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MACROFUNGAL DIVERSITY IN DIFFERENT VEGETATION COMPOSITIONS IN TEGHARI COMMUNITY FOREST, KAILALI, WEST NEPAL

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ABSTRACT

Macrofungi are high-value forest resources that have functionally significant roles in the forest ecosystem. The macrofungal community of three different vegetation compositions, i.e., Sal (*Shorea robusta*) Forest, Tropical Deciduous Riverine Forest, and Tropical Evergreen Forest of Teghari Community Forest were investigated. Systematic random sampling was made where 60 plots (10 x 10 m) were laid in all different forest types (20 plots in each). A total of 102 macrofungi species were reported belonging to 36 families. Polyporaceae (17 species) was the largest family followed by Tricholomataceae (13 species) and saprophytic fungi were more frequent than mycorrhizal and parasitic fungi. The tropical evergreen forest was rich in macrofungi (59 species) followed by sal forest (40 species) and tropical deciduous riverine forest (38 species). Macrofungal diversity was directly related to surrounding host species. Similarly, increased soil moisture and canopy cover intensified the abundance of saprophytic fungi. The species richness was increased with increasing organic carbon, canopy, moisture, pH, and litter cover. However, soil nitrogen, phosphorus, and potassium were less significant in affecting species richness. Also, the disturbance was negatively correlated with the species richness of macrofungi. This study highlights the hidden diversity which is necessary for the conservation of macrofungi, to optimize forest ecosystem integrity and resilience against biotic and abiotic agents.

Keywords: macrofungal diversity, sal forest, species richness, tropical evergreen forest, tropical riverine forest

INTRODUCTION

Biodiversity is simply defined as the presence of the total organism of a particular group at a particular time in a particular area. Conservation of these natural resources is the priority for ecosystem functioning as well as human welfare. Fungi are an enormous usly diverse group of organisms ranging from microscopic to macroscopic forms that grow mostly in the dead and decaying substrate. They appear in all seasons, mostly rainy season, wherever nutrient organic matters or decomposed products are easily available (Jha & Tripathi 2012).

Macrofungi are a group of higher fungi that produce mature spore-bearing fruiting bodies, which are visible to the naked eye (Chang & Miles 1992). They are known to inhabit diverse kinds of habitats varying in the composition of their tree species and substrates. Based on ecology, they are parasitic or saprophytic or may show some mycorrhizal associations with vascular plants (Kumar & Sharma 2011). However, some macrofungi are neutral to the abundance of dominant tree species, in particular, habitat type (Zhang & Zak 1998). The relationship between the tree and fungal communities is reflected in host trees affecting fungal specialization and providing unique habitat availability and different resource quality. The composition and structure of aboveground vegetation are responsible for diverse macrofungi communities (Buee *et al.* 2011).

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Generally, macroscopic fruiting bodies of the fungi is called mushroom which can be epigeous or hypogeous and vary in different shape and sizes. They are fleshy, sub-fleshy, or sometimes leathery and woody and bear their fertile surface either on lamellae or lining the tubes, opening out through pores. The most suitable condition for the growth of carpophores depends upon the high humidity, nutritionally rich substrate, and warm atmospheric temperature (Dickinson & Lucas 1979). Similarly, other environmental conditions such as geographic location, light, and surrounding vegetation types also play a major role in the distribution of the macrofungi (Sibounnavong *et al.* 2008).

Diversity-related studies are carried out in different forests but their relationship with higher plants was poorly explored except these studies such as Pradhan (2013), Baral *et al.* (2015), and Bhandari and Jha (2017). This study aimed to to optimize forest ecosystem integrity and resilience against biotic and abiotic agents, by looking at the effect of different vegetation characteristics and environmental factors on macrofungal species composition and richness in the tropical region of western Nepal.

MATERIALS AND METHODS

Study Sites

The study was carried out in three different vegetation patches within Teghari Community Forest in the tropical riverine belt of Kailali District, West Nepal (Fig. 1). The study area lies between latitudes from 28°50'45" N to 28°51'01" N and longitude 80°33' 3" E to 80°33'13" E, covering an area of 340 ha. The altitude range of the study area is 155 - 254 masl. Meteorological data of the Dhangadi Airport in the year 2019 was obtained from the Department of Hydrology and Meteorology, Government of Nepal which revealed that the study area is represented by a tropical climate and receives an average of 1,406.6 mm annual rainfall with the highest monthly rainfall happens in July (466.9 mm) and the lowest in May (5 mm). The highest monthly mean temperature happens in May (40.41°C) and the lowest in January (6.77 °C).

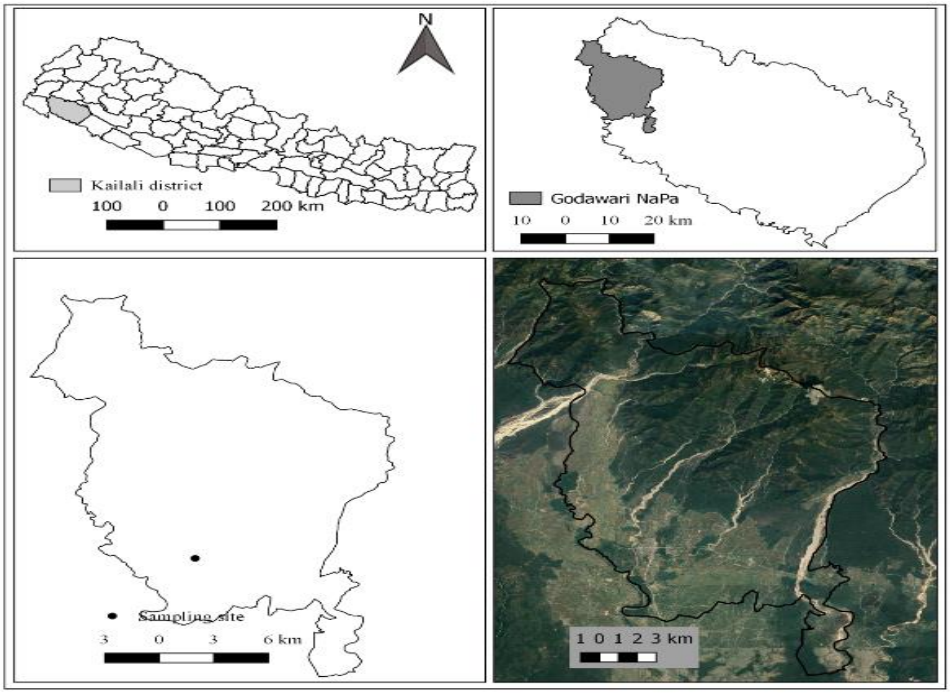

Figure 1 Map of the study area

Study Design

The Teghari Community Forest was selected for the field study as it has three different forest types at the same elevation, i.e., Sal Forest, Tropical Riverine Deciduous Forest, and Tropical Evergreen Forest. *Shorea robusta* (Sal) is the dominant tree species in the Sal Forest which forms magnificent forest stand on the edges of the Godawari River. The tropical deciduous riverine forest is also located similarly and is mainly dominated by *Acacia catechu* and *Dalbergia sissoo* along with *Bombax ceiba*, *Syzygium cumini, Adina cordifolia*, *Hollarrhena pubescens*, *Murraya koenginii, Aegle marmelos,* and *Semicarpus anacardium*. The tropical evergreen forest lies on the Northwest side of Mahakali Highway and is dominated by *Terminalia alata, Lagerstroemia parviflora, Terminalia bellerica, Ficus religiosa*, *Schleichera oleosa, Aegle marmelos,* and *Cassia fistula. Mallotus phlippensis* is present all over the study area. In each forest type, rectangular plots of 10 × 10 m were established. The number of plots to be sampled were determined based on the spatial area of each forest.

Field Sampling

Detailed sampling of macrofungi diversity was made by applying a systematic random method within the period of June - October 2019, where plots were laid in each forest type. A total of 20 plots were laid in each forest type along with the two transects for maintaining an inter-plot distance of at least 20 m (Baral *et al.* 2015). Presence or absence data of macrofungal species were recorded in each plot. Biophysical variables, such as tree canopy cover, litter cover, and anthropogenic disturbances (trampling, fire grazing, non-degradable waste, etc.) were also recorded in each plot. Tree canopy cover and litter cover (in percentage) was estimated visually. For tree canopy cover, observation was made from the middle of each plot. Soil samples were collected at a depth of 15 cm from four corners and at the middle of each plot using a soil digger. The soil samples from each plot were mixed thoroughly. From the mixed soil sample, about 200 g of soil sample was taken and put in a zipper polythene bag. The soil samples were air-dried in shade for a week and stored in airtight plastic bags until laboratory analysis. The physiochemical parameters of soil, such as soil pH, moisture, organic carbon, nitrogen, potassium, and phosphorus were assessed using a standard soil analysis manual (Zobel *et al.* 1987).

Macrofungal specimens were collected, preserved (dry), and taken to National Herbarium (KATH) in Lalitpur, Nepal. Collected specimens were studied based on their morphological characters and ecology with the help of several websites, such as https://www.mushroomexpert.com and http://www.indexfungorum.org. Finally, the identification of specimens was confirmed using relevant literature (Pacioni & Lincoff 1981; Adhikari 2014; Laessoe 2013) along with identification conducted by macrofungi expert. All of the collected macrofungal specimens were deposited in ASCOL herbarium, Amrit Science College, Kathmandu, Nepal.

Data Analysis

All data were entered in Microsoft Excel 2010 for further analysis. Pearson correlation method was used to know the effect of a different environmental variable on macrofungal diversity. Simpson's Diversity Index (Simpson 1949) and Shannon-Wiener Index (Shannon & Weaver 1963) were also calculated. Regression analysis was performed using SPSS Version 20 and Microsoft Excel version 2010. Species composition of different macrofungi species along with different environmental components were evaluated by Canonical Correspondence Analysis (CCA).

RESULTS AND DISCUSSION

Macrofungal Diversity in Different Vegetation Composition

A total of 102 macrofungi consisting of Ascomycetes-5 and Basidiomycetes-97 species were documented, in which 100 species were identified up to species level and 2 species were identified up to genus level. Out of the 36 families, 17 species belonged to the Polyporaceae family, 13 species to Tricholomataceae, 11 species to Marasmiaceae, 9 species to Agaricaceae, 8 species to Coprinaceae, 4 species each to Russulaceae and Xylariaceae, 2 species representing each of the Cortinariaceae, Ento-

lomataceae, Fomitopsidaceae, Ganodermataceae, Hydnangiaceae, Podoscyphaceae, and Suillaceae family and the rest of the family was represented by single species only (Fig. 2).

Tropical evergreen forest harbored the highest macrofungal diversity (59) in all three different substrates (Fig. 3), followed by Sal Forest (40) and tropical deciduous riverine forest (38). The maximum numbers of macrofungi were found growing on the soil, followed by wooden logs. The least number of macrofungal species were found growing on litters in all forest types.

The present study relates to a study conducted in India where macrofungi were reported in various habitats, like wood, litter, and moist soil, among others (Nagaraju *et al.* 2014). As compared to litter and wood, the soil was the most important substrate for maintaining macrofungal diversity in all three forest types studied. In our study, higher number of macrofungi were grown on moist soil compared to those on litter and decaying wood. These findings resemble the previous findings of a study conducted by Bhandari and Jha (2017).

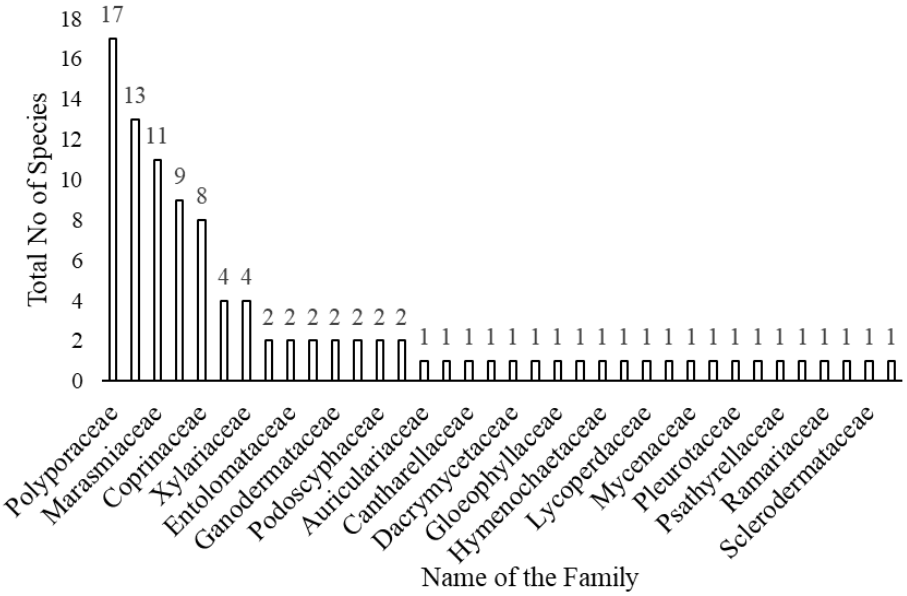

Figure 2 Number of species with their respective family

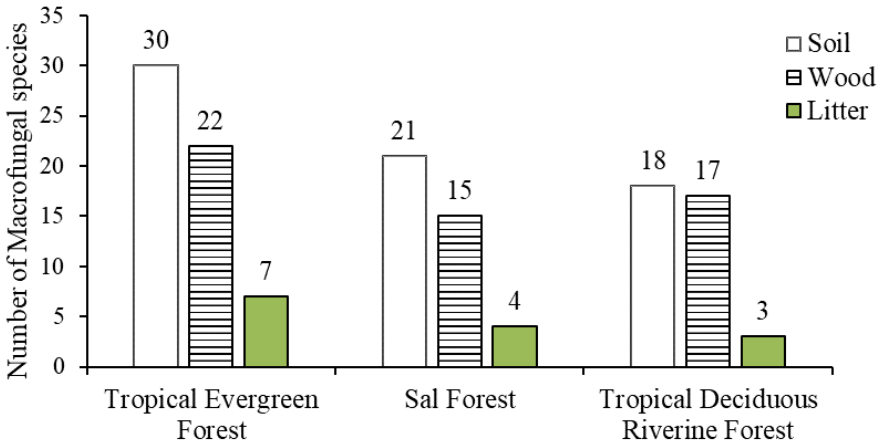

Figure 3 Distribution of macrofungi based on their habitat in different forests

Based on the ecology of macrofungi, the maximum number of macrofungi were consisted of saprophytes, followed by mycorrhizal and parasitic macrofungi, while the least number belonged to termitophilous macrofungi. The mycorrhizal fungi serve as an extension of the plant root system, exploring soil far beyond the roots and transporting water and nutrients to the roots (Tapwal *et al.* 2013). The flourishing of carpophores is enhanced by litter accumulation and decomposition as well as the presence of extracellular microbial enzymes (Pushpa & Purushothama 2012). The rapid change in the weather and high response of mycelia were among the main factors for the increasing number of saprotrophic fungi (Pradhan *et al.* 2012). A similar result was obtained in the research of Topwal *et al.* (2013) and Dey *et al.* (2016). Higher species diversity in Basidiomycota compared to Ascomycota is probably contributed by a higher number of mycorrhizal species found on the soil as studies have shown that soil moisture and decaying litter facilitate many diverse macrofungi (Muller & Schmit 2007).

Simpson's Diversity Index (Table 1) was found to be the highest in Tropical Deciduous Riverine Forest and Tropical Evergreen Forest (0.91) in comparison to Sal Forest (0.88). Similarly, Shannon-Wiener Diversity Index was also found to be the highest in Tropical Evergreen Forest (2.91) followed by Tropical Deciduous Riverine Forest (2.77) and Sal Forest (2.53). The presence of diverse kinds of macrofungi communities is specifically related to the dominant tree species of the forest has been confirmed by many other studies (Straatsma & Krisai-Greilhuber 2003; Gates *et al.* 2011; O'Hanlon & Harrington 2011; Bhandari & Jha 2017; Collado *et al.* 2021; Kutszegi *et al.* 2021). Such variation may be attributed to microclimate conditions (Santos-Silva *et al.* 2011) and forest management practice (Kouki & Salo 2020).

The high macrofungal diversity in the tropical evergreen forest is mainly related to high soil moisture and greater cover of tree species. The high diversity may be also due to suitable habitat, such as soil moisture, litter, and canopy cover which help to maintain sufficient moisture (Trudell & Edmonds 2004). A tropical deciduous riverine forest located on the water edges has a more open canopy and less humidity in the soil which creates a less suitable habitat for the growth of macrofungi. Also, thinning of trees caused a decrease in fruit-body production of the delicate and fragile macrofungi, but this effect varied greatly depending on the season, the macrofungi fruiting pattern and the levels of trees thinning (Luoma *et al.* 2004). Therefore, thinning and pruning, which are common silvicultural activities in the community forests of Nepal (Shrestha *et al.* 2010), might also affect the composition and abundance of macrofungi. Similarly, Sal Forest has a magnificent stand of tall trees and has a more open canopy in comparison to tropical evergreen forest. The growth of macrofungal species like *Pycnoporus cinnarius* and *Scleroderma cepa* was specifically recorded in Sal Forest. A similar finding was also reported by Prasad & Pokhrel (2017) at Amrite community forest, Kapilvastu District (Central Nepal), which might be due to the host specificity of macrofungi with particular plant species. The presence of specific macrofungi communities in the present study may be due to host preferences which were related to the findings by Ding *et al.* (2011) and Lang *et al.* (2011).

Species Richness of Macrofungi and Different Environmental Variables

The Canonical Correspondence Analysis (CCA) revealed the relationship between macrofungi species composition and environmental gradient (Table 2). The analysis results indicated the effective separation of species along the main gradient (Table 3).

Table 1 Diversity indices of macrofungi in different vegetation stands

Forest stands	Simpson's index	Simpson's diversity index	Shannon-Wiener diversity index
Sal Forest	0.12	0.88	2.53
Tropical Deciduous Riverine Forest	0.09	0.91	2.77
Tropical Evergreen Forest	0.09	0.91	2.91

Table 2 Summary of the results of Canonical Correspondence Analysis

Axes	1	2	3	4	Total inertia
Eigenvalues	0.369	0.34	0.279	0.208	11.381
Species-environment correlations	0.886	0.903	0.945	0.88	
Cumulative % variance of species data	3.2	6.2	8.7	10.5	
Cumulative % variance of species-environment data	18.7	35.9	50	60.5	
Sum of all canonical eigenvalues					1.978

Table 3 Relative importance of environmental variables and their significance (P value) on macrofungal species composition derived by using the Monte Carlo permutation test from the Canonical Corrrespondence Analysis with 9999 replications

Environmental variable	Abbreviation	F	P
Organic carbon	Orgcarb	0.914	0.682
pH	pH	1.086	0.227
Moisture	Moist	1.249	0.11
Nitrogen	Nitro	1.057	0.347
Phosphorus	Phosp	1.176	0.182
Potassium	Potas	1.4	0.162
Litter	Litter	1.33	0.046
Canopy cover	Canop	1.033	0.36
Disturbances	Distrb	1.209	0.052

Our study showed that environmental variables, such as moisture, pH, canopy, organic carbon, nitrogen, phosphorus, potassium, and anthropogenic disturbances had significant effect on the distribution and composition of macrofungi. Organic carbon, moisture, pH, canopy, litter, and macrofungal species richness were positively correlated and was comparable with the study of Bhandari and Jha (2017). This finding indicated that these environmental variables play a vital role in shaping macrofungi communities. However, other soil properties such as nitrogen, phosphorus, and potassium were negatively correlated with the species richness. There were no significant effects on the increasing disturbances with the abundance of macrofungi (Fig. 4).

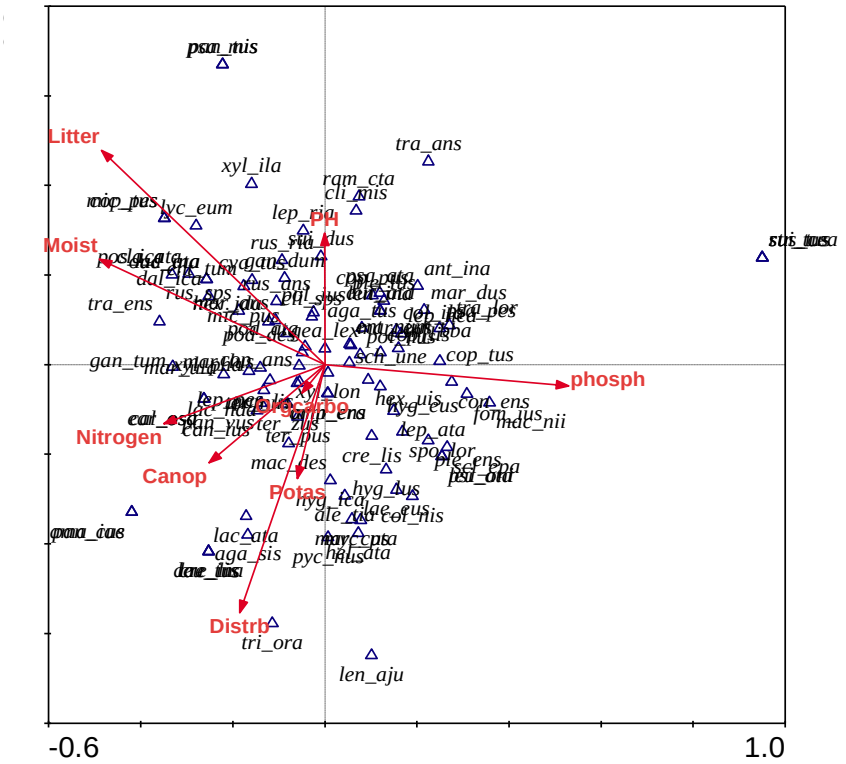

Figure 4 CCA biplot representing the effect of environmental variables on macrofungal species composition

Macrofungi species, like *Pycnoporus cinnarius* (pyc_ius), *Scleroderma cepa* (scl_epa), *Agaricus arvensis* (aga_sis), *Tricholomopsis decora* (tri_ora), *Termitomyces microcarpus* (ter_pus), *Aleuria aurantia* (ale_tia) and *Lentinus sajorcaju* (len_aju), etc. showed strong presence in the Sal Forest and were more resistant to disturbances. Tropical Deciduous Riverine Forest was dominated by the macrofungi species, like *Ramaria stricta* (ram_cta), *Antrodia juniperina* (ant_ina), *Fomes fomentarius* (fom_ius), *Hexagonia tenuis* (hex_uis), *Trametes elegans* (tra_ans), *Clitocybe infundibuliformis* (cli_mis), *Lenzites betulina* (len-ina), *Panus fasciatus* (pan_tus), *Coltricia perennis* (col_nis) and *Microporus xanthopus* (mic_pus), etc. which favored more open canopy. Similarly, Tropical Evergreen Forest harbored macrofungi species, like *Macrolepiota rickenii* (mac_nii), *Marasmius haematocephalus* (mar_lus), *Daldinia concentrica* (dal_ica), *Marasmius androsaceus* (mar_eus), *Lepiota clypeolaria* (lep_ria), *Macrolepiota rhacodes* (mac_des), *Lacrymaria lacrymabunda* (lac_nda), *Lepiota clypeolaria* (lep_ria), *Lycoperdon subcretaceum* (lyc_eum), *Cyathus striatus* (cya_tus) *Microporus xanthopus* (mic_pus) and *Podoscypha multizonata* (pod_ata). Our present study also showed that the species found in the Tropical Deciduous Riverine Forest and Tropical Evergreen Forests were more similar than those in the Sal Forest. However, some macrofungi species, like *Schizophyllum commune* (sch_une), *Polyporus arcularius* (pol_ius), *Termitomyces tylerianus* (ter_nus), *Microporus xanthopus* (mic_pus), and *Geastrum triplex* (gea_lex) were found in all three forest types. The CCA biplot showed environmental variables and aboveground vegetation were the major components for determining macrofungi composition (Fig. 4). The overlapping of macrofungi species was due to a similar ecological niche. As we found in our present study, most of the soil fungi such as *Geastrum triplex* (gea_lex), *Podocypha petaloides* (pod_des), *Suillus granulatus* (sui_tus), and *Russula* species occurred toward the moisture. Our study also showed that the presence of thin canopy cover and low soil moisture seemed to enhance the growth of wood-inhabiting fungi, such as *Spongipollis unicolar* (spo_lar), *Crepodotus mollis* (cre_lis), *Xylaria* sp., *Trametes elegans* (tra_ans), *Antrodia juniperina* (ant_ina), *Fomes fomentarius* (fom_ius), *Hexagonia tenuis* (hex_uis), *Micrporus vernicipes* (mic_pes) and *Lenzites betulina* (len_ina), which were mostly presented opposite direction to the moisture and pH. Organic carbon was also one of the major components which control the distribution pattern of soil fungi. Macrofungi species, like *Termitomyces* sp., *Rusulla* sp., *Macrolepiota rhacodes* (mac_des), *Omphalina umbellifera* (omp_era), and *Marasmiellus ramealis* (mar_lis) were found predominantly toward the direction of organic carbon. In our present study, disturbances seemed to have a poor impact on macrofungi species composition. Most of the fleshy, soft, and gilled macrofungi, like *Clitocybe* sp., *Psathyrella obtusata* (psa_ata), *Agaricus augustus* (aga_tus), *Lepiota clypeolaria* (lep_ria) and *Coprinus disseminates* (cop_tes) were favored by the higher soil pH.

Species richness of macrofungi increased with the increasing soil organic carbon, moisture, pH, litter coverage, and canopy coverage. Soil pH and organic carbon ranged from 4.06 to 7.07 and 0.79 to 5.67, respectively. Similarly, soil moisture, litter cover, and canopy cover ranged from 7.5 to 45.46%, 11 to 49%, and 15 to 95%, respectively. Among all environmental variables, organic carbon, soil moisture, soil pH, litter cover and canopy cover had the most significant positive relationship with macrofungi species richness (Fig. 5). Also, the species richness of macrofungi showed a weak positive relationship with disturbances. Soil nitrogen, phosphorus, and potassium were negatively correlated with macrofungi species richness but the result was statistically insignificant (Table 4).

Species diversity of macrofungi depends on their particular habitat. Geographic location, elevation, temperature, the humidity of air and soil, light, surrounding flora, and anthropogenic activity greatly influence the growth and reproduction of macrofungi (Zervakis & Venturella 2007; Topwal *et al.* 2013). Soil moisture is one of the most important environmental factors responsible for affecting the growth of the macrofungi (Kropp & Albee 2002). Present findings also showed increasing species richness is affected by the increasing moisture content of the soil. This finding was similar to the research of Bhandari and Jha (2017). The fungal diversity studies in Greece and Sicily (Venturella & Zervakis 2000; Zervakis & Venturella 2002) confirmed that fungi require a certain level of moisture; rainfalls,

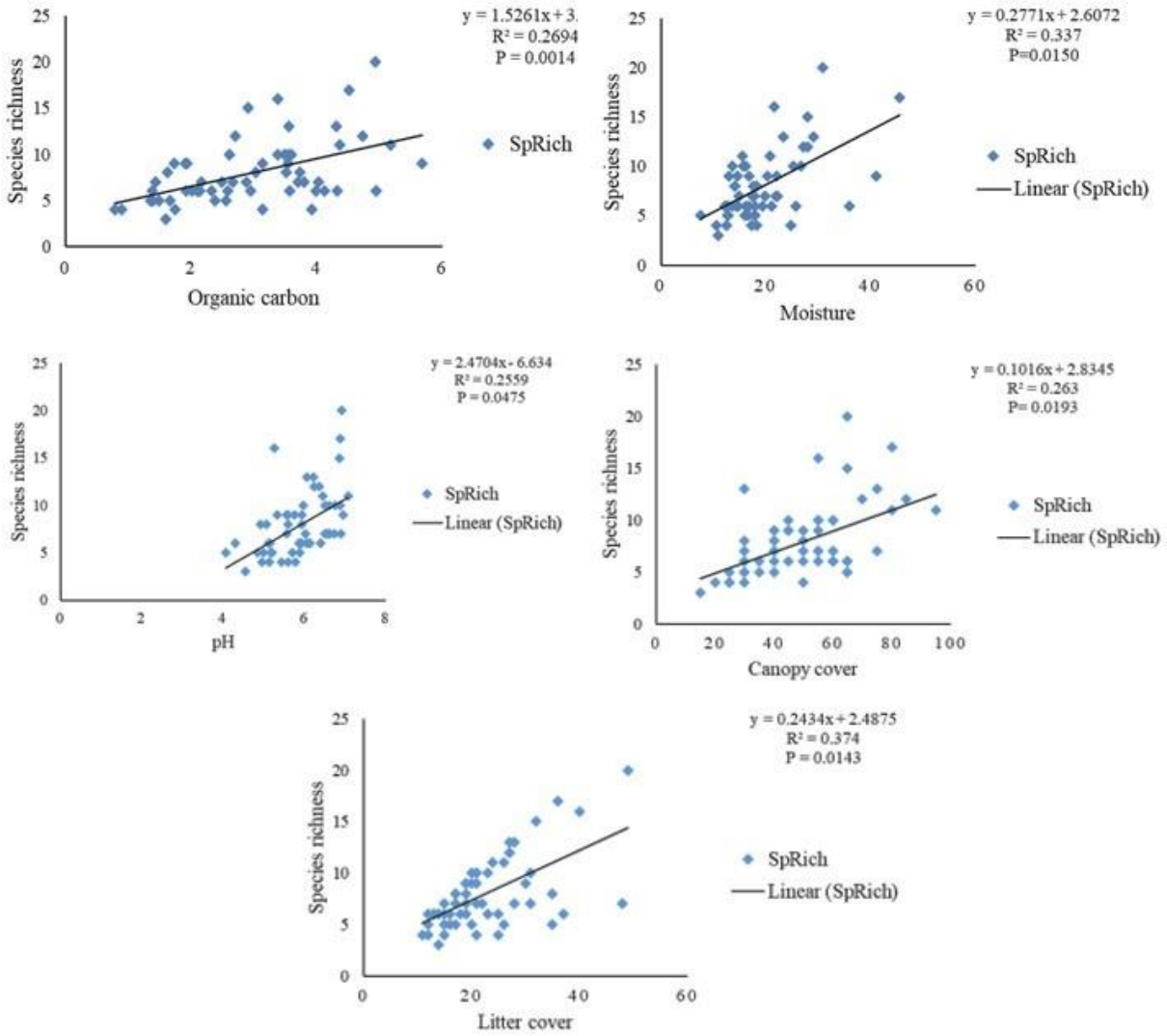

Figure 5 Correlation between macrofungi species richness and organic carbon, moisture, soil pH, canopy cover, and litter cover

Notes: Each point in each figure represents a sampling plot; Total number of sample plots = 60. Less number of points in the figure may be due to the overlapping of the data among the plots. The fitted line is based on the linear regression model for sampling plots.

Table 4 Pearson correlations between environmental variables and species richness of macrofungi

	Soil pH	Organic carbon	Moisture	N	P	K	Canopy cover	Litter cover	Disturbances
Species richness	0.506**	0.519**	0.580**	-0.074	-0.183	-0.14	0.513**	0.612**	0.034

Notes: * = Correlation is significant at P < 0.05 level; ** = Correlation is significant at P < 0.01 level.

air humidity, and soil moisture, which are all significant factors. Canopy cover and litter cover had also provided positive influence on macrofungal species richness in the studied area and a similar result was found by Baral *et al.* (2015). The result unveiled that canopy cover plays a vital role in increasing macrofungal diversity in forests which was also reinforced by other previous findings (Dighton *et al.* 1986; Bonet *et al.* 2004; Sysouphanthong *et al.* 2010; Santos-Silva *et al.* 2011). The reason is likely to be the presence of more substrate on the forest floor and high humidity which favor the growth of more fungal species (Lodge *et al.* 2004). Litter is an important component of every ecosystem and it constitutes the major source of organic matter in the soil. The removal of litter directly affects the diversity and growth of macrofungi (Eaton *et al.* 2004; Sayer 2006). When the forest floor is covered with layers of well-decomposed

leaves, saprotrophic fungi are favored by this organic resource which maintains the temperature and moisture of the surrounding area (Fernandez-Toiran *et al.* 2006).

Also, the abundance of macrofungal species is closely correlated with soil organic matter and other soil parameters (Zamora-Martinez & de Pascual-Pola 1995; Engola *et al.* 2007). The growth of saprophytic fungi was enhanced at pH 7 or 8; while the ectomycorrhizal species showed the peak growth at pH 5 or 6 (Yamanaka 2003). Soil pH was also a major abiotic component responsible for changing macrofungi communities and was found to be positively correlated with macrofungi species richness. The pH range of 5 to 6 favors the growth of soil fungi (Bhandari & Jha 2017; Pavithra *et al.* 2016; Zhang *et al.* 2016).

In this study, it was observed that there were no direct relationships between macrofungi richness and other soil parameters, such as nitrogen, phosphorus, and potassium with macrofungi diversity. However, the importance of forest soil chemistry parameters in fungal species distributions has also been reported by Hansen (1988) and Ruhling & Tyler (1990).

CONCLUSION

The study area is rich in macrofungal diversity with species' richest families being the Polyporaceae followed by Tricholomataceae, Marasmiaceae, Agaricaceae, and Coprinaceae. The presence of diverse kinds of vascular plants and different environmental conditions in different forest types have created unique habitat for the growth and development of a wide variety of macrofungi species. Species diversity is higher in moist and dense canopy forests such as Tropical Evergreen Forests and Sal Forest compared to that in the open and dry Tropical Deciduous Riverine Forests. Soil moisture, organic carbon, soil pH, litter cover, and tree canopy cover are the most important variables affecting macrofungal diversity.

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