Termite mushrooms (*Termitomyces*) in Nepal: Exploring its distribution and diversity across ecological gradients

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Abstract

The distribution and diversity of *Termitomyces* were studied along three different ecological zones within three phytogeographic divisions. The main aim of the study is to assess patterns of species diversity and distribution along the east-west and south-north gradients of Nepal Himalaya. Soil samples from 27 locations were collected; among them four different types of soil (i.e. termite nest, casing soil, vicinity area and forest soil) were fixed for each spot to observe nutrient status of soil and influence of surrounding soil nutrients in the growth of *T. albuminosus*. In total, nineteen taxa have been recorded excluding non-termite mushrooms. The study revealed that species richness was significantly variable within physiographic region, land use, locality, altitude and air temperature for the distribution of *Termitomyces*. Analysis of soil nutrients (N, P, K, OM, Fe, Zn, Cu, Mn and B) revealed that the presences of organic matter and microelement content was apparently higher in most of the natural protected forest with comparisons to the agro-pastoral, plantation and managed forest. Most of the nutrients and their interaction between soil source and eco-zone were significantly different at $p < 0.05$. The study observed that the organic matter content was highest in the Tarai (5.40%) in termite nest (7.90%), comparative to the lowest contain of 2.47% in the Midhill regions of casing soil (1.38%). The study observed that biomass yield of *T. albuminosus* was highest in Tarai, followed by the Siwalik and Mid-hill regions.

Keywords: Biomass, Edaphic factor, Physiography, Phytogeography, Soil nutrient

1. Introduction

Termitomyces are obligatory symbiont fungi. These fungi are among the few organisms that can digest lignin and cellulose. *Termitomyces* is a white-rot fungus [1]. The optimal conditions (high, buffered temperature and high humidity) for these fungi are predominantly found in hot and wet habitats [2], such as rain forests. This fungal white-rot decay requires temperature-buffered, high-humidity environments, which tropical rain forests provide. Optimal growth conditions for *Termitomyces* are relative humidity near the saturation and constant temperatures of about 30ºC [3].

According to Hawksworth [4], fungi are major components of biodiversity, essential for the survival of other organisms, and are crucial in global ecological processes. Contributing to the nutrient cycle as conversion of insoluble inorganic phosphate to soluble organic phosphate and maintenance of ecosystem, fungi play an important role in soil formation, fertility, structure and improvement [5]. Similarly, macro fungi play a principal role in recycling nutrients and influencing plant community composition through symbiotic relationship [6]. There have been a few studies on forest ecology [7] and the relationship of macrofungi with environmental variables [8].

Fungal diversity is a crucial component of biodiversity [9]. They constitute an exceptionally diverse group that is central to the functioning of ecosystem [10], and serves as vital contributors of terrestrial ecosystems because of involvement in nutrient cycling [11, 12]. They also act as principal decomposers of dead organic matter, such as dead wood and litter [13]. Macrofungi together with their mycorrhizae have been shown to have a significant ecological function in the establishment and dynamic succession of plant communities, nutrient cycling and the protection of forest ecosystem, and likely to be crucial to sustainable development, ecological construction and stability [14, 15, 16]. In these aspect, termitophilous fungi represent high value forest resources.

Although factors driving macro fungal diversity remain unclear; climatic conditions such as rainfall, temperatures, evapotranspiration, relative humidity, air, soil, and water deficits or excesses are generally regarded as major factors affecting

macrofungal fructification [17]. Geomorphologic features as slope, aspects and altitude also seem to influence the macro fungal communities [18]. Similarly, biological interactions that occur below the ground, among plants roots, animals and micro-organisms are dynamic and substantially influence the ecosystem processes [19]. Fungi play important role in maintaining soil nutrient like another underground biota [20]. The nature and availability of soil solutes are fundamentally important from the stands point of fungal nutrition. Normally, inorganic solutes are absorbed in ionic forms for normal development, but the amounts of their requirements may vary among different species.

Termitophilic mushrooms are usually observed between 25-32°C during rainy season in tropical to temperate belts. Different species exhibit different fruiting phenologies that vary from year to year across different altitudes and latitudes; the highest richness is observed only for a short period and varies between years. Investigation revel that within a specific geographical region, fruiting is influenced by elevation and latitude, and their influence on temperature and precipitation [21].

Upto 30 species of the genus *Termitomyces* are known and were accepted in the 10th edition of the Dictionary of the Fungi [22, 23]. Though recently a review paper [24] reported 57 species from the globe. Until Heim [25] enlighted the genus *Termitomyces*, early mycologists assigned this genus to widely different genera like *Armillaria* (Fr.) Staude 1857, *Entoloma* P. Kumm. 1871, *Lentinus* Fr. 1825, and *Pluteus* Fr. 1836, as there are some morphological similarities [22] with these genera. One third of the species of the genus *Termitomyces* recorded worldwide are found in Nepal. These species were collected from tropical to temperate region, with a wide distribution throughout the country. Recognizing the significant scope and importance of the issue for ecosystem maintenance and human nutrition, the present investigation was initiated with the objective of documenting the occurrence and distribution of *Termitomyces* in Nepal.

2. Materials and Methods

Soil samples including termite mushrooms were collected from all the studied sites i.e. 27 locations, each representing individual topography as representatives for these comparative sites of habitats (Fig. 1). These soil samples were analyzed in the soil science division of Nepal Agriculture Research Council (NARC), Khumaltar and Soil Management Directorate (SMD), Hariharbhawan, Department of Agriculture, Government of Nepal (GoN). Approximately 500 g of soil was collected from the four corners of each quadrat of $10m^2$ at a depth of 15 cm, thoroughly mixed and placed in a polyethylene bag. In case of termite nest and casing soil of the hump of termatoria, they were simply taken from the ground surface and mixed in the same process before keeping in polyethylene bag. The samples were air dried while in the field and the drying was continued for one week after returning back to the Laboratory. The pH was measured by taking soil sample and mixing it with distilled water (D/W) in a 1:1 ratio. The digital pH meter was calibrated by adjusting in different concentration of buffer (pH 4, 7 & 9) solution to observe the soil pH [26]. Nitrogen concentration was determined in percentage by Kjeldahl digestion method [27], Phosphorus by Olsen's Bicarbonate method [28], and Potash obtained by the adjustment of flame photometer [29]. The filtrated diammonium acetate method [30] was used for determining the organic

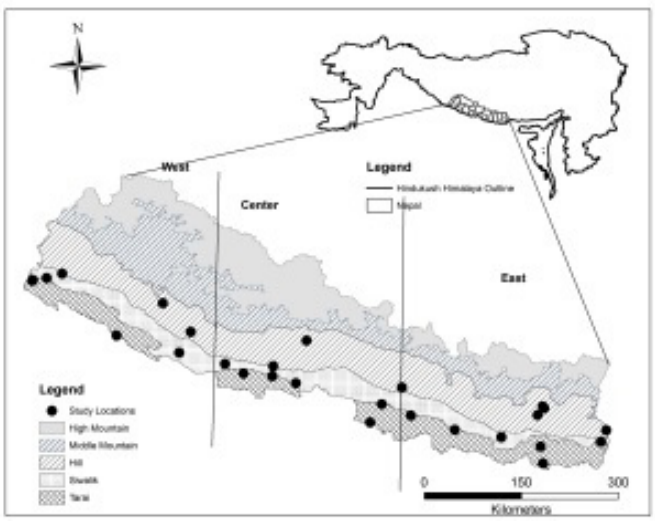

Fig. 1: Sample collection sites

matter (OM) present in the soil, where oxidation of organic carbon in an acid dichromate solution followed by titration of the remaining dichromate with ferrous ammonium sulphate was done [31]. Similarly, micronutrients (Zn, Cu, Mn, & Fe) were analyzed in the NARC by using the DTPA method [32], and Boron by Azometchneh colorimetric method [33].

The yield data of the fruiting bodies for three flushes in a period of 30 days in each year from first flush were recorded in terms of average number, average weight and average weight per fruit body, within a period of three year.

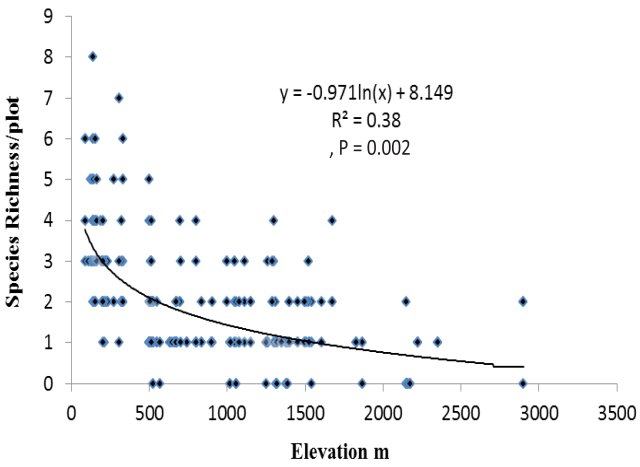

Fig. 2: Relationship between species richness and elevation

3. Results

During investigation 256 wild mushroom species have been collected and identified. Among them termitomycetes were selected for this investigation. These are *T. albuminosus, T. arghakhachensis, T. aurantiacus, T. badius, T. clypeatus, T. eurrhizus, T. fuliginosus, T. globulus, T. heimii, T. le-testui, T. mammiformis, T. microcarpus*, *T. microcarpus f. santalensis*, *T. palpensis, T. robustus, T. schimperi*, *T. striatus f. griseus, T. striatus f. ochraceus* and *Termitomyces umkowaan*, of these, four (*T. albuminosus, T. mammiformis, T. robustus,* and *T. microcarpus*) are mostly common in tropical to subtropical regions of the study area. They were found growing on the tops of termatorium in various localities. The count of individual mushrooms varied, ranging from one individual of *T. umkowaan* to 2823 individuals of *T. microcarpus* termitophilous fungi in Bardiya National park (BNP) area. West Tarai region was rich in *Termitomyces* growth. Species richness decreased as elevation increased in different ecological belts in Nepal (Fig. 2).

Linear regression testing the effects of different environmental parameters on species richness was significantly varied within physiographic regions, land use types, locality and altitude. The number of individuals were significantly influenced by physiography, land use, locality, altitude, air temperature and marginally by (p=0.08) liter cover (Table 1).

The species composition of *Termitomyces* is varied based on altitude and location. When considering the influence of physiography on species composition, Canonical Correspondence Analysis (CCA) revealed a significant (p=0.002) variation in the locality number composition of *Termitomyces* across distinct physiographic regions and altitudes. In total, both physiography and altitude explained 5.86% of the total variation in the data set of species composition. *T. arghakhachensis* and *T. palpensis* were strictly found in Siwalik (between 500 to 1000m), *T. umkowaan*, *T. microcarpus f. santalensis, T. robustus*, and *T. eurrhizus* in Mid-hill (>1000m), and *T. clypeatus, T. microcarpus, T. schimperi* and *T. aurantiacus* were found in Tarai (<500m) region (Fig. 3). Results of the CCA analysis showed that the effect of elevation on species composition of *T. eurrhizus, T. albuminosus* and *T. umkowaan* were found in higher elevation whereas *T. schimperi* and *T. heimii* were found in lower elevation. *T. robustus, T. fuliginosus* and *T. mammiformis* were found everywhere irrespective of any elevation (Fig. 4).

Table 1: Linear regression on species richness of *Termitomyces*

Predictors	df	Deviance	Resid. df	Resid. Dev	F Value	P value	R^2
Physiography	2	21.26051	272	67.43817	65.48979	**<0.001**	0.2397
Region	2	0.34043	270	67.09774	1.04864	0.3526	-
Land use	4	1.92509	266	65.17266	2.96497	**0.0211**	0.0217
Locality	80	26.99966	186	38.173	2.07921	**<0.001**	0.3044
Altitude	1	0.68845	185	37.48455	4.24135	**0.0409**	0.0078
Canopy cover	1	0.01043	184	37.47412	0.06425	0.8002	-
Litter cover	1	0.47766	183	36.99646	2.94272	0.0880	-
Soil moisture	1	0.01661	182	36.97985	0.10232	0.7494	-
Physiography	1	0.01939	181	36.96045	0.11948	0.7300	-
Nitrogen content	1	0.08466	180	36.87579	0.52159	0.4711	-
Phosphorus content	1	0.05183	179	36.82396	0.31932	0.5727	-
Potassium content	1	0.0941	178	36.72986	0.57973	0.4474	-
Organic matter	1	0.00792	177	36.72194	0.04879	0.8254	-
Air temperature	1	0.77839	176	35.94355	4.7954	**0.0299**	0.0088
Air humidity	1	0.00878	175	35.93478	0.05406	0.8164	-

Significant p-values ($p \leq 0.05$) are bold

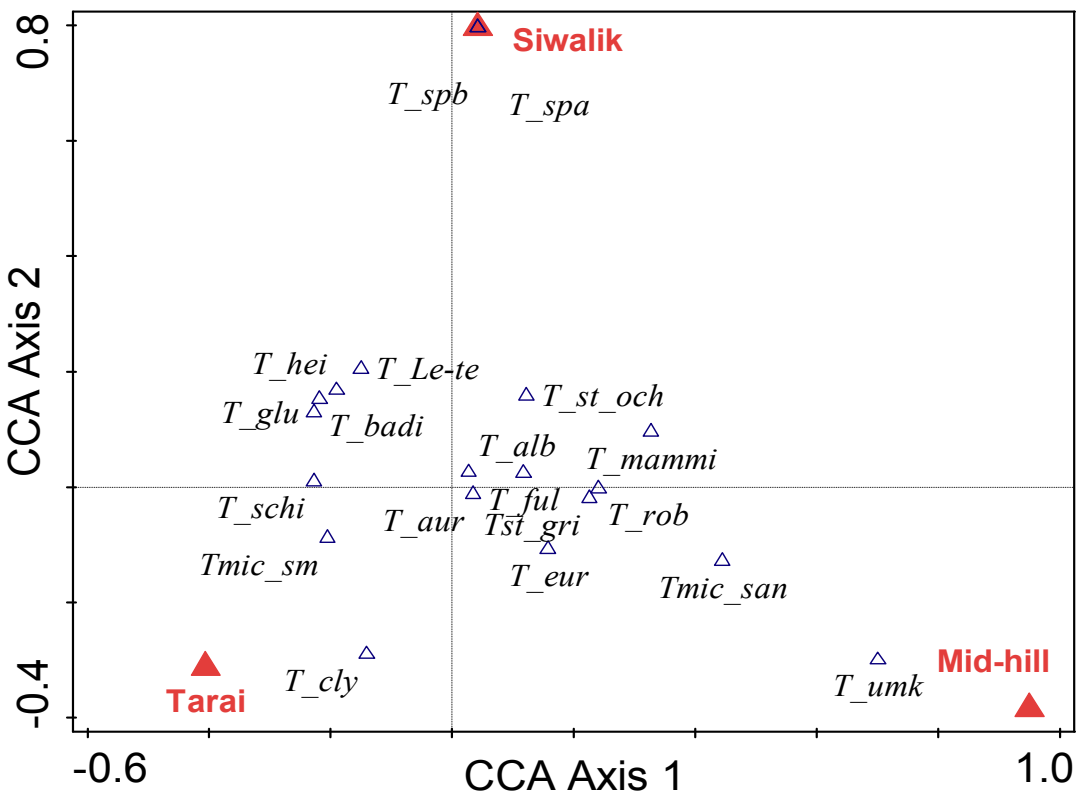

Fig. 3: Effect of physiography on *Termitomyces* species composition (The 1st canonical axis explained 2.39 % and the 2nd explained 0.56 % of the total variation of the data set)

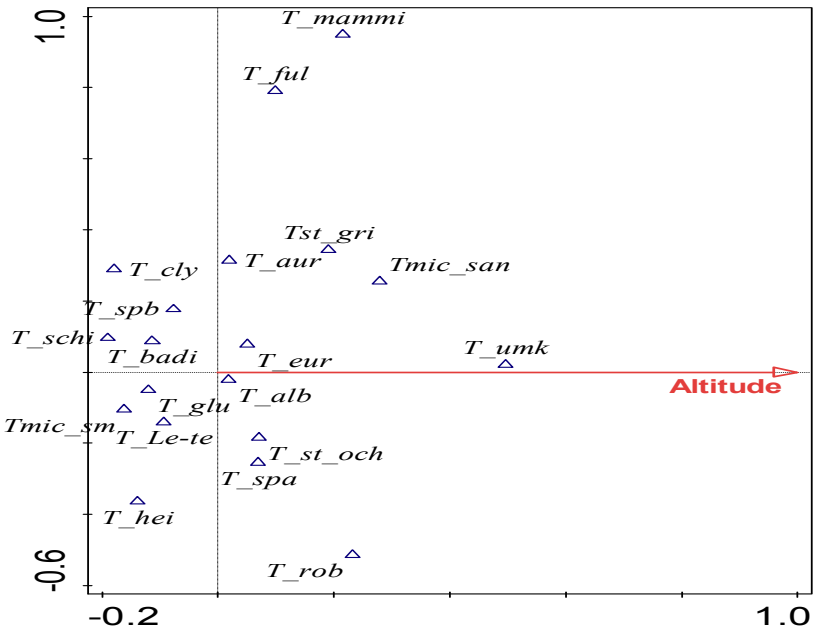

Fig. 4: Effect of elevation on *Termitomyces* species composition (The 1st canonical axis explained 2.06 % and the 2nd explained 0.56 % of the total variation of the data set)

Results of the CCA analysis showed that the relationship between different environmental variable and different species of *Termitomyces* composition, looking at the total relationship between the number of colon of *Termitomyces*, it was found that colon of *T. striatus f. griseus, T. striatus f. ochraceus* and *T. albuminosus* were influenced by soil nutrients such as N, P, K and OM; *T. fuliginosus* and *T. clypeatus* by air temperature, *T. schimperi* and *T. globulus* by pH, *T. heimii* by litter content and *T. microcarpus, T. robustus* and *T. aurantiacus* by soil moisture (Fig. 5).

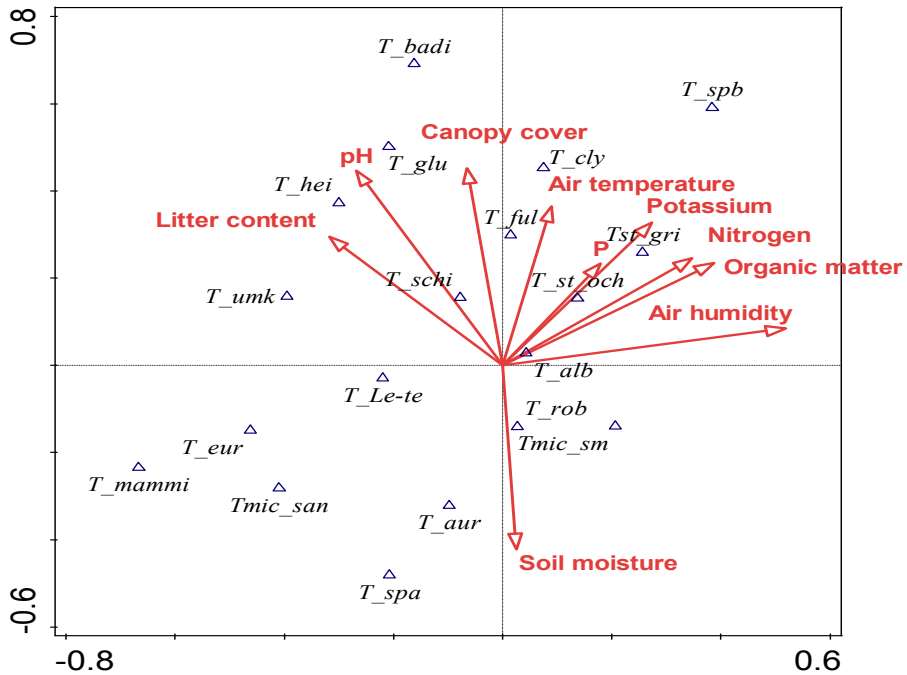

Fig. 5: Relationship between environmental variable and species composition (The 1st canonical axis explained 0.88 % and 2nd canonical axis explained 0.64 % of the total variation of the dataset)

The present organic matter among the 27 locations of sampling habitat features were indicated highest as 40.3 % in natural protected forest with the C: N ratio of 20, (Appendix I) comparative to the least contained of percent organic matter as 0.8% in agro-pastoral land (AGP) with the C:N ratio also as 20. However, the chemical properties of soil based on the level of microelement content (ppm), such as Phosphorus (P), Potassium (K), Iron (Fe), Zinc (Zn), Copper (Cu) Manganese (Mn) and Boron (B) were apparently higher in the most of natural protected forest with comparisons to the AGP land ecosystem and Community Managed Forest (CMF). These differences in obtained results (Table 2) were probably caused by the different in the topography of an areas, soil parent materials, erosion deposited and agricultural practice of the farmers in an area.

The increased biomass yields observed specially in the protected tropical riverine forest areas can be attributed to the favorable environment for termite growth and the longer growing season, especially when compared to higher elevation regions. In Mid-hill, more humid climate and higher rates of soil erosion which may cause a greater loss of organic matter, leads lower the biomass yield (Table 3). The physical properties of soil among the 27 comparative sampling sites were recorded. In a sampling sites, there were no significant difference of p-value of the pH with the soil on the ecozone/ecological region (R) and soil source (Ss) including (R×Ss). So the coefficient of variation is very less (13.51%).

The present organic matter among the 27 sampling habitat features were indicated highest in Tarai

Table 2: Interaction between different eco-zone (R) and soil source (Ss), against different nutrients

Treatment	%		pH	Kg/ha		Ppm				
Regions R	OM	N		P_2O_5	K_2O	Fe	Cu	Mn	Zn	B
Tarai	5.408	0.2733b	6.007	134.608	643.35	31.20b	3.922	162.2a	13.76a	3.939
Siwalik	2.158	0.1025b	6.193	72.7	457.475	33.76b	4.264	3.992c	14.57a	4.078
Mid-hill	2.475	0.6117a	6.164	101.758	651.867	40.14a	4.73	32.64b	8.767b	4.194
P-value	Ns	0.0056	Ns	Ns	Ns	0.001	Ns	0.001	0.001	Ns
LSD 0.05		0.2957				3.792		11.71	2.648	
SEM±		0.1008				1.293		3.992	0.903	
Soil source (Ss)										
Termite nest	7.9	0.6244^{a}	6.011	351.3^{a}	351.3^{a}	63.44^{a}	6.156^{a}	450.8^{a}	11.89^{c}	13.19^{a}
Casing soil	1.389	0.05000^{b}	6.153	5.678^{c}	5.678^{c}	12.56^{d}	3.502^{c}	32.64^{c}	3.578^{d}	0.8878^{c}
Vicinity area	2	0.3156^{ab}	5.783	5.478^{c}	5.478^{c}	46.60^{b}	4.720^{b}	65.82^{b}	15.00^{b}	1.727^{b}
Forest	2.1	0.3267^{ab}	6.538	49.64^{b}	49.64^{b}	17.53^{c}	2.844^{c}	36.11^{c}	19.00^{a}	0.4784^{c}
P-value	Ns	0.0192	Ns	0.001	0.001	0.001	0.001	0.001	0.001	0.001
LSD 0.05		0.3415		0.3415	0.3415	4.379	1.172	13.52	3.058	0.4995
SEM±		0.1164		0.1164	0.1164	1.493	0.3994	4.61	1.043	0.1703
(R)× (Ss)	Ns	Ns	Ns	Ns	0.0349	Ns	Ns	0.001	0.001	Ns
CV%	183.37	105.97	13.51	124.21	49.29	12.78	27.84	9.45	25.3	12.54

Note: Experimentation: Design: 2 factorial RCBD (Randomized Complete Block Deign). Note: (R) = Region, (Ss) = Soil source, CV= Coefficient of variation, (R×Ss) = interaction between region and soil sources.

Table 3: Biomass yield of *Termitomyces* in different ecological region

Regions	Av. No. of fruit	Av. Wt./fruit (gm)	Av. Biomass yield (gm)
Tarai	28	16.66	590.55
Siwalik	22	12.98	337.92
Mid-hill	16.66	10.82	258.26

(5.40%) in termite nest (7.90%) with the C: N ratio of 20, comparative to the low contained of organic matter as 2.47% in Midhill regions of casing soil (1.38%) with the C:N ratio 20. There were no significant difference of p-value and interaction between (R) and (Ss) of the OM, but coefficient of variation is high (183.37%). Nitrogen contents of mound in three regions significantly different at 0.05% level of significance. It is maximum in Mid-hill (0.61%) and the termite nest (0.62%), whereas minimum in Siwalik and Tarai (0.1% & 0.27%) in the casing soil respectively. There were significant differences in both of eco-region (R) (0.0056%) and soil source (Ss) (0.0192%). Interactions between the (R) and (Ss) were not significantly difference at 0.05% level of significance. Its variation of coefficient is high (105.97%).

However, the chemical properties of soil that indicate the level of microelement content (ppm), such as Phosphorus (P), Potassium (K), Iron (Fe), Copper (Cu), Manganese (Mn), Zinc (Zn) and Boron (B), were apparently higher in most of the places (Table 2). For instance, content of phosphorus in Tarai (134.6) is highest in termite nest (351.3), content of potash in Midhills (651.86) is highest in termite nest (351.3), interaction between (R) and (Ss) of the potash is significantly different at 0.05% level of significance. Similarly, P and K on the (Ss) are also significantly different at 0.05% level of significance. Likewise, contents of Fe in Midhill (R) significantly different at 0.05% level of significance. It is maximum in Midhill (40.14ppm) and minimum in Tarai (31.20ppm). Similarly, Fe content in (Ss) is significantly different at 0.05% level of significance. It is maximum in termite nest (63.44 ppm) and minimum in casing soil (12.56 ppm). Cu content is maximum in Midhill (4.730 ppm) but not significantly different at 0.05% level of significance and minimum in Tarai (3.922 ppm), whereas Cu content in (Ss) is significantly different at 0.05% level of significance. It is maximum in termite nest (6.15 ppm) and minimum in forest and casing soil (2.84 & 3.50 ppm) respectively.

Similarly, contents of Mn in (R) significantly different at 0.05% level of significance. It is maximum in Tarai (162.2 ppm) and minimum in Siwalik (3.99 ppm), similarly Mn content in (Ss) is significantly different at 0.05% level of significance. It is maximum in termite nest (450.8 ppm) and minimum in casing soil (32.64 ppm), interaction between (R) and (Ss) of the Mn is significantly different at 0.05% level of significance. Contents of Zn in (R) significantly different at 0.05% level of significance. It is maximum in siwalik and Tarai (14.57 & 13.76 ppm) and minimum in Midhills (8.76 ppm), similarly Zn content in (Ss) is significantly different at 0.05% level of significance. It is maximum in forest soil (19.0 ppm) and minimum in casing soil (3.57 ppm), interaction between (R)and (Ss) of the Zn is significantly different at 0.05% level of significance. B content in (R) is not significantly different at 0.05% level of significance. It is maximum in Midhill (4.19 ppm) and minimum in Tarai (3.93 ppm), but B content in (Ss) is significantly different at 0.05% level of significance. It is maximum in termite nest (13.19 ppm) and minimum in forest and casing soil (0.47 ppm) and (0.88 ppm) interaction between (R) and (Ss) of the B is significantly different at 0.05% level of significance. So, the biomass yield is highest in Tarai, followed by the Siwalik and Midhills. Hence, the soil properties as an edaphic factor influence distribution and abundance of *Termitomyces*.

The present study based on a survey and the literature revealed that the occurrence of 19 taxa of *Termitomyces*, including abundance in responses to land use and other, environmental variable (Table 1). Most of the *Termitomyces* species were found in the forest where the vegetation is dominated by species of the Dipterocarpaceae Combretaceae and Leguminosae families in Tarai and Siwalik. Among them, *T. albuminosus, T. mammiformis, T. microcarpus* and *T. robustus* were frequents; *T. clypeatus, T. eurrhizus, T. heimii, T. le-testui* and *T. schimperi* was common; *T, stratus* (var. griseus & ochraceus), *T. globulus, T. umkowaan, T. arghakhachensis* and *T. palpensis*) were found to be rare.

4. Discussion

4.1 Present diversity status and their propagation

In Global Biodiversity Information Facility database [34], fungi are comparatively less represented group as compared to plants (282 million) and animals (more than one billion). The number of fungal species has been estimated up to 19 million (19,056,194) [34], however, to date, only 150,000 fungal species have been fully explored [35]. Only a small part of total fungal wealth has been subjected to scientific research and mycologist continue to unravel the unexplored and hidden wealth [36]. It is estimated that with the current rate of species description it will take 1170 years to complete the global fungi inventory. So, globally, in recent time, the population of fungi, being a challenge to mycologist all over the world [37]. The total number of mushrooms forming species has been estimated in between 53,000 to 110, 000 which suggest that only 18 to 38% of the total mushrooms have been documented [38]. Upto the present time, approximately 14,000 species have been officially described [39] and 57 species of *Termitomyces* have been reported [24].

There are 2,600 species of termites [40], among them 330 species are responsible for these fungi cultivation [41]. They maintain their fungal symbionts (genus *Termitomyces*, Basidiomycotina) on special structures in gardens inside their colonies, the nest, and the fungus combs, which are housed in specially constructed chambers, either inside a mound or dispersed in soil. Those fungus gardens are continuously provided with plant substrates, whereas older parts that have been well decomposed by fungi are consumed by termites [42, 43]. Termite consumed dry plant materials converted to fecal pellets (primary feces) when added resulted rapidly development of fungal mycelium in the newly added substrate. After a few weeks the fungi start to produce vegetative structure, *nodules* (mushroom) that are consumed by workers. At a later stage, the entire combs structure permeated with mycelium is consumed [44]. The symbiosis between termites and *Termitomyces* fungi is 'symmetric' since both partners are obligatorily interdependent, and this dependence has a single evolutionary origin with no known reversals to non-symbiotic states [41, 45, 22].

Fungus combs require a temperature of 27º-28º C (*Macrotermes jeanneli*); [46] or 30ºC (*Macrotermes bellicosus;* [47]) but the relative humidity is independent. Outside the nests, daily air temperature was 20º-37ºC and surface soil temperature was 21º-45ºC (field observation 2016). Conditions within the mound are therefore more similar to rain-forest climates. Although climate conditions are also buffered to some degree in non-fungus-growing Termitidae, such complex, highly climatically buffered mounds are unique to fungus-growing termites.

4.2 Edaphic factor determines the abundance of fungi

Edaphic factors emerged as the primary driver of termite distribution, while local plant diversity played a lesser role in determining distribution. During investigation the higher the organic matter (OM) and micronutrients present greater the abundance of termitomycetes. The enhancement of soil's physical structure is attributed to the accumulation of mycelium within it. This mycelium binds fine soil particles together, resulting in the formation of stable aggregates that are resistant to water erosion simultaneously, the mycelium penetrates the soil, creating a network that effectively captures and retains small particles [48]. Thus, soil which holds different mycelia should be preserved to maintain the growth of fungi. Conservation of biological diversity has been the subject of intense debate all over the world.

4.3 Symbiotic association between *Termitomyces* and termites

The relationship of termites and fungi has been the subject of many investigations. Fungi in decaying wood provide nitrogen, vitamins, and other substances beneficial to termites, and may also destroy toxic volatile materials or extractives in the

wood [49]. Fungi are also known to produce feeding stimulus. On the other hand, they can remove certain nutrients or produce toxic metabolites. The species of termite and fungus and other variable determine whether fungi are beneficial or not [50]. The higher termites (termitidae) in the fungus combs breakdown the lignin, and it also supply nitrogenous materials and vitamins [49]. In this way, termites provide a constant environment for fungal growth as well as help in the dispersal of spores. In turn, *Termitomyces* provide food for the termites [51].

There are few reports on the ecological aspects of the symbiotic relationship in *Termitomyces* and fungus growing termites. Termite eats dead and sometimes live plant tissues and piles their excrement in a porous structure [52, 53]. Then they make a fungus garden by cultivating *Termitomyces* hyphae on that structure. Finally, they eat the mature hyphae or fungus combs. Termites cannot live without a fungus garden, and *Termitomyces* has been observed only in termite nest [52, 44]. Fruiting bodies of *Termitomyces* have been reported to develop in fungus gardens after termites desert the nest [54]. However, it is unknown whether some aspect of termite behavior prevents the formation of fruiting bodies of *Termitomyces* [55].

4.4 Occurrence of *Termitomyces*

There is association between termites and *Termitomyces* either in permanent termite mounds or in temporary termite colonies. In their combs, a specific microenvironment is created by the termites for cultivation of termitophilic fungi [56]. These are gregarious in and around permanent termite mounds. They are also found in solitary or scattered on ruminant-grazed land, AGP and abandoned bunds of paddy fields. Some species (*T. microcarpus* & *T microcarpus f. santalensis*) prominently erupt wherever ruminant dung lies on the ground or at locations strongly influenced by ruminant activity. Termite (e.g. *Macrotermes natalensis*) colonies take advantage of digested or partially digested lignocellulosic material in ruminant dung for cultivation of termitomycetes. In this investigation *T. clypeatus* mainly preferred grassland in the protected forest (National Park), while it occurs in permanent termite mounds. Permanent termite mounds were preferred by *T. heimii* in protected (CMF) as well as natural forest (National park). Similarly, *T. umkowaan* also preferred the surroundings of permanent termite mounds in the natural forest. The other termitomycetes (*T. eurrhizus, T. microcarpus*) preferred mainly open places in forests and buffer zones (AGP, adjacent to forests) without prominent termite mounds. Physical disruption of lignocellulose by termite mastication supports further degradation by fungal enzymes [57]. Such symbiosis enhances the degradation and turnover of lignocellulosic substrate in grasslands, on agricultural land and in forests. Based on the role of enzymes in tripartite association (bacteria- fungi-termites) [58] considered the termite mound as an external rumen. Besides, some termite workers (e.g. *Odontotermes formosanus*) show higher cellulase activity in their faeces than the symbiotic *Termitomyces* denoting the role of gut-derived and acquired enzymes by the bacteria and fungi [59, 60].

Based on molecular research [61] termite guts and termite combs also revealed that emergence of a particular *Termitomyces* species is due to monoculture by a specific termite population. In view of such specificity, it is necessary to follow the species of termites which exist in forest and buffer zones of National park and the CMF area of the study sites to exploit their beneficial association. Hence field observation implies that, mutualistic association of termite's comb and *Termitomyces* co-exist in a physiologically, ecologically and reproductively active state for long period of time in terrestrial ecosystem.

4.5 Species richness influence by environmental variable

Distribution and fruiting of the basidiome of *Termitomyces* in Tarai to Mid-hill land was determined by the effect of physiography, land use, locality, altitude, soil moisture, organic matter

and soil litter surface thickness. Mostly the soil properties were varied tremendously in an area which greatly affected the land use potentials, vegetation pattern and hydrology. Buol [62] reported that soil parent materials and their weathering products had strongly influenced the soil properties, including the depth of the regolith, texture, stoniness, clay type, nutrient contents and soil pH, they also help for Macrotermetine distribution. Similarly, Schuurman [63] described that the influence of edaphic factors and plant diversity on detrivore community composition were showed that the dominant mound building species, *Macrotermes michaelseni*, consumes both grass and wood litter. As per the physiography, higher biomass was seen in tropical climatic region, this probably implied to the cases of the optimum organic matter and major chemical microelements in protected forest of Tarai and siwalik region which were allocated as the sites of this investigation.

5. Conclusion

Edaphic factors on the termite's distribution and abundance, showed that the soil texture, organic matter content of the soil surface (0-15 cm) and chemical micro- or macro-nutrient composition have played a major role in influencing termites nesting, fungus comb deposition under soil surface and termite's distribution. All of the sampling habitats in both arable and natural ecosystem were given as clump in termite dispersion pattern. The clay-type of soil was observed as the preferable factor shown for predomination termites nesting and termite's fungus combs distribution in protected forest areas. It is difficult to conserve these mushrooms because of limited knowledge on their taxonomy, natural history, and ecology. While information at the individual species level might be limited, the current study demonstrates that a comprehensive understanding exists in a broader context.

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APPENDIX 1: Soil report in different eco-zones

SN	Place	Region	Alt (m)	FT	Soil source	N (%)	P	K	OM (%)	C: N	pH	Fe (ppm)	Zn (ppm)	Cu (ppm)	Mn (ppm)	B (ppm)	Av. No. of fruit	Av. wt. (g)/ Fruit	Av. Biomass yield g
1	Attariya (Kailali)	WT	190	TESF	Termites nest	0.13	356.6	2208.4	2.7	20.8	6.1	60	8	4.73	471.16	12.85	30	14.00	415.00
					Casing soil	0.08	8.9	166.9	1.7	21.25	6.33	9.4	3.6	3.24	17.46	0.64			
					Vicinity area	0.11	1.2	333.1	2.2	20	5	42.3	18.8	3.8	36.2	1.77			
					Forest soil	0.1	93.1	143.2	2.6	26	5.3	17.66	26.724	2.6	31.29	1.051			
2	Chiliya (Rupandehi)	CT	110	CMF	Termites nest	0.25	307.6	2161	5	20	6.3	58	9	5.73	491.16	13.2	28	17.33	666.66
					Casing soil	0.04	8.7	380.6	0.8	20	5.44	13.2	2.8	3.1	37.78	0.62			
					Vicinity area	0.3	1.4	370	2	6.7	5.5	41.4	20	5	38.4	1.25			
					Forest soil	0.05	22	18	2.1	42	7.6	11.37	22.9	2.5	38.12	0.0208			
3	KTWLRC	ET	215	TDsAcF	Termites nest	2.02	796.7	1656.4	40.3	20	5	57	7.5	4.21	432.04	13.85	26	17.66	690.00
					Casing soil	0.07	8.5	170.9	1.5	21,5	5.72	11.8	2.9	4.56	6.56	0.6			
					Vicinity area	0.07	1.7	92.7	2.5	36	6.2	40.9	17.1	4.9	56.2	1.4			
					Forest soil	0.06	8.9	19	1.5	25	7.6	11.36	25.8	2.7	39.8	0.0205			
4	Amaliya (Dang)	WS	510	STDHF	Termites nest	0.22	77.8	594.2	4.4	20	6.75	62	8.5	4.83	466.16	13.7	17	12.05	316.66
					Casing soil	0.05	8.3	380.6	0.9	18	5.5	9	3.6	3	18.4	0.57			
					Vicinity area	0.15	2.2	94.7	1.5	10	6.1	43.3	19.8	3.5	38.2	1.7			
					Forest soil	0.07	23.2	17	1.7	24.28	7.2	13.38	21.9	2.8	38.14	0.02			
5	Thada (Palpa)	CS	1261	STDHF	Termites nest	0.21	361.2	1520	4.2	20	6.4	64	9.5	4.66	445.16	13.4	24	13.06	320.00
					Casing soil	0.06	8	190.7	1.5	25	5.23	16.8	3.4	3.3	31.38	0.86			
					Vicinity area	0.12	1.8	97.7	1.7	15	6.25	45.4	22	6	40.4	1.5			
					Forest soil	0.08	24.2	119.5	1.8	22.5	6.46	18.33	25.632	2.9	40.28	0.8793			
6	Matigadha (Saptari)	ES	507	TDRF	Termites nest	0.15	300.6	902.8	2.5	17	6.34	66	16	8.5	425	13.3	25	13.83	377.10
					Casing soil	0.02	1.4	807.9	1.9	95	6.8	8.3	4.5	3.4	19.2	1.26			
					Vicinity area	0.09	8.9	736.6	1.8	20	4.8	43.9	19.1	5.88	34.2	1.55			

SN	Place	Region	Alt (m)	FT	Soil source	N (%)	P	K	OM (%)	C: N	pH	Fe (ppm)	Zn (ppm)	Cu (ppm)	Mn (ppm)	B (ppm)	Av. No. of fruit	Av. wt. (g)/ Fruit	Av. Biomass yield g
					Forest soil	0.01	54.8	28	2	200	6.48	14.7	20.908	2.4	29.14	0.1925			
7	Puythan-Khalanga	WM	1379	Q-in-Q-Lan-F	Termites nest	0.27	139.1	1662.4	4.7	17.40	4.4	56	6.5	4.12	421.08	11.8	15	9.38	198.60
					Casing soil	0.09	1.2	500	1.6	18	7.14	17.66	3	3.42	65.34	0.55			
					Vicinity area	0.25	24.2	698.2	2	8	6.4	54.4	6.1	4.12	117.7	1.66			
					Forest soil	0.75	93.1	143.2	2.4	3.2	5.3	25.2	6.5	3.6	35.2	1.051			
8	Lele (Lalitpur)	CM	1400	STDHF	Termites nest	1.19	116.1	2137.2	3.7	3.10	5.88	81	24	9.87	460	13.5	15	10.00	265.66
					Casing soil	0.01	2.9	546.7	1.4	140	6.22	15.5	3.3	4	67.34	1.51			
					Vicinity area	0.8	4.2	300	2.4	3	5.8	52.2	5.2	4.36	109.4	3.06			
					Forest soil	0.9	95.1	133.2	2.6	2.88	5.3	29	12.7	1.6	40.2	1.0501			
9	Peepalbot (Ilam)	EM	1290	SCF	Termites nest	1.18	705.9	1069	3.6	3.05	6.93	67	18	8.75	445	13.1	20	13.08	310.53
					Casing soil	0.03	3.2	523	1.2	40	7	11.37	5.1	3.5	30.3	1.38			
					Vicinity area	0.95	3.7	95.7	1.9	2	6	55.6	6.91	4.92	121.67	1.65			
					Forest soil	0.92	32.4	13.8	2.2	2.39	7.6	16.8	7.9	4.5	32.8	0.0203			

Notes:

Koshi Tappu Wild Life Reserves Centre, WT= West Tarai, CT= Central Tarai, ET= East Tarai, WS=West Siwalik, CS=Central Siwalik, ES=East Siwalik, WM=West Midhill, CM=Central Midhill, EM=East Midhill, FT= Forest Type, TESF= Tropical ever green Sal forest, CMF= Community Manage Forest, TDsAcF= Tropical *Dalbergia sissoo-Acacea catechu* forest, STDHF= Sub tropical deciduous hill forest, TDRF= Tropical deciduous riverine forest, Q-in-Q-Lan-F=*Quercus semecarpifolia* in -Quercus- Lauraceae- forest, SCF= *Schima-Castanopsis* forest