

RESEARCH ARTICLE

Evaluation of Plant Species Diversity and Floristic Characteristics in Plant Ecological Group in Relation to Altitudinal Gradient, Kourdkoy Forest, North of Iran

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ABSTRACT

Forest ecosystems have vegetation cover as their fundamental structural element that demonstrates how environmental parameters operate. This study investigated the effect of altitude variations on plant community and diversity patterns in the Kourdkoy Forest located in Golestan province of northern Iran. We established 48 circular plots with an area 1000 m² at each of the six different elevational belts from 500 to 1700 m above sea level at 200 m intervals using random-systematic sampling method. Tree species at each plot were measured, while the herbaceous species survey in 100 m² subplots. Using Two-Way Indicator Species Analysis (TWINSPAN) identified five plant communities known as Cyclameno-Fagetum community, Carexeto-Fagetum community, Athyriumeto-Fagetum community (appeared on two classes), and Galiumo-Fagetum community named according to their dominant and indicator species. among the 60 plant species belonging to 37 families and 55 genera which were found in the studied area. ANOVA test showed that altitude served as the significant variable leading to significant diversity index differences ($P \leq 0.05$) among the communities for Shannon-Wiener, Smith-Wilson, and Margalef. The Raunkiaer method indicated hemicryptophytes prevailed as life-forms over cryptophytes by 43% and 22%, respectively and Euro-Siberian chorotype earned first place position with 40%. The research demonstrated that altitude-caused ecological transformations which occur in a vital forest system enabling understanding for temperate region conservation and management activities.

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1. Introduction

The most vital natural resources that support sustainable economic development are renewable forest resources (Kulakowski et al., 2017; Acwin Dwijendra et al., 2022). Natural resources attribute together with their changing patterns have increased human interest in understanding environmental effects on plant communities (Kulakowski et al., 2017; Acwin Dwijendra et al., 2022). Vegetation cover functions as an essential ecosystem element that both demonstrates environmental characteristics and serves as a secure

information base for ecological examination (Sun et al., 2015; Mucina et al., 2016). Plant communities use species recognition and classification activities at the plant community level to derive valuable health and biodiversity information about their ecosystems (Brosofske et al., 2001). Plants do not arrange themselves at random throughout environments since their spatial patterns align closely with environmental factors (Gao et al., 2015), while multiple important elements substantially influence their organization and species structures (Kraft et al., 2015; Bjorkman et al., 2018). Plant community distribution and species frequency patterns are closely associated with

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topographic and environmental factors, including altitude, slope, climate, and soil conditions (Jafarian et al., 2019). The substantial climatic effects caused by altitudinal gradients guide the formation of various vegetation classes and forest types (Krömer et al., 2013; Sinha et al., 2018).

The creation of different climatic zones across forest ecosystems at different altitudes influences plant diversity because results from Wani et al. (2023) and Rezende et al. (2015) show peak species diversity occurs in intermediate elevation areas. The importance of conducting thorough investigations about these gradients affecting biodiversity becomes clear (Zhang et al., 2021). The most vital natural economic development resource base consists of renewable timber resources along with forests (Kulakowski et al., 2017; Acwin Dwijendra et al., 2022). Researchers accelerate their research on environmental conditions and plant community responses through heightened interest in these resources (Kulakowski et al., 2017; Acwin Dwijendra et al., 2022). Environmental conditions influence vegetation covers and plant community structure, providing an important basis for ecological assessment (Gao et al., 2015; Kraft et al., 2015; Sun et al., 2015; Mucina et al., 2016; Bjorkman et al., 2018). The identification, and classification of plant communities helps scientists understand the biodiversity of ecosystems (Brososke et al., 2001).

Altitudinal gradients are important environmental factors that create climatic variation and provide valuable opportunities to study plant community responses to environmental change that develop unique vegetation groups and forest types (Krömer et al., 2013; Sinha et al., 2018). Research conducted by Wani et al. (2023) and Rezende et al. (2015) indicated that plant species diversity reaches its maximum peak at middle altitude levels across the world. Detailed research needs to focus on the impact of these gradients since they influence biodiversity patterns (Zhang et al., 2021). The Hyrcanian forest regions extend over 1.8 million hectares of north Iranian territory that accounts for 7% of the national landscape (Kooch et al., 2023; Pourghasemi, 2016). This forest ecosystem maintains 80 tree species combined with 50 shrub species (Ali et al., 2020; Bayat et al., 2020). The broadleaved deciduous forests of the Alborz Mountains' northern slopes overlooking the Caspian Sea because they possess wide-ranging climatic elements and terrain characteristics (Pourbabaei & Haghgoy, 2013).

Biological management of variability occurs in forests alongside crucial nutrient cycling processes which support ecosystem development and maintain complete sustainability (Hosseini et al., 2024). Prior studies have reported varying peaks of diversity at different altitudes in the region, such as tree species richness peaking at 500 m in areas like Kourdkey Forest (Ahmadi et al., 2023), while other research indicates peaks at higher elevations, such as 2070 m in adjacent regions (Ataei et al., 2021), suggesting potential regional variation

(Mouquet et al., 2015; Zhou et al., 2020; He et al., 2023; Lu et al., 2023; Rahman et al., 2022; Schroeder et al., 2024; Su et al., 2024; Zhang et al., 2024).

Despite the ecological importance of Hyrcanian forests, detailed information on plant community classification and diversity patterns along altitudinal gradients in the Kourdkey forest remains limited. Research needs to address an essential knowledge gap because scientists have confirmed altitude's impact on worldwide plant diversity (Scherrer & Körner, 2011). Understanding how plant communities change along elevation gradients is important for improving ecological knowledge and supporting forest management and conservation (Noroozi et al., 2016). Therefore, this study aimed to classify plant communities using TWINSPLAN and to evaluate plant diversity patterns along an altitudinal gradient in the Hyrcanian forest ecosystem.

2. Materials and Methods

2.1. Study Area

The study was conducted in Kourdkey Forest where is situated in Golestan Province, northern Iran. It occurs at 36° 39' 00" to 36° 41' 30" N, 54° 04' 30" to 54° 07' 38" E of Golestan Province with an altitudinal gradient of 300 to 2000 m a.s.l. According to estimated climatic data from the nearest stations, annual precipitation, and mean annual temperature were 1240 mm and 12.6°C and also a 2.5-month dry season between June and August. Its temperate climate and varied topography support diverse vegetation typical of the Hyrcanian Forests (Department of Natural Resources and Watershed Management of Golestan Province, 2013).

2.2. Data Collection

Sampling was focused on the elevation range from 500 to 1700 m a.s.l. and the effects of altitude on plant community distribution were determined. The studied area was divided into six altitudinal classes at 200 m intervals according to previous methods (Pourbabaei & Haghgoy, 2013; Kessler et al., 2014). A random-systematic design established eight circular plots with a 1000 m² area in each altitudinal class through the use of 150 m spacing between the plots (Pourbabaei et al., 2019; 2020). The slope percentage and geographic aspect were documented for each plot. The diameter at breast height (DBH \geq 7.5 cm) was measured for all trees (Pourbabaei et al., 2014). The Whittaker's nested plot sampling method provided a basis for choosing appropriate subplot sizes by determining the optimized subplot size to obtain representative herbaceous species data. Results from the analysis led researchers to choose 100 m² subplots as the proper size for herbaceous species estimation. We evaluated herbaceous species occurrence in every subplot using the Braun-Blanquet criterion to measure their cover percentage (Manish et al., 2017; Pourbabaei et al., 2019).

2.3. Data Analysis

Sampling was conducted in two seasons (spring and Summer), the species were dried and pressed before identifying by using Iranica Flora (Rechinger, 1963-1998), Turkey Flora (Davis, 1965-1988), Iran Flora (Assadi, 1988-2003) and the Colorful Flora of Iran (Ghahreman, 1975-2000) in the herbarium of the Faculty of Natural Resources in University of Guilan. Moreover, the chorology of species was determined according to the Zohary and Takhtajan method (Takhtajan, 1986). In order to determine the plant communities, analysis was done with Two-Way Indicator Species Analysis (TWINSPAN) using PC-ORD software for Windows, version 4.17 that the resulting groups are referred to as plant communities based on dominant and indicator species, rather than formal phytosociological associations (Hasanpori et al., 2014). In each community, Species Important Values (SIV) were computed for tree and herbaceous species using equations 1 and 2, respectively (Pourbabaei et al., 2014):

$$\text{SIV} = \text{relative frequency} + \text{relative dominance} + \text{relative density} \quad (1)$$

$$\text{SIV} = \text{relative frequency} + \text{relative dominance} \quad (2)$$

Finally, diversity indices including Shannon-Wiener (H') which is sensitive for comparison among plant communities (Kent, 2011), Smith-Wilson as it is mathematically independent

of species richness and therefore allows a more reliable assessment of abundance distribution patterns among plant communities (Smith & Wilson, 1996), and Margalef indices that diminish the bias associated with differences in total abundance among plant communities (Gómez et al., 2000) were calculated using Ecological Methodology software for Windows, version 6.0 (Hosseinzadeh et al., 2016). Kolmogorov-Smirnov and Levene's tests demonstrated that the data passed the requirements of normality and homogeneity of variances. The analysis consisted of One-way ANOVA at $\alpha = 0.05$ with Tukey's post-hoc tests for mean comparisons between communities and altitudinal classes in SPSS v16.0.

3. Results

3.1. Floristic Composition

It has been identified 60 species belonged to 37 families, and 55 genera (6 trees, and 54 herbaceous species). The prominent families were Rosaceae (5 species), Lamiaceae (4 species), Apiaceae, Aspleniaceae, Asteraceae, Brassicaceae, and Fabaceae (each family with three species), which had the highest frequency. The result of life form classification indicated that hemicryptophytes (26 species), cryptophytes (13 species) were dominance. Other life forms were present with lower frequency, including phanerophytes (11 species), therophytes (7 species), and chamaephytes (3 species) (Figure 1).

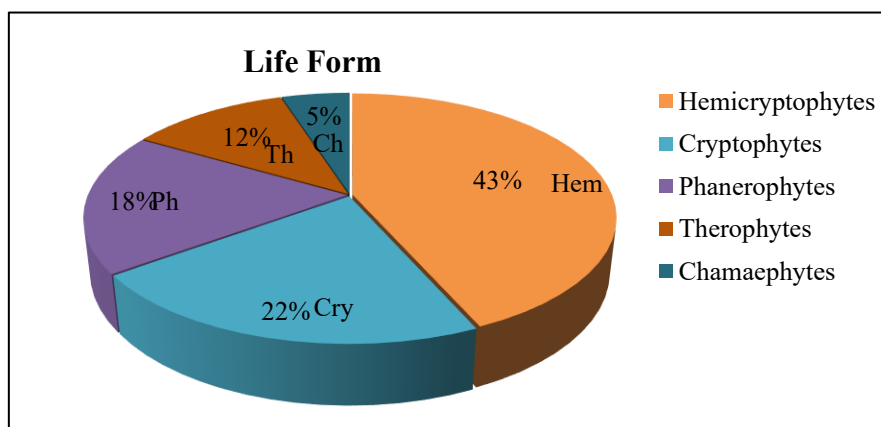


Figure 1. Life forms of trees and herbaceous species in the study area, Hem: Hemicryptophytes; Ch: Chamaephytes; Th = Therophytes; Ph = Phanerophytes; Cry: Cryptophytes.

The chorotype indicated that Euro-Siberia was dominant with 24 species (40%), Euro-Siberian-Irano-Turanian-Mediterranean with 10 species (17%), Euro-Siberian-Irano-Turanian with 7 species (11%), Euro-Siberian-Mediterranean

with 6 species (10%), Polyregional with 5 species (8%), Cosmopolitan with 4 species (7%), and Irano-Turanian with 3 species (5%) (Figure 2).

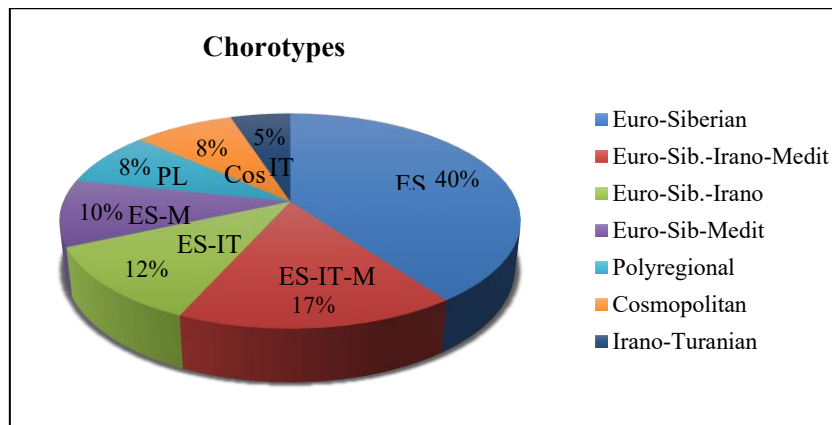


Figure 2. Chorotype of trees and herbaceous species in the study area, note: ES: Euro-Siberian; ES-M-IT: Euro-Siberian-Mediterranean-Irano-Turanian; ES-IT: Euro-Siberian-Irano-Turanian; IT: Irano-Turanian; Cos: Cosmopolitan; ES-M: Euro-Siberian-Mediterranean; Pl: Polyregional; Hyrc: Hyrcani.

3.2. The Classification of Plant Communities

The results of TWINSpan analyses were shown in Figure 3. that five distinct plant communities were identified along the altitudinal gradient, and also their accessory species in each elevation class were listed (Supplementary Table 1). At first level, 48 plots with eigenvalue 0.6059 were divided to two groups. Sixteen plots were located on left group (negative group). *Epipactis* sp. was an indicator herbaceous species in this group. In the right and positive group, there were 32 plots with *Geranium columbinum* as the indicator species. These groups did not have any indicator tree species. In the second level of classification, a group with 16 species, and eigenvalue 0.1826 was divided in 2 groups. In negative group, 8 plots were identified with *Alnus glutinosa* as indicator species without any herbaceous species. The positive group also involved 8 plots with *Euphorbia amygdaloides*. In the next step, two groups were obtained of a group with 32 plots (eigenvalue; 0.5092), 15 plots with *Dryopteris pallida* and 17 plots with *Phedimus stoloniferus* as indicator species were presented in negative and positive group, respectively. In the third level of classification

a group including 15 species and eigenvalue 0.2778 was divided to two groups. 8 plots with *Clinopodium umbrosum* were belonged to negative group and 7 plots with *Geranium* sp. were belonged to positive group. Indicator species for first level of groups has been achieved of plots that the presence of indicator species in those plots was a factor to separation. Classification process was stopped with five communities in third level. Overall, sampling plots were divided into five groups using TWINSpan. First group was belonged to first class of altitude (i.e., from 500 to 700 m a.s.l.) and included plots 1 to 8 (A). The second group had plots from 9 to 16 and was located in altitude from 700 to 900 m a.s.l. (B). The plots 17 to 24 were presented in third group that were located in altitude from 900 to 1100 m a.s.l. (C). The fourth group was belonged to 1100 to 1300 m a.s.l. (D) with 7 plots (from 25 to 31). Finally, the fifth group which was located in altitude range from 1300 to 1700 m a.s.l. (1300-1500= E₁, 1500-1700= E₂), with 17 plots. According to identification of plant ecological group using TWINSpan, each group was considered as a plant community (Abla et al., 2024) and was named by Species Important Value

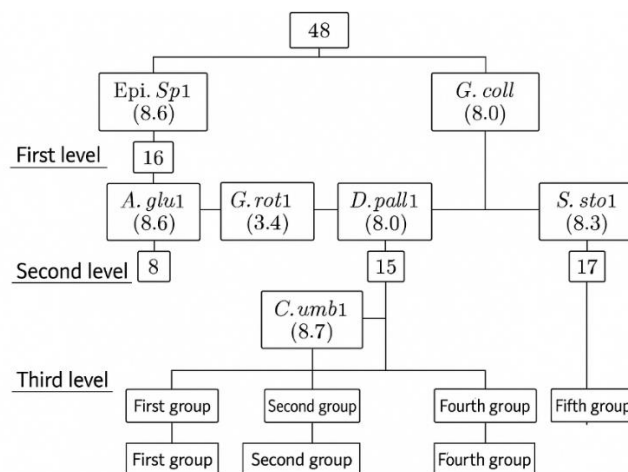


Figure 3. Classification of sampling plots using TWINSpan.

3.3. Diversity, Evenness and Richness Values among Plant Communities

The results of ANOVA test indicated that there was a significant difference among plant communities in terms of Shannon-Wiener, Smith-Wilson and Margalef indices ($P \leq 0.05$) (Table 1). The highest value of Shannon-Wiener was belonged to second community (4.20). While, the fourth

community had the lowest value (2.8) (Figure 4). The highest and lowest value of evenness was obtained for the first and third communities, respectively (Figure 5). Finally, the first community had the highest value of Margalef richness index (5.13) and the lowest was belonged to fifth community (2.39). There was a significant difference between the third community with others (Figure 6).

Table 1. Results of ANOVA test of diversity indices among plant communities.

Variables	DF	MS	Mean± SE	P	F
Shannon-Wiener	4	3.825	3.44±0.11	0.000*	15.54
Smith-Wilson	4	0.133	0.42± 0.02	0.000*	6.46
Margalef	4	14.824	3.46± 0.18	0.000*	52.30

MS: mean square, SE: standard error.

3.4. Diversity, Evenness and Richness Values in Altitude, Slope and Aspect Classes

ANOVA test showed that altitude influenced all indices including Shannon-Wiener, Smith-Wilson and Margalef richness, and returned statistical significance (P -value ≤ 0.05) as indicated in Table 2. The data of this research demonstrated

that altitude influenced diversity ($F = 13.47, P = 0.000$) as well as evenness ($F = 4.23, P = 0.003$) and richness ($F = 44.4, P = 0.000$). The values obtained for these indices showed no significant correlation to slope measurements since their corresponding P -values were 0.252 for diversity, 0.327 for evenness, and 0.121 for richness.

Table 2. Results of ANOVA test of diversity values in altitude, slope and aspect classes.

Variations source	Indices	DF	MS	P	F
Altitude	diversity	5	3.188	0.000*	13.47
	evenness	5	0.095	0.003*	4.23
	richness	5	12.28	0.000*	44.4
Slope	diversity	2	0.76	0.252 ^{ns}	1.42
	evenness	2	0.034	0.327 ^{ns}	1.14
	richness	2	3.20	0.121 ^{ns}	2.21
Aspect	diversity	46	0.036	0.682 ^{ns}	0.17
	evenness	46	0.042	0.221 ^{ns}	0.074
	richness	46	0.309	0.787 ^{ns}	4.54

The results of means comparison revealed that the highest value of Shannon-Wiener was belonged to altitudinal class B (2.4), however, the altitudinal range of E₁ had the lowest value (0.55) (Figure 7). In relation to evenness, the highest and lowest value was gained in altitudinal classes including A and C, respectively (Figure 8). Eventually, the altitudinal range of A had the highest value of Margalef richness index and the lowest was belonged to E₂ class (Figure 9).

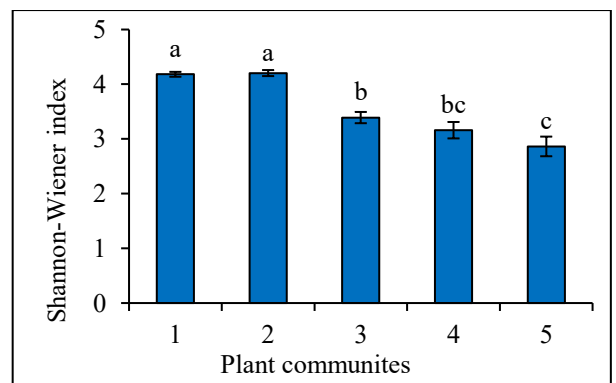


Figure 4. Diversity indices among plant communities.

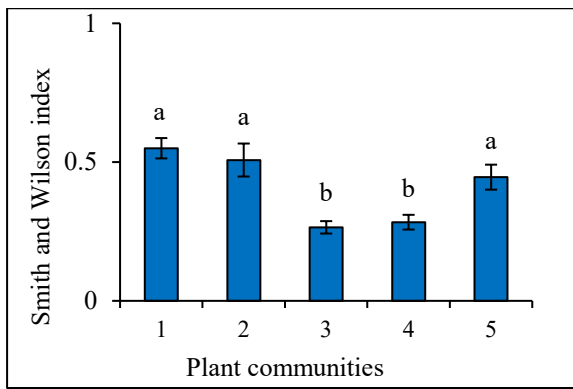


Figure 5. Evenness indices among plant communities.

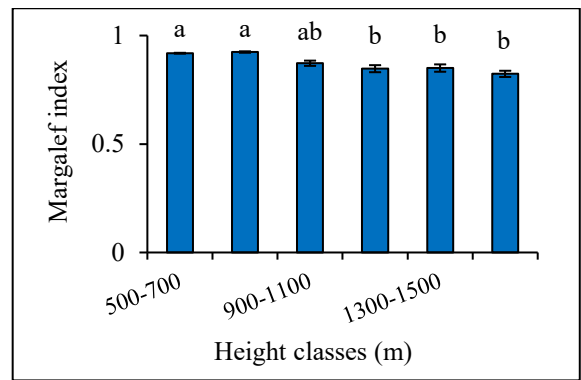


Figure 9. Richness indices of altitudinal classes.

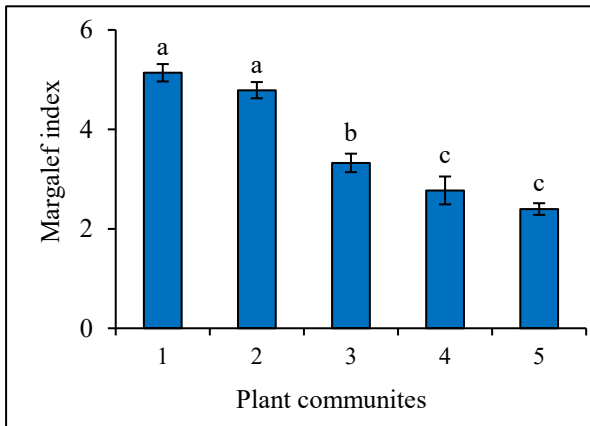


Figure 6. Richness indices among plant communities.

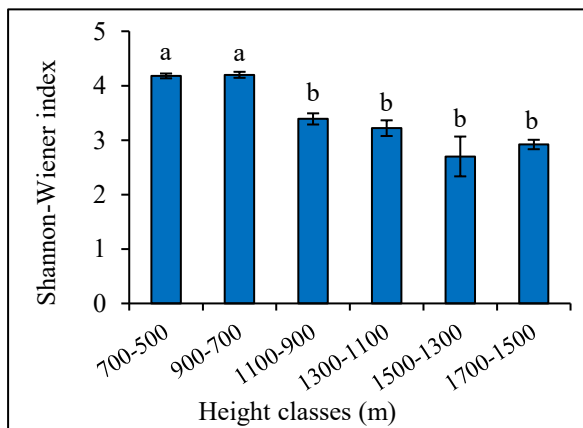


Figure 7. Diversity indices among altitudinal classes.

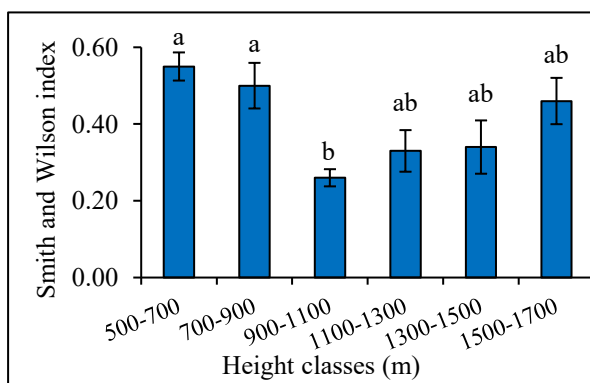


Figure 8. Evenness indices of altitudinal classes.

4. Discussion

In an ecosystem, the study of floristic characteristics, and chorotype form of plant species are effective method for understanding the capacities, management, and conservation of genetic resources which can determine ecosystems position in the natural conservation global network (Haq & Badshah, 2021). According to the results, 60 species were identified in the studied area. The highest number of species was belonged to Rosaceae and Lamiaceae families. It may be related to geographical position as well as topographical heterogeneity in the studied area that possibly provided suitable edaphic conditions for species of these families (Naqinezhad et al., 2010). The results of life form classification showed that Hemicryptophytes and Cryptophytes were dominant. The highest frequency of Hemicryptophytes in an ecosystem can be a result of mountainous, and cold climates (Das et al., 2020; Ghafari et al., 2020; Wagner et al., 2021). Species belonged to this life form are tolerant to these kind of climate by keeping reviver buds in the soil (Naqinezhad et al., 2010).

Kourdkey region is a temperate area with mountainous and cold climate, and dominance of Hemicryptophytes confirm the adaptation of these life form to climatic condition (Midolo et al., 2024). Hemicryptophytes were associated with humid, and cold climate in higher geographical latitude. While, Therophytes and Chamaephytes were tolerant in dry, and unfavorable conditions (Alzamel, 2024; Di Biase et al., 2021). The geographical distribution of plant species in an area is affected by the surrounding vegetation regions (Liu et al., 2016). The temperate forests of Northern Iran belong to the Euxino-Hyrcanian province of the Pontic subregion within the Europe-Siberian region. Therefore, the presence of species belonging to these geographical ranges in the flora is reasonable (Ghorbanalizadeh et al., 2022).

The classification of plant communities indicated that there was a significant difference among diversity values. In addition, altitudinal ranges were significant among topographic factors. Therefore, it can be concluded that TWINSpan has more reasonable in the vegetation unites differentiation by various ecological characteristics than topographic classification.

Moreover, it indicates a favorable description of vegetation in compared to floristic methods (Witte, 2002).

According to TWINSpan, five plant communities were identified. The study of diversity indices among plant communities revealed that the first community had the highest richness, and evenness among groups that can be a result of higher temperature, lower percentage cover of tree species, and also increasing the entrance light in these slopes (Tuomisto et al., 2014). The second community with *Euphorbia amygdaloides* and without an indicator tree species had the highest species diversity. Excessive utilization of tree species and diminishing tree and canopy density are reasons to increase plant species diversity in forests floor (Kashian et al., 2003). On the other hand, the greater density of tree species in higher altitude is an effective factor to limited distribution of different plant species which lead to reduce species diversity (Karami et al., 2008). The third community with *Dryopteris pallida* had the lowest evenness. This species was mainly present in gaps resulting from human activities. In addition, the larger size of these species likely increased competition for light, which may have contributed to reduced richness and percent cover of many understory plants (Ebrahimi et al., 2014). Moreover, this species can diminish growth of other species by allelopathy, and chemical release (Pourbabaei & Ahani, 2004). On the other hand, the high frequency of this species in third group can be an indicative of high humidity in the environment (Chia & Lim, 2022). The presence of this group was demonstrated in the wettest slopes of the study area. The fourth and fifth groups had the lowest value of diversity indices. It may be associated with their establishment at a higher altitude (Rathore et al., 2018; Siles & Margesin, 2016). The highest value of species richness and diversity was found at intermediate altitude. Conversely It was decreased by increasing altitude, and decreasing temperature.

Results of ANOVA test for diversity indices among altitudinal classes indicated that the first, and, second classes had the highest value, and there were significant differences among classes. By increasing altitude, plant growing season will be decreased in climate condition including extreme cold, snow, hail and strong winds which can lead to diminish plant species diversity with absence sensitive species (Zhang et al., 2022; Zhu et al., 2019). Species composition is another factor which can be effective on plant diversity reduction. At lower altitudinal gradient, *Fagus orientalis* was mixed with other tree species. Whereas, the pure communities of *Fagus orientalis* were presented at altitudinal gradient from 1400 to 1700 m a.s.l. Overall, plant species diversity in pure beech communities is lower than mixed communities (Hrivnák et al., 2014; Williams et al., 2017; Benavides et al., 2019). Variation in tree species composition may be associated with differences in light availability in the studied area, possibly reflecting the shade-tolerant characteristics of beech (Chamagne et al., 2016; Zellweger et al., 2016; Driesen et al., 2020). The minimum

value of species richness was belonged to higher altitudinal gradient, which can be attributed to decrease temperature and liquid water availability in this altitude (Sharma et al., 2019). However, environmental variables such as soil properties, and microclimatic conditions were not directly measured in this study, and further research would be useful to better understand the underlying ecological factors.

5. Conclusion

This study identified five plant communities along the altitudinal gradient in the Hyrcanian forest using TWINSpan classification. The results indicated that altitudinal gradient had a significant effect on diversity, richness, and evenness values. Plant community distributions were affected by altitudinal factor which was the most important variability factor among the groups. An altitudinal class can be effective on plant species distribution by temperature, and humidity factors. As the result showed the higher elevation had lower species diversity. Selecting tree species that match the habitat is crucial for forest reconstruction with low diversity at high altitudes. Therefore, it is essential to adjust management strategies for effective care of forest ecosystems.

Conflict of Interest

The authors declare that they have no competing interests.

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Supplementary Information

Supplementary Table 1. Characteristics of identified plant communities according to plant ecological group.

Plant communities	Height class (m.a.s.l.)	SIV of dominant species		Indicator species
		tree	herb	
Cyclameno-Fagetum community	500-700	<i>Fagus orientalis</i> 148.85	<i>Cyclamen coum</i> 109.52	<i>Carpinus betulus</i> L. <i>Ilex spinigera</i> Loes. <i>Myosotis anomala</i> Riedl, <i>Carex sylvatica</i> Huds, <i>Chelidonium majus</i> L. <i>Allium paradoxum</i> G.Don, <i>Hesperis hyrcana</i> Bornm.& Gauba, <i>Polystichum</i> sp. <i>Prunella vulgaris</i> L.
Carexeto-Fagetum community	700-900	<i>Fagus orientalis</i> 204.13	<i>Carex sylvatica</i> 104.66	<i>Carpinus betulus</i> L. <i>Pteris cretica</i> L. <i>Primula heterochroma</i> Stapf. <i>Chelidonium majus</i> L. <i>Epipactis</i> sp. <i>Geum urbanum</i> L. <i>Danae racemosa</i> Moench, <i>Allium paradoxum</i> G.Don, <i>Mentha aquatica</i> L.
Athyriumeto-Fagetum community	900-1100	<i>Fagus orientalis</i> 229.96	<i>Athyrium filix-femina</i> 108.09	<i>Carpinus betulus</i> L. <i>Trifolium</i> sp. <i>Prenanthes cacalifolia</i> Beauverd, <i>Sanicula europaea</i> L. <i>Geranium columbinum</i> L. <i>Geranium</i> sp. <i>Galium odoratum</i> (L.) Scop
Athyriumeto-Fagetum community	1100-1300	<i>Fagus orientalis</i> 236.89	<i>Athyrium filix-femina</i> 112.05	<i>Clinopodium umbrosum</i> (M.Bieb.) Kuntze, <i>Geranium columbinum</i> L. <i>Geranium</i> sp. <i>Dryopteris pallida</i> (Bory) Fomin, <i>Hedera pastuchovii</i> Woronow, <i>Galium odoratum</i> (L.) Scop.
Galiumo-Fagetum community	1300-1500 1500-1700	<i>Fagus orientalis</i> 241.65	<i>Galium odoratum</i> 123.50	<i>Athyrium filix-femina</i> (L.) Roth. <i>Acer cappadocicum</i> Gled. <i>Galium rotundifolium</i> L. <i>Solanum kiesseritzkii</i> C.A.Mey. <i>Lapsana communis</i> L.