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RESEARCH ARTICLE

Environmental variables drive plant species composition and distribution in the moist temperate forests of Northwestern Himalaya, Pakistan

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Abstract

By assessing plant species composition and distribution in biodiversity hotspots influenced by environmental gradients, we greatly advance our understanding of the local plant community and how environmental factors are affecting these communities. This is a proxy for determining how climate change influences plant communities in mountainous regions ("space-for-time" substitution). We evaluated plant species composition and distribution, and how and which environmental variables drive the plant communities in moist temperate zone of Manoor valley of Northwestern Himalaya, Pakistan. During four consecutive years (2015-2018), we sampled 30 sampling sites, measuring 21 environmental variables, and recording all plant species present in an altitudinal variable range of 1932–3168 m.a.s.l. We used different multivariate analyses to identify potential plant communities, and to evaluate the relative importance of each environmental variable in the species composition and distribution. Finally, we also evaluated diversity patterns, by comparing diversity indices and beta diversity processes. We found that (i) the moist temperate zone in this region can be divided in four different major plant communities; (ii) each plant community has a specific set of environmental drivers; (iii) there is a significant variation in plant species composition between communities, in which six species contributed most to the plant composition dissimilarity; (iv) there is a significant difference of the four diversity indices between communities; and (v) community structure is twice more influenced by the spatial turnover of species than by the species loss. Overall, we showed that altitudinal gradients offer an important range of different environmental variables, highlighting the existence of micro-climates that drive the structure and composition of plant species in each micro-region. Each plant community

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along the altitudinal gradient is influenced by a set of environmental variables, which lead to the presence of indicator species in each micro-region.

Introduction

Mountains are the most remarkable landforms on the earth, representing different vegetation zones based on environmental variations [1]. They offer habitat heterogeneity based on microenvironmental variation along the altitudinal gradient [2, 3], where environmental variables (including direct and indirect effects of abiotic and biotic effects) are important factors in determining altitudinal zone boundaries [4, 5]. It is well recognized that altitude is a dynamic gradient along which several environmental variables [6] and species diversity [7, 8] change concomitantly. Within this focus, plant biodiversity is strongly influenced by different environmental variables [9], and certain species can survive on the brink of extinction in high mountains across the world [10-12].

Many mountains across the globe are important hotspots of biodiversity [13–16], with roughly half of all plant species flourishing in hotspot areas [17, 18]. However, despite this high endemism of species greatly influenced by various environmental gradients, such as edaphic, climatic and physiographic variables [19], these areas suffer a major impact from climate change [20, 21]. Plant species present in these gradients have great adaptive power [22, 23]; however, the speed with which climate change is advancing might not be sufficiently achieved by plant species adaptation in these areas, leading to a strong impact on the biodiversity of these areas [24–26], ultimately leading to variations in the community structure [27].

In the face of the current climate change and considering the importance of phytosociological studies for the understanding of biodiversity and species distribution, the Himalayas represent a fundamental piece for these studies. This region is facing a marked increase in temperature [28], which is three times greater than the global average [29]. This unprecedented rise in temperature, and modification of various environmental variables as well, may lead to shifts in species composition [24-26] and variations in community structure [27]. Many researchers have explored the effect of altitude on species composition, diversity, and forests formation structure during the previous two decades, with around half of these studies indicating an inverse association between species richness and altitude. Rahbek [30], on the other hand, did a quantitative investigation of altitudinal gradients of species richness and discovered that among plants, hump-shaped patterns of species diversity with peaks at mid-elevation are the most typically recorded, followed by monotonically declining patterns. Kluge et al. [31] found a hump-shaped diversity pattern for seed plants in the Eastern Himalayas, even though endemic species richness peaks at higher elevations due to increasingly isolated habitats and smaller surface area in mountainous ecosystems, which promotes speciation [32]. Although there has been a considerable increase in the number of phytosociological studies in these altitudinal regions considered hotspots of biodiversity [7, 19, 22, 23, 33], there is still limited knowledge of how and which environmental variables drive the distribution and composition of plant species along altitudinal gradients in specific hotspots regions, such as the Northwestern Himalaya.

By assessing the patterns of composition and distribution of plant species in these biodiversity hotspots influenced by environmental gradients, we greatly advance our understanding of the local plant community and how environmental factors are affecting these communities, which is a proxy for assessing how impacts of climate change can affect plant communities located in mountainous regions [34]. In this context, we evaluated plant species composition and how and which environmental variables drive the plant species distribution of moist temperate zone of the Northwestern Himalaya, Pakistan. Briefly, we assessed (i) the potential plant communities present in the moist temperate zone; (ii) which are the environmental variables that most determine plant community structure in the moist temperate zone; (iii) whether there is variation in plant species composition between plant communities and which are the species that most contributed for species composition dissimilarity; (iv) whether there is variation of diversity indices among communities; and finally (v) which is the beta diversity process that most influence plant community structure in the moist temperate zone.

Materials and methods

Study area

The present study was carried out in the moist temperate zone of Manoor valley (District Mansehra, Khyber Pakhtunkhwa), which is a mountainous valley (34.68165 N to 34.83869 N latitude, and 73.57520 E to 73.73182 E longitude Fig 1) in the Northwestern Himalayan belt of Pakistan [35–39] along an altitudinal range of 1932–3168 m.a.s.l. Monsoon winds are the main source of precipitation as well as the primary force of controlling erosion, topography, climate and vegetation of the Northwestern Himalaya [1].

Ethics approval and consent to participate

This study was approved by the Board of Study (BoS), Committee, Department of Botany and Advanced Study Research Board (ASRB) of Hazara University, Mansehra 21300, KP, Pakistan.

Vegetation sampling and plant identification

In different growing seasons (from March to October), we evaluated 30 sampling sites per year, during four consecutive years, from 2015 to 2018. The line transect method (50 meters) we used for quantitative samplings [40-45], but we never repeated the same transects over years. The surveyed study area was subdivided into 30 stands and three points randomly selected were sampled within each sampling site along 50 meters transect (total = 90 transects).



Fig 1. Map of the study area showing Pakistan, Khyber Pakhtunkhwa (KP) province, and sampling sites for data collection. Points in the right figure represent stands of the four communities identified in the moist temperate zone, Northwestern Himalaya, Pakistan. **CPI:** *Cedrus deodara-Pinus wallichiana-Isodon rugosus*, **IHC:** *Indigofera heterantha- Heracleum candicans-Cynodon dactylon*, **PCP:** *Pinus wallichiana-Cedrus deodara-Parrotiopsis jacquemontiana*, and **VIP:** *Viburnum grandiflorum-Indigofera heterantha-Pinus wallichiana*.

The distance between the sampling sites was kept at 200 meters, while the distance between the transects was kept at 100 meters. The individuals of plant species falling precisely on the line were recorded. The data from each sampling site was calculated using phytosociological characters (*i.e.*, density, frequency, cover and their relative values, as well as importance value) [46–48]. The IV was further used to rank each plant species and species with the highest IV were considered as the dominant species [46, 47]. Similarly, each plant community was named based on three dominant species [49–52]. The slope angle, aspect and exposure were recorded using clinometer; and altitude, longitude and latitude were measured by geographical positioning system (GPS). Plants species collection, labelling, pressing and other herbarium work methodology was adopted following Ijaz [53], Ijaz et al. [54], Amjad et al. [55] and Stefanaki et al. [56]. Identification was done with the aid of Flora of Pakistan [57–59] and submitted to the Herbarium of Hazara University, Mansehra (Pakistan).

Environmental gradients

Soil samples weighting 200 grams were collected (15-30cm depth) randomly from each transect of sampled vegetation site [60, 61]. The replicated samples of each sampling site were thoroughly mixed to form a composite sample [62], which was placed in a sterile polythene bag and labelled accordingly. Raw materials such as rocks, and stones were sieved out and the samples were then shade dried. Each dried soil sample was processed for physicochemical tests [62, 63] to determine soil texture (*i.e.*, clay, sand, silt, loam), pH [64], electrical conductivity (EC) [65], organic matter (OM) [66], nitrogen (N), phosphorus (P), potassium (K) and calcium carbonate (CaCO₃) levels [60, 61, 67]. Other climatic variables such as barometric pressure, dew point, humidity, heat index, temperature, wet bulb (relative humidity and ambient air temperature) and wind speed were also determined using a small remote weather station (Kestrel weather tracker 4000) to record the data at each transect and then average values were calculated at sampling site level [19].

Statistical analyses

The recorded data of vegetation, edaphic, and other environmental variables of sampling sites were compiled in order to determine relationship among them [68, 69]. Matrixes of IV data of all the recorded plant species (244 species) from 30 sampling sites were used in the analyses. Analyses were conducted on PC-ORD [70] and R software 4.0.0 [71]. Packages used in R software are mentioned in each analysis. A georeferenced map was prepared to show the distribution pattern of distinct plant communities (Fig 1).

Sampling effort and cluster analyses. Species area curves (SAC) were used to check the efficiency of the sampling effort, where plant abundance data with Sørensen distance values were used to create species area curves [72]. For classification of the recorded plant species (244) and 30 sampling sites into different plant communities, we used three different cluster analyses: Two-way Indicator Species Analysis (TWINSPAN), and Cluster Analysis (CA). We identified and classified plant species and stands (sampling sites) into major plant communities [73], as well as assess the effects of various factors (such as environmental variables) on such communities by processing clustering method using TWINSPAN [19, 74].

Plant communities and associated environmental variables. Both species and stands (sampling sites) were constrained in relation with the environmental gradients [75, 76], which were divided into geographic, slope aspect, edaphic and climatic gradients. We used non-metric multidimensional scaling (NMDS) and Principal Component Analysis (PCA) ordination biplot to determine the relationship between vegetation communities and environmental gradients using the "vegan" package. In NMDS and PCA, arrows represent the environmental

gradients, in which the length shows the strength of the gradient, and the direction represent the degree of correlation. The direction of gradients on the same axis reveals a positive correlation.

In addition, we performed canonical correspondence analysis (CCA) and variation partitioning tests (partial CCA) [77] to observe how explanatory variables (climatic, edaphic, geographic, and slope) drive the plant species distribution. First we built the best model with the lowest number of variables (those that most explain variance), through the *step* function with permutation using the "stats" package [71]. Next, we also evaluated multicollinearity between variables of the final model using Variance Inflation Factor (VIF), and we removed any variable with VIF>10, one at a time. Finally, with the final model, we carried out CCA and partial CCA to check how much each group of variables (geographic, edaphic, climatic, slope) explain in our model [77]. For these analysis, we used the "vegan" package [78].

Variation of plant species composition among plant communities. To compare whether there is difference in species composition between plant communities, we also used NMDS followed by a Permutational Multivariate Analysis of Variance (PERMANOVA) with Euclidean distance and 999 permutations. After PERMANOVA, we conducted pairwise comparisons between communities with corrections for multiple testing also using Euclidean distance and 999 permutations. We used false discovery rate (FDR) as p-value adjustment method. PERMANOVA and pairwise comparisons were conducted with "RVAideMemoire" package [79]. To observe the contribution of each plant species to overall dissimilarities, we used a similarity percentage analysis based on the decomposition of Bray-Curtis dissimilarity using the package "vegan" [78].

Variation of diversity indices among plant communities. To compare the diversity indices evaluated (species richness, Shannon, Simpson, and Pielou) among the four communities, we also conducted a GLM with Gaussian error distribution, except for species richness, in which we used Poisson distribution followed by Likelihood Ratio test. Pairwise comparisons were conducted with estimated marginal means using the package 'emmeans' [80].

Beta diversity. To evaluate which is the beta diversity process that most influence plant community structure in the moist temperate zone, we decomposed the Sørensen dissimilarity index (β sor), a measure of overall species replacement into two additive components: the spatial turnover (Simpson pairwise dissimilarity, β sim) and nestedness-resultant components (nestedness-fraction of Sorensen pairwise dissimilarity, β sne) [81–83]. Dissimilarity analysis was conducted in the package "betapart" [84].

Results

Sampling effort and plant communities

In total, 244 plant species belonging to 194 genera and 74 families (Table 1) were recorded in the moist temperate forests of the Manoor valley, Northwestern Himalaya, Pakistan. The moist temperate forests ranged from 1932.3m to 3168m. SAC analysis showed that the maximum number of plant species appeared up to stand 26 and the species curve became parallel after it, as no new species were recorded further (Fig 2).

Based on the TWINSPAN analysis (high cluster heterogeneity value–Lambda = 0.4045), we identified four different major plant communities (Fig 3), which were composed of different indicator species (IHC: *Indigofera heterantha-Heracleum candicans-Cynodon dactylon*; VIP: *Viburnum grandiflorum-Indigofera heterantha-Pinus wallichiana*, CPI: *Cedrus deodara-Pinus wallichiana-Isodon rugosus*, and PCP: *Pinus wallichiana-Cedrus deodara- Parrotiopsis jacque-montiana*). The IHC community was primarily found in the lower mountainous ranges (1932.3–2338.4 m.a.s.l), where the dominating flora was a combination of shrub and herb

Table 1. Sp	pecies composition and IV according to each sampling	site and community found along four	years of collection in moist temperate fores	ts of Manoor val-
ley, Northw	vestern Himalaya, Pakistan.			

Plant Species	Abbreviations Family name		Plant Communities			
•			IHC	VIP	СРІ	РСР
Acer caesium Wall. ex Brandis	Ace cae	Sapindaceae	0.00	0.00	0.26	0.56
Achyranthes aspera L.	Ach asp	Amaranthaceae	0.00	0.00	0.00	0.34
Achyranthes bidentata Blume	Ach bid	Amaranthaceae	0.00	0.00	0.00	0.32
Achillea millefolium L.	Ach mil	Asteraceae	0.87	0.50	0.00	0.00
Adiantum capillus-veneris L.	Adi cap-ven	Adiantaceae	0.00	0.00	0.67	1.75
Adiantum indicum J. Ghatak	Adi ind	Adiantaceae	0.00	0.00	0.96	1.84
Adiantum venustum D. Don	Adi ven	Adiantaceae	0.00	0.00	0.49	1.30
Aegopodium burttii Nasir	Aeg bur	Apiaceae	0.00	0.00	0.15	0.34
Ainsliaea aptera DC.	Ain apt	Asteraceae	0.00	0.00	0.00	0.26
Ajuga integrifolia Buch Ham.	Aju int	Lamiaceae	0.00	0.00	0.00	0.27
Alchemilla cashmeriana Rothum.	Alc cas	Rosaceae	0.43	0.00	0.51	0.12
Alcea rosea L.	Alc ros	Malvaceae	0.05	0.00	0.15	0.00
Alnus nitida (Spach) Endl.	Aln nit	Betulaceae	0.45	0.00	0.00	0.29
Amaranthus viridis L.	Ama vir	Amaranthaceae	0.00	0.00	0.00	0.24
Anagallis arvensis L.	Ana arv	Primulaceae	0.00	0.00	0.08	1.15
Anaphalis busua (BuchHam.) DC.	Ana bus	Asteraceae	0.00	0.00	0.20	0.07
Androsace hazarica R.R. Stewart ex Y. Nasir	And haz	Primulaceae	0.00	0.00	0.30	0.39
Androsace rotundifolia Hardw.	And rot	Primulaceae	0.00	0.00	0.06	0.54
Arisaema flavum (Forssk.) Schott	Ari fla	Araceae	0.00	0.00	0.00	0.91
Arisaema jacquemontii Blume	Ari jac	Araceae	0.00	4.24	0.00	0.91
Artemisia absinthium L.	Art abs	Asteraceae	0.36	0.13	0.96	2.25
Asplenium adiantum-nigrum L.	Asp adi-nig	Adiantaceae	0.00	0.00	0.20	0.41
Asparagus fiicinus BuchHam. ex D. Don	Asp fii	Asparagaceae	0.00	0.00	0.00	0.16
Avena sativa L.	Ave sat	Poaceae	0.04	0.09	0.00	0.00
Bauhinia variegata L.	Bau var	Caesalpiniaceae	0.05	0.00	0.00	0.00
Bergenia ciliata (Haw.) Sternb.	Ber cil	Saxifragaceae	0.00	0.00	0.09	0.12
Berberis lycium Royle	Ber lyc	Berberidaceae	0.00	1.42	1.55	0.49
Berberis parkeriana C.K. Schneid.	Ber pac	Berberidaceae	0.00	0.00	0.00	0.24
Bistorta amplexicaulis (D. Don) Greene	Bis amp	Polygonaceae	5.17	1.64	0.00	0.00
Brassica compestris L.	Bra com	Brassicaceae	0.52	0.00	0.00	0.00
Bromus diandrus Roth.	Bro dia	Poaceae	2.00	1.64	0.39	0.19
Bromus secalinus L.	Bro sec	Poaceae	1.83	1.49	0.67	0.59
Bromus tectorum L.	Bro tec	Poaceae	2.20	1.29	0.00	0.06
Bupleurum longicaule Wall. ex DC.	Bup lon	Apiaceae	0.00	0.00	0.10	0.32
Bupleurum nigrescens E. Nasir	Bup nig	Apiaceae	0.51	1.22	0.15	1.48
Caltha palustris var. alba (Cambess) Hook.f. & Thomson	Cal pal	Ranunculaceae	0.00	0.00	0.04	0.46
Calamintha umbrosa (M. Bieb.) Hedge	Cal umb	Lamiaceae	1.11	1.03	1.08	0.61
Campylotropis meeboldii (Schindl.) Schindl.	Cam mee	Papilionaceae	0.00	0.00	0.32	0.04
Cannabis sativa L.	Can sat	Cannabaceae	0.00	0.00	0.00	0.07
Capsella bursa-pastoris (L.) Medik.	Cap bur-pas	Brassicaceae	0.18	0.97	0.53	1.47
Castanea sativa Mill.	Cas sat	Fagaceae	0.00	0.00	0.12	0.14
Cedrus deodara (Roxb. ex Lamb.) G. Don	Ced deo	Pinaceae	0.00	5.16	22.50	16.07
Celosia argentea L.	Cel arg	Amaranthaceae	0.61	0.00	0.00	0.00
Chenopodium album L.	Che alb	Chenopodiaceae	0.75	0.41	0.00	0.24
Chrysanthemum indicum L.	Chr ind	Asteraceae	0.00	0.00	0.00	0.64

Plant Species	Abbreviations	Family name	Plant Communities			
			IHC	VIP	СРІ	РСР
Cichorium intybus L.	Cic int	Asteraceae	0.00	0.00	0.23	0.11
Circaea alpina L.	Cir alp	Onagraceae	0.00	0.00	0.00	0.40
Cirsium arvense (L.) Scop.	Cir arv	Asteraceae	0.79	0.79	0.24	1.55
Circaea cordata Royle	Cir cor	Onagraceae	0.00	0.00	0.21	0.05
Cirsium falconeri (Hook.f.) Petr.	Cir fal	Asteraceae	0.00	0.00	0.00	0.07
Clematis grata Wall.	Cle gra	Ranunculaceae	0.00	0.00	0.96	2.55
Clinopodium vulgare L.	Cli vul	Lamiaceae	1.54	1.10	1.67	3.68
Colebrookea oppositifolia Sm.	Col opp	Lamiaceae	0.00	0.00	0.00	0.06
Commelina benghalensis L.	Com ben	Commelinaceae	0.00	0.65	0.00	0.13
Convolvulus arvensis L.	Con arv	Convolvulaceae	0.00	0.00	0.31	1.14
Conyza japonica (Thunb.) Less. ex Less.	Con jap	Asteraceae	0.00	0.18	0.09	0.40
Corydalis carinata Lidén and Z.Y.Su	Cor car	Papaveraceae	0.10	0.00	0.16	0.07
Corylus colurna L.	Cor col	Betulaceae	0.00	0.00	0.15	0.26
Corydalis cornuta Royle [Syn. Corydalis stewartii Fedde]	Cor cor	Papaveraceae	0.07	0.00	0.46	0.51
Cornus macrophylla Wall.	Cor mac	Cornaceae	0.05	0.00	0.00	0.23
Cornus oblonga Wall.	Cor obl	Cornaceae	0.00	0.00	0.00	0.19
Corydalis virginea Lidén and Z.Y.Su	Cor vir	Papaveraceae	0.00	0.00	0.13	0.18
Cotoneaster acuminatus Wall. ex Lindl.	Cot acu	Rosaceae	0.06	0.00	0.00	0.00
Cuscuta reflexa Roxb.	Cus ref	Cuscutaceae	0.00	0.00	0.08	0.34
<i>Cyanthillium cinereum</i> (L.)H.Rob.	Cya cin	Asteraceae	0.00	0.00	0.08	0.10
Cynoglossum apenninum L.	Cyn ape	Boraginaceae	0.08	0.18	0.00	0.00
Cynodon dactylon (L.) Pers.	Cyn dac	Poaceae	4.59	2.28	4.13	5.28
Cynoglossum glochidiatum Wall. ex Benth.	Cyn glo	Boraginaceae	1.58	0.25	0.33	1.52
Cynoglossum microglochin Benth.	Cyn mic	Boraginaceae	0.14	0.52	0.00	0.00
<i>Cyperus odoratus</i> L.	Cyp odo	Cyperaceae	0.15	0.89	0.08	0.20
<i>Cyperus rotundus</i> L.	Cyp rot	Cyperaceae	0.59	1.09	0.91	0.53
Dactylis glomerata L.	Dac glo	Poaceae	0.41	1.40	0.47	0.40
Daphne papyracea Wall. ex G. Don	Dap pap	Thymelaeaceae	0.00	0.00	0.06	0.25
Desmodium elegans DC.	Des ele	Papilionaceae	1.39	0.00	0.31	0.12
Dicliptera bupleuroides Nees	Dic bup	Acanthaceae	0.00	0.00	0.10	0.93
Dioscorea deltoidea Wall. ex Griseb.	Dio del	Dioscoreaceae	0.13	0.00	0.24	0.03
Diospyros lotus L.	Dio lot	Ebenaceae	0.00	0.00	0.16	0.00
Dipsacus inermis Wall. in Roxb.	Dip ine	Dipsacaceae	0.22	0.67	0.09	0.13
Dryopteris wallichiana (Spreng.) Hyl.	Dry wal	Dryopteridaceae	0.00	2.76	0.52	1.89
Duchesnea indica (Andx) Fake.	Duc ind	Rosaceae	0.00	0.00	0.00	0.54
Dysphania ambrosioides (L.) Mosyakin & Clemants	Dys amb	Chenopodiaceae	0.00	0.40	0.00	0.37
Elaeagnus umbellata Thunb.	Ela umb	Eleagnaceae	0.00	0.21	0.31	0.03
Elsholtzia ciliata (Thunb.) Hyl.	Els cil	Lamiaceae	0.00	0.16	0.18	0.04
Epilobium hirsutum L.	Epi hir	Onagraceae	0.04	0.00	0.17	0.12
Epilobium latifolium L.	Epi lat	Onagraceae	0.00	0.28	0.19	0.07
Epimedium elatum C.Morrenand Decne.	Epi ela	Berberidaceae	0.03	0.18	0.14	0.07
Equisetum arvense L.	Equ arv	Equisetaceae	0.40	0.00	0.00	0.00
Erigeron canadensis L.	Eri can	Asteraceae	0.87	0.00	0.55	0.39
Erysimum melicentae Dunn.	Ery mel	Brassicaceae	0.15	0.12	0.00	0.00
Euphorbia helioscopia L.	Eup hel	Euphorbiaceae	0.11	0.00	0.00	0.05
<i>Euphrasia himalayica</i> Wetts.	Eup him	Orobanchaceae	2.70	0.58	0.00	0.00

Plant Species	Abbreviations		Plant Communities			
-			IHC	VIP	СРІ	РСР
Euphorbia hirta L.	Eup hir	Euphorbiaceae	0.00	0.00	0.09	0.25
Euphorbia prostrata Ait.	Eup pro	Euphorbiaceae	0.00	0.00	0.00	0.07
Euphorbia serpens Kunth	Eup ser	Euphorbiaceae	0.00	0.00	0.08	0.30
Fagopyrum tataricum (L.) Gaertn.	Fag tat	Polygonaceae	0.00	0.21	0.00	0.06
Filipendula vestita (Wall. ex G. Don.) Maxim.	Fil ves	Rosaceae	0.58	2.22	1.26	0.32
Foeniculum vulgare Mill.	Foe vul	Apiaceae	0.70	1.00	0.00	0.68
Fragaria nubicola (Hook. f.) Lindl. ex Lacaita	Fra nub	Rosaceae	0.15	1.95	0.36	4.11
Fumaria indica (Hausskn) Pugsley	Fum ind	Fumaricaceae	0.00	0.00	0.00	0.13
Galium aparine L.	Gal apa	Rubiaceae	0.00	0.00	0.00	0.01
Galium asparagifolium Boiss. & Heldr.	Gal asp	Rubiaceae	0.00	0.00	0.04	0.02
Galium elagans Wall.	Gal ela	Rubiaceae	0.00	0.00	0.05	0.13
Galinsoga parviflora Cav.	Gal par	Asteraceae	0.00	0.00	0.04	0.02
Gentianodes clarkei (Kusn.) Omer	Gen cla	Gentianaceae	0.00	0.00	0.00	0.10
Gerbera gossypina (Royle) Beauverd	Ger gos	Asteraceae	0.00	0.00	0.00	0.21
Geranium nepalense Sweet.	Ger nep	Geraniaceae	1.83	0.28	1.04	2.16
Geranium wallichianum D. Don ex Sweet	Ger wal	Geraniaceae	2.94	1.04	0.66	3.18
<i>Gymnosporia royleana</i> Wall. ex M.A.Lawson	Gym roy	Celastraceae	0.00	0.46	0.00	0.00
Hackelia uncinata (Benth.) C.E.C. Fisch.	Hac unc	Boraginaceae	0.33	0.00	0.00	0.00
Hedera nepalensis K. Koch	Hed nep	Araliaceae	0.00	0.00	0.00	2.61
Helianthus annuus L.	Hel ann	Asteraceae	0.14	0.00	0.00	0.00
Heracleum candicans Wall. ex DC.	Her can	Apiaceae	5.89	1.79	0.00	0.00
Hyoscyamus niger L.	Hyo nig	Solanaceae	0.00	0.00	0.07	0.00
Hypericum perforatum L.	Hyp perf	Clusiaceae	0.00	0.00	0.06	0.22
Impatiens bicolor Royle.	Imp bic	Balsaminaceae	0.12	0.29	2.10	3.56
Impatiens brachycentra Kar. & Kir.	Imp bra	Balsaminaceae	3.98	0.16	0.00	0.00
Indigofera australis Willd.	Ind aus	Papilionaceae	1.15	1.27	0.00	0.00
Indigofera hebepetala Baker	Ind heb	Papilionaceae	0.78	1.83	0.00	0.00
Indigofera heterantha Brandis	Ind het	Papilionaceae	5.08	23.51	2.33	2.83
Inula cuspidata (Wall. ex DC.) C.B. Clarke	Inu cus	Asteraceae	0.00	0.00	0.00	0.24
Inula falconeri Hook.f.	Inu fal	Asteraceae	0.00	0.00	0.05	0.10
Ipomoea nil (L.) Roth	Ipo nil	Convolvulaceae	0.00	0.00	0.26	0.50
Isodon rugosus (Wall. ex Benth.) Codd	Iso rug	Lamiaceae	0.00	0.00	5.77	3.32
Juglans regia L.	Jug reg	Juglandaceae	4.41	0.56	0.00	0.00
Lactuca tatarica (L.) C.A. Mey	Lac tat	Asteraceae	0.35	0.00	0.48	0.41
Lamium album L.	Lam alb	Lamiaceae	0.00	0.00	0.10	0.06
Lamium amplexicaule L.	Lam amp	Lamiaceae	1.23	0.25	0.53	0.88
Lathyrus aphaca L.	Lat aph	Papilionaceae	4.17	0.70	1.65	1.61
Lathyrus odoratus L.	Lat odo	Papilionaceae	0.30	0.74	0.00	0.00
Lathyrus sativa L.	Lat sat	Papilionaceae	0.34	0.90	0.00	0.00
Launaea procumbens (Roxb.) Ramayya and Rajagopal	Lau pro	Asteraceae	0.00	0.00	0.91	0.40
Lavatera cachemiriana Camb. in Jacq.	Lav cac	Malvaceae	0.04	0.00	0.00	0.00
Leptodermis virgata Edgew. ex Hook.F.	Lep vir	Rubiaceae	1.36	0.63	1.24	1.67
Ligularia amplexicaulis DC.	Lig amp	Asteraceae	0.00	0.34	0.00	0.11
Lindelofia sp.	Lin sp	Boraginaceae	0.05	0.00	0.00	0.00
Lomatogonium spathulatum (A. Kern.) Fernald	Lom spa	Gentianaceae	0.00	0.00	0.00	0.22
Lonicera caerulea L.	Lon cae	Caprifoliaceae	0.05	0.28	0.07	0.17

Plant Species	Abbreviations	Family name	Plant Communities		s	
			IHC	VIP	CPI	РСР
Lotus corniculatus L.	Lot cor	Papilionaceae	0.00	0.14	0.03	0.07
Luffa sp.	Luf sp	Cucurbitaceae	0.00	0.00	0.39	0.37
Lyonia ovalifolia (Wall.) Drude	Lyo ova	Ericaceae	0.00	0.00	0.00	0.22
Malus domestica Borkh.	Mal dom	Rosaceae	0.27	0.35	0.05	0.22
Medicago sativa L.	Med sat	Papilionaceae	0.86	0.27	1.04	1.95
Mentha piperita L.	Men pip	Lamiaceae	0.00	0.00	0.00	0.64
Mentha royleana Wall. ex Benth.	Men roy	Lamiaceae	0.00	0.00	0.00	0.63
Micromeria biflora (Ham.) Bth.	Mic bif	Lamiaceae	0.00	0.00	2.17	0.88
Minuartia kashmirica (Edgew.) Mattf.	Min kas	Caryophyllaceae	0.00	0.00	0.00	0.12
Nepeta graciliflora Benth.	Nep gra	Lamiaceae	1.90	0.87	0.00	0.00
Nepeta laevigata (D. Don) Hand Mazz	Nep lae	Lamiaceae	1.00	2.07	0.00	0.00
Oenothera rosea L. Her ex Aiton	Oen ros	Onagraceae	1.36	0.46	0.26	0.44
Olea ferruginea Wall. ex Aitch.	Ole fer	Oleaceae	0.22	0.00	0.00	0.00
Onopordum acanthium L.	Ono aca	Asteraceae	0.00	1.57	0.00	0.00
Onychium contiguum C. Hope	Ony con	Pteridaceae	0.00	0.00	0.00	0.37
Origanum majorana L.	Ori maj	Lamiaceae	0.00	0.00	0.07	0.42
Origanum vulgare L.	Ori vul	Lamiaceae	0.00	0.00	0.74	1.06
Oxalis corniculata L.	Oxa cor	Oxalidaceae	1.28	0.24	1.89	4.94
Oxyria digyna (L.) Hill	Oxy dig	Polygonaceae	0.00	0.00	0.36	1.30
Parrotiopsis jacquemontiana (Decne.) Rehder	Par jac	Hamamelidaceae	0.43	0.00	4.69	10.33
Paspalum dilatatun Poir.	Pas dil	Poaceae	0.40	0.00	0.09	0.00
Pedicularis punctata Decne	Ped pun	Orobanchaceae	2.09	1.10	0.00	0.00
Pennisetum orientale Rich.	Pen ori	Poaceae	2.78	2.83	0.69	0.31
Periploca aphylla Decne.	Per aph	Asclepiadaceae	0.04	0.15	0.13	0.11
Persicaria capitata (BuchHam. ex D.Don) H.Gross	Per cap	Polygonaceae	0.00	0.81	0.31	1.73
Pilea umbrosa Blume	Pil umb	Urticaceae	0.00	0.00	0.27	0.21
Pimpinella stewartii (Dunn) Nasir	Pim ste	Apiaceae	2.11	2.72	0.00	0.40
Pinus wallichiana A.B. Jacks	Pin wal	Pinaceae	0.00	5.30	20.28	16.24
Piptatherum aequiglume (Duthie ex Hook.f.) Roshev.	Pip aeq	Poaceae	0.13	0.00	0.00	0.00
Plantago lanceolata L.	Pla lan	Plantaginaceae	0.85	2.76	0.07	0.36
Plantago major L.	Pla maj	Plantaginaceae	2.06	4.64	0.71	0.79
Pleurospermum stellatum (D. Don) Benth. ex C.B. Clarke	Ple ste	Apiaceae	0.00	0.00	0.21	0.00
Pleurospermum stylosum C.B. Clarke	Ple sty	Apiaceae	0.00	0.00	0.24	0.04
Poa alpina L.	Poa alp	Poaceae	0.00	0.00	0.30	0.00
Poa annua L.	Poa ann	Poaceae	0.45	1.99	0.75	0.00
Poa infirma Kunth	Poa inf	Poaceae	3.08	3.03	1.11	0.03
Polygonum plebeium R.Br.	Pol ple	Convallariaceae	0.49	1.51	0.62	0.60
Polygonatum sp.	Pol sp.	Convallariaceae	0.00	0.00	0.00	0.13
Portulaca oleracea L.	Por ole	Portulacaceae	0.07	0.00	0.00	0.00
Potentilla anserina L.	Pot ans	Rosaceae	0.00	0.67	0.00	0.26
Potentilla nepalensis Hook.	Pot nep	Rosaceae	2.15	1.59	0.00	0.12
Prunus armeniaca L.	Pru arm	Rosaceae	0.07	0.00	0.00	0.00
Prunus cornuta (Wall.ex Royle) Steud	Pru cor	Rosaceae	0.25	0.00	0.00	0.00
Prunus domestica L.	Pru dom	Rosaceae	0.26	0.00	0.00	0.00
Prunella vulgaris L.	Pru vul	Lamiaceae	3.11	4.20	0.00	0.00
Pteridium aquilinum (L.) Kuhn	Pte aqu	Pteridaceae	0.29	0.00	0.00	0.00

Plant Species	Abbreviations	Family name		Plant Co	mmunitie	s
-			IHC	VIP	СРІ	РСР
Pteracanthus urticifolius (Wall. ex Kuntze) Bremek.	Pte urt	Verbenaceae	0.00	0.00	0.07	0.05
Pteris vittata L.	Pte vit	Pteridaceae	1.34	0.00	0.51	0.49
Pyrus pashia BuchHam. ex D.Don	Pyr pas	Rosaceae	0.69	0.00	0.00	0.00
Ranunculus laetus Wall. ex Hook. f. and J.W. Thompson	Ran lae	Ranunculaceae	0.00	0.00	0.00	0.07
Ranunculus muricatus L.	Ran mur	Ranunculaceae	1.61	0.94	0.00	0.23
Reinwardtia trigyna Planch.	Rei tri	Linaceae	0.00	0.00	0.04	0.04
Rhamnus purpurea Edgew.	Rha pur	Rhamnaceae	0.00	0.00	0.09	0.16
Rhynchosia pseudo-cajan Cambess.	Rhy pse	Papilionaceae	0.23	0.00	0.00	0.06
Rosa brunonii Lindl.	Ros bru	Rosaceae	0.00	0.00	0.15	0.07
Rosa webbiana Wall. ex. Royle	Ros web	Rosaceae	0.00	0.00	0.04	0.00
Rubus fruticosus agg.	Rub fru	Rosaceae	0.00	0.00	0.24	0.16
Rubus sanctus Schreber	Rub san	Rosaceae	0.00	0.00	0.00	0.12
Rumex dentatus L.	Rum den	Polygonaceae	0.47	0.00	0.00	0.00
Rumex nepalensis Sprenge	Rum nep	Polygonaceae	0.42	0.77	0.00	0.02
Rydingia limbata (Benth.) Scheen & V.A. Albert [Syn. Otostegia limbata (Benth.) Boiss]	Ryd lim	Lamiaceae	0.00	1.24	0.28	0.00
Saccharum spontaneum L.	Sac spo	Poaceae	0.00	0.00	0.00	0.28
Salvia lanata Roxb.	Sal lan	Lamiaceae	0.15	0.00	0.00	0.00
Salvia nubicola Wall. ex Sweet	Sal nub	Lamiaceae	0.12	0.00	0.00	0.00
Sanicula elata BuchHam. ex D.Don	San ela	Apiaceae	0.00	0.00	0.10	0.25
Sambucus wightiana Wall. ex Wight and Arn	Sam wig	Sambucaceae	1.34	0.00	0.54	0.00
Sarcococca saligna Müll.Arg.	Sar sal	Buxaceae	0.00	0.00	0.00	1.54
Sauromatum venosum (Dryand. ex Aiton) Kunth	Sau ven	Araceae	0.00	0.00	0.00	0.11
Schismus arabicus Nees.	Sch ara	Poaceae	0.00	0.00	0.46	1.24
Senecio analogous DC.	Sen ana	Asteraceae	0.00	0.00	0.05	0.03
Senecio chrysanthemoides DC.	Sen chr	Asteraceae	0.00	0.00	0.15	0.57
Seseli libanotis (L.) W.D.J. Koch .	Ses lib	Apiaceae	0.00	0.00	0.04	0.31
Sida cordata (Burm.f.) Borss	Sid cor	Malvaceae	0.16	0.00	0.05	0.07
Silene conoidea L.	Sil con	Caryophyllaceae	0.37	0.00	0.00	0.05
Silene vulgaris (Moench) Garcke	Sil vul	Caryophyllaceae	0.37	0.23	0.00	0.17
Sisymbrium irio L.	Sis iri	Brassicaceae	0.03	0.32	0.05	0.00
Smilax glaucophylla Koltzsch	Smi glau	Smilacaceae	0.00	0.00	0.00	0.01
Solena amplexicaulis (Lam.) Gandhi	Sol amp	Cucurbitaceae	0.00	0.00	0.05	0.04
Sonchus asper (L.) Hill	Son asp	Asteraceae	0.00	0.00	0.00	0.00
Sorghum halepense (L.) Pers.	Sor hal	Poaceae	0.00	0.00	0.00	0.47
Sorbus tomentosa Hedl.	Sor tom	Rosaceae	0.00	0.00	0.00	0.06
Sorbaria tomentosa (Lindl.) Rehder	Sorb tom	Rosaceae	0.57	3.04	0.00	0.12
Spiraea affinis R.Parker	Spi aff	Rosaceae	0.00	0.00	0.00	0.12
Spiranthes sinensis (Pers.) Ames	Spi sin	Orchidaceae	0.00	0.00	0.00	0.02
Spiraea vaccinifolia D. Don	Spi vac	Rosaceae	0.00	0.00	0.09	0.02
Sporobolus diandrus (Retz.) P.Beauv.	Spo dia	Poaceae	2.48	0.00	0.75	0.26
Stellaria media (L.) Vill.	Ste med	Caryophyllaceae	0.11	0.00	0.00	0.18
Stellaria monosperma BuchHam. ex D. Don	Ste mon	Caryophyllaceae	0.00	0.00	0.00	0.08
Swertia cordata (Wall. ex G. Don) C.B. Clarke	Swe cor	Gentianaceae	0.00	0.00	0.00	0.03
Tagetes minuta L.	Tag min	Asteraceae	0.00	0.00	0.70	2.22
Taraxacum officinale aggr. F.H. Wigg.	Tar off	Asteraceae	0.14	0.66	0.17	0.56
Thalictrum pedunculatum Edgew.	Tha ped	Ranunculaceae	0.00	0.00	0.13	0.05

Plant Species	Abbreviations	Family name	Plant Communities			
			IHC	VIP	СРІ	РСР
Torilis japonica (Houtt.) DC.	Tor jap	Apiaceae	0.00	0.00	0.05	0.08
Trachyspermum amii (L.) Sprague	Tra ami	Apiaceae	0.00	0.00	0.05	0.18
Trifolium repens L.	Tri rep	Papilionaceae	1.55	0.31	0.14	0.69
Urochloa panicoides P. Beauv.	Uro pan	Poaceae	0.61	0.43	0.30	0.00
Urtica dioica L.	Urt dio	Urticaceae	1.52	0.00	1.21	0.53
Valeriana jatamansi Jones	Val jat	Caprifoliaceae	0.00	1.10	0.00	0.00
Verbascum thapsus L.	Ver tha	Scrophulariaceae	1.34	0.00	0.00	0.73
Veronica anagallis L.	Ver ana	Plantaginaceae	0.00	2.12	0.23	0.24
Viburnum grandiflorum Wall. ex DC.	Vib gra	Adoxaceae	0.00	24.43	0.00	1.45
Vicia sativa L.	Vic sat	Papilionaceae	0.36	0.99	0.17	0.00
Vincetoxicum petrense (Hemsl. & Lace) Rech. f.	Vinc pet	Asclepiadaceae	0.00	0.00	0.09	0.13
Viola odorata L.	Vio odo	Violaceae	0.42	0.70	0.15	0.51
Viola serpens Wall. Ex Ging	Vio ser	Violaceae	0.19	0.92	0.38	0.11
Vitex negundo L.	Vit neg	Vitaceae	0.00	0.80	0.00	0.12
Wulfenia amherstiana (Benth.) D.Y. Hong	Wul amh	Plantaginaceae	0.00	0.00	0.04	0.34

IHC: Indigofera heterantha- Heracleum candicans-Cynodon dactylon, VIP: Viburnum grandiflorum-Indigofera heterantha-Pinus wallichiana, CPI: Cedrus deodara-Pinus wallichiana-Isodon rugosus and PCP: Pinus wallichiana-Cedrus deodara- Parrotiopsis jacquemontiana.

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species owing to the existence of a substantial herbaceous layer of *Cynodon dactylon*, which carpeted the landscape alongside *Indigofera heterantha* patches (Table 1). The VIP community was recognized mainly in the foothills and adjacent plains (2390.5–2437.8 m.a.s.l), where the predominant vegetation was shrubland with abundant patches of *Viburnum grandiflorum* and



Fig 2. The Species-Area Curve (SAC) of 244 plant species distributed among 30 sampling sites. The SAC was used to check the adequacy level of the sampling effort, where plant abundance data with Sørensen distance values were used to create the SAC.



Fig 3. Clustering analysis indicates the classification of 30 stands comprised of 244 plant species into four different plant communities. IHC (red triangle): *Indigofera heterantha-Heracleum candicans-Cynodon dactylon*, VIP (blue circle): *Viburnum grandiflorum-Indigofera heterantha-Pinus wallichiana*, CPI (green square): *Cedrus deodara-Pinus wallichiana-Isodon rugosus* and PCP (yellow diamond): *Pinus wallichiana-Cedrus deodara-Parrotiopsis jacquemontiana*. The plant communities are represented by the symbols in the illustration. Letters associated with numbers at the end of each branch of the dendrogram represent the stands evaluated.

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Indigofera heterantha, accompanied by the co-dominant *Pinus wallichiana* (tree species). Nonetheless, the other two plant communities, i.e., PCP and CPI, were significantly dominated by the tree species layer at the middle (2292–2947 m.a.s.l) and higher (2048.2–3168 m.a.s.l) altitudinal ranges alongside shrubby associates (Table 1).

Plant communities and associated environmental variables

NMDS and PCA were used to show the relationship between the plant communities of moist temperate forests and environmental variables (Fig 4A–4D) and PCA (Fig 4E). The ecological and environmental variables like geographic, slope, edaphic, and climatic variables were used to correlate communities (Table 2). The most representative environmental variables that drive the community structure and diversity were altitude, slope angle and aspects (SE, NE, ES, WN), potassium (K), pH, organic matter, loam, silt, sand, clay, temperature, heat index, wind speed and barometric pressure. Environmental variables classify 30 sampling sites into four major plant communities, as shown by the cluster analysis (Fig 3). In constrained PCA ordination, the PC1 axis accounted for the most explanatory variance (20%), while the PC2 axis accounted for the least (14.2%). The profound influence of the environmental variables was revealed by classifying the moist temperate forests vegetation into four communities (Fig 4E), as also shown by CA, TWCA and NMDS.

The PCP community showed positive and significant correlation with northern aspect, silty loamy soil texture, humidity and altitude (Fig 4B and 4C). In contrast, CPI community showed positively significance with southern slope, wind speed, dew point and wet bulb. IHC community showed positive correlation with silty soil texture, electric conductivity and pH. And



Fig 4. Non-Multidimensional Scaling (NMDS) between plant communities in moist temperate forests and environmental gradients. a) geographic, b) slope, c) edaphic and d) climatic. e). Principle Component Analysis (PCA) illustrating the relationship between various measured environmental variables and communities indicated by coloured circles. Large coloured circles show the centroid of each community. NMDS-PCA: Species contribution analysis for community ordination in NMDS is depicted in Table 2. IHC: *Indigofera heterantha- Heracleum candicans-Cynodon*

dactylon, **VIP**: Viburnum grandiflorum-Indigofera heterantha-Pinus wallichiana, **CPI**: Cedrus deodara-Pinus wallichiana-Isodon rugosus and **PCP**: Pinus wallichiana-Cedrus deodara- Parrotiopsis jacquemontiana.

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finally, VPI community revealed positively significant correlation with north-western slope, CaCO₃ and pH. Thus, all the four communities were found separately in clumps with clear differences based on the environmental variables (Fig 4).

The CCA and variation partitioning tests showed that the total inertia results of CCA was 3.023, where our final variables (altitude, temperature, humidity, wind speed, slope angle, slope N, slope NW, slope SW, pH, EC, OM, CaCO₃, K, P, sand, and loam) together explained 66.5% of variation (sum of canonical eigenvalues was 2.011). The first two canonical axes explained 37.1% of variation. CCA model was significant ($\chi^2 = 2.010$; pseudo-F value = 1.613; p<0.001; df = 16; permutations = 999). For the 16 explanatory variables, we tested simple term

Table 2. Mean (SD) of environmental variables and plant species richness per community found along four years of collection in moist temperate forests of Manoor valley, Northwestern Himalaya.

Communities	IHC	VIP	СРІ	РСР
Species Richness	51(8)	53(10)	40(12)	68(13)
Altitude	2251.7(132.7)	2413(19.4)	2588.8(408.8)	2609(167.6)
Latitude	34.7(0)	34.8(0)	34.7(0)	34.7(0)
Longitude	73.6(0)	73.6(0)	73.6(0)	73.6(0)
Temp	23.4(2)	20.7(0.5)	20.8(3.2)	21(3)
Humidity	56.8(6)	54.6(3.7)	54.7(3.6)	56.7(3.7)
Heat index	23.9(2.2)	23.3(2.2)	22.6(2.9)	22.8(3.1)
Wind speed	1.6(0.3)	1.7(0.2)	1.7(0.5)	1.6(0.5)
Dew point	16(0.9)	16.3(0.5)	16.5(1.5)	16.6(2)
Wet bulb	18.2(1.3)	17.3(0.2)	18.2(1.5)	17.3(2.1)
Baro Press	770.2(12.8)	754.6(1.8)	750.4(31.2)	752.9(18.3)
Slope Angle	47.9(16.9)	35(4.1)	56.6(31.7)	46.7(22.2)
Slope ES	0(0)	0(0)	0.3(0.5)	0(0)
Slope N	0(0)	0(0)	0(0)	0.7(0.4)
Slope NW	0.1(0.3)	0(0)	0(0)	0(0)
Slope S	0(0)	0(0)	0.7(0.5)	0.3(0.4)
Slope SW	0.9(0.3)	0.7(0.5)	0(0)	0(0)
Slope W	0(0)	0.3(0.5)	0(0)	0(0)
pH	5.8(0.2)	5.6(0.2)	5.6(0.5)	5.4(0.5)
EC	2.4(1.1)	2(0.6)	1.7(0.8)	1.7(0.9)
ОМ	1.2(0.3)	1.3(0.3)	1.3(0.5)	1(0.4)
CaCO ₃	6.3(1.6)	9.3(1.9)	6.6(2.4)	5.6(2.4)
K	210.9(5.6)	220.3(5)	210.9(3.1)	216(5.2)
Р	13.4(3.2)	11.7(0.5)	11.9(3.2)	10.5(3.8)
Sand	31.2(3.6)	27.6(2.8)	30.5(8.3)	35.2(6.9)
Silt	46.5(6.1)	46.7(3.5)	44.3(7.5)	41.7(7.6)
Clay	22.4(4.1)	25.7(1)	25.2(2.4)	23.2(4)
Loam	0.6(0.5)	0.3(0.5)	0.6(0.5)	0.5(0.5)
Sandy clay loam	0(0)	0(0)	0(0)	0.1(0.3)
Silt loam	0.4(0.5)	0.7(0.5)	0.4(0.5)	0.5(0.5)

IHC: Indigofera heterantha- Heracleum candicans-Cynodon dactylon, VIP: Viburnum grandiflorum-Indigofera heterantha-Pinus wallichiana, CPI: Cedrus deodara-Pinus wallichiana-Isodon rugosus and PCP: Pinus wallichiana-Cedrus deodara- Parrotiopsis jacquemontiana.

effects. Simple term effects showed that Altitude, Slope SW, Slope NW, Slope N, Slope Angle, K, and Humidity (decreasing order of importance) were significant (p<0.05; Table 3). The 16 explanatory variables were grouped into four classes: Climatic (Humidity, Temperature, Wind speed); Edaphic (pH, EC, OM, CaCO₃, K, P, Sand, Loam); Geographic (Altitude); and Slope (Slope Angle, Slope N, Slope NW, Slope SW), and then, we performed variation partitioning tests (partial CCA) for all 15 possible classes (Table 4). Class [b] was the most explanatory variable (104.6%) followed by class [m] (7.2%) (Fig 5).

Variation of plant species composition among plant communities and beta diversity

We found a significant variation in plant species composition among communities (Table 5; Fig 6), in which all communities showed a significant difference in species composition between each other (Table 6). Out of 244 species, six species greatly contributed to the variation in plant species composition between communities, namely *Viburnum grandiflorum*, *Indigofera heterantha, Heracleum candicans, Cedrus deodara, Pinus wallichiana*, and *Parrotiopsis jacquemontiana* (Table 6). Overall, the three species that most contributed for the variation in species composition between communities showed 13.7–29.7% of cumulative contribution (Table 6).

The total beta diversity (β sor) showed a value of 54.7% dissimilarity, of which spatial turnover (β sim) made up 40.5% and nestedness-resultant components (β sne) made up 14.2%. In β sim cluster, we observed 47.8% dissimilarity between PCP-CPI cluster and VIP-IHC cluster (Fig 7). PCP showed a dissimilarity of 9.4% with CPI, and VIP showed a dissimilarity of 21.5% with IHC (Fig 7). In β sne cluster, we found 24.5% dissimilarity between PCP and VIP-C-PI-IHC cluster (Fig 7). VIP showed a dissimilarity of 11.3% with IHC-CPI, and IHC had 4.1%

Variables	Df	ChiSquare	F	p-value
Altitude	1	0.251	3.229	0.001
Slope.SW	1	0.245	3.146	0.001
Slope.NW	1	0.178	2.297	0.001
Slope.N	1	0.214	2.748	0.002
Slope.Angle	1	0.163	2.097	0.009
К	1	0.140	1.798	0.018
Humidity	1	0.119	1.538	0.039
Wind.speed	1	0.114	1.472	0.069
CaCO ₃	1	0.098	1.270	0.151
Temp	1	0.081	1.049	0.376
ОМ	1	0.079	1.017	0.401
EC	1	0.076	0.976	0.474
Р	1	0.070	0.907	0.578
Sand	1	0.069	0.895	0.590
Loam	1	0.056	0.724	0.864
pH	1	0.050	0.647	0.922
Р	1	0.088	0.7295	0.820
К	1	0.075	0.6191	0.922

Table 3. The contribution and ranking of the studied variables in the variation partitioning tests (partial CCA model) to observe how explanatory variables (*i.e.*, climatic, edaphic, geographic, and slope) drive the plant species distribution.

Significant variables are displayed in **bold**.

Individual Fraction	Adjusted R ²	Variation explained (%)	% of all	Df
[a]	0.020	5.5	0.1	1
[b]	0.370	104.6	2.3	4
[c]	0.004	1.2	0.0	8
[d]	0.015	4.3	0.1	3
[e]	0.020	5.7	0.1	0
[f]	-0.091	-25.6	-0.6	0
[g]	-0.005	-1.5	0.0	0
[h]	-0.001	-0.3	0.0	0
[i]	-0.045	-12.9	-0.3	0
[j]	-0.002	-0.6	0.0	0
[k]	0.011	3.1	0.1	0
[1]	0.019	5.3	0.1	0
[m]	0.026	7.2	0.2	0
[n]	0.006	1.7	0.0	0
[0]	0.008	2.3	0.1	0
Total explained	0.354	100.0	2.2	18
All variation	15.835	/	100	

Table 4. Results of variation partitioning tests (partial CCA model) of four environmental variable groups studied (*i.e.*, climatic, edaphic, geographic, and slope) that drives the plant species distribution. For individual fraction letters code see Fig 5.

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dissimilarity with CPI (Fig 7). Thus, plant community structure is twice more influenced by the spatial turnover of species (β sim) than by the species loss (nestedness-resultant, β sne).

Variation of diversity indices among plant communities

We found a significant difference of four diversity indices, species richness (GLM χ^2 = 73.113, df = 3, p<0.001; Fig 8A), Shannon (GLM χ^2 = 35.797, df = 3, p<0.001; Fig 8B), Simpson





	Df	Sums of Sqs	Mean Sqs	F	R ²	Pr(>F)
Communities	3	9961.1	3320.4	13.324	0.6059	0.001
Residuals	26	6479.1	249.2		0.3941	
Total	29	16440.2			1	

 Table 5. PERMANOVA results comparing species composition between the four communities found in Moist temperate forest. This analysis was made with Euclidean distance and 999 permutations. Pairwise comparisons between communities are depicted in Table 6.

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(GLM $\chi^2 = 46.465$, df = 3, p<0.001; Fig 8C), and Pielou (GLM $\chi^2 = 44.093$, df = 3, p<0.001; Fig 8D), between the four communities. PCP showed the highest average number of species (68.1±4.2; mean±SE) followed by VIP (53.3±7.5) and IHC (51.1±3.5), and finally by CPI, with the lowest number of species (40.2±4.5) (Fig 8A). PCP showed a Shannon' value of 3.62±0.08 (mean±SE), followed by IHC (3.53±0.07), VIP (3.29±0.1), and CPI (2.9±0.1) respectively (Fig 8B). IHC showed the highest Simpson' value (0.959±0.01; mean±SE), followed by PCP (0.954±0.01), VIP (0.930±0.01), and CPI (0.898±0.01) respectively (Fig 8C). Finally, IHC showed the highest Pielou' value (0.901±0.01; mean±SE), followed by PCP (0.862±0.01), VIP (0.830±0.01), and CPI (0.797±0.01) respectively (Fig 8D).

Discussion

Mountain ecosystems are characterized by dramatic changes in temperature and abiotic properties over short altitudinal and geographical distances, making them ideal natural laboratories for studying vegetation response to environmental changes [85]. In this study, we evaluated the plant species composition and distribution in a hotspot of biodiversity, the Northwestern Himalayan mountains, Pakistan, assessing how environmental gradients, source of habitat heterogeneity, influence plant community structure and diversity, which might be a proxy for assessing how climate change impacts on plant communities located in mountainous regions



Fig 6. NMDS with PERMANOVA analysis to compare species composition between communities of moist temperate forests. IHC: Indigofera heterantha-Heracleum candicans-Cynodon dactylon, VIP: Viburnum grandiflorum-Indigofera heterantha-Pinus wallichiana, CPI: Cedrus deodara-Pinus wallichiana-Isodon rugosus and PCP: Pinus wallichiana-Cedrus deodara- Parrotiopsis jacquemontiana.

Communities	P-value	Species	Av dis	SD	Ratio	Av Com1	Av Com2	Cum	Cum %	Cont %
IHC-VIP	0.011	Vib.gra	0.1	0	5	0	24.4	0.1	12.2	12.2
		Ind.het	0.1	0	1.9	8.8	23.5	0.2	20.1	7.9
		Her.can	0	0	1.3	6.9	1.8	0.2	22.8	2.7
ІНС-СРІ	0.002	Ced.deo	0.1	0	3.8	0	22.5	0.1	10.9	10.9
		Pin.wal	0.1	0	5.3	0	20.3	0.2	20.8	9.9
		Ind.het	0	0	0.9	8.8	2.3	0.2	24.2	3.5
ІНС-РСР	0.002	Pin.wal	0.1	0	2.7	0	16.2	0.1	6.9	6.9
		Ced.deo	0.1	0	3	0	16.1	0.1	13.8	6.9
		Par.jac	0	0	5.8	0.4	10.3	0.2	18	4.2
VIP-CPI	0.011	Vib.gra	0.1	0	5.1	24.4	0	0.1	11.6	11.6
		Ind.het	0.1	0	5.3	23.5	2.3	0.2	21.5	9.9
		Ced.deo	0.1	0	1.9	5.2	22.5	0.3	29.7	8.3
VIP-PCP	0.006	Vib.gra	0.1	0	3.3	24.4	1.4	0.1	9.5	9.5
		Ind.het	0.1	0	6.6	23.5	2.8	0.2	17.9	8.4
		Pin.wal	0	0	2.6	5.3	16.2	0.2	22.8	4.9
CPI-PCP	0.002	Ced.deo	0	0	1.2	22.5	16.1	0.1	5.4	5.4
		Par.jac	0	0	1.8	4.7	10.3	0.1	9.6	4.2
		Pin.wal	0	0	1.1	20.3	16.2	0.1	13.7	4

Table 6. Pairwise comparisons with FDR p-value adjustment method of species composition and contrast results of the contribution of individual species to the overall Bray-Curtis dissimilarity of species composition between the four communities found in moist temperate forest. We displayed only the three species that most contributed.

Av. dis.–Average dissimilarity; SD–Standard deviation; Av Com1 –Average Community 1; Av Com2 –Average community 2; Cum.–Cumulative; Cont.–Contribution. Vib.gra: *Viburnum grandiflorum*, Ind.het: *Indigofera heterantha*, Her.can: *Heracleum candicans*, Ced.deo: *Cedrus deodara*, Pin.wal: *Pinus wallichiana*, Par.Jac: *Parrotiopsis jacquemontiana*.

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Fig 7. Dissimilarity cluster based on spatial turnover (βsim) and nestedness-resultant components (βsne) of beta diversity components of species dissimilarity between four plant communities of moist temperate forests. IHC: Indigofera heterantha-Heracleum candicans-Cynodon dactylon, VIP: Viburnum grandiflorum-Indigofera heterantha-Pinus wallichiana, CPI: Cedrus deodara-Pinus wallichiana-Isodon rugosus, and PCP: Pinus wallichiana-Cedrus deodara-Parrotiopsis jacquemontiana.



Fig 8. Variation of diversity indices between the four plant communities of moist temperate forests in the Northwestern Himalaya, Pakistan. Figures represent ridgeline plots with raw data (black dots below each density distribution) and the first, second and third quartiles (vertical red lines). Lowercase letters on the left differ from each other by an estimated marginal mean. The Y-axis is displayed in an ascendant altitudinal gradient. **IHC**: *Indigofera heterantha- Heracleum candicans-Cynodon dactylon*, **VIP**: *Viburnum grandiflorum-Indigofera heterantha-Pinus wallichiana*, **CPI**: *Cedrus deodara-Pinus wallichiana-Isodon rugosus*, and **PCP**: *Pinus wallichiana-Cedrus deodara- Parrotiopsis jacquemontiana*.

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[34, 86, 87]. We found that (i) the moist temperate zone in this region can be divided in four different major plant communities; (ii) each plant community has a specific set of environmental drivers; (iii) there is a significant variation in plant species composition between communities, in which six species contributed most to the plant composition dissimilarity; (iv) there is a significant difference of the four diversity indices (species richness, Shannon, Simpson, Pielou) between communities; and finally (v) plant community structure is twice more influenced by the spatial turnover of species (β sim) than by the species loss (nestedness-resultant, β sne). Overall, we showed that altitudinal gradients offer an important range of different environmental variables, highlighting the existence of micro-climates that drive the structure and composition of plant species in each micro-region. In addition, each plant community along the altitudinal gradient has a set of environmental drivers, which lead to the presence of indicator species in each micro-region.

Mountain plant communities are thought to be sensitive to climate change and, thus, able to reveal its effects sooner than others [34, 88]. The four communities found showed a wide range of environmental drivers; however, altitude and temperature showed great prominence, probably making up the main environmental drivers in mountainous plant communities. Similar pattern was observed in the allied area (Nandiar catchment, Battagram) of Northwestern

Himalaya by stating altitude and temperature as the governing gradient [74]. Such variables, which can be strongly correlated [89], modify the diversity and structure of plant communities by creating local micro-climates [90], directly influencing plant community composition and diversity [19, 26, 91, 92].

Indeed, there is no order of importance of environmental variables, but studies are unanimous in showing that there is a consensus on the explanations for the variables' influences. For instance, in two recent studies we showed that the altitude-temperature relationship significantly influenced the physiological attributes of some plant species in the Northwestern Himalayan region [22, 23], which can be a proxy for understanding plant adaptation to climate change. Any change in soil parameters has a significant effect on the growth of plant communities [19]. The studies on mountain forests habitats around the world have also revealed the role of soil structure on species zonation [72, 93, 94]. Furthermore, both chemical and physical attributes of the soil are related to natural soil characteristics, with an impact on plant species composition and distribution of higher vascular plants [95–97]. For instance, some soil variables can have great influence on plant composition and distribution, such as pH. Some studies have shown that pH level on soil can influence nutrient availability, ultimately influencing nutrient uptake for growth [98-100]. However, the availability of some nutrients as a result of pH levels can be detrimental for some plants, since some nutrients are toxic to some plants [98, 101]. Considering that there is a great variability of pH levels and nutrient availability and concentration along altitudinal variables, it is expected a great variability of plant species composition which can be more or less related to specific soil parameters. Since plants are sensitive to small variations of soil characteristics such as pH, minerals, organic matter, among others, and these variables are constantly changing along altitudinal gradients directly and indirectly influencing the presence and availability of other organisms and resources, some plant species might have adapted to specific set of variables.

Variability in plant species diversity is an outcome of species interaction with particular set of environment variables either abiotic and biotic [102, 103], which can occur in both space and time [104, 105]. The concept of changing species composition and vegetation continuum along the ecological gradients emerged as an antithesis model for distinct units [106, 107]. In our study, the moist temperate forest of the studied Northwestern Himalayan region is comprised in an altitudinal gradient of approximately 1500 m. This gradient is subject to strong micro-climatic variation, which results in a set of micro-regions (better discussed above). Each micro-region has certain characteristics, which will influence the set of species that will inhabit these spaces [24-26]. In this sense, it is expected that the plant community structure is more influenced by the spatial turnover of species (β sim) than by the species loss (nestedness-resultant, β sne), *i.e.*, that there are different plant communities along the altitudinal gradient, as shown by our results. The differentiation of species diversity was mainly a consequence of environmental variables which is due to soil factors [108]. Therefore, in addition to the influence of edaphic factors in space on species composition and vegetation continuum, as shown in our study, results from similar studies have shown that the altitude is also important in driving vegetation structure and diversity in plant communities.

We found that environmental heterogeneity among plant communities have significant effects on beta diversity, particularly the spatial turnover. These results indicate that there is not a significant loss of the number of species between the plant community, but a variation in the species composition. This variation may be closely linked to the environmental effects in the area, which induces the appearance of species adapted to environmental variables [109]. The local community composition replacement implied the simultaneous loss and gain of species due to immigration–extinction dynamics and trait-based environmental filtering [110, 111]. This indicates the relationship among plant community types and among species based

on multiple factors. Although we did not find a large variation in β sne (loss of species between plant communities), it is important to note that temporal analysis might be important to consider a notable variation in this component of beta diversity; and β sne variations will be better observed in long-term analysis in future studies. In this sense, it is expected that the plant community structure is more influenced by the spatial turnover of species (β sim) than by the species loss (nestedness-resultant, β sne), *i.e.*, that there are different plant communities along the altitudinal gradient, as shown by our results. Similarly, results were report by Haq et al. [112] from forests of Kashmir Himalaya, India.

We observed a significant variation in plant species composition between communities, in which all communities showed a significant difference in species composition between each other. The measure of Bray-Curtis dissimilarity shows that species composition change that is influenced mainly by abundant species, in our study six species (*Viburnum grandiflorum, Indigofera heterantha, Heracleum candicans, Cedrus deodara, Pinus wallichiana*, and *Parrotiopsis jacquemontiana*) contributed most to the plant composition dissimilarity. These results suggest that the richness and turnover patterns we observed were driven primarily by rare species, which comprise most of the local species pools at these forest communities [113]. These findings are consistent with the idea that less abundant species are more sensitive to climate variability than longer lived and more abundant species [114]. The high level of turnover is common and is an important mechanism by which a large regional species pool buffers site level diversity from interannual variation in climate [115].

Current study provides the baseline and first insights of spatial distribution, vegetation pattern and species contribution in response to environmental gradients in a moist temperate forests, Northwestern Himalaya, Pakistan. Studies that evaluate the distribution and composition of the plant community are fundamental for a better understanding of the local plant community, the conservation status and protection of these communities, as well as providing support for mitigation measures. Especially in the case of Northwestern Himalaya, which represents a biodiversity hotspot, it is even more important that we conduct phytosociological studies in these areas to document and preserve the biodiversity there. In the face of current climate changes, these regions are being heavily impacted [28, 29], where the probability of species extinction may be higher than elsewhere, as these regions are rich in endemic species. Finally, we need to consider that phytosociological studies consider a general profile of the first trophic chains level, *i.e.*, to evaluate the composition, distribution and diversity of plants is to indirectly assess the first level of trophic chains.

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