

Effects of plot size on assessment of tree species diversity in Caspian forests of Iran

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Abstract. Mirzazadeh A, Pourbabaei H, Daryaei MG, Bonyad A. 2022. *Effects of plot size on assessment of tree species diversity in Caspian forests of Iran. Biodiversitas 23: 4879-4886.* Tree species diversity is important to forest managers because any management that reduces tree species diversity may greatly reduce commercial benefits. Therefore, control over tree species diversity is an important aspect of hardwood forest management. An efficient and quick survey method is needed to facilitate the assessment of tree species diversity in forest stands. In this study, the effects of plot size on the assessment of tree species diversity were compared in the uneven-aged hardwood stands in the Hyrcanian forests of Iran. There were selected 50 hectares of these forests representing the regional forests. Sampling procedures were carried out on concentric lozenge plots with different sizes, including 400, 800, 1000, 1200, 1600, 2000, 2500, and 5000 m² in the dimensions of 150×150m. For each plot, the type of the species and the number of trees were recorded. Shannon-Wiener (H'), Simpson (1-D), Mc-Arthur (N₁), Hill (N₂), Camargo (E'), Simpson (E_{1/D}), Nee (E_Q), Smith-Wilson (E_{var}), Menhinick (R₂), Margalef (R₁) indices were used to estimate of tree species biodiversity. The precision and cost criteria (E%²× T) were applied for a better evaluation of different plot sizes. The obtained inventory error values showed that Hill (diversity), Camargo (evenness) and Menhinick (richness) indices had the lowest inventory error in the different plot sizes. Also, the lowest value of inventory error in diversity, evenness and richness indices were related to plot sizes of 2500, 5000, and 5000 m², respectively. Based on the obtained results of diversity and evenness indices, there was a significant difference between the different plot sizes and different indices. The results showed that the lowest value of E%²×T for diversity, evenness and richness was related to Hill (800 m²), Camargo (1000 m²), and Menhinick (400 m²). The finding results revealed that plot size had a significant effect on tree species' evenness.

Keywords: Biodiversity indices, forest management, Hyrcanian forests, precision and cost

INTRODUCTION

One of the most critical issues in natural resource conservation is integrating economic development along with the protection of biodiversity on earth (Mirzaei et al. 2019a). Loss of biodiversity and destruction of habitat has increased due to an increase in human population and increased human demand for natural resources, such as in agricultural practices (Asifat et al. 2019; Mamnun and Hossen 2021). Today, natural ecosystems in different parts of the world are impacted by the destructive activities of humankind (Ebrahimi et al. 2014). The area of the world's forests, particularly tropical forests, has declined and trends indicate that this will continue. As forests are destroyed and their size reduced, the extinction of plant and animal species, and consequently, the decrease of biodiversity around the world are being observed. Biodiversity in forests helps to maintain key ecosystem processes and functions such as atmospheric gas exchange, nutrient cycling, climate regulation, hydrologic cycles, and soil genesis and development. Each species plays a vital and crucial role in the food chains of ecosystems because the extinction of one species might disturb the life balance in nature (Mirzaei et al. 2019b).

Forest production of wood and non-timber products, and other public services will be most likely sustained

when the diversity of their most important components, which are trees and shrubs, are maintained (Solomon 2016). Pasari et al. (2013) reported that tree species diversity promotes a wide array of ecosystem functions and services. In this regard, knowledge of tree species diversity is necessary to manage forest resources sustainably and to better understand the economic consequences of changes in species diversity due to management (Pourbabaei and Rahimi 2016). Sustainable management of these forests to meet the commercial demands on the resource, given the topographic conditions of these forests, requires an efficient and economical forest inventory method to collect data to support sound forest management planning. Sampling plot size is the main factor affecting the accurate estimation of forest parameters commonly used to plan forest management and implement sustainable practices at the stand level (Junttila et al. 2013).

Forests can have high ecological and economic values if they are managed according to a forestry plan that optimizes ecosystem goods and services in a sustainable manner. Yet, the inventory, monitoring and management of these ecosystems are complex due to the biodiversity contained and diverse environmental attributes which are challenging to sustain their quality as productive ecosystems. A focus on tree species diversity may be a simplified way of accounting for other types of ecosystem

diversity. Calculation and comparison of different diversity indices have been recognized as a favorite method for studying biodiversity. These indices estimate the biological and ecological quality of ecosystems through the structure of their communities (Monarrez-Gonzalez et al. 2020); they are also possible indicators for monitoring the level of environmental pollution. In addition to diversity indices, the most important factors for the investigation of biodiversity are sampling plot size, plot shape, number of plots and inventory grid.

A varying number of plots affected the results of studies. In the past, the studies were performed with different plot sizes, plot numbers and inventory grids. For example, Zhang et al. (2014) studied the importance of sampling time in species detectability and suggested that sampling time, combined with sample size and observer effects, should be considered in landscape-scale plant biodiversity surveys. Ebrahimi et al. (2014) used 25 circle plots of 1000-m² to estimate tree and herbaceous species diversity in the north forests of Iran and showed that the effect of land protection on plant diversity was more pronounced for evenness than for species richness and the positive correlation between diversity and evenness indices was higher than that between diversity and richness. Mirzaei et al. (2019a) studied the effects of inventory grids on tree species diversity in semi-arid forests of Iran and showed that based on the results of $E\%^2 \times T$, the inventory grid with dimensions of 200×100m (25 plots) was selected as the most appropriate one for estimating the tree species diversity in semi-arid forests. Mirzaei et al. (2019b) by using 30 circle plots of 1000-m², three variables, including the number of individuals (frequency of species), basal area and volume of tree species, were compared to estimate tree species diversity in broadleaves forests of Iran and showed that basal area and volume variables were selected as more suitable variables in order to estimate of biodiversity indices in northern forests of Iran. Such literature showed a range of optimal plot sizes in forests that varies depending on the response variable of interest, suggesting that relatively small plots can be used to estimate the density of regeneration, while relatively large plots are needed to detect patterns in the spatial structure of trees.

The forests of northern Iran, which have relatively high biodiversity and complex structure, can be managed optimally and sustainably into the future if proper awareness and attention are given to these ecosystems (Payam and Rajabali 2020). In Iran, an important species is an oriental beech (*Fagus orientalis* Lipsky), which is used in different industries. This is the only species of beech that occurs naturally in Iran's northern regions (Moradi et al. 2012). Sustainable management of beech forests includes conserving native biodiversity requires an efficient sampling method to estimate current diversity and monitor its change over time. The main objective of this study was to determine the effect of plot size on biodiversity indices to facilitate the assessment of tree species diversity in beech communities in forests of northern Iran by considering the cost and precision of the sampling method.

We expected to approach an optimal sample plot size to measure tree species diversity under similar conditions.

MATERIALS AND METHODS

Study area

This study was carried out in the northern forests of Iran that known as Caspian forests (Masal City, Guilan Province). The total study area was 50 hectares and located between 48° 55' 19" to 49° 02' 00" E longitude and 37° 14' 00" to 37° 19' 20" N latitude (Figure 1). Elevation ranges between 300 to 200 m above sea level and the study area has a mean slope of 35%, and an east aspect. The mean annual precipitation is 1530 mm and the mean annual temperature is 16.5°C. The bedrock is limestone, shale, and acidic sandstone, and pH is approximately 5.5-6.5. The forests in the study area consisted of deciduous broad-leaved trees of different ages that vary in composition from pure beech to mixtures of beech with other hardwood species. These forests were formerly impacted by disturbances from overgrazing, harvesting by forest dwellers, and illegal logging to supply logs and firewood, all of which have influenced the quantity and quality of forests. However, the study area has been strictly protected since 2006.

Method

Initially, a map of the study area was used to establish an inventory grid of 150×150 m, in a 50-ha area of the compartment. Eight concentric lozenge sampling plots with sizes 400, 800, 1000, 1200, 1600, 2000, 2500 and 5000 m² were established at each sampling point (Figure 2).

In total, 160 sampling plots were measured. In each plot, species, number of species, number of trees and diameter at breast height (DBH≥7.5 cm) were measured and recorded. For the study of tree species diversity, ten frequently used biodiversity indices were selected and their formulae are given in Table 1 (Scott and Anderson 2003):

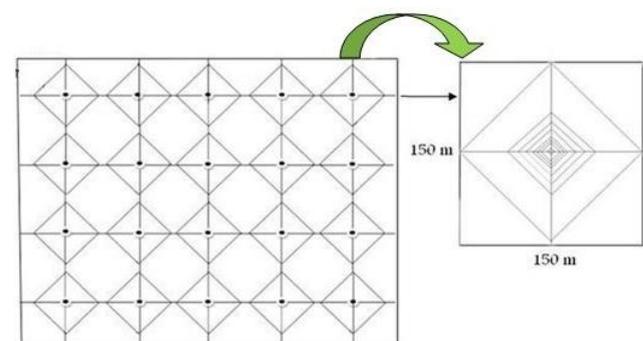


Figure 2. A diagram of sampling plots designed in the studied area. Each plot was indicated with a small block dot (n=20). The right figure shows eight concentric lozenge sampling plots sized 400, 800, 1000, 1200, 1600, 2000, 2500 and 5000 m²

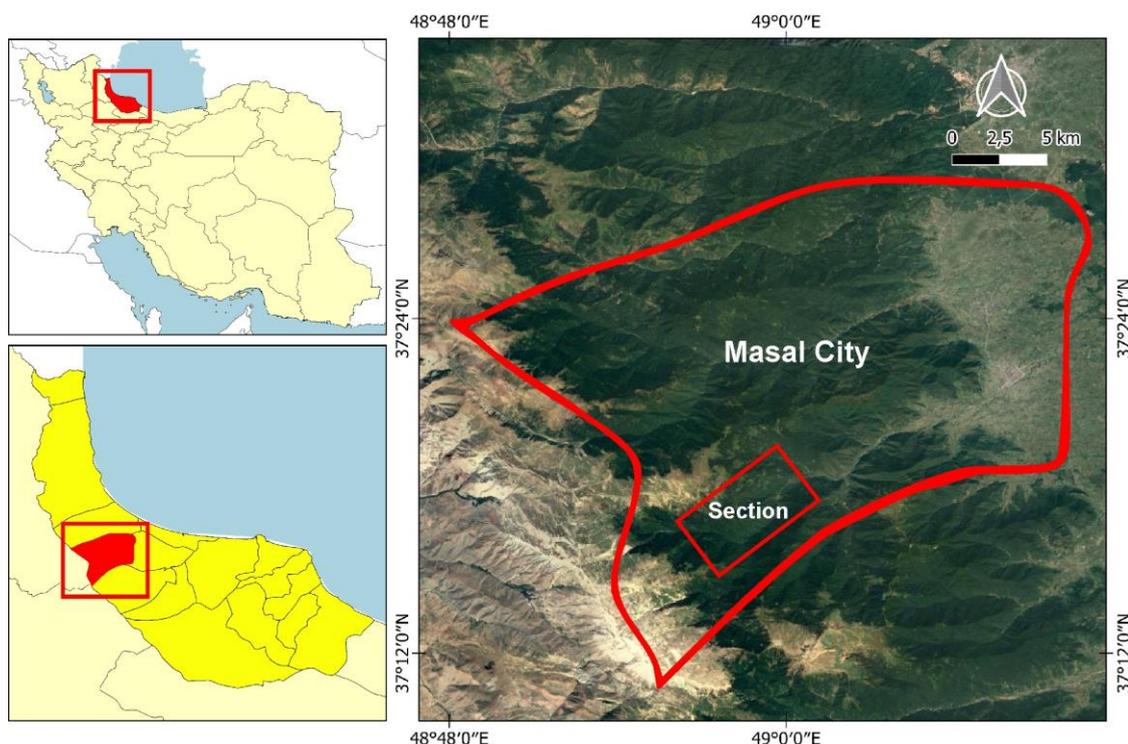


Figure 1. Location of the study area in Masal City, Guilan Province, Iran

Table 1. Biodiversity indices and their equations

Index	Equation
Simpson (1-D)	$1 - D = 1 - \sum_{i=1}^s \frac{n_i(n_i - 1)}{N(N - 1)}$
Shannon-Wiener (H')	$H' = -\sum_{i=1}^s (P_i) (\log_2 P_i)$
Hill (N ₂)	$\frac{1}{D} = \frac{1}{\sum p_i^2}$
Mc Arthur (N ₁)	$N_1 = e^{H'}$
Camargo (E')	$E' = 1.0 - \left(\sum_{i=1}^s \sum_{j=i+1}^s \left[\frac{ P_i - P_j }{S} \right] \right)$
Smith-Wilson (E _{var})	$E_{var} = 1 - \left(\frac{2}{\pi} \left[\arctan \left(\frac{\sum (\log_e(n_i) - \sum \log_e(n_j)/s)^2}{s} \right) \right] \right)$
Simpson (E _{1/D})	$E_{1/D} = \frac{1/\bar{D}}{S - 2}$
Nee (E _Q)	$E_Q = \frac{S - 1}{\pi \arctan(b)}$
Margalef (R ₁)	$R_1 = \frac{S - 1}{\ln(N)}$
Menhinich (R ₂)	$R_2 = \left(\frac{S - 1}{\ln(n)} \right)$

Note: D is dominance index, n_i = the number of individuals of the i^{th} species, N = total number of all individuals, H' =Shanon-Wiener, P_i = the relative frequency of the i^{th} species, P_j = the relative frequency of the j^{th} species, N_1 = an equal number of common species that create diversity similar to the H' , E' = Camargo, S = the total number of species, b = the gradient of dominance - diversity curves, $e = 2.71828$

Comparison of plot size based on $E\% \times T$

Cost and precision are two factors important in forest inventory studies (Cogbill et al. 2018). Since the inventory costs with inventory time have a directional relationship and estimation of inventory costs is difficult, the time was used to estimate the cost. According to Eq. 1, the total time for each sampling grid is calculated:

$$T = (n \times t_i) + (n \times t_j) \quad \text{Eq. (1)}$$

Where, T is the total time for each sampling grid, n is the number of plots in each sampling grid, t_i is the average time taken for measuring the trees of each plot and t_j is the average time taken for movement from one plot to the next plot.

Percentage of inventory error (E%) is calculated in Eq. 2 and 3.

$$E = t \times S_{\bar{x}} \quad \text{Eq. (2)}$$

$$E\% = \frac{E \times 100}{\bar{x}} \quad \text{Eq. (3)}$$

Where, E is inventory error (precision), t is statistic of t -student table, $S_{\bar{x}}$ is the standard error and \bar{x} is mean of diversity index.

In order to determine of appropriate number of plot for estimation of tree species diversity was used $E\% \times T$ criteria. (Zohrevandi et al. 2016).

We determined that the sampling method with the lowest $E\% \times T$ is the most appropriate method for forest inventory. For data analysis, IBM SPSS Statistic 22, Ecological Methodology Version 6, PAST version 1.89 and Excel 2013 software.

RESULTS AND DISCUSSION

Results

In this study, the tree species that had the highest density were *Fagus orientalis*, *Alnus subcordata*, and *Carpinus betulus*. Beech trees comprised 85% of the composition and had the highest amount of basal area. Thus, it can be considered a pure forest. The dominance of beech is, in part, due to its relatively high tolerance to shade and the inability of its shade intolerant competitors to regenerate and persist (Table 2).

The mean of biodiversity indices calculated for different plot sizes is shown in Table 3. The results showed that across different plot sizes, the lowest and highest values of diversity indices are related to 1-D Simpson and N_1 Mc Arthur indices, respectively. The results of evenness indices showed that the lowest and highest values of

evenness indices are related to E_Q modified Nee and $E_{1/\bar{D}}$ Simpson indices, respectively. Also, the results showed that R_2 Menhinick index has the highest value of richness indices.

Inventory error (E%) of biodiversity indices for different plot sizes is shown in Table 4. Based on the obtained results of diversity indices, Hill's N_2 index has the lowest inventory error in all plot sizes, except in a plot with a size of 400 m². The results showed that the Camargo and E_Q modified Nee indices of evenness have the lowest and highest values of evenness indices. Regarding the results, R_2 Menhinick index has the lowest value of richness indices. Also, the results of diversity indices showed that the lowest value of inventory error was observed in a plot with an area of 2500 m². The lowest value of inventory error in the evenness and richness indices is related to a plot with an area of 5000 m² (Table 4).

Table 2. Percentage of the abundance of species in each sample plot size

Species	Percentage of abundance (%) in plot size (m ²)							
	400	800	1000	1200	1600	2000	2500	5000
<i>Fagus orientalis</i>	87.29	89.87	90.83	91.13	90.6	89.91	88.92	83.24
<i>Carpinus betulus</i>	8.05	7.06	6.58	6.42	6.76	7.47	8.46	13.79
<i>Alnus subcordata</i>	4.66	3.07	2.59	2.45	2.64	2.62	2.62	2.97

Table 3. Mean ± standard deviation of biodiversity indices in different plot size

Index	Plot size (m ²)							
	400	800	1000	1200	1600	2000	2500	5000
H'	0.435±0.28	0.423±0.23	0.409±0.22	0.423±0.22	0.459±0.20	0.451±0.20	0.464±0.15	0.628±0.21
1-D	0.180±0.14	0.162±0.11	0.154±0.10	0.155±0.10	0.165±0.09	0.161±0.08	0.166±0.06	0.234±0.09
N_1	1.277±0.50	1.358±0.22	1.345±0.21	1.356±0.22	1.138±0.20	1.381±0.20	1.387±0.14	1.560±0.22
N_2	1.160±0.46	1.219±0.19	1.202±0.17	1.202±0.16	1.233±0.17	1.224±0.15	1.207±0.09	1.323±0.15
E'	0.563±0.21	0.546±0.10	0.514±0.06	0.480±0.08	0.458±0.08	0.445±0.07	0.448±0.08	0.449±0.05
$E_{1/\bar{D}}$	0.580±0.23	0.556±0.11	0.521±0.07	0.485±0.09	0.461±0.08	0.448±0.07	0.450±0.08	0.450±0.05
E_Q	0.165±0.13	0.123±0.06	0.099±0.02	0.097±0.02	0.099±0.02	0.095±0.02	0.098±0.02	0.106±0.02
E_{var}	0.431±0.28	0.315±0.22	0.238±0.12	0.228±0.12	0.228±0.10	0.206±0.08	0.219±0.11	0.246±0.12
R_2	0.391±0.09	0.307±0.10	0.300±0.09	0.290±0.07	0.288±0.05	0.262±0.03	0.241±0.03	0.206±0.02
R_1	0.263±0.11	0.299±0.06	0.282±0.05	0.360±0.12	0.420±0.04	0.407±0.04	0.392±0.04	0.269±0.03

Table 4. Inventory error (E%) of biodiversity indices for different plot size

Index	Plot size (m ²)							
	400	800	1000	1200	1600	2000	2500	5000
H'	29.40	24.50	24.68	23.69	19.68	20.67	14.91	15.05
1-D	35.07	31.11	30.68	29.08	24.73	24.78	18.09	17.47
N_1	17.54	7.46	7.30	7.27	6.63	6.66	4.81	6.36
N_2	17.80	7.00	6.45	6.15	6.22	5.55	3.69	5.19
E'	17.24	8.62	5.88	7.98	7.97	7.20	8.12	5.02
$E_{1/\bar{D}}$	17.81	9.55	6.04	8.33	8.37	7.44	8.46	5.14
E_Q	35.79	23.59	12.14	12.87	10.95	9.96	12.19	11.77
E_{var}	29.53	31.91	22.79	24.75	20.88	19.17	23.76	22.09
R_2	10.42	14.51	12.72	10.16	5.41	6.10	6.44	4.93
R_1	20.34	16.01	16.88	15.03	8.95	7.47	7.51	5.21

The results of the univariate test of biodiversity indices for different plot sizes are shown in Table 5. Based on the obtained results of diversity and evenness indices, there is a significant difference between the different plot sizes and different diversity indices. Also, the results showed that there is a significant difference between the richness indices, but there is no significant difference between plot sizes (Table 6).

Mean comparison of diversity indices showed that there is a significant difference between all of the diversity indices (Figure 3). The results of evenness indices showed that there is no significant difference between Camargo and

Simpson of evenness indices. Also, a significant difference is observed between Margalef and Menhinick indices to estimate species richness.

Mean comparison of diversity indices by Duncan Test across different plot sizes showed that there is a significant difference between plots sized 5000 m² and other plot sizes (Figure 4).

Mean comparison of evenness indices by Duncan Test in different plot sizes showed that there is a significant difference between plots sized 400 and 800 m² with other plot sizes, but there is no significant difference between plots of 1000, 1200, 1600, 2500 and 500 m² (Figure 5).

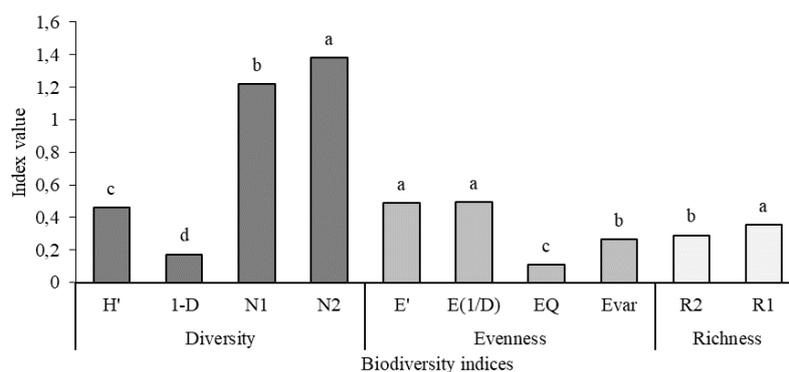


Figure 3. Mean comparison of different diversity, evenness and richness indices

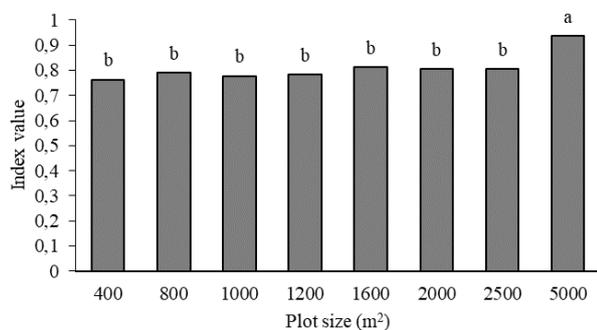


Figure 4. Mean comparison of diversity indices in different plot sizes

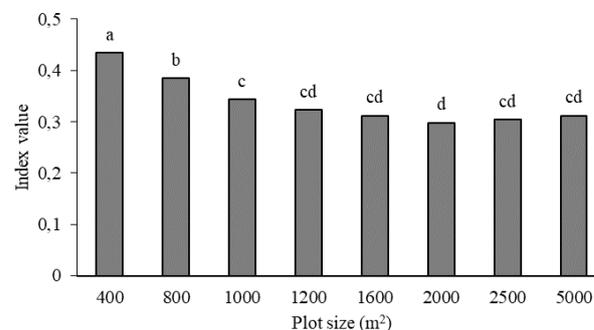


Figure 5. Mean comparison of evenness index across different plot sizes

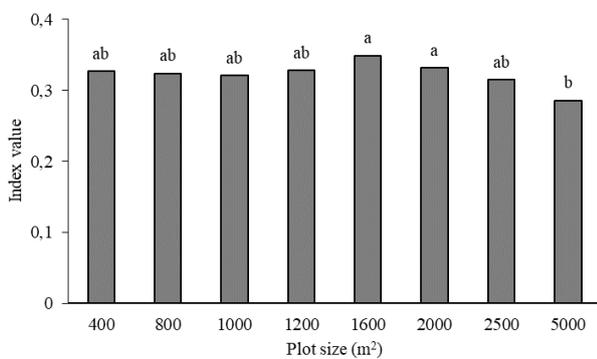
Table 5. Results of univariate test of the biodiversity indices for different plot size

Index	Source	Sum of squares	df	Mean square	F	Sig.
Diversity	Index	163.85	3	54.61	1228.22	0.000 *
	Plot size	1.62	7	0.23	5.21	0.000 *
	Index * Plot size	0.37	21	0.018	0.39	0.993 ns
	Error	27.03	608	0.04		
Evenness	Index	16.66	3	5.55	412.11	0.000 *
	Plot size	1.27	7	0.18	13.52	0.000 *
	Index * Plot size	0.29	21	0.014	1.02	0.426 ns
	Error	8.19	608	0.013		
Richness	Index	0.34	1	0.34	48.98	0.000 *
	Plot size	0.08	7	0.013	1.80	0.085 ns
	Index * Plot size	0.61	7	0.088	12.63	0.000 *
	Error	2.12	304	0.007		

Note: Asterisk mark (*) and ns are significant and not significantly different at the 0.05 probability level respectively

Table 6. Values of $E\%^2 \times T$ criteria of biodiversity indices across different plot sizes

Index	Plot size (m ²)							
	400	800	1000	1200	1600	2000	2500	5000
H'	437816.0	598124.3	868866.8	1069995.0	987618.0	1304535.0	815426.4	1362731.0
1-D	622828.9	964708.8	1341882.0	1612251.0	1532177.0	1873323.0	1200977.0	1838032.0
N ₁	155707.2	55396.7	75814.7	100567.1	109946.8	135459.1	84884.0	243245.0
N ₂	160323.0	48788.4	59159.5	71986.4	96725.9	93799.1	49988.2	162181.0
E'	150490.7	73916.9	49255.5	121395.2	158856.9	158100.3	241828.7	151607.0
$E_{1/\bar{d}}$	160495.3	90871.0	52016.2	132088.5	176483.9	168772.5	262772.0	158617.0
E _Q	648545.2	554606.0	209714.1	315910.4	300666.6	302745.7	545397.2	833521.0
E _{var}	441523.2	1015143.0	740766.2	1167961.0	1092997.0	1120912.0	2073456.0	2937653.0
R ₂	146251.4	258816.5	390205.1	419800.5	193293.0	163410.6	197871.5	163378.0
R ₁	42138.5	173078.3	155413.1	179398.6	94331.1	100189.1	126126.7	145886.5

**Figure 6.** Mean comparison of richness index across different plot sizes

Mean comparison of richness indices by Duncan Test across different plot sizes showed that there is a significant difference between plots sized 1600 and 2000 m² with a plot of 5000 m², but there is no significant difference between plots of 400, 800, 1000, 1200 and 2500 m² (Figure 6).

The results of $E\%^2 \times T$ criterion of biodiversity indices across different plot sizes are shown in Table 6. The results showed that the lowest value of $E\%^2 \times T$ for diversity indices is related to Hill's N₂ index and plot of 800 m². Regarding the obtained results of evenness, the lowest and highest value of $E\%^2 \times T$ are related to the Camargo (1000 m²) and Smith-Wilson (5000 m²), respectively. Also, a comparison of richness indices based on the $E\%^2 \times T$ showed that the lowest and highest value of $E\%^2 \times T$ is related to the Menhinick (400 m²) and Margalef (1200 m²) (Table 6).

Discussion

The most appropriate sampling plot size is the one that produces the highest accuracy in parameter estimates while minimizing the time it takes to collect data. Several research studies on biodiversity used different plot sizes to estimate the biodiversity indices, and so far, no study has been done to compare different plot sizes. So, the results of this study showed that Mc Arthur and Simpson indices had

the highest and lowest values of mean diversity indices and there was a significant difference between diversity indices and different plot sizes. Also, the results showed that with increasing the plot size, there was no trend of regular changes in the values of diversity indices. The mean of evenness indices showed that the lowest values of all indices were observed in the plot size of 400 m². Also, the results of evenness indices showed that with increasing the plot sizes from 400 to 2000 m², the values of all evenness indices had an increasing trend, but with increasing the plot sizes from 2000 to 5000 m², the values of the indices had a decreasing trend. The results of Margalef index showed that with increasing the plot sizes from 400 to 5000 m², the values of this index had a decreasing trend.

We found that a plot size of 400 m² had the lowest value of $E\%^2 \times T$ based on the error percentage of Shannon-Weiner and Simpson indices, which was consistent with the results of the univariate test. These sample plots took the least time to measure compared to other plot sizes, but they had the lowest precision. The time to measure a plot increased as sampling plot size because the number of species increased linearly (Probst 2019). Hence, the value of the Shannon-Weiner and Simpson diversity indices increased when using larger sample plots compared to sample plots of 400 m². Simpson's diversity index is mainly used as an index of dominance because it is more sensitive to general species coverage in the sample plot or overall community, and more sensitive to changes in the evenness of common species (Morris et al. 2014; Roswell et al. 2021; Hillebrand et al. 2018; Di Battista et al. 2016).

The value of this index increased as the sample plot size increased. When the sample plot size was 400 m², the value of error percentage was high and the estimate of diversity was least accurate since the value of Shannon-Weiner and Simpson diversity indices in some plots were lower than average. This resulted mainly because in two of the three replications of this plot size (400 m²) the amount of diversity was zero due to the complete dominance of beech. Tree composition was pure beech on some plots, indicating that the forest was approaching a climax state. Whitfeldm et al. (2014) found that the richness of species increased in late successional stages.

Also, Chai et al. (2015) showed that plant communities that are near climax tend to have species dominance, and consequently, the condition is suitable for higher plant species diversity. This is consistent with the findings of Moridi et al. (2015) who emphasized that the high dominance of beech in mixed stands limited the presence of common hornbeam and other species. Estimates of evenness in our study showed that smaller-sized sampling plots had a greater effect on the value of diversity. Moreover, low richness in the areas due to beech dominance decreased the value of diversity on larger-sized sampling plots, which increased the effect of evenness on changes in diversity. A study on the structural diversity in beech forests in northern Iran using Shannon-Weiner and Simpson's diversity indices showed that in early successional beech habitats, the diversity and richness of tree species would be reduced due to competition from the shade-tolerant beech (Daneshvar et al. 2007). Hájek et al. (2020) reported that evenness had a significant effect compared to richness on diversity.

Hill's N_2 is inherently attractive to ecologists, and Mac Arthur's N_1 index is considered by some to be the best index for measuring diversity because it is sensitive to rare species in a plant community (Pourbabaie et al. 2012). These indices are rarely misleading in comparison to other indices. N_1 and N_2 measure the number of abundant and more abundant species, respectively (Chao et al. 2014). Based on the analysis of $E\%^2 \times T$ using the error percentage of Mac Arthur's N_1 and Hill's N_2 , we identified that the plot size of 800 m² was the best because it had the lowest value with acceptable levels of accuracy compared to the error percentage based on the Simpson and Shannon-Weiner diversity indices. Also, the error percentage of N_1 and N_2 was lower than the Shannon-Weiner and Simpson indices. Similar results were reported by Hassanzad-Navroodi (1992) who found in his study of beech forests in northern Iran that the plot size of 800 m² was best for data collection for forestry projects, providing parameter estimates within desired levels of precision. Estimates of diversity using Camargo, Simpson, Nee's evenness indices had less error percentage and more precision at the plot size of 1000 m² than in plot size of either 400 or 800 m². Using the 1000 m² plot also resulted in more evenness, and took less time and cost in data collection compared to larger plot sizes. Plots of 1000 m² had the lowest value of $E\%^2 \times T$ based on the error percentage of Camargo, Simpson, Nee's indices. The plot size of 400 m² had the lowest value of $E\%^2 \times T$ based on the error percentage of Smith-Wilson's index, but the plot size of 1000 m², which had more precision and less error percentage, was second only because it took twice as long to collect data ($E\%^2 \times T$) as it did on 400 m² plots. Hitherto published empirical studies mostly reported decreases in evenness with increasing plot size (Kwiatkowska and Symonides 1986; Wilson et al. 1999; Small and McCarthy 2002). Our results suggest the opposite trend occurred, i.e., an increase in evenness was observed in an increase in sampling plot size.

We found that the plot size of 400 m² required the least amount of time for collecting data, and it had the lowest value of $E\%^2 \times T$ based on Margalef's error percentage in

comparison to the other sampling plot sizes, i.e., it had the lowest error percentage for estimating Margalef with acceptable accuracy. In contrast, Mastori (2005) found that a plot size of 5000 m² was the most appropriate size for estimating richness in beech communities in northern Iran. The main reason for this inconsistent result was that the forest in our study area consisted of pure beech with low richness, unlike Mastori's study forests. Ottaviani et al. (2019) studied a multifaceted approach for beech forest conservation, and found that as the amount of beech canopy increased as richness decreased. As a result, mature stands of beech forests are generally associated with reduced levels of species diversity (Scolastri et al. 2017; Landuyt et al. 2018). Although estimates of Menhinich diversity index based on plots of 400 m² had a higher error percentage than those from plots of 5000 m², we found that the plot size of 400 m² had the lowest value of $E\%^2 \times T$ because of the difference in time to collect data between the two plot sizes. We recommend that future studies use a different grid dimension with a single large unit to improve the results.

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