Universidade de Évora - Escola de Ciências e Tecnologia

Mestrado em Engenharia Florestal: Sistemas Mediterrâneos

Dissertação

Contribution to the study of holm oak stand dynamics

Eva de Gouveia Barrocas

Orientador(es) | Ana Cristina Gonçalves
Carlos Alexandre

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A dissertação foi objeto de apreciação e discussão pública pelo seguinte júri nomeado pelo Diretor da Escola de Ciências e Tecnologia:

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Évora 2021

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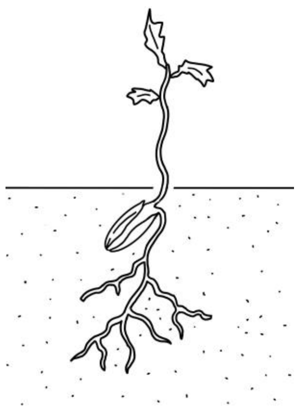

À Natureza, ao Alentejo,

às azinheiras.

Contribution to the study of holm oak stand dynamics

The holm oak is an emblematic species of the montado, a complex and biodiverse multifunctional, agro-silvopastoral system. Signs of the decline of the montado crown cover have been reported. This research followed an integrative approach to investigate how crown cover influences stand structure, natural regeneration, soil conditions, and litter layer in pure holm oak stands. Two new methodologies were created: STRUX Index that facilitates structure classification, and Natural Regeneration Classification that studies the viability of natural regeneration. The results showed that a higher crown cover was linked to uneven-aged structure, higher values of above-ground biomass, higher number of established natural regeneration, and a tendency for higher values of soil organic carbon and litter layer. Furthermore, it was observed a positive correlation between natural regeneration, litter layer, and soil organic carbon together with a negative correlation with soil pH This study brings a positive perspective on the preservation of holm oak stands.

Keywords – montado, stand structure, natural regeneration, silviculture, soil, statistical analysis

Contribuição para a dinâmica de povoamento em montado de azinho

A azinheira é uma espécie emblemática do montado, sistema agro-silvopastoril, multifuncional e de elevada biodiversidade. Como a área de montado regrediu nas últimas décadas, tornou-se urgente compreender a dinâmica dos montados de azinho. Através de uma abordagem integrada, foi estudada a contribuição do grau de coberto para a dinâmica da estrutura dos povoamentos. Duas novas metodologias foram criadas: o Índice STRUX, para a classificação da estrutura do povoamento, e a Classificação de Regeneração Natural, para avaliar a viabilidade e qualidade da regeneração natural. Os resultados indicaram que valores superiores de grau de coberto estão relacionados com a estrutura irregular, com mais biomassa florestal e com mais regeneração natural instalada. Foi observada uma correlação positiva entre a regeneração natural, a manta morta e o carbono orgânico e uma correlação negativa com o pH do solo. Este estudo contribuiu para uma perspetiva positiva sobre a resiliência do montado de azinho.

Palavras-chave – montado, estrutura de povoamento, regeneração natural, silvicultura, solos, análise estatística.

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CHAPTER 1 - INTRODUCTION

1.1 Background

In the Iberian Peninsula there are vast areas of *montado*, ancestral open oak woodland and multifunctional agro-silvopastoral system. In Portugal, there are more than one million hectares of *montado*, mainly in the Alentejo region (Lauw *et al.,* 2013). Common forest species of quercins in the southern Portugal *montado* are the cork oak (*Quercus suber* L.) and the holm oak (*Quercus rotundifolia* Lam also referred in the literature as *Quercus ilex* L: subsp. *rotundifolia Lam*.)

The *montado*s are systems of multiple uses (Figure 1.1), that is, in addition to the use and direct economic value related to cork, agriculture, and livestock, they also generate indirect use values such as landscape, carbon sequestration, interception, and infiltration of rainwater and maintenance of high levels of biodiversity (Pereira *et al.,* 2009). Because of all these ecosystem services can exist in the *montado,* it can be considered a High Nature Value Farming (HNV) (Almeida *et al.,* 2013). Despite the importance of this ecosystem, the area of *montado* has been decreasing (Pinto-Correia *et al.,* 2011). In the latest decades, several signs of decline in the *montado* properties have been identified, including soil degradation, tree mortality, and loss of productive potential (Lauw *et al.,* 2013). Holm oak crown cover has been drastically reducing in the Alentejo region and this phenomenon has been associated with the presence of high cattle numbers and the expansion of more intensive land uses (Acácio, *et al.,* 2021).

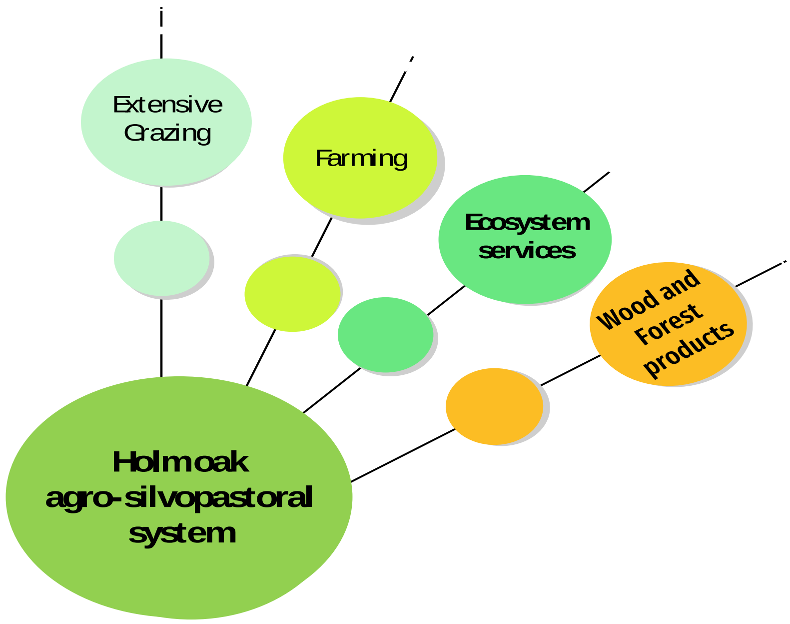


Figure1. 1 Schematic representation of the holm oak agro-silvopastoral multifunctionality (Credit of the author).

Throughout the twentieth century, several policy measures have fostered the decline of the *montado* forest cover in Portugal (Fonseca, 2016). The recession of the *montado* was caused by several interrelated causes, such as management practices concerning pasture use, soil mobilization, and agricultural use of agro-silvopastoral patches or lands (Pinto-Correia *et al.,* 2011). The decline of the montado is also caused by the decrease in tree density. The decrease of adult healthy trees in the *montado* resulted from the reduction in the regeneration rate of oaks and the increase in adult tree mortality (Lauw *et al.,* 2013). The forest cycle of degradation and renewal of this ecosystem is complex and includes dynamic relationships between climate, soil, fauna, and flora.

The importance of the *montado* sustainability in Alentejo during a climate change scenario should be updated considering the benefits that these HNV systems bring to societies. What is more, efforts to maintain the existence of this ecosystem should be reflected in future agriculture policies. As reported by the National Action Program to Combat Desertification (PNAC), approved by the Resolution of the Council of Ministers no. 1/2008 of 04-01-2008, 32.6% of the Portuguese national territory is in a degraded condition. According to the Regional Climate Change Adaptation Strategy 2017/2019

(ERAAC), the territorial combination of the soil susceptibility to desertification and the irregular water availability of the Alentejo will limit the region's future development. In Alentejo, it is pivotal to combine the primary agricultural production, the region's main economic activity, with the ecosystem services necessary for the territory to adapt to the expected climate change scenarios. Both the presence of the forest component and the maintenance of the *montado* areas can be relevant measures in the climate change adaptation strategy to be conceived by policymakers.

1.2 Objectives

The sustainability of any forest system is related to their stand structure dynamics, which include their renewal. Stand structure depends on the regime, composition, and structure of the stands as well as their spatial and temporal arrangements (Forrester, 2019; Looney *et al.,* 2018; Luu *et al.,* 2013). In forest stand dynamics we study tree's interactions with other trees, with disturbances, stand biotic and abiotic factors (Oliver, 1996). The research carried out in this dissertation seeks to deepen the knowledge about the dynamics of holm oak forest pure stands in the *montado* system. The crown cover was chosen as the main factor in this study because it is a relevant indicator of forest structural changes, biological diversity, and biochemical processes (Nakamura *et al.,* 2017; Ribeiro *et al.*, 2004).

Regeneration and recruitment are primordial for the stand sustainability, they depend on the species (growth patterns and shade tolerance), seed production, germination, and spatial arrangement (Gonçalves *et al.,* 2018; Löf *et al.,* 2019). For example, the holm oak canopy nurse effect can play an important role in natural regeneration due to the shade tolerance of the young holm oak individuals. Yet the *nurse effect* is still poorly understood (Badano *et al.,* 2011; Príncipe *et al.,* 2019). In this research, natural regeneration will be analyzed in an integrative way, combining data concerning stand structure and the soil. In this dissertation we tested whether different degrees of crown cover in a holm oak farm contribute differently to the dynamics of the holm oak stand structure (Figure 1. 2).

The holm oak was the species chosen to be the focus of this research because it demonstrates a remarkable drought resistance when compared to the other oaks and can grow in various types of soil, even when the soil is less fertile and mostly composed of limestone (Ferreira *et al.*, 2001; Lauw *et al.,* 2013). As livestock is one of the main sources of economic income for holm oak forests, it was relevant to focus this research on a management unit that is grazed by sheep.

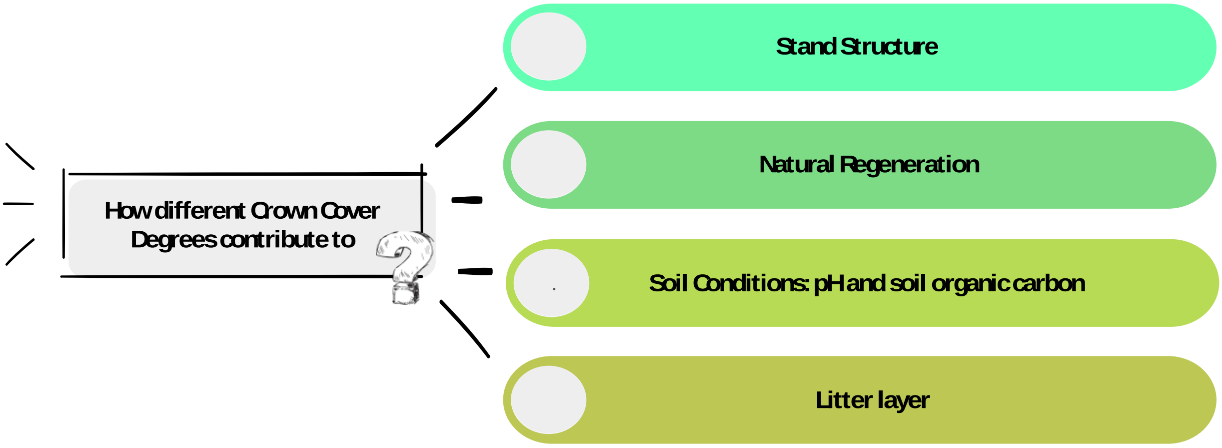


Figure 1. 2 Schematic representation of the problematic (Credit of the author).

The specific objectives in this research are:

1 – Evaluate the structure and its variability as a function of the crown cover.

2 - Evaluate the effect of structure and crown cover on natural regeneration.

3 – Evaluate the effect of litter layer, soil organic carbon, and soil pH on natural regeneration.

1.3 The current state of knowledge

1.3.1 The *montado* and holm oak forests in the Alentejo region

The *montado* is an agrosilvopastoral system characteristic of the Mediterranean Region and extending over more than one million hectares in Portugal (Lauw *et al.,* 2013; IFN6, 2015). The *montado* is a multifunctional system that includes grazing areas, agricultural zones, and sparse tree strata. The *montado* is a manmade landscape that has been characterized by anthropogenic influence since the Neolithic when the Iberian woodlands and forests began to be cleared for agricultural usage (Fonseca, 2016).

To understand the recession of the *montado* it is essential to understand its history and the measures implemented in its management over several years by many generations of owners and stakeholders. Throughout the twentieth century, several policy measures have fostered the decline of the forest cover of the *montado* in Portugal due to the intensification of its agricultural and livestock usage: the Wheat Campaign (1929-1938), the Agrarian Reform (1974), and the Common Agricultural Policy (1992) (Fonseca, 2016).

The loss of holm oak forests and open oak woodlands in the Mediterranean is caused by a complex multifactorial amount of biotic and abiotic stress factors such as changes in land use, abandonment of traditional agricultural activities, soil mobilization, and an increase in livestock density and intensification of agricultural practices (Godinho *et al.,* 2016, Lauw *et al.,* 2013; Pinto-Correia *et al.,* 2011; Plieninger, 2006). In Portugal there was a national tendency to cut down the *montado* tree density in certain regions, to boost agriculture and livestock production (Lauw *et al.,* 2013). According to Freixial (2012), the intensification of annual crop production and livestock exploitation in the Alentejo region led to the degradation of the productive characteristics of the soil and the loss of much of the native herbaceous flora that constituted the natural pastures previously present in this region. The problem is not only the regression of the *montado* areas but also, as stated by Guiomar *et al.* (2020), there has been an increase in the fragmentation level of the *montado* areas. This fragmentation will challenge the preservation of the biodiversity in this ecosystem.

1.3.2 The Socio-economic context and the Ecosystem Services of the *montado*

The multifunctional management of the *montado* commodities involves small-scale production and plans to preserve the longevity of its ecosystem. Both multifunctionality and extensive rainfed production are characteristics of the *montado* and are factors that may be viewed by producers as either weaknesses or strengths, that is why it is necessary to have integrated and dynamic management of the various resources. There is a lack of strategy in the long-term market as the market driving force and government livestock subsidies accelerate the conversion of the *montado* into treeless pastures (Campos *et al.,* 2001) This is particularly true for the holm ok *montado* because the holm oak forest component is not as economically valued as in the case of the cork oak (Vizinho, 2016). The economic viability of the holm oak forest derives mainly from its agricultural and pasture use, enriched with the acorns, an exceptional value of the *montado* as acorn production by oaks is the main food source in winter for both livestock and wildlife (Rodrigues, 2009). The acorn production is abundant every other year and another influential factor in the production is the tree itself (Gea-Izquierdo *et al*., 2006; Rodrigues, 2009). The average production in the *montado* is around 250-600 kg/ha in stands with an average of 50 trees per hectare (Gea-Izquierdo *et al.,* 2006). There are still few studies on the variables involved in acorn production such as pruning, site, and stand structure, and there are interesting relationships still to explore (Gea-Izquierdo *et al.,* 2006; Rodrigues, 2009).

1.3.3 Ecosystem Services of the Montado

Ecosystem services are direct and/or indirect benefits that the human population can enjoy through the existence and maintenance of a given ecosystem. Ecosystem services provide essential functions for human survival, such as climate regulation, ensuring the water cycle, soil conservation, carbon dioxide sequestration, and oxygen production (Madureira *et al.,* 2013). In addition to the use and direct economic value, the *montados* also generate a numerous amount of ecosystem services, values of indirect use, such as landscape, carbon sequestration, rainwater interception, and infiltration as well as maintenance of high levels of biodiversity (Table 1. 1).

Table 1. 1 Types of Ecosystem Services in the Montado

	Types of Ecosystem Services	Literature reference
Water retention	Regulating Services	(Pereira *et al.,* 2009)
Erosion prevention and maintenance of soil fertility	Regulating Services	(Pereira *et al.,* 2009)
Climate Regulation	Regulating Services	(Lauw *et al.,* 2013)
Biodiversity	Habitat and Support	(Pereira *et al.,* 2009) ; (Lauw *et al.,* 2013) ;(Cabral *et al.,* 2005)
Carbon Sequestration and Storage	Regulating Services	(Pereira *et al.,* 2009) ; (Lauw *et al.,* 2013)
Acorns and pasture	Provisioning Services	(Pereira *et al.,* 2009); (Rodrigues, 2009)
Hunting	Provisioning Services /Cultural Services	(Pereira *et al.,* 2009) ; (Lauw *et al.,* 2013)
Wood	Provisioning Services	(Pereira *et al.,* 2009); (Vizinho, 2016)
Tourism	Cultural Services	(Pereira *et al.,* 2009);(Lauw *et al.,* 2013)
Wild Mushrooms	Provisioning Services	(Lauw *et al.,* 2013)
Bee products	Provisioning Services	(Lauw *et al.,* 2013)
Wild plants	Provisioning Services	(Lauw *et al.,* 2013)
Habitat for species	Supporting services	(Pereira *et al.,* 2009) ; (Lauw *et al.,* 2013)

At a time when climate change is beginning to intensify, when we have been dealing with the accumulation of pollution on Earth since the Industrial Revolution of the 18^{th} century, at a time when the human population is increasing and approaching 7.6 million people, it is necessary to evaluate how the Economy is facing nature, and which are the natural goods essential to human survival we need (Sukhdev, 2009). In the past fifty years, human beings have changed ecosystems at a faster rate than in any other period in history (Pereira *et al.,* 2009). Pollution and human action have led to the creation of dead zones, areas where life is no longer possible (Pereira *et al.*, 2009). The current state of ecosystems can be analyzed according to the theory of the *tragedy of commons*, as defined by Garrett Hardin (Hardin, 1968). According to Hardin a common resource will be extinguished if it is managed solely to meet individual interests. Since then, several researchers (Shiva, 2008; Rands *et al.,* 2010; Ehrlich and Daily, 2012; Farley, 2012) have debated and analyzed the paradigm of economic invisibility of ecosystem services. This is of high relevance to the future of the *montado*, since its high value arises from the plentiful and diverse set of ecosystem services (Table 1. 1). The 2006 Red Book of Vertebrates (Cabral *et al.,* 2006) states that several vulnerable or endangered species can still be found in the *montado*s in Portugal, such as: Iberian Imperial Eagle (*Aquila adalberti*): 2 - 5 couples, this species is critically endangered; Short-toed snake Eagle (*Circaetus gallicus*): 250 - 600 couples, this is an almost threatened species; Gray kestrel (*Elanus caeruleus*): 100 - 150 couples, this is also an almost threatened species; Black stork (*Ciconia nigra*): 83 -96 couples, Red kite (*Milvus milvus*), another vulnerable species (Cabral *et al.,* 2005). Maintaining high levels of landscape connectivity is vital for preserving the biodiversity of the *montado* (Machado *et al.*, 2020)

To establish an economic worth to the type of HNV systems like *the montado* is an essential step to guarantee a sustainable future. That is why it is crucial to study and debate the topic of ecosystem services.

1.3.4 The holm oak

In the Mediterranean basin there are both deciduous and evergreen oaks (Gil-Pelegrin *et al.,* 2017). The holm oak belongs to the family *Fagaceae* and the genus *Quercus* and it is referred in the literature as *Quercus rotundifolia* Lam., *Quercus ilex* spp. *rotundifolia* syn. *Quercus ilex* spp. *ballota* (Alves *et al.,* 2012; De Rigo *et al.,* 2016). Different taxonomy scientists discuss if the *Quercus ilex* spp *rotundifolia* should be considered a distinct species (*Quercus rotundifolia)* rather than a subspecies (De Rigo *et al.,* 2016). In the 6th National Forest Inventory in Portugal, the scientific name used for the holm oak was *Quercus rotundifolia* (INF6, 2015). In the Flora Europaea, the holm oak appears divided in two separated species and the homotypic synonyms found in the literature are *Quercus ilex* subsp. *rotundifolia (*Lam.) O. Schwarz ex Tab. Morais, *Quercus* *ballota* var. *rotundifolia* (Lam.) Nyman, *Quercus ilex* var. *rotundifolia* (Lam.), *Quercus ilex* subvar. *rotundifolia* (Lam.) A. Camus (Euro+Med, 2006). The two subspecies or species are recognized by differences in leaf shape (De Rigo *et al.,* 2016). The *Quercus ilex* subsp. *rotundifolia* (also referred to as *Quercus ilex* subsp. *ballota* or as separate species *Quercus rotundifolia*) has more lanceolate leaves with 6-8 veins and occurs mainly in the Iberian Peninsula and in Morocco (De Rigo *et al.,* 2016) (Figure 1. 3).

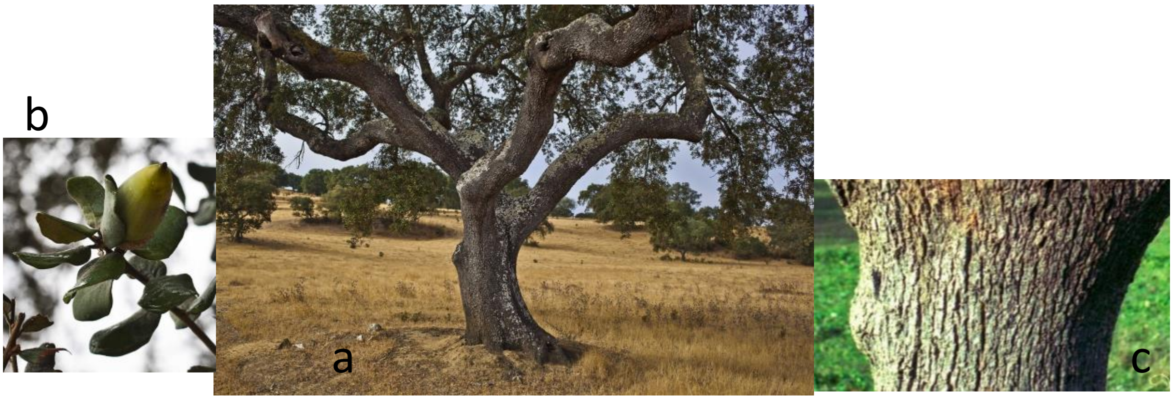


Figure 1. 3 a) Holm oak adult tree, b)- Holm oak acorn and leaves, c) - Holm oak bark (Credit of the author).

Holm oak is a hardwood tree that grows slowly. It is a broadleaved evergreen oak, which can grow up to 30 m in height with over 2 m of diameter at breast height and it can live for hundreds of years (De Rigo *et al.,* 2016). Its leaves are coriaceous, green to dark-green appearance and the upper epidermis and cuticle are much thicker when compared to the other side of the leaves (Terradas, 1999). The holm oak has a broad crown, with ascending branches. The bark is brown and cracked into small, thin plates (De Rigo *et al.,* 2016).

One of the adaptive traits of the holm oak is the species sclerophyllous leaves. The leaves of sclerophyllous trees bear a thickened, hardened foliage that resists the loss of moisture. According to Bussotti *et al.* (2001), holm-oak leaves develop its sclerophyllic characteristrics by the development of an increased thickness of the leaves parenchymatic tissues. The leaf structure of Mediterranean sclerophyllous (hard leaves) is thought to be related to strategies used to adapt to dryland conditions and drought. The holm oak leaves and their sclerophyllic characteristics develop before each summer regardless of each year climate variations (Bussotti *et al.,* 2002).

Holm oak is a monoecious species, which means that the same individual has both male and female reproductive organs (Pulido *et al.,* 2005). The male catkins appear first in early spring and female inflorescences later (Pulido *et al.,*2005). Acorns mature and fall between November and January (Terradas *et al.,* 1999). The holm oak is a common species in the open grazed forests (so-called *montados* in Portugal and *dehesas* in Spain). For the holm oak populations in Portugal, only areas with a crown cover higher than 10% are considered forests (IFN5, 2010). Its natural distribution covers most of the Mediterranean basin (from Portugal, Spain, Turkey, Morocco, also expanding northward up to Italy and France (**Figure 1. 4**). The holm oak distribution map includes the two subspecies, each, shown in indifferent colors and pattern when there is an overlap of the two subspecies created with the data of Caudullo *et al.* (2020). The countries frontiers used in this map were from the UIA World Countries Boundaries (UIA, 2020).

Figure 1. 4 Holm oak (*Quercus ilex* L.) distribution.

The holm oak is a legally protected species in Portugal (Decreto-Lei n.º 169/2001, 25 of March. D.R. n.º 121, Série I-A, updated in Decreto-Lei n.º 155/2004, of the 30th June. D.R. n.º 152, Série I-A). A permission is needed for cutting and pruning the holm oak.

As reported in the Final Report of the 6th National Forest Inventory Data, the total area of holm oak in Portugal decreased between 1995 and 2005, from 366.7 thousand hectares to 335.5 thousand hectares (IFN6, 2015). The holm oak area increased between

2005 and 2010 to a value of 349.2 thousand hectares. In 2015 the holm oak area was 349.4 thousand hectares. Most holm oak occupied areas in Portugal are pure stands, 327.2 thousand hectares, which correspond to 94% (IFN6, 2015).

The holm oak is a common species in agro-silvopastoral ecosystems, where grazing plays an important role and timber is not the main source of income. The lack of interest in oak timber has led to a scarce development of specific growth models to this species (Gea-Izquierdo *et al.,* 2008*)*. Another characteristic of the holm oak, when compared to other species used for timber, is that estimating the tree age by stem analysis is challenging because the eccentric growth and double rings are common (Gené *et al.*, 1993). Therefore, it is necessary to use age-independent models and the growth of an individual oak tree can be represented by a nonlinear sigmoid model with an asymptotic tendency (Gea-Izquierdo *et al.,* 2008; Tomé *et al.,* 2006).

In a climate where drought is common, plants are adapted to survive. In the Mediterranean climate, there are cold winters and hot, dry summers. In the winter *Quercus ilex* presents a photosynthesis rate between 39-99% (Flexas *et al.,* 2014). The Mediterranean evergreen oaks are isohydric, which means that they can control tissue dehydration via stomatal opening/closure and deep root growth thus avoiding drought-induced hydraulic failure (David *et al.,* 2007).

The holm oak species may suffer a cambial stop in summer caused by drought. In the Mediterranean ecosystems, tree-ring growth is limited by soil water availability (Cherubini *et al.,* 2003). Nonetheless, in the research done by Cherubini *et al*. (2003), a low correlation coefficient between total annual precipitation and ring width for *Quercus ilex* was found. Cherubini *et al.* (2003) also found that holm oak reacted significantly to late-summer or early autumnal precipitation of the current year. The relationship between the timing of the growth stimulus, in this case of precipitation, and the quantity of stimulus is complex and dynamic (Cherubini *et al*., 2003). As an example, if the soil has plenty of water early in the growing season, the amount of precipitation has little effect on growth at that same time because there is an abundance of that resource, whereas half that amount of precipitation after a drought can stimulate growth (Kozlowski *et al.,* 1997). According to Gea-Izquierdo *et al.* (2011), *Quercus ilex* stops responding positively to increasing precipitation at levels above 600 mm, regardless of age. The effects of climate change in holm oak populations are going to be more drastic in areas with a high aridity index, with less water and moisture available (Gea-Izquierdo *et al.,* 2011). The holm oak fine roots are mainly in the superficial layers of the soil (top 20 cm as noted by López *et al.* (1998). Roots longevity was 35 to 471 days and it was greater in the control plots than in the thinned plots (López *et al.,* 1998). Understanding the biology of the root system of the oaks is essential to comprehend the decline of the open woodland of the oaks in the Mediterranean. One of the factors of mortaliy of trees is the death of the roots caused by *Phytophthora cinnamoni* (Brasier, 1996; da Clara *et al.,* 2013). The microscopic soil-borne oomycete fungus *Phytophthora cinnamomi* has been associated with the mortality and decline of the cork oak and holm oak in the Mediterranean region (Brasier, 1996; da Clara *et al.,* 2013). Furthermore, other factors are involved in the decline of the oak trees such as soil depth, soil compaction, soil fertility, and cultural practices (da Clara *et al.,* 2013). To prevent the decay of the trees, it is important to enhance root expansion soil conditions and also suitable nutrient and hydric conditions (da Clara *et al.,* 2013).

In the case of the holm oak, the use of the pruning wood as firewood is continuously made during the lifespan of the tree Palacios *et al.*(2009) and not only in the end of rotation age.

The holm oak also has additional importance as an acorn productive tree that will guarantee the nutrient necessities of the cattle and Iberian pigs when there is a shortage of food at the beginning of Autumn (Freixial, 2012). In a silviculture model for acorn production it is important to understand the variables behind the variability in acorn quality and quantity so that the design follows the goal of increasing acorn production. Individually, the holm oaks in low density plots show higher yields per tree (Rodrigues, 2009). However, when analyzing acorn production at stand level and production per hectare, lower densities per hectare had smaller values than populations with higher densities (Rodrigues, 2009). In a study by Alejano *et al.* (2011) the researchers stated that acorns from the south sides of the trees crown were significantly heavier than those at other positions, this is an important result for developing a model of silviculture.

1.3.5 Silviculture practices in the *montado*.

Silviculture practices are sustainable intervention techniques of forestry management based on a deep knowledge of forest ecosystems and considering one or more proposed goals (Gonçalves, 2017a; Alves *et al.,* 2012). Stands are the basic units of forest management. By definition, a stand covers a homogeneous area considering vegetation, site quality, delineated by a combination of stand characteristics including structure and composition (Gonçalves, 2017a; Johnson *et al.,* 2002, Oliver and Larson*.,* 1996, Pommering *et al.,* 2019). According to O´Hara (2013a), there are two types of stand definitions: the *operational* definition, which is focused on defining management units that enable higher efficiency in the silviculture operations towards a defined goal; the *ecologica*l definition of a stand that, is a homogenous unit, defined by climatic, edaphic, or geomorphologic qualities of a given site.

In silviculture, the timeline is decades and centuries because forest systems can survive beyond the life of the professional forester managing it so one of the main challenges of silviculture is to register all the practices and plan decades head (Gonçalves, 2017a; Alves et al., 2012; Johnson et al., 2002). Forest stands can be classified according to their structure in uneven-age stands, that include two or more age classes or cohorts, or even-aged with only one age class of trees. A cohort is a distinct group of trees originating from a single regeneration activity either natural or artificial, that can be considered as a silviculture unit when managing. Common silviculture practices include thinning, pruning and regeneration (Gonçalves, 2017b; Alves et al., 2012).

In the history of silviculture, the greatest silvicultural goals and challenges have been the fulfillment of human needs, such as wood, water, food, energy, fodder, fiber, and recreation. Historically, forestry management in the last two centuries has focused on optimizing the efficiency of commodity production by following the principle that homogenous products are cheaper to produce and easier to manipulate so management practices have typically led to even-aged forests (Puettmann *et al.,* 2015; Johnson *et al.,* 2002). In an even-aged silviculture model, the population is managed to achieve a homogenous cohort of forest trees. An example of an even-age silviculture model for the holm oak is the one described in Louro *et al.* (2000). The Louro *et al. (*2000) silviculture model for the holm oak was designed to manage stands with a high density of planting, over 400 trees/ha. The main silviculture measures prescribed in the first 30 years are: control natural vegetation (especially shrubs) near the trees for understorey competition control; control of branching and forking in the lower bole of the seedlings; pruning to develop a balanced open crown. The first thinning should be done 30 years after planting, selecting the best-shaped trees to obtain a density of 150 trees/ha. After the first thinning and in 10 years it should be done a fructification pruning. Every decade after the 40th year and beyond, thinning is suggested, cutting 20% of the trees (Louro *et al.,* 2000). Promoting and managing natural tree regeneration in the *montado* can be a relevant strategy in the silviculture management practice of oaks (Serradas and San Miguel, 2008; Alves et al., 2012). In the case of artificial regeneration, it is important to consider the planting date and site preparation as these factors can affect the height growth rate (Palacios et al., 2009). The choice of the regeneration method should consider the main goal of the stand and the site index. Seeding can be the cheapest method, with the roots growing in depth, but achieving medium survival rates and its success can be enhanced by using large acorns (González-Rodríguez et al., 2011). Planting one-year seedlings showed best survival rates than direct seeding and planting older seedlings (González-Rodríguez et al., 2011).

In uneven-aged stands, the forester will manage the stands to have different age classes in the same space and this silviculture philosophy is common in different silviculture methodologies (Alves *et al.,* 2012; Puettman *et al.,* 2015). In uneven-aged silviculture management, there is the concern to regulate a forest by managing the stands within it as a mosaic of different age classes and to create a balanced distribution of age classes that are maintained through time (Gonçalves, 2017b; Alves *et al.,* 2012; Johnson *et al.,* 2002). Alternative silvicultural approaches to the even-aged defend the avoidance of clearcutting, an emphasis on structural diversity and small-scale variability, mainly of mixed species with natural regeneration, and eradicating intensive site-preparation methods (Puettman *et al.,* 2015).

According to the International Union of Forest Research Organization (IUFRO), the major silvicultural challenge of today's world is the adaptation of forests and forestry to climate change (IUFRO, 2020) and the holm oak is a species that can succeed in arid sites (Alves *et al.,* 2012).

In the *montado* ecosystem, the area can be divided into different stands and cohorts so that the multifunctionality, sustainability, and longevity of the ecosystem are assured. The silviculture in the *montado* is especially important because it is a complex multifunctional system that benefits from the integrative planning of all its components. In the *montado*, maintaining an uneven-aged stand structure and a continuous forest cover can be a valuable option for the future (Ribeiro *et al.,* 2012; Namora, 2014; Carvalho, 2020).

1.3.6 Natural Regeneration of the Holm oak

Natural regeneration depends on the production of seed and the growth of individuals *in situ*, allows for the preservation of natural genotypes (Harmer, 1994). The success of natural regeneration depends on flowering, acorn/seed production, germination/emergence, seedling establishment, and recruitment. Acorn production per tree is negatively related to stand density (Gea-Izquierdo *et al.,* 2006). However, from a stand analysis point of view Gea-Izquierdo (2008) later concluded that less densely populated plots produce fewer acorns per surface but more per tree. Acorn production and seed availability start to reduce in stands with a number of trees higher than 50 trees per hectare (Gea-Izquierdo *et al.*, 2006). According to Gea-Izquierdo *et al.* (2006) the best objective way of comparing acorn mean values is acorn production per crown unit area of the holm oak is that is around 100 g per $m^2$of crown cover and 15–21 kg per tree.

When comparing natural emergence of the seedling in two different habitats, (forest and *montado*), Pulido *et al.* (2005) found that the natural emergence of seedlings happened frequently in the forest (more dense stands), whereas it was rarer in the *montado*. In the study of Smit *et al.* (2009), it was also found that there is a significant failure in the recruitment process in the savannahs at multiple stages. The survival of the oak seedling is mainly affected by the distribution of suitable habitats, and shrubs may play an important role there (Smit *et al.,* 2009). One of the main challenges of the seedlings is to survive the first summer drought, as observed in sites within the Mediterranean climate regions (Garcia-Fayos *et al.*, 2020; Pulido *et al.,* 2005; Smit *et al.,* 2009). Therefore is important to understand how water stress impact can be diminished.

According to Principe *et al. (*2019) in the Mediterranean dryland region, northern and southern slopes of complex topographic generate different microclimatic conditions. The microclimatic regions develop because of differences in potential solar radiation exposure above the crown cover and due to the effect of the forest stand on climatic regulation below the canopy that was measured with lichens diversity indexes (Principe *et al.,* 2019). Principe *et al.* (2019) observed that holm oak trees growing under the same management unit, but in different microclimates, showed significant changes in their performance indicators. Southern slopes displayed lower tree density and more younger trees whereas northern slopes, showed higher tree density, almost crown closure, and older trees. Principe *et al.* (2019) suggested that such asymmetrical tree spatial pattern was not a consequence of mortality, but it is mainly caused to limitations in germination and/or sapling establishment.

It is very interesting to understand how different ecological conditions can affect sapling establishment, including shade and water availability. In the Benayas *et al.* (2004) study, the holm oak sapling ability to survive according to different treatments (summer irrigation, artificial shading, and combined treatment plots) was tested. The authors found out that the saplings mortality counts were not significantly different between the three treatments and that there were four times less mortality in all treatments than in plots without irrigation and artificial shading. In a previous study by Benayas *et al*. (1998), the authors stated that shade had a significant effect on all growth measurements (stem diameter, height, crown projected area) and irrigation alone only affected stem diameter growth.

The microorganisms of the ecosystem, especially in the soil, may play an important role in the surviving rate of natural regeneration. Gallego *et al.* (1999) observed that seedlings in the presence of pathogenic microorganisms such as *Phytophora cinnamomi,* showed a decline in growth rate and developed root rot and 35.7% died. The decline of the holm oak trees triggers a cascade effect on plant understory and soil microbial communities (Rodriguez *et al.,* 2017), and in this case, the understory species colonize the space created by dead holm oaks. According to the results presented by Dinis (2014) the cork oak seedlings growth did not improve when only fertilization was added and the seedling treatment that presented the better results in terms of growth was the one where a combination of fertilizers, amino acids, and mycorrhizal fungi inoculation were applied. Nocturnal water translocation from plant to mycorrhizal fungi can occur in oak seedlings (*Quercus agrifolia*) thus the mycorrhizal activity can potentially improve the nutrient status of deep-rooted plants during periods when the fertile upper soil is dry (Wang *et al.*, 2019).

Natural regeneration is an important succession process in forest ecosystems and understanding the dynamics of the process is a key part of managing the holm oak stands for a successful future.

1.3.7 Soil importance in the *montado* ecosystem.

The dryland pasture and the montado occupy areas with lower agricultural interest, with less developed soils such as Cambisols, Litosols, and some Luvisols (Cardoso et al., 1971), which correspond, presently to the Reference Soil Groups (IUSS Working Group WRB, 2015) Regosols, Cambisols, Leptosols, and some Luvisols.

Mediterranean lands with low soil organic carbon (SOC) content are at high risk of erosion, land degradation, and desertification. The increase in SOC is relevant in these systems, to build resilience for climate change (Aguilera *et al.,* 2013). Oak crown cover above 30% can be effective in managing erosion risk (Ribeiro *et al.*, 2004). Maintaining the productive potential of the *montado* is especially dependent on the soils (Ferreira *et al.,* 2007).

In the soils occurs the accumulation of carbon over long periods and soil carbon stocks are a tool for decreasing the excess of carbon in the atmosphere and reducing the amount of greenhouse gases, thus mitigating global warming (Lal *et al.,* 2004). The soil organic carbon (SOC) is also an important indicator of soil fertility (Loveland and Webb, 2003). If the SOC is lower than 2%, a relevant decline in soil fertility may occur but according to the literature, there is no single critical threshold value for soil carbon content that can be applied to all soils (Loveland and Webb, 2003). The soil type and composition affect SOC content, soils with higher clay content have a greater SOC (Loveland *et al.,* 2003). SOC sequestration strategies are extremely important in arid lands, such as afforestation, controlled grazing, soil, and water conservation (Lal, 2009).

In the *montado*, the tree cover is associated with increased soil fertility, either by recycling nutrients from deep layers or by the falling leaves' accumulation (Pereira *et al.*, 2009). The importance of tree cover in the production systems of the *montado* where livestock farming is the main function of the farmstead tends to be overlooked. Nevertheless, the presence of holm oaks can have a positive impact on soils and the ecosystem. Holm oaks can provide more moisture and water in the soil by moving water from deeper to shallow ground (Cubera *et al.,* 2007; David *et al.,* 2007). According to Schnabel *et al.* (2013), the trees in the *montado*s lead to an increase in the fertility of the superficial layers of the soil, as their roots reach deeper depths than annual plants. In the study by Simon *et al.* (2013), a positive correlation was found between holm oaks and soil organic carbon stock within an area of 8 m from the stem. Importantly, the largest proportion of organic carbon stored is in the surface layer, and the soil surface layer is most exposed to disturbances induced by livestock management, sowing, harrowing and erosion (Simon *et al.,* 2013). Thus, it is essential to ensure soil protection and conservation practices so tree cover may be a key tool to ensure the longevity of this ecosystem. What's more, the mineralization of organic matter is higher outside the shadowy surface under the tree crowns (Schnabel *et al.,* 2013), which leads to lower values of organic matter in the soil outside the tree crown (Gallardo, 2003; Schnabel *et al.,* 2013).

Decreasing tree numbers and degradation of pasture and soil lead to a decline in carbon sequestration (Aguilera *et al.,* 2013; Lauw *et al.,* 2013). The accumulation of organic matter in the soil through the falling leaves and the development of a superficial organic horizon (usually shallow) in these types of undeveloped soils is important for the pasture development and maintenance of the ecosystem as well as the health of the forest and its herbaceous component. According to Rodrigues *et al.* (2015), trees have a regulatory role in the transformation processes of carbon, nitrogen, and phosphorus in the soil both in natural pastures and in the case of improved pastures, in which more productive species were introduced. The differences found between an improved pasture and a natural pasture were more tenuous under the canopy than in the open field (Rodrigues *et al.,* 2015). Soil management practices are also considered in the silviculture models and play an essential role in the *montado* and may affect stand dynamics.

Understanding organic matter distribution concerning the patchy structure of the landscape of the *montado* should be viewed as an important point for future research and for the silviculture management of these ecosystems (Reyna-Bowen *et al.,* 2020)

CHAPTER 2 - MATERIALS AND METHODS

2.1 The Study area

The study area is located in Ferreira do Alentejo, in the District of Beja, in the Alentejo region (38°00'43.8"N 8°03'36.8"W) southern inland Portugal (**Figure 2. 1**).

The property has 180 ha of holm oak *montado*. The *montado* natural pastures are grazed by a flock of Portuguese merino sheep breed, of on average, 600 sheep, corresponding to 3 animals per hectare. Extensive grazing occurs for 6 months per year on average. According to the data consulted in ICNF geocatalog (https://geocatalogo.icnf.pt/catalogo_tema5.html) there has been no fire event on the property since 1975, there is no information before this data. The last soil mobilization occurred more than a decade ago, there is no precise date.

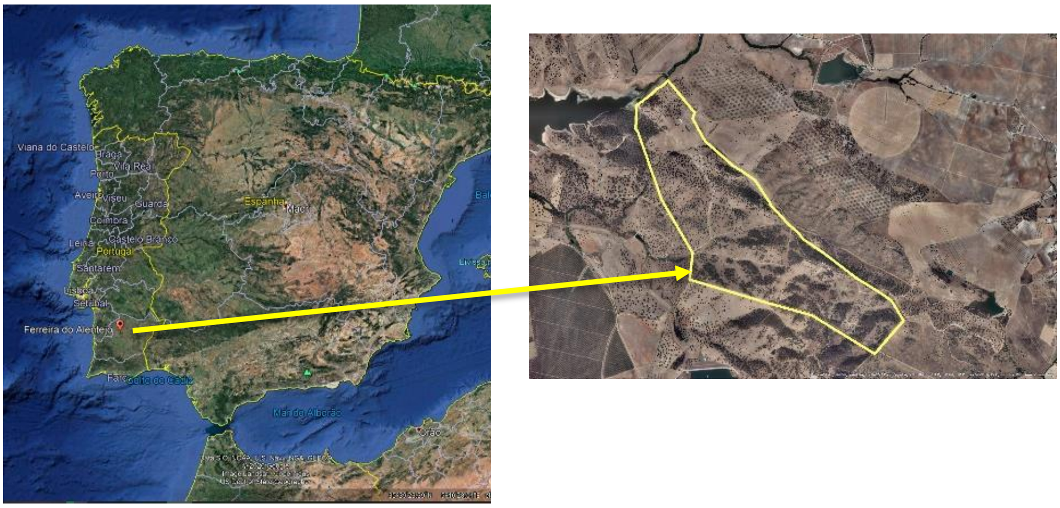


Figure 2. 1 a) Location of the property in study in Ferreira do Alentejo, South of Portugal (Source: Google Earth V 6.2.2.6613 (September 30, 2020). b) Limit of the study area.

The study area is composed of three soil types, classified according to the 1964 Soil Map of Portugal (Map 43 A and C, **Figure 2. 2**): Px (d), Pm, and Vx. Px(d) is the most dominant, occupying 84% of the area. The Px(d) represent the shallow phase of brownish colored soils, with an argic B horizon, that is, a horizon of clay accumulation, typical of Mediterranean climates, derived from schist and greywackes. Both Pm and Vx soils have also an argic B horizon but the Pm soils have a brown B horizon rich in montmorillonite clay and are developed from diorite or quartzodiorite or similar microfaneritic rocks, and the Vx soils have a B horizon red or yellow and are developed from shale or greywackes soils. In the WRBSR classification (IUSS Working Group WRB, 2015) a significant part of the area with the Px(d) soils could belong to the group of the Leptosols (soils with a depth of less than 25 cm or extremely gravelly soils).

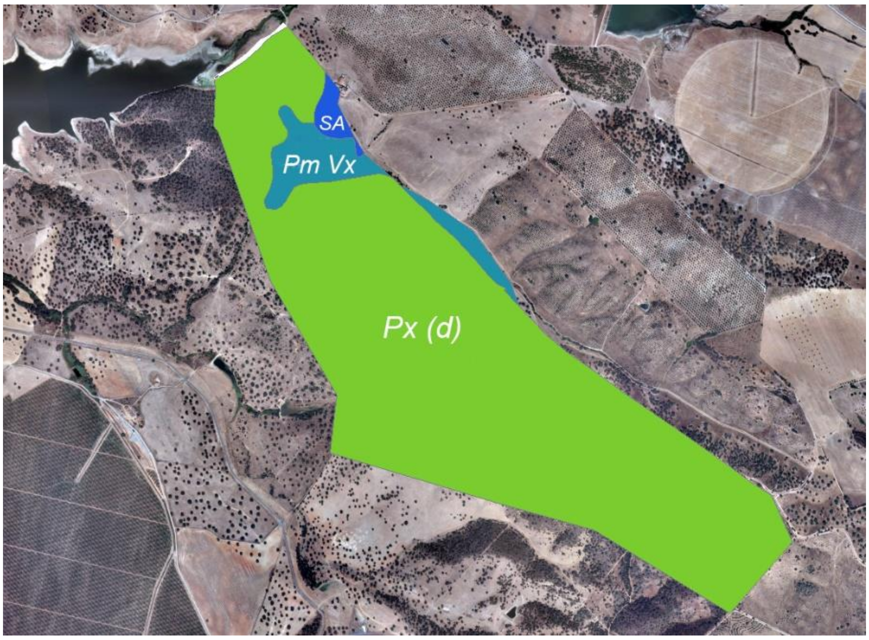


Figure 2. 2 Map of the type of soils Pm Vx and Px (d). SA is the social area of the farm.

In the Köppen classification, Portugal is described as a Mediterranean climate, characterized by hot, dry summers and wet and cool winters. According to the Köppen subclassification system, Ferreira do Alentejo is a Csa, Hot-summer Mediterranean. The climate is characterized as the typical Mediterranean, with a long dry summer where the precipitation occurs mainly between October and April (Figure 2. 3). The tree populations in the *montado* face low winter temperatures and high summer temperatures without any rain and these are challenging climatic conditions for plant development. However, holm oak trees are drought resistant and suitably adapted to this climate, holm oaks transpiration is reduced in the summer and the carbon assimilation processes to the climatic limitations of this species adapts well to such conditions (Vaz, 2005).

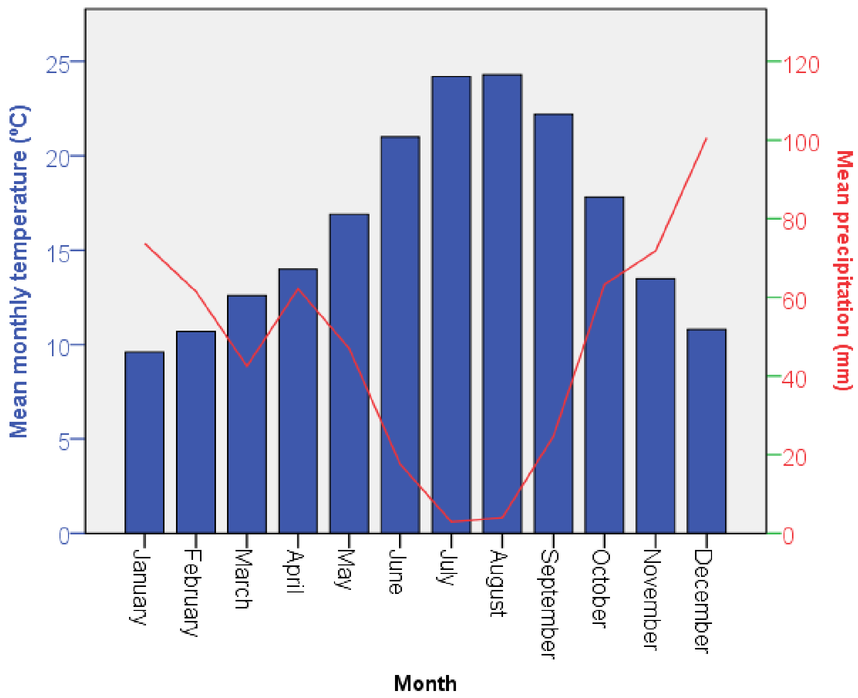


Figure 2. 3 Climogram showing monthly mean precipitation and mean temperature averages for the period 1971-2000, Metereological station of Beja (data from the Instituto Português do Mar e da Atmosfera https://www.ipma.pt)

2.2 The Sampling Design

The main goal of this research is to study holm oak stand structure dynamics by exploring if different degrees of tree crown cover in a holm oak management unit contribute differently to the dynamics of the holm oak stand structure. Holm oak stands may integrate different degrees of crown cover, extending from low crown cover areas to high crown cover areas. Subsequently in this research, the population was stratified by crown cover and a random sampling was applied *per stratum*, to reduce variation within forest subdivisions and increase the precision of population estimates (Kershaw, 2016). This way, a heterogeneous landscape with different degrees of crown cover was divided into subdivisions called *strata*. Therefore, the study area was divided into a square grid of 2000 m^2 (ESRI, 2019). The crown cover degree per grid was estimated according to the protocol of the Portuguese National Forest Inventory (IFN5, 2005/2006) (Figure 2.4, Appendix I). According to the crown cover estimations, 74% of the area had a crown cover between 0 and 30% (Appendix II).

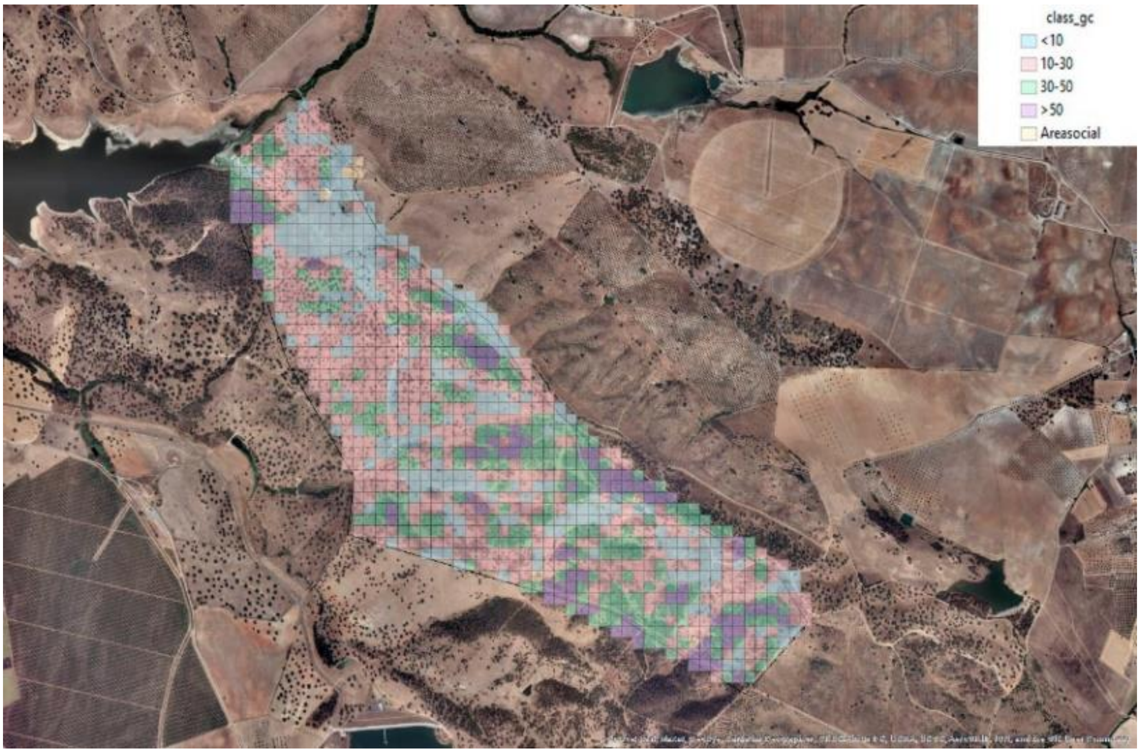


Figure 2. 4 Map of the grid Crown Covers Classes (CC Classes).

2.2.1 The tree sampling

To organize the sampling design process, four Classes of crown cover (CC Classes) were defined: CC Class 1 *Stratum*: < 10% of crown cover (Figure 2.5); CC Class 2 *Stratum*: 10-30% of crown cover (Figure 2.6); CC Class 3 *Stratum*: 30-50% of crown cover (Figure 2.7); CC Class 4 *Stratum* (Figure 2.8): > 50% of crown cover. In this study, 7% of the areas belonged to CC Class 1, 52% to CC Class 2, 24% to CC Class 3 and 17% to CC Class 4 (Appendix III).

A total of 40 plots were randomly selected, 10 plots for each CC Class (Appendix IV). The plots were restricted to the most representative soil type in terms of area (Px(d)). The coordinates of each plot were calculated with ArcGIS 10.6 (ESRI, 2019) and a GPS RTK was used to mark the plot's vertices in the fieldwork. The forest inventory was carried out in these 40 plots (Figure 2.9). The plots were identified as follows: P (number of the plot) and C (number of the correspondent CC Class). For example, plot number 3 of the CC Class 4 has the code P3C4. The field work was undertaken between January of 2020 and May of 2020.

Figure 2. 5 Example of a plot of CC Class 1 (Credit of the author).

Figure 2. 6 Example of a plot of CC Class 2 (Credit of the author).

Figure 2. 7 Example of a plot of CC Class 3 (Credit of the author).

Figure 2. 8 Example of a plot of CC Class 4 (Credit of the author).

2.2.3 The natural regeneration sampling

The natural regeneration of holm oak was sampled in all 40 plots. Two methods were used:

1-Evaluating the amount of established natural regeneration in the plot using regeneration classes: To evaluate the abundance of established natural regeneration (NR) saplings, individuals with *dbh* < 10 cm or *h* ≥ 0.5m in all the plot, were evaluated considering four classes: **NR Class 1**: 0 -25 saplings; **NR Class 2:** 25-50 saplings; **NR Class 3:** 50-75 saplings; **NR Class 4:** 75-100 saplings; **NR Class 5:** > 100 saplings

2- Random transect with 10 m: This method was used to simplify the natural regeneration sampling in plots with a high density of sapling individuals. To define the random transect, an *app* called Random Number (Jhelin corporation, 2020) was used to generate a random number of steps ranging from 0-50. The exact number of steps the *app* generated were given in a random direction and this procedure was repeated 3 times. The sampling of natural regeneration was done in a transect with 10 meters, marked with a measuring tape. A Natural Regeneration Tree Classification (Table 2. 1, Figure 2.9) based on the Assmann Classification of pure stands (Assman, 1970) was created in this research. This procedure was developed for all CC Class 3 and all CC Class 4. In areas where regeneration is low and scattered this method is not suited, such as CC Class 1 and CC Class 2.

Table 2. 1 Natural Regeneration Classification created for evaluating the quality of the regeneration present in each plot.

Superior storey influence
1-Outside the crown projection of an adult tree
1-Inside the crown projection of an adult tree
Degree of crown isolation
1 - Isolated crown
2 - Crown in contact with another tree only in one point
3 - Crown in contact with other tree in two points
4 - Crown surrounded and in contact with other trees
Height (h) Classes
1- $0.5 \leq h < 1.3$m
2- $h \geq 1.3$m
Diameter (dbh) Classes
1 - *dbh* ≤5 cm
2 - 5 cm<*dbh*< 10cm
Tree Shape (Figure 2.12)
1- Shrub shape
2 - Low bifurcation
3 - Tree with one main stem

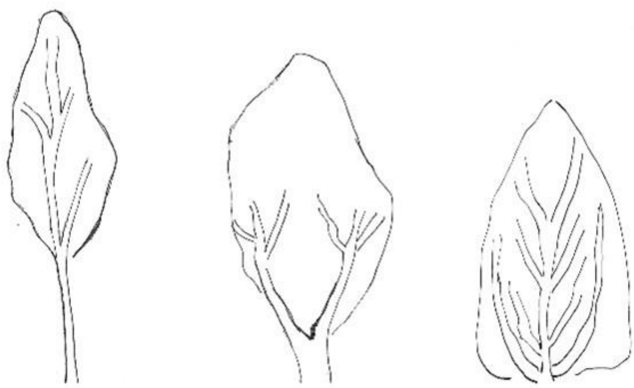


Figure 2. 9 Schematic representation of the tree shape, a) Tree with one main stem, b) Low bifurcation, c) Shrub shaped (Credit of the author).

2.2.4 The soil sampling

For the soil sampling, it was done random subsample of the 40 plots selected initially to do the Forest Inventory (**Figure 2. 10**). For each CC Class, 5 plots were randomly selected for soil sampling, a total of 20 plots (**Figure 2. 10**). Inside each plot and outside the crown of any tree, two areas were defined according to the presence or absence of natural regeneration. All the samples were taken outside the tree's crown projection. So, two sampling areas inside each plot were defined (**Figure 2. 11**): One area with more than 3 saplings alive and with a height≥ 1m (these areas were marked with (+) sign) and another area with no natural regeneration event (these areas were coded with a minus (-) sign (**Figure 2. 11**)). The soil and litter layer samples were carried out within a 25cmx25cm frame (the sampled area was 625 cm^2). The entire amount litter layer was collected within this frame and collected in a plastic bag. After this, the first 10 cm of soil were collected in the defined square area in a different bag. All bags were labeled with the code of the plot and with a + or -. All bags remained semi-opened until taken to the laboratory.

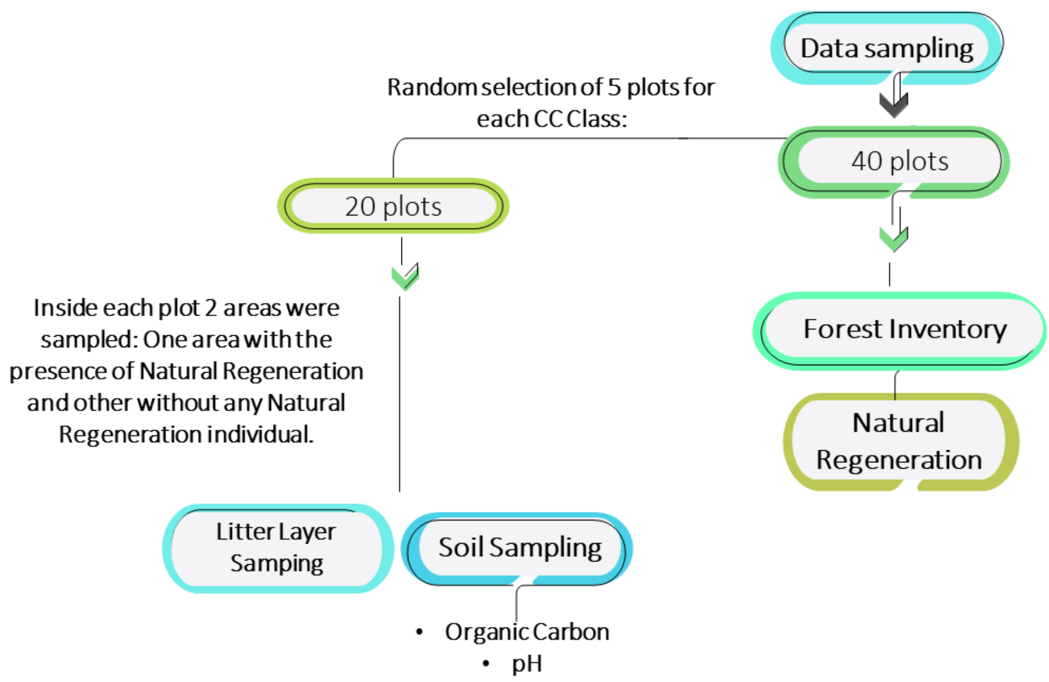


Figure 2. 10 Schematic representation of the sampling process (Credit of the author).

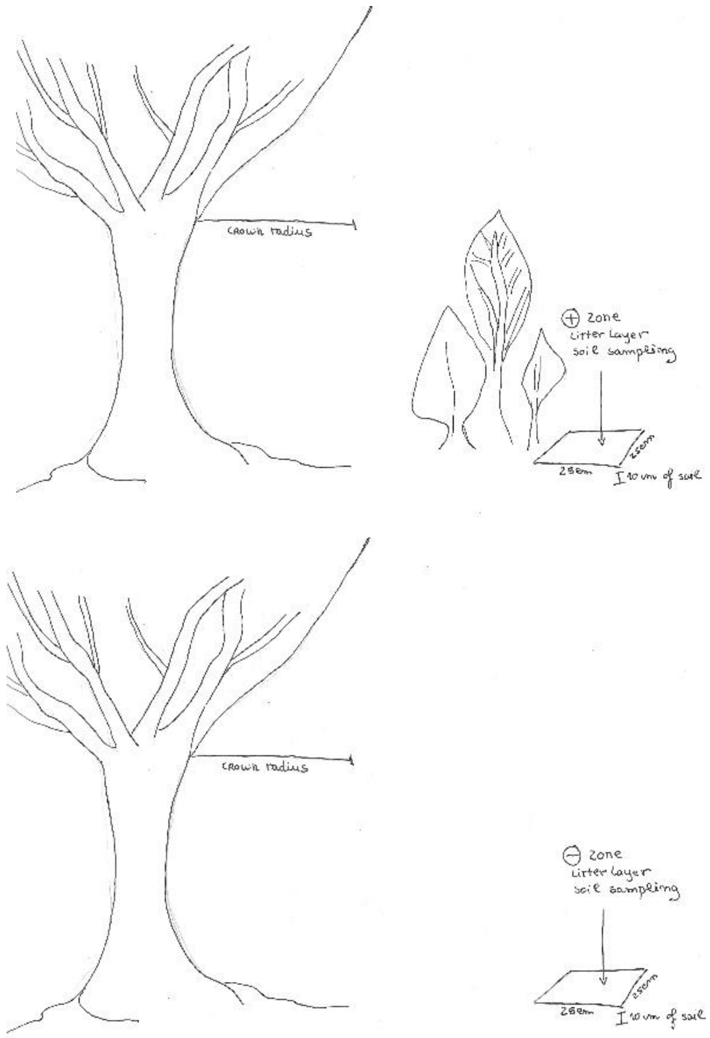


Figure 2. 11 Litter Layer and Soil Sampling Scheme: a) Litter layer and soil sampling in a regeneration zone. b) Litter layer and soil sampling in the absence of regeneration (-) (Credit of the author).

2.3 Materials and Methods

2.3.1 Dendrometric variables

All trees with a diameter at breast height above 10 cm and located inside the selected plots were considered to belong to the main stand. The following dendrometric variables were measured (Philip, 1994; Kershaw, 2016; Gonçalves, 2017a): the **circumference at breast height (*cbh*, m),** the perimeter the outside bark at a height of 1.3 m above ground level was measured with a measuring tape; the **total height (*h*, m)**, the vertical distance from the ground to the top of the crown, was measured using the Vertex IV ultrasound instrument system; the **bifurcation height (*hbif*, m)** the vertical distance from the ground to the point where there is the division of the main stem in two or more secondary branches; the **crown radius (RN, RS, RW, RE, m)**, the horizontal distance from the stem center of a tree to the edge of the crown was measured in the four cardinal directions: north, south, east, and west using a compass and a measuring tape; the **height to live crown base (*hlc*, m),** the vertical distance from the ground to the point where the live crown starts.

The measured dendrometric variables were used to calculate the following parameters (Philip, 1994; Kershaw, 2016; Gonçalves, 2017a):

The **diameter breast height (*dbh*, cm)** was calculated $dbh = \frac{cbh}{\pi} \times 100$. The **linear crown ratio (*lcr, adimensional*)** was calculated as the ratio between the mean crown diameter and the diameter at breast height $lcr = \frac{2mrc \times 100}{dbh}$. The **crown length (*lc*, m)** was calculated as the difference between the total height and the height of the beginning of the live crown $lc\ (m) = h - hlc$. The **crown ratio (*cr, adimensional*)** was calculated as the percentage of the crown length to the total height $cr = \frac{lc}{h} \times 100$. The **mean crown radius (*mcr*, m)** was calculated as the mean of the four cardinal crown radius measured $mrc(m) = \frac{(RN+RS+RE+RW)}{4}$. The **basal area (*g*, $\boldsymbol{m^2}$)** was calculated as area of the circle with diameter equal to *dbh* (cm) for each tree, g=((*dbh*/2)*100)^2)* π.The ***hd* ratio (*hd*, *adimensional*)** is the ratio between the total height and its diameter at breast height, with both variables in the same units (m).The **above ground biomass (*wa*, kg)** was calculated as the sum of three parameters: the stem biomass (***ww***, **kg),** the bark biomass (***wb***, **kg),** is the crown biomass (***wc***, **kg),** using the allometric functions of Tomé *et al.* (2006).

Table 2. 2 Biomass Equation for the Holm oak.

Biomass Equation *Quercus rotundifolia* Lam.		
$wi = \beta_0 \, dbh^{\beta 1} (i = w, b, c, r)$		
Tree Component (wi)	β_0	$\beta 1$
Stem (ww)	0.164185	2.011002
Bark (wb)	0.600169	1.3559597
Crown (wc)	1.909152	1.200354
Above-ground (wa)	*wa =ww+wb+wc*	

The parameters above were used to calculated the absolute density measures (Philip, 1994; Kershaw, 2016; Gonçalves, 2017a): the **number of trees per hectare** (N, trees/ha); The **basal area per hectare**, (G, m^2/ha); the **above-ground biomass per hectare** (Wa, kg/ha) and the **mean quadratic diameter** (*dg*, cm) defined as the diameter corresponding to the tree with the average basal area**; the above-ground carbon** (AGC) per hectare (AGC, ton/ha) was calculated as 50% of the above-ground biomass per hectare (Cotillas *et al.,* 2016).

2.3.2 Development of a Structure Index for holm oak stands

The structure analysis is a relevant tool to diagnose the current stage of the populations in the *montado* and to have an holistic approach for developing a *montado* management unit. Developing objective measures to study structure and tree size diversity independent of the age are important tools to develop silvicultural management plans that integrate ecological and economical values in stands with an unknown past.

Old growth forests often have complex disturbance histories that can result in stands with widely varying structure and the use of chronosequence can be problematic because identifying the time since last stand-replacing disturbance often cannot be determined (Lorimer *et al*., 2014). Therefore, although the importance of diagnosing structure is irrefutable, the diagnosis of the structure in stands of trees with high longevity as the oaks and with unknown history can require a complex of approach and the knowledge of an experienced forest engineer. Structure is often evaluated visually or with *dbh* distributions (Johnson *et al.,* 2002), however, it requires practice and experience. Besides, the evaluation of stands such as those of this study, diameter distributions are sometimes rather difficult to interpret and the classification in even-aged or uneven-aged structure is not straightforward. Considering this difficulty, an original quantitative methodology, through an index, was developed in this research.

The structure index (STRUX, Eq. 1) is the combination of two alpha diversity indices (Jost, 2006; Lexerød *et al.*, 2006) namely Simpson (D) and Weaver (H) indices and the coefficient of variation (CV), all calculated for *dbh*.

$$\text{STRUCTURE INDEX } (STRUX) = (1 - D) * 10 + H * 10 + CV \text{ (Eq. 1)}$$

The Simpson index, D (Simpson, 1949) is based on the probability that any two trees, drawn at random from an infinitely large population, belong to the same size class. The Simpson index is inversely proportional to diversity and it is bounded between 0 and 1 (Eq. 2 where $N'$is the total number of trees and N'_i is the number of trees for each *dbh* class.). The Simpson Index is close to 0 when variation is high and close to 1 when variation is low.

$$D = \sum_{i=1}^{N} \left(\frac{N'_i(N'_i - 1)}{N'(N' - 1)} \right) \text{ (Eq. 2)}$$

The use of this index was standardized using **1-D** that corresponds to Gini-Simpson Index, so that the increase of the values of all the indices used correspond to the increase of variability (Jost, 2006; Lexerød, 2006).

The Shannon-Weaver index, H (Shannon and Weaver, 1949) is based on the probability that an individual chosen randomly from an infinitely large community belongs to a certain species. Shannon and Weaver index is proportional to diversity (Eq. 3, where p_i corresponds to the $p_i = \frac{N'_i}{N'}$ and it mainly varies between 1,5 and 3,5 (Gonçalves, 2018b)

$$H = -\sum_{i=1}^{k} p_i \times \ln pi \text{ (Eq. 3)}$$

Coefficient of Variation, CV is a statistical measure of the dispersion of data points around the mean.

$$\boldsymbol{CV = \frac{sd}{\bar{x}} \times 100}$$

The three parameters of the STRUX were chosen because they quantify the variability of the diameter and thus of the structure in distinct ways. The Shannon-Weaver index (H) is more sensitive to rarer species/sizes and the Simpson index responds more to abundant species (Hakkenberg *et al.,* 2016). The Simpson index was standardized using (1-D), so that the increase of the values of both indices correspond to the increase of variability (Lexerød, 2006). Also, (1-D) and H were multiplied by 10 so that they had a similar range to that of CV. Classes of 2.5 cm for diameter at breast height were used. The *CV* is an interesting parameter to use in this study because it allows the comparison free from scale effects (Brown, 1998).

The threshold between even-aged and uneven-aged structures was set at 20. The results of the application of the structure index and the visual analysis of *dbh* distribution had a high level of agreement of 100%, for the plots of this study.

$$\underline{\boldsymbol{Even-aged\ stand}}$$

$$Structure\ Index\ \leq\ 20$$

$$\underline{\boldsymbol{Uneven-aged\ stand}}$$

$$Structure\ Index\ >\ 20$$

The inclusion of the three variables in the index calculated for total height in 1 m classes was also tested. However, the discrimination between the two structure classes resulted in an overlapping of the two classes. This might be due to pruning practices in holm oak trees.

2.3.3 Soil and litter layer laboratory work.

The dry litter layer was separated from coarse elements through a 1mm sieve, and the fine elements were disposed of. The litter layer was placed in an oven for 48 hours at 60ºC to attain its dry weight. Then the samples were weighed. All the soil laboratory work was done between the 15 of June and 30 of June of 2020.

The soil laboratory procedure was done as follows:

1 - After air drying the soil sample, the entire sample was weighted.

2- The separation of the coarse fraction (> 2mm) from the fine fraction (<2 mm) was done: Using a stone mortar the clay aggregates were broken. After this, the sample was sieved through the 2 mm sieve. The material that passed through the sieve (fine fraction) was collected in a clean jar and identified with the respective sample reference label and stored. The material that stayed in the sieve was also collected and corresponds to the coarse fraction. The coarse fraction was washed directly on the 2 mm sieve, under the tap, until no cloudy water came out. Then it was transferred to a glass cup and placed in the oven at 105ºC overnight, removed from the oven, and weighed.

3- Preparation of the soil sample for pH tests and organic carbon laboratory tests: It was done a subsample of 50 gr of the fine fraction of the soil, which was labeled and kept in plastic bags.

4 - Laboratory pH tests: From the 50 g bags with the fine fraction soil sample, 10g of soil were weighted and were mixed with 25 ml of distilled water for 1 hour. This solution was mixed 4 times in 15 minutes intervals during that hour. The soil pH in water was measured with pH Meter GLP 22. The same procedure was used to measure the pH in a KCl solution (1N).

5 - Soil organic carbon (SOC) is commonly used as a measure for organic matter in soils. To determine the organic carbon values, 0.35 g of the fine fraction of the soil (<2 mm) were weighed, incinerated at 1300 °C and the CO_2 released was measured in an elementary analyzer.

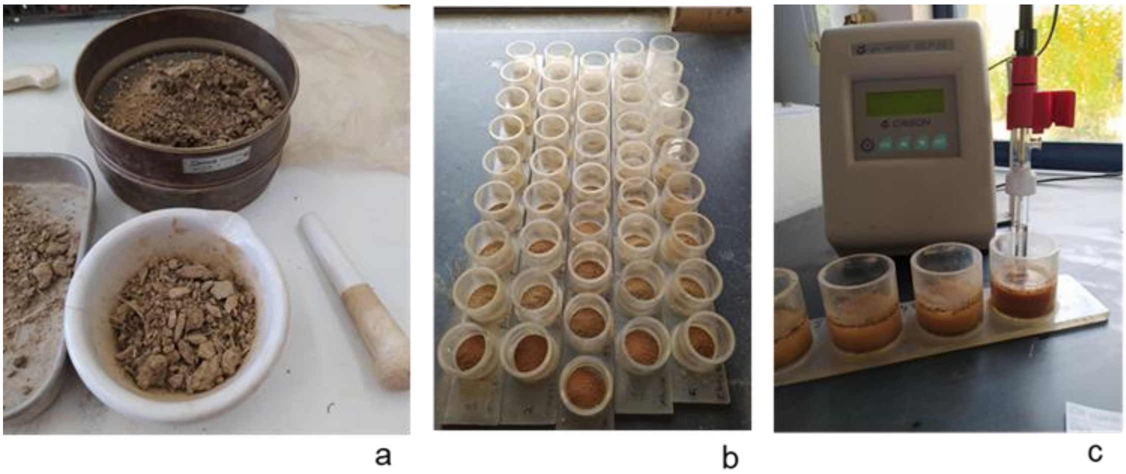


Figure 2. 12 Soil samples being prepared for laboratory analysis. a) Stone mortar and 2mm sieve, b) Fine Fraction Samples organized by plots, c) Measuring the pH of the soil (Credit of the author).

2.3.4 Data processing and database analysis

The data were analyzed at two levels: the class of crown cover and plot. The former enables the evaluation of the variability in each crown cover class and between the different crown cover classes, that is by *strata*. The latter enables further detail of the variability in each plot and between plots. For each level, the descriptive statistics were calculated, including histograms, kurtosis and skewness. The test of *Kolmogorov-Smirnov (KS)* was performed to check the normality of the distribution and Levene's test was used to test the homogeneity of variance of the data distribution for every parameter (Figure 2. 13) (Afonso and Nunes, 2019).

To find out if there were significant differences in the studied variables grouped by CC Classes, the statistical analysis was carried on through parametric and non-parametric tests. If the normality and homogeneity of variance assumptions were not violated, the parametric tests were used: ANOVA and Pearson Correlation were used (Afonso and Nunes, 2019). In the case of parametric test ANOVA, it is often desirable to know more about the specific groups to find out if they are significantly different or similar, so a post-hoc analysis was needed. The method of post-hoc analysis used was the Tukey's Test. If the normality assumption was violated but the distribution kept the homogeneity assumption, non-parametric tests were used: Kruskal-Wallis (*KS*); Mann-Whitney U test (*MW* also known as Wilcoxon rank-sum) and Jonckheere-Terpstra test (Figure 2.15). The most common distribution-free hypothesis tests used are the Kruskal-Wallis and the Mann-Whitney U ones. In all these tests the null hypothesis was that all *k* populations sampled were identical, or equivalently, meaning that all the samples were originated from the same population (Glover and Mitchell, 2008). A 95% confidence level was used to accept or reject the null hypothesis of the test. When studying the plots, the Shapiro-Wilk (SW) test was used to check if normality was assumed since the samples were small (less than 30 trees per plot) (Afonso and Nunes, 2019).

The data were analyzed using IBM SPSS Statistics, version 21.0 (IBM, 2016) IBM SPSS v 21.0, RStudio Team (R Core Team, 2020).

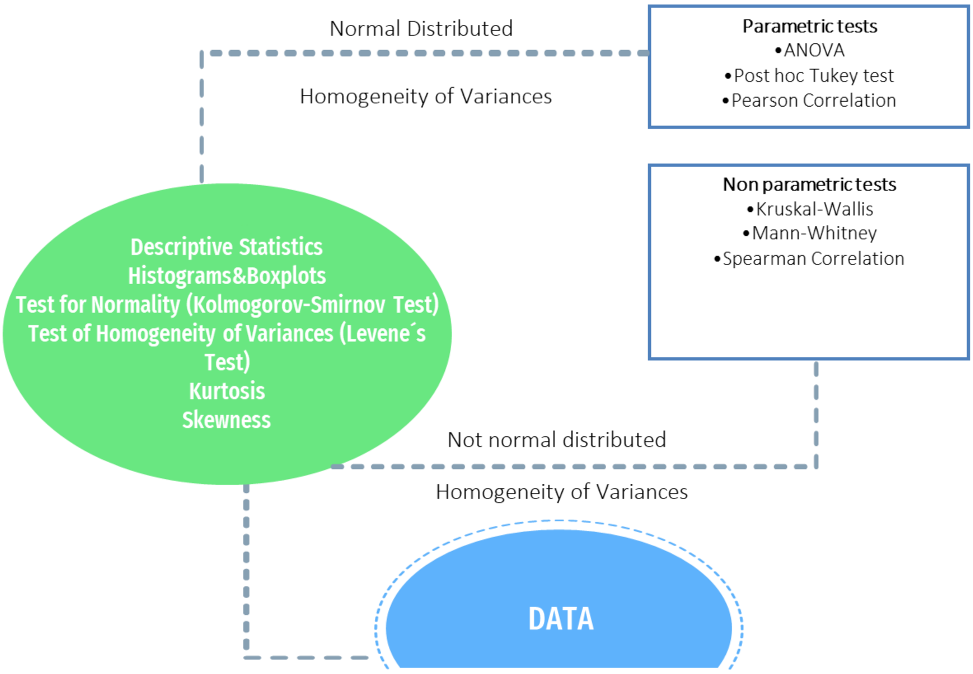


Figure 2. 13 Schematic organization of the Statistics Methodology on this study

CHAPTER 3 - RESULTS

Understanding the structure is essential for deciding on silvicultural practices and for estimating the yield of different products and services existing on a stand. The population in a study can be characterized by a set of parameters, the descriptive statistics. When exploring the stand structure, there can be used vertical and horizontal parameters, the most used is *dbh* (cm) as a horizontal factor and *h* (m) as a vertical factor. The *hd* ratio, the mean crown ratio (*mrc*, m), the crown length (*lc*, m), and the linear crown diameter (*lcr)* were also used as parameters to describe the structure. The absolute density measures applied were the number of trees per hectare (N, trees/ha), the basal area per hectare (G, m^2/ha) the above-ground biomass per hectare (WA, kg/ha), and mean quadratic diameter (*dg*, cm). First, when analyzing structure, a tree level analysis grouped by Crown Cover Class was done, which means that all trees in each CC Class were considered together. Secondly, an analysis at the plot level was carried out. The STRUX Index was used as a method to evaluate structure at a plot level.

The soil parameters, litter layer (LL, kg/m^2), soil organic carbon (SOC, %), and pH in H_2O and KCL were firstly studied by CC Class and then studied grouped by the Absence (-) or Presence (+) of Natural Regeneration.

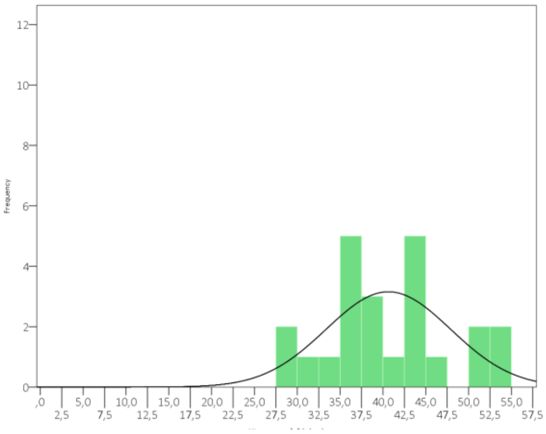
The natural regeneration results were presented by CC Class, including first the results of the NR Classes and then the results of the random transect sampling method and NR Classification.

3.1 Analysis per Crown Cover Class Strata

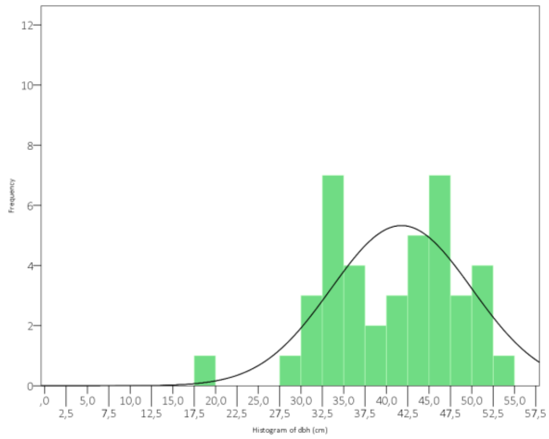
3.1.1 The diameter distribution analysis

The distribution of diameters is a parameter used to analyze the horizontal age structure of the stand. An even-aged stand is represented by a Normal Gaussian Distribution and in an uneven-aged stand, the diameters distribution follows a non-parametric distribution (Kershaw, 2016). Conforming to the *Kolmogorov-Smirnov* (KS) test results CC Class 1 (KS p=0.2), 2 (KS p=0.2), 3(KS p=0.27) follow a Gaussian Distribution, suggesting these CC Classes are represented by even-aged populations. The CC Class 4 (KS p=0.01) does not follow a Gaussian Distribution in *dbh*. So, considering the *dbh* distribution and the results of the normality test for all Classes, there is an indication that CC Class 1, Class 2, and Class 3 behave more closely as even-aged stands and CC Class 4 as uneven-aged structure.

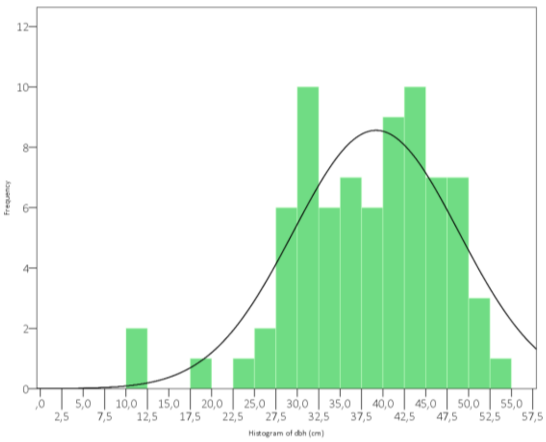
According to Kershaw *et al.* (2016) and Plieninger *et al.* (2003), older holm oaks stands show more accentuated platykurtic diameter distributions. CC Class 1 (k=-0.486) and 2 (k=-0.195), show platykurtic diameter distributions (Figure 3. 1), which means the distribution is less peaked than the normal distribution, it is flatter, so there are fewer individuals in the central size classes when compared to a standard gaussian distribution and also thinner tails are present. Both CC Class 4 (k=1.503) and 3 (k= 0.430) *dbh* distributions have a positive kurtosis that means that the distributions are leptokurtic, showing heavier tails than the normal distribution (Figure 3. 1). To understand the tails of these distributions the skewness was studied. The skewness values *dbh* distributions were negative for CC Class 2 (s=-0.285), 3 (s=-0.3910), 4 (s=-0.622)] which means that these *dbh* distributions have the left side tale longer, which corresponds to the lower size of classes. When considering the marginal size classes, or the tales, the asymmetry identified by the skewness values in the left tail of the distributions can suggest a future cohort differentiation (Figure 3. 1). The CC Class 1 *dbh* distribution skewness is an exception in this study, it showed a positive skewness value CC Class 1 (s=0.2409), which means the data are skewed right, the tale correspondent to the bigger size classes is longer, the existence of trees with high diameters is influencing the distribution. It is also relevant to analyze the continuity of the distribution in *dbh* size classes. Discontinuity of the distributions represented by sudden drops or peeks in the frequency of size classes, suggests the possible existence of differentiated cohorts. To study the cohort differentiation, it is significant to look at the modal tendencies of the distribution. There can be patterns of cohorts differentiation indicated by multimodal distributions of the *dbh* and by suddenly decreases or gaps in frequencies of size classes, as showed between size class [37.5-40cm] in Class 2 and in the drop represented by the size classes [32.5-35;37.5-40] between the bimodal classes in Class 3 (**Figure 3. 1**). Nevertheless, to be able to do a more detailed analysis of the stand dynamics it is necessary to look at structure in a sublevel, in the plots.



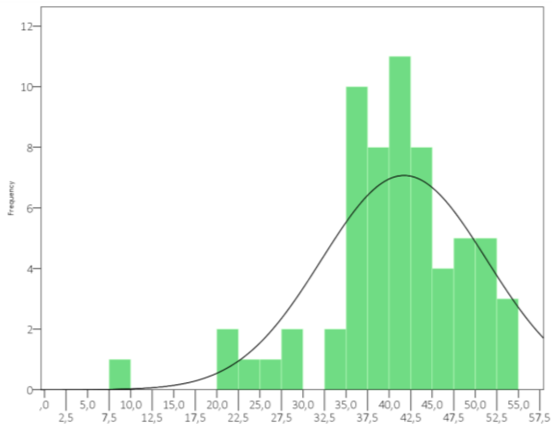
a) CC Class 1



b) CC Class 2



c) CC Class 3



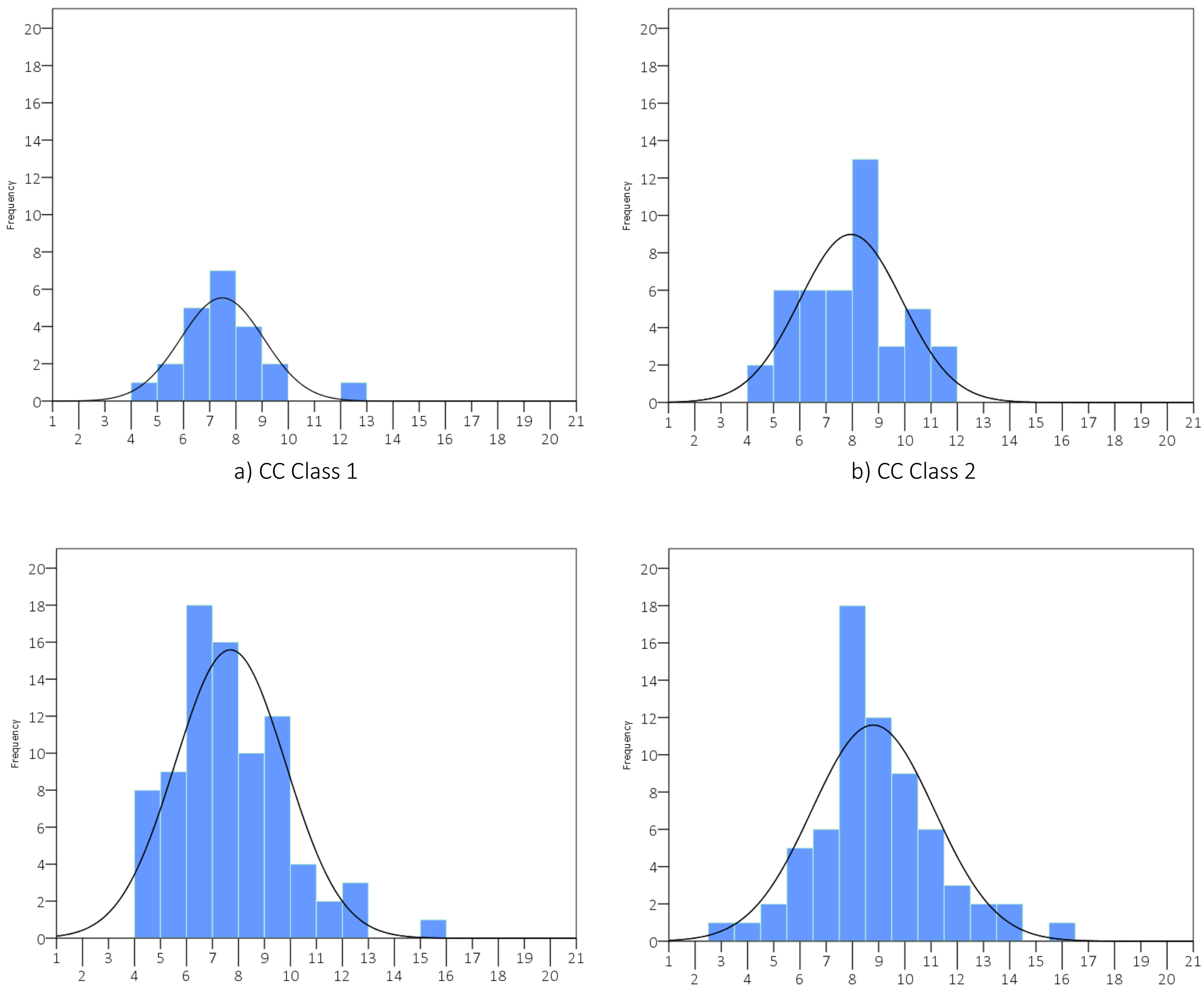
d) CC Class 4

Figure 3. 1 Histogram *dbh* (cm) Distribution for all CC Classes

As the data did not met the criteria of normality and showed homogeneity of variances (Levene's test for *dbh* p=0.18), the Kruskal-Wallis test was used to test if there were significant differences between the *dbh* distribution by CC Class and, it was observed that there were no significant statistical differences (all, $p < 0.05$).

3.1.2 The height distribution analysis

CC Class 1 (*KS* p=0.04) was not normally distributed and CC Class 2, 3 and 4 (*KS* p=0.2) were. All Classes showed a positive skewness in the *h* Distribution, represented by a long tail in the right side of the distribution, which means that despite the high concentration of data in the central size values, individuals with higher height values are influencing the distribution (**Figure 3. 2**).



a) CC Class 1

b) CC Class 2

d) CC Class 4

c) CC Class 3

Figure 3. 2 a) Total Height (h, m) Distribution for Class 1

Because not all the data was normally distributed but showed homogeneity of variances (Levene's test for *h* p=0.223), the Kruskal-Wallis and Mann-Whitney tests were applied to test if there were statistical differences between *h* distribution of CC Classes. According to the Kruskal-Wallis test result, there were significant statistical differences between CC Classes (p=0.02). To test where the differences were the Mann-Whitney test was used to compare pair CC Classes. There were significant statistical differences in *h* distribution between CC Class 1 vs Class 4 (p= 0.003) and Class 3 vs Class 4 (p=0.001).

3.1.3 The *dbh* and *h* distributions grouped by CC Class.

There were significant differences in *h* distribution between CC Classes and no significant differences in the *dbh*. To analyze the *h* variation according to the *dbh* a linear graph was produced (Figure 3. 2). There were accentuated variations in *h,* with a range of 10 meters, in the individuals with a *dbh* value between 34 cm and 50 cm (Figure 3. 2).

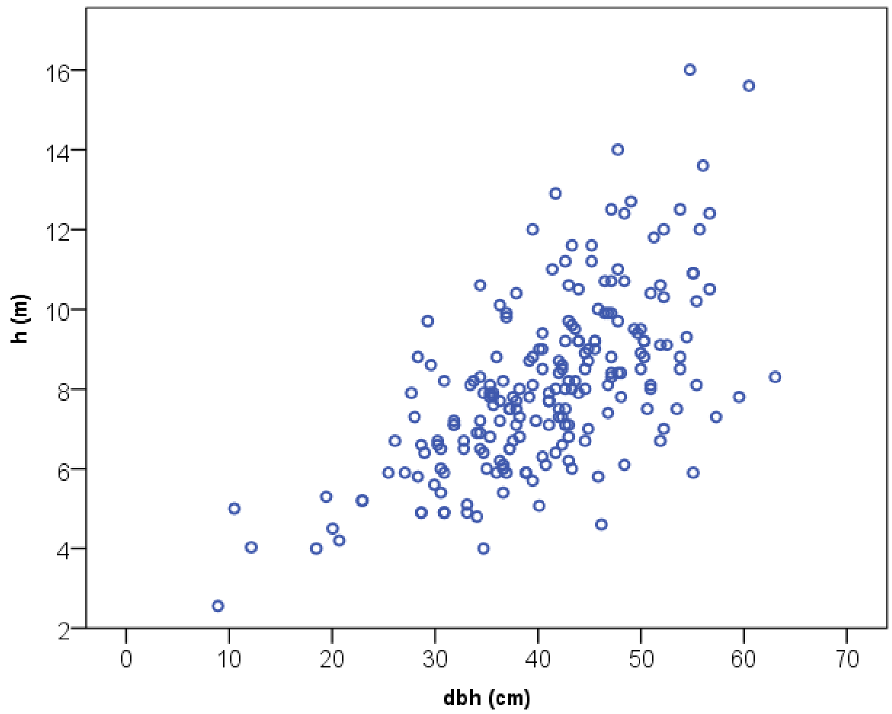


Figure 3. 2 Linear Graph with *dbh* and *h* for the whole sample of trees.

To further study this relation between *dbh* and the *h* variation a linear graph with *dbh* and *h* was created. It was grouped by CC Class and it was verified that CC Class 4 had the maximum *h* peak values while CC Class 2 had the minimum *h* peak values (Figure 3. 3).

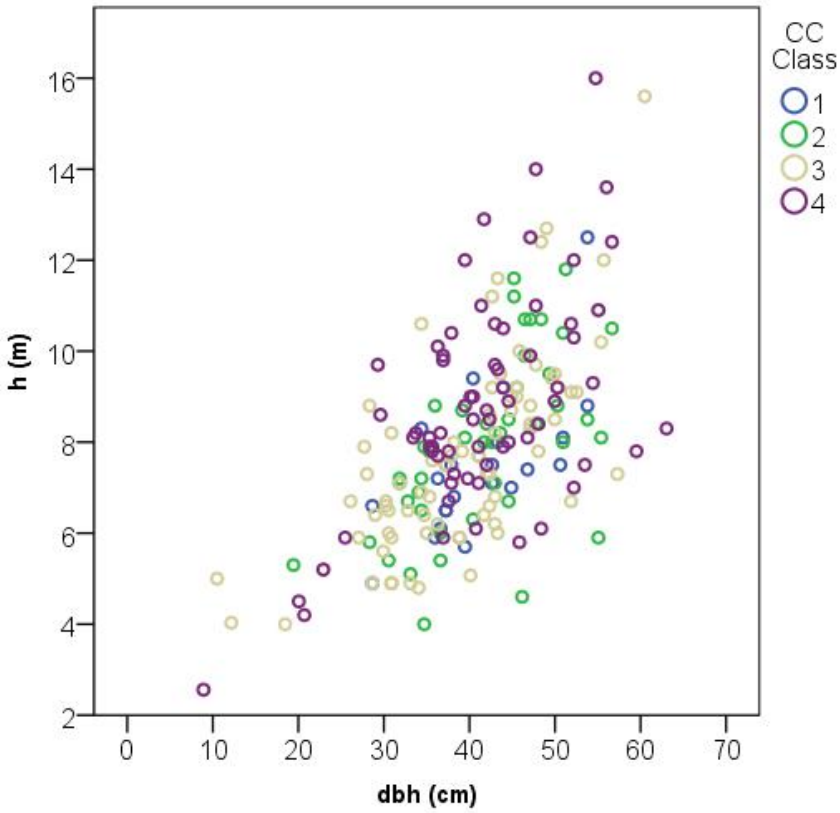


Figure 3. 3 Linear Graph of *dbh* and *h* for the whole sample of trees grouped by CC Class.

3.1.4 The Tree Biomass grouped by CC Class.

The analysis of the tree biomass according to CC Classes gaves us vital information concerning the sequestration of above-ground forest biomass in different crown cover environments. In the histograms (**Figure 3. 4**) in CC Class, 1 the smallest above-ground biomass (*wa*) class (0-200 kg/tree) is absent while those with the highest frequencies are between 400 and 600 kg/tree. The *wa* class of 400-600 kg/tree is also the most common in CC Class 3 and 4. In CC Class 2 the modal *wa* class is the one ranging from 600-700 kg/tree. The *wa* was normally distributed (*KS* CC Class 1 p=0.2, *KS* 2 p=0.17, *KS* 3 p=0.2, *KS* 4 p=0.18) and showed homogeneity (Levene's test p= 0,417) in all CC Classes. There were no significant differences between CC Classes means (ANOVA p= 0.3).

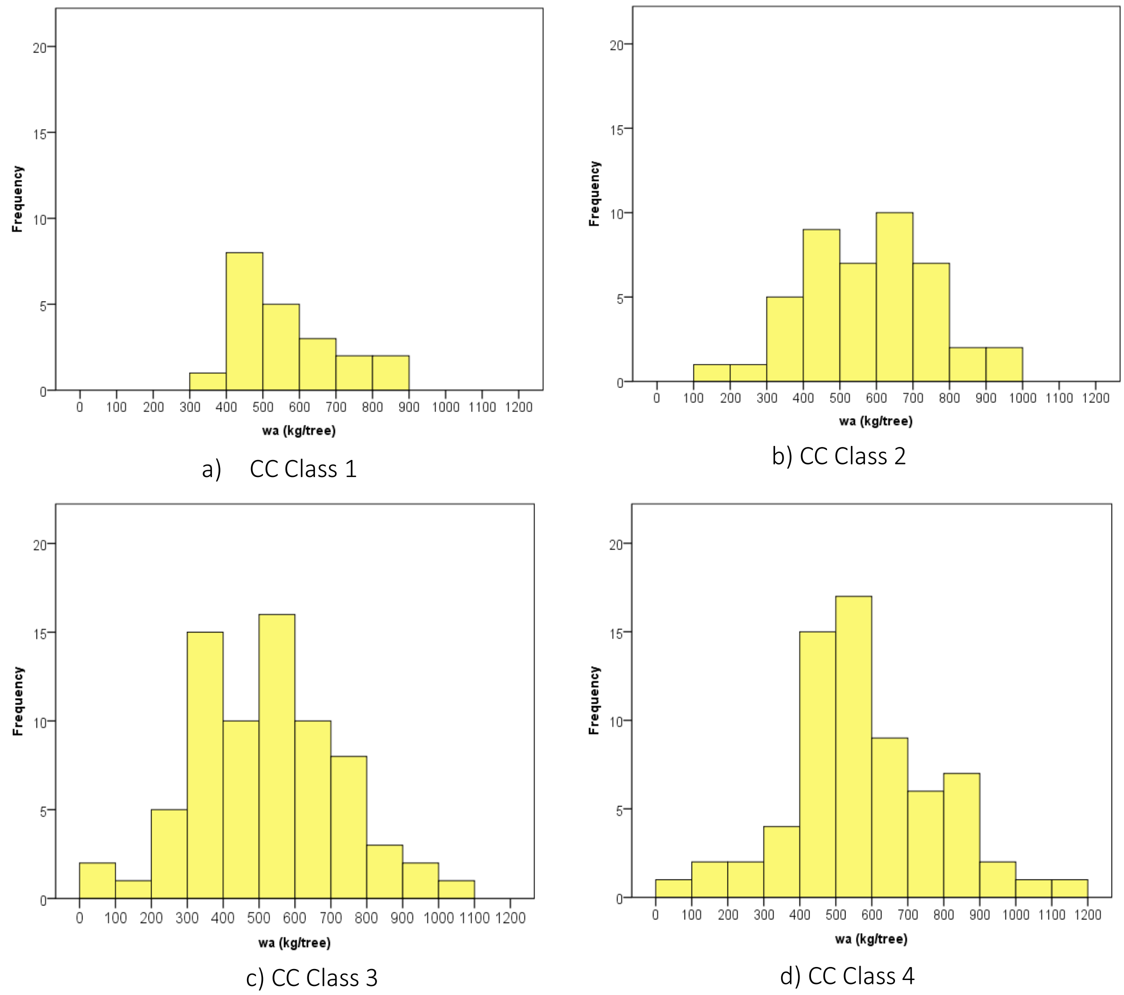


Figure 3. 4 Histogram of Above Ground Biomass (*wa*, kg/tree) for all CC Classes.

3.1.5 The *hd* ratio

The higher the competition the higher the *hd,* young trees can also have higher *hd* values (Gonçalves, 2018a; Saha *et al.,* 2014; Rinn, 2015). All trees in this study had *hd* ratio < 45 indicating free growth and tree stability except for one tree, which corresponds to a young individual with a *dbh*=10,5 cm and *h*=5,00 m (Figure 3. 5, represented with *). CC Class 2 and CC Class 4 were normally distributed (*KS 2 p*=0.85 and *KS* 4 *p*=0.95). For CC Class 1 and 3 *hd* did not meet the normality criteria (KS 1 *p*=0.01 and *KS* 3 *p*=0.0. The data showed homogeneity of variance (Levene's test p=0.597).

When testing for differences in *hd* distribution with the Mann-Whitney test according to CC Classes, there was a significant difference between Class 1 and Class 4 (*p*=0.005). The CC Class 1 *hd* ratio mean was 18.6 and CC Class 4 was 21.5 and analyzing Figure 3. 5 it is possible to see that there is a higher variability in the *hd* ratio values in CC Class 4 (*sd*= 5.14) than in CC Class 1 (*sd*= 3.29). CC Class 3 and CC Class 4 have trees with smaller *dbh* and with higher *hd* ratio values (Figure 3. 6). CC Class 3 and CC Class 4 showed higher values of *hd* ratio when compared with CC Class 1 and CC Class 2 for the same *dbh*.

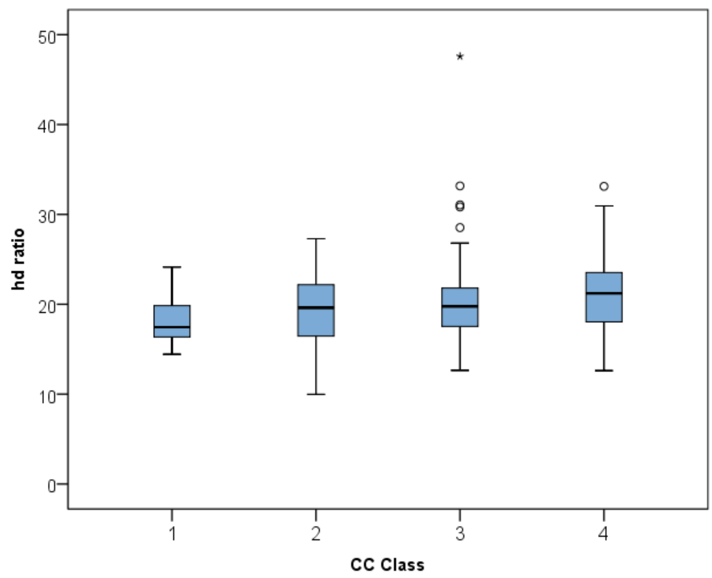


Figure 3. 5 Boxplot for the *hd* ratio for all four CC Classes.

When analyzing *hd ratio* in **Figure 3. 6** it was evident that CC Class 4 had the maximum peak values when compared with the other CC Classes in the same *dbh* values and that CC Class 2 had the minimum values.

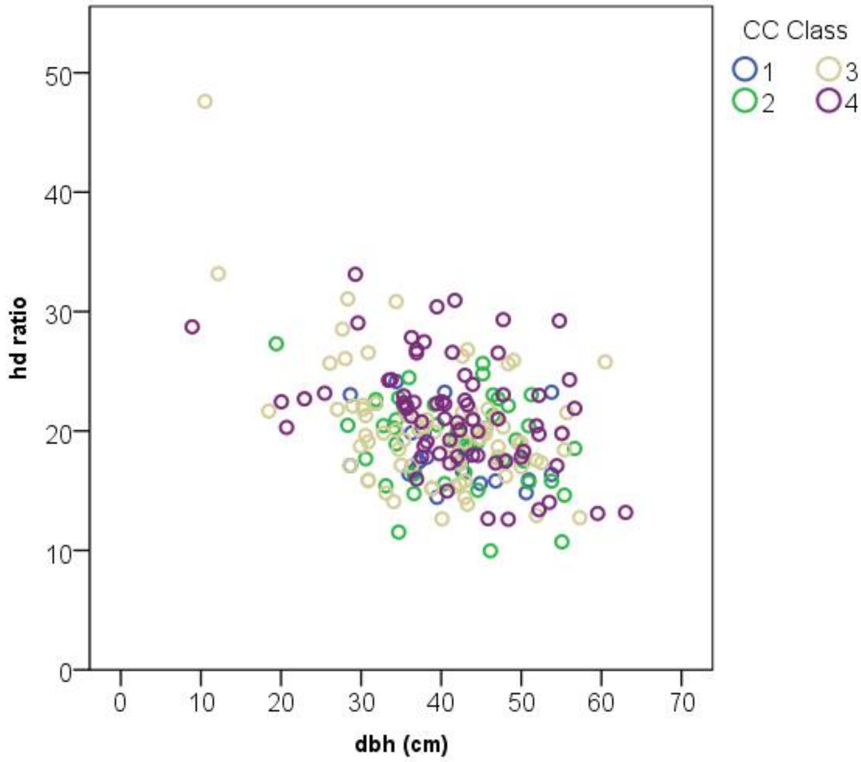


Figure 3. 6 Linear Graph with *hd* ratio and *dbh* for the whole sample of trees grouped by CC Class.

3.1.6 The Crown Parameters

In this section the crown parameters were studied namely the mean crown **radius** (*mrc*), crown length (*lc*), crown ratio (*cr*), the linear crown diameter (*lcr*).

Analyzing the mean crown radius (*mrc*, m) of the trees in each CC Class, it is possible to see that in CC Class 4 variability is higher than in the other ones (**Figure 3. 7**). Noteworthy is that CC Class 4 and Class 3 have high variability in *mrc* but no significant differences were found between CC Classes (all CC Classes KS > 0,05, Levene's test *p*=0,8 and One way ANOVA test *p*=0,36).

The crown length *lc* distribution in CC Class 1,3,4 did not follow a gaussian distribution (*KS* 1 *p*=0.001, *KS* 2 *p*=0.002, *KS* 3 *p*=0.04) but *lc* had homogeneity of variance (Levene's test *p*=0.07). There were not statistically significant differences between all CC Classes for *lc* (Kruskal-Wallis *p*=0.075). Analyzing the *lc* boxplot (**Figure 3.8**)

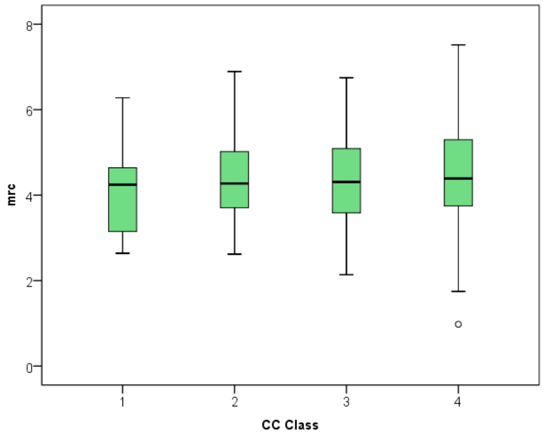


Figure 3. 7 Boxplot for *mrc* per CC Class.

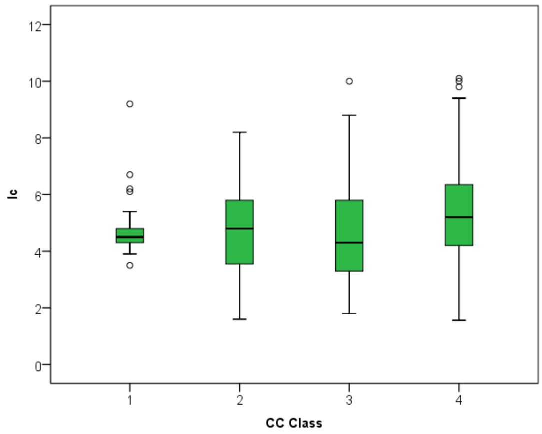


Figure 3. 8 Boxplot for *lc* per CC Class.

Most individual trees present in the population show a well-balanced crown ratio because its *cr* value is higher than 30%, even higher than 50% (Gonçalves, 2018b). Only two trees had a cr < 30%. There was no statistically significant difference between CC classes when considering the crown ratio (**Figure 3. 9**, *KS* CC Class 1 *p*=0.6, *KS* 2 *p*=0.0, *KS* 3 *p*=0.24, *KS* 4 *p*=0.23, Levene's test *p*=0.65, KW *p*=0.24).

The *lcr* did not follow a gaussian distribution in all CC Classes (*KS* CC Class 1 *p*= 0,82, *KS* 2 *p*=0.87, *KS* 3 *p*=0.0, *KS* 4 *p*=0.11) but had homogeneity of variances (Levene's test *p*=0.07). There was a statistically significant difference between CC Class 1 and CC Class 4 detected with Mann-Whitney test (*p*= 0.012). The CC Class 4 has a *lcr* mean equal to 21.87, variance of 9.99 and minimum of 14.47, maximum of 32.00. The CC Class 1 has a mean of 19.94, variance of 10,4, minimum of 15,04 and maximum of 27.27. So there is more variance in CC Class 4 than in 1, that can be an indicator of the presence of different cohorts in CC Class 4 (**Figure 3. 10**).

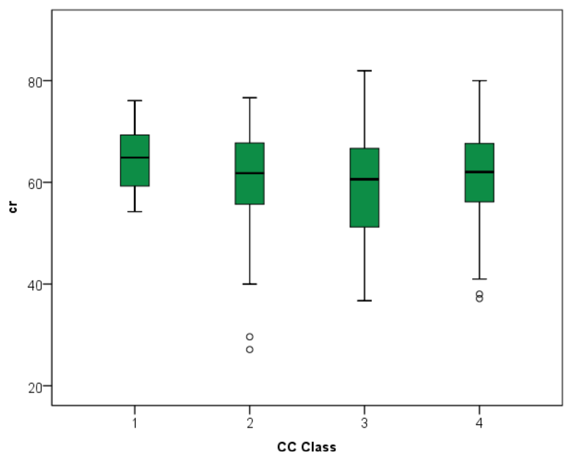


Figure 3. 9 Boxplot for cr for all CC Classes

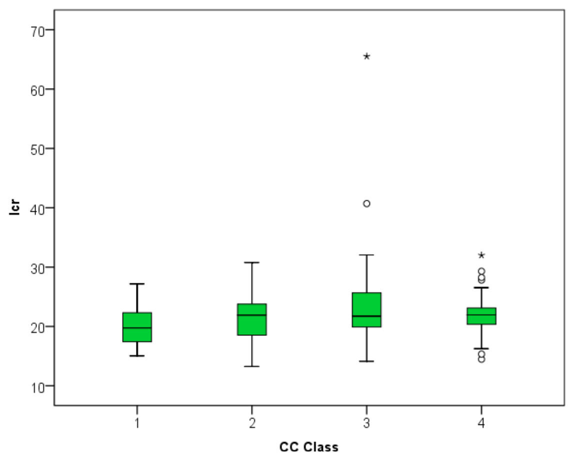


Figure 3. 10 Boxplot for lcr for all CC Classes

The *lcr* (**Figure 3. 10**) depends mainly on the aerial growing space. To test if there was any correlation between CC Class and the *lcr* (*p*=0.03) a Spearman Correlation was done. What we found out was that there is a positive correlation between CC Class and *lcr* (Spearman *rho*=0.12, *p*=0.04) , although it is positive, it is weak. So the *lcr* is slightly increasing with the increasing of CC Class, this result turned out to be the opposite of the expected hypothesis that the *lcr* decreases as competition and canopy closure increases. This increase in the lcr can be both caused by a decrease in the *dbh* or an increase in the *mrc.* This result can also be caused by different intensities of pruning in the past and/or by ecological relationships established between the individual tree of each plot. This can also be an indicator that stand structure is different between Crown Cover classes. In **Figure 3. 11** it is possible to detect that the *lcr* a tendency to be higher in CC Class 4 and Class 3 than in CC Class 1 and CC Class 2 between the 20 and 40 cm of *dbh,* so it is not only the presence of younger trees in CC Class 3 and 4 that is influencing the *lcr* tendency to be higher in this CC Classes.

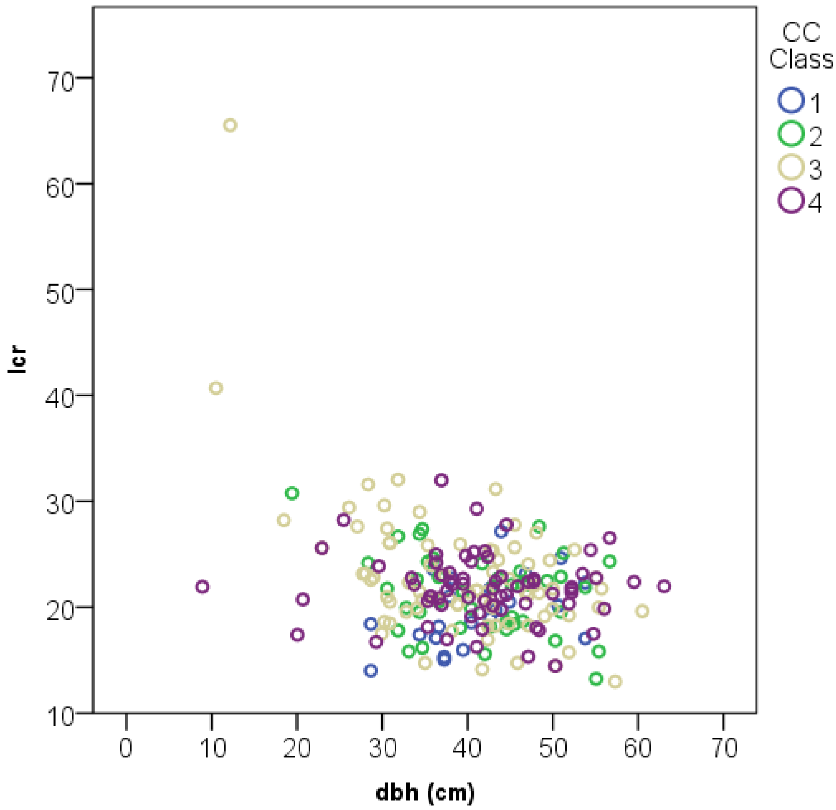


Figure 3. 11 Mean *lcr* and *dbh* per by CC Class

3.3 The Absolute density measures analysis by CC Class

Analyzing the absolute density measures per CC Class it was observed an increase of *N, G* and *Wa* from CC Class 1 to 3 and a stabilization from CC Class 3 to 4 were observed (Table 3. 1, Figure 3. 12, Figure 3. 14, Figure 3. 15). The *dg* suffered less variation when analyzed per CC Class (Figure 3. 13).

Table 3. 1 G, N and Wa mean per crown cover class

CC Class	*G* (m^2/ha)	*N* (trees/ha)	*Wa* (ton/ha)	*AGC (ton/ha)*	*dg* (cm)
1	1.9	15	7.6	3.8	41.9
2	3.8	26	15.4	7.7	41.4
3	5.6	43	22.7	11.35	40.6
4	5.6	38	22.4	11.2	42.1

The *Wa, G* and *N* were normal distributed in all CC Classes (*KS* p>0.05) and homogeneity of variance criteria was met (Levene's test p> 0.05).

There was a very strong correlation between variables *Wa* and *G* with a Pearson's correlation (r=0.998) and with *Wa* and *N* (r=0,942)

When testing if there were significant differences between Classes in these two variables with an One-Way ANOVA test (Table 3. 2) it was verified that there was significant statistically differences between CC classes for *G, N, Wa* and *AGC* (all, p=0.0) and but not for mean quadratic diameter (*dg*, p=0.4). The fact that there was no significant statistically differences in the mean quadratic diameter (*dg*) between CC Classes, indicate that is not the diameters of trees that are responsible for the differences in the biomass per hectare between CC Classes. The tree with average basal area had similar values for all CC Classes

A Post hoc - Tukey HSD was done and it showed that there were statistically differences in *G, N,Wa*, and *AGC* between CC Classes 1 and 3, 1 and 4, 2 and 3 and 4 and 2 (Table 3. 3). There was a significant statistical difference between consecutive CC Classes 2 and 3, and no significant difference between other consecutive CC classes. It is possible that this result indicates that 30% Crown Cover can be a threshold value for a higher *Wa N, G* per hectare and an indicative value for a relevant increase in forest biomass in the *montado* because the increase in *Wa* is significant when the 30% Crown Cover threshold is achieved (Figure 3. 12). The AGC ditributions matched the Wa distributions, as it was expected because the AGC is dependent on the Wa (Appendix V, Appendix VII).

Table 3. 2 ANOVA between CC Classes for parameters G, N, *dg* and Wa

ANOVA	F	Sig.
G	10.28	0.00
N	15.7	0.00
dg	1.0	0.40
Wa	11.2	0.00
AGC	11.1	0.00

Table 3. 3 Multiple Comparisons ANOVA Post hoc - Tukey HSD

CC Classes	2	3	4	2	3	4
	G			*dg*		
1	0.178	0.000	0.001	0.499	0.499	0.499
2	-	0.035	0.115	-	1.000	1.000
3	-	-	0.948	-	-	1.000
4	-	-	-	-	-	-
	N			*Wa and AGC*		
1	0.136	0.000	0.000	0.162	0.000	0.000
2	-	0.001	0.070	-	0.021	0.103
3	-	-	0.397	-	-	0.900

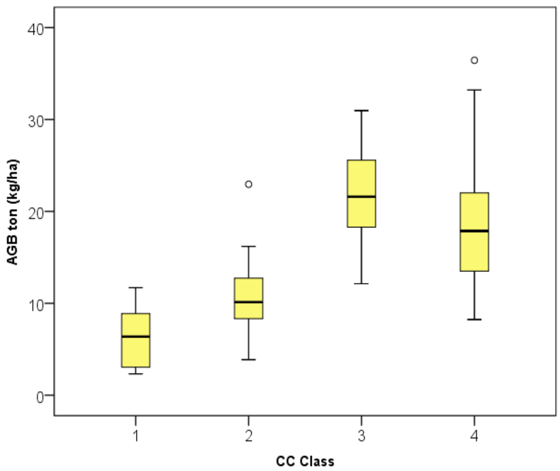


Figure 3. 12 Boxplot of the Above Ground Biomass per hectare (Wa) grouped by CC Class

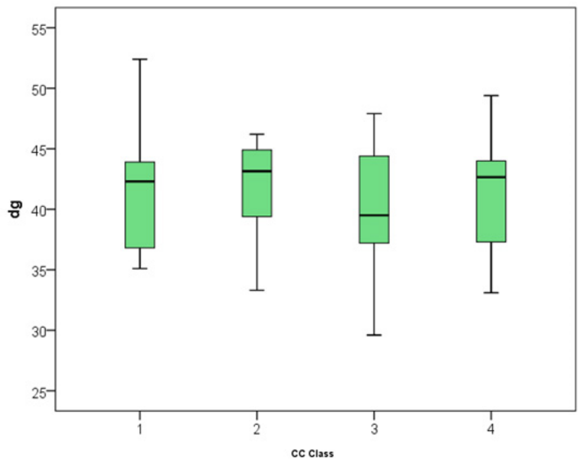


Figure 3. 13 Boxplot of the mean quadratic diameter grouped by CC Class

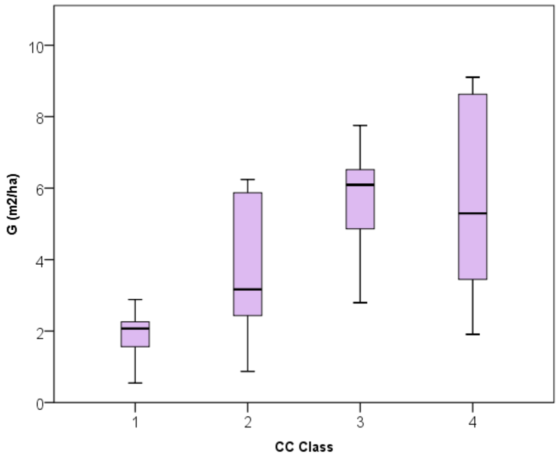


Figure 3. 14 Boxplot of Basal Area per hectare grouped by CC Class

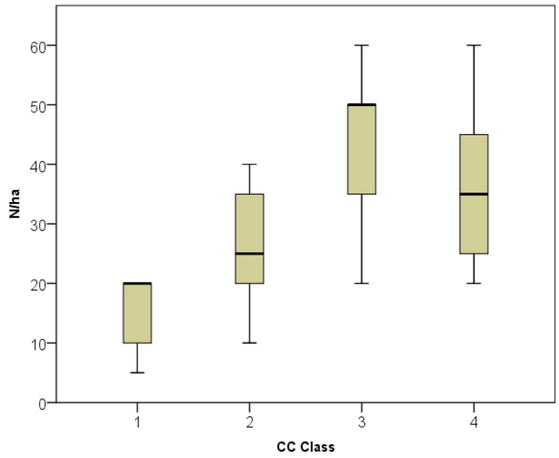


Figure 3. 15 Boxplot of Number of trees per hectare grouped by CC Class

3.4 STRUX Index - Plot Analysis

The STRUX index was an innovation created in this research to evaluate the age structure of the plots. In this part of the study the data is grouped by CC Class and by plots. Evaluating the stand structure is an important step to understand the landscape dynamics of the property. The spatial heterogeneity of the *montado* promotes a variety of ecological niches (Lauw *et al.,* 2013). Even-aged and low density *montado* stands are associated with agriculture uses and direct services such as grazing or intercropping. Uneven-aged structure higher density stands can support other ecosystem services like habitat for forest species, higher biomass per hectare, soil conservation and a diversity of forage wild food like mushrooms (Pereira *et al*., 2009).

3.4.1 CC Class 1 Plots

Analyzing the plots structure in CC Class 1, makes it possible to detect that P1C1 and P6C1 have a higher *dbh* variation when compared with the other Class 1 plots (Figure 3. 16) that have more concentrated value distribution around the mean, both in *dbh* and *h* distributions (Figure 3. 17). The boxplot (Figure 3. 16) for *dbh* distribution of CC Class 1 plots, there are two plots that show higher variability than the rest, P1C1 (min *dbh*=28.65cm, max *dbh*= 53.79cm) and P6C1 (min *dbh*=38.20, max *dbh*=50.61). P1C1 also shows higher variability in *h* distribution (min h=5.7, max h=12.50, Figure 3. 17). The *dbh* and *h* did not meet the normality criteria and (Normality test SW *dbh* and *h p* > 0.05 to all plots with more than one tree, Levene's test *dbh p*=0.4 *h p*=0.1). There were not statistically significant differences between CC Class 1 plots in *dbh* and in *h* distribution (*KW dbh* p=0.13, *h p*=0.54)

The STRUX index for the *dbh* was applied to each plot so that an evaluation of the plot structure could be executed. In the plots where there was only one individual the index was not calculated (P4C1, P7C1, P10C1 and P11C1) and these plots were considered even-aged plots (Table 3.4). The P1C1 and P6C1 are the only uneven-aged stands of CC Class 1 (Table 3.4, (STRUX) > 20). In CC Class 1, 80% of the plots can be considered even-aged.

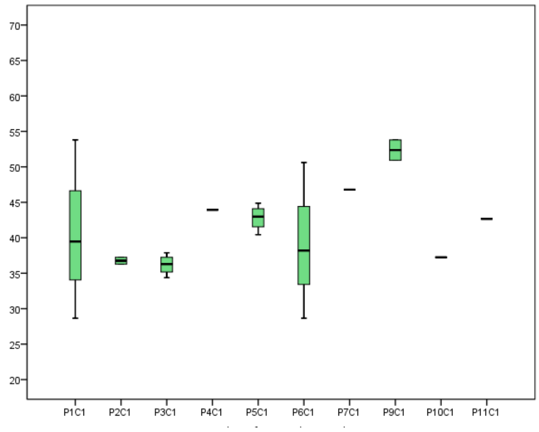


Figure 3. 16 Boxplot of *dbh* of plots from CC Class 1.

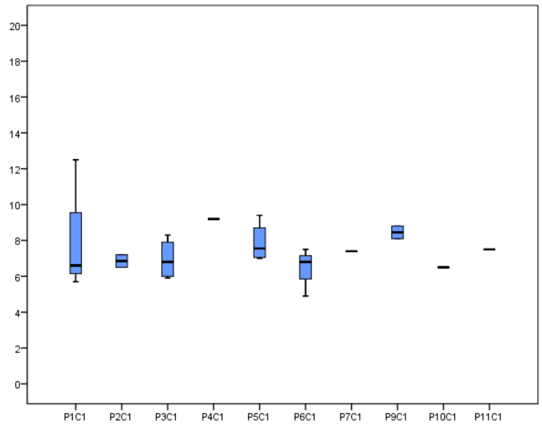


Figure 3. 17 Boxplot of *h* of plots from CC Class 1.

Table 3. 4 Structure index (STRUX) for CC Class 1 (where *D is the* Index of Simpson; *H* the Index of Shannon-Weaver, *CV* the Coefficient of Variation for *dbh*)

Plots	CC Class	D_*dbh*	H_*dbh*	*dbh*_cv%	*STRUX_dbh*
P1C1	1	0.3	1.1	17.9	36.1
P2C1	1	1.0	0.0	1.3	1.3
P3C1	1	0.3	1.0	2.0	19.0
P4C1	1	1.0	0.0	ND	ND
P5C1	1	0.6	0.6	2.1	11.7
P6C1	1	0.3	1.1	15.6	33.8
P7C1	1	1.0	0.0	ND	ND
P9C1	1	0.4	0.7	2.7	15.2
P10C1	1	1.0	0.0	ND	ND
P11C1	1	1.0	0.0	ND	ND

3.4.2 CC Class 2 Plots

The analysis of the *dbh* distribution (Figure 3. 18) showed that there is one plot that has higher variability, that is P2C2 (min *dbh*=30,56 cm, max *dbh*=53.59 cm). The plot P3C2 also behaves interesting, there are six trees, four of them have very close *dbh* values and two extreme values as case number 33 (*dbh*=28.33cm) and case number 38 (*dbh*=42.65cm). The normality criteria was not meet in all plots (Normality test SW *dbh* p > 0,05 for all except P5C2 (p=0.42) and SW *h* p > 0,05 for all except P1C2 and P5C2, Levene's test *dbh p*= 0.32, *h p*=0.13). There were no significant differences between plots of CC Class 2 in *h* (Figure 3. 19) and *dbh* distributions *KW dbh* p=0.18 and *KW h* p=0.18). Conforming to the STRUX results, P6C2 is an even-age plot, and all the other stands behave as uneven-aged plots (Table 3. 5).

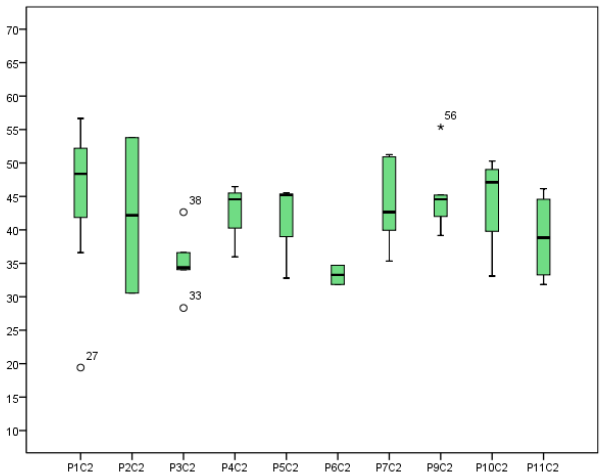


Figure 3. 18 Boxplot of *dbh* of plots from CC Class 2.

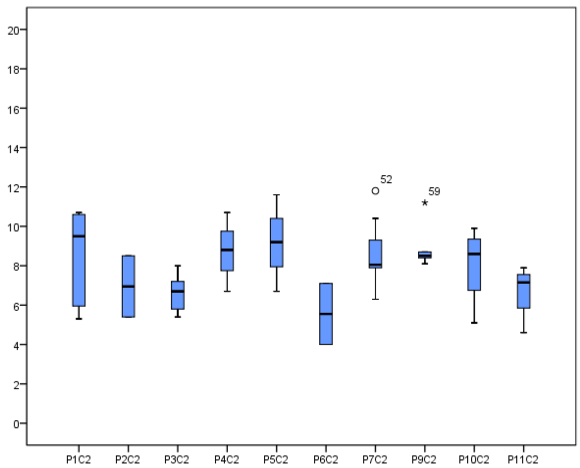


Figure 3. 19 Boxplot of *h* of plots from CC Class2.

Table 3. 5 Structure index (STRUX) for CC Class 2 (where *D is the* Index of Simpson; *H* the Index of Shannon-Weaver, *CV* the Coefficient of Variation for *dbh*)

Plots	CC Class	D_*dbh*	H_*dbh*	*dbh*_cv%	*STRUX_dbh*
P1C2	2	0.2	1.6	10.9	34.4
P2C2	2	0.4	0.7	27.5	40.0
P3C2	2	0.3	1.2	5.4	24.7
P4C2	2	0.3	1.1	7.6	25.8
P5C2	2	0.5	0.6	10.2	21.3
P6C2	2	0.4	0.7	4.3	16.8
P7C2	2	0.2	1.5	4.9	27.5
P9C2	2	0.2	1.6	6.1	30.5
P10C2	2	0.2	1.4	8.6	30.4
P11C2	2	0.2	1.4	8.7	30.5

3.4.3 CC Class 3 Plots

The *dbh* distribution was analyzed and revealed that there is one plot that has higher variability, that is P2C3 (min *dbh*= 12.15 cm, max *dbh*=48.38 cm,**Figure 3. 20**) and in h distribution (min *h*= 4 m, max *h*= 15.60 m,**Figure 3. 21**). The research concerning whether there were differences inside CC Class 3 plots demonstrated there were differences in the medians both in *dbh* as in *h* (Normality test SW dbh $p > 0.05$ for all except P6C3 (p=0.02), P7C3 (p=0.01) and SW h p > 0,05 for all except P7C3 (p=0.01), Levene's test *dbh* p= 0.01, h p=0.00). Furthermore, there were statistically significant differences among the plots in h distribution (KW *dbh* p=0.12 KW *h* p=0.014). According to the STRUX results for CC Class 3 plots, all plots behave as uneven-aged stand (**Table 3. 6**).

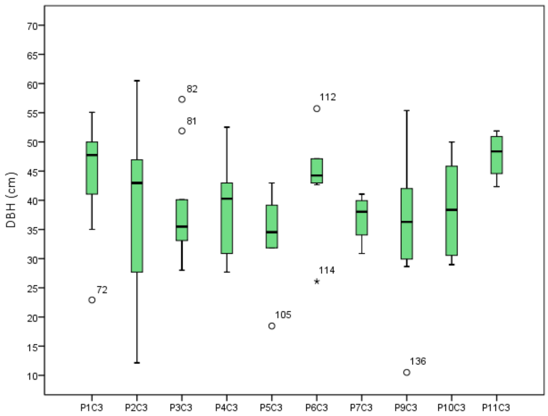


Figure 3. 20 Boxplot of *dbh* of plots from CC Class 3.

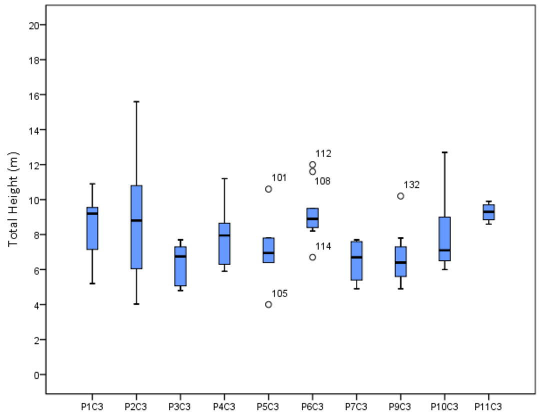


Figure 3. 21 Boxplot of *h* of plots from CC Class 3.

Table 3. 6 Structure index (STRUX) for CC Class 3 (where *D* is the Index of Simpson; *H* the Index of Shannon-Weaver, CV the Coefficient of Variation for *dbh*)

Plots	CC Class	D_*dbh*	H_*dbh*	*dbh*_cv%	*STRUX*_*dbh*
P1C3	3	0.2	1.8	9.6	35.5
P2C3	3	0.2	1.6	16.2	40.6
P3C3	3	0.1	2.1	3.4	33.7
P4C3	3	0.2	1.7	7.6	33.3
P5C3	3	0.2	1.6	11.1	34.7
P6C3	3	0.3	1.2	5.3	23.5
P7C3	3	0.2	1.4	6.2	27.7
P9C3	3	0.1	1.9	12.1	39.6
P10C3	3	0.1	1.9	6.7	34.2
P11C3	3	0.2	1.4	4.4	26.1

3.4.4 CC Class 4 Plots

The analysis of the *dbh* distribution showed that there are three plots with higher variability and whiskers in the lower *dbh* observations that are P1C4, P2C4 and P10C4 (Figure 3. 22). P9C4 is a plot that shows the structure of an aging stand, with a minimum *dbh* of 42.97cm (Figure 3. 22). The study in the matter if there were differences in variance distribution inside CC Class 4 plots (Normality test SW *dbh* p > 0.05 for all, SW *h* p > 0.05 for all except, Levene's test *dbh* *p*=0.04, *h* *p*=0.44), demonstrated that there were statistically significant difference between the plots in *dbh* distribution (*KW dbh p*=0.01, *KW h p*=0.27). In the plots P3C4, P4C4, P5C4, P6C4, P7C4, P9C4 there was more variabilityin the *h* distribution (Figure 3. 23) when compared with the *dbh* (Figure 3. 22). All plots behave as uneven-aged stands with *STRUX* > 20 (Table 3. 7).

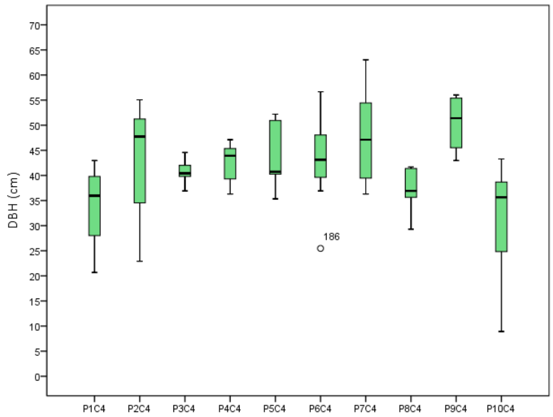


Figure 3. 22 Boxplot of *dbh* of plots from CC Class 4.

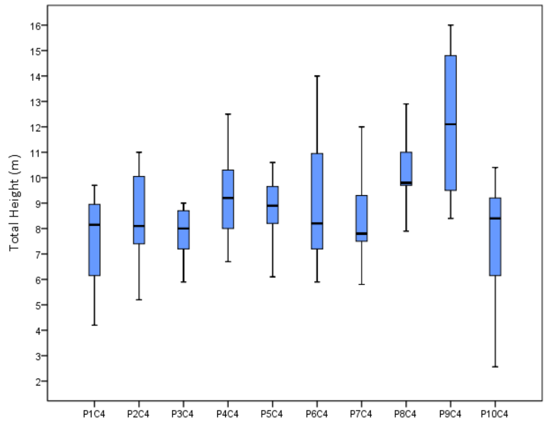


Figure 3. 23 Boxplot of *h* of plots from CC Class 4.

Table 3. 7 Structure index (STRUX) for CC Class 3 (where *D* is the Index of Simpson; *H* the Index of Shannon-Weaver, CV the Coefficient of Variation for *dbh*)

Plots	Plot cod	CC Class	D_*dbh*	H_*dbh*	*dbh*_cv%	*STRUX_dbh*
P1C4	14	4	0.3	1.0	13.9	30.8
P2C4	24	4	0.2	1.8	10.6	36.5
P3C4	34	4	0.3	1.3	3.1	23.9
P4C4	44	4	0.2	1.6	3.8	27.3
P5C4	54	4	0.3	1.3	5.8	25.7
P6C4	64	4	0.1	2.0	5.3	33.7
P7C4	74	4	0.2	1.7	6.6	32.3
P8C4	84	4	0.3	1.1	6.1	23.3
P9C4	94	4	0.2	1.4	7.8	29.5
P10C4	104	4	0.2	1.7	13.0	37.7

3.5 Natural Regeneration

The natural regeneration (NR) is the indicator of the natural renovation of a population of a stand. The NR method created in this study made it possible to implement an efficient evaluation in each plot.

3.5.1 Results of the evaluation of the amount of established natural regeneration in the plot using regeneration classes

The CC Class 2 and CC Class 3 have a very similar natural regeneration distribution (**Figure 3. 24**). The NR grouped by CC Class did not behave normally for any CC Class (KS $p < 0.05$). In CC Class 1 most of the plots had several individuals of natural regeneration in the NR Class 1, only plot P3C1 in the NR Class 2 (**Figure 3. 24**). The CC Class 2 and CC Class 3 had a higher variability with plots with the natural regeneration in NR Class1, Class 2 and Class 3 and both CC Classes have a higher percentage of plots in NR Class 2. The CC Class 4 have most of the plots with a natural regeneration higher than 50 saplings for plot (**Figure 3. 24**).

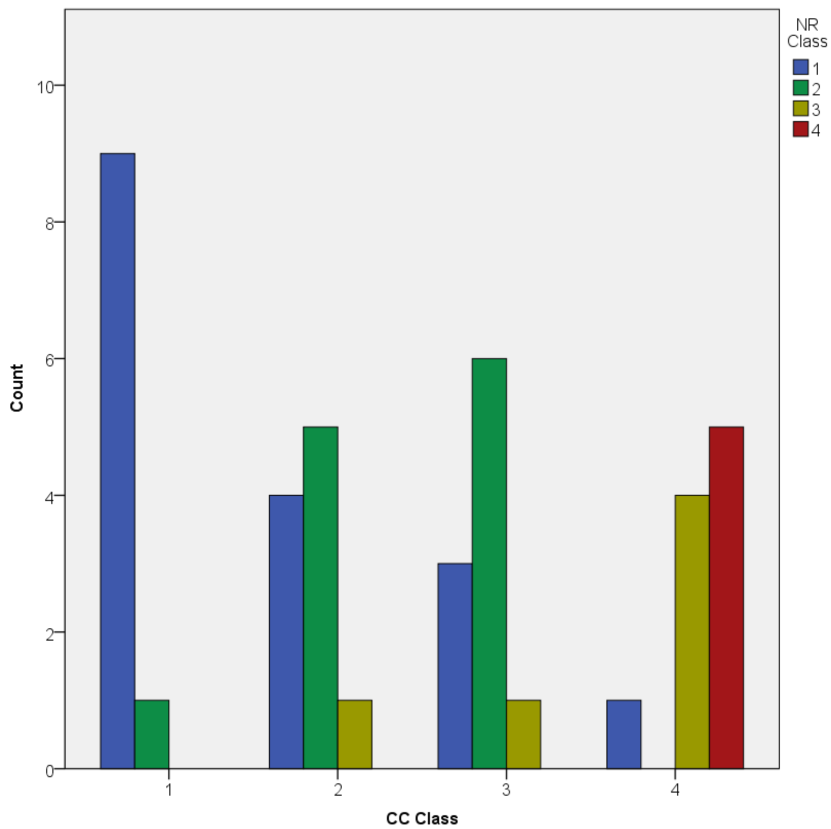


Figure 3. 24 NR Class per CC Classes.

The Levene's test was used to check the homogeneity of variances (p=0.49) and the Krustal-Wallis test (p=0.0) was applied to check if there were differences between CC Classes concerning the amount of natural regeneration. Consequently, to identify the pairwise comparisons it was done the Mann-Whitney test. There were statistically differences in the amount of natural regeneration between the CC Class 1 and CC Class 4 (p=0.00) and between CC Class 2 and CC Class 4 (p=0.023), so the differences found were between the two CC Classes with lower crown cover and the CC Class with the higher crown cover.

3.5.2 Results of Random Transect Sampling & NR Classification

For this study, the objective of the method of Random Transect Sampling and the NR Classification was to evaluate the possibility of recruitment of natural regeneration in plots with a high crown cover.

The NR method created in this study was very useful in the field work and facilitated the evaluation of the NR in stands with high density crown cover and in challenging terrain. In this research, the parameter superior storey influence was the same for all the trees because all the transects were marked outside the crown of adult trees since we were only focused on the regeneration that was able to occur beyond the age when shade tolerance starts to diminish. Nevertheless, this parameter would be useful in research considering all the regeneration of the plot, under and outside the crown of adult trees.

Considering the size of the saplings evaluated with the natural regeneration classification (NR classification), most of it had a *dbh* <5cm, 73% were in the *dbh* Class 1 and 27% in *dbh* Class 2 (Figure 3. 25). In the case of *h*, *h* class 1 and *h* class 2 had similar values of representation in the sample, although *h* class 2 had a higher value of 56% (Figure 3. 26). Furthermore, 33% of the individuals in *dbh* class 1 were in *h* class 2, so there was more variability in *h* in *dbh* class 1 than in *dbh* class 2 (Figure 3. 27). So, until the sapling achieves a *dbh* of 5cm there is more variability in height growth.

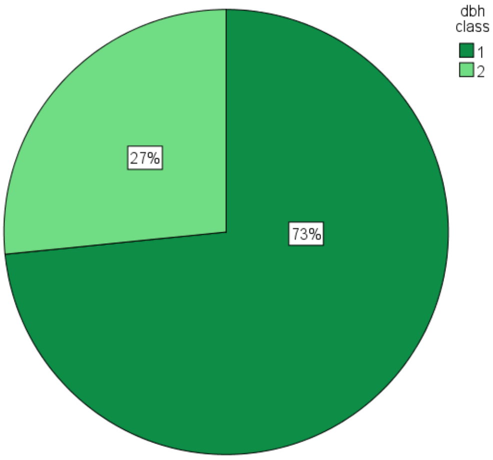


Figure 3. 25 The NR *dbh* Class results

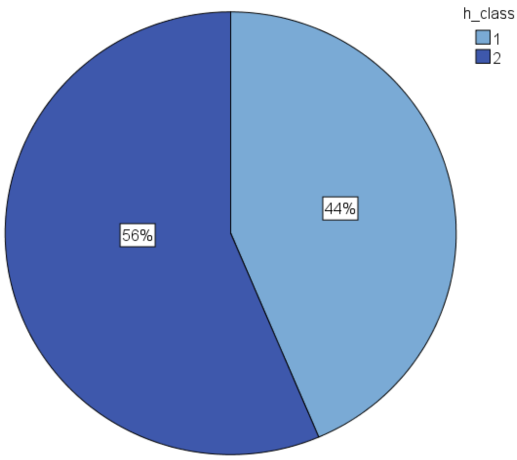


Figure 3. 26 The NR *h* Class results

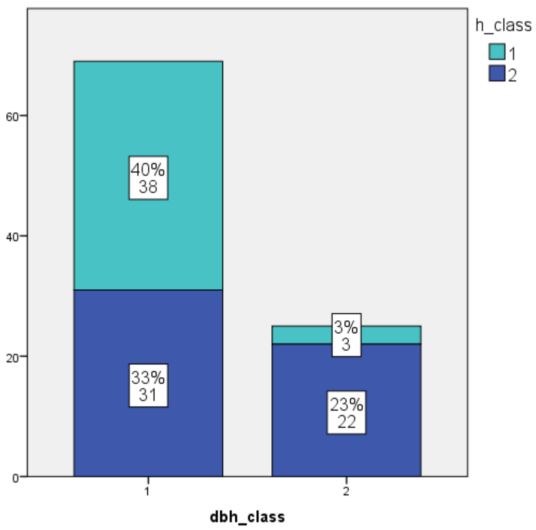


Figure 3. 27 The *h* class per *dbh* class results

Also, 50% of the saplings were growing isolated from another sapling, 41% the crown was in contact with another tree in one point, 9% were touching in two point (**Figure 3. 28**). In addition, 72% of the saplings had only one main stem, corresponding to the shape 3 of the NR Classification (**Figure 3. 29**). The low bifurcation (tree shape 2) was rare, corresponding to 5% of the total sample and the shrub shape (tree shape 1) was only present in 22% (**Figure 3. 29**).

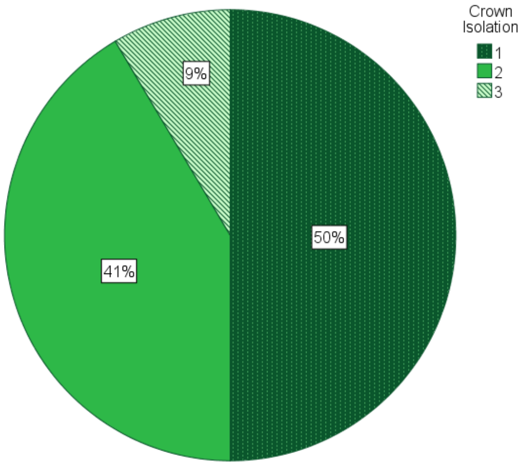


Figure 3. 28 The NR crown isolation class results

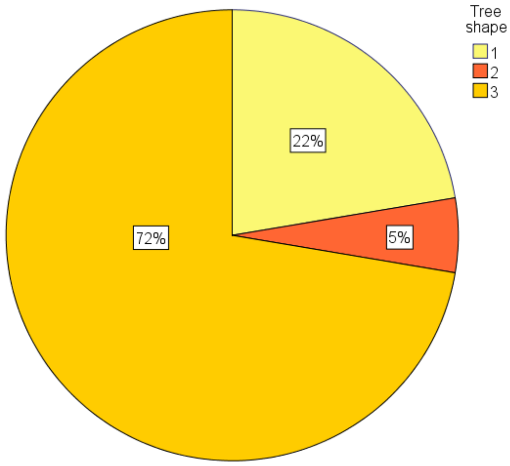


Figure 3. 29 The NR tree shape class results

The NR Classification created allowed for a fast evaluation of the quality of NR present in each plot, the result of the method was a Table with a code with four factors (Appendix - NR Random Transect Results for CC Class 3).

Most of the plots had NR individuals that could be recruited, except for plots P3C3, P11C3 and P1C4 that were dominated by *Cistus ladanifer* and had almost no regeneration present (Appendix - NR Random Transect Results for CC Class 3 and 4). In 20 plots, there were two plots where the NR Classification method with the transect did not detect the NR present in the plot because the natural regeneration was not uniform.

The use of NR Classification Table (Table 2. 1) allows for an effective method to select saplings for future recruitment, it is possible to apply filters to each NR parameter (for example it could be done a selection of the bigger sapling in the *dbh*, that corresponds to a 2 in that parameter, and with an arboreal tree shape, that corresponds to a 3 in the parameter tree shape).

3.6 Soil Results & Analysis

3.6.1 Litter Layer (LL) and Organic Carbon of the soil (SOC) grouped by CC Class

The LL and SOC distributions were mainly not normally distributed when grouped by CC Class and there was homogeneity of variance (**Appendix VIII**, **Appendix IX**). The LL median is lower in CC Class 1 than in all other CC Classes (**Figure 3. 30**). The LL variability is also higher in all other CC Classes than in CC Class 1 (**Figure 3. 30**). In CC Class 2 there is an extreme, it is plot P1C2 that has a very high value of LL of 3.5 kg/m^2, and this plot also has a high value of Organic Carbon (SOC, %) of 5.7% (**Figure 3. 31**). There were no statistically significant differences between CC Classes in the SOC (KW SOC p=0.5, **Figure 3. 31**). In CC Class 4 two extremes had very high values of SOC, 33 - P7C4 and 29 - P6C4.The SOC in the CC Classes shows a higher variability of values in CC Class 1 and less variability in CC Class 4. There was a positive correlation between LL and SOC and it was tested using the non-parametric Spearman Correlation test (Correlation Coefficient=0.5 and p=0.002). There were no statistically significant differences between CC Classes considering LL (*KW* LL p=0,299).

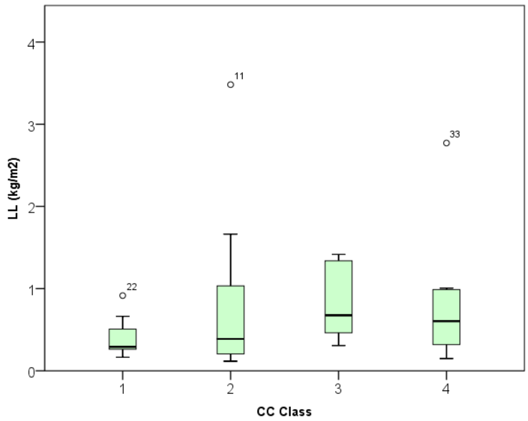


Figure 3. 30 Boxplot of Litter Layer (g) grouped by CC Class.

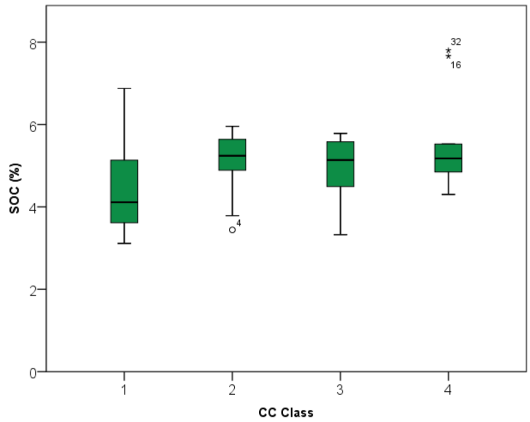


Figure 3. 31 Boxplot of Organic Carbon % grouped by CC Class

The mean of LL in CC Class 1 is lower than the other CC Classes and that CC Class 3 and CC Class 4 had the same mean (**Figure 3. 32**). CC Class 4 SOC mean was higher than other CC Classes. CC Class 2 and CC Class 3 behaved very similarly in the LL and SOC. CC Class 3 had the same LL mean as CC Class 4 but the mean of SOC was higher in CC Class 4 (**Figure 3. 32**).

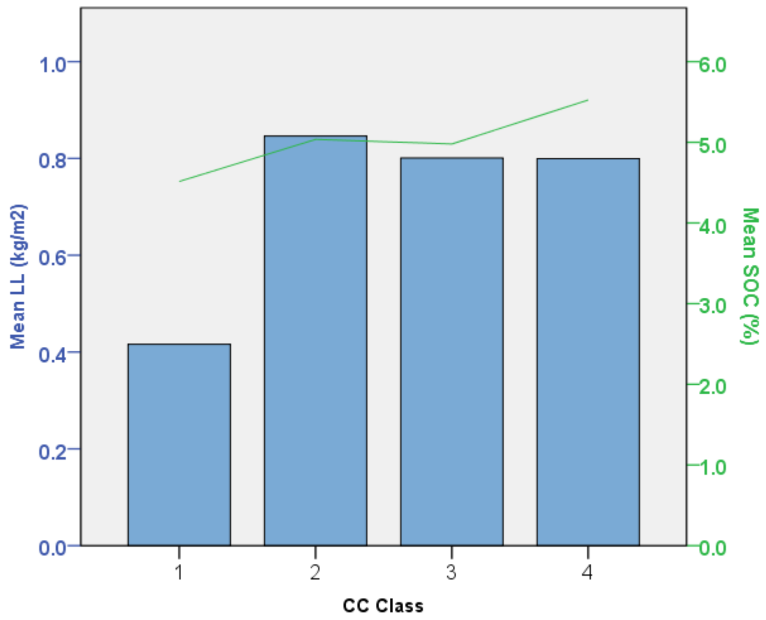


Figure 3. 32 Mean litter layer (LL, bars) and the mean organic carbon (SOC, lines) by CC Class.

3.6.2 Litter Layer and Organic Carbon grouped by the Absence (-) and Presence (+) of Natural Regeneration (NR)

The LL was not normally distribute (KS LL NR+ and NR- *p*=0.0) but there was homogeneity of variance (Levene's test *p*=0.8). So, the Mann-Whitney (MW) test was used to determine if there were differences in the LL according to the absence or presence of NR, and there was a meaningful statistical difference in LL (MW *p* =0.024).

The SOC was normally distributed (KS SOC NR+ *p*=0.2 NR- *p*=0.2) and there was homogeneity of variance (Levene's test p=0.5). There was a significant difference in SOC in the presence and absence of NR (ANOVA *p* =0.036). The SOC demonstrated a tendency to increase with the increase of the CC Class in the absence of regeneration and the opposite tendency in the presence of regeneration (Figure 3. 33). In the presence of NR, the LL values of CC Classes were less differentiated (Figure 3. 34). The LL in the presence of NR had a mean of 1.2 kg/m^2 and a standard variation of 0.18, also, it showed a tendency to increase with the increase of CC Class, except for CC Class 2, which showed the highest value. In the absence of NR, the LL had a mean value of 0.3 kg/m^2 and a standard variation of 0.03. Both the LL and SOC were higher in the presence of natural regeneration (Figure 3. 35). These results suggest that LL, SOC, and NR are related.

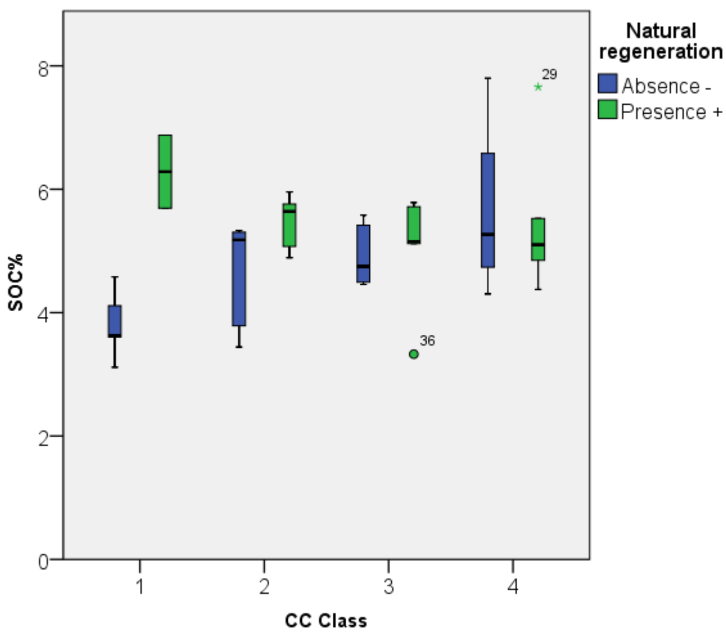


Figure 3. 33 The SOC (%) distribution according to NR and CC Class

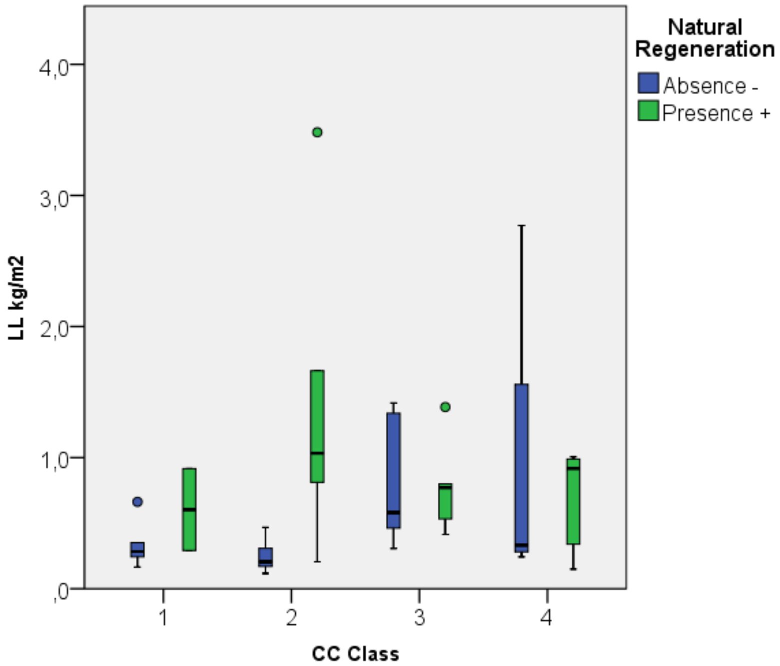


Figure 3. 34 The LL distribution (kg/m²) according to NR and CC Class.

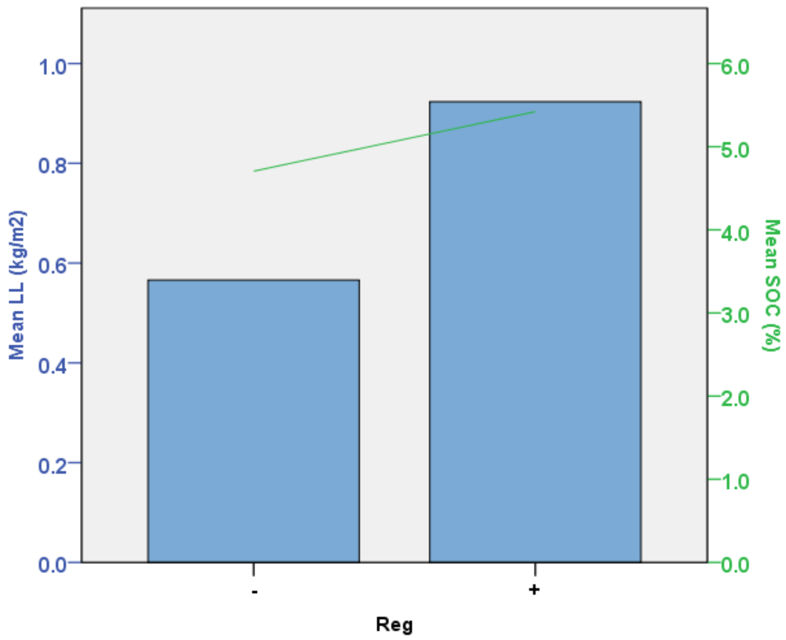


Figure 3. 35 Mean litter layer (LL, bars) and line graph of the mean organic carbon (SOC, lines) grouped by the absence (-) and presence (+) of natural regeneration.

3.6.3 The pH of the soil grouped by CC Class

The pH results, both pH(H_2O) and pH(KCl), suggest a tendency for decreasing as CC Class increases (**Figure 3. 37**, **Figure 3. 36**).

The pH(H_2O) and the pH(KCl) grouped by CC Classes was normally distributed (*KS* H_2O CC Class 1 p=0.12, CC Class 2,3,4 p=0,2, *KS* KCl all CC Classes p=0.2) and had homogeneity of variance (pH(H_2O) Levene's test p=0,6; pH(KCL) Levene's test p=0.79).

The ANOVA results for pH(H_2O) (p=0.167) did not show any statistical significance differences between CC Classes but the p for the ANOVA for pH(KCl) (p=0.07) was very close to 0.05.

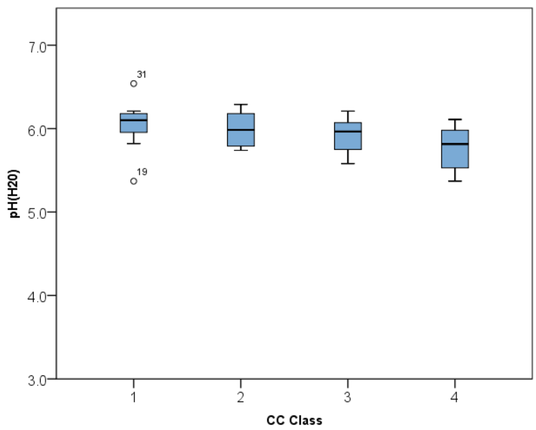


Figure 3. 36 Boxplot of pH(H2O) by CC Class

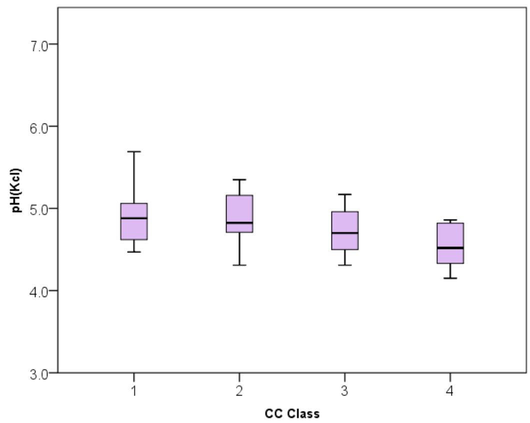


Figure 3. 37 Boxplot of pH(KCl) by CC Class

There was a strong positive correlation (Pearson Correlation of 0.917) between pH(H_2O) and pH(KCl).There was also a significant correlation between CC Class and pH(H_2O) (Pearson Correlation=-0.361 and p =0.028) and between CC Class and pH(KCl) (Pearson Correlation=-0.420 and p =0.01). The pH(H_2O) means were: CC Class 1= 6.04; CC Class 2 = 5.99; CC Class 3=5.92; and CC Class 4=5.77.

3.6.4 The pH of the soil grouped by the Absence (-) and Presence (+) of Natural Regeneration (NR)

The samples where regeneration was present had 75% of the value under the pH of 6.00 in H_2O and 75% under a pH(KCl) of 5.00. The median of pH (H_2O) in the presence of regeneration was 5.86 while when regeneration was absent it was 6.0 (**Figure 3. 38**). When analyzing the boxplot of pH (H_2O) (**Figure 3. 38**). The variability of pH measured was higher in the absence of regeneration in pH(KCl) (**Figure 3. 39**). It is possible to see that the absence of regeneration (-) has a higher maximum value than the presence of regeneration (+) and the same happens in the pH(KCl).

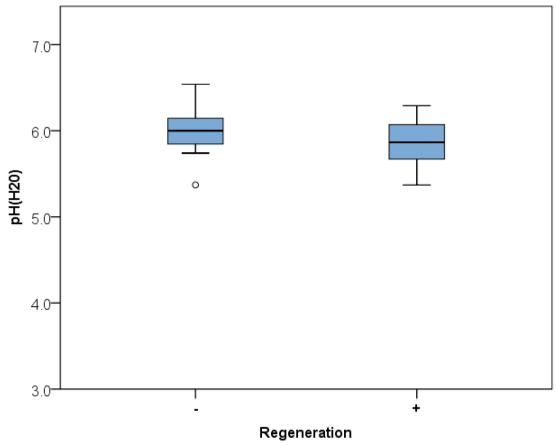


Figure 3. 38 Boxplot of pH(H2O) grouped by the absence (-) or presence (+) of natural regeneration

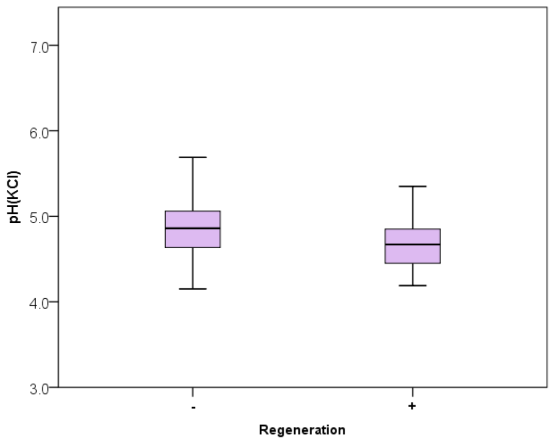


Figure 3. 39 Boxplot of pH(KCl) grouped by the absence (-) or presence (+) of natural regeneration

The pH(H_2O) and the pH(KCl) grouped by the absence (-) or presence (+) of established NR, were normally distributed (*KS* pH in H_2O + p=0.18 and – p=0.2 ;*KS* pH(KCl) + p=0.2 and – p=0.2) and the homogeneity of variance of assumption was met (pH in H_2O Levene's test p=0,6; pH(KCL) Levene's test p=0.137). An One-way ANOVA test was done for both pHs. There was a significant statistical difference in the means of pH(H_2O) in the absence (-) or presence (+) of established NR (ANOVA p=0.032). There were no significant differences when the pH(KCL) was studied (ANOVA p=0.137).

In CC Class 1 the difference in the pH between the absence and presence of NR was more accentuated. The pH(H_2O) was always higher in the absence of natural regeneration (**Figure 3. 40**).

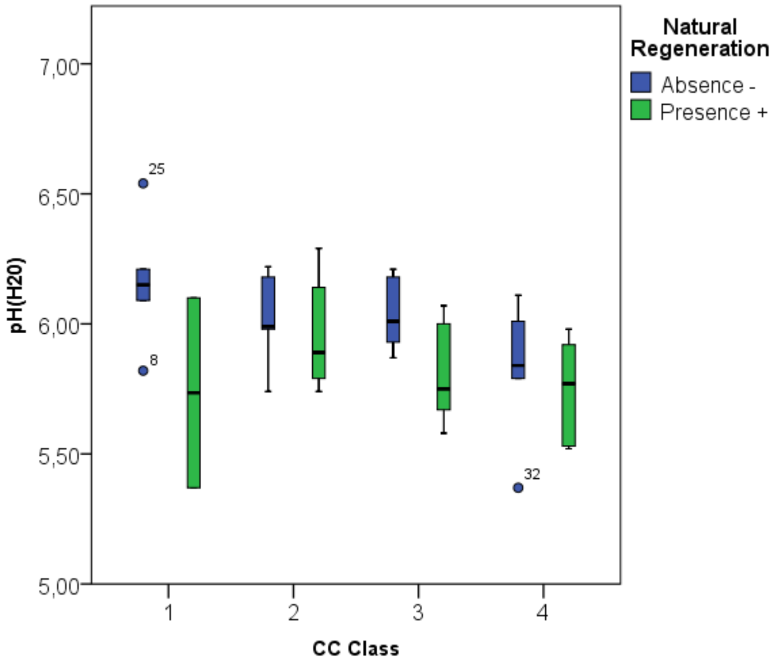


Figure 3. 40 The pH in H_2O grouped by CC Class

A non-parametric Spearman correlation test was done between the pH(H_2O) and SOC values considering all sampled soils. No significant correlation between pH(H_2O) and SOC (Spearman *rho*= -0.192, *p*=0.26) and pH(KCl) and SOC (Spearman rho= -0.07, p=0.69) was found.

CHAPTER 4 –DISCUSSION

- **The *dbh* and *h* distribution and above-ground biomass by CC Class**

In areas with a crown cover less than 10 %, the *dbh* distribution was influenced by the presence of older individuals in the higher *dbh* classes. The opposite tendency was found in higher CC Classes where the *dbh* distribution reflected the presence of younger individuals. These findings conform to what was stated by Pulido *et al.* (2001), Plieninger *et al.* (2003) and Principe *et al.* (2019). The holm oak diameter distributions, when grouped by crown cover, appeared to reflect a tendency to be bimodal. This result opened the window for a future deeper research on the development of oak stand structure because bimodality in monospecific populations can also indicate interaction of different factors (Houston and DeAngelis, 1987). It can also reflect moments of episodic disturbance and recruitment in the past (Vlam *et al.*, 2017) and/or asymmetric competition among oak individuals (Kang *et al.,* 2017).

The *h* distribution significantly differed with the crown cover degree. CC Class 4 had the maximum *h* peak values when comparing trees in the same *dbh* class. The height of the holm oaks could have been affected by competition in dense stands (Gonçalves, 2018a; De Groote *et al.*, 2018) or/and by pruning because pruning scars were detected on the adult trees during the fieldwork. Heavy pruning could affect the vigour and vegetative status of adult trees (Martin *et al.,* 2015). Plots with higher crown cover and with higher tree density are more difficult to get access to, therefore it is possible that plots with a high crown cover were less pruned in the past than plots in the other CC Classes.

The above-ground biomass (*wa*) classes with higher frequencies in this study ranged between 400kg and 600kg per tree and individual tree biomass distribution was not affected by crown cover. The *wa* class of 400-600kg represented 60% in CC Class 1, 36% in CC Class 2, 40% in CC Class 3 and 42% in CC Class 4. Although significant differences between CC Classes in the *wa* were not detected, it was possible to observe that there was a higher variability in tree sizes in areas with a crown cover higher than 30%. The above-ground biomass tree variability in areas with higher crown cover is a relevant result because conjugates both diameter and total height, horizontal and the vertical dimensions (Gonçalves, 2018).

- **Tree level parameters**

The *hd* ratio for all CC Classes was lower than 45 which indicates good stability and free growth of the individual trees. Yet, both between and within the CC Classes a large variability was observed, which is most like due to the structure of the size classes. The younger the trees have the higher the *hd* ratio (Saha *et al.,* 2014; Gonçalves, 2018; Rinn, 2015), which is plausibly a result of the tree growth strategies, as trees first invest in the height growth and after in the diameter growth (Alves *et al*., 2012). In plots with higher crown cover the trees showed higher values of *hd* ratio for the same *dbh*, this could be related to the higher density in these plots, as *hd* ratio significantly increases with aggregate and intraspecific competition (Saha *et al.,* 2014, Gonçalves, 2018; Rinn, 2015). The difference in *hd ratio* between CC Classes 1 and 4 also results from the different type of structures, even-aged for the former and uneven-aged for the latter, which was also observed at plot level.

- **Crown parameters**

There were no significant differences between CC Classes in mean crown diameter, crown length and crown ratio. Most trees in the study exhibit a well-balanced crown ratio. There was a positive correlation between CC Class and the *lcr*, the *lcr* was slightly increasing with the increasing of CC Class. This result can be explained by different parameters such as the intensity of pruning in the past and/or by ecological relationships between the individual tree of each plot, tree age, height to the base of the crown, crown length, as well as competition (Burkhart, *et al.,* 2012; Attocchi *et al.,* 2015).

- **Absolute Density measures**

There was a significant statistical difference between consecutive CC Classes 2 and 3, and no significant difference between other consecutive CC classes. This result suggests that 30% Crown Cover can be a threshold value for a higher*, N*, *G, Wa* and *AGC* and per hectare and an indicative value for a relevant increase in forest biomass in the *montado* because the increase in *Wa* was only significant when the 30% Crown Cover threshold was achieved. CC Class 3 had a higher *N* and higher *Wa* than CC Class 4. There was no significant statistically differences in the tree with the average basal area of CC Classes, the *dg*. The mean *G* value presented in the last Portuguese Forest Inventory (IFN6, 2015) for the pure holm oak stands was 3.48. The *G* CC Class 1 was 1.9, lower than 3.49, the *G* for CC Class 2 was 3.8 and both *G* for CC Class 3 and 4 was 5.6 m^2/ha. The mean *N* value (IFN6, 2015) to pure holm oak stands was 42. The *N* for CC Class 1,2 and 4 was lower than 42 (15, 26, 38 respectively), only CC Class 3 had a higher value of 43.

- **Creation of an age structure index – STRUX**

The STRUX index was created to facilitate the age structure classification and it was an efficient way of classifying the plot age structure using only the *dbh* data. The innovation of the STRUX was the conjugation of the two diversity indices (Simpson and Shannon and Weaver Indices) and the coefficient of variation in only one value to characterize structure. The use of the Shannon index to measure the diversity of tree size using diameter had already been tested by other authors (Buongiorno *et al.,* 1994; Staudhammer *et al.,* 2001). The use of the Simpson Index measuring the size diversity was also explored in other articles (Lexerød, 2006). The STRUX allowed us to do the structure classification using *dbh* classes of 2.5 cm. When focusing on the plot as the study unit, it was possible to get a more detailed portrait of the characteristics of the holm oak stands using the STRUX Index. In CC Class 1 72.7% of the plots were classified as even-aged ones. In contrast, in CC Class 2 90% of plots were classified as uneven-aged. In CC Class 3 and CC Class 4 100% plots were classified as uneven-aged stands. It would be interesting in the future to test the index with different *dbh* classes and with different species to evaluate its performance. So according to these results, a crown cover higher than 10% and a non-normal diameter distribution was linked to an uneven-aged structure. Also, more data is known about even-aged stands than uneven-aged stands

(Oliver and Larson, 1996) and our STRUX results suggest that a deeper study of uneven-aged stand structure in holm oaks is a relevant step in researching the longevity and the sustainability of the holm oak ecosystems. Uneven-aged forest systems present an increasing resilience, which puts forward the possibility that continuous cover forestry can be a protective approach to the *montado* forest management (Ribeiro *et al.,* 2012; O'Hara, 2006; O'Hara *et al.,* 2013b). These results highlight the potential of the *montado* ecosystem for multifunctionality because increased structural heterogeneity would promote the supply of multiple ecosystem services (Felipe-Lucia *et al.,* 2018; Valdés *et al.,* 2020).

- **Natural Regeneration Results & Methodology Evaluation**

Due to long life cycles, an analysis of the regeneration dynamics of tree populations is hard but an essential part of the study of holm oak stand dynamics. The new natural regeneration classification method created in this research allowed for a quick and efficient evaluation, essential for the development of a future silviculture plan. All put together, this methodology was a valuable step for an accessible and practical study of natural regeneration of oaks and could be relevant for other common Mediterranean forest species.

Plots with a crown cover of less than 30% showed lower values of established natural regeneration when compared to plots with higher crown cover. Our observations suggested that the density of established regeneration increased as the degree of crown cover increased, and it was significantly more evident when crown cover was higher than 50%. This result was expected as shading and forest conditions promote the oak acorn germination and primary development, reinforcing the importance of the *nurse effect* (Badano *et al.,* 2011; Broncano *et al.*, 1998; Espelta *et al.,* 1995; Gea-Izquierdo *et al.*, 2006; Pulido *et al.,* 2005). Also, sapling density appeared to be higher when the crown cover was also higher and this tendency was the same as the one described in the literature (Espelta *et al.*, 1995; Pulido *et al.,* 2005; Principe *et al.* 2014). The fact that vigorous natural regeneration was found in stands where grazing occurs frequently is interesting and reinforces the idea that is possible to manage the *montado* considering the success of the oak trees regeneration and recruitment and extensive periodic grazing.

This result conforms to what was stated by Carmona *et al.* (2013), but contradicts what was found by Plieninger (2007).

According to our results of the NR Classification, 85% of the plots, with a crown cover higher than 30%, had good quality saplings suited to be recruited. Only 15% of the plots with a crown cover higher than 30% were dominated by *Cistus ladanifer* and had almost no oak saplings. Hence, we found out that natural regeneration of holm oak is a valuable tool. This result is relevant because these areas are often categorized as shrubland pastures and the oak natural regeneration is not considered as a value or an ecosystem service of these dense plots.

- **Soil**

Areas with high crown cover presented higher mean values of LL and SOC. Although the data suggested that both LL and the SOC would increase proportionally with the increase of crown cover, no significant statistical differences were found between CC Classes. To further investigate this result, a more detailed spatial measurement of the LL and SOC in each plot could be done in the future because litter layer accumulation is distributed in patches and its spatial distribution may be dependent not only on the crown cover of the area but on the canopy spatial arrangement (Boeken *et al.*, 2001; Reyna-Bowen *et al.,* 2020). We found out that both LL and SOC had higher values when natural regeneration was present. This result showed that litter layer and natural regeneration were interconnected, as referred to in previous studies (Collins and Good, 1987; López-Barrera and Gonzalez-Espinosa*,* 2001; Rinkes and McCarthy 2007; Loydi *et al.*, 2013; Gavinet, *et al.,* 2018). According to Collins *et al.* (1987) there were more oak seedlings in areas where the litter layer was deeper. The study by López-Barrera *et al.,* 2001 stated that oak seedlings growing through oak litter allocated more biomass to roots than to the shoot as litter depth increased. As stated by Lunt *et al.* (2004) the addition of organic matter produced a two to threefold increase in oak seedling dry weight. In the study by Loydi *et al.* (2013), the authors described a similarly positive result between regeneration and litter layer in big seeds like acorns. Gavinet *et al.* (2018) also found out that the litter layer improved soil humidity and seemed to be relevant for oak seedling development in the Mediterranean. Evrendilek, *et al.* (2004) observed that SOC is positively correlated to water retention in the soil, this could be relevant for the development of natural regeneration. There was a relevant negative correlation between crown cover and pH(H_2O). The mean of pH(H_2O) decreased between the mean value of 6.04 (CC Class 1) and 5.77 (CC Class 4). Similarly, in the study by Flores-Rentería *et al.* (2018) the researchers found out that there were significantly lower values of soil pH under the oak canopy when compared to open areas in holm oak woodlands. There was a significant statistical difference in the means of pH(H_2O) in the absence (-) or presence (+) of established natural regeneration. When regeneration was present 75% of the samples had a value of pH(H_2O) under 6 but always higher than 5. In the absence of regeneration, 50% of soil sampled had a pH(H_2O) higher than 6. The CC Class 4 was the CC Class with a higher presence of natural regeneration, and it was also the CC Class with lower soil pH(H_2O). The soil pH(H_2O) decreased as the crown cover increased and the natural regeneration increased. It is known that the presence of organic matter in the soil can result in a pH(H_2O) decrease (Botelho da Costa, 1995) so the slightly higher soil acidity observed under the higher crown cover areas and in areas with a higher density of established natural regeneration can be linked to higher soil organic matter.

- **Management implications**

The restoration of oak forest depends on advanced oak regeneration. In our research, stands with low crown cover areas were linked to lower number of established regeneration, so it would be relevant to enhance the presence of younger trees, so that tree population is renewed. This stand rejuvenation could be obtained in two ways: favoring the existing natural regeneration or using artificial regeneration. In the case of the artificial regeneration, both seeding and planting are options to consider (Lauw *et al.,* 2013; Löf *et al.,* 2019; Martínez-Muñoz *et al.,* 2019). As it was showed in this research, organic matter plays an essential role in natural regeneration. For this reason, it is a relevant measure to ensure that the soil remains covered with pasture and litter all year, even when grazing occurs and especially in areas where tree crown cover is low. In addition, a generous amount of litter layer from the local oak trees can be added to the place of seeding or transplanting. If possible, it would be pertinent to create rotational grazing areas, so that the areas where seeding was recently done are less intensively grazed in the first years.

After a successful primary growth of regeneration, a reduced growth of saplings due to light competition can occur, when shade-tolerance starts to reduce (Espelta *et al.,* 1995; Ward, 1992). This fact is of extreme importance in areas with high crown cover. Consequently, it is important to implement silviculture practices to assure the holm oak restoration in dense stands and that light competition is mediated, this can be achieved by selective thinning. If there are suppressed saplings, all the aerial part can be removed to promote sprouting. Therefore, it is relevant to detect when saplings are prepared to be recruited in dense stands and the new methodologies created within the scope of this research are meant to ensure such diagnosis as the NR Classification. When grazing occurs, different strategies are helpful to ensure that regeneration is protected by using individual tree protectors made of metal grids, piles of pruned branches or shrubs (Costa *et al.,* 2017; Martínez-Muñoz *et al.,* 2019). Formation pruning can be useful to guide the growth of the saplings, when they reach 15 cm of *dbh* (Serradas *et al.,* 2008).

CHAPTER 5 –CONCLUSION

Our research showed that a higher crown cover was linked to an uneven-aged structure, higher values of above-ground biomass per hectare, higher number of established natural regeneration, and a tendency for higher values of soil organic carbon and litter layer. We found out that there was structure variability as a function of crown cover, and a tendency of a slight decrease in soil pH, between 5 and 6, in the 0-10 cm layer.

One of the goals of this research was to evaluate the interconnectivity of factors involved in oak natural regeneration. We concluded that exists a positive correlation between natural regeneration, litter layer and soil organic carbon together with a negative correlation with soil pH.

The use of crown cover as the grouping stratifying factor allowed for a closer look in the heterogeneity of the landscape and, to ensure this goal, this investigation included areas with all degrees of crown cover, from the open *montado* to the dense holm oak forests. Crown cover can be an essential tool to enable large scale assessment of ecosystem services, connecting empirical data with satellite and aerial photography data.

The development of specific methodologies was pivotal to make it possible to embark upon this scientific journey and, that resulted in the innovative STRUX Index and the Natural Regeneration Classification. Both methods are now available for future research on the topics.

Conclusively, our results deepened the need for the development of environmental and farming policymaking that will create strategies for a successful management of the holm oak ecosystems, with all its complexity, where natural regeneration, structure diversity and ecosystem services will then be a seen as fundamental and economical relevant.

This investigation highlighted a positive overview on the resilience of holm oak ecosystems in the Mediterranean. In this study, holm oaks stands are inarguably proven as having high value in the Mediterranean territory.

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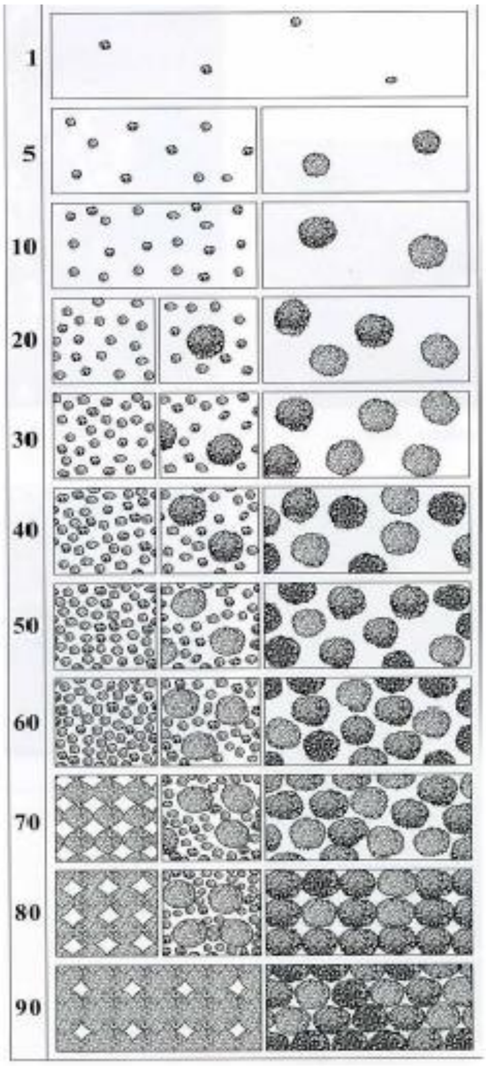
APPENDICES

Appendix I – Crown cover estimated according to the protocol in IFN5 (2005/2006)



Appendix II - Crown Cover occupation (%) in the total area of the property.

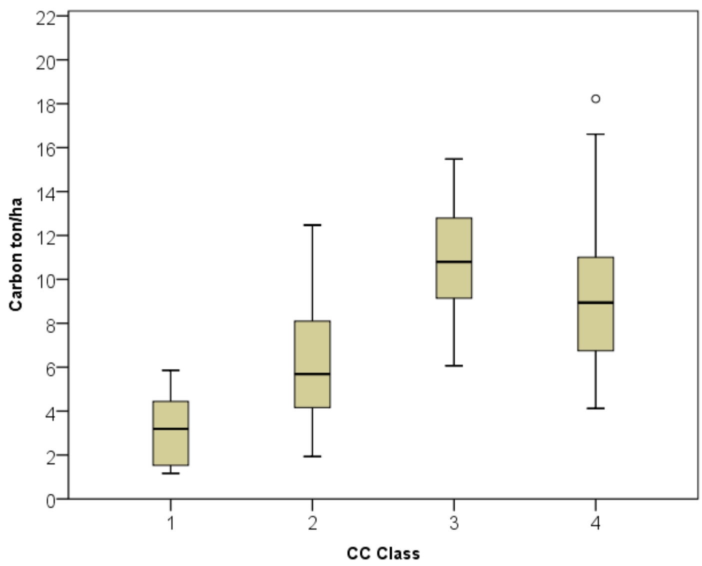
Crown Cover	Number of plots	%
0	52	7
10	191	24
20	220	28
30	119	15
40	66	8
50	50	6
60	44	6
70	28	4
80	10	1
90	3	0
100	0	0
Total	783	100

Appendix III CC Class occupation (%) in the total area of the property.

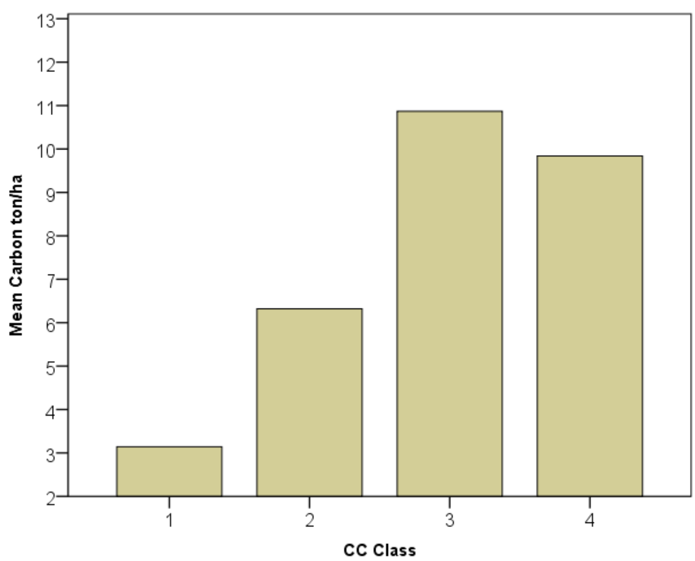
Crown Cover	Number of plots	%
CC1	52	7
CC2	411	52
CC3	185	24
CC4	135	17
Total	783	100

Appendix IV Map of randomnly selected plots

Appendix V – Boxplot of the above-ground carbon per hectare per CC Class.



Appendix VI - The above-ground carbon per hectare per CC Class.



Appendix VII – Natural Regeneration Transect Results

Plots	Plot cod	CC Class	NR Class	N of trees
P1C1	11	1	1	0-25
P2C1	21	1	1	0-25
P3C1	31	1	2	25-50
P4C1	41	1	1	0-25
P5C1	51	1	1	0-25
P6C1	61	1	1	0-25
P7C1	71	1	1	0-25
P9C1	91	1	1	0-25
P10C1	101	1	1	0-25
P11C1	111	1	1	0-25
Plots	Plot cod	CC Class	NR Class	N of trees
P1C2	12	2	3	50-75
P2C2	22	2	1	25-50
P3C2	32	2	2	0-25
P4C2	42	2	2	25-50
P5C2	52	2	1	0-25
P6C2	62	2	2	25-50
P7C2	72	2	1	0-25
P9C2	92	2	1	0-25
P10C2	102	2	2	25-50
P11C2	112	2	2	25-50
Plots	Plot cod	CC Class	NR Class	N of trees
P1C3	13	3	2	25-50
P2C3	23	3	2	25-50
P3C3	33	3	1	0-25
P4C3	43	3	2	25-50
P5C3	53	3	2	25-50
P6C3	63	3	3	50-75
P7C3	73	3	1	0-25
P9C3	93	3	2	25-50
P10C3	103	3	2	25-50
P11C3	113	3	1	0-25
Plots	Plot cod	CC Class	NR Class	N of trees
P1C4	14	4	1	0-25
P2C4	24	4	3	50-75
P3C4	34	4	3	50-75
P4C4	44	4	4	75-100
P5C4	54	4	3	50-75
P6C4	64	4	4	75-100
P7C4	74	4	4	75-100
P8C4	84	4	4	75-100
P9C4	94	4	4	75-100
P10C4	104	4	3	50-75