# Structural heterogeneity and old-growthness: A first regional-scale assessment of Sardinian forests

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Abstract Mediterranean forests have been altered by several human activities. Consequently, relatively intact forests that have been unmodified by humans for a relatively long time (i.e., old-growth forests) are often reduced to isolated and fragmented stands. However, despite their high conservation value, little is known about their features and even presence several Mediterranean areas. First steps of their investigation are based on the identification of old-growth features such as amount of large-size and old trees, tree species composition, canopy heterogeneity, occurrence and amount of deadwood. The Structural Heterogeneity Index (SHI) is commonly used to summarise features of old-growthness in one single value. Here, the SHI was derived for 68 plots included in 45 forest stands within the 4,297 km<sup>2</sup> of territory that is covered by forests in Sardinia. SHI values were affected by variables that are likely to be related to forest age and structural complexity, such as presence of cerambycids, canopy cover, forest layers, location and three old-growthness classes. Results confirm a high structural variability among forests with old-growth features, determined by the presence, or lack, of given living and deadwood features. Our findings identified, for the first time, most of the forest stands that need special protection in Sardinia for the presence of old-growth features. In this sense, the SHI was confirmed useful for improving their management and conservation, although more specific and deeper studies are necessary for better understanding their species composition and dynamics.

**Keywords:** old-growth forests; forest ecosystems conservation; Mediterranean Basin; Structural Heterogeneity Index

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# Introduction

Forest ecosystems have been subject to human exploitation for millennia, since the establishment of the first agricultural societies in the mid-Holocene, mainly for fuel wood production, food, cropland, pasture, and construction materials (Parviainen 2005, Kaplan et al. 2009). The impact is more pronounced where the stable presence of humans is historically documented, like in Europe and especially in the Mediterranean Basin (Grove & Rackham 2001). Despite being one of the most threatened by current changes (Gauquelin et al. 2018), plant diversity in the Mediterranean Basin is still high, primarily as a result of habitat diversity (Fois et al. 2017). Among them, thermophilous deciduous, broadleaved evergreen and coniferous forests and woodlands represent a wide range of habitats of conservation concern in the Mediterranean (Gauquelin et al. 2018). In this context, human disturbance has, among others, caused simplification of forest structure, changes in plant composition, abundance and distribution (Blasi et al. 2010, Abadie et al. 2018). Moreover, forest conversions into managed systems have been proven to decrease the capacity of storing carbon below ground due to simplification of foliage and root litter inputs (Błonska et al. 2020).

A long-lasting history of land use has reduced forests without visible human interventions to cover in Europe about 0.25% of land (Sabatini et al. 2018). This is particular evident in the southern shore of the Mediterranean Basin, where forest degradation, mainly as a result of intensive wood collection and livestock grazing, is still intense and leads to their fragmentation or disappearance (Gauquelin et al. 2018) or in countries, like Italy, where forest structure since Roman times has been greatly influenced by man (Chiavetta et al. 2012). Exotic trees species such as *Pinus* spp., *Eucalyptus* spp. and *Acacia* spp. have been largely used to restore degraded Mediterranean ecosystems, which are now modifying the natural structure and composition of plant cover and the vegetation successions (Gauquelin et al. 2018). This situation is even exacerbated in the southern Mediterranean by global warming, which is expected to result in increased frequency, intensity and duration of drought, heat stress and wildfires (Cardil et al. 2014, Gauquelin et al. 2018, Arar et al. 2020).

Naturally regenerated forests of native species, where there are no clearly visible signs of human activities and ecological processes are not significantly disturbed, are generally called primary forests (FAO 2015). These often isolated primary forest stands recover compositional, structural and functional features and can be also defined old-growth forests or long untouched forests (Buchwald 2005. Chirici & Nocentini 2013). As a forest ages, peculiar structural features develop, for instance through the accumulation of high quantities of large trees and deadwood, which determines an increase in general complexity (=heterogeneity) of the forest structure (Burrasacano et al. 2018). Besides being extremely complex in structure (Motta et al. 2015), recent researches has highlighted that old-growth forests, especially in the Mediterranean, are characterised by hosting an especially high number of species of conservation concern for several taxonomic groups such as invertebrates, bryophytes, lichens, and vascular plants (Janssen et al. 2009, Brunialti et al. 2010, Vicol 2016). However, the response of biodiversity to the cessation of management remains in many aspects unclear, due to the interdisciplinary and time-consuming efforts required, joined to site-specific socio-ecological features that differently drive forest dynamics in different contexts (Paillet et al. 2010, Burrascano et al. 2018).

Due to the low knowledge level about such old-growth forests and woodlands in several Mediterranean landscapes, first steps towards their understanding focus on indices or proxies of "old-growthness". Structural components of

forests are often used for the relative simplicity in measuring, calculating and interpreting their variability (McElhinny et al. 2006, Sabatini et al. 2015). Among forest features that have been used to rank the old-growthness of forests and stands, the most common are: occurrence and amount of large-size and old trees, tree species composition, canopy heterogeneity, occurrence and amount of deadwood, lack of human disturbance, and gap dynamics (Burrascano 2010, Ziaco et al. 2011). Because no one single proxy indicator is able to be a measure of old-growthness alone, various indices have been elaborated in order to summarise various forest characteristics in one single value (Storch et al. 2018). Ideally, this would allow a quantification and comparison of old-growthness of different stands and provide an indication of their potential contribution to biodiversity (Parkes et al. 2003). Measures of structural complexity were elaborated in several contexts (e.g., McElhinny et al. 2006, Sabatini et al. 2015); these were based on a core of attributes collected from standardised plots arranged within each stand. Each attribute is quantified proportionally to the observed level and combined with others through an additive index. The rationale of this approach is that forest ecosystems containing stands with a variety of structural components are considered likely to have a variety of resources and species that utilise these resources. In fact, there is often a positive correlation between elements of biodiversity and measures of the variety and/or complexity of structural components within forest ecosystems (McElhinny et al. 2006, Parisi et al. 2016).

In the Mediterranean Basin, Amici et al. (2013) showed that light-demanding species richness decreased with increasing successional age of forest. Plant species in old-growth forests are often specialists, with short distance dispersal mechanisms and no persistent soil seed bank, which makes them more vulnerable to forest habitat loss (Burrascano et al. 2009, Campetella et al. 2016, Abadie et al. 2018).

Saprobic fungi diversity is often particularly high in temperate and boreal old-growth forests (Ohlson et al. 1997), even though this was not confirmed in drier conditions, like in Corsica, where ectomycorrhizal fungi were dominant (Richard et al. 2004). Also, some lichens, such as Lobaria pulmonaria (L.) Hoffm., Antitrichia curtipendula Bridel-Brederi, and Homalothecium sericeum (Hedw.) Schimp. (Brunialti et al. 2010), and invertebrates, like Cerambyx cerdo L. (Casula 2017), are found as indicators of old-growth features in Mediterranean forests. Despite such a kind of promising results, compared to temperate contexts, old-growth forests have been much less explored in the Mediterranean (Abadie et al. 2018). Patched and uncomplete information is present at this scale, and exhaustive literature on old-growth dynamics and features is available for only few Mediterranean territories, such as the Cilento, central Italy or eastern Pyrenees (e.g., Marchetti et al. 2010, Lombardi et al. 2015, Fortuny et al. 2020).

Such first-step research work, towards a knowledge improvement about old-growth forests, is therefore needed in Sardinia, the second largest island of the Mediterranean Basin. Although a recent increase in forest cover occurred mainly due to the spontaneous colonisation of marginal agricultural areas, following the abandonment of crops (INFC, 2015), the extension of old-growth forests in Sardinia was estimated to be considerably reduced during the nineteenth century, mainly due to deforestation, subsequent livestock, coupled with fire to maintain pastures (Pungetti 1995, Caterini 2013). Nonetheless, several small and scattered stands remained untouched in remote mountainous areas of the Island. Despite their high conservation value, there is not much knowledge about the distribution, composition, structure and function of the residual fragmented stands of old-growth forests in Sardinia (hereafter forest stands). In this study, our goals were thus to: i) identify forests stands with old-growth

features in Sardinia and evaluate their degree of structural heterogeneity; ii) investigate how the structural heterogeneity index varies across biological, environmental or human-related factors; iii) identify strengths and limitations of the structural heterogeneity index as an informative tool for the conservation and management of Mediterranean old-growth forest.

# Materials and methods

# Study area

The reference area of this study is the island of Sardinia (Fig. 1), the second Mediterranean island after Sicily by extension, with a surface area of about 24,090 km<sup>2</sup>. Sardinia is located in the central-western part of the Mediterranean Sea and, together with Corsica and the Tuscan Archipelago, it constitutes an independent biogeographical province (Fenu et al. 2014). The island is underpopulated compared to other Italian and European regions: it has a population density of 68 inhabitants per  $km^2$ , compared to the average of 200 people per km<sup>2</sup> for Italy (ISTAT 2019). Around 40% of Sardinians live in urbanised areas in the North of the island (Sassari) and in the South (Cagliari).

The mountainous inland areas of Sardinia are still relatively isolated and sparsely populated where the island preserves many natural environments with difficult access, partly wild and relatively well preserved (Fois et al. 2019). For the high concentration of endemic species (especially plants and invertebrates), it has been identified as a biodiversity hotspot of global and regional importance (Biondi et al. 2013, Fois et al. 2018).

Forests in Sardinia have been estimated to cover a surface of 4,297 km<sup>2</sup> (Puddu et al. 2012), which mainly consists of holm oak (*Quercus ilex* L.) and cork oak (*Quercus suber* L.) evergreen forests and forests of deciduous oak woods (*Quercus congesta* C. Presl and *Q. ichnusae* Mossa, Bacch. & Brullo), with a wide ecological range and a widespread distribution throughout the island, from coastal areas up to 1400 m above sea level. These formations constitute a wide range of potential climax forests, some of them endemic to the island, such as the *Glechomo sardoae-Quercetum congestae* or the *Prasio majoris-Quercetum ilicis quercetosum virgilianae* (Bacchetta et al. 2004a,b, 2009).

Furthermore, particular soil-climatic conditions support the instauration of several formation types, which make the island very diverse in this aspect. One of the most interesting examples are relic yew (Taxus baccata L.) forests, which can be found mainly in the central-northern sectors of Sardinia, often limited to gorges or high slopes, in soils often rich in skeleton (Farris et al. 2012). Other potential natural forests are summarised in Bacchetta et al. (2009) and are represented, in peculiar soil-climatic conditions, by



Figure 1 Plots distribution map and SHI ranges

formations of hop hornbeam (e.g., Cyclamino repandi-Ostrvetum carpinifoliae), junipers Chamaerono humilis-Juniperetum (e.g., turbinatae), phillyreas (e.g., Prasio majoris-Ouercetum phillvretosum latifoliae) and olive trees (e.g., Asparago albi-Oleetum svlvestris). Riparian forests are mainly constituted by black alders (Alnus glutinosa (L.) Gaertn.), willows and poplars (Salix spp. and Populus spp.), tamarisks (Tamarix spp.) and oleanders (Nerium oleander L.). Planitial woods of silver poplars (Populus alba L.), raywoods (Fraxinus oxycarpa Willd.) and field elms (Ulmus minor Mill.) are also present in few and small alluvial planes.

### Selection of study forest stands

The investigated forest stands were identified through a questionnaire sent to local experts from research institutes universities and public forestry agencies. Forest stands were reported by indicating the location and describing main forest characteristics, including the presence of large old trees, dead wood, and human disturbance. The full questionnaire is available on supplementary material (Table S1, Supp. Info.). Each stand was considered to be inventoried or not, after a first screening in the field of the following features: presence of dead wood, large trees, and negligible signs of recent human presence. In other words, the forest stands reported in the questionnaires that showed clear signs of management, like pure coppices, were not inventoried.

# Variables selection and collection

The selected forest stands were sampled following methods described in the National Inventory of Forests and forest Carbon reserve INFC2015 (Gasparini et al. 2016). Within each surveyed stand, circular plots of 13 m radius (approx. 530 m<sup>2</sup>) were positioned. Such plot surface was considered as enough for providing a reliable inventory in old-growth

forest conditions (Lombardi et al. 2015).

The sampling effort (i.e., number of plots per stand) was determined by the need to survey the most representative as possible number of stands reported by the questionnaires on a regional scale. Random plots within each oldgrowth stand were positioned as following: hiking tracks were walked and, once inside the old-growth stand, a random direction was selected and a constant distance of about 100 m walked to reach the centre of the plot. In very small stands, this distance was reduced to 50 m to avoid old-growth forest edges. In any case, plots were positioned at least 100 m from edges. At least one plot per each reported forest stand was surveyed and, where forest stand was suitable and topography allowed it (i.e., time to reach localities, accessibility, forest stand extension), up to 10 plots per stand and four plots per day were investigated.

In each plot, the following parameters were considered: heights of all live trees, with a minimum diameter of 9.5 cm at breast height (DBH), were measured with a Blume-Leiss ipsometer for an as much as possible number of individuals and at least for 10% of the total number. These were used as standard comparison for measuring the remaining trees. Length and diameters (minimum, maximum and average) of the three different kinds of deadwood (deadwood debris, stumps and standing dead trees) were recorded, as well as their decay level according to the 1-5 scale proposed by Hunter (1990). Deadwood debris on the ground was measured with a minimum diameter > 9.5 cm. The volume was calculated according to Huber's formula (Fridman & Walheim 2000):

$$V = S0.5 x h$$

where S0.5 is the median sectional area, h = length. We considered as stumps the truncated stems at a height of less than 1.30 m from the ground, whose diameter on the cutting plane is equal or greater than 9.5 cm. The volume of

stumps was computed by equation:

$$V = \pi x h/3 x [(D/2) + (d/2) + (D/2) x (d/2)]$$

where V is the volume; h is the height; D is the maximum diameter, and d is the minimum diameter (Lombardi et al. 2013).

Since no volume tables are available, the estimate of the volume of live trees was performed using the model tree method, applying the general equation of standing trees for each plant:

 $V = \pi/4 x H x F = G x H x F$ 

in which D is the diameter with bark measured at 1.30 m from the ground, G is the basal area and corresponds to the surface of the cross section at 1.30 m, H is the estimated height of the plant and F is the reduction coefficient. The reduction coefficient (0 < X < 1) can be calculated exactly only on felled plants which are the model trees. As this is not foreseen in our study, it was estimated based on a representative sampling of the average shape of one or more plants of the stand (Bernetti & La Marca 1983).

Table 1 Variables identified measured (M) and estimated (E) in the field or derived (D)

n	Code	Variable description	Data typology
1	BA	Basal area	D
2	BASnagST	Basal area of standing dead trees and stumps	D
3	CC	Canopy cover %	E
4	CWD	Coarse woody debris volume	D
5	DensNLiv	Living trees density	D
6	DensStuDW	Standing dead trees including stumps density	D
7	Dw/Liv	Deadwood/living wood volume ratio	D
8	Dwtot	Total deadwood volume	D
9	GSDBH	DBH diversity (calculated using the Gini-Simpson index)	D
10	QMDDBH	Quadratic mean DBH	D
11	HM	Maximum height	E/D
12	Hm	Average height	D
13	Hsd	Height standard deviation	Е
14	Lam	Lichens and mosses layer %	Е
15	LenghtCWD	Total length of coarse woody debris	М
16	Liv	Living trees volume	Е
17	NDBH	Number of diameters classes	М
18	NDC	Number of decay classes occurring in the plot	М
19	NHt	Number of hollow trees	М
20	NstuStDW	Number of standing dead trees including stumps	М
21	RDBH	Range of diameters distribution	D
22	STDw	Standing dead trees volume	D
23	STUMPS	Stumps volume	D
24	STUStDw	Standing dead trees including stumps volume	D
25	T>40	Number of living trees with dbh> 40 cm	М
26	TreeRich	Tree species richness	М

Data collected in the field and derived in the laboratory (26 structural variables) were used for the elaboration of an index of structural heterogeneity (Table 1). These variables comprise those commonly found in national forest inventories and reported in the literature of old-growth forests and forest structural complexity (McElhinny et al. 2005, Sabatini et al. 2015).

Other indicators of old-growthness, such as number of hollow trees (Lindenmayer et al. 2000, McElhinny et al. 2006, Rozas 2006), the type of layering of the canopy (single, double, multi) (Franklin & Van Pelt 2004), the percentage of canopy (Jennings et al. 1999, Franklin & Van Pelt 2004) and lichen-muscinal covers (Lesica et al. 1991, Ódor & Standovár 2001) were also recorded. Furthermore, the presence of the large longhorn beetles (Cerambyx cerdo, Aegosoma scabricorne (Scopoli) and Prinobius mvardi Mulsant), generally associated with mature trees and aging forests (Parisi et al. 2016, Casula 2017), was also recorded. This was established by searching for exit holes of longhorn beetle adults (larger diameter of about 20 mm) in the lower part of the trunks. The presence of livestock grazing was evaluated by the searching for faecal material, presence of grazing signs and by interviewing local people. Finally, forest types were defined based on the relative dominance (basal area) of the recorded tree species. Mixed forest stands were those without a species representing more than 75% of the total basal area. Such mixed stands were defined by species representing at least 25% of the total basal area (Gauthier et al. 1996).

# Elaborating and evaluating the Structural Heterogeneity Index (SHI)

A simplified structural index was elaborated according the methodology developed by McElhinny et al. (2005) and Sabatini et al. (2015). The number of variables was reduced from 26 to eight, identifying a single attribute that best represented each of the eight categories of complexity recognised for the old-growth forests in Italy (Sabatini et al. 2015): (1) vertical heterogeneity (VH); (2) compositional diversity (CH); (3) uneven agedness (UA); (4) density of large living trees (LLT); (5) growing stock (GS); (6) total deadwood volume (DW-TOT); (7) deadwood decay classes (DW-DC); (8) standing deadwood, dead trees and stumps (DW-ST).

Each of the eight variables was selected according to the following criteria: (1) to have a kurtosis < 2, in order to avoid attributes with a non-normal distribution and a low discrimination ability: for those attributes that showed a high Kurtosis value (> 2), their distribution was corrected through the logarithmic or the square root transformation; (2) to be not correlated with other attributes: strengths of correlation were estimated by Spearman correlation coefficients. Accordingly, the number of attributes was reduced by choosing only one among those having a correlation coefficient <0.50; (3) to have the highest as possible contribution of variations retained by two principal components PC1 and PC2. Principal component analysis (PCA) was made in R (ver.R-3.6.1) using R Package FactoMineR version 1.34 (Le et al. 2008).

Total contribution of each variable on explaining the variations retained by the two principal components was calculated by using R package factoextra version 1.0.5 (Kassambara et al. 2017). Among the correlated variables with a Kurtosis values < 2. the variable showing the higher contribution of variation was retained; (4) to have good field sampling efficiency: following the example of Sabatini et al. (2015), each attribute was classified in three sampling efficiency classes, 1 (low), 2 (medium) and 3 (high). Attributes with higher values were preferred. Kurtosis values were calculated in Microsoft Excel 2013, while the rest analyses were carried out in R environment Version 3.5.2 (R Core Team 2018).

A score ranging from 0 to 10 was assigned to each one of the eight retained variables. We first set a score of 2.5, 5, 7.5 and 10 to the quartile midpoints (corresponding to the 12.5, 37.5, 62.5 and 87.5 percentiles, respectively) of the raw attribute distribution. Then, a linear regression through quartile values was fitted to ensure that the attribute scores were evenly distributed between 0 and 10. This regression equation was used to associate a score with each observation (Table 2). Finally, a Structural Heterogeneity Index (SHI) was obtained by summing the scores in the range 0-10 assigned to each variable in the core set, and then expressed as a percentage.

To evaluate the descriptive capacity of the SHI, it has been related to variables that are likely to be related with forest age and structural complexity, such as presence of cerambycids (higher presence associated with mature forest), canopy cover (higher canopy cover in older forests), forest layers (higher complexity of layers in mature forests), grazing (lower structural heterogeneity in forest with regeneration strongly affected by grazing) and location (easy coastal access favours the economic exploitation of the wood and the drier climate slows down its growth). Moreover, each site was classified according to the three old-growthness classes proposed for different types of Sicilian forests (Badalamenti et al. 2018). Such classification was based on field measurements of four internationally recognised indicators of oldgrowthness: deadwood, tree size, structural characteristics and tree species richness. In particular, for the proxy indicator "deadwood" was assigned a score ranging from 0 to 2 based on the total volume of dead tree values (DW) and the number of decay classes (DC): 0 for  $DW \le 0.09$ , 1 for  $0.09 < DW \le 0.5$ , and 2 for DW > 0.5, attributing one class higher if  $DC \ge 4$ . For "structural characteristics", was assigned a suitability (1/0) based on the type of coverage (single, double or multi), on the value of the standard deviation and on the range of diameters. Also, for "tree size" and for the "tree species richness" was assigned only the suitability (1/0), based on the presence of trees with DBH > 40 cm and on the number of species detected, respectively. On the basis of these 0-5 scores, each site was classified into three classes of old-growthness, as high (H, with scores  $\geq$  4) medium (M, with scores = 3) and low (L, with scores < 3). Differences among all the above-described characteristic parameters were graphically represented by box plots and tested using Kruskal-Wallis rank sum tests for pairwise comparisons and Dunn's test for multiple comparisons.

 Table 2 Regression equations used to assign a score to attributes on a scale of 0-10

Structural indicator	Regression equation R <sup>2</sup>
Hsd: Height standard deviation	$y = 1.010x + 1.720$ $R^2 = 0.838$
TreeRich: Tree species richness	$y = 1.446x + 1.821$ $R^2 = 0.939$
DensNLiv: Stem density, LOG+1	$y = 7.789x - 15.23$ $R^2 = 0.937$
T>40: Number of living trees with DBH>40cm	$y = 0.535x + 0.892  R^2 = 0.900$
BA: Basal area, LOG	$y = 9.368x - 12.30$ $R^2 = 0.896$
Dw/liv: Deadwood/living wood volume ratio, RAD	$y = 9.281x + 2.316$ $R^2 = 0.886$
LenghtCWD: sum of lengths of every coarse woody debris piece, LOG+1	$y = 4.436x + 2.014$ $R^2 = 0.930$
DensStuDW: Density of standing deadwood, LOG+1	y = 2.790x + 2.106 R <sup>2</sup> = 0.945

# Results

After field surveys, eight areas reported by questionnaires were excluded, as they showed clear signs of forest management. Accordingly, a total of 68 plots included in about 45 forest stands were selected for this study. Most of these plots (67.7%) were in central-northern Sardinia (Fig. 1), while five plots each were sampled in the South-East and South-West side of Sardinia.

Two different forest types were mostly represented in this study: pure or mixed forests

with *Quercus ilex* (42) and pure or mixed forests with *Taxus baccata* (15). Eleven plots where representative of persistent formations dominated by *Juniperus* sp.pl. (n=5), *Phillyrea latifolia* L. and *Arbutus unedo* L. (n=2, each), and *Quercus congesta* and *Alnus glutinosa* (n=1, each).

Eight structural indicators were identified (Table 3). The basal area (BA) and the density of standing deadwood (DensStuDW) were the most contributing variables. The reliability of these variables was confirmed by their high sampling efficiency.

 Table 3 List of categories of structural complexity and the corresponding identified structural indicators with selection criteria

Structural indicator	Sources of structural complexity	Function as a surrogate (significant ρ≤0.5)	Contribution of variations by first two principal components		Sampling efficiency
Hsd: Height standard deviation	Vertical heterogeneity (VH)	HM (0.689)	1.670	1.807	2
Treerich: Tree species richness	Compositional diversity (CH)	-	0.782	-0.615	3
DensNLiv: Stem density LOG+1	, Uneven-agedness (UA)	NDBH (0.632); NstuStDW (0.510); DensStuStDw (0.510)	4.561	-0.037	3
T>40: Number of living trees with DBH>40cm	Density of large living trees (LLT)	NHt (0.522)	3.563	0.283	3
BA: Basal area, LOG	Growing stock (GS)	RDBH (0.698); Liv (0.868).	6.483	1.287	3
Dw/liv: Deadwood/living wood volume ratio, RAD	0	StDw (0.529); StuStDw (0.565); BasnagSt (0.543); CWD (0.661); Dwtot (0.786)	4.338	0.607	1
LenghtCWD: sum of lengths of every coarse woody debris piece, LOG+1	Deadwood decay classes (DW-DC)	CWD (0.843); NDC (0.633); Dwtot (0.719)	4.542	0.317	3
DensStuDW: Density of standing deadwood, LOG+1	Standing deadwood, dead trees and snags (DW-ST)	BasnagST (0.781); STDw (0.814); StuStDw (0.814); Dwtot (0.517); DensNLiv (0.510); GSDBH (0.531)	5.611	-1.287	3

Notes: Sampling efficiency: 1 - poor, 2 - medium, 3 - high. See Table 1 for abbreviations.

The SHI showed a normal distribution (Appendix B, supplementary Fig. 1), ranging from 25.6% to 76.6% (median=50.31). Results showed that the plots with the highest SHI values are those located in mountainous areas. All specific values related to each site, with ADSXX codes and toponyms, are reported in Table 4. Three sites located within the Gennargentu massif of central Sardinia (ADS29, ADS31, ADS32) accounted for high SHIs (65.5%, 67.4%, 67.60%, respectively). Another massif where several sites with high SHI were recorded is the Limbara in northern Sardinia. Here, SHIs range from 58.3% (ADS58) to 61.5% (ADS41). In the southeastern Sardinian mountains of Sarrabus, plots with high SHIs were found in the locality of Buddidorgia (ADS06, ADS08, ADS09), with values of 61.7%, 65.3%, 72.5%, respectively. In general, the 20 plots with the highest SHI values were often characterised by a high number of large trees (mean=10.15), high

density of live trees (mean=517.89) and standing deadwood (mean=78.15), and high tree species richness (mean=3.10). Coastal plots in the south-west (ADS38, ADS39) and in the centre (ADS11, ADS12) had lower SHI values (33.3%, 36.2%, 41.8%, 47.5% respectively) and were characterised by a low amount of large trees (mean=1.75), tree species richness (mean=2.00) and coarse woody debris (mean=0.24 m).

No significant difference (p> 0.05) was found by relating forest type with SHI. On the other hand, SHI significantly increased with higher percentages of canopy cover and with the number of canopy layers. SHIs were higher in absence of livestock grazing and when large longhorn beetles were present. Inland stands showed significantly higher SHIs than coastal. A significant correspondence among oldgrowthness classes and SHIs was found (Fig. 2).



**Figure 2** Boxplot of SHI across grazing, canopy cover, canopy layers (single, double, multi-layered), cerambycid presence, old-growthness class location plots. Ns= P > 0.05; \* =  $P \le 0.05$ ; \*\* =  $P \le 0.01$ ; \*\*\* =  $P \le 0.001$ . Differences were tested using Kruskal-Wallis rank sum tests (pairwise) or Dunn's test (multiple comparisons; R-package "dunn.test"; Dinno 2016) using rank sums with Bonferroni correction

					Canopy			Dana	Davia	N	т	CWD	Old-	
Name	ID site	Loc	.Graz	.Cer.	cover	CL	T>40	Dens	Dens		Tree		, growth	SHI
					(%)			StuDW	Liv	Liv	Rich	(m)	class	
Arala	ADS 01	Ι	No	Yes	76-100	D	6	37,66	583,80	31	4	10.0	M	48.72
Aranzos	ADS 02	Ι	Yes	No	51-75	S	16	18,83	338,98	18	1	51.0	М	57.06
Baccu Addas 1	ADS 03	Ι	No	Yes	51-75	М	5	75,33	357,82	19	1	45.0	Н	49.78
Baccu Addas 2	ADS 04	Ι	Yes	No	51-75	М	8	56,50	376,65	20	3	24.0	Н	56.48
Bacu sa Figu	ADS 05	Ι	No	Yes	51-75	М	3	131,83	395,48	21	4	25.0	Н	56.11
Buddidorgia 1	ADS 06	Ι	No	No	76-100	М	11	0,00	508,47	27	4	75.5	Н	61.67
Buddidorgia 2	ADS 07	Ι	No	No	76-100	М	11	0,00	451,98	24	2	8.0	М	46.24
Buddidorgia 3	ADS 08	Ι	No	Yes	76-100	М	13	131,83	602,64	32	5	20.0	Н	65.30
Buddidorgia 4	ADS 09	Ι	No	Yes	76-100	М	13	207,16	696,80	37	5	117.0	Н	72.49
Carracana	ADS 10	Ι	No	Yes	51-75	S	9	18,83	225,99	12	1	18.1	М	43.08
Cala Fuili	ADS 11	С	No	No	51-75	М	2	56,50	659,13	35	2	8.0	L	41.80
Puntali Mannu	ADS 12	С	No	No	51-75	М	4	18,83	866,29	46	3	10.0	Н	47.53
Dortesi	ADS 13	Ι	Yes	Yes	51-75	S	7	37,66	131,83	7	1	9.0	М	40.82
Goloritzè 1	ADS 14	Ι	Yes	No	51-75	М	5	0,00	131,83	7	3	12.0	М	38.37
Goloritzè 2	ADS 15	Ι	Yes	No	51-75	S	3	0,00	56,50	3	1	4.0	L	25.64
Gorroppeddu	ADS 16	Ι	Yes	No	51-75%	S	9	0,00	301,32	16	3	18.7	Н	44.49
Is Arridelaxius	ADS 17	Ι	No	No	51-75	М	8	18,83	376,65	20	3	34.0	Н	49.02
Is Cannoneris	ADS 18	Ι	No	No	76-100	S	10	508,47	1996,23			25.0	М	63.79
Is Cioffus	ADS 19	Ι	No	Yes	51-75	М	6	18,83	828,63	44	5	15.5	Н	54.79
Janne 'e Cori	ADS 20	I	Yes	Yes	76-100	М	10	225,99	470,81	25	1	11.2	Н	54.25
La Signora	ADS 21	Ι	Yes	Yes	51-75	М	7	94,16	659,13	35	2	59.6	Н	59.66
Li Femmini	ADS 22	I	No	Yes	51-75	М	4	56,50	753,30	40	2	15.1	М	48.64
Lispedda	ADS 23	Ι	Yes	Yes	51-75	М	5	37,66	640,30	34	3	29.0	Н	52.30
Canali mau 1	ADS 24	I	No	No	76-100	M	7	0,00	338,98	18	4	7.5	М	43.31
Canali mau 2	ADS 25	I	No	No	76-100	M	10	75,33	244,82	13	3	59.5	Н	62.05
Mandra Brujata	ADS 26	I	Yes	Yes	51-75	D	5	0,00	414,31	22	5	3.0	L	40.38
Marmurata	ADS 27	I	Yes	No	51-75	S	4	0,00	320,15	17	3	14.5	M	42.33
Barbudu	ADS 28	Ι	No	No	51-75%	М	5	18,83	414,31	22	2	53.0	Н	53.66
Donnianigoro 1	ADS 29	I	Yes	Yes	76-100	S	18	94,16	433,15	23	1	71.0	М	65.52
Fumai	ADS 30	I	Yes	No	51-75	S	9	18,83	188,32	10	1	4.8	М	43.25
Mereu	ADS 31	I	Yes	Yes	51-75	M	15	75,33	583,80	31	2	100.0		67.41
Tureddu	ADS 32	I	No	Yes	51-75	D	8	56,50	451,98	24	3	241.0	Н	67.60
Monti di Deu_1	ADS 33	I	No	No	76-100	D	12	18,83	301,32	16	1	11.0	М	45.36
Monti di Deu 2	ADS 34	Ι	No	Yes	51-75	М	7	56,50	338,98	18	1	51.4	М	50.06
Mularza Noa	ADS 35	I	No	No	76-100	M	11	94,16	659,13	35	6	60.0	Н	76.59
Nieddoni	ADS 36	I	Yes	Yes	76-100	S	11	37,66	621,47	33	1	18.5	М	50.57
Oruddulè	ADS 37	I	Yes	Yes	51-75	S	9	18,83	244,82	13	4	31.0	Н	54.59
Piscinas 1	ADS 38	С	Yes	No	51-75	S	1	94,16	244,82	13	1	3.5	L	33.32
Piscinas 2	ADS 39	C	Yes	No	51-75	S	0	37,66	414,31	22	2	2.0	Ĺ	36.25
Rio Aratu	ADS 40	I	Yes	No	51-75	S	4	94,16	207,16	11	2	2.0	M	49.63
Rio Columbano	ADS 41	I	No	Yes	51-75	M	5	94,16	1167,61	62	3	49.2	Н	61.46
Correboi_1	ADS_42	I	Yes	No	0-50	М	9	37,66	188,32	10	5	11.5	Н	55.85
Correboi 2	ADS 43		Yes	No	0-50	S	5	0,00	112,99	6	2	0.0	L	31.28
Rodè	ADS_44		No	Yes	76-100	М	7	37,66	564,97	30	4	10.2	H	55.62
Salbatore Logu	ADS 45		No	No	76-100	D	5	56,50	772,13	41	4	17.0	Н	53.12
Punta Salinas	ADS_46		Yes	Yes	51-75	M	7	18,83	357,82	19	5	14.0	Н	57.22
Sambuco Giuntura	ADS 47		No	No	76-100	M	6	37,66	451,98	24	2	50.7	Н	55.79
Sambuco Nord	ADS_47 ADS_48		Yes	Yes	0-50	D	8	0,00	188,32	10	2	16.7	M	44.00
Trattalas 1	ADS_48 ADS_49		Yes	Yes	0-50	M	9	18,83	225,99	12	1	7.5	M	43.44
Trattalas 2	ADS_50		Yes	No	0-50	S	10	0,00	188,32	12	1	11.0	L	40.67
iianaias_2	AD3_30	1	105	110	0-30	3	10	0,00	100,52	10	1	11.0	L	-10.07

# Table 4 Specific values related to each site

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Research article

Trattalas_3	ADS_51	Ι	Yes	Yes	51-75	D	4	0,00	131,83	7	1	4.5	L	35.89
Sos Nibberos	ADS_52	Ι	Yes	No	76-100	S	12	0,00	527,31	28	2	0.0	L	42.74
Sos Nibberos_2	ADS_53	Ι	Yes	No	76-100	S	14	56,50	451,98	24	2	10.0	М	55.97
Su Irove	ADS_54	Ι	No	Yes	51-75	Μ	7	112,99	772,13	41	4	13.0	Η	58.66
Su Prappare	ADS_55	Ι	No	Yes	76-100	Μ	11	37,66	433,15	23	1	46.8	Η	59.51
Su Suercone Ilex	ADS_56	Ι	No	Yes	51-75	D	5	0,00	131,83	7	2	67.3	Η	51.71
Su Suercone tassi	ADS_57	Ι	No	No	76-100	Μ	10	301,32	414,31	22	1	63.9	Η	63.46
Suliana	ADS_58	Ι	No	Yes	51-75	Μ	8	94,16	583,80	31	3	25.2	Η	58.26
Tedderieddu_1	ADS_59	Ι	Yes	No	51-75	Μ	6	18,83	131,83	7	4	10.8	Η	51.19
Tedderieddu_2	ADS_60	Ι	Yes	No	0-50	Μ	6	0,00	131,83	7	1	0.0	L	29.25
Tedderieddu_3	ADS_61	Ι	Yes	No	76-100	S	9	0,00	169,49	9	2	4.0	L	41.50
Tuones	ADS_62	Ι	Yes	No	0-50	S	3	0,00	94,16	5	2	0.0	L	27.49
Tuviois_1	ADS_63	Ι	Yes	No	51-75	S	5	18,83	112,99	6	1	14.2	L	35.18
Tuviois_2	ADS_64	Ι	Yes	Yes	76-100	D	8	0,00	150,66	8	2	0.0	L	33.03
Arcosu_Su Corteddu	ADS_65	Ι	Yes	No	76-100	S	5	0,00	451,98	24	1	8.0	М	41.78
Arcosu_Lattias	ADS_66	Ι	No	No	76-100	Μ	5	56,50	1544,26	82	4	16.0	Η	56.97
Arcosu_Longufresu_1	ADS_67	Ι	No	Yes	76-100	Μ	4	0,00	772,13	41	2	46.5	Η	50.83
Arcosu_Longufresu_2	ADS_68	Ι	No	No	76-100	S	11	0,00	414,31	22	3	12.0	М	46.68
		***	(			~ `	-		4 ( 100)		4 (20)		-	

Notes: Name sites detected and ID (ADS\_XX), location (Loc.) of each site (coastal (C)/inland (I)), presence of grazing (Graz.) and cerambycids (Cer.); estimated % of canopy cover; structure of canopy layers (CL): Multi (M), Double (D), Single (S); tree species richness; Old-growthness class estimated according to Badalamenti et al. (2018): High (H), Medium (M), Low (L). See Table 1 for the rest of abbreviations

### Discussion

# Factors affecting forest heterogeneity in Sardinia

Proxies related to growing stock and standing deadwood, namely BA and LenghtCWD, were themost contributing variables in discriminating the examined plots. This confirmed again that the structural complexity of a forest stand is roughly equally defined by the co-presence of different characteristics related to both living and deadwood. For instance, plots with lower SHI values are characterised by a low number of large trees (T>40) and coarse woody debris (LenghtCWD). Under these conditions, the presence of few and majestic old trees, although they are quite important for the maintenance of saproxylic entomofauna, was unable to determine high structural heterogeneity. Low SHI values associated with low LenghtCWD are mainly explained by the removal and exploitation of dead wood by the local populations. In this sense, morphology and socio-economic factors are likely to be crucial drivers in inhibiting or favour the use of the land and consequently the permanence of old-growth forest stands. In particular, steep and mountainous morphologies have generally favoured the conservation of most of mature forest areas in the mountainous areas of Limbara and Gennargentu of central-northern Sardinia.

As regards the distribution of SHI values among plots, our results confirmed that the SHI is influenced by variables that are likely to be related with structural complexity, which was, in this case, measured by means of number of canopy layers. Although their management is debated, evidences that live-tree structural complexity is generally higher in old-growth than in mature and early transitional stands are ample. Among others, including also geomorphological aspects, big old trees have a crucial role in shaping a high structural complexity by determining a lower tree density and increased tree size heterogeneity (Zenner 2004). Moreover, coastal plots appear with lower SHI values than inland ones, probably as a consequence of higher

human accessibility, economic interest and, possibly, to slower forest growth associated with dry climatic conditions and general low forest resilience of Mediterranean coastal areas (Mayor et al. 2007). As already reported for different European countries, livestock grazing (Bergmeier et al. 2010) and human activities (Motta 2002), appear to be crucial factors for shaping the structural variability of the forests in Sardinia and consequently old-growthness. Furthermore, our results showed significant differences of the SHI among the three levels of canopy cover, as well as among the number of canopy layers, confirming that such structural characteristics are discriminant characters for old-growthness definitions. Indeed, old growth forests have generally multiple or continuous canopy layers and are "bottom loaded" in foliage mass, in contrast to the top loaded canopies of young stands (Franklin & Van Pelt 2004). The presence of large longhorn beetles was also weakly associated with higher structural complexity of forests, generally associated with mature trees and aging forests (Buse et al. 2008, Casula 2017).

# The SHI as a proxy forest of old-growthness and conservation value

Although a large part of the Sardinian forests preserves traces of ancient human activities, such as paths and small plateaus for the coal production, and woodlands are currently still used for livestock grazing and firewood production, most of the investigated plots showed at least some characteristic or even a high level of old-growthness, which make them priority areas for conservation. In some cases, mature forests were maintained by the local communities who preserved them from cuts, recognising their cultural value, besides their productive function (Heatherington 2001) or, such as the plots with a high SHI value in "Buddidorgia" (ADS09, SHI up to 72.5%), forest stands are interdicted under military jurisdiction since 1956.

However, signs of human impact, such as livestock grazing, were significantly influencing the SHI (i.e. a lower SHI in grazed stands). Even though grazing may in some cases favour forest development by, for instance, reducing litter and flammable biomass (Diaci et al. 2010), this is one of the main threats to the conservation of oldgrowth forests in Sardinia (Farris et al. 2012) and in other several Mediterranean areas (e.g., Bergmeier et al. 2010, Motta et al. 2015). The excessive trampling and/or herbivory of seedlings does not allow replacement of dead trees in the long term. An illustrative case is the secular Taxus baccata forest of "Sos Nibberos", where the presence of several large old trees is associated with the lack of renewal due to overgrazing. Moreover, the historical presence of human modifications was still visible in several investigated plots. For instance, small plateaus built by the charcoal burners are still visible, even if such practices were generally abandoned (Scotti & Cadoni 2007). Following the definitions of Buchwald (2005), who developed an array of levels of naturalness of natural forests, we could classify part of the forests found in Sardinia as "relatively intact forest (stand level) that has been essentially unmodified by human activity for the past sixty to eighty years or for an unknown, but relatively long time", as in the case of high SHI values measured for ADS09 (SHI 72.5%) or ADS32 (SHI 67.6%). This was supported by the significant differences of SHIs among forest stands classified under three classes of old-growthness. Even though the SHI is not conceived for classifying oldgrowth forests, like the one proposed by Badalamenti et al. (2018), it can be used as a preliminary conservation priority-ranking measure of old- growth forests and persistent woodlands in Sardinia and other poorly investigated territories. Forests with high SHI stands should be prioritised for conservation and investigations aimed at understanding local processes underlying forest structural

complexity and dynamics.

### Potential and limitations of the SHI

One of the main advantages of the application of this methodology is that it comes from the sum of scores for each structural attribute. omitting the subjectivity due to a human evaluation. In this study, it was chosen to include in the SHI only those structural variables that can be easily measured in a plot of approx. 530 m<sup>2</sup>, which are proxies of other attributes very difficult and expensive to measure, such as biodiversity, the fraction of the gaps, or the interactions among biotic and/ or abiotic components. The structural attributes considered are a compromise between the relevance of information to include in the index and its cost, due to the large extent of the study area and the low available resources and information. Surprisingly, no significant differences were identified among the investigated forest and persistent woodlands types. On one side, this confirms that the SHI is unusable for comparisons among forest types. On the other side, the rarity of some forests such as the ones dominated by Phillyrea latifolia and Arbutus unedo, which does not allow achieving statistical significance in comparisons, suggests more specific and deeper studies on the structure dynamics of such uncommon typologies.

Although the SHI does not discriminate the process (anthropogenic or natural) that has determined the accumulation of a certain structural heterogeneity in a stand, humanrelated processes, such as livestock grazing, were confirmed to lower SHI values. Moreover, the index showed that, in some cases, plots with a different structure might have similar SHI values. An example can be the comparison of ADS18 (SHI 63.7%), characterised by a high number of live trees and low values of tree species diversity, with ADS08 (SHI 65.3%), characterised by a smaller number of live trees, but a high diversity of tree species. Despite these differences, both plots showed roughly the same SHI value (Table 4). This suggests that old-growthness and maturity does not necessarily take into account the arboreal layer diversity, in accordance with McElhinny et al. (2005), which stated that structural complexity is a relative concept rather than absolute. Similarly, remarkable SHI values can be obtained in forest areas modified by silvicultural practices such as coppice with standards and retention forestry (Fedrowitz et al. 2014). An example is ADS45 (SHI 53.1%), a typically biplane forest where a limited number of tall and large *Quercus ilex* trees dominate the lower layer of *Phillyrea coppice*.

# Conclusion

The structural heterogeneity, in terms of SHI, of a representative sample of natural forests in Sardinia was for the first time elaborated. Specifically, a total of 68 plots included in 45 forest stands were here studied. Pure or mixed forests with Ouercus ilex and pure or mixed forests with Taxus baccata were the most represented types. Similarly to other studies (e.g., Storch et al. 2018), the SHI was applied for analyses at a relative large spatial scale and for different types of forests, in an attempt to obtain an adequate assessment of the structural diversity of several forest types that co-occur in Sardinia. The SHI was mostly defined by the co-presence of both living and deadwood characteristics, namely basal area and density of standing deadwood, respectively. SHI increased with the number of canopy layers and canopy cover and it was likely to be disfavoured by overgrazing and by the soil-climatic and human accessibility conditions of coastal areas. Although a large part of the Sardinian forests preserves traces of ancient human activities, such as paths and small plateaus for the coal production, and woodlands are currently still used for livestock grazing and firewood production, most of the investigated plots showed at least

some characteristic or even a high level of oldgrowthness, which make them priority areas for conservation.

This work produced a first characterisation of the ancient residual forests of Sardinia, which could be followed by more in-depth studies. On a small spatial scale, it would be interesting investigating which species respond as a maturity qualitative indicator. Among them, the presence of specific nemoral herbs/geophytes, such as Viola sp.pl., Glechoma sardoa (Bég.) Bég. or several ferns like Polystichum setiferum (Forssk.) Woyn. and Drvopteris oreades Fomin (Bacchetta et al. 2004a,b, Farris et al. 2012), moss-lichen assemblages with, for instance, Lobaria pulmonaria (Brunialti et al. 2010), fungi such as Fomes fomentarius (L.) Fr. (Bernicchia et al. 2008) and bats like Myotis mystacinus (Kuhl) and Plecotus sardus Mucedda, Kiefer, Pidincedda and Vieth (Mucedda et al. 2002, Kaňuch et al. 2008) are just few of the rich array of species that might be investigated in this sense. On a large scale, the use of remote sensing techniques as the object oriented classification (Johansen et al. 2007), might allow an automated mapping and monitoring of their evolution through the identification of spatial structures typical of old growth forests, such as gaps or dead branches in the forest canopy. Furthermore, finding out which and how environmental and anthropogenic factors affect SHI might provide useful insights for forest conservation and management.

Even if it represents just a first step toward oldgrowth forests identification, considering the very sparse knowledge in the Mediterranean Basin, we believe that this method can be replicated in order to preliminarily assess the distribution and structure of old-growth forests and persistent woodlands at Mediterranean scale.

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