

# Macrofungal diversity and distribution in Kishtwar High Altitude National Park, Jammu and Kashmir, India

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The present study was conducted at 10 sites in Kishtwar High Altitude National Park (KHANP), Jammu and Kashmir, India, with the objective to analyse the diversity and distribution of macrofungal communities. A total of 40 permanent plots (four plots in each site) were established and macrofungal fruiting bodies were recorded monthly from each plot between July 2015 and October 2017. Diversity indices and canonical correspondence analysis were applied to determine the composition and environmental factors responsible for structuring the macrofungal communities in the study area. In total, 83 wild macrofungal species were identified belonging to 35 genera, 24 families and 9 orders. Humicolous fungi were the most dominant group of macrofungi contributing 71.8% of the total dominance, followed by lignicolous fungi (11.8%). The distribution of fruiting bodies of macrofungal species was mainly in groups, i.e. aggregated pattern (75.9%). The diversity indices varied from 20 to 37 (richness), 2.04 to 3.16 (Menhinick), 4.14 to 7.25 (Margalef), 0.03 to 0.06 (Simpson's dominance), 2.91 to 3.49 (Shannon-Wiener's diversity), 0.86 to 0.95 (evenness), 7.69 to 16.29 (Fisher's alpha) and 0.05 to 0.12 (Berger-Parker diversity). Canonical correspondence analysis revealed that *Scleroderma verrucosum*, *Boletus granulatus* and *Ramaria formosa* were the most important species, and that mean temperature and rainfall were the key environmental factors responsible for the diversity and distribution of macrofungi in the present study.

**Keywords:** Agaricomycetes, diversity and distribution, environmental factors, macrofungal communities, National Park.

FUNGI play a pivotal role in litter degradation in forest ecosystems during humus formation by assimilating the lignocelluloses present in the litter<sup>1-3</sup>. They are part of the forest ecosystem as mutualists, saprotrophs or pathogens. These different modes of nutrition along with associated interactions influence nutrient cycling and improve nutrient uptake by plants, which directly or indirectly help in

maintaining biodiversity and good health of a forest. Therefore, measuring the macrofungal richness and diversity helps in monitoring of the health of an ecosystem<sup>4</sup>. Moreover, macrofungal diversity is significantly correlated with the total diversity of a site and, therefore, its quantification helps in the assessment of priorities for the conservation of an area<sup>5</sup>.

The diversity and community composition of macrofungi and their relationship with the environmental variables have been studied worldwide for both ectomycorrhizal and terricolous communities. Ectomycorrhizal communities are mainly structured by soil properties, viz. nutrients, pH, temperature and moisture, season and species composition of the forests<sup>6-15</sup>. Terricolous saprotrophic communities are, however, structured by the effects of the quantity of litter and pH<sup>16-20</sup>, soil nutrients<sup>21</sup>, temperature<sup>22,23</sup>, tree species composition<sup>14</sup>, and phyto-geomorphic features and climatic conditions<sup>24</sup>.

The Kishtwar High Altitude National Park (KHANP) is located in Kishtwar district, Jammu and Kashmir, India. The terrain of KHANP is generally rugged with steep slopes and narrow valleys surrounded by high ridges culminating in glaciers. It lies in the Central Crystalline strip of the Himalayas, and has rocks strongly folded in places and composed mainly of schist, granite and gneiss, with sporadic belts of marble. The soil is shallow and slightly alkaline having alluvial composition along with gravel deposits<sup>25</sup>. Vegetation of KHANP mainly comprises *Cedrus deodara*, *Pinus wallichiana*, *Aesculus indica*, *Juglans regia*, *Populus ciliata*, *Corylus cornulatum* and *Taxus wallichiana* in the altitudinal range 1700–2400 m amsl. Altitudes between 2400 and 3000 m amsl are dominated by *Abies pindrow*, *Picea smithiana*, *Pinus wallichiana* and *Pinus gerardiana*. The higher reaches (3000–3700 m amsl) up to the tree line are occupied primarily by *Betula utilis*. A few reports on macrofungal diversity in the outer areas of KHANP have been published<sup>26</sup>. However, no ecological study vis-à-vis macrofungi has been conducted in this Park.

The main objectives of the present study were to: (i) document the macrofungal diversity of KHANP, (ii) understand various associations and interactions of the macrofungi and (iii) assess the impact of environmental factors

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**Table 1.** Location and environmental parameters of various sites of Kishtwar High Altitude National Park (KHANP), Jammu and Kashmir, India

Site	Latitude	Longitude	Altitude (m amsl)	Mean temperature (°C)	Humidity	Rainfall (mm)
Sonder	33°28'19.11"N	75°49'29.05"E	2056.6 (±105.5)	17.9 (±0.9)	63.1 (±2.2)	83.2 (±4.3)
Loopara	33°28'32.71"N	75°45'59.67"E	3134.2 (±125.2)	16.6 (±0.4)	54.1 (±1.0)	77.3 (±5.4)
Janakpur	33°30'7.08"N	75°48'4.49"E	2133.6 (±154.5)	15.1 (±0.2)	61.3 (±3.1)	79.3 (±3.7)
Palmar	33°27'20.01"N	75°41'5.65"E	2438.8 (±82.1)	14.7 (±0.1)	60.1 (±5.4)	81.8 (±5.6)
Loharna	33°31'24.70"N	75°48'5.34"E	2420.1 (±187)	14.6 (±0.4)	55.9 (±3.5)	81.2 (±8.2)
Deharna	33°35'41.32"N	75°44'2.46"E	2253.4 (±61.5)	14.0 (±0.5)	58.4 (±4.8)	81.6 (±6.0)
Qaderna	33°38'18.65"N	75°42'12.39"E	2403.6 (±213.3)	12.9 (±0.3)	55.8 (±4.2)	77.6 (±5.7)
Marwah	33°40'12.17"N	75°42'1.00"E	2497.8 (±55.5)	11.9 (±0.5)	54.8 (±0.6)	78.4 (±4.6)
Nath	33°33'33.15"N	75°47'16.71"E	2256.0 (±84.3)	11.5 (±0.6)	56.1 (±3.6)	82.8 (±7.2)
Ekhala	33°27'38.67"N	75°43'56.52"E	1847.1 (±100.3)	10.8 (±0.3)	57.6 (±5.2)	71.7 (±4.0)

on the diversity and distribution of macrofungal species in this Park.

## Materials and methods

### Study site

The study was conducted at 10 sites in KHANP (Table 1). The Department of Forest, Environment and Ecology, Government of Jammu and Kashmir, had declared KHANP as a National Park on 4 February 1981 (notification no. 21/FST of 1980–1981). The Park, with an estimated area of 425 sq. km, is situated at a high altitude, i.e. sub-alpine and alpine zones. The altitude range of KHANP is 1720–6000 m amsl and the tree line lies at 3300 m amsl. The area receives snowfall during winter and rainfall during summer. Mean annual precipitation and annual temperature are 975 mm and 11°C respectively.

### Sampling design

Macrofungi diversity and distribution were analysed by establishing four permanent plots of 100 m × 100 m each in 10 different sites of KHANP (Figure 1). The plots were laid randomly, located at least 10 m from each other and a minimum of 30 m from the edge of the forest. The number of macrofungal fruiting bodies was counted from the 10 random quadrats of 2 m × 2 m plotted in each 1 ha plot. The count values or abundance of macrofungal fruiting bodies of these 10 quadrats were then pooled for each plot. Monthly sampling was done for two years, between July 2015 and October 2017. However, in the rainy season (July–October), fortnightly surveys were conducted.

### Macrofungal sampling

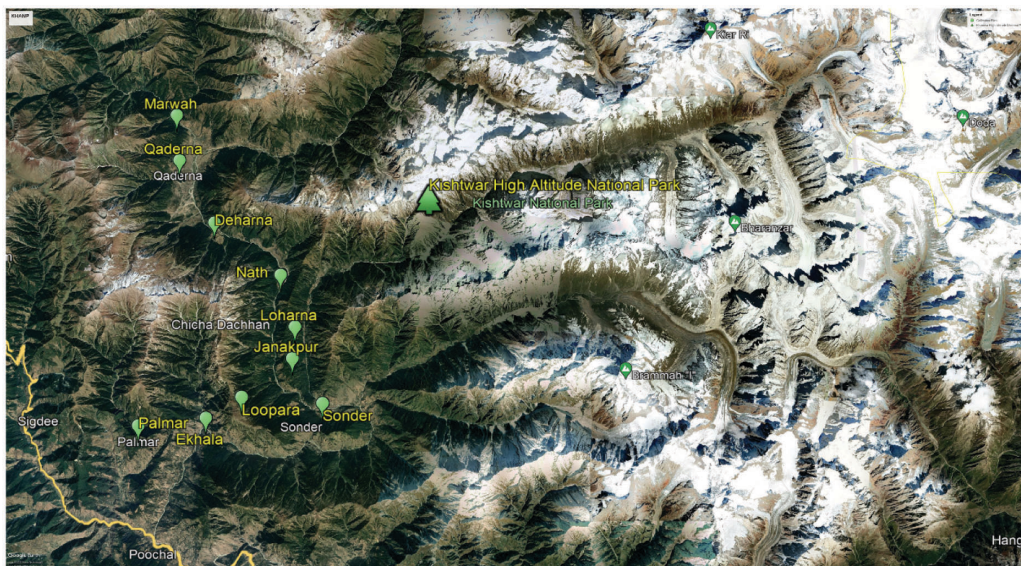
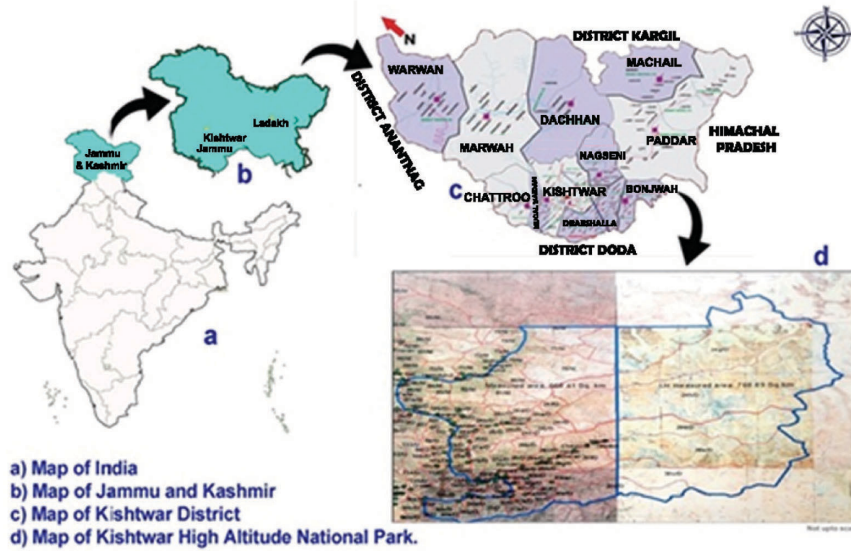
The fruit bodies were photographed from the sites mentioned in Table 1 using a digital camera (SONY D3400) and their morphological features were documented in their natural habitat. Specimens were collected, documented

and preserved. Macroscopic features were studied from fresh material and microscopic structures were observed in dried material using 5% KOH and Congo Red. Micro-characters were recorded with a microphotographic unit (Nikon 4.11.00 (Build 871) LO, 32 bit). Image capturing was done using NIS-Elements D imaging software. Further identification of the macrofungal species was done using pertinent keys, monographs and books<sup>27–34</sup>. Websites like [www.mycology.com](http://www.mycology.com) and [www.mushroomexpert.com](http://www.mushroomexpert.com) were also used for identification and related information. All the identified specimens were submitted to the Herbarium of the Department of Botany, University of Jammu, India.

### Data analysis

The explanatory variables recorded once from each plot of KHANP were geographical coordinates, altitude, and soil carbon and pH. Additionally, we collected data on minimum and maximum temperature, precipitation, humidity and soil moisture on a monthly basis. Climatic data (mean maximum and minimum temperature, precipitation and humidity) were extracted for each plot with the help of high-resolution interpolated database using ArcGIS software<sup>35</sup>. Soil moisture was studied by collecting soil samples in aluminium boxes and with further estimations in the laboratory. For soil pH and carbon, three soil samples were collected from each quadrat at 0–15 cm depth. Soil pH was estimated using a Systronics pH meter (Type 335), India and carbon analysis was done using the method of Kalra and Maynard<sup>36</sup>. To normalize the data, all the attributes like altitude, soil moisture, pH and carbon were log-transformed. In the case of minimum temperature, the log transformation was done after adding a constant to each number to make the values positive and non-zero<sup>37</sup>. To down weight the effect of rare species in the fungal community, data was transformed using the Hellinger equation<sup>38</sup>.

Richness of macrofungal species was determined as the total number of species observed in each study site. Other indices of alpha diversity were calculated according to the following formulas.



**Figure 1.** Location map of Kishtwar High Altitude National Park (KHANP), Jammu and Kashmir, India, with GPS locations.

Fisher’s alpha<sup>39</sup>:

$$S = \alpha * \ln(1 + n/\alpha),$$

where  $S$  is the number of taxa,  $n$  the number of individuals and  $\alpha$  is the Fisher’s alpha.

Shannon–Wiener Index<sup>40</sup>:

$$H' = -\sum_{i=1}^s p_i \ln p_i,$$

where  $p_i$  is the proportion of the  $i$ th species and  $s$  is the number of individuals of all the species.

Concentration of dominance<sup>41</sup>:

$$C_d = \sum_{i=1}^s (p_i)^2.$$

Margalef index<sup>42</sup>:

$$R_1 = S - 1/\ln(n),$$

where  $S$  is the number of species and  $n$  is the number of individuals.

Menhinick index<sup>43</sup>:

$$R_2 = S / \sqrt{n}.$$

Evenness<sup>44</sup>:

$$J = H' / \ln(s),$$

where  $H'$  is the Shannon–Wiener diversity index and  $s$  is the number of species.

Beta diversity ( $\beta$ ) was computed to measure the rate of species change across sites using the following formula<sup>45</sup>

**Table 2.** Macrofungal description, habitat and distribution in KHANP

Macrofungal taxon	Species abbreviation	Family	Accession number	Habitat	Distribution
<i>Gyromitra esculenta</i> (Pers.) Fr.	<i>Gyro escu</i>	Discinaceae	HBJU-583	Humicolous	Aggregated
<i>Helvella acetabulum</i> (L.) Quél.	<i>Helv acet</i>	Helvellaceae	HBJU-580	Ectomycorrhizal	Random
<i>Helvella atra</i> J. König	<i>Helv atra</i>	Helvellaceae	HBJU-581	Ectomycorrhizal	Aggregated
<i>Helvella macropus</i> (Pers.) P. Karst.	<i>Helv macr</i>	Helvellaceae	HBJU-582	Bryophilous	Aggregated
<i>Morchella crassipes</i> (Vent.) Pers.	<i>Morc cras</i>	Morchellaceae	HBJU-619	Humicolous	Aggregated
<i>Morchella deliciosa</i> Fr.	<i>Morc deli</i>	Morchellaceae	HBJU-585	Humicolous	Random
<i>Morchella elata</i> Fr.	<i>Morc elat</i>	Morchellaceae	HBJU-584	Humicolous	Aggregated
<i>Morchella esculenta</i> (L.) Pers.	<i>Morc escu</i>	Morchellaceae	HBJU-586	Humicolous	Aggregated
<i>Peziza ampliata</i> Pers.	<i>Pezi ampl</i>	Pezizaceae	HBJU-587	Bryophilous	Aggregated
<i>Peziza badia</i> Pers.	<i>Pezi badi</i>	Pezizaceae	HBJU-588	Bryophilous	Aggregated
<i>Peziza succosa</i> Berk.	<i>Pezi succ</i>	Pezizaceae	HBJU-589	Humicolous	Aggregated
<i>Geopora arenicola</i> (Lev.) Kers	<i>Geoparen</i>	Pyrenomataceae	HBJU-590	Humicolous	Random
<i>Agaricus arvensis</i> Schaeff	<i>Agar arve</i>	Agaricaceae	HBJU-591	Humicolous	Random
<i>Agaricus californicus</i> Peck	<i>Agar cali</i>	Agaricaceae	HBJU-592	Humicolous	Aggregated
<i>Agaricus langei</i> (F.H. Moller) F.H. Moller	<i>Agar lang</i>	Agaricaceae	HBJU-620	Humicolous	Aggregated
<i>Bovista colorata</i> (Peck) Kreisel	<i>Bovi colo</i>	Agaricaceae	HBJU-621	Humicolous	Random
<i>Bovista minor</i> Morgan	<i>Bovi mino</i>	Agaricaceae	HBJU-595	Humicolous	Random
<i>Bovista plumbea</i> Pers	<i>Bovi plum</i>	Agaricaceae	HBJU-622	Humicolous	Random
<i>Bovista pusilla</i> (Batsch) Pers.	<i>Bovi pusi</i>	Agaricaceae	HBJU-623	Humicolous	Random
<i>Calvatia elata</i> (Massee) Morgan	<i>Calv elat</i>	Agaricaceae	HBJU-624	Humicolous	Aggregated
<i>Calvatia lycoperdoides</i> A. H. Sm.	<i>Calv lyco</i>	Agaricaceae	HBJU-625	Humicolous	Aggregated
<i>Calvatia</i> sp.	<i>Calv sp.</i>	Agaricaceae	HBJU-626	Humicolous	Aggregated
<i>Calvatia bovista</i>	<i>Calv bovi</i>	Agaricaceae	HBJU-662	Humicolous	Aggregated
<i>Chlorophyllum molybdites</i> (G. Mey.) Massee	<i>Chlo moly</i>	Agaricaceae	HBJU-593	Humicolous	Aggregated
<i>Coprinus comatus</i> (O. F. Mull.) Pers.	<i>Copr coma</i>	Agaricaceae	HBJU-596	Bryophilous	Random
<i>Lepiota procera</i> (Scop.) Grey	<i>Lapi proc</i>	Agaricaceae	HBJU-627	Humicolous	Aggregated
<i>Lepiota sistrata</i> (Scop.) Grey	<i>Lapi sist</i>	Agaricaceae	HBJU-628	Humicolous	Aggregated
<i>Leucoagaricus rubrotinctus</i> (Peck) Singer	<i>Leuc rubr</i>	Agaricaceae	HBJU-629	Humicolous	Aggregated
<i>Lycoperdon molle</i> Pers	<i>Lyco moll</i>	Agaricaceae	HBJU-630	Humicolous	Aggregated
<i>Lycoperdon pedicellatum</i> Batsch	<i>Lyco pedi</i>	Agaricaceae	HBJU-631	Humicolous	Random
<i>Lycoperdon perlatum</i> Pers	<i>Lyco perl</i>	Agaricaceae	HBJU-632	Humicolous	Aggregated
<i>Lycoperdon pyriforme</i> Pers	<i>Lyco pyri</i>	Agaricaceae	HBJU-618	Humicolous	Aggregated
<i>Lycoperdon rimulatum</i> Peck	<i>Lyco rimu</i>	Agaricaceae	HBJU-633	Humicolous	Aggregated
<i>Lycoperdon umbrinum</i> Pers.	<i>Lyco umbr</i>	Agaricaceae	HBJU-634	Humicolous	Aggregated
<i>Macrolepiota procera</i> (Scop.) Singer	<i>Macr proc</i>	Agaricaceae	HBJU-594	Humicolous	Aggregated
<i>Gymnopilus sapineus</i> Fries	<i>Gymn sapi</i>	Cortinariaceae	HBJU-598	Lignicolous	Random
<i>Gymnopilus</i> sp.	<i>Gymn sp.</i>	Cortinariaceae	HBJU-635	Lignicolous	Aggregated
<i>Flammulina velutipes</i> (Curtis) Singer	<i>Flamm velu</i>	Physalacriaceae	HBJU-599	Lignicolous	Aggregated
<i>Coprinellus domesticus</i> (B.) Vilg. Hop. & Jacq.	<i>Copr dome</i>	Psathyrellaceae	HBJU-636	Lignicolous	Aggregated
<i>Coprinellus micaceus</i> (Bull) Fr.	<i>Copr mica</i>	Psathyrellaceae	HBJU-637	Coprophilous	Aggregated
<i>Coprinopsis atramentarius</i> (Bull.)Fr.	<i>Copr atra</i>	Psathyrellaceae	HBJU-597	Humicolous	Aggregated
<i>Pholiota squarrosa</i> (Oeder) Kumm.	<i>Phol squa</i>	Strophariaceae	HBJU-600	Humicolous	Aggregated
<i>Pholiota</i> sp.	<i>Phol sp.</i>	Strophariaceae	HBJU-638	Humicolous	Random
<i>Amanita flavoconia</i> G.F. Atk.	<i>Aman flavo</i>	Amanitaceae	HBJU-601	Humicolous	Aggregated
<i>Amanita pantherina</i> (DC) Krombh	<i>Aman pant</i>	Amanitaceae	HBJU-639	Humicolous	Aggregated
<i>Amanita phalloides</i> Secr.	<i>Aman phal</i>	Amanitaceae	HBJU-640	Humicolous	Aggregated
<i>Amanita vaginata</i> (Bull.) Lam.	<i>Aman vagi</i>	Amanitaceae	HBJU-602	Humicolous	Aggregated
<i>Pleurotus ostreatus</i> (Jacq. Ex. Fr.) P. Kumm	<i>Pleu ostr</i>	Pleurotaceae	HBJU-641	Lignicolous	Aggregated
<i>Pleurotus pulmonarius</i> (Fr.) Quel.	<i>Pleu pulm</i>	Pleurotaceae	HBJU-642	Lignicolous	Aggregated
<i>Pleurotus squarrosulus</i> (Mont.) Singer	<i>Pleu squa</i>	Pleurotaceae	HBJU-603	Lignicolous	Aggregated
<i>Boletus edulis</i> Bull	<i>Bole edul</i>	Boletaceae	HBJU-604	Humicolous	Aggregated
<i>Boletus formosus</i> Corner	<i>Bole form</i>	Boletaceae	HBJU-643	Humicolous	Random
<i>Boletus granulatus</i> L.	<i>Bole gran</i>	Boletaceae	HBJU-644	Humicolous	Aggregated
<i>Boletus luridus</i> Schaeff	<i>Bole luri</i>	Boletaceae	HBJU-605	Ectomycorrhizal	Aggregated
<i>Suillus cavipes</i> (Opat.) A. H. Sm. & Thiers	<i>Suil cavi</i>	Boletaceae	HBJU-645	Ectomycorrhizal	Aggregated
<i>Scleroderma citrinum</i> Pers.	<i>Scle citr</i>	Sclerodermataceae	HBJU-606	Ectomycorrhizal	Aggregated
<i>Scleroderma geaster</i> Fr.	<i>Scle geas</i>	Sclerodermataceae	HBJU-646	Humicolous	Aggregated
<i>Scleroderma verrucosum</i> (Bull.) Pers.	<i>Scle verru</i>	Sclerodermataceae	HBJU-647	Humicolous	Random
<i>Cantharellus cibarius</i> Fr.	<i>Cant ciba</i>	Cantharellaceae	HBJU-607	Humicolous	Random
<i>Cantharellus infundibuliformis</i> (Scop.) Fr.	<i>Cant infu</i>	Cantharellaceae	HBJU-648	Humicolous	Aggregated

(Contd)

Table 2. (Contd)

Macrofungal taxon	Species abbreviation	Family	Accession number	Habitat	Distribution
<i>Clavaria vermicularis</i> Scop.	<i>Clav verm</i>	Clavariaceae	HBJU-649	Humicolous	Aggregated
<i>Sparassis crispa</i> (Wulfen) Fr.	<i>Spar cris</i>	Sparassidaceae	HBJU-608	Humicolous	Aggregated
<i>Sparassis radiata</i> (Weir)	<i>Spar radi</i>	Sparassidaceae	HBJU-650	Humicolous	Aggregated
<i>Ramaria apiculata</i> (Fr.) Donk	<i>Rama apic</i>	Ramariaceae	HBJU-609	Humicolous	Aggregated
<i>Ramaria aurea</i> (Schaef.) Quel	<i>Rama aure</i>	Ramariaceae	HBJU-610	Humicolous	Aggregated
<i>Ramaria flavobrunnescens</i> var <i>aurea</i> (Fr.) Donk	<i>Rama fl_au</i>	Ramariaceae	HBJU-651	Humicolous	Aggregated
<i>Ramaria flavobrunnescens</i> var. <i>longisperma</i>	<i>Rama fl_lo</i>	Ramariaceae	HBJU-652	Humicolous	Aggregated
<i>Ramaria formosa</i> (Pers.) Quel.	<i>Rama form</i>	Ramariaceae	HBJU-653	Humicolous	Aggregated
<i>Lactarius deliciosus</i> (L.) Gray	<i>Lact deli</i>	Russulaceae	HBJU-612	Humicolous	Aggregated
<i>Lactarius deterrimus</i> Groger	<i>Lact dete</i>	Russulaceae	HBJU-654	Humicolous	Aggregated
<i>Lactarius vellerreus</i> (Fr.) Fr.	<i>Lact vell</i>	Russulaceae	HBJU-655	Humicolous	Aggregated
<i>Lactarius volemus</i> (Fr.) Fr.	<i>Lact vole</i>	Russulaceae	HBJU-611	Humicolous	Random
<i>Russula annulata</i> var. <i>evanescens</i> var. nov	<i>Russ annu</i>	Russulaceae	HBJU-656	Humicolous	Random
<i>Russula atropurpurea</i> (Krombh.) Britzelm.	<i>Russ atro</i>	Russulaceae	HBJU-657	Ectomycorrhizal	Aggregated
<i>Russula cynoxantha</i> (Schaeff.) Fr.	<i>Russ cyno</i>	Russulaceae	HBJU-658	Humicolous	Random
<i>Russula lepida</i> Fr.	<i>Russ lepi</i>	Russulaceae	HBJU-613	Ectomycorrhizal	Aggregated
<i>Hericium erinaceus</i> (Bull.) Persoon	<i>Heri erin</i>	Hericiaceae	HBJU-614	Lignicolous	Aggregated
<i>Schizophyllum commune</i> Fr.	<i>Schi comm</i>	Shizophyllaceae	HBJU-615	Lignicolous	Random
<i>Auricularia auricula-judae</i> (Bull.) Quel	<i>Auri auri</i>	Auriculariaceae	HBJU-616	Lignicolous	Aggregated
<i>Geastrum campestre</i> Morgan	<i>Geas camp</i>	Geastraceae	HBJU-659	Humicolous	Aggregated
<i>Geastrum saccatum</i> Fr.	<i>Geas sacc</i>	Geastraceae	HBJU-617	Humicolous	Aggregated
<i>Geastrum triplex</i> Jungh	<i>Geas trip</i>	Geastraceae	HBJU-660	Humicolous	Aggregated
<i>Geastrum velutinum</i> Morgan	<i>Geas velu</i>	Geastraceae	HBJU-661	Humicolous	Aggregated

$\beta = S_c/S$ , where  $S_c$  is the total number of species encountered in all communities and  $S$  is the average number of species per community.

The dominance–diversity curves, representing resource distribution among the species and contrasting patterns of species richness, were plotted between the log values of abundance and species sequences. Abundance is simply the count of macrofungal fruiting body in each site.

The relationship between fungal species and environmental variation was assessed using canonical correspondence analysis (CCA)<sup>46</sup>. In this analysis, species values are weighted averages of an eigenvector. The importance of each CCA axis is represented by an eigenvalue, which measures the variation in species data and explains environmental variables for the axis<sup>47</sup>. Statistical significance of the environmental factors was tested by the Monte Carlo permutation test with 999 permutations<sup>48</sup>. CCA was executed using CANOCO 4.5 (ref. 48) and diagrams were drawn using CanoDraw 3.1 (ref. 49).

## Results

### Species composition and distribution

A total of 83 macrofungal species were identified from KHANP (Table 2). They belonged to 35 genera spread over 24 families and 9 orders of 2 classes (Agaricomycetes and Pezizomycetes). Agaricales (44%) was the largest order followed by Pezizales (14%), Russulales (10%), Boletales (9%), Cantharalles, Gomphales and Geastrales

(6% each), and Schizophyllales and Auriculariales (1% each) (Figure 2). The most represented families were Agaricaceae (23 species, 27.7%) and Russulaceae (eight species, 9.6%). Other important fungal families (Figure 3) were Boletaceae (two genera and five species), Ramariaceae (one genus and five species), and Amanitaceae, Geastraceae and Morchellaceae (one genus and four species each). The nature of macrofungal species collected was mainly humicolous (71.8%), followed by lignicolous (11.8%) and ectomycorrhizal (10.8%) (Figure 4). The macrofungal species were mainly distributed in aggregated arrangement (75.9%), and the only other distribution pattern was random (24.2%). Most (77.8%) of the ectomycorrhizal fungi had a clumped or aggregate distribution.

### Species diversity

Loharna recorded the highest species richness (37 species and 7.25 Margalef index value), while Loopara had the lowest species richness (20 species and 4.16 Margalef index value). According to the Menhinick value, the highest and lowest species-rich sites were Qaderna (3.16) and Loopara (2.04) respectively (Table 3). The highest Simpson's index ( $D$ ) was recorded in Deharna and Loopara (0.06), and lowest in Loharna (0.03). The Shannon–Wiener diversity index ( $H'$ ) varied between 2.91 (Loopara) and 3.49 (Loharna). Fisher's alpha recorded maximum values in Loharna (16.19), whereas Berger–Parker values were highest for Nath (0.12). The values of evenness ( $E$ ) ranged from 0.86 (Nath) to 0.95 (Qaderna).

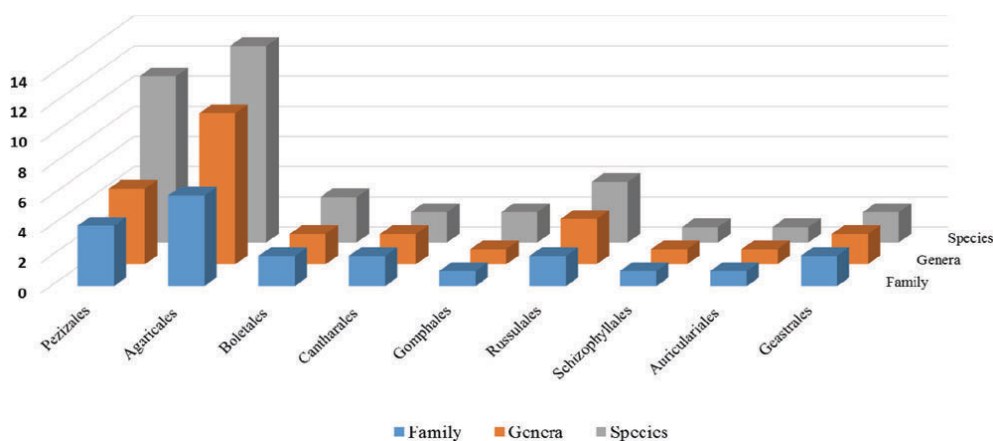


Figure 2. Diversity of families, genera and species in various orders of macrofungi in KHANP.

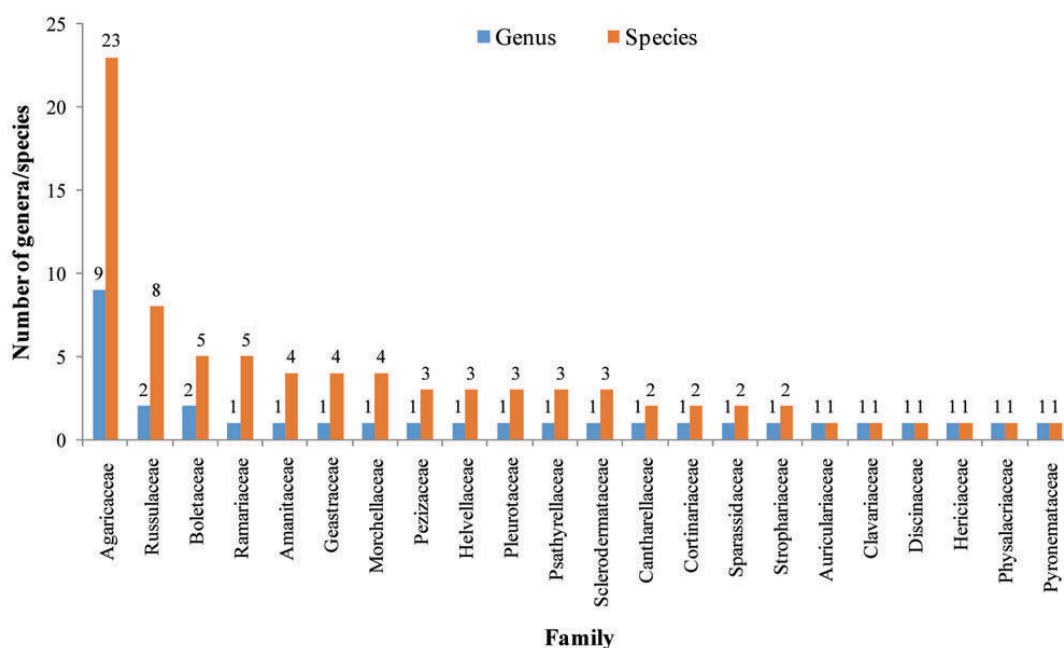


Figure 3. Important families and number of genera and species of macrofungi in KHANP.

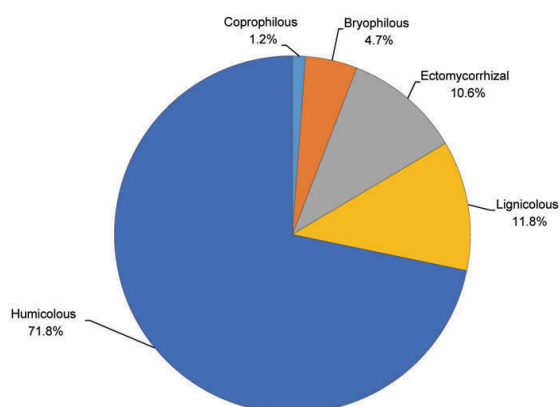


Figure 4. Percentage contribution of various habitats of macrofungi in KHANP.

Dominance–diversity curves of the 10 sites reveal that in all the sites, except Loharna and Ekhala, the top species followed a geometric pattern, whereas rest of the species showed broken-stick model. In Loharna and Ekhala, only broken-stick model was followed by the macrofungal species. In this model, the relative abundance of more than one species is present in a linear scale on the y-axis (Figure 5).

Whittaker’s  $\beta$ -diversity showed that maximum similarity of 95% existed between Janakpur and Loopara. Other important associations and species turnovers were found between Janakpur and Palmar (88%), as well as Loharna and Deharna (86%). Least percentage of association (52 each) was found between Janakpur and Sonder, as well as Marwah and Ekhala (Table 4).

**Table 3.** Species richness and diversity indices in 10 different sites of KHANP

Site	Richness	Menhinick	Margalef	$C_d$	$H'$	Evenness	Fisher alpha	Berger-Parker
Sonder	27	2.50	5.46	0.05	3.16	0.87	11.00	0.10
Loopara	20	2.04	4.16	0.06	2.91	0.92	7.69	0.09
Janakpur	23	2.20	4.69	0.05	3.01	0.88	8.90	0.11
Palmar	28	2.49	5.57	0.05	3.20	0.88	11.11	0.10
Loharna	37	3.09	7.25	0.03	3.49	0.89	16.19	0.06
Deharna	22	2.54	4.86	0.06	2.98	0.90	10.48	0.11
Qaderna	29	3.16	6.32	0.04	3.32	0.95	15.68	0.06
Marwah	32	3.07	6.61	0.04	3.35	0.89	15.26	0.07
Nath	31	3.06	6.47	0.04	3.29	0.86	15.05	0.12
Ekhala	31	2.73	6.17	0.04	3.33	0.90	12.95	0.05

$C_d$ , Simpson's dominance index and  $H'$ , Shannon-Wiener's diversity index.

**Table 4.** Whittaker's  $\beta$ -diversity of different sites in KHANP

	Sonder	Loopara	Janakpur	Palmar	Loharna	Deharna	Qaderna	Marwah	Nath	Ekhala
Sonder	–	0.83	0.52	0.82	0.72	0.71	0.57	0.56	0.72	0.59
Loopara		–	0.95	0.63	0.54	0.76	0.76	0.65	0.73	0.53
Janakpur			–	0.88	0.80	0.69	0.65	0.71	0.63	0.74
Palmar				–	0.69	0.60	0.72	0.70	0.66	0.66
Loharna					–	0.86	0.76	0.62	0.59	0.59
Deharna						–	0.84	0.59	0.70	0.70
Qaderna							–	0.77	0.63	0.53
Marwah								–	0.71	0.52
Nath									–	0.74
Ekhala										–

### Interaction of macrofungal species with environmental variables

Monte Carlo test of CCA for all the canonical axes was significant at  $P = 0.032$ , and showed a significant correlation between macrofungal species and the environmental variables. The first two canonical axes explained 40.2% cumulative variance and displayed strong species-environment correlations ( $r = 0.99$ ). The most important species in axis 1 were *Scleroderma verrucosum* (Bull.) Pers. and *Boletus granulatus* L., and in axis 2 *Ramaria formosa* (Pers.) Quel. (Figure 6). The main environmental factors in axis 1 and axis 2 were mean temperature and rainfall respectively.

### Discussion

Eighty-three macrofungal species were identified from KHANP and more than two-thirds of them belonged to the orders Agaricales (44%), Pezizales (14%) and Russulales (10%). Agaricales and Russulaceae were the most represented fungal families. The dominance of these macrofungal orders and families has ensured the dominance of humicolous (71.8%), lignicolous (11.8%) and ectomycorrhizal (10.8%) fungi in KHANP. Higher percentage of saprophytes in the present study may be compared with that of Salerni *et al.*<sup>50</sup> and Pradhan *et al.*<sup>20</sup> in the Mediterranean region and Eastern Himalayas respectively. These

authors have mentioned that rapid change in weather and the response of saprophytic mycelia to these changes are the possible reasons for the high diversity of saprophytes. Saprophytic fungi play a significant role in the cycling of soil nutrients, as they are one of the most active degraders of forest litter<sup>11</sup>. The high percentage of humicolous and lignicolous macrofungi shows that, at present, the forests of KHANP are in good health and have (i) good amount of decomposable litter, (ii) an undisturbed forest floor and (iii) less anthropogenic interference. This could also be described in terms of the decomposing capability of macrofungi for many intractable substrates found in the forests. However, Ortega and Lorite<sup>51</sup> have emphasized the priority for the conservation of ectomycorrhizal fungi that act as a nutritional support system and buffer for environmental stress for the host plants, rather than saprophytes because the latter represent a potential pool of pathogens if the forest area declines.

Most of the macrofungal species were from Basidiomycota (85.5%). Similar results were reported by Reverchon *et al.*<sup>21</sup> in pine-oak forests of Mexico (96% basidiomycetes) and Bhandari and Jha<sup>52</sup> from various forest types of Nepal (89.5% basidiomycetes). According to Dix and Webster<sup>53</sup>, basidiomycetes are vital for organic matter degradation as they produce a variety of lignocellulolytic enzymes. The higher species diversity in Basidiomycota may probably be due to accumulation of the substrate in temperate forests as a result of low decomposition rates<sup>54</sup> and higher number of mycorrhizal species belonging to

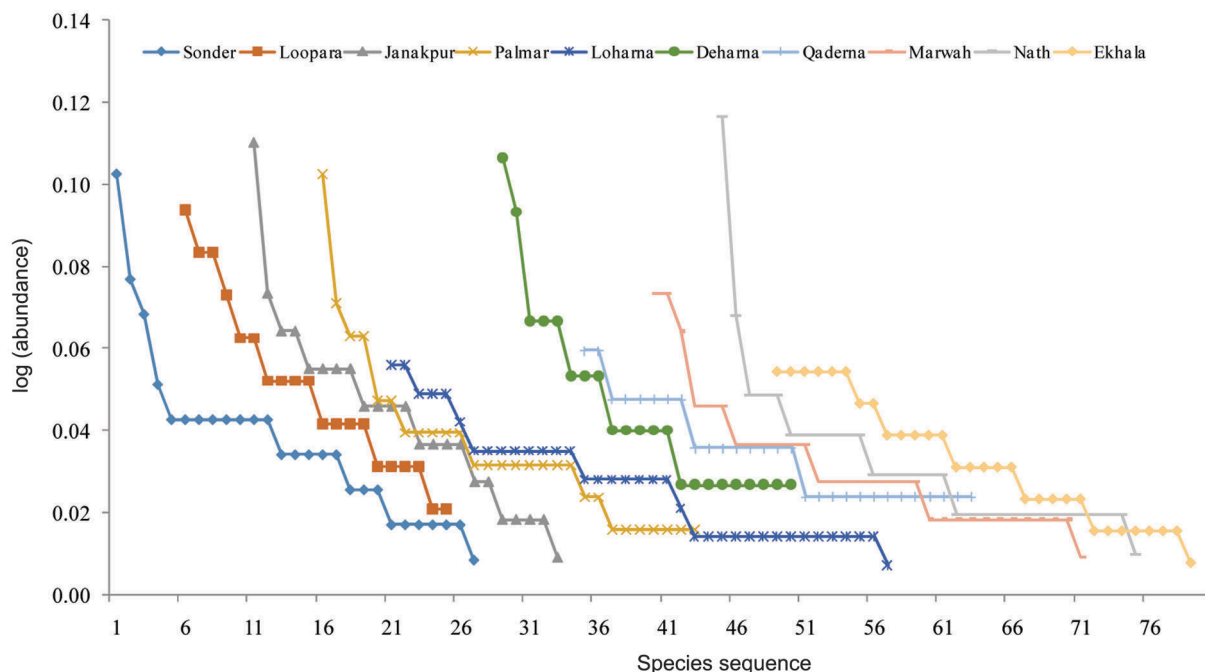


Figure 5. Dominance diversity curves of various sites in KHANP.

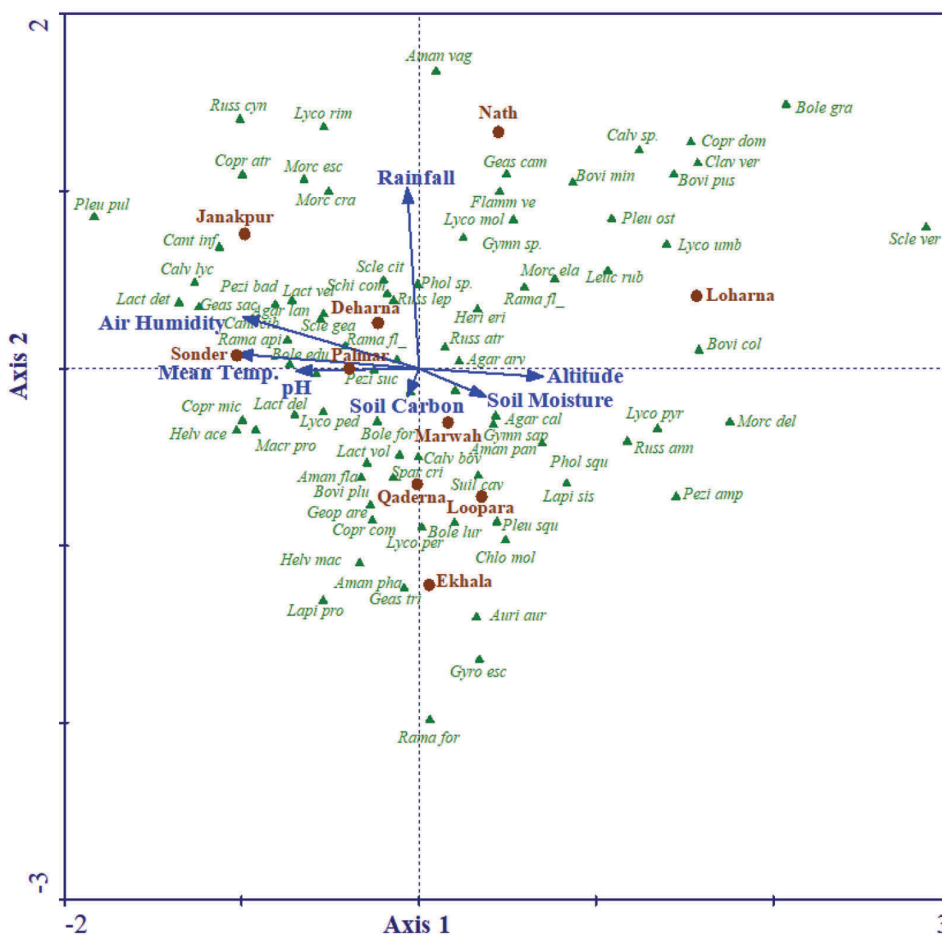


Figure 6. Canonical correspondence analysis ordination diagram with sites (•), fungal species (▲) and environmental variables (arrows). See Table 2 for a complete list of fungal species.



Basidiomycota found on soils with decaying litter<sup>55</sup>. The noticeable sporocarps may also influence the results towards basidiomycetes. The mycelium of members of this fungal class is reported to be omnipresent in forest soils<sup>56</sup> and plays a pivotal role in nutrient cycling<sup>57</sup>.

The pattern of dispersion of a species is indicative of habitat heterogeneity, distribution of nutrients and environmental conditions of an ecosystem. Plants growing in forests generally follow aggregate and random patterns. In the present study, the macrofungal species followed aggregate (75.9%) and random (24.1%) patterns. The aggregate distribution of mycelia in the forest floor could be a response to the diverse environment as the mycelia proliferate profusely in nutrient-rich patches<sup>58</sup>. Higher percentage of aggregate patterns among ectomycorrhizal fungi may be due to higher localized activity of mycelia and mycorrhizae with respect to soil heterogeneity coupled with distribution of roots of the host<sup>59</sup>. Kent and Dress<sup>60,61</sup> explained various models of spatial patterns in natural forests, and mentioned that both random and contiguous spatial patterns are conserved over a period of time and uniform pattern also transforms into a random pattern.

The most important characteristic of biodiversity assessment for fungi is species richness because insights into species richness of fungi are pivotal for biodiversity management, especially during the evaluation of their conservation status<sup>62-64</sup>. In the present study, values of various alpha-diversity indices varied from 20 to 37 (richness), 2.04 to 3.16 (Menhinick), 4.14 to 7.25 (Margalef), 0.03 to 0.06 (Simpson's dominance), 2.91 to 3.49 (Shannon–Wiener's diversity), 0.86 to 0.95 (evenness), 7.69 to 16.29 (Fisher's alpha) and 0.05 to 0.12 (Berger–Parker diversity). A significant difference was found between the sites for most of these indices. This clearly shows that species composition of the forests and environmental variables are key factors controlling the structure and diversity of macrofungal communities. According to Richard *et al.*<sup>59</sup> fungal diversity is strongly associated with forest composition and structure, whereas Piepenbring *et al.*<sup>65</sup> have reported that different fungal species develop in association with a wide range of host plants or on various substrata. Some studies have also confirmed the subsistence of distinctive macrofungal communities and diversity associated with the dominant tree species of a forest<sup>66,67</sup>.

In most sites, the species with maximum abundance contributed more than 40% of the total fruiting bodies and exhibited geometric series distribution. As reported by Whittaker<sup>68</sup>, the curves representing geometric series confirm niche pre-emption hypothesis and are indicative of low competition among the species. The utilization of resources follows a hierarchical fashion and a single dominant species pre-empts a large portion of the resources while the next most successful species pre-empts a lesser fraction of the leftover resources, and so forth. The other species follow the broken-stick model. May<sup>69</sup> concluded

that with the broken-stick distribution, it is apparent that an important ecological factor is being shared more or less evenly between the species.

CCA of macrofungal species revealed that *S. verrucosum* and *B. granulatus* (axis 1), and *Ramaria formosa* (axis 2) were the most important species of KHANP. All these species are humicolous in nature. These results not only justify our findings of dominance of humicolous fungi in KHANP but also show that they are the driving variables for these forests. Also they do not face any sort of competition with the ectomycorrhizal species probably because of the huge availability of slowly decomposing litter and less humus<sup>18,20,54</sup>.

CCA of the data showed that the distribution of species was mainly regulated by temperature and rainfall in axis 1 and axis 2 respectively. Some studies have reported that temperature and precipitation along with plant diversity are the chief determinants of distribution of macrofungal flora<sup>51,70,71</sup>. In addition to these factors, soil organic carbon also contributes to the general availability of macrofungi, as most of the fungal species are distributed along with low organic carbon concentration, i.e. sites having low organic carbon values. It has been reported that higher fungal diversity may lead to enhanced decomposition rates and, therefore, less organic matter<sup>21,72</sup>. In general, different fungal species show different relationships with the climatic and edaphic factors, as evident from the CCA diagram.

Many macrofungal species encountered during the present study have not been identified and are still under observation. The two-year survey could not give an assurance of comprehensive analysis of the macrofungi in KHANP. Complete knowledge of the fungi for any region requires periodic observations and collection of data over many years because diversity and the occurrence of macrofungal species increase with increasing number of visits over a period of time<sup>19,73</sup>. Moreover, gathering environmental data from these far-flung areas is also a big challenge for the researchers. Hence, studies should be carried out longer to record adequate data on macrofungal richness, diversity and distribution in KHANP.

## Conclusion

A good number of macrofungal species inhabit KHANP and most of them are humicolous, lignicolous and ectomycorrhizal macrofungi. These species are indicative of good diversity of nutrient cycling-regulating species. The results of studies on community structure of macrofungal species with respect to environmental variables show that mean temperature and rainfall are the two main driving factors responsible for the distribution and community organization of macrofungi in KHANP. The present study will provide baseline information for further assessment of macrofungal diversity in KHANP. Nevertheless, a

detailed study for a longer duration is required to ascertain these findings.

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