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Ecological gradient analysis and community structure of macrofungi in different climatic zones of Western Himalayas, Pakistan

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Key words: Community ecology, Cluster analysis, Indicator species

Abstract

This paper communicates the analytical exploration of macrofungi of the western Himalayan region of Pakistan. The study was aimed to explore species diversity and community structure of macrofungi based on edaphic, topographic and climatic factors. Monte Carlo test were used for indicator species (P value ≤ 0.05). Sixty species of macrofongi were recorded from 65 stands during 2016-2018. The diversity index and species richness were highest in the humid temperate zone. Multivariate analysis ranked the diversity of macrofungi from 65 stands to 5 myco-communities based on indicator species. Cluster analysis showed that among the environmental variables, the significant result was the contribution of temperature and pH (P= 0.0002), followed by moisture (P= 0.0004), organic matter (P= 0.0006), phosphorus (P= 0.04) and saturation (P= 0.12). *Amanita oblongospora, Amanita pantherina, Bovista plumbea, Lycoperdon alpinum, Lycoperdon excipuliforma* and *Russula ilicis* have shown significant results with more than two environmental variables. The diversity index of myco-communities significantly responds to climatic, edaphic, topographical and anthropogenic stimuli.

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Introduction

The Fungi kingdom constitute a large and diverse group of organisms that have more than 5.1 million species (Kirk *et al.*, 2008; Blackwell 2011). It can be divided into four phyla i.e. Chytridiomycota, Zygomycota, Ascomycota and Basidiomycota (Webster and Weber 2007). Macrofungi consist of mushrooms, puffballs, bracket fungi, false-truffles, and cup fungi, differentiated by spore-bearing structures visible to the naked eye (Razaq *et al.*, 2014). Most macrofungi are Ascomycota or Basidiomycota, but some are Zygomycota. Most terrestrial macrofungi are saprobes or mycorrhizal symbionts, but some are plant pathogens and sporocarp abundance is dependent on weather conditions (Muller *et al.*, 2001).

Fungi on a global scale are an important source of food (Sultana *et al.*, 2007). They yield vital chemicals such as hormones, pheromones, toxins, carcinogenic enzymes, antibiotics, anticarcinogens and pigments (Redhead 1997). Dried mushrooms contain about 36.7% protein. In addition to their good protein value, they are also a good source of fat, phosphorus, sodium, potassium, iron, thiamin B1, riboflavin B12, niacin and biotin. The species differ in the amount of specific proteins (Sultana *et al.*, 2007). Macroscopic fungi play an important role in the nutrient cycling and pH balance of harmful natural resources through mycorrhizal association (Razaq *et al.*, 2014; Gates *et al.*, 2011).

The ecological research provides the basic knowledge that explains the variation in species data under different abiotic factors and to answer applied questions of importance to the management of the natural world (Tavili and Jafari 2009; Haq *et al.*, 2015a; Haq *et al.*, 2015b). Multivariate analysis helps in categorizing the effects of environmental variables across the entire species group (Haq *et al.*, 2017). Multidimensional field data can be summarized into a small number of dimensions through classification and ordination techniques (Haq *et al.*, 2015a).

Fungi were considered a strange group of organisms, poorly understood and difficult to study because of their hidden nature, sporadic and short-lived spore carps. Mushrooms have therefore been ignored in national conservation actions. However, thanks to the research of professional mycologists and field observers in recent decades, our knowledge of mushrooms has increased considerably. It is now possible to assess the current rank and future of fungal species and how human activities, such as land management procedures, will affect fungal diversity. No such studies have been conducted on fungal biodiversity in Pakistan.

The study aimed to develop an experimental model of fungal flora using species composition and community type in the region. The Himalayan regions have not yet been studied for such study. The study was therefore proposed to explore the species diversity and community structure of macrofongs based on edaphic, topographic and climatic factors. The study contributes to expanding efforts to systematically describe mountainous fungal communities using a phytosociological approach supported by robust statistical analysis that will form the basis of strategic conservation planning.

Materials and methods

Study area

The Nandiar Valley is located between 34° 33'and 34° 47'N, and 72° 55' and 73° 14 'E in Pakistan's Khyber Pakhtunkhwa Province (Haq 2015; Haq *et al.*, 2017). The area is mountainous, with elevations ranging from 525 m at subtropical zone to 3817 m at alpine zone above mean sea level (Haq *et al.*, 2011; Haq 2018). Haq *et al.* (2010) classified the Nandiar Valley vegetation into six zones under moist temperate category of the Western Himalayan moist temperate ecology (Champion *et al.*, 1965). The valley is underlined by metamorphic and plutonic igneous rocks, pegmatite, aplite and quartz veins. The soil under fir and spruce is deep and fairly rich in humus, whereas it is shallow and poor under pines and scrub areas, including wasteland (Haq 2018). The present study recorded the diversity and taxonomic richness of macrofungi in different stands. Classification and ordination were also used to establish relationships between species and to account for climatic, edaphic and topographic variables.

Samples collection and identification

Macrofungi samples were collected from 65 stands in the Nandiar Valley for analytical and synthetic features in different seasons of 2016 to 2018. Phytosociological approaches have been used to test the effect of climatic, edaphic and topographic variables on macrofungi population (Laurance *et al*., 2004, Haq *et al*., 2017). Species of macrofungi were identified based on the macroscopic and microscopic morphologies using the illustrated literature (Demoulin and Mirriott 1981; Buczacki 1989; Shibata 1992; Murakami 1993; Swann and Taylor 1993; Ahmed *et al*., 1997; Leelavathy and Ganesh 2000; Fiaz 2013).

Data collection

The linear transect method was used for quantitative sampling and points were collected at 20 m intervals along 200m long transects (Buckland *et al*., 2007; Brown *et al*., 2011). Altitude, latitude and longitude were determined by GPS. The clinometer was used for the aspect of the slope and the angle (Haq *et al*., 2015a). The weather station was used to record temperature, wind speed, humidity, dew point and barometric pressure (Haq *et al*., 2015b). One kilogram of soil was taken from each stand and stored in polyethylene bags for physico-chemical characteristics. Soil texture was determined by the hydrometer method and classified according to the textural triangle (Ghani and Amer 2003). Electrical conductivity was measured using an electrical conductivity meter and soil pH by pH meter. The organic matter concentration was calculated by the Walkley and Black titration method. The concentration of phosphorus and potassium was determined by atomic absorption spectrophotometer (Fonge *et al*., 2011).

Multivariate analysis

Data from sixty-five stands have been transferred to an MS Excel spreadsheet. Species presence and absence data as well as climatic, edaphic and topographic variables were used for multivariate analysis. TWINSPAN has classified species and samples by reciprocal mean defined by pseudo-species levels (Hill 1979). Significant habitat and community types were identified through cluster analysis and indicator species analysis. Indicator species values for species in each group were obtained and tested for statistical significance using the Monte Carlo test. A threshold of 30% with a significance of 95% (p value <0.05) was chosen as a threshold for indicator species. The indicative value of plant species was calculated using the Phi coefficient method of Tichý and Chytrý (2006). DCA and CCA ordination were used to determine the relationship among vegetation, stands and environmental variables (Hill 1979; ter Braak, 1986, 1994).

Results

Species Composition

In the study sum of 60 species of macro fungi which belongs to 37 genera and 23 families were reported. The dominant family was Agaricaceae contributing 9 genera and 16 (26.6%) species. It was followed by Russulaceae with 10 (16.7%) species, Amanitaceae by 5 (8.3%) species and by Morchellaceae 4 (6.6) species. The diversity index and taxonomic richness values were maximum in the least distubed forests of the moist temperate zone of the area and were manimum in the alpine and subtropical zones. The variation in diversity index and taxanomic richness was due to slope aspect, vegetation structure, anthropogenic disturbance and grazing factors.

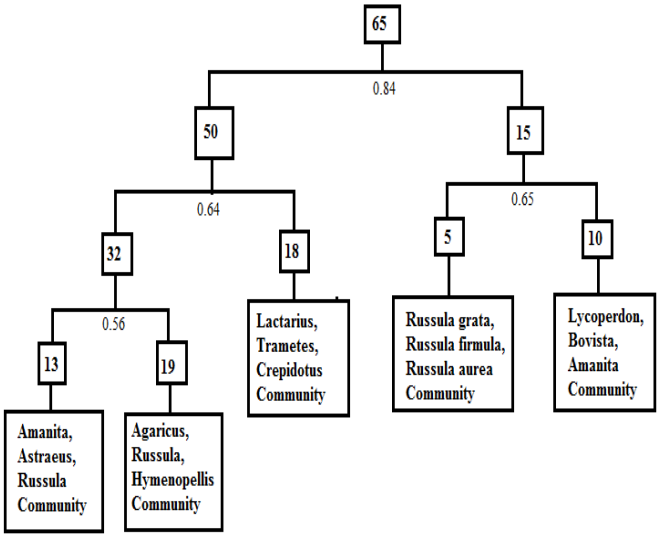

Fig. 1. Summary of TWINSPAN classification of macrofungi of Nandiar valley.

Classification

On the basis of indicator species TWINSPAN classified the data of 65 stands into 5 macrofungi communities. The primary indicator species were *Lycoperdon excipuliforma* with eigenvalue 0.84. In division first the data of 50 stands were in group *0 and 15 were in group *1. The indicator species in division 2 was *Agaricus campestris, Pisolithus tinctorius, Lactarius pterosporus* and *Amanita oblongospora* with eigenvalue 0.64. In division 3 the indicator species were *Bovista plumbea*, *Amanita pantherina* and *Russula albanoides* with eigenvalue 0.65. TWINSPAN analysis were also confirmed through indicator species analysis and two ways cluster analysis. The diversity index ranges from 1 to 3.5. The communities are described below (Figs. 1, 2 and 3).

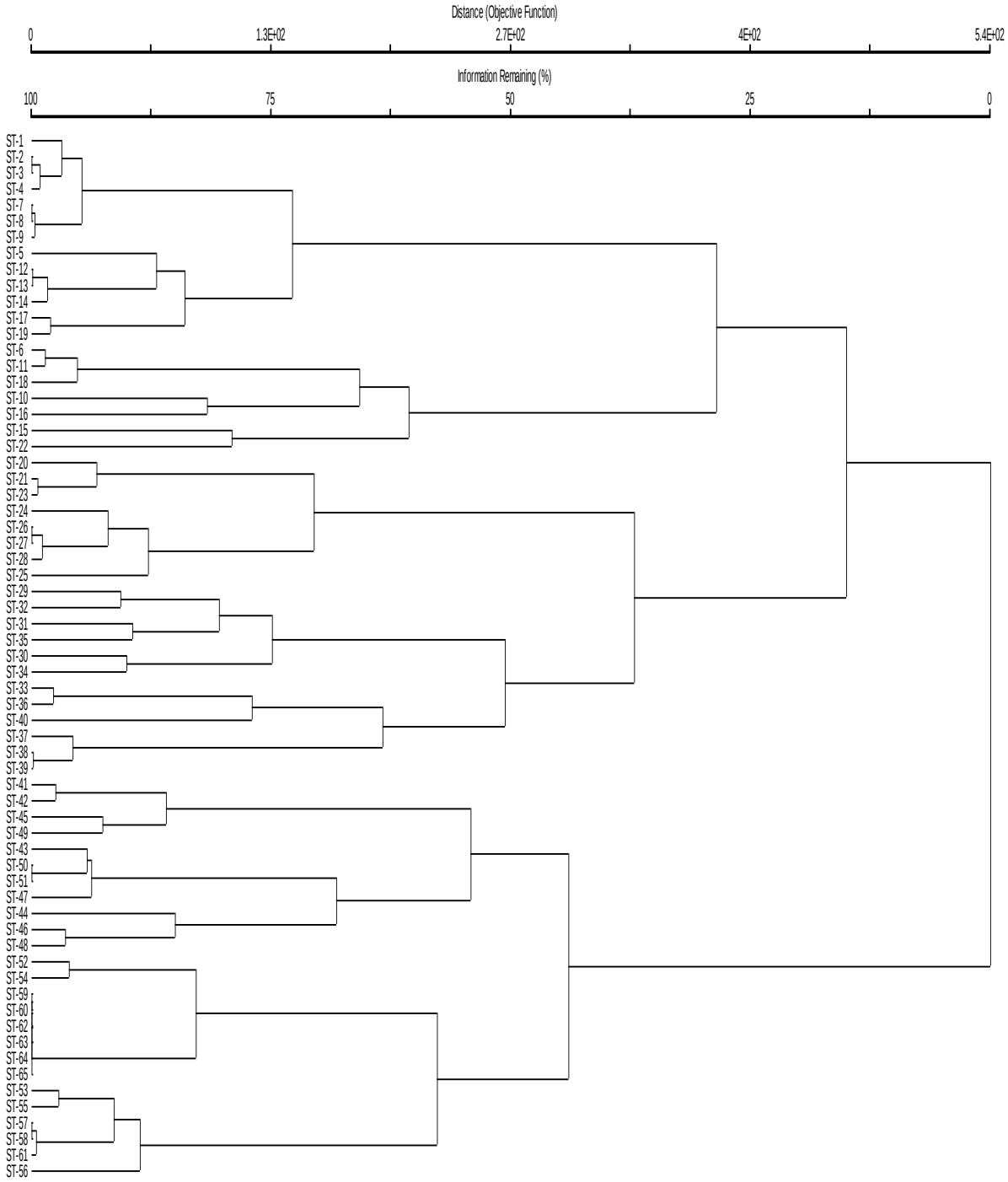

Fig. 2. Summary of cluster analysis of macrofungi of Nandiar valley.

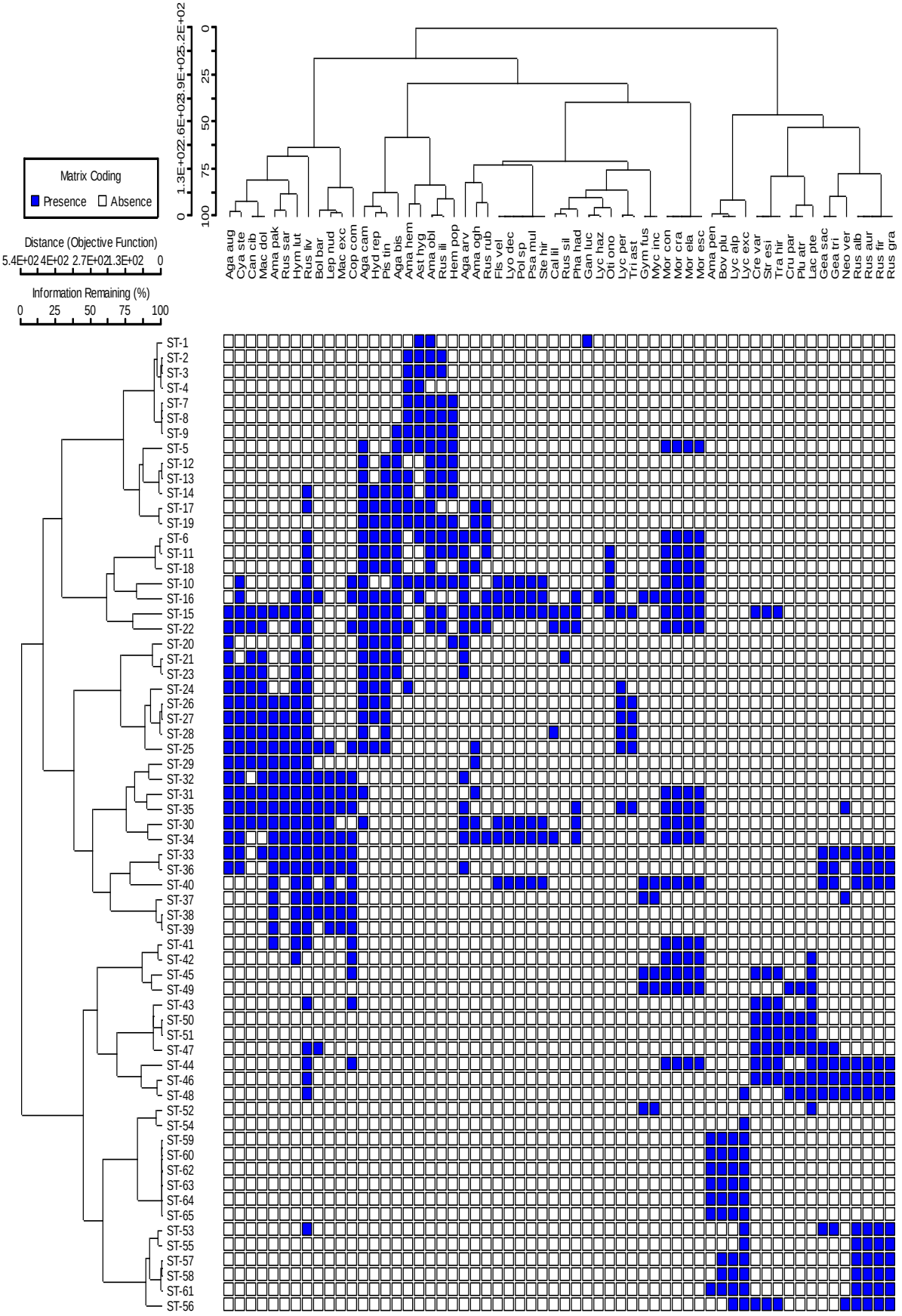

Fig. 3. Two ways cluster dendogram of stands and species of macrofungi.

Amanita, Astraeus, Russula community

Amanita oblongospora, Astraeus hygrometricus, Russula ilicis community was recorded from 13 stands between altitudinal ranges of 539 to 1650m. In this community 17 species were recorded. The associated species included *Amanita hemibapha, Hemipholiota populnea* and *Agaricus bisporus*. The species of this community were sensitive to high temperature.

Agaricus, Russula, Hymenopellis community

Agaricus augustus, Russula sarcodina, Hymenopellis luteus community were recorded between 1400 to 2100m from 19 stands having 46 species. *Pisolithus tinctorius, Cyathus stercoreus* and *Hydnum repandum* were the codominant species. This community occurred at moderate environmental conditions.

Lactarius, Trametes, Crepidotus community

Lactarius pterosporus, Trametes hirsuta, Crepidotus variabilus community was recorded from 18 stands between an elevations of 2000 to 2800m contributing 40 species. *Strobilurus esculantus, Pluteus astromarginatus* and *Crucibulum parvulum* were the codominant species. The species of this community varied with atmospheric humidity and soil electrical conductivity.

Russula grata, Russula firmula, Russula aurea community

This community wase recorded from 5 stands between altitudinal range of 2300 to 2900m contributing 16 species. The codominant species were *Russula albanoides, Russula livescens, Neohygrophorous verrucosporus, Geastrum saccatum* and *Geastrum triplex*. This community was sensitive to soil Phosphorous and saturation.

Lycoperdon, Bovista, Amanita community

Lycoperdon excipuliforma, Bovista plumbea Amanita pantherina community were recorded between an elevations of 2800 to 3780m with 8 species. The other codominant species were *Lycoperdon alpinum* and *Russula albanoides*. This community was found at high altitude having low temperature.

Cluster analysis

The cluster analysis showed that among environmental variables the significant result were contribution by temperature and pH (P=0.0002), followed by humidity (P=0.0004), organic matter (P=0.0006), phosphorous (P=0.04) and soil saturation (P=0.12) (Table 1). *Amanita oblongospora, Amanita pantherina, Bovista plumbea, Lycoperdon alpinum, Lycoperdon excipuliforma* and *Russula ilicis* showed significant result with more than two environmental variables. The other species were significantly correlated with specific environmental variables (Table 2).

Table 1. Summary of cluster analysis of environmental variables.

Variables	Observed Indicator value	Indicator value from randomized groups		P
		Mean	S. Dev	
Temperature	0.41	0.28	0.10	0.0002
pH	0.25	0.13	0.08	0.0002
Humidity	0.41	0.31	0.10	0.0004
Organic Matter	0.18	0.10	0.07	0.0006
Phosphorous	0.45	0.38	0.15	0.04
Saturation	0.58	0.53	0.16	0.12

Ordination

The result of DCA ordination showed that the variance in species data was 4.74. The eigenvalue and gradient length was maximum for axis 1 i.e. 0.85 and 10.05 respectively. It was followed by axis 2 with 5.29 gradient length and 0.36 eigenvalue. DCA ordination explains that the entire data set is dominated by a single dominant gradient with decreasing in its value on other axis. The result showed that maximum species were common in stands and stands having common species. However variance in species diversity was observed in ordination space. Species clusters in ordination space at different locations on the basis of habitat types (Fig. 4).

Table 2. Significant value of species with environmental variables.

Variables	Botanical Name	Observed Indicator value	Indicator value from randomized groups		P
			Mean	S. Dev.	
Temperature	*Agaricus bisporus*	0.45	0.25	0.09	0.003
	Amanita oblongospora	0.40	0.25	0.09	0.006
	Amanita pantherina	1.00	0.31	0.11	0.0002
	Bovista plumbea	0.84	0.29	0.10	0.0002
	Ganoderma lucidum	1.00	0.35	0.12	0.0008
	Hemipholiota populnea	0.62	0.27	0.09	0.0004
	Hymenopellis luteus	0.40	0.24	0.08	0.0004
	Lycoperdon alpinum	0.78	0.28	0.10	0.0002
	Lycoperdon excipuliforma	0.62	0.27	0.09	0.0004
	Russula ilicis	0.47	0.25	0.09	0.0022
	Russula livescens	0.27	0.21	0.03	0.0002
Humidity	*Agaricus augustus*	0.48	0.28	0.08	0.0002
	Amanita oblongospora	0.37	0.28	0.07	0.0028
	Bovista plumbea	0.70	0.31	0.10	0.0002
	Canthrallus cibarius	0.63	0.29	0.10	0.0002
	Cyathus stercoreus	0.48	0.28	0.08	0.0002
	Hymenopellis luteus	0.36	0.27	0.05	0.0002
	Lycoperdon alpinum	0.64	0.30	0.10	0.0002
	Lycoperdon excipuliforma	0.53	0.29	0.10	0.0002
	Macrolepiota dolichaula	0.63	0.29	0.10	0.0002
	Russula ilicis	0.45	0.29	0.10	0.0002
	Russula livescens	0.22	0.20	0.006	0.0014
Organic Matter	*Amanita pantherina*	0.27	0.09	0.08	0.02
	Crepidotus variabilus	0.29	0.10	0.07	0.04
	Lactarius pterosporus	0.34	0.10	0.07	0.01
	Strobilurus esculantus	0.29	0.10	0.07	0.04
	Trametes hirsuta	0.29	0.10	0.07	0.04
Saturation	*Russula silvicola*	0.95	0.48	0.11	0.02
pH	*Amanita hemibapha*	0.53	0.13	0.07	0.0006
	Amanita oblongospora	0.43	0.13	0.08	0.0014
	Astraeus hygrometricus	0.50	0.13	0.07	0.0004
	Hemipholiota populnea	0.48	0.13	0.07	0.0016
	Hydnum repandum	0.42	0.13	0.07	0.0092
	Lycoperdon excipuliforma	0.40	0.13	0.08	0.002
	Russula ilicis	0.50	0.13	0.08	0.0016
	Russula silvicola	0.47	0.12	0.06	0.0004
Phosphorous	*Amanita pantherina*	0.78	0.35	0.13	0.009
	Bovista plumbea	0.95	0.36	0.13	0.0002
	Lycoperdon alpinum	0.94	0.37	0.13	0.0002
	Lycoperdon excipuliforma	0.89	0.38	0.15	0.0008
	Pluteus astromarginatus	0.61	0.35	0.13	0.03

In CCA ordination the maximum eigenvalue was recorded for axis 1 (0.76) followed by axis 2 (0.37) and axis 3 (0.18). Pseudo-canonical correlation for axis 1, 2 and 3 were 0.96, 0.78 and 0.63 respectively. The permutation test results for all axes was pseudo-F=3.2, P=0.002. The CCA ordination indicates that the species are linearly distributed along different environmental variables (Figs. 5, 6).

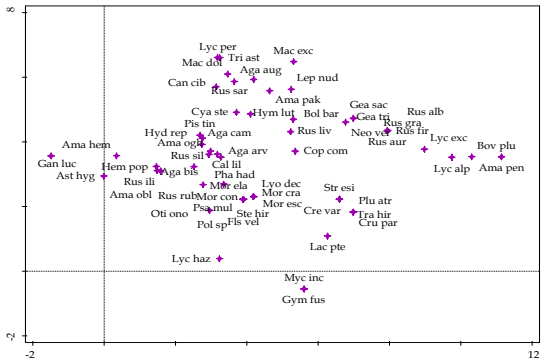

Fig. 4. DCA ordination of species of macrofungi.

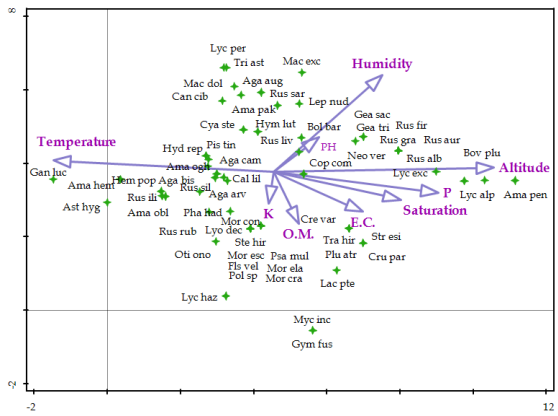

Fig. 5. CCA ordination of macrofungi species along environmental variables.

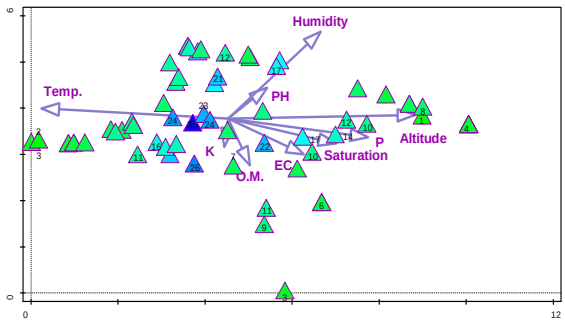

Fig. 6. CCA ordination of stands along environmental variables.

Discussion

Compared to research in the field of phytosociology, mycosociology is a branch of science rarely practiced in the world. This lack of research is explained by the complexity of the myco-communities, the large number of species they contain, the dynamics and seasonal variation in the production of fruit bodies, the life cycle of ectomycorrhizal fungi and the absence of systematic parcel networks. Hawksworth (2001) pointed out that the considerable difference between the number of known and estimated mushroom species could be attributed to the unfortunately inadequate mushroom sampling in many parts of the world. Ecological classification aims to manage natural resources efficiently and is often used to effectively solve environmental problems.

Sixty species of macrofongi have been recorded from Nandiar valley. The humid temperate ecosystem was almost stable having maximum diversity index and species richness values. Such species occurrence has also been noted in other mountainous regions (Ahmad 2009; Haq 2012; Haq 2015; Haq *et al.*, 2017). Variation in diversity index and taxonomic richness was due to slope appearance, vegetation structure, anthropogenic disturbance, and grazing factors. Analogous result were also presented by Vujnovic *et al.* (2012) in central Alberta, western Canada.

Multivariate analysis ranked the diversity of macrofungi from 65 stands to 5 myco-communities based on indicator species. The vegetation was classified based on climatic, edaphic and topographic variables, which allowed us to compare our result with the result already undertaken in adjacent areas of the same vegetation type (Khan *et al.*, 2014; Haq *et al.*, 2017; Haq 2018). Our results in each vegetation zone resemble the results of other researchers in adjacent areas (Ahmed *et al.*, 2006; Siddiqui *et al.*, 2009; Saima *et al.*, 2009; Akbar *et al.*, 2010; Pharswan *et al.*, 2010; Ilyas *et al.*, 2012; Haq *et al.*, 2015a). Peter and Erik (1992) from Senegal, Jurisic *et al.* (2014) Posavina Flood Forests in Serbia, Chen *et al.* (2015) from Xiamen City.

The Monte Carlo significance test specifies that the slope proportion, the distorted aspect, and soil thickness can be used to clarify the distribution of all leading species and species in each environmental gradient (Guan *et al.*, 2000; Aytok *et al.*, 2013).

The DCA clearly signposts that the whole statistics set is dominated by a single dominant gradient with decreasing in its value on other axis. This show that maximum species are shared in stands. Though variance in species diversity was observed in ordination space. Species clusters in ordination space at diverse locations on the basis of habitat types. The CCA ordination indicates that the species are virtually frequently scattered along different environmental variables. Our results in each vegetation zone resemble the results of other researchers in adjacent areas (Saima *et al.*, 2009; Akbar *et al.*, 2010; Ilyas *et al.*, 2012; Haq *et al.*, 2015a).

The analysis of the indicator species revealed that the significant result was the contribution of temperature,

pH, moisture, organic matter and phosphorus ($p \leq 0.05$) determining the composition of the myco-communities and the indicator species in each habitat. Similar results were also presented by Khan *et al.* (2016) of the Thandiani Forest Subdivision in the Western Himalayas.

Conclusion

The diversity index of myco-communities significantly responds to climatic, edaphic, topographical and anthropogenic stimuli. Biotic disturbances such as intensive seasonal grazing, trampling and medicinal plant harvesting have been identified as potential threats resulting in the degradation and regression of pastures and affecting the natural diversity and community structure of the Nandiar Valley biome. The biodiversity and community structure of the western Himalayas is declining due to uncontrolled degradation and disruption processes. Thus, to protect Himalayan biodiversity, regional conservation strategies are needed.

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