Hindawi
International Journal of Forestry Research
Volume 2017, Article ID 2818132, 13 pages
https://doi.org/10.1155/2017/2818132

Research Article

Comparison of T-Square, Point Centered Quarter, and N-Tree Sampling Methods in *Pittosporum undulatum* Invaded Woodlands

Lurdes Borges Silva,[1,2,3] Mário Alves,[2] Rui Bento Elias,[4] and Luís Silva[1]

[1] *InBIO, Rede de Investigação em Biodiversidade e Biologia Evolutiva, Laboratório Associado, CIBIO-Açores, Universidade dos Açores, Açores, 9501-801 Ponta Delgada, Portugal*
[2] *NATURALREASON, LDA, Caminho do Meio Velho, 5-B, Açores, 9760-114 Cabo da Praia, Portugal*
[3] *3CBIO, Universidade dos Açores, Faculdade de Ciências e Tecnologia, Ponta Delgada, Portugal*
[4] *Centre for Ecology, Evolution and Environmental Changes (CE3C), Azorean Biodiversity Group and Universidade dos Açores, Faculdade de Ciências Agrárias e do Ambiente, Açores, 9700-042 Angra do Heroísmo, Portugal*

Correspondence should be addressed to Lurdes Borges Silva; lurdesborgesilva@gmail.com

Received 1 August 2016; Accepted 15 November 2016; Published 11 January 2017

Academic Editor: Guy R. Larocque

Tree density is an important parameter affecting ecosystems functions and management decisions, while tree distribution patterns affect sampling design. *Pittosporum undulatum* stands in the Azores are being targeted with a biomass valorization program, for which efficient tree density estimators are required. We compared T-Square sampling, Point Centered Quarter Method (PCQM), and N-tree sampling with benchmark quadrat (QD) sampling in six 900 m^2 plots established at *P. undulatum* stands in São Miguel Island. A total of 15 estimators were tested using a data resampling approach. The estimated density range (344–5056 trees/ha) was found to agree with previous studies using PCQM only. Although with a tendency to underestimate tree density (in comparison with QD), overall, T-Square sampling appeared to be the most accurate and precise method, followed by PCQM. Tree distribution pattern was found to be slightly aggregated in 4 of the 6 stands. Considering (1) the low level of bias and high precision, (2) the consistency among three estimators, (3) the possibility of use with aggregated patterns, and (4) the possibility of obtaining a larger number of independent tree parameter estimates, we recommend the use of T-Square sampling in *P. undulatum* stands within the framework of a biomass valorization program.

1. Introduction

In ecological research, the basic objective of sampling is to obtain a descriptive estimate of some attribute of a plant population. This estimate should be a relatively accurate representation to allow detection of real differences among plant populations. Quantitative data are essential to adequately characterize the woody component of forest communities [1]. Some form of sampling is required because total counts of individuals in naturally occurring plant populations are generally impractical without an exhaustive expenditure of energy and resources [2]. A number of sampling techniques are available to quantify forest community traits. These techniques vary in their underlying assumptions (e.g., random distribution of target population), equipment required, and time necessary to obtain an adequate sample for statistical analysis [3].

One of the most commonly sampled parameters is density [4]. Stand density or tree density, expressed as the number of trees per unit area, is an important forest management parameter. Together with other forest structure parameters such as crown closure and crown diameter, it is used by foresters to evaluate regeneration, to assess the effect of forest management measures or as an proxy variable for other stand parameters such as age, basal area, and volume [5]. Thus, density is considered as one of the most important numerical indices to explain quantitative values of tree and shrub

communities. By affecting many aspects of the ecosystem, it can be used in a wide variety of situations.

One of the oldest techniques of data collecting is quadrat counting [6] but this analysis may be insufficient to distinguish certain point patterns and the size of quadrats may affect the results [7]. Obtaining adequate information with minimum effort and time is a major concern when sampling vegetation [3]. In sampling for indices of forest structure, distance sampling (plotless sampling) can be very efficient [8]. Continued technological advances in range measurements and field computing add to the attraction of distance sampling because measurements can be completed ever faster. Also, in natural populations characterized by an irregular, possibly clustered, distribution of trees, the precision of a density estimate from distance sampling can be better than the precision obtained with fixed area plot sampling [9]. Distance sampling has attracted the attention of researchers over the past 50 years as a means of estimating density. Its main attraction is that it is fast and easy to use, and one or more distances are always recorded at each sample point. In contrast, plot sampling can sometimes be a very time consuming process, boundary trees may be overlooked, and some plots may have no tallies [10]. Distance sampling has a long history in forest inventories where the distance and attributes of interest are measured on the k-trees closest to a sample point [9]. When quadrat sampling is difficult or too costly (*e.g.*, in low density populations or mountain areas) distance sampling is favored [11]. Distance sampling is often faster than fixed area plot sampling, since the number of measurements to take at each location can be fixed in advance. This is because there is no need to sample all the trees present in a plot and therefore measurement efforts are independent of local tree density. Expediency that is faster data collection in stand-wise forest management inventories has been another motivating factor [12].

A variety of methods using distance measures have been developed [1]: T-Square sampling [13, 14], Point Centered Quarter Method [15], N-tree sampling [16], random pairs [17], variable area transect [18], closest individual [15], ordered distance [19], compound estimators [20, 21], corrected point-distance [22], nearest neighbor [15], quartered neighbor [23], and angle order [24]. All these methods are essentially based on the distance measures from event to event or from point to event [1, 25].

A variety of density estimators have been proposed for different spatial patterns and modifications to improve their robustness, especially when nonrandom spatial patterns are assumed [15, 20, 24, 27]. Some of the distance methods can be used to determine the general spatial pattern or distribution of a population [13, 28] and are useful to determine inter- and intraspecific relationships in plant communities [29]. Advantages arise from ease of use calculation and interpretation by foresters [25]. Their great disadvantage is connected with the loss of detailed information about spatial patterns at different spatial scales [30], describing the spatial structure only at the fine scale of nearest neighbors [25].

Forest stand structure is a key element in understanding forest ecosystems and many methods have been developed for its study [31]. One of the major components of forest stand structure is the spatial arrangement of tree positions [8]. Spatial information for individual trees is increasingly sought by forest managers and modelers as a means to improve the spatial resolution and accuracy of forest models and management scenario [32]. There are three general categories of two-dimensional point patterns, uniform (hyperdispersed), random, and clumped (aggregated). Random type of spatial distribution means that trees are distributed independently of each other and the probability of finding trees in the whole population is the same. In aggregated populations, individuals occur in clumps of different densities and sizes, and in a regular distribution objects are evenly spaced in a population over a given area [25]. We can assume that the spatial pattern reflects the major trends in stand dynamics. For example, regular spatial structures indicate high competition, whereas aggregate patterns indicate massive regeneration without subsequent strong self-thinning [29].

According to Elias et al. [33], in Azorean native mountain forests, the spatial pattern of tree species is largely explained by disturbance regimes and the regeneration strategies of each species. However, factors such as habitat related patchiness, competition, and dispersion limitation may also explain many of the observed patterns. Meanwhile, spatial patterns in Azorean exotic woodland have not been studied. Invasive alien plants are one of the most relevant threats to the long term integrity of biodiversity, affecting the Azores archipelago, with a relatively high rate of nonindigenous species, among which are several top invaders [34–36]. *Pittosporum undulatum* Vent. (Pittosporaceae) is one of the top invasive trees in the Azores, affecting protected areas and indigenous species, and was targeted by the Azorean Regional Program for Control and Eradication of Invasive Plants in Sensitive Areas (PRECEFIAS) [36–40]. Accurate data about abundance and distribution of *P. undulatum* is needed for its control and management, including the evaluation of the energetic potential of its biomass [38, 41]. This information will be crucial for the implementation of a large scale survey of *P. undulatum* stands in the Azores.

Therefore, the aims of this study are (i) to evaluate the most accurate and precise method to estimate tree density in Azorean exotic woodland; (ii) to test plotless density estimators that have been recently validated for other species and regions; (iii) to compare results with those from quadrat counts; (iv) to determine tree distribution pattern in exotic woodland.

2. Material and Methods

2.1. Study Area. We investigated *P. undulatum* dominated forests located in São Miguel Island (Azores) situated in the North Atlantic Ocean, 1500 km west from Portugal (Figure 1). The archipelago of the Azores, scattered across 615 km on a WNW–ESE alignment, covering a total of 2323 km^2, comprises nine volcanic islands. The climate is temperate oceanic with a mean annual temperature of 17°C at sea level. Relative humidity is high while rainfall ranges from 1500 to 3000 mm, depending on the altitude and increasing from east to west [42]. The topography of the island is characterized by volcanic craters, large catchments, ravines, and seasonal water streams

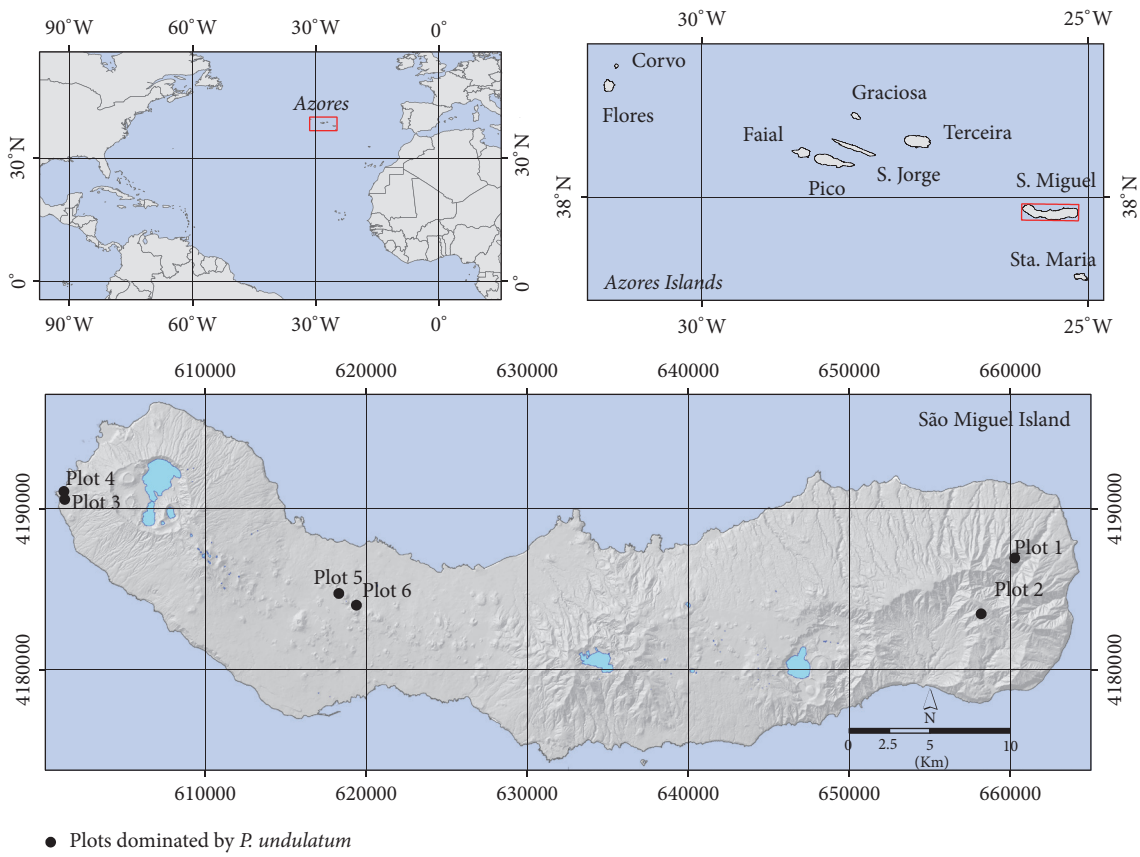

Figure 1: Study sites location in São Miguel Island, Azores archipelago, Portugal. Plot 1, Ribeira do Guilherme, Nordeste; Plot 2, Mata dos Bispos, Povoação; Plot 3, Pico das Camarinhas, west slope; Plot 4, Pico das Camarinhas, Caldera; Plot 5, Pico da Furna, São Vicente Ferreira; Plot 6, Picos de Lima, Fenais da Luz.

Table 1: Main features of the studied *Pittosporum undulatum* stands.

Plot number	Location	Maximum elevation (m a.s.l.)	Area (ha)	Other species
1	Ribeira do Guilherme	700	120.0	*Laurus azorica, Morella faya*
2	Mata dos Bispos	500	2.5	*Laurus azorica, Erica azorica, Clethra arborea*
3* 4**	Pico das Camarinhas	250	10.0	*Morella faya*
5	Pico da Furna	311	2.2	*Acacia melanoxylon*
6	Picos de Lima, Fenais da Luz	312	2.3	*Acacia melanoxylon*

*West slope, outside volcanic crater, and **Caldera, inside volcanic crater.

with a maximum altitude of 1105 m above sea level and a surface area of 745 km^2.

2.2. Stand Characterization. The studied stands (Figure 1) are located within geomorphological formations [43], namely, Nordeste Volcanic System and Povoação Volcano (Plots 1 and 2), Sete Cidades Volcano (Plots 3 and 4), and Picos Fissural Volcanic System (Plots 5 and 6). Owing to their volcanic origin, soils are generally young Andosols, formed under a humid climate on relatively recent lava flows and pyroclastic deposits. Moreover, Ribeira do Guilherme is a protection area for the management of habitats or species and Pico das Camarinhas is a geological monument [44]. The characterization of each plot is presented in Table 1.

2.3. Plot Sampling. The field work was carried out in May and June 2014. At each stand we marked a plot with 30 × 30 m (900 m^2). We divided each plot into 36 5 × 5 m quadrats. At each quadrat, the total number of trees was thoroughly counted. Saplings less than 2 cm of diameter at breast height were excluded because most likely they would not survive. All woody species that were within the plots were included in the measurements.

Regarding plant density obtained with plot counts we estimated the confidence interval using the usual formula for the mean:

$$\lambda \pm z_{\alpha/2} \mathrm{SE}_{\lambda}, \quad (1)$$

where λ is the mean density and SE_{λ} is the respective standard error.

2.4. Plotless Sampling. Thirty random points were selected at each plot. Distances from point-tree and tree-neighbor were measured at 1.30 m high, at the center of each tree. Three methods were used.

In T-Square [1, 13, 14, 20, 26], Figure 2(a), a random point is chosen and the distance to nearest tree is measured. A second distance is measured from the nearest tree to its nearest neighbor constrained to be in the hemisphere to the left of the dashed line. Four density estimators were used (Table 2). For T-Square sampling the 95% confidence interval for the reciprocal of tree density is calculated as

$$\frac{1}{\lambda_2} \pm t_\alpha \,[\mathrm{SE}]$$
$$\mathrm{SE} = \sqrt{\frac{8\left(\bar{t}^2 S_r{}^2 + 2\bar{r}\bar{t}S_{rt} + \bar{r}^2 S_t{}^2\right)}{n}}, \tag{2}$$

where SE is the standard error given by Diggle [31], λ_2 is the density estimator for T-Square (Table 2), $\bar{r}$ and $\bar{t}$ are the respective distance means, $S_r{}^2$ is the variance of r, $S_t{}^2$ is the variance of t, S_{rt} is the covariance of r and t, and t_α is the value taken from a Student distribution with $n-1$ degrees of freedom.

In Point Centered Quarter Method (PCQM; [15, 19, 24]; Figure 2(b)), the area around the random point is divided into four 90° quadrants and the distance to the nearest tree is measured in each quadrant. Three density estimators were used (Table 2). For PCQM confidence limits are given by Seber [45]:

$$\sqrt{\lambda_5} = \frac{\sqrt{16n-1} \pm z_{\alpha/2}}{\sqrt{\pi \sum \left(r_{ij}{}^2\right)}}, \tag{3}$$

where λ_5 is the density estimator for PCQM (Table 2), r_{ij} is the distance from random point i to closest individual in quarter j ($j = 1, 2, 3, 4$; $i = 1, \ldots, n$), and $z_{\alpha/2}$ is the value taken from the standard normal distribution, for an error α.

In N-tree sampling (K-tree sampling; [16, 27]; Figure 2(c)), a number of trees, N, closest to a random point are selected and the respective distances measured. Plot shape is circular and is based on a radius from the sampling point to the centre of the Nth tree closest to the point. In our study we considered a value of $N = 6$ (see Table 2 for estimators).

2.5. Spatial Pattern Analyses. Since the 1950s, several spatial methods of analysis have been developed and modified to improve our ability to detect and characterize spatial patterns [46]. We used five indexes of spatial pattern, applied to our data from T-Square [47, 48], PCQM [28, 49], and quadrat sampling [50].

For T-Square we used the index recommended by Ludwig and Reynolds [48]:

$$C = \frac{\sum \left[r_i{}^2 / \left(r_i{}^2 + (1/2)\, t_i{}^2\right)\right]}{n},$$
$$z = \frac{C - 0.5}{\sqrt{1/(12n)}}. \tag{4}$$

The value of C is approximately 0.5 for random patterns, significantly less than 0.5 for uniform patterns, and significantly greater than 0.5 for clumped patterns. To test the significance of any departure of C from randomness, we compute a value for z (see [48], pp. 57-58 for details). We also used the statistic recommended by Hines and O'Hara Hines [47]:

$$H_t = \frac{2n\left[2\sum\left(r_i{}^2\right) + \sum\left(t_i{}^2\right)\right]}{\left[\left(\sqrt{2}\sum r_i\right) + \sum t_i\right]^2}. \tag{5}$$

This test statistic is compared with reference values (see [6], pp. 210 for details). Randomness corresponds to 1.27; smaller values indicate a uniform pattern while larger values indicate clumping.

We used our data from PCQM by selecting the tree closest to the sample point [6, 25, 48] and using the index of dispersion described by Johnson and Zimmer [49]:

$$I = (n+1)\,\frac{\sum\left(r_i{}^2\right)^2}{\left[\sum\left(r_i{}^2\right)\right]^2},$$
$$z = \frac{I - 2}{\sqrt{4(n-1)/(n+2)(n+3)}}. \tag{6}$$

The results are compared with the value defined for a random pattern, 2, with lower values suggesting a uniform pattern and larger values suggesting a clumped pattern (see [48], pp. 58-59 for details); and z is compared to a table of critical values for the standard normal distribution to detect significant departures from randomness [48]. The second procedure used for PCQM was suggested by Eberhardt [50] and analyzed further by Hines and O'Hara Hines, [47]:

$$I_E = \left(\frac{s}{\bar{r}}\right)^2 + 1, \tag{7}$$

where s and $\bar{r}$ are, respectively, the standard deviation and mean of the point to nearest individual distances. Critical values have been computed by Hines and O'Hara Hines (see [6], p. 210 for details). The expected value in a random population is 1.27, lower values suggest a regular pattern, and larger values indicate clumping.

Regarding quadrat counts, the data comes from 5 × 5 m quadrats. Morisita's index of dispersion, I_d, has been extensively used to evaluate the degree of dispersion/aggregation of spatial point patterns [51]. It is based on random or regular quadrat counts and is closely related to the simplest and oldest measures of spatial pattern, the variance to mean ratio [6, 30]. We used a standardized version (I_p) of the index which is

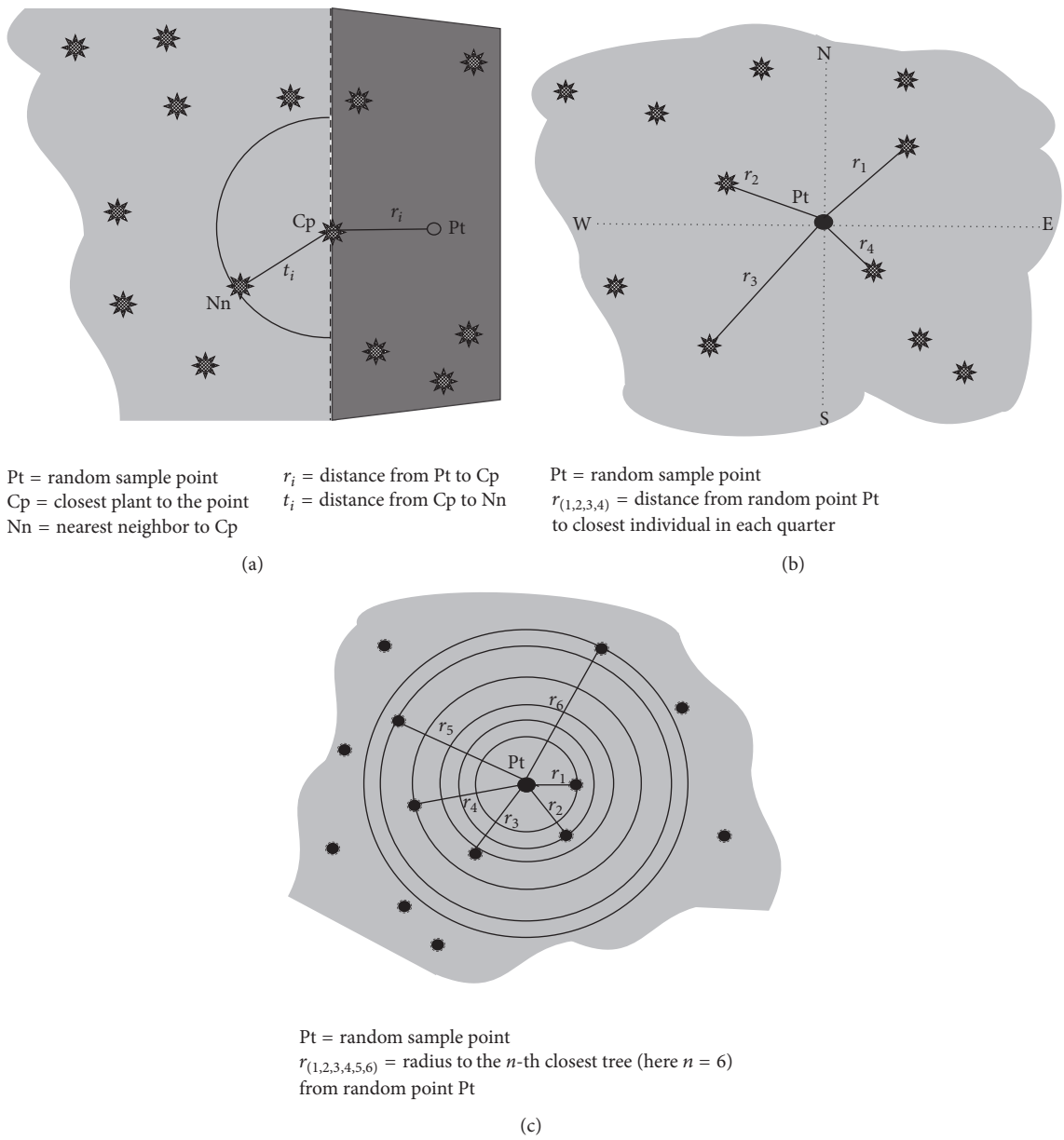

Figure 2: Application of T-Square sampling (a); Point Centered Quarter Method (b); and N-tree sampling method (c).

robust to variation in sample number and size [51] and is obtained by first calculating the raw index:

$$I_d = n\left[\frac{\sum x_i^2 - \sum x_i}{\left(\sum x_i\right)^2 - \sum x_i}\right], \tag{8}$$

where n is the number of samples and x_i is the number of individuals per sample. The standardized Morisita index of dispersion (I_p) ranges from −1 to +1, with 95% confidence limits at +0.5 and −0.5. Random patterns give an I_p of zero, clumped patterns above zero, and uniform patterns below zero (see [6], p. 217 for details). The second index used for quadrat counts is the variance to mean ratio, where a random pattern, described by the Poisson distribution, corresponds to the value 1:

$$I_c = \left(\frac{s^2}{\bar{x}}\right). \tag{9}$$

If the variance is larger than the mean, distribution is clumped; if it is smaller than the mean, distribution is regular [6]. To test significant departures from the random expectation, confidence envelopes using the χ^2 test for $n - 1$ degrees of freedom are calculated [30].

2.6. Statistical Analyses. To compare the different sampling methods, we used a resampling approach [52, 53]: (i) 25 sample points were randomly selected from the 30 available

Table 2: Different sampling methods and estimators used in this study (adapted from [1]).

Sampling methods	Estimators		Characteristic
T-Square	$\lambda_1 = \frac{2n}{\pi \sum(t_i^2)}$	[13]	λ = estimated density n = sample size t_i = distance from closest individual to nearest neighbor r_i = distance from random point to closest individual
	$\lambda_2 = \frac{n^2}{[2\sum(r_i)(\sqrt{2}\sum t_i)]}$	[14]	
	$\lambda_3 = \frac{2n}{[\pi \sum(r_i^2) + 0.5(\pi \sum(t_i^2))]}$	[20]	
	$\lambda_4 = \frac{n}{[\pi(\sum(r_i^2) + 0.5\sum(t_i^2))1/2]}$	[26]	
Point Centered Quarter	$\lambda_5 = \frac{4(4n-1)}{\pi \sum(r_{ij}^2)}$	[15]	λ = estimated density n = sample size g = number of individuals to be located in each sector of the area around the random sampling point r_{ij} = distance from random point i to closest individual in quarter j
	$\lambda_6 = \frac{1}{[\sum(r_{ij})/4n]}$	[3]	
	$\lambda_7 = \left(\frac{12}{\pi n}\right)\sum\left[\frac{1}{\sum\left(r_{ij}^2\right)}\right]$ for $g = 1$	[24]	
N-tree	$\lambda_8 = \frac{1}{n[\sum(2.5/A_i)]}$	For 3-tree	n = sample size λ = estimated density A_i = plot area in each plot is calculated separately using the following equation: $A_i = \pi r_i^2$ r_i = plot radius and in normal method prolongs to mid diameter of nth tree but in adjusted methods prolongs between n and $n+1$ trees.
	$\lambda_9 = \frac{1}{n[\sum(3/A_i)]}$	3-tree adjusted	
	$\lambda_{10} = \frac{1}{n[\sum(3.5/A_i)]}$	For 4-tree	
	$\lambda_{11} = \frac{1}{n[\sum(4/A_i)]}$	4-tree adjusted	
	$\lambda_{12} = \frac{1}{n[\sum(4.5/A_i)]}$	For 5-tree	
	$\lambda_{13} = \frac{1}{n[\sum(5/A_i)]}$	5-tree adjusted	
	$\lambda_{14} = \frac{1}{n[\sum(5.5/A_i)]}$	For 6-tree	
	$\lambda_{15} = \frac{1}{n[\sum(6/A_i)]}$	6-tree adjusted [16]	

for each plot; (ii) the different estimators were used to calculate tree density; (iii) this procedure was repeated 100 times; (iv) a similar approach was followed to estimate tree density using quadrat counts, where 30 out of 36 were used at each iteration. This procedure allowed simulating the variability in tree density estimates obtained using the different methods. Using this simulated dataset of tree densities, we compared all the estimators using a generalized linear model (ANOVA). Standard contrasts were then used to compare tree densities obtained using quadrat counts (the benchmark method) with all the other methods/estimators (λ_1–λ_{15}). In order to further compare the departure of each estimator from the benchmark method, we calculated the relative mean difference (for each of the six plots) between tree densities obtained by each estimator and the quadrat method and represented those values using a bar plot. In the same sense, the estimators were ranked, according to their distance to the benchmark method, and the ranks for the six plots were represented using a box-plot. To analyze estimator precision, we calculated the coefficient of variation (CV) of tree density for each estimator and divided it by the CV obtained using quadrats. Statistical analyses were all performed within the R software, version 3.2.3 (R Development Core Team, 2014) [54].

3. Results

3.1. Estimated Tree Densities. Tree densities ranged from 344 to 5056 trees/ha and *P. undulatum* tree density ranged from 267 to 4233 tree/ha (Table 3). According to the general results, T-Square sampling methods tended to underestimate the tree densities obtained with quadrats (Figure 3).

3.2. Comparison of Sampling Methods. The results of ANOVA, based on 100 simulations, revealed significant differences for the estimation of tree density among the different estimators: plot 1, $F = 8963$ and $P < 0.001$; plot 2, $F = 794$ and $P < 0.001$; plot 3, $F = 3040$ and $P < 0.001$; plot 4, $F = 3653$ and $P < 0.01$; plot 5, $F = 3037$ and

Table 3: Calculation of tree density at six *Pittosporum undulatum* dominated stands in São Miguel Island, sampled using a total of 36 5 × 5 m quadrats, corresponding to six 900 m^2 plots.

Plot	Total number of trees	*P. undulatum* number (%)		Total density (trees/ha)	*P. undulatum* (trees/ha)
1	31	24	77.4	344	267
2	124	84	67.7	1378	933
3	388	381	98.2	4311	4233
4	81	76	93.8	900	844
5	455	366	80.4	5056	4067
6	351	266	75.8	3900	2956

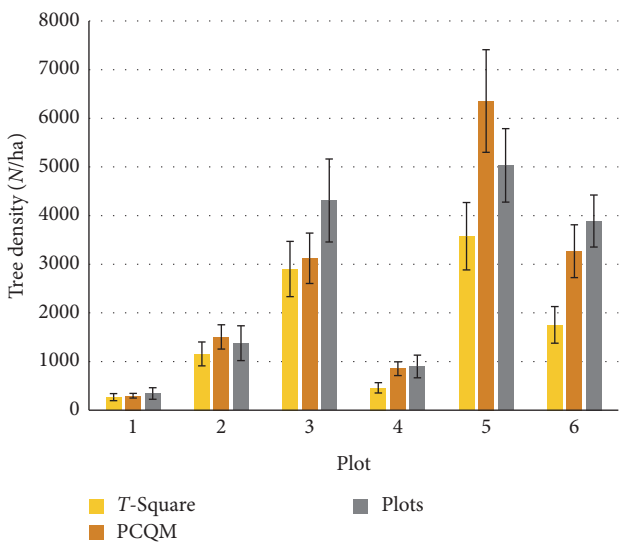

Figure 3: Tree density estimates and 95% confidence intervals obtained using the data collected at each plot, for T-Square sampling (λ_2), Point Centered Quarter Method (λ_5), and quadrat counts.

$P < 0.001$; and plot 6, $F = 2904$ and $P < 0.001$. In a more detailed analysis, comparing each distance based estimator with the quadrat estimates, T-Square and PCQM were consistently more accurate than N-tree (Figures 4–6). T-Square estimators λ_1, λ_2, and λ_3 were much more accurate and precise than estimator λ_4 that greatly overestimated tree density (Figures 4–7).

The PCQM estimators performed with mix results. In general λ_5 and λ_7 produced more accurate estimates than λ_6 (Figure 4). Overall, the best estimators, in terms of originating a consistently low difference towards the benchmark, include λ_1 and λ_5, (Figure 4). Also, the estimators that showed the lowest differences towards the benchmark, based on the results for the six plots, were λ_1 to λ_3, λ_5, and λ_7 (Figure 5). In the same sense, the estimators that scored the best approximations towards the benchmark more often, based on the six plots, were again λ_1 to λ_3 and λ_5 (Figure 6).

Regarding precision, the more accurate estimators, mentioned above, were also among those with lower CV as compared to the values obtained with quadrat counts (Figure 7).

3.3. Spatial Pattern Analysis. Regarding T-Square sampling, the test of significance for C was above the critical value (1.96) for plot 2 only. For H_t, according to critical values for this test statistic, plots 3 and 5 showed regular patterns, plots 1 and 4 showed aggregation, and plots 2 and 6 showed a random pattern (Table 4). Regarding PCQM, for plots 1 and 4, z was above the critical value (1.96). For I_e, according to critical values for this test statistic, plots 5 and 6 showed regular pattern, plots 1 and 4 an aggregated pattern, and plots 2 and 3 a random pattern (Table 4).

For quadrat counts, I_p indicated random patterns for all plots, although with several values close to the limits of the 95% confidence interval (i.e., −0.5 to 0.5), for which I_c already indicated a significant departure from randomness (Table 4).

4. Discussion

4.1. Tree Densities. The tree density values (trees/ha of *P. undulatum*) obtained in the present research are within the range of the results obtained for *P. undulatum* stands in Graciosa Island in a previous survey using PCQM [41]. This confirms the wide variation in tree density that can be found in the Azorean exotic woodland dominated by *P. undulatum* and will have implications in estimating and managing biomass production [38, 41]. Such density variations are dictated by the underlying physical environment and past disturbance history. The results also confirm the potential for the biomass valorization of this species, which together with other uses, such as honey and compost production [55, 56] and reforestation measures, could be included in a broader management program for this invasive plant.

4.2. Sampling Methods. In terms of accuracy of results, when compared with quadrat counts, the T-Square and PCQM estimators provided the best results. T-Square estimators evolved as methods to remove some bias due to nonrandomness associated with the nearest neighbor distance measurement [13]. The T-Square estimators performed generally slightly better and globally more consistently than the other estimators. The usual T-Square estimator, λ_2, showed a similar performance regarding λ_1 and λ_3. However, in studies performed by Engeman et al. [21], λ_2 performed slightly better than λ_3 and λ_4. In our study λ_4 appeared to be inaccurate and should therefore not be used in Azorean forests dominated by *P. undulatum*. The two compound estimators λ_2 and λ_3 suggested by Byth [14] and Diggle [20], respectively, were developed to create a more robust estimator using T-Square distance measurements that would perform better in a variety of spatial patterns, relative to more simple estimators utilizing only one distance measurement [13]. Both estimators showed a similar performance in our study, while some authors argue in favor of λ_2 [14]. T-Square measurements are essentially nearest neighbor measurements modified only by restricting the direction in which measurements are made but have been shown to be accurate in many situations [29]. However, in our study T-Square estimates tended to underestimate the results obtained with the quadrat method (used as a benchmark). This should be further analyzed and taken into account in future studies, although T-Square sampling is simple to

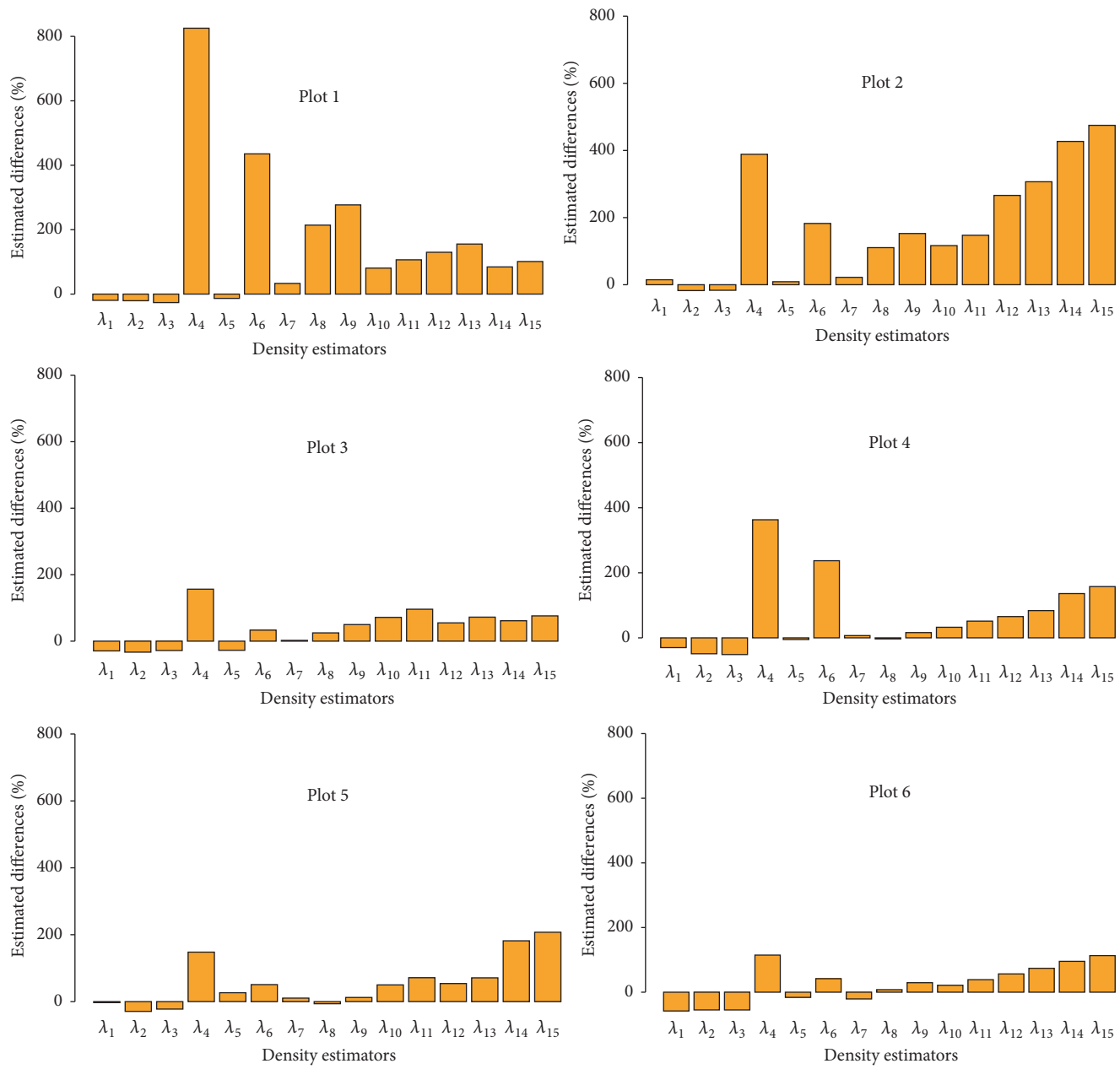

Figure 4: Comparison of distance methods and respective estimators (T-Square, λ_1–λ_4; PCQM, λ_5–λ_7; N-tree, λ_8–λ_{15}) with quadrat counts, for tree density estimates in all six *Pittosporum undulatum* stands. The bars represent the estimated relative difference between each distance method and the estimated tree density using quadrat counts, as obtained from contrast analysis applied after ANOVA (divided by tree density estimated by quadrat counts and expressed as a percentage).

Table 4: Summary of the analysis performed regarding the spatial distribution patterns in the six plots sampled at *Pittosporum undulatum* stands. Significant deviations from a random pattern are indicated in bold (aggregated) or in italic (regular).

Plots	T-Square			PCQM			Quadrats			
	C	z	H_t	I	z	I_e	I_d	I_p	I_c	χ^2
Plot 1	0.57	1.34	**1.37**	**3.12**	**3.38**	**1.61**	0.93	−0.07	0.94	32.87
Plot 2	**0.62**	**2.29**	1.23	2.13	0.39	1.26	1.34	0.50	**2.20**	**76.90**
Plot 3	0.51	0.10	*1.19*	2.27	0.81	1.26	1.27	0.50	**3.94**	**137.89**
Plot 4	0.58	1.55	**1.31**	**2.98**	**2.97**	**1.39**	1.18	−0.49	1.41	49.22
Plot 5	0.59	1.64	*1.15*	1.35	−1.96	*1.08*	1.13	0.50	**2.66**	**93.04**
Plot 6	0.55	0.96	1.28	1.39	−1.83	*1.07*	1.07	0.50	**1.73**	**60.40**

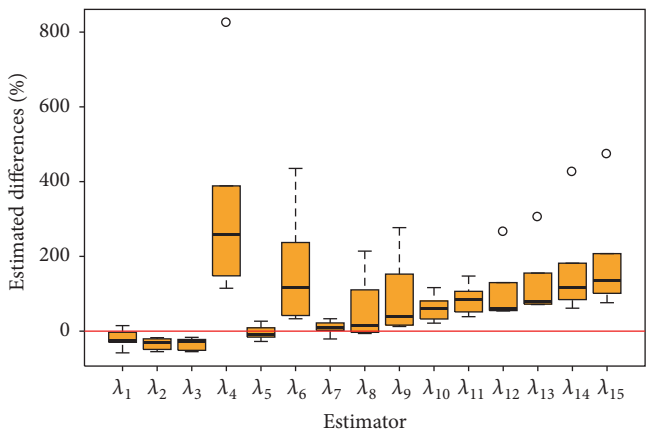

Figure 5: Box-plot representing the distribution of mean relative differences in tree density estimation, between each estimator and the quadrat counts, based on the data from the six *Pittosporum undulatum* plots.

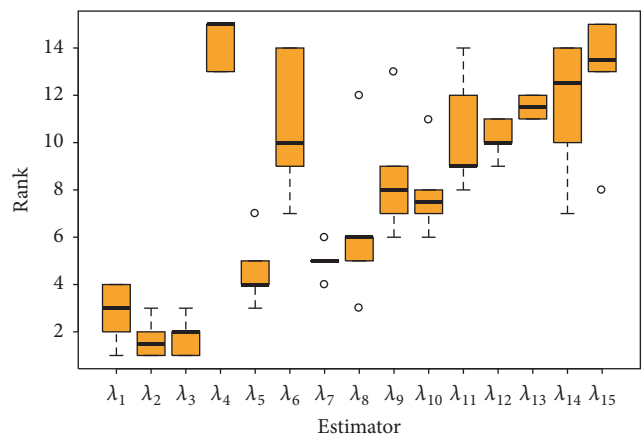

Figure 6: Box-plot representing the ranks of each estimator, based on the results for the six *Pittosporum undulatum* plots. A high rank (i.e., a maximum of 1) indicates that a particular estimator was closest to the benchmark more often.

implement in the field, and this simplicity is often preferred by field workers.

Point Centered Quarter Method is often used in forestry, and we found that the estimator λ_5, given by Pollard [15], was among the most accurate among those tested. The advantage of this estimator is that it is very efficient in field sampling. Previous work [2, 3] concluded that PCQM was capable of yielding accurate results and was least susceptible to subjective bias than other methods [1]. However, it has the disadvantage of being very susceptible to bias whenever the spatial pattern is not random [15].

Our results showed that N-tree method performed badly in all plots, showing a considerable overestimation of tree density. Klein and Vilkco [57] proposed two new estimators but it seems that these estimators still need more field work validation. Zobeiry [58] showed that N-tree sampling techniques are not suitable for the natural forest of northern Iran because they consistently underestimated the true population parameters, a clumped pattern being the most reasonable cause of this behavior. However, N-tree sampling performed well in others studies [1].

4.3. Spatial Pattern. In temperate forests, tree species can have random, aggregated, and even regular spatial patterns, to different degrees, and the spatial pattern of a given species may vary from place to place [33]. The major weakness of density estimators is that their bias is dependent on the spatial distribution of the population [10]. Some distance estimators have been shown to be unbiased over a wide range of spatial patterns if the estimators are adjusted according to the spatial pattern. However, this adjustment would need additional tests to determine a population's spatial distribution before estimating density [10]. T-Square sampling has been largely recommended for spatial pattern analysis based on both simulated patterns and mapped field data [26, 29, 31, 48]. However, the statistics used in our study showed somewhat irregular results. This might be explained if *P. undulatum* stands correspond to a spatial distribution with a slight deviation from randomness, that is, a small degree of aggregation. This was evident in the results obtained for the parameters used for spatial pattern analysis from quadrat counts, with several values very close to the limit for randomness in the standardized Morisita index, which already corresponded to a significant deviation from randomness when using the variance to mean ratio. Therefore, the studied plots are more likely to correspond to random or slightly aggregated distribution patterns. Among Azorean native trees, the aggregate pattern is predominant in saplings but adults are mostly randomly distributed [33]. Aggregate patterns of distribution are reported as the most commonly observed in nature [6, 59], caused by environmental heterogeneity [6] and nonlinearity of biological processes [60] and density-dependent mortality/self-thinning [33]. The main reasons leading to a clustered pattern in a population are the behavioral characteristics of the species and intra- and interspecific relationships [61]. Intraspecific aggregation should limit the mean species richness of the communities to a level less than what it would be if individuals were randomly distributed among the communities [59]. Spatial structure of forest is a complicated characteristic due to the complex factors influencing it [25]: climate, micro-site mosaic, regeneration methods, natural mortality of individuals, biological and ecological characteristics of organisms, natural disturbance (e.g., fire, wind, and landslides), and human activities.

Human activity is an important factor in managed stands, such as the case of *P. undulatum* that was widely cultivated throughout the world, including Atlantic (e.g., Azores, Jamaica) and Pacific islands (e.g., Hawaii) and South Africa [62]. It has become dominant in moist disturbed secondary forests and some primary forests from low to middle elevations [36–39]. Perhaps the main effect of *P. undulatum* comes from its ability to penetrate natural ecosystems by the competitive exclusion of native species [63], regenerating readily and competitively in forest gaps [64] and having dense shade tolerant seedlings that outcompete native seedlings, which affects its distribution pattern [65]. Seedling and sapling

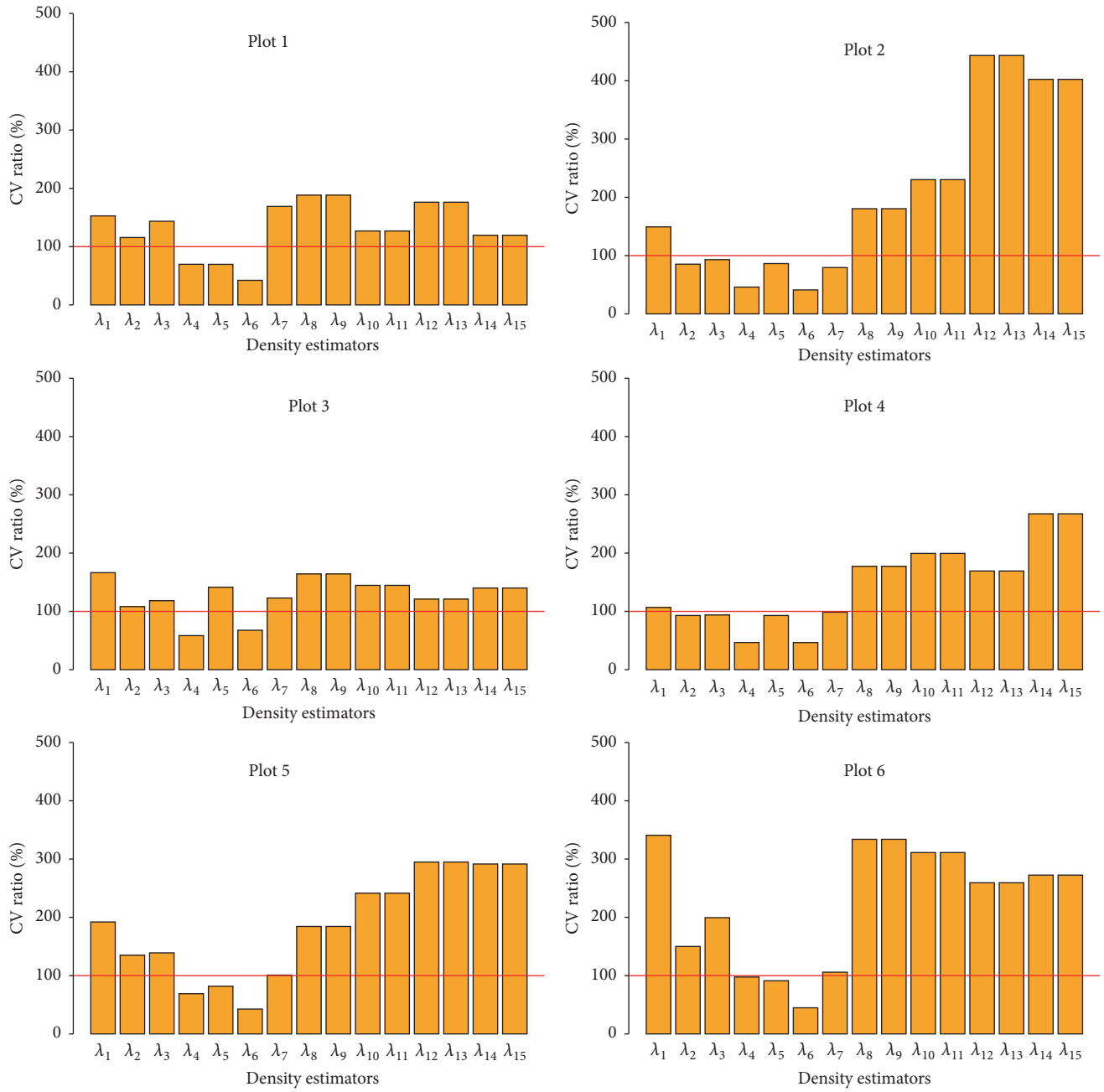

Figure 7: Comparison of the precision obtained when using distance methods or quadrat counts to estimate tree density. The bars represent the ratio between the coefficient of variation (CV) obtained for each estimator and the CV obtained when using quadrat counts. The 100% red line corresponds to the same CV for both the distance estimator and the quadrats.

aggregation may also result from the type of dispersion and seed rain [66]. Seed and seedling concentration may occur, for example, in places where animals frequently defecate [67] which is especially important since most Azorean tree species are dispersed by birds [68].

5. Conclusion

Distance methods also known as plotless sampling techniques were introduced because of the practical difficulties sometimes raised by quadrat sampling [69]. Plotless sampling is considerably more efficient than quadrat sampling, since searching and counting individuals in a large quadrat are very time consuming. However, accuracy and precision are important factors in determining an appropriate sampling method. This is because bias or imprecision in initial density estimates will propagate through derived metrics, such as basal area, biomass, and carbon storage. Among the tested methods, T-Square sampling ranked more often as the closest estimator to the benchmark method. It is recommended

for aggregated spatial patterns (a limitation for the use of PCQM). According to our results aggregation might occur in some of the stands. Moreover, T-Square sampling provides more independent tree measures than PCQM (only 2 per sampling station, instead of 4), which might be useful when other parameters are also being sampled. Therefore, we suggest that T-Square sampling might be used to estimate tree density in management programs dedicated to *P. undulatum* in the Azores.

Competing Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Acknowledgments

The first author would like to acknowledge NATURAL-REASON, LDA, a company devoted to biofuels production, for supporting her research grant and for its financial contribution to the ongoing research project. This work is also funded by FEDER funds through the Operational Programme for Competitiveness Factors (COMPETE) and by National Funds through FCT (Foundation for Science and Technology) under the UID/BIA/50027/2013 and POCI-01-0145-FEDER-006821.

References

[1] B. Kiani, A. Fallah, M. Tabari, and S. M. Hosseini, "A comparison of distance samling methods in Saxaul (*Halloxylon Ammodendron* C.A. Mey Bunge) shrub-lands," *Polish Journal of Ecology*, vol. 61, pp. 207–219, 2013.

[2] S. L. Beasom and H. H. Haucke, "A comparison of four distance sampling techniques in South Texas Live oak mottes," *Journal of Range Management*, vol. 28, no. 2, pp. 142–144, 1975.

[3] G. Cottam and J. T. Curtis, "The use of distance measures in phytosociological sampling," *Ecology*, vol. 37, no. 3, pp. 451–460, 1956.

[4] Y. Askari, E. S. Kafash, and D. Rezaei, "Evaluation of different sampling methods to study of shrub density in Zagros forest," *European Journal of Experimental Biology*, vol. 3, pp. 121–128, 2013.

[5] V. Lieven, F. Vancoillie, and R. D. Wulf, "Object based forest stand density estimation from very high resolution optical imagery using wavelet based texture measures," *International Archives of Photogrammetry, Remote Sensing and Spatial Information Sciences*, vol. 36, no. 4-C42, article 13, 2006.

[6] C. J. Krebs, *Ecological Methodology*, Addison Wesley Longman, New York, NY, USA, 2nd edition, 1999.

[7] K. Mitchell, *Quantitive Analysis by the Point-Centered Quarter Method*, Department of Mathematics and Computer Science Hobart and William Smith Colleges, Geneva, Switzerland, 2007.

[8] V. Kint, D. W. Robert, and L. Noël, "Evaluation of sampling methods for the estimation of structural indices in forest stands," *Ecological Modelling*, vol. 180, no. 4, pp. 461–476, 2004.

[9] S. Magnussen, C. Kleinn, and N. Picard, "Two new density estimators for distance sampling," *European Journal of Forest Research*, vol. 127, no. 3, pp. 213–224, 2008.

[10] D. Unger, J. P. Stovall, B. P. Oswald, D. Kulhavy, and I.-K. Hung, "A test of the mean distance method for forest regeneration assessment," *Mathematical and Computational Forestry and Natural-Resource Sciences*, vol. 6, no. 2, pp. 54–61, 2014.

[11] N. Picard, A. M. Kouyaté, and H. Dessard, "Tree density estimations using a distance method in Mali Savanna," *Forest Science*, vol. 51, no. 1, pp. 7–18, 2005.

[12] V. C. Lessard, D. D. Reed, and N. Monkevich, "Comparing n-tree distance sampling with point and plot sampling in northern Michigan forest types," *Northern Journal of Applied Forestry*, vol. 11, pp. 12–16, 1994.

[13] J. E. Besag and J. T. Gleaves, "On the detection of spatial pattern in plant communities," *Bulletin of the International Statistical Institute*, vol. 45, pp. 153–158, 1973.

[14] K. Byth, "On robust distance-based intensity estimators," *Biometrics*, vol. 38, no. 1, pp. 127–135, 1982.

[15] J. H. Pollard, "On distance estimators of density in randomly distributed forests," *Biometrics*, vol. 27, no. 4, pp. 991–1002, 1971.

[16] M. Prodan, "Punktstichprobe für die forsteinrichtung für die forsteinrichtung. (A point sample for forest management planning)," *Forst Holzwirt*, vol. 23, pp. 225–226, 1968.

[17] G. Cottam and J. T. Curtis, "A method for making rapid surveys of woodlands by means of pairs of randomly selected trees," *Ecology*, vol. 30, no. 1, pp. 101–104, 1949.

[18] K. R. Parker, "Density estimation by variable area transect," *The Journal of Wildlife Management*, vol. 43, no. 2, p. 484, 1979.

[19] M. Morisita, "Estimation of population density by a spacing method," *Memoirs of the Faculty of Science, Kyushu University*, vol. 1, pp. 187–197, 1954.

[20] P. J. Diggle, "Robust density estimation using distance methods," *Biometrika*, vol. 62, pp. 39–48, 1975.

[21] R. M. Engeman, R. T. Sugihara, L. F. Pank, and W. E. Dusenberry, "A comparison of plotless density estimators using Monte Carlo simulation," *Ecology*, vol. 75, no. 6, pp. 1769–1779, 1994.

[22] W. G. Warren and C. L. Batcheler, "The density of spatial patterns: robust estimation through distance methods," in *Spatial and Temporal Analysis in Ecology*, R. M. Cormack and J. K. Ord, Eds., pp. 247–270, 1979.

[23] X. Zhu and J. Zhang, "Quartered neighbor method: a new distance method for density estimation," *Frontiers of Biology in China*, vol. 4, no. 4, pp. 574–578, 2009.

[24] M. Morisita, "A new method for the estimation of density by spacing method applicable to non-randomly distributed populations," *Physiology and Ecology*, vol. 7, pp. 134–144, 1957.

[25] J. Szmyt, "Spatial statistics in ecological analysis: from indices to functions," *Silva Fennica*, vol. 48, no. 1, pp. 1–31, 2014.

[26] P. J. Diggle, "Note on the Clark and Evans test of spatial randomness," *Appendix to Hodder and Orton*, pp. 246–248, 1976.

[27] C. Kleinn and F. Vilčko, "Design-unbiased estimation for point-to-tree distance sampling," *Canadian Journal of Forest Research*, vol. 36, no. 6, pp. 1407–1414, 2006.

[28] P. J. Clark and F. C. Evans, "Distance to nearest neighbor as a measure of spatial relationships in populations," *Ecology*, vol. 35, no. 4, pp. 445–453, 1954.

[29] H. B. Özel, "The spatial pattern analyses of natural pure oriental beech (*Fagus orientalis* Lipsky.) stands in the bartin-yenihan district in Turkey," *Biyoloji Bilimleri Araştırma Dergisi*, vol. 7, pp. 34–37, 2014.

[30] M. R. T. Dale, P. Dixon, M.-J. Fortin, P. Legendre, D. E. Myers, and M. S. Rosenberg, "Conceptual and mathematical relationships among methods for spatial analysis," *Ecography*, vol. 25, no. 5, pp. 558–577, 2002.

[31] P. J. Diggle, *Statistical Analysis of Spatial Point Patterns*, Mathematics in Biology, Academic Press, London, UK, 1983.

[32] M. A. Wulder, J. C. White, K. O. Niemann, and T. Nelson, "Comparison of airborne and satellite high spatial resolution data for the identification of individual trees with local maxima filtering," *International Journal of Remote Sensing*, vol. 25, no. 11, pp. 2225–2232, 2004.

[33] R. B. Elias, E. Dias, and F. Pereira, "Disturbance, regeneration and the spatial pattern of tree species in Azorean mountain forests," *Community Ecology*, vol. 12, no. 1, pp. 23–30, 2011.

[34] L. Silva and C. W. Smith, "A quantitative approach to the study of non-indigenous plants: an example from the Azores Archipelago," *Biodiversity and Conservation*, vol. 15, no. 5, pp. 1661–1679, 2006.

[35] L. Silva, E. Ojeda-Land, and J. L. Rodríguez-Luengo, *Invasive Terrestrial Flora and Fauna of Macaronesia. Top 100 in Azores, Madeira and Canaries*, ARENA, Ponta Delgada, Portugal, 2008.

[36] H. Costa, V. Medeiros, E. B. Azevedo, and L. Silva, "Evaluating ecological-niche factor analysis as a modelling tool for environmental weed management in island systems," *Weed Research*, vol. 53, no. 3, pp. 221–230, 2013.

[37] J. Hortal, P. A. V. Borges, A. Jiménez-Valverde, E. B. de Azevedo, and L. Silva, "Assessing the areas under risk of invasion within islands through potential distribution modelling: the case of *Pittosporum undulatum* in São Miguel, Azores," *Journal for Nature Conservation*, vol. 18, no. 4, pp. 247–257, 2010.

[38] P. Lourenço, V. Medeiros, A. Gil, and L. Silva, "Distribution, habitat and biomass of *Pittosporum undulatum*, the most important woody plant invader in the Azores Archipelago," *Forest Ecology and Management*, vol. 262, no. 2, pp. 178–187, 2011.

[39] H. Costa, S. C. Aranda, P. Lourenço, V. Medeiros, E. B. D. Azevedo, and L. Silva, "Predicting successful replacement of forest invaders by native species using species distribution models: the case of *Pittosporum undulatum* and *Morella faya* in the Azores," *Forest Ecology and Management*, vol. 279, pp. 90–96, 2012.

[40] H. Costa, M. J. Bettencourt, C. M. N. Silva, J. Teodósio, A. Gil, and L. Silva, "Invasive alien plants in the azorean protected areas: invasion status and mitigation actions," in *Plant Invasions in Protected Areas: Patterns, Problems and Challenges*, vol. 7 of *Invading Nature—Springer Series in Invasion Ecology*, pp. 375–394, Springer, Amsterdam, The Netherlands, 2013.

[41] A. Teixeira, C. Mir, L. Borges Silva, and L. Hahndorf, "Invasive woodland resources in the azores: biomass availability for 100% renewable energy supply in graciosa Island," in *Proceedings of the 23rd European Biomass Conference and Exhibition*, pp. 14–23, Vienna, Austria, 2015.

[42] E. B. Azevedo, "Condicionantes Dinâmicas do Clima do Arquipélago dos Açores. Elementos para o seu AÇOREANA," *Boletim da Sociedade de Estudos Açoreanos Afonso Chaves*, vol. 9, no. 3, pp. 309–317, 2001.

[43] J. L. Gaspar, J. E. Guest, G. Queiroz et al., "Eruptive frequency and volcanic hazards zonation in São Miguel Island, Azores," in *Volcanic Geology of São Miguel Island (Azores Archipelago)*, J. L. Gaspar, J. E. Guest, A. M. Duncan, F. J. A. Barriga, and D. K. Chester, Eds., vol. 44 of *Geological Society, London, Memoirs*, pp. 155–166, The Geological Society, 2015.

[44] E. A. Lima, M. Machado, and J. Ponte, "Geological heritage management: monitoring the Azores Geopark geosites," *Comunicações Geológicas*, vol. 101, no. 3, pp. 1295–1298, 2014.

[45] G. A. F. Seber, *The Estimation of Animal Abundance*, Charles Griffin and Company, London, UK, 2nd edition, 1982.

[46] M. J. Fortin, M. R. T. Dale, and J. V. Hoef, "Spatial analysis in ecology," in *Encyclopedia of Environmetrics*, vol. 4, pp. 2051–2058, John Wiley & Sons, Chichester, UK, 2002.

[47] W. G. S. Hines and R. J. O'Hara Hines, "The Eberhardt statistic and the detection of nonrandomness of spatial point distributions," *Biometrika*, vol. 66, no. 1, pp. 73–79, 1979.

[48] J. A. Ludwig and J. F. Reynolds, *Statistical Ecology: A Primer on Methods and Computing*, John Wiley & Sons, New York, NY, USA, 1988.

[49] R. B. Johnson and W. J. Zimmer, "A more powerful test for dispersion using distance measurements," *Ecology*, vol. 66, no. 5, pp. 1669–1675, 1985.

[50] L. L. Eberhardt, "Some developments in 'distance sampling,'" *Biometrics*, vol. 23, no. 2, pp. 207–216, 1967.

[51] M. Morisita, "I_σ-index, a measure of dispersion of individuals," *Researches on Population Ecology*, vol. 4, no. 1, pp. 1–7, 1962.

[52] B. Efron and R. J. Tibshirani, *An Introduction to the Bootstrap*, vol. 57 of *Monographs on Statistics and Applied Probability*, Chapman & Hall, New York, NY, USA, 1993.

[53] A. C. Davison and D. Kuonen, "An introduction to the bootsrap with applications in R," *Statistical Computing & Statistical Graphics Newsletter*, vol. 13, pp. 6–11, 2002.

[54] R Core Team, *R: A Language and Environment for Statistical Computing*, R Foundation, 2014.

[55] J. Tavares and J. Baptista, "Cultura do Ananás em Estufa Ilha S. Miguel—Açores (Portugal)," in *Ponta Delgada: Produtos—Cooperativa de Produtores de Frutas; Produtos Hortícolas e Florícolas de S. Miguel*, p. 195, 2004.

[56] Azores Government, "Caderno Especificações DOP Mel dos Açores," 2016, https://www.azores.gov.pt/NR/rdonlyres/10DBC106-63C8-4ED8-BD69-9FB765D835BB/452723/Caderno-EspecificaesDOPMeldosAores4.pdf.

[57] C. H. Klein and F. Vilkco, "On estimation in *k*-tree sampling," in *Proceedings of the 7th Annual Forest Inventory and Analysis Symposium*, pp. 203–208, Portland, Ore, USA, 2005.

[58] Y. M. Zobeiry, "Tree sampling in natural forests of northern Iran," *The Forestry Chronicle*, vol. 54, no. 3, pp. 171–172, 1978.

[59] J. A. Veech, "Analyzing patterns of species diversity as departures from random expectations," *Oikos*, vol. 108, no. 1, pp. 149–155, 2005.

[60] S. W. Pacala and S. A. Levin, "Biological generated spatial pattern and the coexistence of competing species," in *Spatial Ecology: The Role of Space in Population Dynamics and Interspecific Interactions*, D. Tilman and P. Kareiva, Eds., pp. 204–232, Princeton University Press, Princeton, NJ, USA, 1997.

[61] D. S. Matteucci and A. Colma, *Metodologia para el Estúdio de la Vegetación*, Secretaria General de la Organizacion de los Estados Americanos, Washington, DC, USA, 1982.

[62] P. Binggeli, *An Overview of Invasive Woody Plants in the Tropics*, vol. 13 of *School of Agricultural and Forest Sciences Publication Number*, Unversity of Wales, Bangor, UK, 1998.

[63] T. Goodland and J. R. Healey, *The invasion of Jamaican montane rainforests by the Australian tree* Pittosporum undulatum, School of Agricultural and Forest Sciences, University of Wales, Bangor, UK, 1996.

[64] R. B. Elias and E. Dias, "Gap dynamics and regeneration strategies in *Juniperus-Laurus* forests of the Azores Islands," *Plant Ecology*, vol. 200, no. 2, pp. 179–189, 2009.

[65] P. J. Bellingham, E. V. J. Tanner, and J. R. Healey, "Hurricane disturbance accelerates invasion by the alien tree *Pittosporum*

undulatum in Jamaican montane rain forests," *Journal of Vegetation Science*, vol. 16, no. 6, pp. 675–684, 2005.

[66] A. F. Souza and F. R. Martins, "Spatial distribution of an undergrowth palm in fragments of the Brazilian Atlantic Forest," *Plant Ecology*, vol. 164, no. 2, pp. 141–155, 2003.

[67] P. J. van der Meer, F. J. Sterck, and F. Bongers, "Tree seedling performance in canopy gaps in a tropical rain forest at Nouragues, French Guiana," *Journal of Tropical Ecology*, vol. 14, no. 2, pp. 119–137, 1998.

[68] B. Rumeu, M. Nogales, R. B. Elias et al., "Contrasting phenology and female cone characteristics of the two Macaronesian island endemic cedars (*Juniperus cedrus* and *J. brevifolia*)," *European Journal of Forest Research*, vol. 128, no. 6, pp. 567–574, 2009.

[69] P. J. Diggle, *Statistical analysis of spatial and spatio-temporal point patterns*, vol. 128 of *Monographs on Statistics and Applied Probability*, CRC Press, Boca Raton, Fla, USA, Third edition, 2014.