

## The influence of environmental variables on *Punica granatum* L. assemblages in subtropical dry temperate woodland in the district of Lower Dir, Khyber Pakhtunkhwa, Pakistan

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**Abstract:** *Punica granatum* L. (Wild) is an important fruit-yielding species of the world and a source of income for people, particularly in the Hindukush-Himalayas of Pakistan. Considering its ecological and commercial importance, an attempt was made to provide a primary assessment of its compositional pattern with relation to environmental variables for ecologically unexplored *Punica granatum* forests located in a subtropical dry temperate zone using multivariate techniques. The vegetation data were collected from 40 *Punica granatum* forest stands along with the associated environmental data (12 variables) at different locations. Classification of the stands was carried out using Ward's agglomerative cluster analysis. In total, 20 tree species belonging to 13 families of 19 genera with 78 understories were surveyed. Finally, nonmetric multidimensional scaling ordination with associated Monte Carlo permutation tests was performed to explore the patterns of variation in vegetation distribution explained by the environmental variables. Four community types were identified in different altitudinal and microclimatic thickets that significantly varied in species composition. The soil physical properties, i.e. sand and clay, forming an amalgam with chemical properties, i.e. N<sup>+</sup> and K<sup>+</sup> concentrations, were the most influential variables responsible for distribution of *Punica granatum* and associated species and compositional variation in the subtropical dry temperate areas of Pakistan. The present study will help in the understanding of conservation and management of this ecologically and commercially important species and will provide baseline information for other forests species growing in the area.

**Key words:** *Punica granatum*, natural forests, fruit-yielding, commercial importance, classification and ordination, species conservation and management

### 1. Introduction

*Punica granatum* L. belongs to Punicaceae and its forests constitute important natural communities in the subtropical dry temperate areas of the Hindukush-Himalayas of Pakistan. These forests have provided a wide variety of ecological and economic services to the local human populations over centuries (Khan et al., 2011). The species is known for its edible fruits with fleshy red, pink, or whitish external layers (Lama et al., 2001). It has supplied wood for fire, construction, and medicinal products to different ethnic groups from prehistoric times to the present (Hazrat et al., 2007).

There is debate regarding the center of origin of the species (Adhikari and Adhikari, 2010); however, it is frequently reported from the Mediterranean belt, Arabia, Iran, Bengal, China, Japan, and Central and Western Asia. In addition, the species is found in small pockets of the Hindukush and Himalayan region, where pure wild forests

exist in hot, dry valleys (Champion et al., 1965). The species has been introduced into the East and West Indies and is cultivated in all countries where the climate is warm (between the subtropical and subtemperate regions), including in Asia and especially the eastern Himalayas and southern Europe (Adhikari and Adhikari, 2010). Similar to Nepal, only one species of *Punica* (*Punica granatum* L.) has been reported in the Hindukush and Himalayan ranges of Pakistan in wild and cultivated form, grown on the open and dry slopes of the relatively warm valleys and the outer hills at an altitudinal range of 700 to 2300 m (Bista et al., 2001; Khan et al., 2010). It is frequently distributed below the pine zone and is rarely found in association with *Pinus roxburghii* (personal observations). *Monothecha buxifolia* and *Olea ferruginea* are the most common and economically important species associated with *Punica granatum* forests (Khan et al., 2011) between 700 and 1800 m of altitude, whereas *P. roxburghii* grows between 700

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and 2200 m above sea level and has no association with *Punica*.

*Punica* seeds have a high content of fat (41%) and protein (37%) while the fleshy layer is edible and the juice is highly nutritious for patients (Adhikari and Adhikari, 2010). In Pakistan and neighboring countries, the fruit is very expensive and commercial extraction every summer (August and September) provides the main source of income for local inhabitants (Rawat et al., 2010). The dried seeds is locally known as anardana in Pashto/Urdu and is available on the market for the price of 500–800 rupee per kilogram (Ibrar et al., 2003) depending on the quality. However, there is a growing concern that the uncontrolled harvest of fruits for human consumption depletes the seed bank and threatens the natural regeneration of the species, as the commercial source of anardana is said to be the wild trees of *Punica* (Khan et al., 2011). Other overriding factors leading to low population recruitment are the natural low production of fruits per adult tree each year (personal observation), insect damage to fruits (Orwa et al., 2009), climate change, and primary forest fragmentation and anthropogenic perturbation, including expansion of the agricultural frontline (Siddiqui et al., 2009). Hence, forest types are considered to be at great risk of losing plant diversity, including *Punica* natural forests in Pakistan (Khan et al., 2010).

Owing to the importance of the species there is a growing concern to take initiatives for the grafting of Kabali pomegranate (Kandahari Anar) to wild *P. granatum* in some areas of Pakistan, regrettably without any proper research or studies. Generally, *P. granatum* growing in Pakistan has the potential to grow well, like the species in other countries, particularly in Afghanistan and Iran. However, adequate silvicultural treatments are needed in order to produce trees and graft better varieties to elevate the fruit production. Before silvicultural treatments and grafting, environmental factors that affect the distribution, establishment, and growth of *Punica granatum* reproduction must be understood on a scientific basis. Very little is known about the quantification of prominent ecological relationships of environmental factors, and particularly soil physicochemical factors, in determining the distribution of *Punica granatum* communities in subtropical dry temperate forests of Pakistan.

Despite the growing concern about grafting the species under the presence of such climatic and anthropogenic stresses, the remaining primary *Punica granatum* forests of Pakistan lack studies that explain their relationships with environmental factors in a quantitative manner, apart from a few studies conducted on the mixed broadleaved and conifer forests in the northern areas (e.g., Ahmed et al., 2011; Khan et al., 2011, 2013, 2014; Khan, 2012; Ali et al., 2014). Most of the previous studies provided no

information on the relationships between *Punica granatum* communities and environmental parameters that could help identify ecological requirements of the species in its current niches in the Hindukush region. As a result, the Forest Department and other agencies are lagging far behind in implementing any effective monitoring and conservation strategies. Therefore, the present study was conducted with the objectives of providing a preliminary assessment of the numerical classification and investigating relationships between environmental variables in an ecologically unexplored natural forest of *Punica granatum* (wild) in District Dir (Lower) of northern Pakistan, using multivariate statistical methods to assist in sound conservation and management plans for its natural population.

## 2. Materials and methods

### 2.1. Study site

The subtropical dry deciduous mixed broadleaf forest, geographically located at 35°50'N and 34°22'N and 71°2'E and 72°3'E longitudes, enriched with *Punica granatum*, is one of the remnant natural forests in the foothills of the Malakand Division of Khyber Pakhtunkhwa, Pakistan (Figure 1). The forests generally form an ecotone with subtropical dry temperate pine forests and lie under the jurisdiction of the Dir Forest Division. The forests are within the continental type of climate zone without monsoons with four different seasons, where June is the hottest month (maximum average temp is 32.52 °C and minimum is 15.67 °C) and January is the coldest (max is 11.22 °C and min is -2.39 °C), with total rainfall of 242.22 mm in March.

The available data of the nearest meteorological station located in Timergara (866 m a.s.l.) show that average monthly rainfall substantially varies throughout the year depending on the elevation gradient (Khan et al., 2010). Relative humidity remains moderate (30%–70%) throughout the year, with the highest (80.37% to 86.27%) during the months of November to February. The mountains receive snowfall from late November to mid-March. The topography of the area is uneven and varies from medium to steep slopes, hillocks, plains, and massive mountains of different elevations (700 to 3500 m a.s.l.) with undulating valleys, ridges, and water channels with many tributaries joined to the rivers Panjkora and Swat. The mountainous soil texture is loamy to slightly sandy, having a high proportion of rock fragments, and acidic to slightly alkaline in nature. In addition, the soil of plains or lowlands is alluvial to clay-loam to sandy-loam in texture with an alkaline nature and is mostly under agriculture practices. The areas are generally composed of igneous, sedimentary, and metamorphic rocks with various subtypes.

## 2.2. Vegetation survey

After repeated reconnaissance of the entire region of the Dir Lower District (ca. 500 km<sup>2</sup>), 40 sites were selected for the sampling of *Punica granatum* populations that covered the total range of vegetational variation during 2012 and 2013 (Figure 1). At each site quadrates of 20 × 20 m were taken systematically along a line transect (at 50-m intervals) for the enumeration of all tree species, including *P. granatum*, which were measured for girth (>2 m height). Data on understory shrubs and herbs including saplings (0.5-2 m) and seedlings (0.5 m) of the dominant species were obtained from subplots of 5 × 5 m in size and 2 × 2 m established at the center of individual main plots. Trees, saplings, and seedlings were classified based on height following Wang et al. (2006) and Gairel et al. (2010).

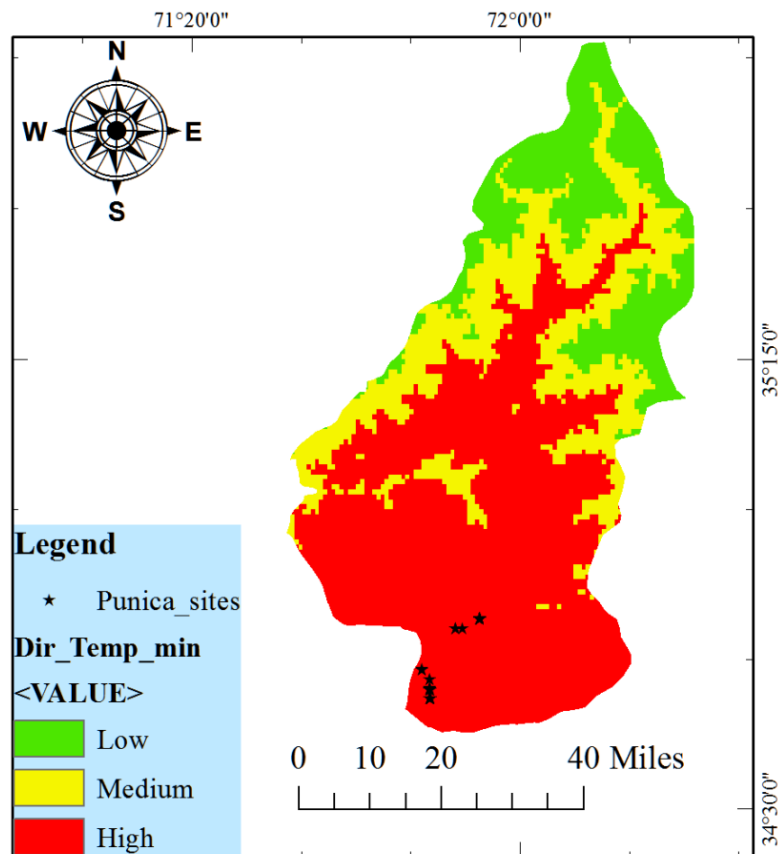
All plants were identified to species level with the help of a senior taxonomist following the nomenclature of *Flora of Pakistan* (Ali and Qaiser, 1995), and voucher specimens were deposited for future reference in the Laboratory of Plant Ecology, Botanical Garden and Herbarium, University of Malakand. The data were analyzed with

computer programs, MS Excel and XL-Stat ver. 2.10, for quantitative phytosociological attributes, i.e. relative frequency, relative density, and relative dominance (cover) (Curtis and McIntosh, 1950). These attributes were added together to obtain importance values for constituent tree species (Ahmed and Shaukat, 2012).

## 2.3. Environmental data

We measured 12 environmental variables including topographic, edaphic, and soil physical and chemical variables in order to ecologically interpret the main vegetation gradients. Among the topographic variables, the elevation and aspect of individual sites were recorded using a handheld Vista German Global Positioning System and slope was recorded with the help of a Suunto PM-5 handheld clinometer. The soil pH was measured immediately in the field making a suspension of 1:5 fresh soil:water (Khan et al., 2014) by a dynamic soil pH (model P9565-1EA) and moisture content by a digital moisture meter (VG-Meter-200).

For soil physical and chemical constituents, five freshly dug soil samples randomly located in 20-cm pits



**Figure 1.** Study area: map showing *Punica granatum* strata along the minimum temperature classes. The red area indicates the approximate location and extent of the initial escarpment of the study area from south to north and east to west where wild *Punica granatum* predominate.

from nearly level or gentle slopes were collected from lower, middle, and upper forest floors. They were bulked together, thoroughly mixed in the field, and air-dried at room temperature in the laboratory. The finer particles (silt and clay) were assessed using the hydrometer method, whereas total nitrogen was determined following the Kjeldahl method (Bremner and Mulvaney, 1982) and oxidation of soil organic matter by  $H_2SO_4$  and Se reagent mixture (catalyst), conversion to ammonium, distillation, and titration with HCl. Soil organic matter content was determined following the method of Jackson (1958). The Kjeldahl procedure of Bremner (1965) was followed for the determination of total nitrogen (N) and available soil phosphorus (P). Exchangeable potassium ( $K^+$ ) was estimated using a Jenway (PFP7) flame photometer following standard procedures.

#### 2.4. Classification of vegetation

The stands (i.e. the sampled forest sites) were clustered with two-way hierarchical agglomerative clustering techniques (McCune and Grace, 2002) using importance values for tree species. Ward's Euclidean (Pythagorean) distance measure was used as a group linkage method. Hierarchical clustering was selected over other classification methods due to poor performance with heterogeneous datasets containing more than one important gradient and the loss of information from quantitative data inherent in the 'pseudospecies' concept (Belbin and McDonald, 1993; Legendre and Legendre, 1998).

The importance values of all species were standardized by relativization through the maximum option of the PC-ORD software before starting the analysis. This procedure is recommended as clustering of data with Ward's method and Euclidean distance is often improved (McCune and Grace, 2002). Importance values of species in each cluster were summarized and the first two species having the highest mean values were used in naming the community types.

The overall environmental characteristics of the groups defined by Ward's agglomerative cluster analysis were analyzed using univariate analysis of variance (ANOVA) to determine whether environmental characteristics significantly ( $P \leq 0.05$ ) varied among the communities. Furthermore, the mean vectors of these variables were statistically evaluated by Hotelling's  $T^2$  multivariate statistics and normality was checked following Liang et al. (2009). In this analysis 40 forest stands, 20 plant species, and 13 environmental variables were used after the standardization and conversion of some variables (i.e. aspect, i.e. north = 1, west = 2, south = 3, east = 4, and so on) into ordered scale variables for quantitative analysis as recommended by Palmer (2005) and Khan et al. (2013). In classification and ordination we abbreviated the species names (e.g., *Punica granatum* = Pg; *Monothea buxifolia*

= Mb), forest stands (stand 1= St. 1; stand 2 = St. 2), and variables (elevation = Elev; organic matter = OM).

#### 2.5. Diversity analysis

We estimated Margalef's ( $D_{Mg}$ ) Fisher's alpha, Shannon diversity indices ( $H' = -\sum p_i \ln(p_i)$ ) (Magurran 1988), and Simpson diversity ( $D' = 1/\sum (p_i)^2$ ) in different groups. Evenness or equitability ( $J = H'/H'_{max} = H'/\ln S$ ) indices (Pielou, 1969) of abundance were also calculated using the Biodiversity online calculator. These diversity measures are very attractive to ecologists because these are simple, easily calculated, readily appreciated, and easy to communicate to policy makers and lay people for various ecological settings (Purvis and Hector, 2000).

In order to determine the compositional similarities among different vegetation groups the Morisita–Horn index was applied using a two-way probabilistic approach following Chao et al. (2008). This abundance-based index is generally a recommended technique and gives statistically valid results affected by common species in the communities (Chao et al., 2008). For estimation of compositional similarity a bootstrap technique was used to obtain the 95% confidence interval with 200 simulations using SPADE software Ver. 2.56 following Chao and Shen (2010).

#### 2.6. Ordination of vegetation

All 40 forest stands (sample plots) and 20 species were subjected to nonmetric multidimensional scaling (NMS) ordination using PC-ORD software package 5.10 (McCune and Mefford, 2006). Importance values of species were used to ordinate the sampled forests to illustrate their relationships with corresponding environmental variables. A Sorensen distance measure of 50 runs (250 maximum iterations) with real data indicated that the minimum stress of a 2-D solution was lower than would be expected by chance ( $P = 0.00001$ ). Thus, a satisfactory 2-D NMS ordination solution was obtained with final instability of 17.91 and 0.00481, respectively. Quantitative Sorensen (Bray–Curtis) and varimax rotation was used because this distance method permits ready comparison of results with those of hierarchical cluster analysis (Perrin et al., 2006).

Pearson correlation coefficients were used to test the intracorrelation among the variables and NMS-ordination axis to expose one or more variables to describe the strongest patterns in species composition with the Monte Carlo permutation test ( $P = 0.05$ ) to statistically evaluate whether NMS extracted stronger axes than expected by chance.

Since species exhibited a bell-shaped rather than a linear response to environmental gradients, stepwise discriminant analysis using SPSS 16.10 was performed for the texture parameter and nitrogen content considering Ward's clusters as groups. This procedure is generally used to model the value of a dependent categorical variable

based on its relationship to one or more predictors and has been successfully used in ecology to determine the habitat characteristics that allow or prevent a species' existence (Tabachnik and Fidell, 1996). The use of classification and ordination methods in vegetation ecology has been advocated by several authors (McCune and Grace, 2002) for yielding complementary results.

### 3. Results

#### 3.1. Species composition of the *Punica granatum* forests

A total of 400 quadrates were measured during this study, resulting in a total survey area of 16,000 m<sup>2</sup>. Forests were selected with at least 1–2 ha in area that were free from extreme disturbances. Forest stand inclines were from 0° to 40° and altitude ranged from 836 to 1570 m a.s.l. Specific site variables and geographical coordinates were recorded. The vegetation survey of 40 stands identified 20 tree species belonging to 13 families of 18 genera with a total of 78 understories comprising 74.35% herbs and 25.65% shrubs. Among the understories the frequency of

perennials was high as compared to annuals and biennials (Table 1).

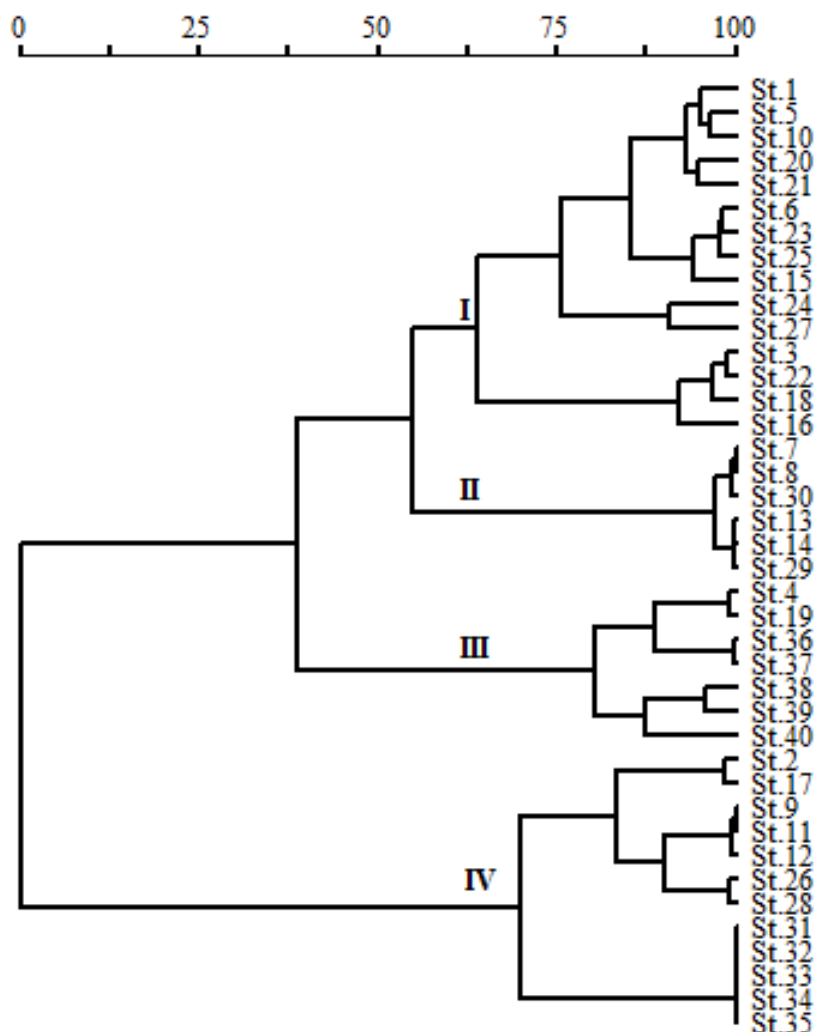
Tree vegetation was satisfactorily clustered into four groups at 65% of the information where 4 distinct groups (I–IV) representing a compromise between homogeneity of the groups and the number of group were defined (Figure 2). With the help of Ward's agglomerative cluster analysis, various stands were subjectively assigned to these four community types that were ecologically interpretable with different environmental variables, i.e. topographic, edaphic, and soil variables, located in geographically close locations. All of the structural traits and floristic compositions were significantly different ( $F = 46.031$ ;  $P < 0.01$ ) among the four vegetation types.

Community type I is dominated by *Punica granatum* and *Olea ferruginea* with an average importance value of  $60 \pm 1.48$  and  $10 \pm 2.1$ , respectively (Table 2). Among the understories rare species were abundant, followed by the seldom present category, whereas constantly present species were negligible in this community. This community

**Table 1.** Summary of the species recorded in the forests of *Punica granatum* in District Dir, Malakand Division, Pakistan.

S. no.	Family name	No. of species	Herbs	Shrubs	Annuals	Biennials	Perennials	S. no.	Family name	No. of species	Herbs	Shrubs	Annuals	Biennials	Perennials
1	Berberidaceae	1	-	1	-	-	1	26	Anacardiaceae	1	1	-	-	-	1
2	Budlijaceae	1	-	1	-	-	1	27	Asteraceae	9	9	-	2	3	4
3	Cornaceae	1	-	1	-	-	1	28	Saxifragaceae	1	1	-	-	-	1
4	Thymeleaceae	1	-	1	-	-	1	29	Nyctaginaceae	1	1	-	-	-	1
5	Urticaceae	2	1	1	-	-	2	30	Cannabaceae	1	1	-	1	-	-
6	Sapindaceae	1	-	1	-	-	1	31	Chenopodiaceae	3	3	-	2	-	1
7	Celastraceae	1	-	1	-	-	1	32	Cucurbitaceae	1	1	-	1	-	-
8	Papilionaceae	2	1	1	1	-	1	33	Poaceae	3	3	-	-	-	3
9	Oleaceae	1	-	1	-	-	1	34	Ericaceae	1	1	-	-	-	1
10	Acanthaceae	1	-	1	-	-	1	35	Fagaceae	1	1	-	1	-	-
11	Euphorbiaceae	2	1	1	1	-	1	36	Fumaricaceae	1	1	-	1	-	-
12	Myrsinaceae	1	-	1	-	-	1	37	Rubiaceae	2	1	-	1	-	1
13	Myrtaceae	1	-	1	-	-	1	38	Hypericaceae	1	1	-	-	-	1
14	Apocynaceae	1	-	1	-	-	1	39	Lauraceae	1	1	-	-	-	1
15	Orobanchaceae	1	-	1	-	-	1	40	Linaceae	1	1	-	-	-	1
16	Asclepiadaceae	2	1	1	-	-	2	41	Malvaceae	1	1	-	1	-	-
17	Rosaceae	2	1	1	-	-	2	42	Onagraceae	1	1	-	1	-	-
18	Solanaceae	3	2	1	1	-	2	43	Boraginaceae	1	1	-	-	1	-
19	Rutaceae	1	-	1	-	-	1	44	Oxalidaceae	1	1	-	1	-	-
20	Rhamnaceae	1	-	1	-	-	1	45	Plantaginaceae	1	1	-	-	-	1
21	Amaranthaceae	2	1	-	2	-	-	46	Polygonaceae	2	2	-	1	-	1
22	Polypodiaceae	1	1	-	-	-	1	47	Scrophulariaceae	2	2	-	1	-	1
23	Limiaceae	6	6	-	1	-	5	48	Verbinaceae	1	1	-	1	-	-
24	Alliaceae	1	1	-	1	-	-	49	Leguminaceae	1	1	-	1	-	-
25	Primulaceae	1	1	-	1	-	-	50	Violaceae	1	1	-	-	-	1
Σ=		38 <sup>a</sup>	12 <sup>a</sup>	20 <sup>a</sup>	8 <sup>a</sup>	0 <sup>a</sup>	30 <sup>a</sup>			40 <sup>b</sup>	33 <sup>b</sup>	0 <sup>b</sup>	16 <sup>b</sup>	4 <sup>b</sup>	20 <sup>b</sup>
Σ <sup>a+b</sup> =		78 <sup>a+b</sup>	58 <sup>a+b</sup>	20 <sup>a</sup>	24 <sup>a+b</sup>	4 <sup>b</sup>	50 <sup>a+b</sup>								
%			74.3	25.6	30.8	5.2	64.1								

a: Species in column a, b: Species in column b



**Figure 2.** Ward's agglomerative cluster analysis of 40 forest stands dominated by *Punica granatum* L. showed 4 major community types.

type was located at an average elevation of  $1271 \pm 55$  m on extremely steep ( $27 \pm 2.21^\circ$ ) rocky northeast prone sites with high species richness, Shannon–Wiener diversity, and equitability (Table 3). The group members extend their distribution to the pine zone where *P. roxburghii* is a minor associate with an importance value of  $<5\%$ . The soil textural characteristics demonstrate that the soil is sandy loam, comprising a high percentage of sand particles (70.86%) as compared to silt (15%) and clay (13.63%) (Table 4). Soil pH is slightly acidic with a small fraction of lime, which is often used to reclaim the acidic soil. Organic matter was comparatively low, resulting in the low availability of phosphorus and nitrogen. Potassium ( $K^+$ ) content was generally high as compared to the proceeding community (Type II).

Type II is dominated by *Punica granatum* (importance value =  $66.16 \pm 0.3$ ) and *Monotheca buxifolia* (importance

value =  $26 \pm 1.4$ ) located at comparatively high elevations ( $1509 \pm 21$  m) and degree of slope, forming an association with *Pinus roxburghii*. Understory vegetation shows a similar pattern with the prior community, though no species occurred in the constant category. Alpha diversity parameters within this group were lower than those of the other identified vegetation types. The soil associated is somewhat gritty, slightly plastic and acidic in nature, with a low content of organic matter and percent lime (Table 4). Nitrogen and potassium were lowest while phosphorus was comparatively high in this group.

Type III has the second highest species richness, diversity, and equitability, dominated by *P. granatum* and *Quercus ilex* with mean importance values of  $58 \pm 2.6\%$  and  $22 \pm 2.9\%$ , respectively (Table 2). The codominant species at middle elevations (mean  $\pm$  SE =  $1414 \pm 29$  m) with gentle slope ( $19 \pm 2.8^\circ$ ) lie in between the prior

**Table 2.** Mean ( $\pm$ SE) importance values for the main tree species in four communities of subtropical dry temperate *Punica granatum* (Wild) forest.

Species	Group I	II	III	IV
	Mean $\pm$ SE	Mean $\pm$ SE	Mean $\pm$ SE	Mean $\pm$ SE
<i>Punica granatum</i>	60 $\pm$ 1.48	66.16 $\pm$ 0.3	58.07 $\pm$ 2.6	89.24 $\pm$ 2.9
<i>Monothecha buxifolia</i>	9 $\pm$ 1.9	26.26 $\pm$ 1.4	1.62 $\pm$ 0.9	4.04 $\pm$ 1.8
<i>Olea ferruginea</i>	10.8 $\pm$ 2.1	2.9 $\pm$ 1.13	6.74 $\pm$ 2.8	2.8 $\pm$ 1.7
<i>Robinia pseudoacacia</i>	0.83 $\pm$ 0.54	-*	-	-
<i>Pinus roxburghii</i>	4 $\pm$ 1.2	1.2 $\pm$ 0.8	-	0.9 $\pm$ 0.6
<i>Quercus ilex</i>	3 $\pm$ 1	-	22.7 $\pm$ 2.9	0.3 $\pm$ 0.3
<i>Celtis europea</i>	1.5 $\pm$ 0.4	-	4.8 $\pm$ 1.9	-
<i>Acacia modesta</i>	0.5 $\pm$ 0.27	-	4.3 $\pm$ 2	-
<i>Pyrus communis</i>	-	-	0.53 $\pm$ 0.35	-
<i>Ficus carica</i>	1.8 $\pm$ 0.64	-	0.24 $\pm$ 0.24	0.34 $\pm$ 0.34
<i>Ailanthus altissima</i>	4 $\pm$ 1.7	3 $\pm$ 1.17	-	0.72 $\pm$ 0.5
<i>Melia azedarach</i>	1 $\pm$ 0.56	-	-	-
<i>Morus alba</i>	1.35 $\pm$ 1	0.49 $\pm$ 0.49	0.24 $\pm$ 0.24	0.44 $\pm$ 0.44
<i>Diospyros kaki</i>	0.29 $\pm$ 0.2	-	0.24 $\pm$ 0.24	-
<i>Juglans regia</i>	0.2 $\pm$ 0.2	-	0.25 $\pm$ 0.25	0.6 $\pm$ 0.4
<i>Platanus orientalis</i>	-	-	0.27 $\pm$ 0.27	-
<i>Morus nigra</i>	0.67 $\pm$ 0.51	-	-	0.29 $\pm$ 0.29
<i>Eucalyptus</i>	1 $\pm$ 0.7	-	-	-
<i>Mallotus philippensis</i>	0.14 $\pm$ 0.14	-	-	-
<i>Zanthoxylum armatum</i>	-	-	-	0.29 $\pm$ 0.29

\*: Absent.

**Table 3.** Alpha diversity patterns within different groups isolated by Ward’s cluster analysis.

Alpha diversity	Group I	Group II	Group III	Group IV	Overall
Species richness	17	7	13	10	20
Margalef’s index (M)	2.076	0.940	1.674	1.260	2.211
Simpson’s index (1/D)	1.95	1.588	2.122	1.124	1.730
Shannon–Wiener index (H')	1.242	0.697	1.071	0.301	1.068
Pielou’s index (J)	0.439	0.358	0.418	0.131	0.357

community types. Rare, seldom present, and constant species were predominant as compared to other groups. The soil in this group is composed of a high percentage of sand particles (67.41%) followed by sand and silt, resulting in a sandy loam texture. Soil pH was acidic and organic matter was high with moderately high potassium and phosphorus content.

Type IV is a monospecific community led by *Punica granatum* (importance value = 89  $\pm$  2.9), though 10 species were associated with negligible (<5%) importance

values. The frequency of rare and seldom present species was higher as compared to other categories, indicating low diversity of the understories. The alpha diversity parameters were also comparatively lower than those of prior communities, except Type II (Table 2). This type was located just above the *Punica–Olea* and below the *Punica–Quercus* community types on northeast gentle slopes (Table 4). Two exotic species, *Robinia pseudoacacia* and *Ailanthus altissima*, were subordinate species with very low importance values. The vegetation in this group

**Table 4.** Mean ( $\pm$ SE) values of topographic, edaphic, and soil parameters for four *Punica granatum* communities in the subtropical dry temperate forest.

Factors	Group I	II	III	IV
	Mean $\pm$ SE	Mean $\pm$ SE	Mean $\pm$ SE	Mean $\pm$ SE
Elevation (m)	1271 $\pm$ 55	1509 $\pm$ 21	1414 $\pm$ 29	1393 $\pm$ 39
Slope ( $^{\circ}$ )	27 $\pm$ 2.1	31 $\pm$ 2.28	19 $\pm$ 2.8	21 $\pm$ 3.5
Aspect	6 $\pm$ 0.56	6.7 $\pm$ 0.7	7.3 $\pm$ 0.5	6.6 $\pm$ 0.7
Clay %	13.6 $\pm$ 0.6	11 $\pm$ 0.70	16 $\pm$ 1.30	14 $\pm$ 1.0
Silt %	15 $\pm$ 1.16	11 $\pm$ 0.39	15 $\pm$ 1.81	15 $\pm$ 1.0
Sand %	70 $\pm$ 1.29	75 $\pm$ 0.80	67 $\pm$ 1.93	68.9 $\pm$ 1.6
pH (1:5)	6.8 $\pm$ 0.13	6.7 $\pm$ 0.25	6.7 $\pm$ 0.15	6.78 $\pm$ 0.1
Organic matter %	1.9 $\pm$ 0.34	0.79 $\pm$ 0.30	2.5 $\pm$ 0.63	2.3 $\pm$ 0.4
Lime %	5.5 $\pm$ 0.97	4.3 $\pm$ 1.34	5.2 $\pm$ 1.77	7.04 $\pm$ 1.0
N %	0.13 $\pm$ 0.02	0.07 $\pm$ 0.02	0.16 $\pm$ 0.04	0.16 $\pm$ 0.03
P (mg/kg)	5.1 $\pm$ 0.16	5.2 $\pm$ 0.29	5.2 $\pm$ 0.25	5.5 $\pm$ 0.18
K (mg/kg)	96 $\pm$ 11	66 $\pm$ 7.20	117 $\pm$ 15	118.5 $\pm$ 14

generally grows on sandy loam soil with comparatively high contents of organic matter, lime (%), phosphorus, and potassium.

### 3.2. Analysis of variance

Twelve environmental variables of each community type (Table 2) were analyzed using ANOVA. Mean elevation ((F 3, 36) = 3.794; P = 0.0183) and slope ((F 3, 36) = 2.596; P = 0.067) of the forest stands were significantly different. Among the textural properties, percent sand exhibited significant difference ((F 3, 36) = 3.73; P = 0.20), whereas other topographic, edaphic, and soil physical and chemical variables did not differ significantly in the group means.

### 3.3. Hotelling T<sup>2</sup> test

The Hotelling T<sup>2</sup> test, a multivariate statistic, was applied for the comparison of groups derived from Ward's clustering technique on the basis of mean vectors of environmental variables. The three categories of environmental variables, i.e. topographic, edaphic, and soil nutrients, were compared between pairs of groups with respect to the first set of variables. Groups 1 and 2 were significantly different (F = 3.4, df 1 = 7, df 2 = 4, P < 0.05) while the mean vectors for Groups 1 and 3 and 2 and 3 were not significantly different. Edaphic variables were similar for Groups 1 and 2 (F = 0.9, df 1 = 7, df 2 = 4, Ns), Groups 1 and 3 (F = 0.1.5, df 1 = 7, df 2 = 7, Ns), and Groups 2 and 3 (F = 0.1.33, df 1 = 4, df 2 = 7, Ns) (Ns = nonsignificant.). None of the nutrient concentrations in soils differed between the groups.

### 3.4. Ordination and gradient analysis

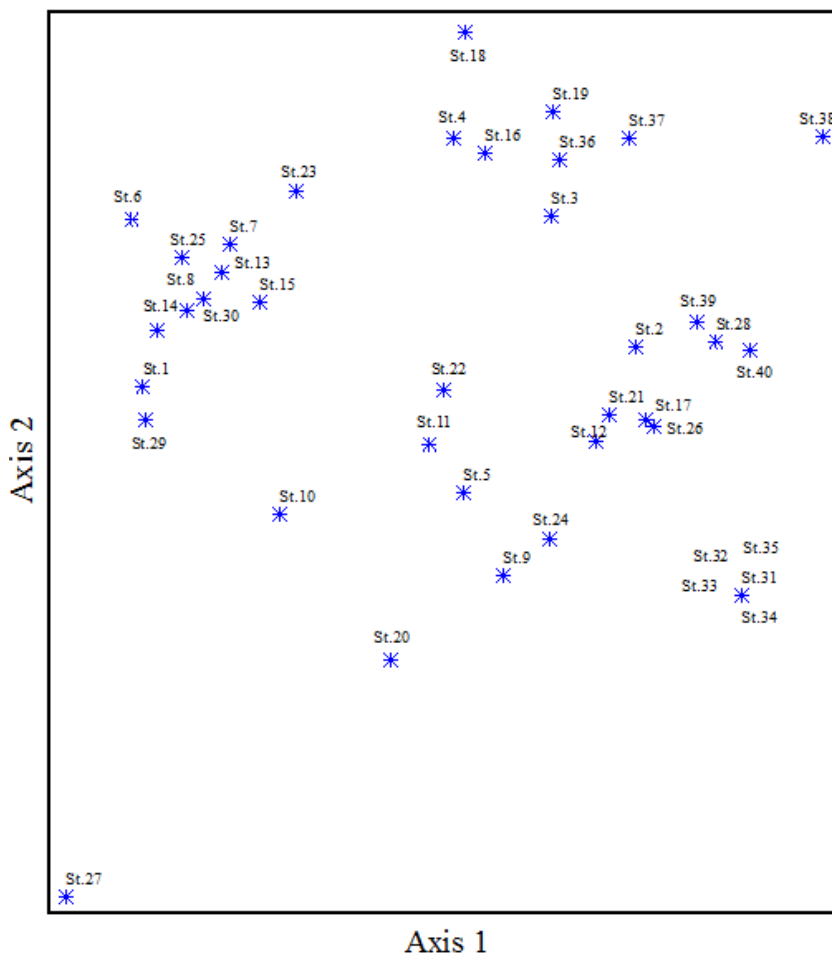
NMS produced a 2-dimensional solution (Figure 3) with a stress value of 14.27 (P < 0.01 based on 250 runs with randomized data) and a final instability of 0.000010. The

ordination explained 89% of the variance in the original dissimilarity matrix, with axes 1 and 2 accounting for 33% and 56% of the total, respectively. In the NMS four groups identified by cluster analysis were superimposed onto the graph configuration quite neatly and could be distinguished along both axis 1 and axis 2. However, considering the difference in species composition the four forest community types were maintained and the most important and codominant species are presented in Table 2.

Generally, the gradients do not necessarily have a physical reality on spatial or temporal scales, but rather are abstract dimensions of an ecological space used for explaining the distributions of organisms and their combinations in space and time. Although intraset correlation among the variables showed a strong relationship (Table 5), in NMS ordination axes 1 and 2 were positively related to the proportion of clay (R = 0.3093; P < 0.05) and proportion of sand correlated strongly (R = 0.4147; P < 0.001) with ordination axis 2 (Table 3). In addition, axis 2 exhibited a strong relationship with shifts in soil nitrogen (R = 0.4685; P < 0.001) and potassium contents (R = 0.3844; P < 0.01) (Table 6). Topographic variables, i.e. elevation, slope, and aspect, did not yield any significant relationships. The majority of the edaphic factors, i.e. pH, organic matter, lime, and nitrogen, also remained active in the distribution of vegetation in the current study area.

The relationships of soil texture coupled with nitrogen and potassium contents with community types identified by Ward's agglomerative clustering techniques were tested by discriminant analysis. The results explained a total of 70.6% and 36.8% variance with discriminant functions 1





**Figure 3.** NMS ordination between axes 1 and 2 of 40 stands dominated by *Punica granatum*. The 4 major groups obtained by Ward's agglomerative cluster analysis are superimposed on the 2-D ordination configuration.

**Table 5.** Intraset correlations among the different topographic, edaphic, and soil variables.

	Elev	Slope	Aspect	Clay	Silt	Sand	pH	OM	Lime	N	P	K
Elev (m)	1											
Slope (°)	0.35*	1										
Aspect	0.001	-0.49	1									
Clay %	-0.071	-0.39**	0.10	1								
Silt %	-0.16	-0.40**	0.27	0.13	1							
Sand %	0.13	0.50***	-0.28	-0.67***	-0.81***	1						
pH (1:5)	-0.28	-0.23	0.15	0.03	0.21	-0.15	1					
Org matter	-0.14	-0.38**	0.31*	0.49***	0.51***	-0.64***	0.28	1				
Lime %	0.03	-0.25	0.04	-0.17	0.34*	-0.14	0.39*	-0.09	1			
N %	-0.08	-0.30*	0.15	0.25	0.35*	-0.42**	0.163	0.50***	0.12	1		
P (mg/kg)	0.07	-0.20	0.03	-0.15	0.11	0.002	-0.02	-0.25	0.67***	0.04	1	
K (mg/kg)	-0.142	-0.426**	0.283	0.459***	0.765***	-0.82***	0.13	0.57**	-0.004	0.30*	-0.02	1

\*P < 0.05; \*\*P < 0.01; \*\*\*P < 0.001.

**Table 6.** Correlation coefficient (R) between different environmental variables and 2-D NMS ordination axes 1 and 2.

Variables	Axis 1 P-value	R-value	Axis 2 P-value	R-value
Elevation (m)	0.19	Ns	0.22	Ns
Slope (°)	0.13	Ns	0.20	Ns
Aspect	0.05	Ns	0.03	Ns
Clay %	0.30*	P > 0.05	0.30*	P > 0.05
Silt %	0.16	Ns	0.27	Ns
Sand %	0.32*	P > 0.05	0.41***	P > 0.001
pH (1:5)	0.002	Ns	0.01	Ns
Org matter %	0.14	Ns	0.24	Ns
Lime %	0.05	Ns	0.19	Ns
N %	0.03	Ns	0.46***	P > 0.001
P (mg/kg)	0.10	Ns	0.17	Ns
K (mg/kg)	0.13	Ns	0.38**	P > 0.01

Org matter = organic matter; N = nitrogen; P = phosphorus; K = potassium;  
\* = P < 0.05; \*\* = P < 0.01; \*\*\* = P < 0.001; Ns = nonsignificant.

and 2, respectively. Most of the sites (80%) were correctly classified while the remaining 20% were misclassified, maybe due to other biotic disturbances.

#### 4. Discussion

##### 4.1. Classification of vegetation

The numerical analysis used in the present study has proved to be useful in classifying the *Punica granatum*-dominated vegetation into four main community types (Groups I–IV). These vegetation types are linked to certain topographic, edaphic, and soil factors. *Punica granatum* did occur in all of the sample plots and was ultimately present in all of the communities as a dominant species, which reflects its broad distribution in these forests. Generally, these communities vary significantly in species composition and were clearly segregated by cluster analysis that revealed a major dichotomy of vegetation composition into two elevation phases, i.e. lowland (Groups I and III) and upland (Groups II and IV) (Figure 2).

The lowland communities, namely *P. granatum*-*O. ferruginea* (Group I) and *P. granatum*-*Q. ilex* (Group III), are comparatively more species rich than the valley upland communities (Table 2). These species-rich communities are located at low elevations on northeast hill slopes. However, *P. granatum* and *M. buxifolia* that dominate the upland drier sites with comparatively steep slopes are species poor compared to the other communities in the area. Species in this group were characterized by shrubs as the most common understories are similar apart from *M. buxifolia* that frequently coexists with *Punica* in the

lower hills of Dir (Khan et al., 2010). The first cluster with species groups was generally existing in the northwest stands from the nearly monospecific *Punica granatum* vegetation, which is located above the *Punica*-*Olea* and below the *Punica*-*Quercus* community types on northeast gentle slopes (Table 4).

Species like *Ficus carica*, *Juglans regia*, and *Morus alba* lead the list of minor associates intermingling with *Pinus roxburghii* although the *P. granatum* and *Q. ilex* community type forms an ecotone with *Pinus roxburghii* at upper elevations. These facts were more clearly shown by the importance values of the sites for different species (Table 2) and were similar to findings reported by Khan et al. (2010, 2011), who reported the occurrence of *Pinus roxburghii* with broadleaved and deciduous species at higher elevations in the study area.

Generally, *P. roxburghii* starts at comparatively high elevations and *Punica granatum* is frequent below *Quercus* and rarely found with *Pinus* (Ahmed et al., 2006). This may be due to precipitation in the form of snow at high elevations that remains on the slopes for extended periods in winter and early spring and increases soil moisture contents, which supports typical high mountain slope vegetation. In *Punica* forests two exotic species, *A. altissima* and *Robinia pseudoacacia*, were subordinate species with very low importance values at low elevations. The occurrence of these two species in the natural vegetation may be due to the forestation practices of the forest department or local inhabitants to meet the need for fuel wood for domestic purposes as reported by Hazrat et al. (2007).

Compared with other studies from subtropical dry and moist temperate forests, i.e. the Swat and Muree adjoining hilly areas, the *Punica granatum* forests are low in species richness (Shaheen et al., 2010). This may be due to the variation in climate, physiographic, and soil physical and chemical properties (Ali et al., 2014). The current results also emphasize the importance of soil mechanical and physiochemical properties in influencing tree species composition and richness from lower to high elevation with species being tolerant to higher pH and least acidic sandy-loam soils. This may explain the postulated lower to high elevation gradient of species composition in the study area. Khan (2012) noted prominent variations in species distribution in these mountain forests in respect to soil moisture regime and other environmental variables, but the elevation gradient was overriding in the vegetation distribution of northern mountains as reported by several authors (e.g., Ahmed et al., 2011; Khan et al., 2011, 2013; Khan, 2012; Ali et al., 2013).

Contrary to the tree stratum, the understory vegetation was diverse and showed clear similarities in the groups identified by cluster analysis. Most of the species were rare and seldom present, whereas few individuals were in the categories of often, mostly, and constantly present according to Raunkiaer (1934). The close similarities in the understories may be due to the influence of similar environmental factors and a long history of anthropogenic disturbances as few factors showed significant differences using univariate and multivariate tests.

#### 4.2. Relationship between plant communities and environmental variables

Axes 1 and 2 of the NMS stand ordination (Figure 3) were used to generate hypotheses in identifying probable environmental attributes in the distribution of individual stands and species. NMS ordination of 40 sampled stands revealed significant stand group segregation along both axes 1 and 2. The zonation of *Punica granatum*-dominated vegetation with respect to elevation is conspicuous in the study area. Topographic factors, i.e. elevation and slope, remain active in organizing the final grouping of stands and species as none of these variables exhibited a strong correlation with either of the ordination axes. However, previous studies showed that most of the vegetation distribution in the northern Himalayan and Hindukush mountain ranges are generally linked with physiographic factors that produce characteristics locally or even as microclimates (e.g., Ahmed et al., 2011; Khan, 2012; Khan et al., 2013, 2014). Champion et al. (1965) reported that the ecological attributes of vegetation largely depend on elevation, which determined species distribution and vegetation associations in northern Pakistan.

In the present study the results of NMS ordination suggest that soil textural parameters like sand and clay

contents have great impacts on the distribution of *Punica granatum* dominated communities. Total nitrogen (N) and potassium (P) contents are also significantly correlated with the second axis of ordination, indicating that soil contents also play a key role in the distribution pattern of *Punica granatum* and associated tree species in the study area (Table 6). These results indicated that soil physical (textural properties) and chemical parameters combine to play an important role in controlling the distribution of *Punica granatum*-dominated vegetation in the region. However, soil textural properties were overriding factors responsible for the distribution in the current forests as explained by the discriminant functions. It was argued that soil texture governs most of the properties of soil, i.e. permeability, water retention capacity, degree of aeration, nutrient storage in the clay-humus complex available to plants, ability to withstand mechanical working of the top soil, and, finally, ability to support a permanent plant cover.

Chahouki et al. (2008) stated that soil texture and geomorphology control the distribution of plant species by affecting moisture availability, ventilation, temperature, and distribution of plant roots (Chuang-Ye et al., 2009). The principal reason for weak or no correlation with many other environmental variables seems to be the disturbance regime of vegetation, primarily owing to legal or illegal logging, cutting of trees for fuel, and grazing of domestic animals. Soil texture and biotic stress may synergistically influence patch turnover while other anthropogenic disturbances like grazing may cause a decrease in clay content and an increase in sand content (Dagar, 1987), trampling may enhance the loss of soil through surface runoff (Novikoff, 1983), and the removal of vegetation may lead to soil loss, leading to the shallowness of soil as observed in the *Punica granatum* forest floors. Soil depth began to increase considerably at the upper *Pinus roxburghii* ecotone with a thick layer of litter while shallow soil was underneath this zone in *Punica granatum* and associated vegetation. Shallow soil does not store abundant water and more water is stored in deeper soils, which can support relatively large trees, such as *Pinus roxburghii*, *Pinus wallichiana*, etc. Hence, it was concluded that shallowness of soils may be an important factor in the development and distribution of *Punica granatum* in these areas and could be considered as a factor of interest in future studies in these forests.

Among the chemical elements potassium is also one of the effective factors in the distribution of vegetation types, which is an indicator of the separation of grassland and scrublands, i.e. a high K/Mg ratio is suitable for shrub growth (Jensen et al., 1998; Xu, 2010). These facts are clear from the current forest survey where most of the trees were scrubs and *Punica granatum* was rarely growing as

huge trees (personal observation). The role of potassium, nitrogen, and soil textural properties as controlling factors in vegetation distribution have been documented in the past (e.g., Udoh et al., 2007; Arshad et al., 2008; Khan et al., 2013).

#### 4.3. Implications and future directions

Results of the multivariate analysis (classification and ordination) using vegetation environmental data support previous studies in northern Pakistan that certain abiotic factors, i.e. soil textural and chemical properties, significantly affect the distribution of *P. granatum* and associated species. *P. granatum*, *O. ferruginea*, *M. buxifolia*, and *Quercus ilex* were the strong indicator species in Groups I, II, III, and IV, respectively.

These assemblages in the study area may be representative of vegetation and landscape conditions on high mountain slopes throughout northern Pakistan. However, distributions of species are strictly related to environmental variables, but nevertheless correlation between two variables does not necessarily mean that a cause-and-effect relationship exists between them. Understanding additional ecological attributes or processes is essential for developing a local model that can accurately predict species and community distribution and abundance (Ali, 2015). Establishment of long-term plots, as well as experimental, physiological, and ecosystem approaches, are necessary to determine cause-and-effect relationships between the distributions of *Punica granatum* and associated vegetation with associated abiotic factors in northern Pakistan. Other future ventures could be the

understanding of the species response to the changing climate, as climate change might be the most significant factor controlling, for example, the soil properties and thus the distribution of the species.

Relationships between *Punica granatum* communities and environmental variables, soil textural parameters like sand and silt, and the combination of total nitrogen and potassium contents are overriding factors affecting the distribution of *Punica granatum* and associated tree species. The results showed that *Punica granatum* is often associated with broadleaved species and seldom occurs in the pine zone (*Pinus roxburghii*). The species is heavily exploited by local inhabitants for various domestic needs and is thus depleting at an alarming rate. Based on the results, it is recommended that other factors, i.e. spatial and biological factors, may also be considered for further detailed studies on *P. granatum* that will provide a broader framework for identifying important factors related to species distribution. These findings will prove to be very helpful in the management and conservation of *P. granatum* woodlands.

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