isolate JP34	6	8	2	1.00	5.33	5.33	1.60	1.88	1.55	1.68
72	<i>Punctularia atropurpurascens</i> isolate JP69	3	0	0	0.33	1.00	3.00	0.53	0.35	0.87	0.59
73	<i>Ramaria sp.</i> isolate JP70	8	12	6	1.00	8.67	8.67	1.60	3.05	2.53	2.39
74	<i>Geastrum triplex</i> isolate JP96	5	6	0	0.67	3.67	5.50	1.07	1.29	1.60	1.32
75	<i>Geastrum xerophilum</i> isolate JP106	4	7	0	0.67	3.67	5.50	1.07	1.29	1.60	1.32
76	<i>Itajahya roseae</i> isolate JP140	0	5	3	0.67	2.67	4.00	1.07	0.94	1.17	1.06
77	<i>Stereopsis radicans</i> isolate JP43	8	3	0	0.67	3.67	5.50	1.07	1.29	1.60	1.32

Diversity Profiling

Figure 1 illustrates the outputs of alpha diversity (species richness). Alternative mathematical diversity indices, including the Shannon–Wiener index, Simpson index of dominance, and evenness, were employed to evaluate fungal community structure across various sites, with the corresponding results presented in **Table 2**. MAWS had the greatest species variety and evenness, signifying a more intricate and stable fungal environment. The Simpson Index of Dominance quantifies the likelihood that two randomly selected individuals from a sample are from distinct species. All three locations have exceptionally high values (0.98), indicating minimal dominance and elevated diversity. This indicates that no single species predominates at any of the locations; all exhibit balanced community patterns. The Shannon–Wiener Index (H') quantifies both species richness and evenness. Elevated values signify increased diversity. MAWS (4.20) has the highest score, indicating it is the most diversified location for both species richness and individual distribution among those species. KWS (3.93) and SWS (3.84) exhibit somewhat reduced diversity, perhaps attributable to the dominance of a limited number of taxa or a reduced total species count (**Figure 2**). The Evenness Index (E) indicates the uniformity of individual distribution throughout the existing species. MAWS (0.90) demonstrates the most equitable distribution, hence corroborating the elevated Shannon index. KWS and SWS have a little lower evenness (0.86), however still suggest rather balanced societies. Consequently, MAWS seems to be the most ecologically stable and diversified environment, presumably sustaining a robust and

well-distributed fungal population. KWS and SWS exhibit considerable variety; nevertheless, they have somewhat reduced evenness, indicating potential domination by a limited number of species or less intricate ecosystems.

The overall pattern suggests that species richness diminishes as alpha diversity increases. This may first appear paradoxical, although it is characteristic of diversity profiles (such as Rényi or Hill numbers), where an increase in the alpha parameter accentuates dominance and diminishes the significance of uncommon species. A comparative investigation of richness indicated that MAWS consistently has the highest species richness, implying it sustains a higher number of macrofungal species throughout all diversity orders, whereas KWS exhibits intermediate richness, and SWS displays the lowest richness at all levels of alpha diversity. Ecologically, our data suggest that the pronounced decrease in species richness with rising alpha in SWS implies a community potentially dominated by a limited number of species. Conversely, MAWS has a more uniform species distribution, preserving more richness while the diversity profile increasingly reflects species abundance distribution. The picture clearly illustrates site-specific variations in fungal species richness and community evenness using alpha diversity profiles. It indicates that Mount Abu Wildlife Sanctuary contains the most diversified macrofungal community, corroborating previous findings using Shannon, Simpson, and evenness indices.

The results of beta diversity (β) are shown in **Table 3**. SWS and KWS exhibited more similarity, with a β diversity of 0.24, whilst MAWS and KWS shown lesser similarity, with a β value of 0.16.

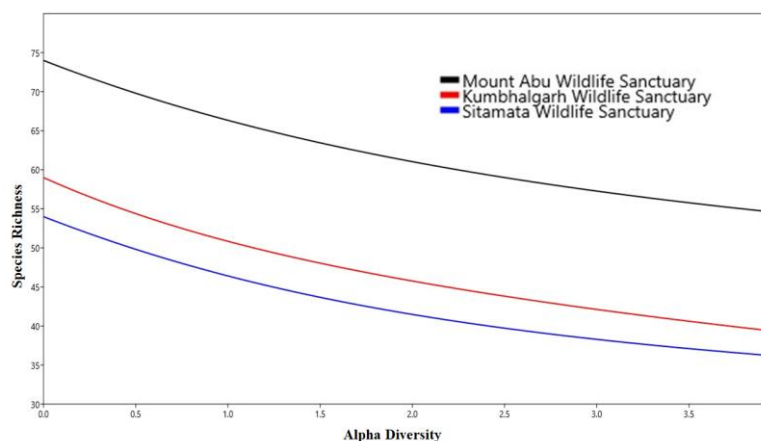


Figure 1: Alpha diversity at three studied sites

Table 2: Diversity Profiling at the Studied Sites

Diversity Parameters	MAWS	KWS	SWS
Simpson Index of Dominance	0.98	0.98	0.98
Shannon -Weiner index	4.20	3.93	3.84
Evenness Index	0.90	0.86	0.86

Table 3: Whittaker Beta Diversity Among different Studied Sites

Sites	MAWS	KWS
KWS	0.16	-
SWS	0.19	0.24

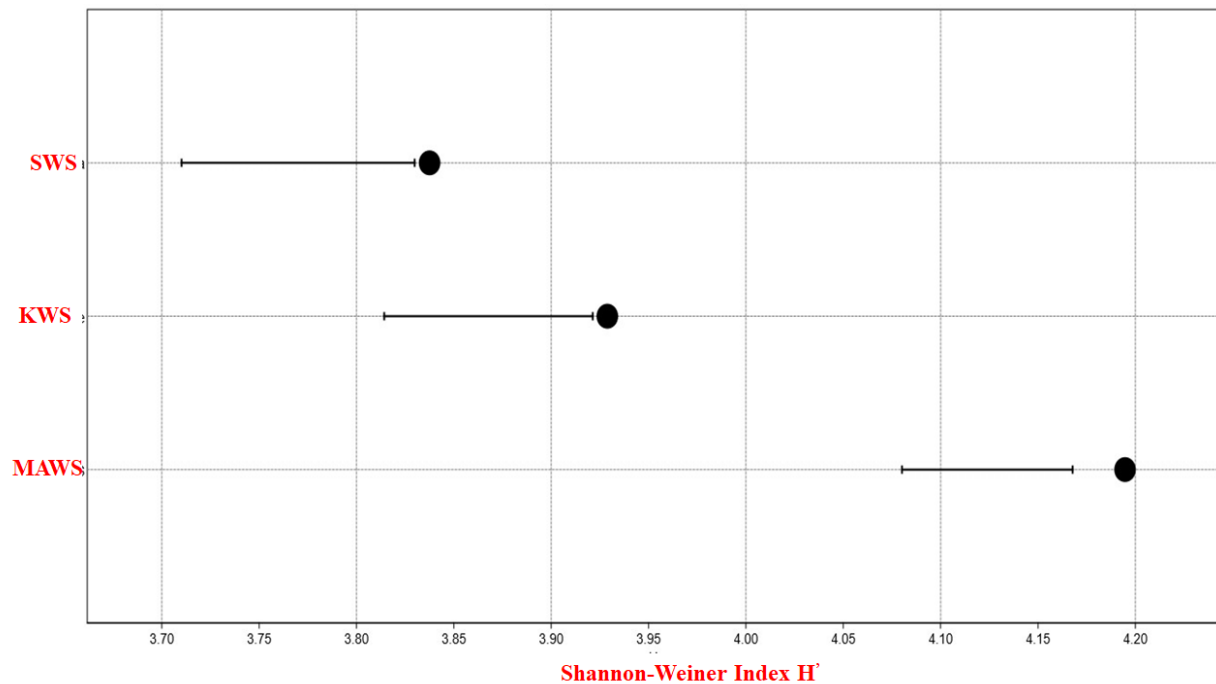


Figure 2: Shannon-Weaver Diversity Index at three studied sites

Diversity Dominance Curve (DD Curve)

The Rank-Abundance Curve, or Whittaker plot, is frequently employed in community ecology to illustrate species diversity and dominance patterns within biological groups. The X-axis (Rank) displays species listed from most to least numerous on a logarithmic scale, while the Y-axis (Abundance) represents the logarithmic scale of the observed abundance of each species. Rank-abundance curves were employed to assess the relative abundance and evenness of fungal communities at several sample locations (MAWS, KWS, SWS, **Figure 3**). The pronounced slope in the KWS curve indicates less community evenness and increased dominance by select fungal species, whereas the gentler slopes of MAWS and SWS reflect a more equitable distribution. The graph

indicated that MAWS exhibit intermediate abundance with many dominating species, KWS demonstrate lower evenness with fewer dominant species and a rapid decline in abundance, whilst SWS display better evenness and a less pronounced decline, signifying a more equitable distribution of species. From an ecological perspective, the species richness, shown by the length of the curve, suggests that all three samples encompass a similar range along the x-axis (about up to rank 90), implying equal richness in terms of total taxa. The slope of the curve suggests that SWS and MAWS have a flatter slope, implying more evenness, as species are more equally frequent. In contrast, KWS has a steeper decline, showing that a few species dominate while others are rare, reflecting poor evenness. Domination signifies that MAWS has

greater abundances at higher ranks compared to SWS and KWS, suggesting a more pronounced species domination by select taxa. KWS seems to be increasingly dominated by a limited number of species, while the others are rapidly disappearing, sometimes associated with disturbed or stressful settings near MAWS, *Schizophyllum commune*, *Scleroderma albidum*, and *Termitomyces* sp. were the most prominent species, whereas *Ramaria* sp., *Podaxis pistillaris*, and *Omphalotus olivascens* were observed near the apex of the KWS curve. Likewise, *Entoloma luteodiscum*, *Coprinus* sp., and *Fuscoporia gilva* were the predominant species at SWS. Similarly, the least dominant species at all three studied sites varied, including *Agaricus*

parviniveus - *Marasmius leveilleanus*, *Bolbitius* sp - *Disciseda hyalothrix* - *Marasmius leveilleanus* - *Gerronema keralense* - *Hymenopellis furfuracea* - *Rhodophana* sp - *Porostereum fulvum* - *Efibula tuberculata*, and *Xanthagaricus epipastus* - *Leucocoprinus cepistipes* - *Conocybe aurea* - *Calvatia holothurioides* - *Scleroderma albidum*, which were the least dominant species at MAWS, KWS, and SWS, respectively, as these species are positioned at the lower end of the dominance-diversity curve. The results indicate that each of the three analyzed locations have a distinct mechanism, specifically in soil and plant species composition, that governs the composition and dominance of Macro-fungi species.

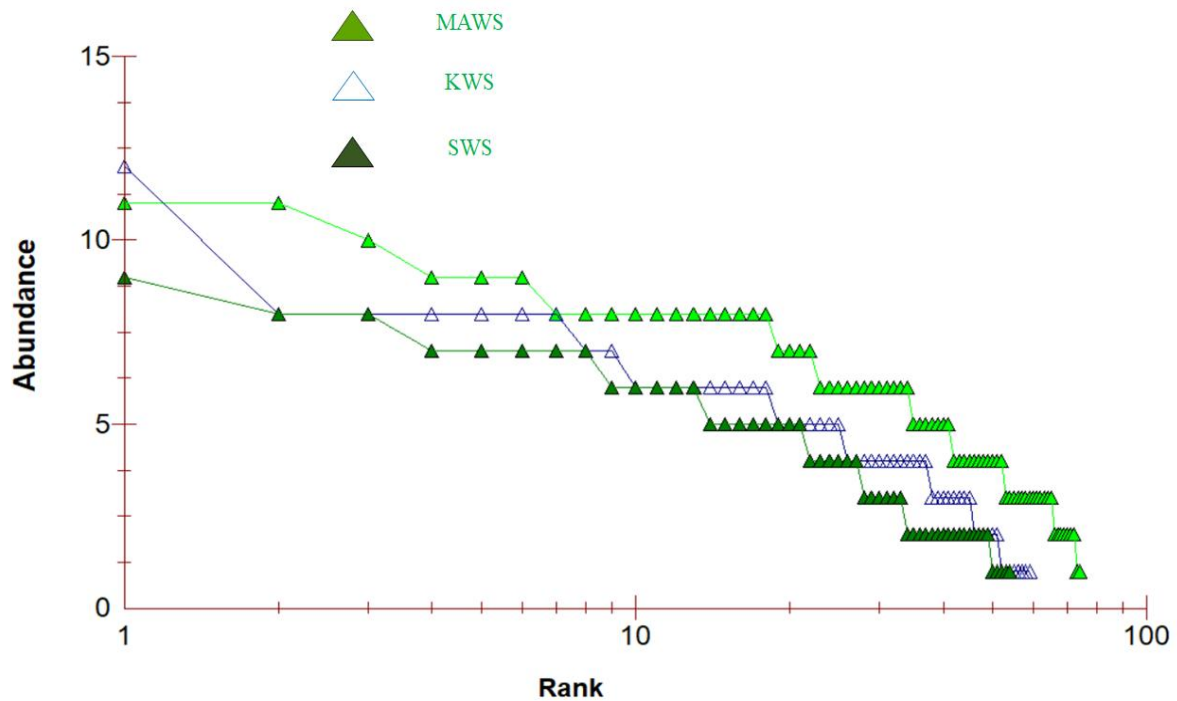


Figure 3: Diversity Dominance Curves

K-Means Clustering Analysis

K-means cluster analysis classified the abundance-based dataset into three groups containing 22, 32, and 23 species, respectively (Figure 4). The table delineates the characteristics of each cluster. Within Cluster Sum of Squares (WCSS) quantifies cluster compactness- lower values generally signify tighter clusters. In this study, cluster 2 exhibited the lowest WCSS (180.84), suggesting the most compact structure, whereas cluster 3 displayed the highest WCSS (384.00), indicating a more dispersed arrangement of data points. The Average Distance signifies the mean distance between points within the cluster and the cluster center (centroid). Cluster 2

exhibits the smallest average distance (2.21), underscoring its compactness, whereas cluster 3 displays the largest average distance (3.94), corroborating the WCSS result that it is the least compact. Ultimately, Maximum Distance refers to the distance from the centroid to the most distant point inside the cluster, with cluster 1 exhibiting the greatest maximum distance of 6.29, indicating the presence of at least one outlier or a point significantly removed from the center. Conversely, cluster 2 demonstrates the most compact grouping, with a maximum distance of 4.88.

Consequently, study revealed cluster 2 is the most compact and homogenous, whereas cluster 3 is the

most scattered and likely less cohesive; cluster 1 has moderate compactness but contains a probable outlier. The application of this technique for fungal classification aligns with the previous studies which advocate for the utilization of clustering analyses of fungal community composition across ecosystems, biogeographic regions, and community profiling through bioinformatics tools to investigate fungal community structure.

Our interpretation indicates that Cluster 1 (Black = 22) possessed - *Agaricus subrufescens* isolate JP24; *Microspalliota* sp. isolate JP59; *Conocybe utricystidiata* isolate JP38; *Crepidotus dentatus* isolate JP66; *Crepidotus indicus* isolate JP81; *Psathyrella roqueiana* isolate JP113; *Lycoperdon pratense* isolate JP54; *Porothelium* sp. isolate JP15; *Gerronema keralense* isolate JP52; *Amanita manicata* isolate JP8; *Entoloma luteodiscum* isolate JP102; *Pisolithus albus* isolate JP30; *Scleroderma citrinum* isolate JP76; *Trametes ellipsospora* isolate JP5; *Trametes pavonia* isolate JP12; *Trametes elegans* isolate JP21; *Ganoderma multipileum* isolate JP22; *Lentinus* sp. isolate JP205; *Phlebiopsis lacerata* isolate JP47; *Fomitiporia bannaensis* isolate JP62; *Fuscoporia senex* isolate JP77. While cluster two have 32 species belongs to *Agaricus glabriusculus* isolate JP206; *Agaricus parviniveus* isolate JP202; *Agaricus* sp. isolate JP27; *Xanthagaricus epipastus* isolate JP42; *Leucocoprinus birnbaumii* isolate JP29; *Leucocoprinus cepistipes* isolate JP-31; *Chlorophyllum palaeotropicum* isolate JP201; *Bolbitius* sp. isolate JP109; *Conocybe aurea* isolate JP88; *Stropharia* sp. isolate JP49; *Coprinellus aureogranulatus* isolate JP74; *Candolleomyces asiaticus* isolate JP98; *Calvatia holothurioides* isolate JP58; *Disciseda hyalothrix* isolate JP207; *Marasmius brunneoaurantiacus* isolate JP16; *Marasmius hypochroides* isolate JP51; *Marasmius leveilleanus* isolate JP101; *Collybiopsis menehune* isolate JP41; *Hymenopellis furfuracea* isolate JP61; *Pluteus* sp. isolate JP23; *Amanita nana* isolate JP208; *Entoloma* sp. isolate JP25; *Rhodocybe brunneoaurantiaca* isolate JP40; *Rhodophana* sp isolate JP139; *Scleroderma bovista* isolate JP37; *Gyrodontium sacchari* isolate JP44; *Earliella scabrosa* isolate JP90; *Porostereum fulvum* isolate JP99; *Efibula tuberculata* isolate JP91; *Auricularia srilankensis* JP204; *Punctularia atropurpurascens* isolate JP69 and *Itajahya roseae* isolate JP140.

Finally, cluster 3 (Blue = 23) had have *Coprinus sterquilinus* isolate JP103; *Coprinus* sp. JP203; *Tulostoma operculatum* isolate JP45; *Podaxis pistillaris* isolate JP73; *Omphalotus olivascens* isolate JP26; *Mycena* sp. isolate JP56; *Pleurotus djamor* isolate JP82; *Clitopilus prunulus* isolate JP33; *Tricholosporum* sp. isolate JP48; *Termitomyces* sp. isolate JP97; *Schizophyllum commune* isolate JP112; *Scleroderma albidum* isolate JP84; *Microporus xanthopus* isolate JP17; *Coriolopsis trogii* isolate JP65; *Hexagonia tenuis* isolate JP80; *Podoscypha petalodes* isolate JP57; *Auricularia nigricans* isolate JP-32; *Fuscoporia gilva* isolate JP107; *Phylloporia* sp. Isolate JP34; *Ramaria* sp. isolate JP70; *Geastrum triplex* isolate JP96; *Geastrum xerophilum* isolate JP106.

Venn Diagram Analysis

With binary data -set, Venn diagram (Figure 5 left) revealed that 7 (9.1%) species are exclusively pertains to MAWS only and these includes: *Agaricus glabriusculus* isolate JP206; *Agaricus* sp. isolate JP27; *Leucocoprinus birnbaumii* isolate JP29; *Chlorophyllum palaeotropicum* isolate JP201; *Candolleomyces asiaticus* isolate JP98; *Gyrodontium sacchari* isolate JP44; *Punctularia atropurpurascens* isolate JP69. While only *Rhodophana* sp isolate JP139 is specifically related to KWS, however, no such wild life specific species was identified at SWS. 15 Species or 19.5% were common to both MAWS and KWS, 2 (2.6%) common between KWS and SWS and 11 (14.3%) were common between MAWS-SWS. On the basis of presence-absence data-set we can say that 41 (53.2%) species were common at all three studied site.

On the basis of numerical abundance, Venn diagram revealed that habitat specific dominance of studied fungi (Figure 5 right). We found that 68, 55 (38%), (30.7%) and 48 (26.8%) species have habitat specific abundance. Based on this, only 2 species were common between MAWS -KWS (*Conocybe aurea* isolate JP88; *Marasmius leveilleanus* JP101) and KWS-SWS (*Agaricus parviniveus* isolate JP202; *Auricularia nigricans* JP-32), and 4 species were common abundance between MAWS-SWS:- *Conocybe utricystidiata* isolate JP38; *Coprinellus aureogranulatus* isolate JP74; *Tricholosporum* sp. isolate JP48; *Auricularia srilankensis* JP204.

Table 4: Parameters of K- Mean Cluster Analysis

	Number of Observations	Within Cluster Sum of Square	Average Distance	Maximum Distance
Cluster1	22	192.05	2.73	6.29
Cluster2	32	180.84	2.21	4.88
Cluster3	23	384.00	3.94	5.77

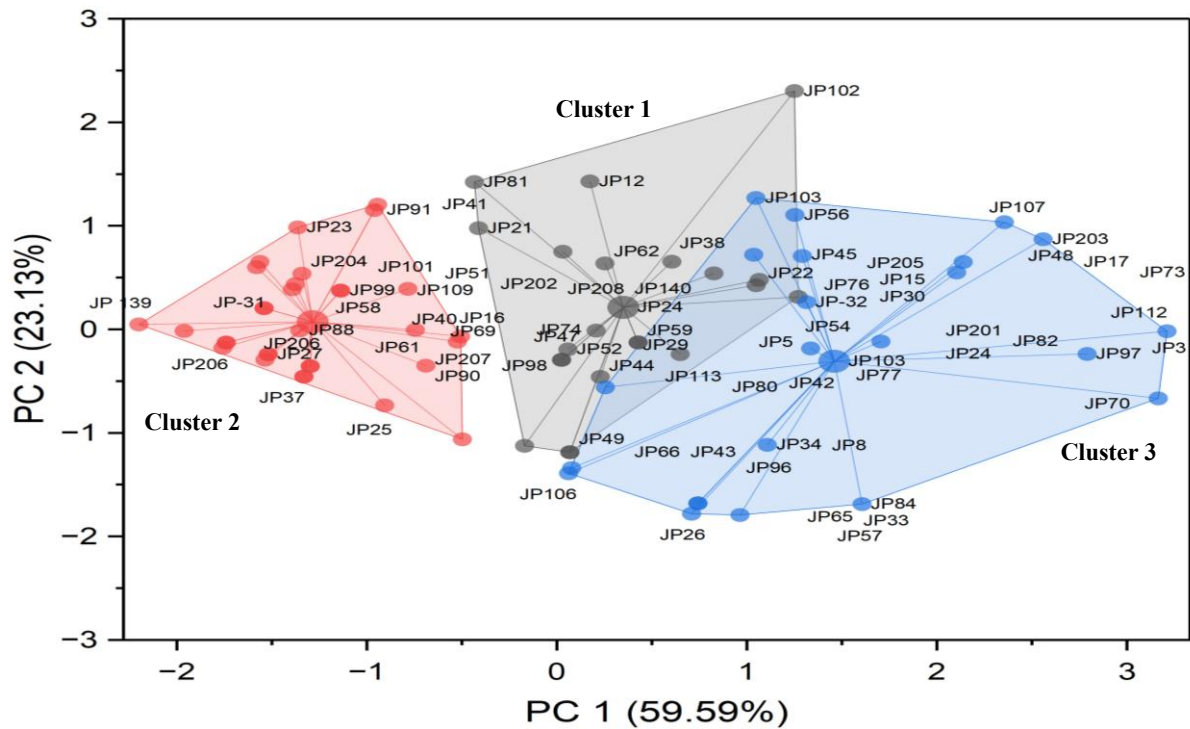


Figure 4: K- Mean Cluster Analysis

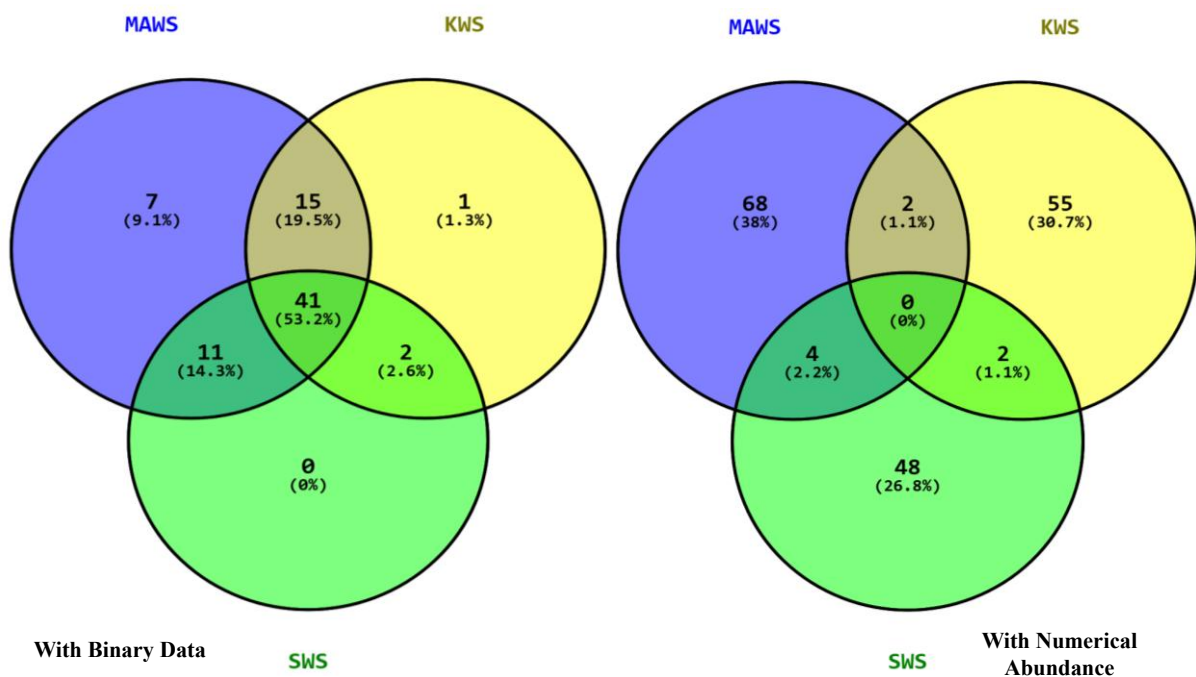


Figure 5: Venn diagram representing comparative analysis of fungal community dynamics based on with binary data-set (Left side) and with numerical abundance (right side)

DISCUSSION

This study offers detailed insights into the macrofungal diversity and community organization within three biologically diverse wildlife sanctuaries: Mount Abu Wildlife Sanctuary (MAWS), Kumbhalgarh Wildlife Sanctuary (KWS), and Sitamata Wildlife Sanctuary (SWS) in Rajasthan, India. Through a synthesis of qualitative observations, quantitative ecological indices, diversity profiling, and clustering techniques, we discerned notable regional heterogeneity in species richness, dominance, and distribution patterns. The documented macrofungal species richness (77 species) across the three locations indicates a reasonably high variety in this dry deciduous forest environment, particularly at MAWS, which had the highest species richness (74 species), evenness, and Shannon–Wiener diversity index ($H' = 4.20$). The results align with observations in other forest ecosystems, indicating that microclimatic variability and vegetation variety enhance fungal richness and community evenness (Baldrian et al. 2012, Tedersoo et al. 2014). Diversity metrics are often utilized in fungal ecology to assess richness, dominance, and dispersion along environmental gradients (Baldrian et al. 2012; Tedersoo et al. 2014).

Notably, 53.24% of species were common across all three locations, suggesting a fundamental fungal population potentially suited to local ecological circumstances. Significant site-specific species were also recorded, notably at MAWS (e.g., *Agaricus glabriusculus*, *Leucocoprinus birnbaumii*), indicating niche specialization. These trends reflect findings by Nguyen et al. (2016), who identified habitat filtering as a crucial factor influencing fungal community composition in tropical and subtropical areas.

MAWS consistently exhibited elevated diversity indices and evenness values across all diversity profiles, indicating a more ecologically stable and intricate macrofungal community. KWS and SWS demonstrated somewhat reduced Shannon diversity (3.93 and 3.84, respectively), with KWS displaying a more pronounced diversity dominance curve and diminished evenness, signifying more domination by select species. These findings align with those of Peay et al. (2016), who revealed that fungal diversity in forest ecosystems often diminishes with heightened habitat disturbance and simplification. Shade et al. (2012) emphasize that diversity

profiles indicate ecological stability and community dynamics in microbial, including fungal, assemblages. The global research of fungal diversity conducted by Tedersoo et al. (2014) utilized alpha diversity indices and species richness curves to illustrate spatial trends. Nguyen et al. (2016) employed alpha diversity indices to analyze fungal community composition across various environments and functional groupings.

Beta diversity (β) analysis indicated a higher similarity between KWS and SWS ($\beta = 0.24$) compared to MAWS and the other sites, indicating a tighter ecological relationship between KWS and SWS, may be attributable to analogous plant types, soil characteristics, or climatic conditions. These findings correspond with the conclusions of van der Heijden et al. (2008), who associated microbial (including fungal) community similarities with vegetation shape and edaphic circumstances. Our findings are consistent with those of Manoharachary et al. (2005), van der Heijden et al. (2008), Baldrian et al. (2012), and Tedresso et al. (2014). Manoharachary et al. (2005) examined diversity measures (Shannon, Simpson) for evaluating fungal communities across diverse settings. Van der Heijden et al. (2008) examined the correlation between microbial (including fungal) diversity, as quantified by several indicators, and ecosystem functioning. Baldrian et al. (2012) utilized Simpson and Shannon indices to demonstrate depth-related stratification of fungal communities in soil, whereas Tedersoo et al. (2014) employed diversity indices to compare fungal communities across global biomes.

The rank-abundance curves (Whittaker plots) provide more understanding of the composition of fungal communities. MAWS and SWS had flatter slopes, signifying more evenness, whereas KWS demonstrated a steeper curve, indicative of a reduced number of dominating species and likely heightened environmental stress or anthropogenic influence. These patterns align with findings from other fungal ecological research (Tedersoo et al., 2010; Baldrian et al., 2012). The findings align with Tedersoo et al. (2010), who utilized rank-abundance analysis to differentiate between main and secondary forests in tropical environments. He also utilized rank-abundance plots to illustrate variations in the dominance and richness of tropical mycorrhizal fungi across different sequencing methods and settings, while Baldrian et al. (2012)

employed rank-abundance and diversity metrics to distinguish between active and dormant fungal species. Nguyen et al. (2016) employed this method to investigate guild-level dominance patterns and analyze the functional roles of fungal species.

K-means clustering of species abundance data categorized fungal communities into three separate groupings. Cluster 2 appeared as the most compact and uniform, indicating a well integrated collection of co-occurring species. The utilization of clustering strategies to investigate community structure has been employed by Oono et al. (2017) and Nilsson et al. (2019) in microbial ecology, nevertheless it remains inadequately implemented in regional macrofungal investigations, underscoring a methodological development in our research. The application of this technique for fungal classification aligns with the studies of Tedersoo et al. (2014), Peay et al. (2016), Oono et al. (2017), and Nilsson et al. (2019), which advocate for the utilization of clustering analyses of fungal community composition across ecosystems, biogeographic regions, and community profiling through bioinformatics tools to investigate fungal community structure.

Binary and abundance-based Venn diagram studies highlighted the existence of habitat-specific fungus. Species such as *Chlorophyllum palaeotropicum* and *Gyrodontium sacchari* were restricted to MAWS, indicating distinct ecological niches. In contrast to several other studies that concentrate exclusively on presence-absence data (e.g., Manoharachary et al. 2005), we integrated abundance data into the Venn analysis, yielding a more sophisticated comprehension of species distribution and dominance across environments. Although several prior studies have examined macrofungal diversity in tropical and temperate environments (Tedersoo et al. 2014), less research has concentrated on semi-arid, seasonally dry deciduous forests such as those in Rajasthan. This work is unique in its integrative technique, merging traditional diversity indices with contemporary multivariate and cluster analysis to define fungal communities. Furthermore, the thorough habitat-level comparison provides one of the initial complete baselines for macrofungal diversity in this little studied region. Unlike research performed in mesic or evergreen forest types (Peay et al. 2013; Baldrian et al. 2012), which typically demonstrate a strong correlation between

fungal richness and moisture availability as well as complex canopy structure, our study indicates that dry deciduous forest ecosystem can also sustain rich and structurally diverse fungal communities, given adequate microhabitat diversity.

CONCLUSION

The macrofungal communities in the three sanctuaries display considerable regional variation, influenced by habitat traits, climatic conditions, and ecological interactions. MAWS emerged as the most diversified and ecologically balanced location, fostering more species richness and evenness. This work enriches the biogeographical comprehension of fungal diversity in dry environments and emphasizes the significance of habitat variation in maintaining fungal biodiversity. Our findings provide new baseline data and analytical methodologies for forthcoming mycological and ecological research in analogous areas.

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REFERENCES

- Allen, E.B. 2007. Restoration of Artemisia shrublands in the Mojave Desert with native shrubs and mycorrhizae. *Restoration Ecology*, **15(s1)**: S47–S52; doi: 10.1111/j.1526-100X.2007.00204.x.
- Baldrian, P., Kolařík, M., Stursová, M., et al., 2012. Active and total microbial communities in forest soil are largely different and highly stratified during decomposition. *The ISME Journal*, **6**:248–258; doi: 10.1038/ismej.2011.95.
- Borg Dahl, M., Brejnrod, A.D., Unterseher, M., et al., 2017. Genetic barcoding of darkspored myxomycetes (Amoebozoa) – identification, evaluation and application of a sequence similarity threshold for species differentiation in NGS studies. *Molecular Ecology Resources*, **18(2)**: 306-318.
- Cannon, P.F. and Kirk, P.M. 2007. Fungal Families of the World. *CAB International, United Kingdom, Wallingford*, 456 p.
- Chaithaisong, P., Alcantara, M.J.M., Song, L., et al., 2022. Generalists and specialists

- determine the trend and rate of soil fungal distance decay of similarity in a 20-ha Subtropical Forest. *Forests*, **13**(8):1188; doi: 10.3390/f13081188.
- Clark, K.R. 1990. Comparisons of dominance curves. *Journal of Experimental Marine Biology and Ecology*, **138** (1):143-157.
- Cody, M.L. 1975. Towards a theory of continental species diversities. In: *Ecology and Evolution of Communities* (Eds.: Cody M. L. and Diamond, J. M.). Belknap Press of Harvard University. Cambridge, pp.214-257.
- Cody, M.L. 1993. Bird diversity patterns and components across Australia. In: *Species Diversity: Historical and geographical aspects* (Eds.: Ricklefs R. E. and Schluter, D.). University of Chicago Press, pp.147-158.
- Gehlot, P., Agrawal, D.K., Sudaramoorth, S., *et al.*, 2016. Diversity Spectrum of Soil Fungi in the Indian Thar Desert. *Annals of Arid Zone*, **48**(2):113-117.
- Jordan. 2004. The Encyclopedia of Fungi of Britain and Europe. *Francis Lincoln Publishers Ltd., London*, 334 p.
- Manoharachary, C., Sridhar, K., Singh, R., *et al.*, 2005. Fungal biodiversity: distribution, conservation and prospecting of fungi from India. *Current Science*, **89**(1):58–71.
- Margalef, D.R. 1958. Information theory in Ecology. *Generla Systematics*, **3**:36-71.
- Mathur, M. 2005. Ecology and prospecting of some medicinal plants of aphrodisiac potential. *Ph.D. thesis, Jai Narain Vyas University, Jodhpur, India*, 465p.
- Mathur, M., Tak, A., Gehlot, P. 2019. Distance based analysis of soil mycoflora communities of hot arid region of the India. *International Journal of Ecology and Environmental Sciences*, **45**(1):45-58.
- Mohanani, C. 2011. *Macrofungi of Kerala*. Kerala Forest Research Institute, Peechi, Kerala, India, 597 p.
- Morris, E.K., Caruso, T., Buscot, F., *et al.*, 2014. Choosing and using diversity indices: insights for ecological applications from the German Biodiversity Exploratories. *Ecology and Evolution*, **4**(18):3514-24; doi: 10.1002/ece3.1155.
- Nguyen, N.H., Song, Z., Bates, S.T., *et al.*, 2016. FUNGuild: An open annotation tool for parsing fungal community datasets by ecological guild. *Fungal Ecology*, **20**:241–248; doi: 10.1016/j.funeco.2015.06.006.
- Nilsson, R.H., Larssonm K.H., Taylor, A.F.S., *et al.*, 2019. The UNITE database for molecular identification of fungi: handling dark taxa and parallel taxonomic classifications. *Nucleic Acids Research*, **47**(D1):D259–D264; doi: 10.1093/nar/gky1022.
- Oliveros, C. 2007-2015. Venny. An interactive tool for comparing lists with Venn's diagrams. <https://bioinfogp.cnb.csic.es/tools/venny/index.html>.
- Oono, R., Lefèvre, E., Simha, A., *et al.*, 2017. A comparison of enrichment methods for effective detection of diverse fungal endophytes from leaves. *Fungal Ecology*, **28**:39–46; doi: 10.1016/j.funeco.2017.04.002.
- Parihar, K., Gehlot, P., Mathur, M., *et al.*, 2022. Species composition and diversity dynamics of actinomycetes in arid and semi-arid salt basins of Rajasthan. *Current Microbiology*, **79**:1-13; doi: 10.1007/s00284-022-02851-
- Peay, K.G., Kennedy, P.G., Talbot, J.M. 2016. Dimensions of biodiversity in the Earth mycobiome. *Nature Reviews Microbiology*, **14**(7):434–447; doi: 10.1038/nrmicro.2016.59.
- Peet, R.K. 1974. The measurement of species diversity. *Annual Review of Ecology and Systematics*, **5**:255-307.
- Pegler, D.N. 1977. A preliminary agaric flora of east Africa. *Kew Bulletin Additional Series*, Kew, 615 p.
- Philips, R., 2006. Mushrooms. Pan Macmillan, London, 384 p.
- Pielou, E.C. 1965. Species-diversity and pattern-diversity in the study of ecological succession. *Journal of Theoretical Biology*, **10**(2):370–383.

- Rillig, M.C. 2004. Arbuscular mycorrhizae, glomalin, and soil aggregation. *Canadian Journal of Soil Science*, **84**(4): 355–36; doi: 10.4141/S04-003.
- Routledge, R.D. 1977. On Whittaker's components of diversity. *Ecology*, **58**:120-127.
- Shade, A., Peter, H., Allison, S.D., *et al.*, 2012. Fundamentals of microbial community resistance and resilience. *Frontiers in Microbiology*, **3**:417; doi: 10.3389/fmicb.2012.00417
- Sharma, M.P. and Adholeya, A. 2004. Diversity of arbuscular mycorrhizal fungi and their distribution in semi-arid region of Rajasthan. *Mycorrhiza* **14**(3):167–174; doi: 10.1007/s00572-003-0268-4.
- Singer, R. 1986. The Agricales in modern taxonomy. 4th edn. *Koeltz Scientific Book: Koenigsteiin*, 981 p.
- Sultana, A., Hussain, M.H., Rathore, D.K. 2014. Diversity of tree vegetation of Rajasthan, India. *Tropical Ecology*, **55**: 403-410.
- Tedersoo, L., Nilsson, R.H., Abarenkov, K., *et al.*, 2010. Pyrosequencing and Sanger sequencing of tropical mycorrhizal fungi provide similar results but reveal substantial methodological biases. *New Phytologist*, **188**(1):291–301; doi: 10.1111/j.1469-8137.2010.03373.x.
- Tedersoo, L., Bahram, M., Pölme, S., *et al.*, 2014. Global diversity and geography of soil fungi. *Science*, **346**(6213):1256688; doi: 10.1126/science.1256688.
- Uddin, M., Chowdhury, F.I., Hossain, M.K., 2020. Assessment of tree species diversity, composition and structure of Medha Kachhapia National Park, Cox's Bazar, Bangladesh. *Asian Journal of Forestry*, **4**: 15-21.
- van der Heijden, M.G.A., Bardgett, R.D., van Straalen, N.M. 2008. The unseen majority: soil microbes as drivers of plant diversity and productivity. *Ecology Letters*, **11**(3), 296–310; doi: 10.1111/j.1461-0248.2007.01139.x
- Verma, R.K. and Gupta, A.K. 2022. Diversity of Soil Fungi in the Arid and Semi-Arid Regions of Rajasthan, India. *Journal of Arid Environments*, **193**:104605; doi: 10.1016/j.jaridenv.2021.104605.
- White, T.J., Bruns, T., Lee, S., *et al.*, 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: *PCR Protocols: a guide to methods and applications*, (Eds. Innis, M.A., Gelfandm, D.H. Sninsky, J.J., White, T.J.) Academic Press, pp.315-322.
- Whittaker, R.H. 1960. Vegetation of the Siskiyou Mountains, Oregon and California. *Ecological Monographs*, **30**:279–338.
- Whittaker, R.H. 1972. Evolution and measurement of species diversity. *Taxon*, **21**: 213–251.
- Whittaker, R.H. 1977. Evolution of species diversity in land communities. *Evolutionary Biology*, **10**:1- 67.