CORK OAK (*Quercus suber* L.) ROOT SYSTEM: A STRUCTURAL-FUNCTIONAL 3D APPROACH

Sistema Radical do Sobreiro (*Quercus suber* L.): Uma Abordagem Estrutural-Funcional 3D

Cati Oliveira Dinis

Tese apresentada à Universidade de Évora
para obtenção do Grau de Doutor em Gestão Interdisciplinar da Paisagem

ORIENTAÇÃO: *Professora Doutora Maria do Rosário Gamito de Oliveira*
Professor Doutor Nuno Manuel Cabral de Almeida Ribeiro
Doutor Peter Surový

ÉVORA, NOVEMBRO 2014

INSTITUTO DE INVESTIGAÇÃO E FORMAÇÃO AVANÇADA

A doutoranda Cati Oliveira Dinis beneficiou de uma Bolsa de Doutoramento pelo Programa Bento Jesus Caraça, concedida pela Universidade de Évora (Instituto de Investigação e Formação Avançada), entre o período de Janeiro de 2011 a Dezembro de 2013

Agradecimentos

No decorrer desta tese muitas foram as pessoas que direta ou indiretamente colaboraram para que a sua conclusão fosse possível. O meu sincero agradecimento a todas elas. Contudo, não poderia deixar de destacar e agradecer, particularmente:

- À minha orientadora e mentora, Professora Maria do Rosário Oliveira, por todo o seu apoio, disponibilidade, sentido crítico e conhecimento transmitido quer a nível científico quer a nível pessoal.
- Aos meus co-orientadores Doutor Peter Surový e Professor Nuno Ribeiro por me terem "lançado" este desafio e pelo seu acompanhamento incansável ao longo destes anos de trabalho conjunto! Pela transmissão de conhecimentos técnicos e científicos, *brainstorms*, apoio, confiança, paciência, entusiasmo e, amizade.
- A todos os colegas que passaram pela equipa de investigação: Susana, Rita, Paulo, João, Marco, Isilda, Constança, Radek, Marketa, Ana, Gabriela, Chris, Hugo, Manuela. O vosso apoio foi essencial nos trabalhos de campo assim como nas temporadas laboratoriais, sempre com motivação, profissionalismo e…boa disposição!
- A toda a equipa da empresa TRA pelo apoio prestado, garantindo a execução de parte desta investigação.
- Ao Professor Cermak por me ter recebido em Brno, República Checa, partilhando o seu conhecimento, em ambiente de campo, acerca dos métodos de escavação de raízes. "*Quem estuda raízes tem que ter um pouco de loucura dentro de si!"*. E ao Professor Robert Marusak por ter cedido emprestar o equipamento necessário ao tratamento laboratorial de raízes finas.
- À Doutora Teresa Soares David pela sua disponibilidade e transmissão de conhecimentos na área das raízes.
- Às técnicas D. Zetinha e D. Maria das Dores, pelo inestimável apoio nas longas horas passadas no laboratório.
- Aos vários docentes e investigadores da Universidade de Évora, principalmente do Departamento de Fitotecnia, que me acompanharam durante este processo. Quer seja ao nível dos ensinamentos transmitidos nos mais variados temas abordados nesta investigação, quer seja pelas palavras sábias, experientes e motivacionais nos momentos certos, o meu Muito Obrigado! Um especial Obrigado ao Professor Rui Machado e ao Professor Alfredo Gonçalves Ferreira.

- À D. Fátima pela sua eficiência profissional e pelo apoio pessoal transmitido durante as “nossas conversas”.
- À minha Amiga Susana Palminha que me acompanhou nos primeiros tempos desta aventura… muitos meses passados a escavar raízes! E embora nos tenhamos separado a nível profissional a sua amizade incondicional, apoio, compreensão, espírito crítico e motivação ao longo do tempo de elaboração desta tese ajudaram-me incansavelmente a seguir em frente.
- A todos os meus amigos do coração, eles sabem quem são! Obrigada por compreenderem as minhas ausências, os momentos de impaciência e, por vezes o mau humor momentâneo; pelas palavras amigas e por nunca deixarem de acreditar em mim. Um agradecimento especial às minhas “5 chacras” (Jaqueline, Raquel, Sara, Su, Maria Inês) por me terem sempre ajudado a manter o equilíbrio, cada uma à sua maneira… e um agradecimento especial também aos meus amigos da “La Família”! Tenho um lugar no coração para cada um de vocês!
- À minha família! Obrigada por tudo e principalmente obrigada pela vossa paciência e compreensão. Obrigada por nunca me deixarem esquecer quem sou, de onde venho e que o mundo é do tamanho dos meus sonhos. Um obrigado carinhoso aos meus sobrinhos Tomás e André pelo seu amor… e desculpem por tantas vezes terem dito “Oh, a tia está sempre a trabalhar!”.
- Aos meus pais, as minhas RAÍZES! Os que acompanharam verdadeiramente este processo do início ao fim… os bons e os maus momentos, as dúvidas, as incertezas, as quebras de força, os momentos de ânimo… foram vocês que sempre estiveram lá! Obrigada por me terem incutido os valores e princípios de que eu tanto me orgulho e que me fizeram chegar aqui! Ao meu Pai, o meu pilar de segurança, que por mais que eu abane nunca me deixa cair ou levantar de mais, sempre com as palavras certas no momento certo, essencial para manter a mente e o espírito sãos! E à minha Mãe, uma força da natureza, uma guerreira da vida…um exemplo que quero seguir! O seu amor foi um porto de abrigo para mim neste processo. Muitas vezes fazendo-me acreditar que tenho todas as forças do mundo para zarpar e chegar onde quiser e outras vezes, puxando-me para a “costa” e fazendo-me ver a realidade como ela é! Apesar de muitas vezes eu ter sentido que gostavam de me ter ajudado mais, acreditem… vocês estiveram sempre lá quando eu precisei! E mais importante que tudo, obrigada por nunca terem deixado de acreditar em mim! Esta tese é dedicada a vocês!

Abstract

The last decades have witnessed the decline and sudden death of the cork oak (*Quercus suber* L.) in *Montado*. The complexity of this production system management has lead to a large set of solutions which have been absent by scientific based research findings (such as those related with the cork oak root system) deriving mainly from empirical knowledge application. The present integrated research approach permits a better understanding of the production system vulnerabilities that can result in management modification proposals which will be useful in a near future. To contribute to a more realistic and integrated forest management and planning, a study relying on a morphological evaluation of cork oak root system in a Cambissoil soil, using a 3D digitizing method was performed. Cork oak showed a dimorphic root system with a relative high quantity of sinkers distributed all over the soil profile, one root subsystem at a superficial level until 40 cm depth and another at a deeper level, around 1.20 m depth. Tree biomass allocation was similarly distributed between aerial and root systems. Previously, a study on methodologies for roots excavation - profile washing with water and excavation through high pressure air jet – was carried out. Results showed that for sandy soils the most suitable method is the excavation by high pressure air jet. Both methods showed to be inadequate for clayed soils. Following the evidence that soil compaction could be an important factor for root growth, a study was conducted, in a greenhouse, with cork oak seedlings. Results showed that tap root length and total root biomass (coarse and fine roots) are negatively affected by soil compaction in depth. In regard to the low survival rate of cork oaks regeneration that has been observed in *Montado*, another complementary study was conducted in a greenhouse where fertilisation, inoculation with mycorrhizal fungi and aminoacids supply were tested. It was concluded that seedlings subjected to fertilisation and inoculation had a more equilibrated growth between shoot and root components. It is expected that the research developed in the present thesis can provide an essential tool for future forest planning and management and for the natural and artificial regeneration processes in cork oak stands, ensuring the maintenance of the typical *Montado* landscape.

Key-words: Cork oak; 3D root system architecture; *Montado* decline; Tree regeneration

Sistema Radical do Sobreiro (*Quercus suber* L.): Uma Abordagem Estrutural Funcional 3D

Resumo

Desde as últimas décadas que se tem vindo a testemunhar o declínio e a morte súbita do sobreiro (*Quercus suber* L.) no *Montado*. A complexidade da gestão deste sistema de produção engloba um grande conjunto de soluções que, por terem sido ausentes de validação científica (tal como a relacionada com o sistema radical do sobreiro), tem vindo a ser suportado, principalmente, pela aplicação do conhecimento empírico. A presente abordagem integrada permite uma melhor compreensão das vulnerabilidades deste sistema que pode resultar em propostas de alteração de gestão que serão úteis num futuro próximo. De forma a contribuir para uma gestão e planeamento florestal mais realistas e integrados, foi realizado um estudo acerca da avaliação morfológica do sistema radical do sobreiro num cambissolo, usando o método de digitalização 3D. O sobreiro mostrou um sistema radicular dimórfico com uma elevada quantidade relativa de *sinkers* distribuídos por todo o perfil do solo. Foi observado um subsistema à superfície, até aos 40 cm de profundidade e outro mais profundo, a cerca de 1.20 m. Observou-se também que a biomassa da árvore foi distribuída de forma similar entre os sistemas aéreo e radical. Anteriormente foi conduzido um estudo sobre as metodologias de escavação de raízes - lavagem de perfil com água e escavação por meio de jato de ar de alta pressão. Os resultados mostraram que para solos arenosos, o método mais adequado é o método por meio de jato de ar de alta pressão e, que ambos os métodos mostraram ser inadequados para os solos argilosos. Após a observação de que a compactação do solo pode ser um fator importante para o crescimento das raízes, um estudo foi realizado em ambiente de estufa com plântulas de sobreiro. Os resultados mostraram que o comprimento da raiz principal e a biomassa total de raízes (raízes grossas e finas) foram negativamente afetados pela compactação do solo em profundidade. No que diz respeito à baixa taxa de sobrevivência da regeneração dos sobreiros, outro estudo complementar foi realizado em ambiente de estufa onde a fertilização, a inoculação com fungos micorrizos e o suplemento de aminoácidos foram testados. Concluiu-se que as plântulas submetidas à fertilização e inoculação tiveram um crescimento mais equilibrado entre as componentes aéreas e radicais. Espera-se que a investigação apresentada nesta tese possa proporcionar uma ferramenta essencial para o planeamento e gestão florestal futuros e, contribuir para o sucesso da regeneração natural e artificial dos povoamentos de sobreiro, garantindo a manutenção da paisagem típica do *Montado.*

Palavras-chave**:** Sobreiro; Arquitetura 3D do sistema radical; Declínio do *Montado*; Regeneração

CONTENT

LIST OF FIGURES

CHAPTER 1 GENERAL INTRODUCTION

CHAPTER 2 COMPARISON OF TWO METHODS TO ASSESS THE ROOT ARCHITECTURE AS A POTENTIAL FACTOR INFLUENCING THE DIVERSITY OF A STAND

CHAPTER 3 MORPHOLOGICAL EVALUATION OF CORK OAK ROOT SYSTEM USING A 3D APPROACH

CHAPTER 4 THE EFFECT OF SOIL COMPACTION AT DIFFERENT DEPTHS ON CORK OAK SEEDLINGS GROWTH

CHAPTER 5 CORK OAK SEEDLINGS GROWTH UNDER DIFFERENT SOIL CONDITIONS FROM FERTILISERS, MYCORRHIZAL FUNGI AND AMINOACIDS APPLICATION

LIST OF TABLES

CHAPTER 2 COMPARISON OF TWO METHODS TO ASSESS THE ROOT ARCHITECTURE AS A POTENTIAL FACTOR INFLUENCING THE DIVERSITY OF A STAND

CHAPTER 3 MORPHOLOGICAL EVALUATION OF CORK OAK ROOT SYSTEM USING A 3D APPROACH

CHAPTER 4 EFFECTS OF SOIL COMPACTION DEPTHS ON CORK OAK SEEDLINGS GROWTH

CHAPTER 5 CORK OAK SEEDLINGS GROWTH UNDER DIFFERENT SOIL CONDITIONS FROM FERTILIZATION, MYCORRHIZAL FUNGI AND AMINOACIDS APPLICATION

Preface

This thesis is structured in seven chapters. Chapter 1 consists of a general introduction with the main objectives of the research highlighted. This chapter also provides a relevant literature review about the decline of *Montado*, tree root systems and the role of cork oak root system as a structural and functional element in the *Montado* landscape dynamic, justifying the relevance of this study at the landscape level. This additional literature is focused mainly on topics that are not extensively mentioned in the literature reviews of the remaining chapters. Chapters 2, 3, 4 and 5 correspond to the main research studies, which are presented through scientific articles format (published, submitted or ready for submission for future publication), including a theoretical framework (introduction), methods, results, discussion and conclusions. A number of illustrations that were not included in the articles, due to high publication costs, are presented in this thesis in order to emphasize important research points. The studies presented in these chapters integrate the research project: "Determination of Methodologies for Adult Cork Oak Trees Transplantation". Chapter 2 includes a study about comparison of methods to access tree root systems. Specifically, soil profile washing with water and high pressure air jet methods were tested. The results of this study provide important information regarding the choice of which method should be applied according to soil characteristics. Chapter 3 presents one of the most important parts of the research undertaken, consisting of an intensive study about the functional structural evaluation of the cork oak root system development in a Cambissoil soil. The approach applied in this study was the use of three-dimensional scanning techniques and the evaluation of important variables to describe the morphology of the roots (biomass, length, volume, etc.). One of the major contributions of this study is the quantity and quality of information generated, non-existent to date, which can be used as a basis for future decision support systems in planning and management of cork oak stands. The experimental study described in Chapter 4 emerged from the evidence observed during the study of cork oak root system (Chapter 3), that is, that soil compaction in depth can be a determining factor for the tree root system morphology and, consequently, contribute to the lack of natural regeneration observed in *Montado*. Following these observations, an experimental study was carried out under greenhouse conditions. This study assessed the effects of soil compaction at different depths in the cork oak seedlings growth. Still regarding the lack of natural regeneration, and taking into account the studies that have been conducted in the main research project about the determination of methodologies for cork oak transplantation, an additional study on cork oak seedlings was carried out in a controlled environment (Chapter 5) . This study served to test the influence of fertilization, mycorrhizal fungi and aminoacids applications on cork oak seedlings growth. The main purpose was to promote a reduction in the time required for regeneration and, simultaneously, to reduce the stress effects of post-transplant. Chapter 6 presents the general discussion and conclusions regarding all the results from the previous chapters. The final remarks and some technical improvements, suggested for future forest management and planning, are in chapter 7, which also highlights the relevance of this work and some perspectives for future research lines.

CHAPTER 1	**General Introduction**
CHAPTER 2	**Comparison of Two Methods to Assess the Root Architecture as a Potential Factor Influencing the Diversity of a Stand**
CHAPTER 3	**Morphological Evaluation of Cork Oak Root System Using a 3D Approach**
CHAPTER 4	**Effects of Soil Compaction Depths on Cork Oak Seedlings Growth**
CHAPTER 5	**Cork Oak Seedlings Growth under Different Soil Conditions from Fertilisers, Mycorrhizal Fungi and Aminoacids Application**
CHAPTER 6	**General Discussion and Conclusions**
CHAPTER 7	**Final Remarks**

CHAPTER 1

GENERAL INTRODUCTION

1.1 Background

The awareness that the dependence of the relationship between the use of territory by human and the natural cycle's dynamics (water and carbon) is what enables and supports the existence of life on earth, has promoted a growing concern about environmental issues by the society. Nowadays, notions about scarcity of natural resources are increasingly present and the search for sustainable solutions to conservation is rising. Globally, targets and measures to ensure the sustainability of these resources for future generations have been imposed.

Existing concerns about water and greenhouse gases, have taken a growing relevance worldwide. To achieve the desired goals in the regulation of these parameters it is essential to increase the knowledge not only about the natural cycle's dynamics but also about the influence of ecosystem management in these cycles. Forests are recognized as having a crucial role in sustaining water and nutrient cycles. An elevated biodiversity, expressed through different degrees of tree cover (trees of different ages), shrub and herbaceous entitling a wide variety of vertical and horizontal vegetation structure, benefits the functionality of the referred cycles. The maintenance of these vegetation structures through low impact forestry operations allows a stability that is crucial to the quality and durability of ecosystems.

With the Water Framework Directive (Directiva 2000/60/CE - Jornal Oficial das Comunidades Europeias, 2000) Portugal, as all member states, aims to achieve by 2015 a good qualitative status of its water resources through a series of ambitious goals. These goals, which are reflected at watershed management plans scale and include the polluter-payer principle, are quite demanding. The two major goals outlined in this policy are the characterization of the maximum ecological potential conditions for the main water reservoirs, and the creation of a method for potential ecological evaluation. For environmental protection purposes this document also dictates a better integration of the qualitative and quantitative aspects of surface and ground water, regarding the natural water flow conditions within the hydrological cycle. Thus, the purposed challenge requires a greater knowledge about the hydrological balance functioning within the forest systems. The specific issue of quantification of how much water the forest uses and resets to the system constitutes a stimulating challenge that could be integrated into watershed management plans.

The same can be applied to carbon sequestration. It is generally known that forests capture and sequester more carbon compared to what they release to atmosphere, but the knowledge about the quantification and therefore the attempt to assign real value to indirect use of this service is minimal. The European carbon markets in functioning since 2005 and, at the same year, the Kyoto Protocol (http://www.apambiente.pt/), bring new dynamics and interest regarding carbon cycle knowledge.

For Portugal to adopt a competitive strategy it is essential and fundamental to know how much carbon is captured and sequestered by our forest ecosystems. However to accomplish this target it is necessary to understand the forest systems dynamics, assuming the multifunctionality of their uses. It is also important to highlight that the management of forest multifunctionality is what enables forest goods and services provision to society, both facilities or amenities.

In the south of Portugal co-exist, among others, two different vegetation stand structures. Where the tree density is high and the goal is forest productivity (cork, wood, etc.) the system is defined as cork oak (*Quercus suber* L.) or holm oak (*Quercus rotundifolia* Lam.) stands. When the system integrates several functions it is called cork oak or holm oak woodland referred in Portugal as *Montado* (*Dehesa* in Spain). The *Montado* supports multiple land uses, combining the exploitation of tree cover, and a rotation of grazing, cultivation and fallow in the undercover. This agro-silvo-pastoral system has existed for centuries in a more or less developed and intensively managed form (Surova & Pinto-Correia, 2008). *Montado* ecosystem has a variety of different vegetation structures, the most predominant of which is a savannah-type landscape mainly composed by scattered trees of *Quercus* species, cork oak (*Quercus suber* L.) and holm oak (*Quercus ilex* subsp. *rotundifolia* Lam), without shrubs and only with an herbaceous substrate. It is common to use this herbaceous layer with cereal production. In areas of less accessibility, such as in high slopes, stands are more closed and the shrub layer develops under the tree canopies.

Cork oak (*Quercus suber* L.) is a large and long-living tree, spatially distributed across the Mediterranean basin, which is part of the Mediterranean *Montado* structure. This ecosystem has a high national importance not only due to the production of cork, but also because of their vegetation formations that have an extraordinary ability to adapt to the specific climatic conditions of the Mediterranean climate (Moreno & Oechel, 1995). These formations have the capacity to support large climatic variations over the year, a long period of dry conditions in summer and cold and humid conditions in the winter (Otieno *et al.*, 2006).

In Portugal, the current area occupied by *Montado* of cork oak is about 715 922 ha of which 601 90 6ha are in the south, in the Alentejo region (AFN, 2010). This area represents 22% of the total country forested area and 33% of the total world area occupied by cork oaks (Ribeiro *et al*., 2010). Due to the high density of this species in the country, Portugal is currently the world leader in cork production and exportation (representing 54% of the cork annual average world production). Portugal is also responsible for the transformation, by the cork industry, of 70% of the cork produced globally.

The *Montado* ecosystem is classified as a "High Nature Value Farming System" according to the European classification proposed by the European Environment Agency (Paracchini *et al*., 2008). Ecologically, this ecosystem promotes a high amount of benefits and services. The elasticity and resilience of *Montado* is good, but can be disturbed by extreme changes of various nature. According to Ribeiro *et al.* (2004) this may happen both by (1) random, external variables that relate to tree mortality, tree damage and intensity of natural regeneration, and (2) management-based variables that can affect the system at tree level physiology (debarking, crown pruning and root pruning) and at site level mainly by soil structure modifications (soil mobilization, erosion risk, organic matter depletion, fertility loss, etc.).

For Portugal, *Montado* ecosystem has a high ecological representativeness contributing to biodiversity, watershed protection, CO_2 fixation, soil improvement and flood protection, among others (Millenium Ecosystem Assessment, 2003). *Montado* benefits rely on nutrients and soil formation cycles hence their trees have the ability to capture large quantities of nutrients not only at superficial soil layers but also at major depths, which otherwise would be inaccessible to vegetation (Millenium Ecosystem Assessment, 2003). Biota of the ecosystem plays an important role in nutrients storage and recycling. The trees provide a lot of material that rots in the soil as humus, enriching it with nutrients. As mentioned before an important *Montado* benefit and regulatory function is carbon fixation through the reduction of greenhouse gas emissions (all plants store carbon dioxide - CO_2 - captured from atmosphere). Healthy cork oak stands with reasonable tree cover can sequester annually 1 – 3 tons of carbon per hectare (Pinto-Correia *et al*., 2013). These values are close to the Central European forest's mean, although the variability is very high. Carbon balance values tend to decrease with aging and sick trees (after reaching a maximum); water deficit; fire and high impact forestry operations which increase soil erosion. Each time cork is removed and cork oak trees are pruned, they absorb more CO_2 (Pinto-Correia *et al*., 2013). Other benefit of this system is the control of erosion, by wind and rainfall. Forest vegetation increases the rate of infiltration and evapotranspiration (namely rainfall interception and transpiration), decreasing the amount of streamflow and aquifer recharge, compared to short vegetation.

Montado is also presented as a singular Mediterranean forest ecosystem, extremely valuable in biodiversity and is identified as of upmost importance for nature conservation at national and European level. What is the contribution of the *Montado* for water cycle and carbon sequestration? How does forest management of this multifunctional system can enhance this contribution? And how can the trees role in these dynamic cycles be studied? Knowing that the tree water and nutrients uptake is made through its root system, the functioning of the referred cycles in *Montado* can be evaluated through the understanding of the relationship between tree root system and the surrounding matrix.

In an attempt to valuate these benefits and functions it is necessary to increase the resolution scale to an individual level scale – the tree. Only after understanding the relationship between roots and the surrounding matrix it is possible to assess the tree contribution to the water and carbon cycles. Expectedly, the ideal would be to extrapolate these models to *Montado* and watershed scale.

Current climate change scenarios point to an intensification of dry periods in Mediterranean climate regions (Vaz, 2005). This fact will lead to an intensification of vegetation drought stress in these regions and thus carbon assimilation and transpiration will be severely restricted (Pereira *et al*., 2004) which may jeopardize the survival of the trees. Otieno *et al.* (2006) assume that, in regard to *Montado* ecosystem, the establishment of only certain specific species, their distribution, and their mortality appear to be partially controlled by edafoclimatic conditions. Plants that have the ability to keep physiologically active for long periods of summer dryness must have access to deep soil layers which remain wet for a longer period of time (Rambal, 1984). Also the plants must have the ability to exploit large volumes of soil (Breda *et al*., 1995) and redistribute water within the soil profile through roots (Ryel *et al*., 2003; David *et al*., 2013).

Trees act as propulsion pumps of nutrients, enabling the uptake of nutrients from deeper soil areas and lateral areas underneath the canopy projection, depositing them in the most superficial soil layers (Joffre, 1999). In general, the trees rooting depth is greater in dry environments with seasonal drought (Canadell *et al*., 1999; Otieno *et al.*, 2006; Schenk & Jackson, 2005). David *et al.* (2007, 2013) and already in 2005, Lubczynski and Gurwin reported that the evergreen trees from Mediterranean *Montado* depends greatly on groundwater tables.

Ecophysiologicaly the processes of resistance to the summer drought stress by the cork oaks are certainly related to maintaining internal water homeostasis (Losch & Schulze, 1995; Kurz - Besson *et al*., 2006; Otieno *et al*., 2006; Palace *et al*., 2009) and to ensure the carbon balance (Pereira *et al*., 2004; Unger *et al*., 2009). Lima (2008) in his review about cork oak root system, listed the following strategies for this species: 1) interruption of shoot growth during the summer when the dawn water potential in the upper soil layer (where more fine roots are present) is about -1.5MPa, 2) emission of deeper fine roots to capture the water available in the soil, 3) secondary production of narrower conducting vessels in the summer, to replace the large vessels produced earlier in the spring, preventing the xylem cavitation, 4) maintenance of an efficient transport of water between roots and leaves and; 5) extraction of water from the soil at a reduced mean transpiration rate associated to minimal seasonal stomatal conductance. In this ecosystem that depend on the groundwater, the lowering of the water table level can increase the tree water stress and, consequently, promote the leaf fall which may lead to the death of trees (Zencich *et al*., 2002; Cooper *et al*., 2003).

As mentioned, cork oak must have structural, functional and ecophysiological response patterns which allow the withstanding of the seasonal effect of intense water stress, imposed by the Mediterranean climate during summer (Aranda *et al*., 2007; Costa *et al*., 2009; David *et al*., 2004; 2013; Gouveia & Freitas, 2008; 2009; Ja *et al*., 2011; Kurz-Besson *et al*., 2006; Otieno *et al*., 2007). It is thus crucial to understand the access of cork oak root system to groundwater to evaluate the water balance of the tree and consequently of the *Montado* (Lacambra *et al*., 2010). For that purpose it is necessary to identify what structural and functional mechanisms occur in the root system and what is the relationship between roots and the surrounding matrix, at different growth stages (seedling and adult stages).

Seedling stage is one of the crucial steps in the tree development. The behavior of mature shoot and root systems are the result of the growth conditions at the initial phase. In addition to the intrinsic factors inherent to plant growth, extrinsic factors are decisive for the development of the tree. Essentially, the morphological structure of mature root system is a result of the roots soil exploitation strategy at the first stages. This initial root growth strategy is related with the rhizosphere characteristics where the plant develops. During their growth, roots explore the soil searching for water and essential elements which promote the structural functional support of the tree (Coder, 2007). Is this initial growth and development strategy that will define the morphological structure of mature root and shoot system.

Knowing that the root system is responsible not only for the tree fixing and support but also for water and nutrients uptake and transport to the shoot, it is easy to understand the importance of root systems on the dynamics of *Montado*. Because we are not dealing with a static and timeless ecosystem, to understand the root system development it is necessary to relate the belowground system (root) with the aerial system (shoot); with the biophysical parameters of the surrounding matrix (soil type, water and nutrients availability); with ecophysiological parameters (water and nutrients uptake and transport and carbon assimilation); and with the soil tillage applied (disking, clear cuts, traction) (Figure 1). It is important to mention soil tillage techniques because bad forest management practices or aggressive farming techniques can induce the destruction of vegetation, roots and fungi which are essential to provide a good state of *Montado* conservation. These facts, together with soil erosion, can lead to the weakening and diminish of the tree water and nutrients absorption capacity promoting the tree water stress (internal dry) which consequently will affect directly the water balance.

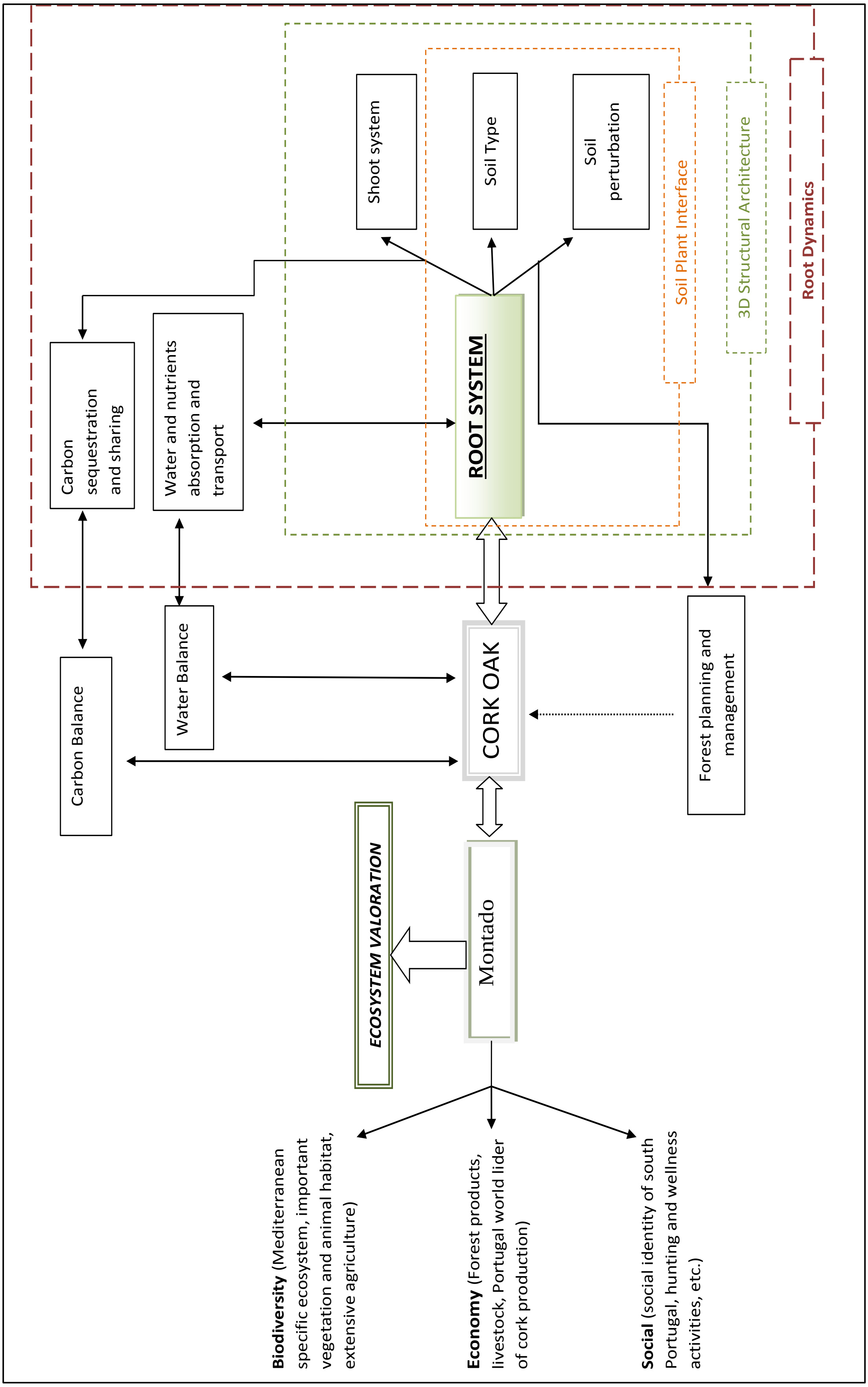


Figure 1. Explanatory model of research background.

The knowledge about this topic is almost nonexistent either at national or international level. The difficulty and time consuming nature of obtaining root data, as well as the costs associated with this type of research, explains the lack of research focusing on this topic. However, as mentioned before, the need to improve the knowledge of root systems and their relations with the subsoil, which are clearly conditioned by the surrounding matrix processes, is increasingly relevant. In the specific case of cork oak, forests researchers but also plant physiologists have studied more intensively the shoot system. The only studies identified that relate to adult cork oak root system are: i) Surový *et al.* (2011) where 12-years old root systems were partially accessed to verify the influence of field installation process (planting and seeding) on the morphology of root systems. These authors compared the distribution of roots along the soil depth profile; ii) Metro and Sauvage (1957) where the root systems of some adult cork oaks of different ages from Mamora forest were fully described in terms of horizontal and vertical distribution; iii) Kurz-Besson *et al.* (2006); Nadezhdina *et al.* (2008) and David *et al.* (2013) who focused their studies on the structural and/or functional aspects of cork oak root system, namely on root hydraulic redistribution.

Tree root systems can be studied having different approaches, according to the specific objectives of the study. A structural functional approach is essential to understand the development strategy of roots in the soil. Recent methods to evaluate root systems, such as tridimensional digitizing, have the possibility to bring new insights about this research area. This method is largely discussed in Chapter 3 which represents the main research study of this thesis.

1.2 Objectives

This research was developed under the research project “Determination of Methodologies for Adult Cork Oak Transplantation". This project was financed by a consortium between the University of Évora and a private company, TRA (Transplant Trees, Ltd). This project presents itself as innovator and pioneer in the research area of cork oak root system as it will not only add to the existent studies but will also potentially enable the development of a new range of scientific and technological research studies.

Also the understanding of these underground structures will influence different research fields, such as environment, ecology, ecosystem valuation, economy, etc...

The main objectives of this thesis are:

i) To evaluate the morphological, structural and architectural characteristics of cork oak root system through the three-dimensional digitising method;

ii) To understand the root distribution pattern on depth and extend and accordingly to soil conditions;

iii) To relate the cork oak root system behavior with the surrounding matrix focusing mainly on soil type, soil mechanical impedance and effects of nursery cultivation for future field establishment (fertilisation, mycorrhizal fungi inoculation and aminoacids supply).

For a common and universal evaluation of the results obtained during this research process, it was decided to adopt the world soil classification from FAO (2006).

1.3 Review of Relevant Literature

1.3.1 *Montado* Landscape

The definition of landscape has been evolving over time. According to the review made by Filho (1998) the concept of landscape has changed due to an attempt to integrate all the system´s constituents. In the abovementioned review the author emphasizes the Bertrand (1968) landscape definition as "*a particular portion of space that results from the dynamic combination of physical, biological and human elements which interact dialectically on each other form a single and inseparable set in perpetual evolution*". In 1972, Zonneveld (*in* Filho, 1998) described landscape "*as a part of space on Earth's surface covering a systems set characterized by geological, air, plants, animals and human and their resulting physiognomic forms, which may be recognized as entities*". More recently, according to the European Landscape Convention (2000), landscape was defined as "*the result, observed by Man, of a complex and dynamic system of many natural and cultural factors that influence each other and change over time*". All the definitions abovementioned identify the need of thinking about the landscape as a dynamic system in constant variation of their multiple system "components".

A landscape can be in equilibrium or exist in different states of equilibrium, depending on its resistance level to disturbance and also on the ability to recover from a disturbance process. Moderate disorders of the landscape elements (such as vegetation) provide more patches in the landscape. However, severe disturbances of those elements can eliminate the presence of patches, resulting in a complete landscape change. For instance, adverse climatic and environmental conditions and certain soil uses may promote a patched distribution that is totally distinctive from the original landscape distribution.

Biodiversity is an integral part of the landscape allowing for variability. This variability can be expressed into economic, social and environmental improvements. Bioindicators are essential tools for planning and according to Quine and Watts (2009) and Di Giulio *et al*. (2009) these bioindicators should be applied at different spatial scales, describing the variation of the landscape functions, and taking into account the specifications of the spaces that are to be managed. The application of bioindicators at different temporal scales should also be taken into account in the context of the landscape multifunctionality.

For instance, in agricultural systems the management can be made in certain cases at short-medium term, but if we are dealing with a forest system management it should be planned for a medium - long term. It is of the upmost importance to face the composition of a space as a set of existent processes that occur in that space. It is also necessary to be aware that the loss of a certain potential at any of the system levels will be expressed as a loss of value on the entire space.

Forest ecosystems are one of the existing landscape types. The forest ecosystem can be considered as a set of biological communities and the abiotic environment in which they live. These ecosystems are places of high metabolic activity where the water and energy fluxes are strongly influenced by the existence of trees and by their density. Tree canopies absorb large amounts of solar radiation for food production, but can also shade other plant species, which will influence the type and diversity of species occurring below the canopy. Trees also modify substantially the forest ecosystem’s microclimate. In this type of ecosystems the relations with the soil are strong, not only because the soil serves as a source of raw materials for the tree, but also because the level of organic processes occurring in it will influence the soil quality and the productivity of trees. The main characteristics of the forest ecosystem are the strong dependence of the natural environment, the difficulty to distinguish between productive capital and incomes, the production of long-term benefits, productive externality, residual value of products, goods and services, production variability, impact of forest operations on the vegetation and fauna and management of marginal productive spaces, among others. The human species although sheltered by culture and technology from the immediate effects of environment, is ultimately dependent on a number of ecosystem services (Millennium Ecosystem Assessment, 2003). In this context ecosystem services are defined as all the benefits that people get from ecosystems: food, fiber and water; regulation services; cultural services (relating to aesthetic, spiritual or recreational experiences); and support services (such as biogeochemical cycles, soil formation and primary production of ecosystems) (Millenium Ecosystem Assessment, 2003). However in these multifunctional ecosystems (forests) there are still issues in the definition of multifunctionality for each space. To focus on forest multifunctionality it is necessary to know previously what model space will be evaluated and particularly, concerning to forest planning and management, it is necessary to be aware of the temporal scale.

There are few natural forest systems still in existence worldwide. Most of them have been influenced by humans thus becoming humanized such as the case of *Montado*. The *Montado* ecosystem is a specific landscape type that is constantly changing without being noticeable on the scale of human life. This type of landscape is characterized by a savanna forest type with the existence of open spaces, allowing a high biodiversity, both locally and at a regional level. The presence of many endemic species in these landscapes is also highlighted. *Montado* can be divided into three categories: *Montado* of cork oak (*Quercus suber*), dominated by cork oak species; *Montado* of holm oak (*Quercus rotundifolia*) in which the predominant species is the holm oak; and mixed *Montado* in which the coexistence of the two species is observed in the same space. Additionally to cork oak and holm oak their association with other species, such as the stone pine (*Pinus pinea*) and olive trees (*Olea europaea*) is often observed. The *Montado* ecosystem is legally protected in Portugal by the Law No. 169/200137 and in Europe through the Directive 92/43/CEE38.

Due to the desertification observed essentially in the south of the country, the components of ecological biodiversity linked to the trees and land use management, turned the *Montado* ecosystem into a heterogeneous, dynamic and sustainable landscape with increasing contrast mosaics (Pinto-Correia *et al*., 2011). As mentioned before, cork oak *Montado* in Portugal are dominated by cork oak tree which is a native oak species, evergreen and is distinguished from other oak species because of their typical bark – the cork.

1.3.2 Cork Oak Decline

The last decades (since the 80’s) have been witnessing the decline and sudden death of the cork oak in Portugal (Ja *et al*., 2011; Ribeiro & Surový, 2008; Sousa *et al*., 2007). However, the factors involved in this phenomenon are not fully understood. Inadequate control of shrub removing operations in the *Montado* during the second half of the 20th century; pathogens establishment and increased aggressiveness; and drought stress have been suggested as some of the factors related to the cork oak vulnerability (Azul, 2011).

However as early as the 50's, Natividade (1950) highlighted the importance of facing and evaluating this typical ecosystem through a new perspective: "*The intensive tree and soil exploration, a seductive and misleading cultural technique, badly adjusted to the agro climate conditions of the country; the disinterest on the deciduous trees replacement; the premature cut or the no use of very valid and promising wood; the enlargement of crop cultures; the expansion of pine and olive forests; the diseases and pests and the climate fatalities, weakened and continuous to weaken day after day the Portuguese cork heritage and threaten its future"*. Nowadays, the declining signs of cork oak trees patches throughout the country are indisputable and the lack of natural and/or artificial regeneration is visible. These trends may result, among many other factors, from various aspects carried out for decades, many of which still continue to be observed nowadays in the conduction of *Montado*. The over exploration of the tree constitutes a threat with the excessive pruning to produce firewood, wood and coal as well as the bad practices applied on the debarking causing irreparable mutilation of trees, leading them to death. The soil over exploration through intensive cultural practices; the use of heavy machinery; the soil mobilization with impact on tree roots; misuse of pesticides and an excess on the number of animals (especially occurring due to the previously replacement of pigs and sheeps for cows, causing a major impact) lead to the weakening and decreasing of the tree´s ability to absorb water and nutrients, creating a situation of water “stress" on the tree (internal dry) and consequently, causing its death (Matias, 2008). Other causes of cork oak decline include bad management practices with the use of aggressive farming techniques destroying vegetation, roots and fungi, essential to a good soil preservation. This fact together with soil exploration enhances the soil erosion, decreasing soil water availability. These factors will interfere on the tree water, carbon and nutrient cycles.

Initially cork oak decline was only associated to pest and disease infestation (*Phytophthora cinnamomi* Rands and *Armillaria mellea* (Vahl:Fr.) Kumm on the root system; *Botryosphaeria* spp., *Biscogniauxia mediterranea* (De Not.) Kuntze (Syn *Hypoxylon mediterraneum* (De Not.) Mill.), *Coryneum modomium* (Sacc.) Griff & Maubl. *and Endothiella gyrosa* Sacc. on the trunk and branches) . However due to the high complexity of the ecosystem, many other factors that could act directly on the imbalance of the system, including physical and chemical properties of soil and climatic factors (mainly the drought periods) are starting to be considered as well. These factors have been reported to be determinants for cork oak decline evaluation (David *et al*., 2004;

Sousa *et al.*, 2007). Sousa *et al.* (2007) also identified the human activities on forest and stands management, the air pollution and the forest fires as possible factors related to cork oak decline (Figure 2).

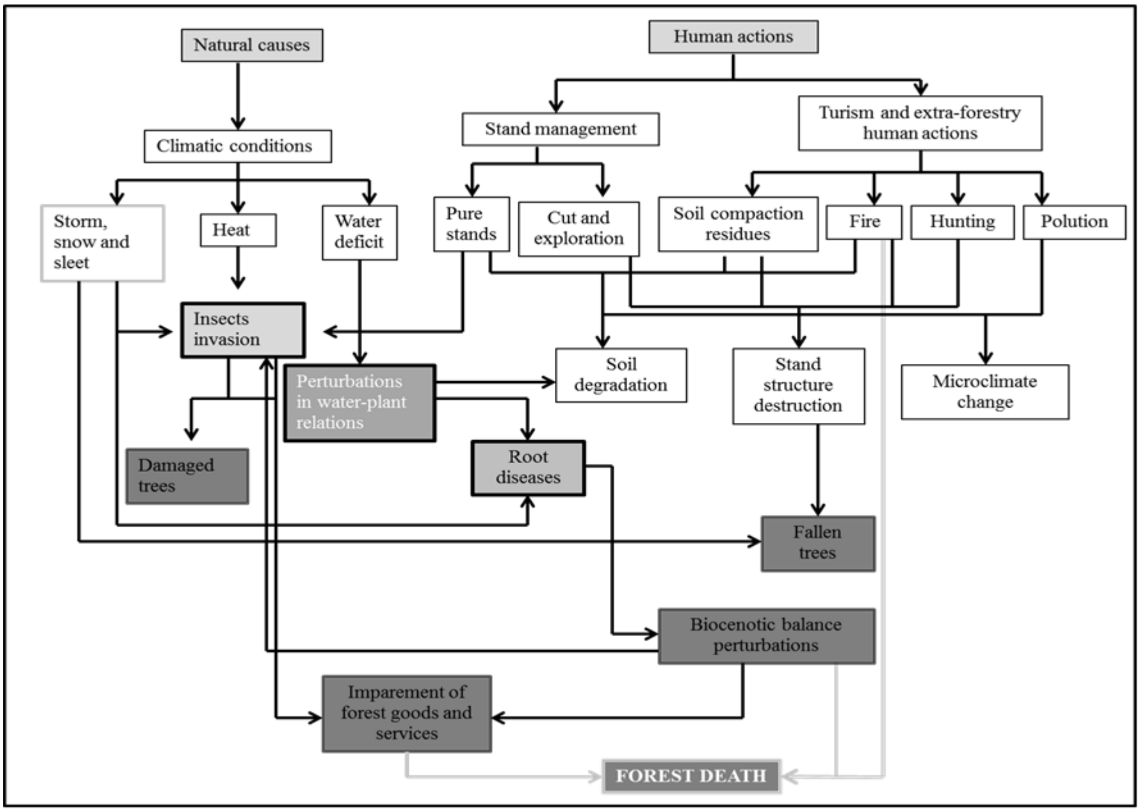


Figure 2. Main factors associated to cork oak stands decline in Portugal (adapted from: Sousa *et al*,. 2007).

The misconception that cork oak trees presenting thermophilic and xerophilic characteristics would be better adapted to extreme conditions of temperature, associated with climate changes, and the consequent potential increase of the area of this species has been discarded. Matias (2008) states that, given the scenarios of increased droughts and taking into account that, currently, many stands are already debilitated, this increase could lead to a further decline in the stands, especially in warm and dry areas of the interior of the country.

Regarding human actions on the management of the stands, in the second half of the century they were characterized by bad farming practices and excessive use of heavy machinery. These actions probably caused an intensive damage on tree roots, especially in dry regions or during the drought seasons when trees become more dependent on their extensive shallow root systems to survive (Acácio, 2009; David *et al.*, 2004). The destruction of acorns and regeneration plants, due to the excessive pressure of cattle in settlements observed in recent decades (Campos *et al.*, 1998), combined with the factors mentioned before, contributed decisively to the observed cork oak decline.

Ribeiro and Surový (2008) in their study regarding the cork oak mortality in Portugal, identified a set of interactions occurring between the trees and the biophysical environment, based on diagnostic features. They observed that a close relationship exists between these interactions and the observed decline. Some conclusions of this study are: 1) on land with slopes between 15 and 35%, cumulative effects of soil erosion (conditioned by shrubs control made with mobilization of soil) led to a loss of soil thickness usable by trees; 2) on sloping land (between 15 and 35%) combined with a depth soil limit (textural discontinuity, effective thickness and expandable depth), the effects of soil tillage are more intensively felt limiting even more the volume of soil available for trees; and 3) soil tillage creates a debilitating interaction between soil volume loss (slow process and dependent on the slope value) and the loss of a significant amount of the root system (quick process that occurs periodically during the moment of soil mobilization). The referred root loss is an important issue hence cork oak trees have a surface and deep thick root distribution in which about 80% of fine roots are distributed in the first 30cm depth. Ribeiro and Surový (2008) also referred that the effects of cuts, further healing and later replacement of damaged roots consume a significant amount of tree resources facilitating the appearance of multiple infection points, especially by *Phytophora cinnamomi*.

1.3.3 The Tree Root Systems – A Complementary Review

1.3.3.1 Terminology

Throughout this thesis some specific terms about root systems will be used. Thus, in order to clarify the interpretation and harmonize the terms used, a short summary of the definitions is presented (adapted from Lyford, 1980).

Root system: The whole structure of fine and coarse roots that extends horizontally, vertically and diagonally in the subsoil. Tap root: first root to be formed. It is an individual and central root that presents a vertical growth through depth. This root emerges from the acorn. Initially, during the acorn germination phase, it is called radicle. This root is often regarded as the extension of the trunk in the subsoil. Lateral root: any root originated from another root. The primary lateral roots originate in the tap root. Parental root: root that originates another root. Replacement root: any lateral root that was formed due to injuries in the parental root. For most species, the replacement roots follow its elongation growth in the same direction that the injured root was taken. The replacement roots are usually classified with the same order of the root that was previously damaged. Root cap: terminal root zone that protects the apical meristem from the abrasive action of the soil during the root elongation. Root tip: terminal portion of the root that contains live cortical tissue that extends from the terminus of the root cap back to either the first lateral root or if, lateral is non-existent, until the parent root. May or may not have mycorrhiza. These root portions are divided into 4 distinct areas: 1) cap; 2) Region of cell division (production zone of new cells promoting the root growth); 3) elongation region (zone where cells grow and elongate in size towards the root axis); and 4) maturation region (zone where differentiation of root tissues occurs and where root hairs appear, promoting an increase in the surface area available for water and nutrients absorption and uptake).

Branching order: degree of bifurcation. Lateral roots of first order originate from tap root; second order lateral roots originate from 1st order lateral roots and so on (Figure 3). Longitudinal spacing: distance between lateral roots along the root axis. Central root system): group of roots located on the central tree basis which can extend laterally and in depth, about 2meters. In this system are included the tap root and a high portion of lateral and vertical coarse roots. This system includes the majority of first order roots and is responsible for the tree fix and support in the ground. Outer root system: all the rest of roots that extend far behind the central root system. Due to the high distance between these roots and tap root, they present smaller diameters becoming more flexible and consequently, more subject to an easier break in adverse conditions (eg., strong winds).

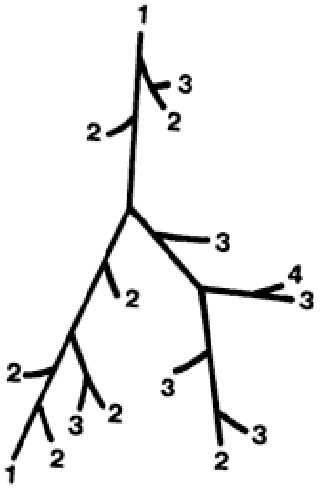


Figure 3. Example of a schematic diagram of root system composition, representing the branching order classification according to root formations.

Woody or coarse roots: woody roots with rough and thick structure due to secondary xylem. Usually woody or coarse roots of forest trees present diameters greater than 2mm. Non-woody or fine roots: fine and flexible roots with diameters less than 2mm. Structural roots: thicker roots that are responsible for tree supporting (structure). These roots can be simultaneously structural and functional. Functional roots: roots that have as primordial role the maintenance and functionality of the tree. These roots are responsible for water and nutrients transport, from water caption in the root/soil interface to water achieving the trunk column, promoting the balance between root and shoot system. Sinker roots: roots usually from secondly or thirdly order originated from parental roots that develop horizontally. These specific roots present a woody structure and develop vertically into depth and parallel to tap root. Normally they can reach higher depths than tap root. Their main function is to capture water from low water table levels and transport it to the trunk or, when necessary, transport the water from deepest levels and release it in first horizon layer, process called *hydraulic lift.*

Root grafts: small structures formed by the conexion of two coarse roots that grow closely to each other or that in some point cross each other. This conexion can be so strong that sometimes roots can even share their internal tissues (xylem, phloem and cambium), resulting in a root intergrowth. These root grafts can occur between roots of the same tree or can be formed by the conexion with roots from neighboring trees. Turnover: life time of fine roots since their formation until their death. Turnovers vary according to tree species, root diameters, soil type, etc.

1.3.3.2 Roots Formation and Functions

Generally, the initial root systems of all seeds develop along a single axis that grows in depth, the tap root, from which the lateral roots develop. These roots will form a complex root network of various orders that develop either horizontally, vertically and diagonally, originating the root system of the adult tree (Hodge, 2009). However, in most of the tree species the tap root dominance will decrease as tree grows, with that dominance replaced by secondary roots (Sutton, 1980).

All the roots from the most various species of plants have a common characteristic: the continuous root growth takes place from the division and subsequent extension or elongation of the cells from the apical meristem (Oliveira, 1988; Hodge, 2009; Pacheco-Villalobos & Hardtke, 2012). During the formation of the lateral roots a group of pericycle cells become meristematic and form a salient lateral root primordium that grows through the endodermis, cortex and epidermis (Guyomarch *et al.*, 2012; Nibau *et al.*, 2008). Before breaking through the surface tissue of the parental root the lateral root develops a well-defined apical meristem and a root cap. Both the surrounding tissue digestion and the mechanical pressure seem to be involved in the lateral roots growth through the cortex (Kozlowski, 1971; Malamy, 2005).

Despite the lack of knowledge concerning root growth, opportunism seems to be the main cause of its growth both in terms of time and in terms of orientation. Growth occurs when and where water, oxygen, nutrients and minerals are available (Guyomarch *et al*., 2012; Malamy, 2005). The roots grow most of the year stopping only when soil temperatures are too low (Kozlowski, 1971). The roots can be woody and perennial (thick roots) or with absorptive characteristics and annuals (fine roots). Fine roots die and are replaced by others also with absorptive functions. These roots are usually horizontally arranged forming a network mainly in the first soil layers. Woody roots become thicker every year (Gautam *et al*., 2003) and can grow to a greater or lesser depth, in order to enable the tree establishment on the soil.

In a simplistic way the root systems structural architecture consists of a tap root; lateral coarse roots (greater than 2mm diameter) which can have horizontal or vertical direction and different orders; and fine roots (with less than 2mm diameter). Each order is characterized by its unique set of parameters that describe the frequency of branching, elongation rate, growth direction, biomass deposition rate and time. These parameters will determine firstly the functional properties in terms of support, water and nutrient uptake and transport; and secondly determine its influence on the rhizosphere (Tobin *et al*., 2007).

The first studies about root systems, as the one of Büsgen *et al.* (1929 *in* Perry, 1982), already identified that in some species the tap root persists until adult stage and that these structures present higher diameters just below the trunk zone. A drastic decrease of tap root diameters was verified with depth. These authors identified three types of root systems. Despite the fact that these definitions are considered as generalist, they continue to be use nowadays: i) sharped root systems, characterized by the dominance of a primary root that develops vertically in depth on the same axis as the trunk (eg., *Quercus robur* L., *Pinus sylvestris* L. and *Abies alba* Mill); ii) fasciculated root systems, characterized by the presence of thick roots of different diameters that descended diagonally in relation to stem axis (eg., *Betula* spp., *Fagus* spp., *Larix* spp., *Tilia* spp., *Acer platanoides* L.); and iii) surface root systems, characterized by the existence of thick roots that grow both horizontally and vertically, just below the soil surface line, and from which smaller roots develops vertically in depth (eg., *Fraxinus* spp., *Populus* spp., *Picea abies* Karst., *Pinus strobus* L.).

To understand the root growth pattern level it is necessary to know how the roots explore the subsoil area. Generally, root soil penetration occurs through the pores presenting greater or equal diameters as the root end section (Coder, 2007). When the pore diameter is less than the root diameter and if the soil is easily deformed, roots will exert a certain pressure on the surrounding particles enabling an increase on pores diameter. This will allow the continuous root growth through soil until mechanical soil impedance gets too high (Taylor & Gardner, 1960; Kristoffersen & Riley, 2005). When the soil structure presents a greater rigidity, disabling the movement of the soil particles, the root does not penetrate. Instead, roots become ticker and invest on the branching process (Bengough & Mullins, 1990; Day *et al*., 2010; Hisinger *et al*., 2009; Hodge *et al*., 2009). If the soil pores size is too small even for lateral roots growth the entire root system becomes atrophied.

The reason for how or why only some thick roots develop from a range of numerous fine roots of first order, originated from the taproot, remains unexplained. The process by which some roots develop to thick roots occurs due to the increased growth of the primary tissue. It is noteworthy that the rate and time duration of root growth may vary, either seasonally or daily, according to rhizosphere environment, species or tree age.

Roots have a mechanism involving signal emissions to shoot system (Aiken & Smucker, 1996; Dodd, 2005). Basically, the responsible molecules for this mechanism are the abscisic acid (ABA), the aminocyclopropane carboxylic acid (ACC), the cytokinins (CKs), the gibberellins and the nitrates. This mechanism is extremely important because it is through the responses to these signals that plants can change their growth and development. According to Davies (2007) these signals emitted by the roots "contain" information that allow the plant to change its pattern of growth according the water and nutrients availability in the soil, or soil mechanical impedance. The same author also highlighted that shoot system development and the behavior of leaf stomata can be determined by the signals emitted by the roots. Signals allow the plant to maintain or even increase the canopy development rate when the soil presents a water deficit. On the other hand, roots are dependent of shoot system at carbohydrates, growth regulators and some organic compounds levels (Aiken & Smucker, 1996).

Generally, the main root functions of terrestrial plants are the absorption and transport of resources (mainly water and nutrients) and the support of all aerial plant structure

(Brunner & Godbold, 2007; Danjon & Reubens, 2008; Eissenstat & Volder, 2005; Fitter *et al.*, 2002; Malamy, 2005; Pregitzer, 2008). Pagès *et al.* (2004) also mentions that root systems act as a storage, deposition and excretion of biochemical components source forming associations with symbiotic organisms. Coarse roots also contribute to the tree stability, and provide a network of vessels for water and nutrients transport and for other metabolic components. These coarse roots also act as a backup power system during dormancy periods (Danjon & Reubens, 2008; Pagès, 2002). However, to have an effective ability to use the water and nutrients for the shoot system at the same time they provide an increase on support structure, the root system has to establish an intimate and robust interface with the surrounding soil matrix. This interface is possible due to the production of fine roots and root hairs that significantly increase the surface area contact between root and soil (Harris, 1992). Fine roots are also mainly responsible for the water and nutrients uptake from soil (Brunner & Godbold, 2007; Danjon & Reubens, 2008; Jackson *et al.*, 1997). These fine structures constitute the most dynamic portion of the root system. Due to its constant replacement because of turnover, fine roots contribute to subsoil biomass increase containing carbon and nutrients. These turnovers, which may last from a few weeks up to more than 8 years (Hendrick & Pregitzer, 1992), make costs for fine roots formation very high when compared to leaves formation. Fine roots are generally found in most abundance at surface layer (Pagès, 1999). This abundance at superficial layers is justified by the high availability of nutrients and water but also due to the high microbial activity present at this level (Kucbel *et al.*, 2011). It is important to highlight that according to Kucbel *et al.* (2011) review, fine roots production is extremely sensitive to the amount of biomass needed for the canopy structure maintenance because these are the final structures that act as a carbohydrates reserve source. The deepest fine roots, despite being non representative for total tree biomass, can play a crucial role in the extraction of deeper soil mixture during dry periods (Canadell *et al.*, 1996; Hendrick & Pregitzer, 1996), which is a typical characteristic of the Mediterranean climate. Yet, Brunner & Godbold (2007) defend that mycorrhizal symbiosis are important for the soil carbon flux, as well as for the subsurface nutrients recycle such as nitrogen (N), phosphorus (P), magnesium (Mg) and calcium (Ca).

Under normal conditions and even considering that tree shoot system can be usually higher in terms of dry weight, plant roots, as previously mentioned, are capable of supplying water and nutrients to all the aerial system as well as storing certain carbohydrates and plant growth regulators. Most plants have the ability to adapt to environmental changes conditions if the changes are not too drastic or quick. For instance, if the shoot is subject to pruning, more carbohydrates are used to restore the tree top and less stay available for the roots. Contrarily, if roots suffer any damage or if the water and nutrients availability become scarcer, carbohydrates will be used for roots maintenance. Harris (1992) also points out that responses to the availability or not of certain nutrients, at tree structure level, has led to the notion that nitrogen stimulates the shoot growth instead of root growth; and that phosphorus stimulates root growth. However, more recent studies indicated that both nutrients (nitrogen and phosphorus) stimulate both tree systems growth (Rubio *et al.*, 2002; Trubat *et al.*, 2006).

1.3.3.3 Root Soil Interface

Roots grow following a strategy of searching for available resources to maintain the tree functionality and survival. Therefore, biotic and abiotic factors have a deep influence on root growth and consequently on its structure (Cuesta *et al.*, 2010).

A crucial key factor for root growth is water availability which will influence all the tree maintenance (Yu *et al.*, 2007). The process of water absorption and transport from roots to the shoot system occurs because of pressure gradient differences (Steudle, 2000; Wiegers *et al.*, 2009). When the water is released by transpiration occurring in the leaves, the gradient difference provides a replacement of the water loss in the leaves by the water existent on the xylem of other aerial structures and consequently, on the xylem of roots. The consequent reduction of the roots water potential forces water uptake from the soil and the flow process to go back, upwards. If transpiration rates are not very high and if the trees are well rooted in a soil with high water availability, this movement occurs relatively quickly promoting a balance between the water released by transpiration and the one captured by roots. However, when transpiration rates are high and subsoil water availability is low and slowly restored, areas of water and nutrients "shortage" may occur near the roots (Davies, 2007). In these areas the water movement can be drastically reduced causing a consequent tree restriction on water and nutrients uptake rate. Water availability and consequent absorption is usually a component with a simplified approach given the frequent lack of data about the distribution of roots in soil. However, in 2006, Kurz-Besson *et al.* found that the daily water fluctuations on the topsoil under oaks canopy (0.35m depth), observed during the summer season, suggest that part of the still existent water on deepest soil horizons is pulled by the sinkers (during night when no transpiration occur) and is released (in early morning) in the upper layers of the soil (due to the water potential gradient). After, this water is reabsorbed by the superficial roots and used in next day tree transpiration. This process is known as *hydraulic lift*. According to the review made by Jackson *et al.* (1999) a relative frequency of this process was verified on trees belonging to the Mediterranean biota.

Another factor influencing significantly the growth and development of roots is the soil mechanical impedance, resulting in an induced soil compaction (Bejanaro *et al.*, 2010; Ganatsas & Spanos, 2005; Kozlowski, 1999). This unsaturated soil compression modifies the original soil structure, essentially the structure of large soil pores. This modification causes a decrease in soil porosity, aeration and infiltration rate (Kristoffersen & Riley, 2005). This process can occur naturally by the soil sedimentation or may be artificially induced by heavy machinery, cattle and fire, among several others factors (Kozlowski, 1999). For a given root point mechanical impedance is defined as the ratio between the forces exerted at that point and his dislocation speed (Portas, 1970). Bengough (2003) states that the increase in soil strength, as it becomes dryer, can have a significant impact on root branching ability and, normally, more lateral roots per unit length of the parental root axis are found. However, Goss (1997) found that the total number of lateral roots may decrease with the increase of soil mechanical impedance. The plant benefit or loss effects of soil compaction have caused some disagreement among experts in this area. For example, Bassett *et al.* (2005) and Kozlowski (1999) showed in their studies, that tree growth is adversely affected by soil compaction. On the other hand, Alameda & Villar (2009) and Tubeileh *et al.* (2003) obtained positive results in terms of growth with low intensity levels of soil mechanical impedance.

Soil temperature might also influence the root growth and functioning (Domisch *et al.*, 2002; Lahti *et al.*, 2005; Oliveira, 1988; Pregitzer *et al.*, 2000). According to the review of Pregitzer *et al.* (2000), which compiled several studies findings regarding the influence of soil temperature on plants growth, this factor was found to have the potential to alter the root morphology and the quality of root tissues, reducing its absorption capacity. This reduction may be a result of an increase in the water viscosity, a decrease on the soil/root hydraulic conductivity and a lower permeability of the membrane cell (Voorhees *et al.*, 1981). The way this factor affects the growth of root structures is directly related not only to the tree species and nutrients availability, but to the climatic conditions where they develop as well. The root growth rate increase almost linearly with temperature until it achieves a maximum value, after which root growth decreases drastically. The same situation occurs when soil temperatures are too low (eg., Apostol *et al.*, 2007; Lathi *et al.*, 2005; Peng & Dang, 2003; Vappavuori *et al.*, 1992). For most species there is an optimum soil temperature value, corresponding to the maximum root growth rate. However, as referred by Pregitzer *et al*. (2000), the studies concerning to the influence of soil temperature on plant root growth, are scarce.

1.3.3.4 Root Models

Since the last decade the architectural models of root systems have received a relevant importance in the understanding of the tree functioning. The necessity to integrate these models in the already existent for tree growth is known, thus the knowledge about forest ecosystems has been seen as crucial in the current reality of preservation and valuation of natural resources. In the actual context of climate changes its relevance is also highlighted since, in general, roots act as dioxide carbon sinks through photosynthetic processes, mitigating the consequences of an anthropogenic increase of gases inherent to greenhouse effect (Vaz, 2005).

Root systems can be modeled considering different basic principles, different scales and different levels of detail. Studies about roots usually rely on geometry, topology or biomass estimations (Danjon & Reubens, 2008). Specifically, studies on root modeling through biomass estimation focus on tree species and on its relation with the forest management activities (Bolte *et al.*, 2004; Le Goff & Ottorini, 2001; Nielsen & Hansen, 2006). Although some authors have chosen to use the root homogeneous density (Feddes *et al.*, 1978), others represented a decrease in root density along the soil profile through a linear function (Heidmann *et al.*, 2000), a potential function (Monteith *et al*., 1989), or through an exponential function (Williams *et al*., 2001). Other authors used specific values of relative root density for each soil layer considered (eg., Tiktak & Bouten, 1992).

Studies about root distribution usually are based on root biomass and/or root length, through soil depth (Lynch, 1995). Physiologically, root biomass is a parameter used to evaluate the role of roots as carbon sinks, while root length is a direct indicator of the water and nutrients absorption capability (Atkinson, 2000). However, Canadell *et al*. (1996) argue that for models using these parameters the main roots have a limited significance, besides their important role on the survival strategy during drought period.

The simplest root distribution models were developed with the purpose of evaluate the roots distribution on depth under non-limiting growth conditions (Pagès *et al*., 2000). The model used by Monteith *et al*. (1989) defines the root length density (root length per unit of soil volume) as an inverse function of depth square root. Drexhage & Gruber (1998) used a similar approach to describe the biomass decomposition as a function of the horizontal distance from the tree trunk base. More flexible models have been adjusted to root distributions at plant community level (Schenk & Jackson, 2002). The parameterization obtained by fitting a model with a certain root distribution allows a simple characterization of their rooting pattern.

Bengough *et al*. (2000) found that, due to a high root systems diversity existent in natural conditions, the approaches focusing the individual plant must be favored. Models at individual plant scale allow the study of determinant factors that influence root branching pattern hence root distribution is directly influenced by these factors. Nevertheless, a considerable quantity of important information is overlooked with these simplifications, such as the spatial distribution of root system. According to Mulia *et al*. (2010) models including the root spatial distribution can be aggregated in four classes: i) Models that ignore the root dynamics and that use the root spatial distribution independently from time variable; ii) models that incorporate a simple root dynamics described by a generic distribution model independently of shoot system processes and soil conditions; iii) models that simulate the root growth as a response of shoot system conditions but without considering the interaction of soil; iv) models that simulate root growth considering soil conditions and characteristics as well as shoot system conditions. Dupuy *et al*. (2010), on their review work, considered another type of classification, specifically into three categories: 1) models of rooting depth defined as continuous models showing the root length density distribution; 2) architectural models based essentially on the root length description and bifurcation pattern in 1,2 or 3 dimensionally aspect; 3) spatial models of root dynamics that are continuous models describing the patterns of growth, whether in 2D or 3D, using length and meristems densities. According to a recent literature review, the emerging models presenting better and more relevant results are the ones that focus on root system representation as a dynamic structure (Kalliokosky *et al.*, 2010). This dynamic structure follows a growth pattern defining types of roots with specific morphology (defined according to their differentiation state and development pattern) (Jourdan & Rey, 1997). These structures may also be related and evaluated according to the characteristics of soil profile horizon (Pagès *et al.*, 2004), basing their growth on fractal modeling. In this type of modeling root systems are represented as static structures, resulting from similar repetitions of branching patterns (following Gravelius codification) on similar "sub-structures”. In these models the morphological characteristics of the “new roots” formed, derive exclusively from the dimensions of parental roots (van Noordwijk *et al.*, 1994; Ozier Lafontaine *et al.*, 1999; Smith, 2001; van Noordwijk & Mulia, 2002; Richardson & zu Dohna, 2003). Yet and according to the review made by Huang *et al.* (2010), some studies indicate that root bifurcation patterns shows great morphological heterogeneity and different orders may have significant differences at physiological activity, chemical composition and functions (Pregitzer *et al.*, 1997; Guo *et al.*, 2011). Roots characterization by their bifurcation pattern is considered an essential method approach to identify the root functions inside the complex networks of root systems.

Wagner *et al.* (2010) mentioned that the perception of tree support, development processes and possible carbon exchanges between shoot and root systems would provide a significant advance of scientific knowledge on root modeling through the integration of root annual behavior. This author also highlighted that most of the studies about this subject only focused on individual samples and that there is a urgent necessity to model the entire root system, providing the reconstruction of the exact position of roots on the underground. In the future, this would allow justifying and understanding many of the actions and functions that this important belowground system has, both in the functional and structural ecosystem dynamics, and on the understanding of the role of roots on the water and carbon balances. Thus and citing the author (Wagner *et al*., 2010) "*the exposure of the root system is essential for future research*".

However, the efforts that have been made to quantitatively characterize the root systems have been low mainly concerning to coarse roots. The majority of the studies relying on root systems have been conducted on agricultural species and on forest tree seedlings. This is probably justified by the fact that the efforts necessary to access roots systems of adult trees are quite cost and time consuming.

1.4.4 The Role of Cork Oak Root System on the Landscape Dynamics

Throughout all that was already mentioned, the concern with the decline signs and consequently with the change of the landscape as we know it, is increasing also due to an increase about environmental awareness and biodiversity maintenance. The intensification of the *Montado* decline verified since the mid 80's throughout the Mediterranean region including southwest Portugal, together with the general decline of oaks in Europe and North America, lead to concerns about their overall sustainability (Costa *et al*., 2009). This vulnerability exposes the need to meet new biological and ecological parameters that may influence the vitality of cork oak tree. Sousa *et al*. (2007) suggested that future lines of research, to understand the causes of decline, should focus on the physiology of trees and on an accurate mapping of the root system, both laterally and in depth.

The role of the cork oak root system in landscape is preeminent. As a landscape element of the *Montado* structure, the cork oak survival strategy and growth dynamics will influence the landscape dynamics. The root system, along with its function of tree support and uptake and transport of water and nutrients to the shoot, is essential for water and carbon cycles dynamics in the ecosystem. A break or deficiency in these processes, at a high scale, will certainly cause a deep change in the landscape multifunctionality which can lead to a loose of the landscape identity. Therefore, knowing and understanding the structures that develop in the underground of this landscape is crucial and essential to maintain such a distinctive landscape as *Montado*. Despite the almost non-existent information about the root system dynamic and about the relationships that occur at the underground level, this "hidden landscape” may bring some answers to the observed cork oak decline. In addition, studies on the best conditions to promote and facilitate the natural and/or artificial regeneration establishment will also promote the maintenance of the *Montado* structure as we know it, guaranteeing the functioning of ecosystem services promoted by these trees.

References

Acácio, V. (2009). *The dynamics of cork oak systems in Portugal: the role of ecological and land use factors*. Doctoral Thesis, Wageningen University,Wageningen, Netherlands. ISBN 978-90-8585-504-0. Available at: http://edepot.wur.nl/ 14120. Accessed 12 Oct 2012.

AFN (2010). *Inventário Florestal Nacional 2005-2006, Autoridade Florestal Nacional*. Lisboa, Portugal. ISBN: 978-972-8097-76-9

Aiken, R.M., & Smucker, A.J.M. (1996). Root system regulation of whole plant growth. *Annual Review of Phytopathology*, 34, 325–46.

Alameda, D., & Villar, R. (2009). Moderate soil compaction: Implications on growth and architecture in seedlings of 17 woody plant species. *Soil and Tillage Research*, 103, 325–331.

Apostol, K.G., Jacobs, D.F., Wilson, B.C., Salifu, K.,F., Dumroese, R.K. (2007). Growth, gas exchange, and root respiration of *Quercus rubra* seedlings exposed to low root zone temperatures in solution culture. *Forest Ecology and Management*, 253, 89–96.

Aranda, I., Pardos, M., Puertolas, J., Jimenez, M.D., Pardos, J.A. (2007). Water-use efficiency in cork oak (*Quercus suber*) is modified by the interaction of water and light availabilities. *Tree Physiology*, 27, 671-677.

Atkinson, D. (2000). Root characteristics: why and what to measure. In Smit, A.L., Bengough, A.G., Engels, C., van Noordwijk, M., Pellerin, S., van de Geijnk, S.C. (Eds.) *Root Methods: A handbook.* (Pp:2–32). Springer-Verlag. Berlin. Heidelberg. Germany.

Azul, A.M. (2011). *Diálogos e modos de actuação colectiva com vista à sustentabilidade do sobreiro em Portugal*. Available at: *http://www.ces.uc.pt/e-cadernos/media/ecadernos10/3%20%20Anabela %20Marisa%20Azul.pdf*

Bassett, I.E., Simcock, R.C., Mitchell, N.D. (2005). Consequences of soil compaction for seedling establishment: implications for natural regeneration and restoration. *Austral Ecology*, 30, 827–833.

Bejanaro, M.D., Villar, R., Murillo, A.M., Quero, J.L. (2010). Effects of soil compaction and light on growth of *Quercus pyrenaica* Willd. (Fagaceae) seedlings. *Soil and Tillage Research*, 110, 108-114.

Bengough, A.G., & Mullins, C.E. (1990). Mechanical impedance to root growth: a review of experimental techniques and root growth responses. *Journal of Soil Science*, (41)3, 341-358.

Bengough, A.G., Castrignano, A., Pagès, L., Van Noordwijk, M. (2000). Sampling strategies, scaling and statistics. In Smit, A.L., Bengough, A.G., Engels, C., Van Noordwijk, M., Pellerin, S., Van de Geijn, S.C. (Eds.) *Root Methods, a Handbook*. (Pp: 147-173). Springer Verlag, Berlin.

Bengough, A.G. (2003). Root growth and function in relation to soil structure composition and strength. In Kroon, H., & Visser, E.J.W. (Eds.) *Root Ecology*. (Pp: 151–171) Springer.

Bolte, A., Rahmann, T., Kuhr, M., Pogoda, P., Murach, D., Gadow, K. (2004). Relationships between tree dimension and coarse root biomass in mixed stand of European beech (*Fagus sylvatica* L.) and Norway spruce (*Picea abies* [L.] Karst.). *Plant and Soil*, 264,1-11.

Breda, N., Granier, A., Barataud, F., Moyne, C. (1995). Soil water dynamics in oak stand. Soil moisture, water potentials and water uptake by roots. *Plant and Soil*, 172, 17-27.

Brunner, I., & Godbold, D.L. (2007). Tree roots in a changing world. *Journal Forest Research*, 12, 78–82.

Campos, P., Díaz, M., Pulido, F.J. (1998). Las dehesas arboladas. Un equilibrio necesario entre explotación y conservación. *Quercus*, 147, 31–5.

Canadell, J., Jackson, R.B., Ehleringer, J.R., Mooney, H.A., Sala, O.E., Schulze, E.D. (1996). Maximum rooting depth of vegetation types at the global scale. *Oecologia*, 108, 583-595.

Canadell, J., Djema, A., López, B., Lloret, F., Sabaté, S., Siscart, D., Gracia, C.A. (1999). Structure and dynamics of the root system. In Rodà, F., Retana, J., Gracia, C.A., Bellot, J. (Eds.) *Ecology of Mediterranean Evergreen Oak Forests*. (Pp: 48–59) Springer-Verlag, Berlin, Heidelberg.

Coder, K.D. (2007). *Soil Compaction Stress & Trees: Symptoms, Measures, Treatments*. Warnell School Outreach Monograph.WSFNR07-9.

Cooper, D.J., D'Amico, D.R., Scott, M.L. (2003). Physiological and morphological response patterns of *Populus deltoides* to alluvial groundwater pumping. *Environmental Management*, 31, 215–226.

Costa, A., Pereira, H., Madeira, M. (2009). Landscape dynamics in endangered cork oak woodlands in Southwestern Portugal. *Agroforestry Systems*, 77, 83-96.

Cuesta, B., Veja, J., Villar-Salvador, P., Rey-Benayas, J. (2010). Root growth dynamics of Aleppo pine (*Pinus halepensis* Mill.) seedlings in relation to shoot elongation, plant size and tissue nitrogen concentration. *Trees*, 24(5), 899-908.

Danjon, F., & Reubens, B. (2008). Assessing and analyzing 3D architecture of woody root systems, a review of methods and applications in tree and soil stability, resource acquisition and allocation. *Plant and Soil*, 303, 1-34.

David, T.S., Ferreira, M.I., Cohen, S., Pereira, S., David, J.S. (2004). Constrains on transpiration from an evergreen oak tree in southern Portugal. *Agricultural and Forest Meteorology*, 122,193–205. doi:10.1016/j.agrformet.2003.09.014

David, T., Henriques, M., Kurz-Besson, C., Nunes, J., Valente, F., Vaz, M., Pereira, J.S., Siegwolf, R., Chaves, M.M., Gazarini, L.C., David, J.S. (2007). Water-use strategies in two co-occurring Mediterranean evergreen oaks: surviving the summer drought. *Tree Physiology*, 27,793-803.

David, T.S., Pinto, C.A., Nadezhdina, N., Kurz-Besson, C., Henriques, M.O., Quilhó, T., Cermak, J., Chaves. M.M., Pereira, J.S., David, J.S. (2013). Root functioning, tree water use and hydraulic redistribution in *Quercus suber* trees: A modeling approach based on root sap flow. *Forest Ecology and Management*, 307, 136–146.

Davies, W.J. (2007). Root growth response and functioning as an adaptation in water limiting soils. In Jenks, M.A. *et al.* (Eds.) *Advances in Molecular Breeding Toward Drought and Salt Tolerant Crops*. (Pp: 55–72). Springer.

Day, S.D., Wiseman, P.E., Dickinson, S.B., Harris, J.R. (2010). Tree Root Ecology in the Urban Environment and implications for a sustainable rhizosphere. *Arboriculture & Urban Forestry*, 36(5),193–205.

Di Giulio, M., Holderegger, R., Tobias, S. (2009). Effects of habitat and landscape fragmentation on humans and biodiversity in densely populated landscapes. *Journal of Environmental Management*, 90(10), 2959-2968.

Directiva 2000/60/CE do Jornal Oficial das Comunidades Europeias (2000). file:///C:/Users/Flor%20linda/Desktop/Directiva_Quadro_Agua.pdf. Accessed 7 February 2012

Dodd, I.C. (2005). Root-to-shoot signalling: assessing the roles of “up” in the up and down world of long distance signalling *in planta*. *Plant and Soil*, 274, 251-270.

Domisch, T., Finer, L., Lehto, T., Smolander, A. (2002). Effects of soil temperature on nutrient allocation and mycorrhizas in Scots pine seedlings. *Plant and Soil*, 239, 173-185.

Drexhage, M., & Gruber, F. (1998). Architecture of skeletal root system of 40-year-old *Picea abies* on strongly acidified soils in the Harz mountains (Germany). *Canadian Journal of Forest Research*, 28,13–21.

Dupuy, L., Gregory, P., Bengough, A. (2010). Root growth models: towards a new generation of continuous approaches. *Journal of Experimental Botany*, 61, 2131-2143.

Eissenstat, D.M., & Volder, A. (2005). The efficiency of nutrient acquisition over the life of a root. In Bassirirad, H. (Ed.) *Ecological Studies. Volume 181. Nutrient Acquisition by Plants: An Ecological Perspective.* (Pp: 185-220). Springer-Verlag.

European Landscape Convention (2000). Available at: http://www.gddc.pt/siii/docs/dec4-2005.pdf. Accessed 12 Dec 2010.

FAO (2006). *World reference base for soil resources*. Food and Agriculture. Organization of the United Nations, Rome. ISBN: 92-5-105511-4

Feddes, R.A., Kowalik, P.J., Zaradny, H. (1978). *Simulation of field water use and crop yield.* Simulation monographs PUDOC. Wageningen. Netherlands. Pp:189.

Filho, B. (1998). Análise da paisagem: fragmentação e mudanças. Available at: *http://www.csr.ufmg.br/dinamica/publications/apostila.pdf.* Accessed 4 Feb 2012

Fitter., A., Williamson, L., Linkohr, B., Leyser, O. (2002). Root system architecture determines fitness in an Arabidopsis mutant in competition for immobile phosphate ions but not for nitrate ions. In *Proceedings of the Royal Society of London, Series B–Biological Sciences. 269.* (Pp: 2017–2022).

Ganatsas, P., & Spanos, I. (2005). Root system asymmetry of Mediterranean pines. *Plant and Soil*, 278, 75–83.

Gautam, M.K., Chang, S.X., Mead, D.J., Clinton, P.W., Roberts, E.H. (2003). First order lateral root characteristics reflect the competitiveness of radiata pine genotypes in agroforestry systems. In *Proceedings of Australasia Forestry Conference,* Queenstown, New Zealand.

Goss, M.J. (1977). Effects of mechanical impedance on root growth in barley. 1. Effects on the elongation and branching of seminal root axes. *Journal of Experimental Botany*, 28, 96–111.

Gouveia, A.C., & Freitas, H. (2008). Intraspecific competition and water use efficiency in *Quercus suber*: evidence of an optimum tree density? *Trees*, 22, 521-530.

Gouveia, A.C., & Freitas, H. (2009). Modulation of leaf attributes and water use efficiency in *Quercus suber* along a rainfall gradient. *Trees*, 23, 267-275.

Guo, Y., Fourcaud, T., Jaeger, M., Zhang, X., Li, B. (2011). Plant growth and architectural modelling and its applications. *Annals of Botany*, 107, 723–727.

Guyomarch, S., Léran, S., Auzon-Cape, M., Perrine-Walker, F., Lucas, M., Laplaze, L. (2012). Early development and gravitropic response of lateral roots in Arabidopsis thaliana. *Philosophical Transactions Royal Society*, 367, 1509-1516.

Harris, R.W. (1992). Root:shoot ratios. *Journal of Arboriculture*, 18(1), 39-42.

Heidmann, T., Thomsen, A., Schelde, K. (2000). Modelling soil water dynamics in winter wheat using different estimates of canopy development. *Ecological Modelling*, 129, 229-243.

Hendrick, R.L. &, Pregitzer, K.S. (1992). Spatial variation in tree root distribution and growth associated with minirhizotrons. *Plant and Soil*, 143, 283–288.

Hendrick, R.L., & Pregitzer, K.S. (1996). Temporal and depth-related patterns of fine root dynamics in northern hardwood forests. *Journal of Ecology*, 84, 167-176.

Hinsinger, P., Bengough, A.G., Vetterlein, D., Young, I.M. (2009). Rhizosphere: biophysics, biogeochemistry and ecological relevance. *Plant and Soil*, 321, 117-152.

Hodge, A. (2009). Root decisions. *Plant, Cell & Environment*, 32, 628–640.

Huang, G., Zhao, X., Zhao, H. (2010). Linking root morphology, longevity and function to root branch order: a case study in three shrubs. *Plant and Soil*, 336, 197–208.

Ja, K.M., Han, L.S., Y, W.S. (2011). Cork oak (Quercus suber L.) forest decline in Tunisia: A linkage between physiological adaptation and stress. *Scientific Research and Essays*, 6(6), 1143-1146.

Jackson, R.B., Mooney, H.A., Schulze, E.D. (1997). A global budget for fine root biomass, surface area, and nutrient contents. *Proceedings of National Academy of Sciences. USA*, 94, 7362–7366.

Jackson, R.B., Moore, L.A., Hoffmann, W.A., Pockman, W.T., Linder, C.R. (1999). Ecosystem rooting depth determined with caves and DNA. *Proceedings of National Academy of Sciences. USA*, 96, 11387-11392.

Joffre, R., Rambal, S., Ratte, J. (1999). Dehesa system in Spain and Portugal as a natural ecosystem mimic. *Agroforestry Systems*, 45, 57-79.

Jourdan, C., & Rey, H. (1997). Architecture and development of the oil-palm (*Elaeis guineensis* Jacq.) root system. *Plant and Soil*, 189, 33-48.

Kalliokoski, T., Sievanen, R., Nygren, P. (2010). Tree roots as self-similar branching structures: axis differentiation and segment tapering in coarse roots of three boreal forest tree species.*Trees*, 24, 219-236.

Kozlowski, T.T. (1971). *Growth and Development of Trees. Volume II – Cambial growth, Root growth, and Reproductive Growth*. Academic Press.

Kozlowski, T.T. (1999). Soil compaction and growth of woody plants. *Scandinavian Journal of Forest Research*,14, 96–619.

Kristoffersen, A.O., & Riley, H. (2005). Effects of soil compaction and moisture regime on the root and shoot growth and phosphorus uptake of barley plants growing on soils with varying phosphorus status. *Nutrient Cycling in Agroecosystems*, 72, 135–146.

Kucbel, S., Jaloviar, P., Špišák, J. (2011). Quantity, vertical distribution and morphology of fine roots in Norway spruce stands with different stem density. *Plant Root*, 5, 46-55.

Kurz-Besson, C., Otieno, D., Lobo do Vale, R., Siegwolf, R., Schmidt, M., Herd, A., Nogueira, C., David, T.S., David, J.S., Tenhunen, J., Pereira, J.S., Chaves, M. (2006). Hydraulic lift in cork oak trees in a savannah-type Mediterranean ecosystem and its contribution to the local water balance. *Plant and Soil*, 282, 361–378.

Lacambra, L., Andray, A., Francés, F. (2010). Influence of the soil water holding capacity on the potential distribution of forest species. A case study: the potential distribution of cork oak (*Quercus suber* L.) in central-western Spain. *European Journal of Forest Research*, 129, 111-117.

Lahti, M., Aphalo, A.J., Finer, L., Ryyppö, A., Lehto, T., Mannerkoski, H. (2005). Effects of soil temperature on shoot and root growth and nutrient uptake of 5-year-old Norway spruce seedlings. *Tree Physiology*, 25, 115–122.

Le Goff, N., & Ottorini, J.-M. (2001). Root biomass and biomass increment in a beech (*Fagus sylvatica* L.) stand in North-East France. *Annals of Forest Science*, 58, 1-13.

Lima, J.C. (2008). *Contributo para a determinação do coeficiente cultural em árvores jovens de sobreiro (Quercus suber L.) com cinco e seis anos de idade - um estudo de eco-hidrologia agroflorestal*. Dissertação para a obtenção do grau de Doutor. Universidade de Évora. Évora.

Losch, R. & Schulze, E. (1995). Internal coordination of plant responses to drought and evaporative demand. In Schulze, E., & Caldwell, M. (Eds.) *Ecophysiology of Photosynthesis*. Berlin, Heidelberg, New York. Springer-Verlag.

Lubczynski, M.W., & Gurwin, J. (2005). Integration of various data sources for transient groundwater modeling with spatio-temporally variable fluxes—Sardon study case, Spain. *Journal of Hydrology*, 306, 71–96.

Lyford. W. (1980). Development of the root system of northern red oak (*Quercus rubra* L.). *Harvard Forest Paper 21*. Harvard university. Pp:1 – 29. Accessed on July

2012 at: http://harvardforest.fas.harvard.edu/sites/harvardforest.fas.harvard.edu/files/publications/pdfs/HFpubs/paper21.pdf.

Lynch, J. (1995). Root architecture and plant productivity. *Plant Physiology*, 109, 7–13.

Malamy, J.E. (2005). Intrinsic and environmental response pathways that regulate root system architecture. *Plant, Cell & Environment*, 28, 67–77.

Matias, M. (2008). *A importância do montado para o desenvolvimento rural. Que futuro no distrito de Beja?* Available at: http://www.geogra.uah.es/web_11_cig/cdXICIG/docs/01-PDF_Comunicaciones_coloquio/pdf-2/com-P2-17.pdf Accessed 12 Jul 2012.

Mètro, A., & Sauvage, C.H. (1957). Observações sobre o raizame do sobreiro em Mamora (Marrocos ocidental). *Annales de la Recherche Forestière au Maroc*.

Millenium Ecosystem Assessment (2003, 2005) *Available at: http://www.unep.org/maweb/ documents/document.356.aspx.pdf*. Accessed 12 Dec 2010.

Monteith, J.L., Huda, A.K.S., Midya, D. (1989). RESCAP: a resource capture model for sorghum and pearl millet. In Virmani, S.M., Tandon, H.L.S., Alagarswamy, G. (Eds) *Modeling the growth and development of sorghum and pearl millet. ICRISAT Res Bull, 12* (Pp: 30–34)

Moreno, J., & Oechel, W. (1995). *Global change and Mediterranean-type ecosystem. (Ecological Studies), Volume 117*. Pp:527.

Mulia, R., Dupraz, C., van Noordwijk, M. (2010). Reconciling root plasticity and architectural ground rules in tree root growth models with voxel automata. *Plant and Soil*, 337, 77-92.

Nadezhdina, N., Ferreira, M.I., Silva, R., Pacheco, C.A. (2008). Seasonal variation of water uptake of a *Quercus suber* tree in Central Portugal. *Plant and Soil*, 305(1-2), 105-119.

Natividade, J.V. (1950). *Subericultura*. Ministerio da Economia, Direcção Geral Serviços Florestais e Aquícolas, Lisboa.

Nibau, C., Gibbs, D.J., Coates, J.C. (2008). Branching out in new directions: the control of root architecture by lateral root formation. *New Phytologist*, 179, 595–614.

Nielsen, C.C.N., & Hansen, J.K. (2006). Root CSA-root biomass prediction models in six tree species and improvement of models by inclusion of root architectural parameters. *Plant and Soil*, 280, 339–356.

Oliveira, M.R.G. (1988). *Comportamento do sistema radical de algumas espécies forrageiras e pratenses com interesse para a modernização dos sistemas de* *agricultura no Alentejo*. Dissertação para a obtenção do grau de Doutor. Universidade de Évora. Évora

Otieno, D., Kurz-Besson, C., Liu, J., Schmidt, M., Vale-Lobo, R., David, T., Siegwolf, R., Pereira, J.S., Tenhunen, J.D. (2006). Seasonal variations in soil and plant water status in a *Quercus suber* L. stand: roots as determinants of tree productivity and survival in the Mediterranean-type ecossystem. *Plant and Soil*, 283, 119-135.

Ozier-Lafontaine, H., Lecompte, F., Sillon, J. (1999). Fractal analysis of the root architecture of Gliricidia sepium for the spatial prediction of root branching, size and mass: model development and evaluation in agroforestry. *Plant and Soil*, 209(2), 167-180.

Pacheco-Villalobos, D., & Hardtke, C.S. (2012). Natural genetic variation of root system architecture from *Arabidopsis* to *Brachypodium*: towards adaptive value. *Philosophical Transactions Royal Society*, 367, 1552-1558.

Pagès, L. (1999). Root system architecture: from its representation to the study of its elaboration. *Agronomie*, 19, 295–304.

Pagès, L., Asseng, S., Pellerin, S., Diggle, A. (2000). Modelling root system growth and architecture. In Smit, A.L., Bengough, A.G., van Noordwijk, M., Pellerin, S., van de Geijn, S.C. (Eds.) *Root Methods: A Handbook.* (Pp:113-145). Springer-verlag.

Pagès, L. (2002). Modelling root system architecture. In Weisel, Y., Eshel, A., Kafkafi, U. (Eds.) *Plant roots: the hidden half. 3rd edition*. (Pp:175–186). Marcel Dekker, New York.

Pàges, L., Vercambre, G., Drouet, J., Lecompte, F., Collet, C., Le Bolt, J. (2004). Root type: a generic model to depict and analyse the root system architecture. *Plant and Soil*, 258(1-2), 103-119.

Paracchini, M.L., Petersen, J.E., Hoogeveen, Y., Bamps, C., Burfield, I., van Swaay, C. (2008). *High nature value farmland in Europe: an estimate of the distribution patterns on the basis of land cover and European Commission*. Joint Research Centre, Institute for Environment and Sustainability, Office for Official Publications of the European Communities, biodiversity data. Luxembourg.

Peng, Y.Y., & Dang, Q.L. (2003). Effects of soil temperature on biomass production and allocation in seedlings of four boreal tree species. *Forest Ecology and Management*, 180, 1–9.

Pereira, J., Silva, T., Correia, A. (2004). Florestas e alterações climáticas – o sequestro de carbono nas florestas. *Ingenium*, 84, 62-63.

Perry, T.O. (1982). The ecology of tree roots and the practical significance thereof. *Journal of Arboriculture*, 8, 197–211.

Pinto-Correia, T., Ribeiro, N., Sá Sousa. P. (2011). Introducing the montado, the cork and holm oak agroforestry system of Southern Portugal. *Agroforestry Systems*, 82, 99-104.

Pinto-Correia, T., Ribeiro, N., Mira Potes, J. (Coord.). (2013). *Livro Verde dos Montados*. ICAAM, Universidade de Évora

Portas, C.A.M. (1970). *Acerca do sistema radical de algumas culturas hortícolas*. Universidade de Luanda. Luanda.

Pregitzer, K.S., Kubiske, M.E., Yu, C.K., Hendrick, R.L. (1997). Relationships among root branch order, carbon, and nitrogen in four temperate species. *Oecologia*, 111, 302–308.

Pregitzer, K.S., King, J.S., Burton, A.J., Brown, S.E. (2000). Responses of tree fine roots to temperature. *New Phytologist*, 147, 105-115.

Pregitzer, K.S. (2008). Tree root architecture – form and function. *New Phytologist*, 180, 562–564.

Quine, C.P., & Watts, K. (2009). Successful de-fragmentation of woodland by planting in an agricultural landscape? An assessment based on landscape indicators. *Journal of Environmental Management*, 90, 251-259.

Rambal, S. (1984). Water balance and pattern of root water uptake in Quercus coccifera L. evergreen shrub. *Oecologia*, 62, 18-25.

Ribeiro, N., Dias, S., Surovy, P., Gonçalves, A.C., Ferreira, A.G., Oliveira, A.C. (2004). The importance of crown cover on the sustainability of cork oak stands: a simulation approach. *Advances in Geoecology*, 37, 275-267.

Ribeiro, N., & Surový, P. (2008). *Inventário nacional de mortalidade de sobreiro na fotografia aérea digital de 2004/2006*. ISBN : 978-989-8132-01-7. Pp. 82.

Ribeiro, N., Surový, P., Pinheiro, A. (2010). Adaptive management on sustainability of cork oak woodlands. In Manos, B., Paparrizos, K., Matsatsinis, N., Papathanasiou, J. (Eds.) *Decision support systems in Agriculture, food and the environment – Trends, applications and advances*. (Pp:437-449). Hershey. New York. Information science reference.

Richardson, A., & zu Dohna, H. (2003). Predicting root biomass from branching patterns of Douglas-fir root systems. *Oikos*, 100, 96-104.

Rubio, G., Walk, T.C., Ge, Z., Yan, X.L., Liao, H., Lynch, J. (2002). Root gravitropism and below-ground competition among neighbouring plants: a modelling approach. *Annals of Botany*, 88, 929–940.

Ryel, R., Leffler, A., Peek, M., Ivans, P., Caldwell, M. (2003). Water conservation in Artemisia tridentata through redistribution of precipitation. *Oecologia*, 141, 335-345.

Schenk, H.J., & Jackson, R.B. (2002). Rooting depths, lateral root spreads, and belowground/aboveground allometries of plants in water limited ecosystems. *Journal of Ecology*, 90, 480-494.

Schenk, H., & Jackson, R. (2005). Mapping the global distribution of deep roots in relation to climate and soil characteristics. *Geoderma*, 126, 129-140.

Smith, D. (2001). Estimation of tree root lengths using fractal branching rules: a comparison with soil coring for Grevillea robusta. *Plant and Soil*, 229(2), 295-304.

Sousa, E., Santos, M., Varela, M., Henriques, J. (2007). *Perda de vigor dos montados de sobro e azinho: Análise da situação e perspectivas*. Documento síntese. Available at: http://www.inrb.pt/fotos/editor2/inia/manuais/livro_causas_doc_ sintese.pdf. Accessed 12 Jul 2012

Steudle, E. (2000). Water uptake by plant roots: an integration of views. *Plant and Soil*, 226, 45–56.

Surova, D., & Pinto-Correia, T. (2008). Landscape preferences in the cork oak Montado region of Alentejo, southern Portugal: Searching for valuable landscape characteristics for different user groups. *Landscape Research*, 33(3), 311-330. DOI: 10.1080/01426390802045962

Surový, P., Ribeiro, N., Brasil, F., Pereira, J.S., Oliveira, M.R.O. (2011). Method for evaluation of coarse cork oak root system by means of digital imaging. *Agroforestry Systems*, 82(2), 111-119.

Sutton, R.F. (1980). Root system morphogenesis. *New Zealand Journal of Forest Science*, 10, 265–292.

Taylor, H., & Gardner, H. (1960). Use of wax substrates in root penetration studies. *Soil Science Society of America*, 24, 79-81.

Tiktak, A., & Bouten, W. (1992). Modelling soil water dynamics in a forested ecosystem. III: Model description and evaluation of discretization. *Hydrol. Proceedings*, 6, 455-465.

Tobin, B., Cermak, J., Chiatante, D., Danjon, F., Di Orio, A., Dupuy, L., Eshel, A., Jourdan, C., Kalliokoski, T., Laiho, R., Nadezhdina, N., Nicoll, B., Pagès, L., Silva, J., Spanos, I. (2007). Towards developmental modelling of tree root systems. *Plant Byosystems*, 141(3), 481-501.

Trubat, R., Cortina, J., Vilagrosa, A. (2006). Plant morphology and root hydraulics are altered by nutrient deficiency in *Pistacia lentiscus* L. *Trees*, 20, 334–339.

Tubeileh, A., Groleau-Renaud, V., Plantureux, S., Gucker, A. (2003). Effect of soil compaction on photosynthesis and carbon partitioning within a maize–soil system. *Soil and Tillage Research*, 71, 151–161.

Unger, S., Maguas, C., Pereira, J.S., Aires, L.M., David, T.S., Werner, C. (2009). Partitioning carbon fluxes in a Mediterranean oak forest to disentangle changes in ecosystem sink strength during drought. *Agricultural and Forest Meteorology*, 149, 949-961.

van Noordwijk, M., Spek, L., Dewiligen, P. (1994). Proximal root diameter as predictor of total root size for fractal branching models. 1. Theory. *Plant and Soil*, 164(1), 107-117.

van Noordwijk, M., & Mulia, R. (2002). Functional branch analysis as tool for scaling above- and belowground trees for their additive and non-additive properties. *Ecological Modelling*, 149, 41-51.

Vapaavuori, E.M., Rikala, R., Ryyppo, A. (1992). Effects of root temperature on growth and photosynthesis in conifer seedlings during shot elongation. *Tree Physiology*, 10, 217-230.

Vaz, M. (2005). *Balanço do carbono e transpiração num montado alentejano de sobro e azinho*. Dissertação para a obtenção do grau de Doutor. Universidade de Évora. Évora

Voorhees, W.B., Allmaras, R.R., Johnson, C.E. (1981). Alleviating temperature stress. In Arkin, G.F., & Taylor, H. (Eds.) *Modifying the root environment to reduce crop* stress. (Pp: 217-266). Monograph 4. Am. Soc. Agric. Eng. St. Joseph. Michigan.

Wagner, B., Gartner, H., Ingensand, H., Santini, S. (2010). Incorporating 2D tree-ring data in 3D laser scans. *Plant and Soil*, 334, 175-187.

Wiegers, B.S., Cheer, A.Y., Silk, W.K. (2009). Modeling the hydraulics of root growth in three dimensions with phloem water sources. *Plant Physiology*, 150, 2092-2103.

Williams, W., Bond, B.J., Ryan, M.G. (2001). Evaluating different soil and plant hydraulic constraints on tree function using a model and sap flow data from ponderosa pine. *Plant, Cell & Environment*, 24, 679–690.

Yu, L.Z., Ding, G.Q., Shi, J.W., Yu, S.Q., Zhu, J.J., Zhao, L.F. (2007). Effects of fertilization on fine root diameter, root length and specific root length in *Larix kaempferi* plantation. *Chinese Journal of Applied Ecology*, 18, 957–962.

Zencich, S.J., Froend, R.H.,Turner, J.V., Gailitis, V. (2002). Influence of groundwater depth on the seasonal water sources of water accessed by Banksia tree species on a shallow, sandy coastal aquifer. *Oecologia*, 131, 8–19.

[on-line] *www.apambiente.pt/index.php? ref=16&subref=81&sub2ref= 119&sub3ref = 500*. Accessed 7 February 2012.

CHAPTER 2

COMPARISON OF TWO METHODS TO ASSESS THE ROOT ARCHITECTURE AS A POTENTIAL FACTOR INFLUENCING THE DIVERSITY OF A STAND

Dinis, C., Surovy, P., Ribeiro, N.A. (2011). Comparison of two methods to assess the root architecture as a potential factor influencing the diversity of a stand. *Proceedings of metody inventarizace a hodnocení biodiverzity stromové složky*. (Pp: 57-64). ISBN: 978-80-213-2244-8

2.1 Abstract

In this article we describe two methods for acquisition and examination of the root architecture of trees in order to evaluate the possible influence on stand diversity and horizontal structure. The roots and belowground biomass in semi-arid areas of south Portugal can be understood as the main competition area for the trees. Evaluation of root architecture is essential for understanding plant growth, interaction among plants and finally the diversity of the stand.

2.2 Introduction

The rooting strategy is of major importance in understanding the dynamics of species over forest succession and maturation (Curt & Prévosto, 2003). If a determined tree has the ability to, for some reason, acquire a greater proportion of water and nutrients, they turn to be more competitive in producing biomass and allocating assimilates in ways that maximize its survival and growth. Researchers that try to explore knowledge in the area of root systems recognized the difficulties associated with the study of roots in the soil (Box, 1996; Atkinson, 2000; Pàges *et al.*, 2004; Tobin *et al.*, 2007; Dupuy *et al.*, 2010). Besides the growing of technological innovations that already can be used actually, the study of plant root systems still continue to be a challenge often refused, because of the small relation between the obtained results and the invested effort (Dupuy *et al.*, 2010; Mulia *et al.*, 2010; Kalliokosky *et al.*, 2010). Whatever the type of modeling applied, root system must be exposed in a way of that it is possible to capture and analyze its volume and tridimensional structure. To have access to the belowground structure several researchers applied different methodologies, e.g., Hruska *et al.* (1999) used the “ground penetrating radar”. However, in a more recent study, Stokes *et al.* (2002) showed that the resolution obtained is unsatisfactory plus the demanding requirements necessary to apply this method (homogeneous soil, dry soil, etc.), make this technique inappropriate in the majority of studies of root systems behavior. Danjon & Reubens (2008) used another technique, laser digitizing, referring that it’s the best technique available to describe the surface and the shape of roots. The authors recognize that the integration of lift perception, the development processes and carbon exchanges in modeling works should also integrate the annual behavior of root system (Wagner *et al.*, 2010). This author also refers, that many studies only analyze samples of individual roots but the necessity of construction on new modeling approaches of the entire root system is urgent. Acquisition of the exact spatial distribution of the roots in belowground, will allow the explanation and understanding of many actions and functions of these systems, so little studied, in terms of functional and structural dynamic of tree, allowing the ecosystem understanding also in terms of water and carbon cycles. Quoting the referred author “the exposition of root system is crucial to future researches”.

According to Danjon & Reubens (2008), the collected information about the availability for coarse roots can be achieved through invasive and non-invasive techniques. In the application of non-invasive methods, the accessibility to root system does not implicate its exposure, being the measurements made through X-ray (Pierret *et al*., 1999) or MRI (Asseng *et al*., 2000). In these techniques the quantity of available parameters is low, comparing with value cost of equipment acquisition and, also the demand of specific soil conditions, as temperature, texture, water, presence of coarse soil material, etc. make this technique less viable for applications in tree root system. In invasive techniques, it’s assumed that the exposition of tree root system should be made by pulling or lifting of roots with heavy machinery help, by profile excavation or excavation of total volume of soil occupied by the roots. After root system exposition, the coarse root measurements can be achieve by the techniques: (1) manually using a frame and plumb (Khuder *et al*., 2006); (2) using computational programs to reconstruct the geometry analyzed manually by measurements recovered with the use of a ruler and compass (Dupuy *et al*., 2003) and (3) semi automated method using a digital compass or a 3D digitiser (Danjon *et al*., 1999; Oppelt *et al*., 2000). Nowadays, the method more often used is the 3D digitizing, as the Polhemus Fastrak of low magnetic field and the analysis made through specific software’s (Danjon *et al*., 1999; Danjon & Fourcaud, 2005; Tamasi *et al*., 2005; Nicoll *et al*., 2006). By the research made until this moment, the measurements can be done in situ (Oppelt *et al*., 2001; Khuder *et al*., 2006; Danjon & Reubens, 2008) or in excavated root systems (Danjon *et al*., 1999; Danjon & Fourcaud, 2005; Nicoll *et al*., 2006). The in situ method is the better technique to apply resulting in a major quantity of information despite expensive and time consuming.

In this paper we try to demonstrate the application of two different methodologies in excavation of tree root systems (*Pinus pinea* L. and *Quercus suber* L.) representing a technical component of a project of tridimensional architecture modelation of tree root system.

2.3 Methodology

When the main goal is to evaluate the morphology and root system architecture, it is necessary to have access to this system in a complete and integral way in all the extension, horizontally and in depth. For this experimental work, two healthy trees were selected, a stone pine (*Pinus pinea* L.) and a cork oak (*Quercus suber* L.). Both of trees were located in Canha, in central Portugal (38º45'31.94"N 8º38'30.93"O and 8º45'38.38"N 8º 38'22.22"O, respectively). The predominant type of soil in the study area is a Cambissoil soil (Vt) with sandy-loam characteristics with clay congregations.

For both method application, a shortly biophysics analysis of the surrounding matrix was made, soil condition, slope of the surface, density stand conditions and stand type characteristics of the study area (trees, shrubs and herbs layers) (Table 1).

In this comparative experiment, we tested two methods for excavation of total volume soil occupied by the root system: (1) by washing the root with water and, (2) cleaning the root by high pressure air spade. In both methods, initially dendrometric evaluation of tree samples and the collection of soil cores (two soil cores minimum per tree) for textural and chemical analysis of the different soil layers of the profile, were made.

The stone pine was located in a pure stand installed by seedlings in 1998, with 4x4m distance. Cork oak was located in a mixed stand with juvenile cork oaks, stone pines and essentially eucalyptus (*Eucalyptus globulus* Labill.), main competitor for subsoil resources. The surface layer was also occupied by some sage-leaved *cistus* shrubs (*Cistus salvifolius* L.) and some annual herbs. These were removed in advance to facilitate the excavation process. The cork oak was young (around 20 years) and never had been debarked.

Table 1. Evaluation of study location characteristics.

	Stone pine	**Cork oak**
Stand	Pure	Mixed
Neighborhood	Stone pines	Cork oaks, stone pines, eucalyptus
Shrub layer	No	Sage-leaved cistus
Slope (%)	0	0

1 - Root system excavation by profile washing with water

We use the following steps: (1) initially a hole was open with two meters of depth and in a distance of three meters from the tree trunk (this distance was estimated as the maximum horizontal root spread), to function as a deposit of water and flowed sediments, from the washed profile; (2) Proceeded to the opening of the main trench with water washing; being careful with the position and distance of the water jet, because it can cause the movement and displacement of mainly more fine structural roots (diameter between 0.2 – 0.5 cm) from the original position (Figure 1). In our case, the loss of structural fine roots was imminent, so we use a fine net in the top of the hole in the expectation of collecting these roots for further biomass evaluation.

Simultaneously, after the opening/washing of each 10 cm of vertical soil layer, in the demand of not losing the original position of the roots, we proceed with the digitalizing with 3D digitizer (*Polhemus Fastrak*), collecting the 3D representation of roots, codifying each root, collecting and storing them to future laboratory analysis. Because of the complex “net” form by root system, we decided to label the roots for easier identification along the excavation process.

For the first layers (layers AP and beginning of A) (Table 2) this method of excavation with water works well, however for deeper layers, because of the embedded clay features we decide to use the manual excavation method which turned the process harder and time consuming.

In the final stage of excavation, when only the central part of root system wasn't exposed, it was necessary to fix the root system with sticks and strings avoiding the movement and displacement of the rest of the system while the soil was being removed. The purpose of this step is trying to maintain the 3D spatial distribution the most close to reality as possible.

For fine roots samples (less than 2mm of diameter) we used the method of wall profile where we collected soil cubes with 15x15x15 cm dimensions, in a soil wall at 0.75 m deeper and with one meter of length. These soil cubes were codified and store in cold environment, for future laboratory treatment.

Figure 1. Stone pine root system excavation through profile washing with water method.

2 - Root system excavation by high pressure air jet

For the method of excavation by high pressure air jet after dendrometric evaluation of cork oak aerial component, similar to the technique adopted to wash profile excavation a deposit of sediments was open with two meters of depth and at a distance of four meters from the trunk of the tree. With the use of the jet air connected to a compressor we began the excavation of the first layer – topsoil – from the trunk in the direction of the crown edge's horizontal projection. This technique was well succeed in the first 15 cm of soil (high percentage of sand), however as we were reaching more depth, soil characteristics became more clayey, what made us change to the manual option (by and with help of archaeological material used in excavations) with the aid of a pneumatic hammer when was necessary (characteristics of high bulk density) (Figure 2).

With the excavation of one quarter of the horizontal projection of the root system we began the 3-dimensional digitizing of the roots, applying the same methodological proceeding used for the stone pine, to cut, label and store of the roots for further analysis always aiming the total exposure of each root for proper digitizing. In the final stage of cork oak root system excavation, the central part of the root was supported and fixed with wooden sticks, strings and also with the support of a tractor to avoid displacement of the remaining root system when the rest of soil was being removed. After total exposure, the rest of the root system was carried to laboratory where we finished the task of 3D digitizing, cutting and storing the root samples.

Figure 2. Cork oak root system excavation by high pressure air jet metod.

2.4 Results

The results show that both methods are able to obtain the expected results, i.e., the ability to have full access to the entire root system. However according to the soil characteristics of the study area also supported by the results obtained by chemical and textural analysis (see Table 2), both methods only worked well up to 20 cm depth where the predominant texture was sandy. After this depth the only method possible to use in our profiles was the manual excavation method plus the pneumatic hammer, when necessary.

Table 2. Profile layers description of the soil in the study area.

Layers	**Depth (cm)**	**Texture**	**Structure**	**Consistency**
Ap	20	Sandy	Independent particles	Loose, not sticky
A	12	Sandy-loam	Weak fine blocky	Mild, friable, not sticky
AB	23	Sandy with clay congregations	Weak fine blocky	Loose, friable, not sticky
C	92	Sandy-loam	Weak medium blocky	Loose, friable, not sticky
R	Sandstone			

According to what was possible to evaluate with this work, the comparative results of the two methodologies are shown in Table 3.

Table 3. Comparative results of application of both methods evaluated.

	Excavation method	
	Water washing	**High pressure air jet**
Opening deposit sediment trench	Yes	Yes
Opening main drainage trench	Yes	No
Cleaning the deposit sediment using the pressure pump	Yes	No
Cleaning the deposit sediment using heavy machinery	Yes	Yes
Removal of coarse material	+++	+
Structural fine roots loss	+++	++
Displacement of coarse roots	+++	+
Time requirements	+++	++

For root system excavation by profile washing with water, the total money budget spend between days of work, men per day for the excavation process and digitizing (100 man/days for excavation), heavy machinery work (5 hours) for open the deposit sediment trench and for cleaning the deposit sediment during the process, acquisition of excavation equipment (1500€) and water spended (700€), was about 7000€. In case of excavation method by high pressure air jet it was necessary 150 man/days, 5 hours of heavy machinery work. The comparison is shown in Table 4.

The values presented in Table 4 indicate that the excavation types might have similar costs. However to obtain the precise comparison we should divide the total amount of air excavation costs by two due to the fact that the total volume of excavation was twice as big as in the water case. So in such a way, when multiplying the man/day values by index price 35 we obtain for water excavation total 5105 (3500+105+1500+500) and for air excavation 3177.5 (5250+105+1000)/2. So we can conclude that the air excavation is approximately 40% cheaper than water.

Table 4. Comparison of total costs for water washing and high pressure air jet methods.

	Excavation method	
	Water washing	**High pressure air jet**
Excavation time man/days	100	150
Heavy machinery hours	5	5
Additional equipment costs (index)	1500	1000
Additional costs (water)	500	0

2.5 Discussion and Conclusion

In this article we describe two methods for excavation of structural root systems of trees. The first one is based on water washing of root system aiming to remove the surrounding soil by water current. Second method uses air pressure jet. For the application of both methods it is advisable to make a brief analysis of biophysical environmental matrix, such as, soil conditions, the slope of the surface, conditions of stand density and characteristics of forestry layers of the stand (trees, shrubs, herbs). For both methods before beginning the excavation of volume soil occupied by the roots its necessary to remove the aerial part of the tree, which could cause displacement of roots during topsoil removal. In the case of sandy texture soils the method of high pressure air jet, will probably have good results when the main goal is having complete access to the root systems. This method besides being logistically easier to install is faster and less costly. In cases of clayey textural soil characteristics both methods are likely to be inefficient to clean soil layers. The manual option is the best choice to achieve best results, although time-consuming and expensive.

Taking into account the criteria evaluated in this work of comparison of root excavation methodologies and making the balance of costs/time versus quality of results, we conclude that for sandy soil types with embedded clay features, the method of excavation by high pressure air jet together with manual excavation is the best technique to apply when the main goal is to achieve the complete root system of a tree. The growing interest that has been observed in this research area – large trees root systems – justify an imminent and necessary research focus on excavation methods, testing and creating new alternatives. Results obtained within this study provide a better understanding on the competition that may exist belowground, dependent on rooting patterns, contributing to explain stand diversity. The net formed by the root systems, and its functions and spatial distributions justify the survivor of the trees enabling the anchorage, the acquisition and transport of nutrients and water for aerial growth and survival.

References

Asseng, S., Aylmore, L.A.G., McFall, J.S., Hopmans, J.W., Gregory, P.J. (2000). Computer-assisted tomography and magnetic resonance imaging. In Smit, A.L., Bengough, A.G., Engels, C., van Noordwijk, M., Pellerin, S., Geijn, S.C. (Eds.) *Root methods – a handbook*. (Pp: 343-363). London. Springer.

Atkinson, D. (2000). Root characteristics: why and what to measure. In Smit, A.L., Bengough, A.G., Engels, C., van Noordwijk, M., Pellerin, S., and van de Geijn, K.S.C. (Eds.) *Root Methods: A handbook*. (Pp: 2–32). Springer-Verlag. Berlin. Heidelberg. Germany.

Box, J.E. (1996). Modern methods for root investigations. In Waisel, Y., Eshel, A., Kafkafi, U. (Eds.) *Plant Roots: The Hidden Half*. (Pp:193–237). Marcel Dekker. New York.

Curt, T., & Prévosto, B. (2003). Rooting strategy of naturally regenerated beech in silver Birch and Scots pine woodlands. *Plant and Soil*, 255, 265-279.

Danjon, F., Sinoquet, H., Drexhage, M. (1999). Characterisation of structural tree root architecture using 3d digitising and AMAPmod software. *Plant and Soil*, 211, 241-258.

Danjon, F., & Fourcaud, T. (2005). Root architecture and wind firmness of mature *Pinus pinaster*. *New Phytologist*, 168, 387-400.

Danjon, F., & Reubens, B. (2008). Assessing and analyzing 3D architecture of woody root systems, a review of methods and applications in tree and soil stability, resource acquisition and allocation. *Plant and Soil*, 303, 1-34.

Dupuy, L., Fourcaud, T., Lac, P., Stokes, A. (2003). Modelling the influence of morphological and mechanical properties on the anchorage of root systems. In Ruck, B., Kottmeier, C., Mattheck, C., Quine, C., Wilhelm, G. (Eds.) *Wind Effects on Trees*. (Pp:315-322). Universitat Karlsruhe, Germany.

Dupuy, L., Gregory, P., Bengough, A. (2010). Root growth models: towards a new generation of continuous approaches. *Journal of Experimental Botany*, 61, 2131-2143.

Hruska, J., Cermak, J., Sustek, S. (1999). Mapping tree root systems with ground-penetrating radar. *Tree Physiologist*, 19, 125-130.

Kalliokoski, T., Sievanen, R., Nygren, P. (2010). Tree roots as self-similar branching structures: axis differentiation and segment tapering in coarse roots of three boreal forest tree species. *Trees*, 24, 219-236.

Khuder, H., Danjon, F., Stokes, A., Fourcaud, T. (2006). *Growth response and root architecture of black locust seedlings growing on slopes and subjected to mechanical perturbation. Proc. 5th. Plant Biomechanics Conference.* Stockolm: Salmen L. Pp: 299-303.

Mulia, R., Dupraz, C., van Noordwijk, M. (2010). Reconciling root plasticity and architectural ground rules in tree root growth models with voxel automata. *Plant and Soil*, 337, 77-92.

Nicoll, B., Berthier, S., Achim, A., Gouskou, K., Danjon, F., van Beek, L. (2006). The architecture of *Picea sitchensis* structural root systems on horizontal and sloping terrain. *Trees*, 20, 701-712.

Oppelt, A., Kurth, W., Dzierzon, H., Jentschke, G., Godbold, D. (2000). Structure and fractal dimensions of root systems of four co-occurring fruit tree species from Botswana. *Annals of Forest Science*, 57, 463-475.

Oppelt, A., Kurth, W., Godbold, D. (2001). Topology, scaling relations and Leonardo's rule in root systems from African tree species. *Tree Physiologist*, 21, 117-128.

Pàges, L., Vercambre, G., Drouet, J., Lecompte, F., Collet, C., Le Bolt, J. (2004). Root type: a generic model to depict and analyse the root system architecture. *Plant and Soil*, 258(1-2), 103-119.

Pierret, A., Capowiez, Y., Moran, C.J., Kretzschmar, A. (1999). X-ray computed tomography to quantify tree rooting spatial distributions. *GEODERMA*, 90, 307–326.

Stokes, A., Fourcaud, T., Hruska, J., Cermak, J., Nadezdhina, N., Nadyezdhin, V., Praus, L. (2002). An evaluation of different methods to investigate root system architecture of urban trees in situ: I. ground-penetrating radar. *Journal of Arboriculture*, 28, 2-10.

Tamasi, E., Stokes, A., Lasserre, B., Danjon, F., Berthier, S., Fourcaud, T., Chiatante, D. (2005). Influence of wind loading on root system development and architecture in oak seedlings (*Quercus robur* L.). *Trees*, 19, 374-384.

Tobin, B., Cermak, J., Chiatante, D., Danjon, F., Di Orio, A., Dupuy, L. (2007). Towards developmental modelling of tree root systems. *Plant Byosystems*, 141(3), 481-501.

Wagner, B., Gartner, H., Ingensand, H., Santini, S. (2010). Incorporating 2D tree-ring data in 3D laser scans. *Plant and Soil*, 334, 175-187.

CHAPTER 3

MORPHOLOGICAL EVALUATION OF CORK OAK ROOT SYSTEM USING A 3D APPROACH

Dinis, C., Surovy, P., Ribeiro, N.A., Oliveira, M.R.O. (2014). Morphological Evaluation of Cork Oak Root System using a 3D Approach. *Submitted to Trees - Structure and Function.*

3.1 Abstract

The knowledge and understanding of the cork oak (*Quercus suber* L.) root system and its capacity to reach the groundwater, which is crucial for the water balance of the tree and consequently for woodlands sustainability is very important. The architecture of a root system besides determines its exploration of distinct spatial domains in the soil also reveals the root system ability to respond dynamically to the localized availability of soil resources (which are highly nonuniform within a root system space). Yet, root function in mechanical support of the shoot system is also determined by root architecture. The purpose of this study was to show how the root system of cork oak (*Quercus suber* L.) developed structurally (3D architecture) under a Cambissoil soil profile with 1.4 m depth. A 19 years old cork oak was intensively studied with focus on its structural and morphological aspects. The entire root system was exposed and digitized by magnetic motion tracker device Fastrak POLHEMUS. The dimorphic root system was observed as well as the presence and location of sinkers roots, important structures for drought resistance in the Mediterranean ecosystems. Through this work several spatial distribution aspects were evaluated. Evaluation of root lengths, biomass and volumes, among others, were performed. It was verified that the higher root volume distribution is located on the first 20 cm depth. The horizontal canopy projection was 2 times minor than the root system horizontal spread. In North-East direction some roots between 5 and 40 cm depth were found at 10 meters distance from the trunk, four times the line canopy projection. The biomass evaluated for shoot and root system were similar with 65 Kg allocated at the aerial compartment and 55 Kg allocated at the root system. We believe that this work, also supported by others about cork oak hydraulic distribution, will reinforce less intensive tillage techniques in order to avoid the tree decline and mortality of cork oak woodlands.

Key-words: cork oak (*Quercus suber* L.); root system; tridimensional architecture; root volume

3.2 Introduction

Climate change scenarios suggest an intensification and an increase of time periods of dryness for the regions of Mediterranean climate (Vaz, 2005) which will cause an increase of drought stress in these ecosystems (eg., cork oak woodlands). The carbon assimilation and transpiration will be severely restricted (Pereira *et al*., 2004) and may jeopardize the survival of trees. Otieno *et al.* (2006) refer that in cork oak woodlands, the establishment of only certain specific species, their distribution, as well as its mortality appears to be partially controlled by edafoclimatic conditions and may be the product of an optimum of environmental water availability. Plants that have the ability to remain physiologically active for long periods of dryness during the summer must have access to deep soil layers (David *et al*., 2004; 2013*)*, which stays moist longer (Rambal, 1984), as well as the ability to explore large volumes of soil (Breda *et al*., 1995) or have efficient mechanisms redistributing water within the soil profile (Ryel *et al*., 2003; David *et al*., 2004; 2013; Kurz-Besson *et al*., 2006). One of the main specie occurring in these woodlands is the cork oak (*Quercus suber* L.). Generally cork oak is adapted to well-aerated soils, avoiding compacted and permanently flooded soils. According to Montero *et al*. (1988), the most abundant soil texture where this specie occurs is loamy (74 percent of studied cases), followed by sandy (14 percent), and within the loamy texture group about 30 percent are sandy loam, 28 percent loam, and 16 percent silty loam. Also most cork oak woodlands occur on moderately acidic to slightly acidic soils, with pH generally in the range of 4.7 to 6.5 and, more rarely, 3.4 to 7.8.

Several researchers (David *et al*., 2007; 2013; Kurz-Besson *et al*., 2006; Lacambra *et al*., 2010; Oliveira *et al*., 1992; Otieno *et al*., 2006) advises that it is of upmost importance the knowledge and understanding of the cork oak root system and its capacity to achieve the groundwater, which is crucial for the water balance of the tree and consequently for woodlands sustainability. Nevertheless only few studies focus on this system, mostly because of the difficulty associated to the access to belowground compartment. Root researchers recognize the difficulties associated with the study of roots in soil (Kummerov, 1981; Box, 1996, Atkinson, 2000; Pagès *et al*., 2004, Tobin *et al*., 2007; Dupuy *et al*., 2010). Even taking into account technological innovations that can be used today still remains a challenge often refused, given the very low benefit between results and effort invested (Silva *et al.*, 2002, Dupuy *et al.*, 2010; Mulia *et al.*, 2010; Kalliokoski *et al.*, 2010).

Root systems are complex structures, typically being composed of thousands of individual root axis that vary developmentally, physiologically, and morphologically (Lynch, 1995). Roots, like shoots, are dynamic branched structures originating from the collar of the plant. They really form a "system" in the sense that their components are connected in an organized network (Pàges, 1999). Their functioning seriously depends on the entire root system structure or architecture (Danjon & Reubens, 2008). Roots with different orders play different roles in absorbing (radially) and transporting (longitudinally) water and nutrients (Doussan *et al.*, 1998).

Tree architecture

Tree architecture (structure) has been subject of research since long time (see review article from Barthelemy & Caraglio, 2007; Surový *et al.*, 2011a; 2012) creating the physical framework for different functions. Tree structure has to ensure resource capture and transport, and mechanical stability continuously during its life span (Kalliokoski *et al.*, 2010). In order to accomplish these functions trees need to adjust their structure and functions according to the surrounding environment. Tree architecture describes the spatial configuration of a tree, i.e. the geometric dimensions, shapes, and explicit locations of botanical units in 3D space (Barthélémy & Caraglio, 2007; Danjon & Reubens, 2008) and the constructional organization of the branching system (Godin, 2000). The functioning of both shoots and roots depends on the entire structure or architecture of the system, including the topological arrangement of components and their geometric characteristics. However, Danjon & Reubens (2008) refer that roots deviate from shoots in many ways: they are functionally less differentiated, do not have similar kinds of morphological markers (no leaves, leaf petioles, branches or stem, as in shoots) and have more irregular, opportunistic growth as a response to soil heterogeneity.

Full, detailed quantitative 3D plant architecture assessment was initiated in 1979 by de Reffye and Fisher & Honda (Danjon & Reubens, 2008). Several methods for description and modeling of 2D and 3D shoot and root architecture are revised in Godin (2000), Pagès *et al*. (2000) and Danjon & Reubens (2008).

According to Surový *et al.* (2011a) shoot architecture, as a specific sub-area of plant architecture, has been intensively studied to evaluate the reappearance of geometrical structures or to analyze carbon allocation. Although studies about root system architecture are scarce due to the complexity to access the entire root systems and to obtain their architecture (Cermak *et al.*, 2006; Collet *et al.*, 2006; Dupuy *et al.*, 2007; Fitter & Stickland, 1991; Hruska *et al.*, 1999; Kalliokoski *et al.*, 2008; Lintunen & Kalliokoski, 2010; Nicoll *et al.*, 2006, Stokes *et al*., 2002; Surovy *et al.*, 2011b). Despite this scarcity some authors advise different approaches when dealing with root systems. Asaah *et al.* (2010) defend that studies on root systems should concentrate more on root architecture and distribution than on individual root numbers. Root system architecture plays an important role in the acquisition of the edaphic resources, which are in subject to local depletion (Zhang *et al*., 2003). These kinds of studies, usually do not include fine structural details, such as root hairs, instead they rely in an entire root system or a large subset of the root system of an individual plant (Lynch, 1995). Root architecture refers to the spatial configuration of the root system presenting the explicit geometry deployment of root axes. Yet, root system spatial configuration (number and length of lateral organs), vary greatly depending on the plant species, soil composition, and particularly on water and mineral nutrients availability (Malamy, 2005; Hodge, 2009). It results of the extension growth of individual root axes, the appearance of lateral roots along root axes, the direction of root axis elongation, the senescence or mortality of root axes, and the plasticity of these processes in response to environmental conditions such as soil strength, nutrient availability, water status, and oxygen status (Lynch, 1995).

When root system architecture is studied there are several aspects that can be explored such as root typology, topology, geometry of root elements and their spatial distribution in soil (Lynch, 1995). Specifically, root dry weight and length are the most studied characteristics, respectively for plant partitioning and the uptake of water and nutrients from the soil (Amato & Pardo, 1994).

Root branches are usually classified as coarse or fine roots based on the root diameter; most typically, roots with a diameter ≥ 2 mm are defined as coarse roots and thinner root branches (less than 2 mm diameter) as fine roots (Kalliokoski *et al.*, 2010). The two most distinctive groups are shallow and sinker roots (Danjon & Reubens, 2008). Shallow root axes of mature trees spread several meters horizontally in the uppermost soil layers, determining the limits of the horizontal influence area of the root system. Sinker roots extend obliquely or vertically in soil determining the vertical influence area of the root system.

3D Digitizing

Exhaustive 3D plant digitizing is presently considered to be the most accurate way to describe plant architecture (Sonohat *et al.*, 2006). The 3D digitizing method has been used since the 70’s. Firstly, an articulated arm measuring rotation angles was employed (Lang, 1973 *in* Godin & Sinoquet, 2005). Although more advanced technologies are applied nowadays. Semiautomatic measurement of 3D aerial tree architecture using a 3D digitizer was first proposed by Sinoquet and Rivet (1997) and Sinoquet *et al.* (1997), and was adapted to root system by Danjon *et al.* (1999). A detailed review about comparisons with other techniques, equipments and different software are presented in Danjon & Reubens (2008) work. A precise and detailed tree reconstruction can be achieved using special equipment of 3D digitizing which record the 3D position of sensors inside a magnetic field created around the target (Lintunen, 2013). Polhemus “3Space Fastrack”, manufactured by Polhemus (Polhemus, Colchester, Vermont), is an alternating current low frequency magnetic field digitizer, consisting of an electronic unit, a magnetic transmitter and a small receiver (pointer) fitted with a switch. The whole is connected to a computer. According to Danjon & Reubens (2008) the “3Space Fastrack” provides 6-degree of freedom (X Y Z Cartesian coordinates and azimuth, elevation as well as roll orientation of the receiver) digitizing of single points in a 1.5 m radius sphere. The size of the sphere can be extended to 5 m with the “Long Ranger” option. The magnetic digitizer requires that no metallic objects are situated in the measurement field (Surovy *et al.*, 2011a).

Aim of study

The purpose of this study is to show how the root system of cork oak develops structurally (3D architecture) under a specific soil type – Cambissoil soil (FAO, 2006). We believe that the results will open new insights to the research of this underground system.

To our knowledge, only the works from Metro and Sauvage (1957) analysed the full root system of cork oak trees of different ages from the Mamora forest (in Marocco occidental). Surovy *et al.* (2011b) accessed partially the root system with the main purpose of comparing rooting patterns from seeded and planted juvenile trees; David *et al.* (2013) mapped the root system of an adult cork oak tree till the lowest water table level at the end of summer (4.5 m depth) and modeled the contribution of different water sources to tree transpiration based on root structure and functioning. Results from this work showed a dimorphic root system with a network of superficial roots linked to sinker roots, and a tap root diverging into tangles of deep fine roots submerged for long periods. Kurz-Besson *et al*. (2006) and Nadezhdina *et al*. (2008) have also found a dimorphic root system and identified the occurrence of hydraulic lift.

From our knowledge, in terms of detailed 3D cork oak root architecture, using the referred digitalization technology, this work is unique. This study will only focus on one sample tree. But we consider that the results that will be obtained together with the information already existent from the abovementioned studies, will allow a general description of the structural-functional approach for the root system development of this species. The tree specific pattern of roots distribution in soil is tree self-dependent on its own genetic and fenology, and completely dependent on the soil conditions and effects of the surround matrix.

3.3 Material and Methods

3.3.1 Field site and environmental conditions

This study was carried out in Canha, in SW Portugal (38’38.38”N 8º 38’22.22”O). The field work was executed between September 2008 until November 2010. Soil profile description and evaluation was made *in situ* and soil samples from each layer were collected for physical and chemical analysis. Also specific soil samples were taken for bulk densities and porosity analysis. For this purpose, we used cylindrical metallic samplers (with 5 cm Ø and 3 cm width).

Data from Pegões climatic station was used on climatic conditions evaluation, due to the proximity to field work site. Data was collected from MAMAOT (2012) publication and climatic parameters were analyzed. Assuming the mean monthly temperature, the year can be divided in two semesters. The warmer semester occurs between May and October. The mean monthly temperature ranges from 9.8ºC in January and 22.5ºC in August. The warmer months, consequently, are the ones with lower values of precipitation, July and August; with values less than 10 mm (Figure 1). The months where more precipitation is observed are December and January with values of 99.3 and 100.8 mm, respectively. The mean annual value verified for Pegões station is about 703 mm. This region according to hidric index is evaluated as sub-humidic dry (C1) (MAMAOT, 2012). Maximum values of evaporation occur in summer, with August and July presenting the higher values (202.2 mm and 191.7 mm, respectively). By year, evaporation in Pegões, achieve 1347.2 mm. Potential evapotranspiration (PET) was calculated according to Thornthwaite method which assumes PET as a result of mean temperature, with a correction in function of day time and number of days per month. Results presented in Figure 2 shows that for this region values goes from a minimum of 22.7 mm in January and a maximum of 126 mm in July. The start increase of this value is verified in February and it continues almost in a mean of 20 mm per month until July. From August until January it decreases in mean almost 30 mm per month. Annual potential evapotranspiration assumed is of 797.4 mm.

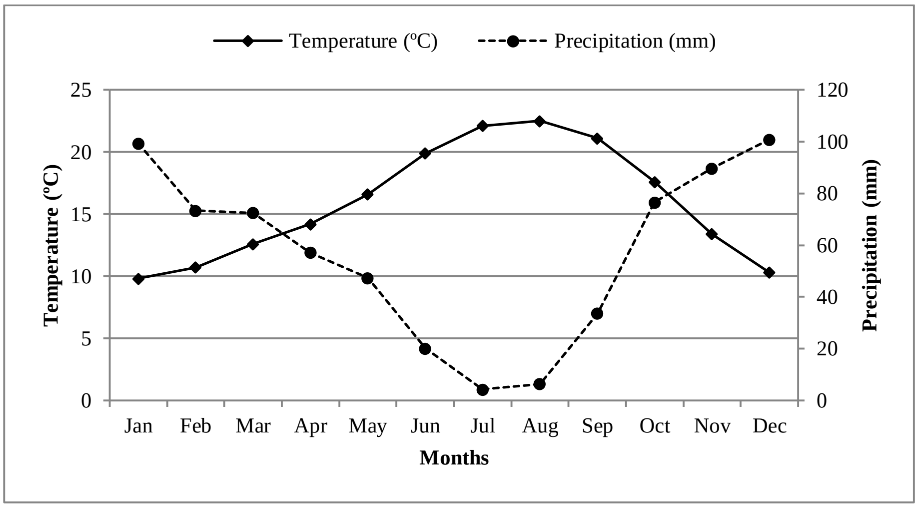
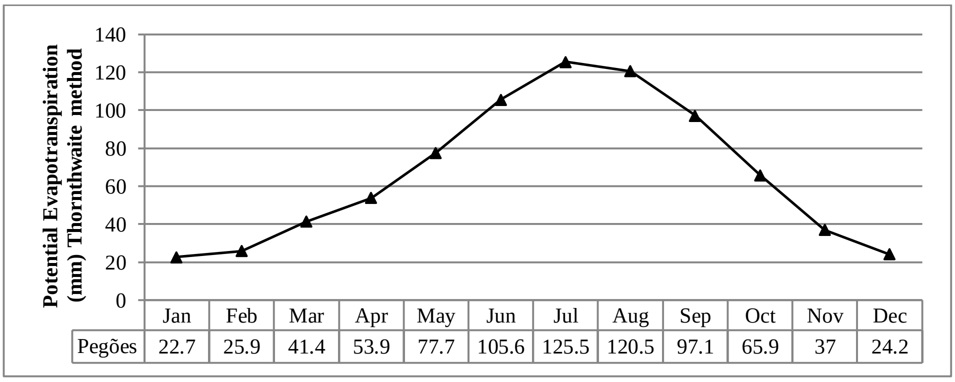


Figure 1. Ombrotermic diagram for Pegões station (mm).



	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Pegões	22.7	25.9	41.4	53.9	77.7	105.6	125.5	120.5	97.1	65.9	37	24.2

Figure 2. Mean monthly potential evapotranspiration (mm) evaluated by the Thornthwaite method, for Pegões station.

3.3.2 Plant material

A cork oak of 6 m height (3.82 m of trunk and 2.18 m of canopy height) with a diameter at breast height (1.3 m) of 19.8 cm was used in this study (Figure 3). It had19 years old and had never been debarked. The tree was located in a flat terrain (0º slope) and the last management practice – disking – occurred 8 years ago. Young cork oaks, eucalyptus and young maritime pines were present in the neighborhood.

Figure 3. Cork oak used in the study**.**

3.3.3 Field survey

3.3.3.1 3D Digitizing

The digitizing process began with the tree identification into individual branches, leaves and trunk for shoot system and individual root segments for belowground system. For the digitizing process we use the methodology described in Surový *et al.* (2012) work. We used the FASTRAK Polhemus magnetic digitizer that enables the acquisition of Cartesian coordinates in relation to a chosen origin, or 0,0,0 reference point. To transfer information from FASTRAK into the database we used PiafDigit (Dones *et al*., 2006) and an experimental software written in Delphi 2009 Professional (Embarcadero, California, USA) for visualizing and editing datasets. Yet, for branches, some lateral roots and central root system, where the digitizing process *in situ* was impossible due to displacement, were marked reference points before they were cut. After they have been removed and placed in a new site, these reference points are then digitized again enabling the reconstruction of the original position of the segments in the tree architecture (for a detailed description see Surový *et al*., 2011a).

The semi automated 3D digitizing methodology was made by a team of 2 persons. As advised by Danjon & Reubens (2008) one person was responsible for clicking the appropriate positions with the receiver and for measuring the root diameters, and other person was responsible for entering diameters and topological indications on the PC as well as controlling the measurement process through the root system image on the screen. For diameters, two perpendicularly measurements were taken with a plastic caliper. Yet, recommendations from the abovementioned authors were followed for definition of segments: they advise that very short segments will result in an overestimation of root length and volume because of proportionally large imprecision in XYZ pith position. When a 3D digitizer is used, in large axes, digitizing should be made with the same receiver direction, for example clicking all the time on the roots from the north direction. From 3D digitizing process in the field, the following parameters were obtained: root length from 3D (RL_{3D}) and root diameter from 3D (RD_{3D}).

3.3.3.2 Plant material collection

Specific parameters for shoot system as total height, trunk height, and circumference at breast height were measured. Identification and codification of branches was done according to tree graph structure. The first branches originated from trunk (first order) were considered of second order; the ones originated from second order were identified as third order and so on. Seven orders were identified in aboveground system. The aerial system was divided into leaves (which weren't 3D digitized), new branches (last year grow), branches and trunk.

A first digitizing was made from the trunk base until the top of the tree. The two main branches were tridimensional digitized avoiding any disturbance from their original spatial position. Due to tree height, it was impossible to make the digitalization *in situ* or with standing tree, because the cable length from 3D equipment was not enough. We decided to cut the branches and by so, several spatial reference points were marked in each branch before cutting. Each main branch was carefully cut and transported to specific place where the conditions allowed a complete digitalization. Through these reference points it was possible to join the several files, maintaining the original spatial position in the tree architecture.

After the digitizing process, each individual segment (branches and small branches) was collected, codified and stored in hermetic plastic bags in a fridge (4ºC), for biomass analysis. Respective group of leaves from each branch were also collected, codified and stored at low temperatures. The trunk was maintained in the field in its original position until the root system excavation process was completed. After ¾ of root system excavation and due to the instability of the trunk, caused by soil removing, some ropes were used to prevent trunk displacement from the original position. At the end of root system excavation, trunk was separated from main root and carried out to laboratory.

The methodology described at our previous work for cork oak (see Dinis *et al.*, 2011) was adopted to access the entire root system. At the beginning, a deposit of sediments was opened with a machine. This deposit was 2 m depth and was at 6m distance from tree trunk. The excavation by high pressure air jet method started in the topsoil (first layer) following the orientation from trunk to the crown projection line. These first 18 cm had an elevated sand percentage and by so this technique was well succeeded. However for deepest layers, it was difficult to remove the soil only through the air pressure. Deepest layers became less sandy and more clayed. Since these characteristics were not suitable for the use of this method and in accordance with the result of earlier work (see Dinis *et al.*, 2011), we also excluded the method of profile washing with water. Thus, the only viable option for the excavation of deepest layers was the manual (by hand, with the aid of material used in archaeological excavations and sometimes with the aid of a pneumatic hammer, in the cases where the soil characteristics were very compacted with a high bulk density).

The excavation was conducted in different phases. After the excavation of one quarter of root system, and with the roots exposed and maintained at the original position, digitizing began. Each root segment was identified, codified and digitized. After digitalization, the respective root segment was cut, labeled and stored in hermetic plastic bags in cold environment. The respective smaller fine roots (less than 1mm diameter) spread through all root segment were also stored. When all the roots from this section (one quarter) were exposed, digitized and removed, the excavation continued. At each complete root exposure the digitalization was made. These two processes occurred simultaneously until achieve the final stage of root system excavation. When only the central part of root system was left, to avoid movement and displacement of roots, the trunk was supported and fixed with wooden sticks, strings and also with the aid of a tractor. Through this method it was possible to remove the soil from the main root. After total exposure of root system, this central system was carried to laboratory where the tasks of 3D digitizing, cutting and storing the root segments were completed. All the roots were labeled, following the graphic tree codification, where roots that were originated from tap root received the code of order 1; the ones originated from order 1 receive the code of order 2 and so on. Seven orders were identified in this cork oak root system. All the root segments were stored in fridge (4ºC) for further laboratory biomass analysis.

3.3.4 Laboratory measurements

3.3.4.1 Shoot system

Aboveground structures, such as leaves, new branches, branches and trunk were analyzed at laboratory and different procedures were applied for the different components.

35 bags of leaves were randomly selected for leaf area measurements. Leaf area measures were obtained through image analysis software (ImageJ) from the scanned images made with a scanner (HP Scanjet 4850), with 254 dpi resolution. After area measurements, leaves were dried at 75ºC during 48 hours and dry weight was taken. With these results, a linear regression model was tested between biomass and areas (Figure 4).

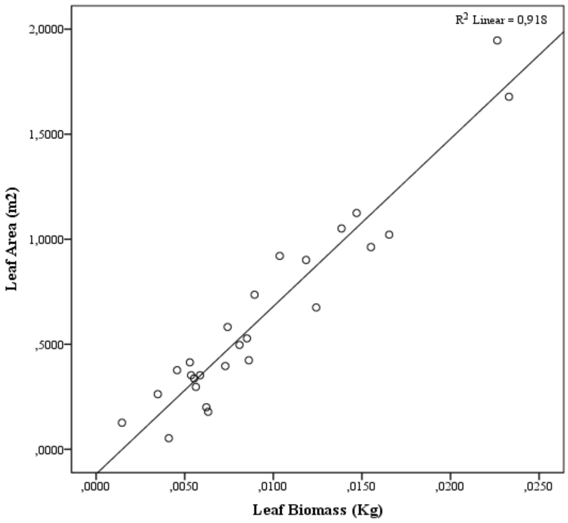


Figure 4. Linear regression model between leaf area (dependent variable) and leaves biomass (independent variable).

Through this model (1) it was possible to achieve leaf area values for all sets of leaves collected on the tree. All the fresh leaves sets were dried in an oven at 75ºC, during 48 hours and dry weights were registered.

$$Leaf\ area\ (m2) = 79.798 * Leaf\ Biomass\ (Kg) - 0.118 \qquad R^2 = 0.918 \tag{1}$$

Wood structures (new branches, branches and trunk) were individually analyzed. Lengths and minimum and maximum diameters were recorded. Biomass, expressed in terms of dry weight, was obtained after a drying process at 103ºC, during 48 hours. For shoot system the follow variables were obtained:

LB – Leaf biomass (Kg)

LA – Leaf area (m^2)

NbB – New branches biomass (Kg)

NbL - New branches length (m)

BB – Branches biomass (Kg)

BL - Branches length (m)

TB – Trunk biomass (Kg)

TH – Trunk height (m)

SB – Shoot biomass (Kg)

3.3.4.2 Root system

Root system laboratory analysis was divided in two phases, one concerning to the coarse roots and other to the fine roots. Assuming that coarse roots are all the roots that present a diameter greater than 2 mm, the laboratory procedure applied for each root segment was: (1) root segment division by diameter classes, (2) length measurement using a metric tape, (3) evaluation of fresh weight, (4) drying of roots in an oven at 103ºC, during 48 hours, (5) registration of biomasses expressed as dry weights. For fine roots analysis: (1) fine roots were separated from the respective root segment and washed carefully with water enabling the separation of soil aggregates and were conserved at a water and alcohol solution, at lower temperatures, (2) Lengths of fine roots were measured using Comair Root Length Scanner (Commonwealth. Aircraft Corp. Ltd. Melbourne, Australia) and metric tape. The Comair root length scanner is one automated device that is based on the line-intercept method (Newman, 1966). For Comair root length scanner fine roots were individually spread on the circular glass plate, avoiding overlapping and water bubbles (Figure 5). Due to that, sometimes it was necessary to repeat the process several times to complete only one sample.

Figure 5. Measurements of fine roots using Comair Root Length equipment.

The total length was then calculated by sum of the several measurements. This equipment does not allow the measurement of areas and diameters. (3) Fine roots fresh weights were taken, (4) fine roots were dried, on an oven, during 48 hours at 103ºC and (5) registration of biomass, expressed in terms of dry weight, for each sample.

The size-related root variables obtained from laboratory analysis were:

RL –Root length (m)

RB – Root biomass (Kg)

RD – Root diameter (cm)

CRL – Coarse root length (m)

CRB – Coarse root biomass (Kg)

FRL - Fine root length (m)

FRB – Fine root biomass (Kg)

Through the previous variables, functional morphological root parameters were also calculated:

RSA – Root surface area (m^2) = 2Π R (m) * Root Length (m)

SRA – Specific Root Area ($m^2\ Kg^{-1}$) = Root Surface Area (m^2) / Root Biomass (Kg)

SRL –Specific Root Length ($m\ Kg^{-1}$) = Root Length (m)/Root Biomass (Kg)

RV – Root Volume (m^3) = Π*Length (m) * [R_{max}^2(m) + (R_{max} (m) * R_{min}(m)) + R_{min}^2(m)] / 3

RF – Root Fineness ($cm\ cm^{-3}$) = Root Length (cm) / Root Volume (cm^3)

FRTD – Fine Root Tissue Density ($g\ cm^{-3}$) = Fine Root Biomass (g)/Fine Root Volume (cm^3)

R:S ratio – Root shoot biomass ratio = Root Biomass (Kg) / Shoot Biomass (Kg)

According to several authors such as Eissenstat and Yanai (1997), Ostonen *et al.*, (2007), Pregitzer *et al.* (2002) these morphological parameters, especially SRL and to a lesser extent SRA, have been used as indices of root benefit to root cost, assuming that resource acquisition is proportional to length or surface area and that root cost (construction and maintenance) is proportional to mass. Yet, Ostonen *et al.* (1999) assumes that higher SRA indicates a higher allocation of assimilates to roots. Root volume and root fineness are parameters that allow a major perception of root structure strategy. From tridimensional architecture, which included the minimum and maximum diameter and length variables taken in the field during the digitizing method, root volume (RV_{3D}) was calculated.

3.4 Results and Discussion

3.4.1 Soil characteristics

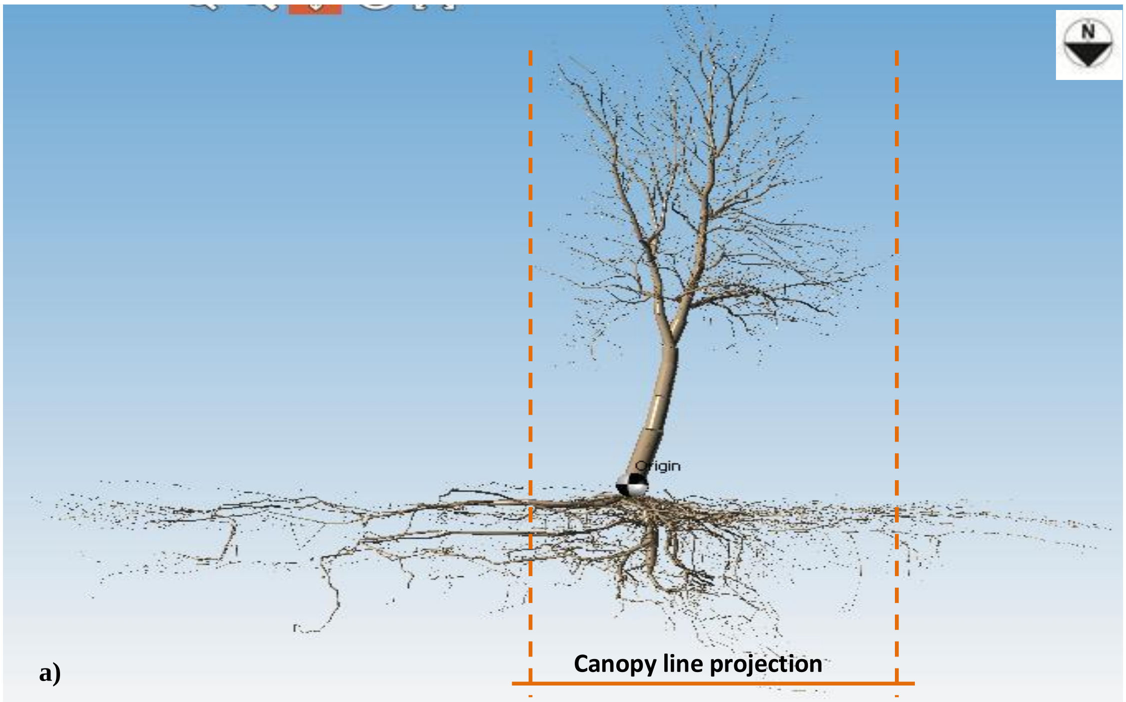
The soil type where the cork oak was developing was a Cambissoil soil with sandy-loam characteristics with clay congregations. This type of soil is originated from unconsolidated materials, with exception of materials with fluvic properties or with very rough or stony texture. It is characterized by not having clear diagnostic layers besides a umbric A and without hidromorphic properties within 50cm of the surface. The results of soil profile evaluation made in field, through wall visualization showed that this soil was composed by 3 different layers (Table 1). In the first layer (A horizon), from 0 to 8cm depth, was observed the presence of many roots from herbs, shrubs and eucalyptus. This high root presence is due to topsoil characteristic where the higher quantity of organic matter was available (1%) when compared to the other depth layers. Its physical evaluation showed a higher presence of sand (75%) and through chemical analysis the higher values for nutrients available in soil (phosphurus (0.0103%), potassium (0.056%) and nitrogen (0.03%)), were verified in this layer. A second homogeneous layer (B horizon) was verified at 20-95 cm depth. The presence of fine roots from shrubs was verified until approximately 35 cm depth. Below this depth only roots from cork oak and eucalyptus were verified. The organic matter available was only of 0.20% and nitrogen was not detected in the chemical analysis. The presence of sand continued very high in this layer (75.1%) although some clay aggregations were observed. The last homogeneous layer (bedrock) was observed between 95 and 140 cm depth, were only few tree roots (from cork oak and eucalyptus) were present. This layer was completely different from the previous when observed in the field. The presence of clay aggregations was high and easily recognized by visualization. The physical soil analysis confirmed this aspect. The presence of clay increase 229% compared to previous layer. This was the layer, in the entire soil profile, where a higher percentage of potassium was found (0.0126%). Below the 1.40 meters it was impossible to continue the soil profile description due to bedrock conditions. pH ranged from 4.9 to 5.1 (moderated acidic soil) which is in accordance to soil type preferences for cork oak development (Montero *et al.*, 1988). As expected, soil bulk density increase with depth increase. A range from 1.62 to 1.76 g cm^{-3} was obtained. The second layer (B horizon) presented a value of 1.73 g cm^{-3}.

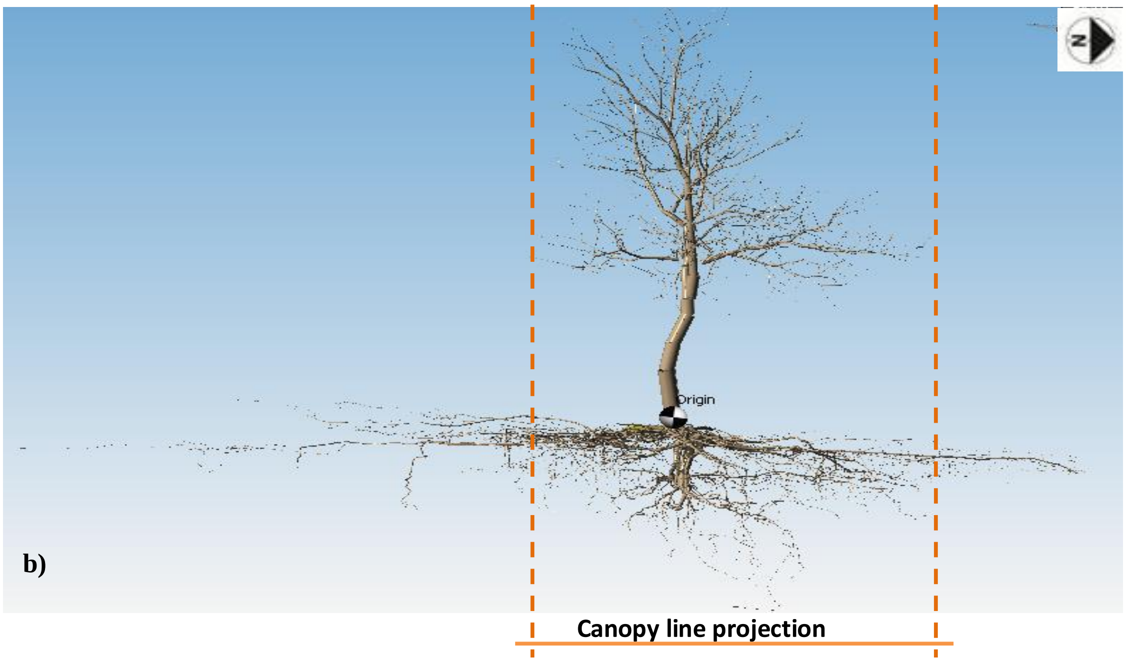
Table 1. Physical and chemical characteristics and field observations of soil profile.

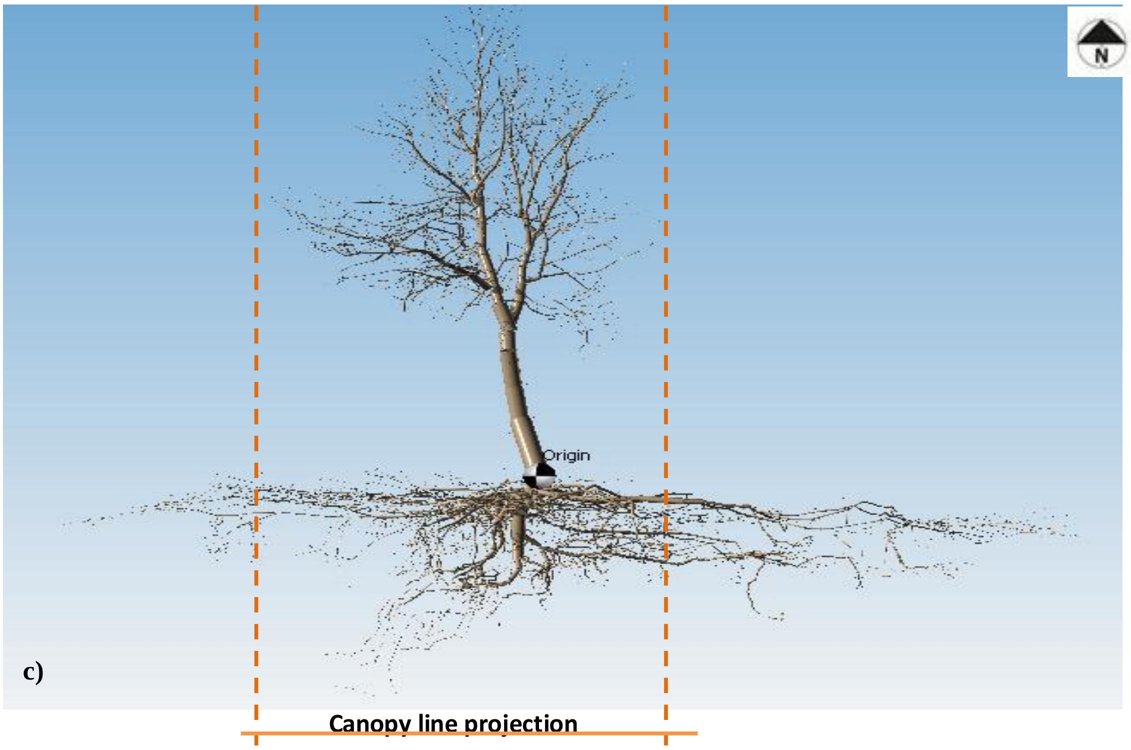
Depth, cm	0-20	20-95	95-140
Texture	Sandy-loam	Sandy-loam	Sandy-clay-loam
Sand, %	75.2	75.1	58.7
Silt, %	16.4	14.6	7.5
Clay, %	8.4	10.3	33.9
Bulk density, g cm^{-3}	1.62	1.73	1.76
pH (H_2O)	4.90	5.00	5.10
Organic matter (%)	1.00	0.20	0.30
Phosphorus (P), %	0.0103	0.0035	0.0018
Potassium (K), %	0.0056	0.0056	0.0126
Nitrogen (N), %	0.03	Non detectable	0.01
Field observations	Many roots from herbs,shrubs and eucalyptus	Some fine roots from herbs and shrubs	No roots from herbs and shrubs

3.4.2 Cork oak tree architecture and morphological evaluation

In cork oak 3D architecture the segments of roots, new branches, branches and trunk are represented with real diameters and lengths evaluated *in situ*. 3D cork oak architecture shows a root system that spread horizontally far away from horizontal canopy projection. From the longest distance between outsider’s branches (largest horizontal canopy projection) the diameter reaches 4.80 m. The shoot system is formed by a trunk of 3.82 m height with 19.8 cm Ø at breast height (1.30 m), several branches, new branches (last annual growth) and leaves. Two main branches with a growing pattern mostly in height and, several branches from different orders growing diagonally, constitute the shoot system structure. Total height measured for this cork oak was of 6 m. Although leaves are not presented in the 3D architecture, each new branch formed (at the final of each branch presented in the architecture) contains a set of leaves. According to spatial position, root system could reach until 10 m distance measured horizontally from trunk, and in vertically, tap root reached 1.40 m depth. Only few small roots were observed bellow this depth. Figures 6a, b, c and d represent the different views of cork oak architecture.




N
Origin
b)
Canopy line projection


N
Origin
c)
Canopy line projection

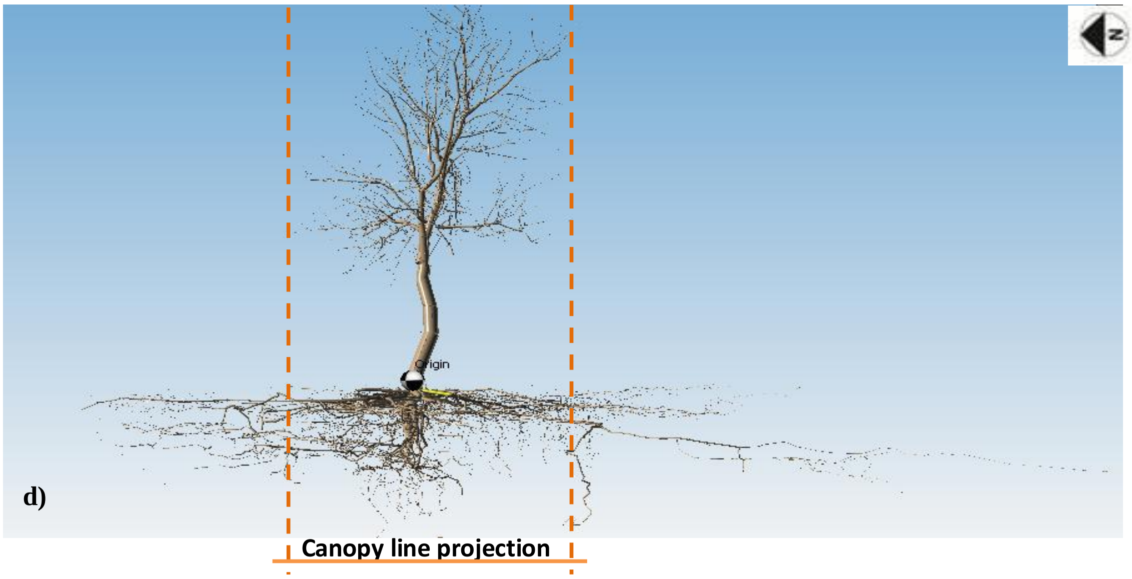


Figure 6. Different angle views from cork oak 3D architecture (aerial and root system) a) from North, b) from East, c) from South and d) from West.

By the representation presented on Figure 7 it is possible to verify that cork oak root system spread much more than the aerial system. These spatial distributions occurs possibly due to the soil conditions and specifically due to the high bulk density of the deepest layers (see Table 2), forcing the horizontal development of roots instead of their growth in depth. Also Metro and Sauvage (1957) in their work, verified through an observation of a 40 years old cork oak growing in a sandy profile soil with a maximum depth also of 1.40 m, that root surface area extended a lot more the horizontal line of canopy system. David *et al.* (2013) in their recent study observed that root system of a mature cork oak growing on a deep Haplic Arenosoil, with high permeability and a low water retention capacity, only extended at least into the crown limits. However they related this limit with probably physical restrictions by the occurrence of prior soil tillage.

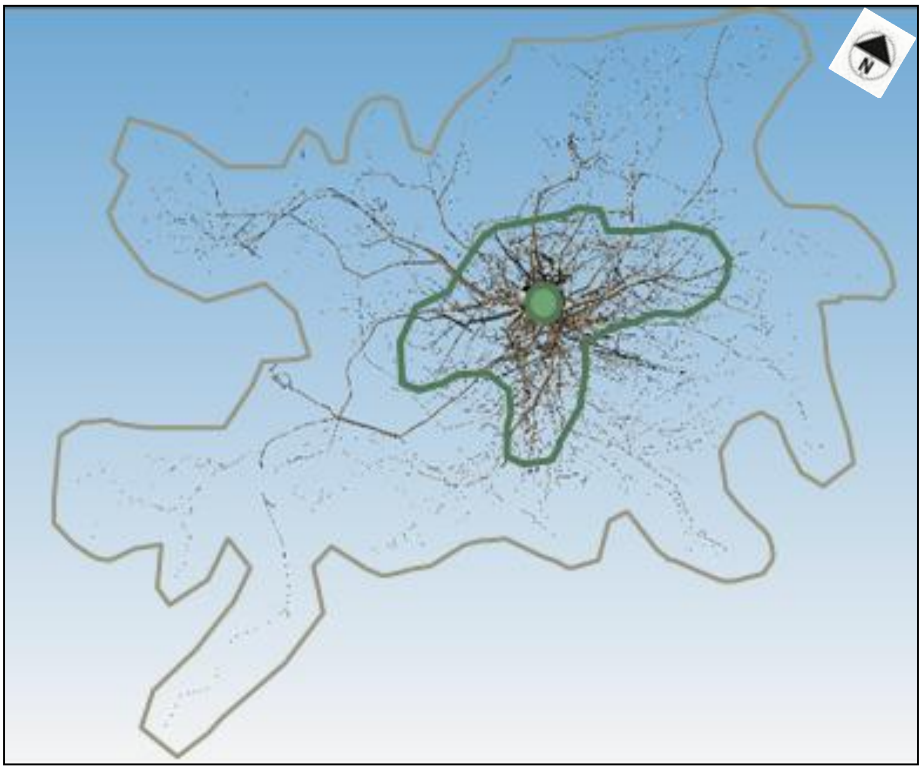


Figure 7. Spatial distribution of cork oak systems. Green color: shoot system, brown color: root system.

Evaluation of cork oak morphology shows that biomass allocation is similarity distributed between both systems (Table 3). Shoot system represents 54.1% of the total biomass distribution, with 64.7 Kg and root system presented 55 Kg. In total was verified that cork oak allocated 119.7 Kg of biomass, expressed in terms of dry weight. For total length, measured trough axes (see Figure 8) of trunk, branches, new branches and roots, a result of 5426 m was obtained. Root system for this parameter is more representative with 1815 m measured through root axis, mainly due to fine root structures. Structural root system composed only by the coarse roots, presented a total of 753 m length.

Table 2. Total tree biomass and length (aerial and root system).

	Shoot System	Root system	Total
Biomass (Kg)	64.74	54.94	**119.68**
Length (m)	1 610.48	1 815.44	**3 425.92**

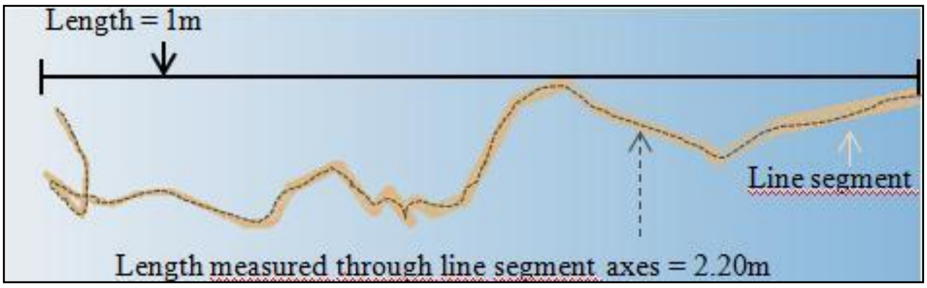


Figure 8. Difference between typical length and length measured through line segment axes (method adopted on Fastrak Polhemus 3D digitizer).

3D architecture of cork oak canopy system is presented on Figure 9. Biomass allocation of cork oak aerial system (Table 3 and Figure 10) indicates that, as expected due to its constitution, woody structures allocate more biomass than leaves. Tree trunk has more weight in terms of biomass (33 kg) representing 51.6 % of total aboveground system. Branches represent approximately 29.6%, with about 19 Kg of biomass evaluated. The structures that allocate less biomass are the new branches and leaves. Respectively, the values obtained for these compartments were 2.5 Kg and 6 kg. In percentage, new branches allocate 4% and leaves 15% of aboveground system biomass. The total length reported for the various tree aerial structures; show that branches and new branches together present 1607 m of length and the trunk measure 3.82 m (Table 2).

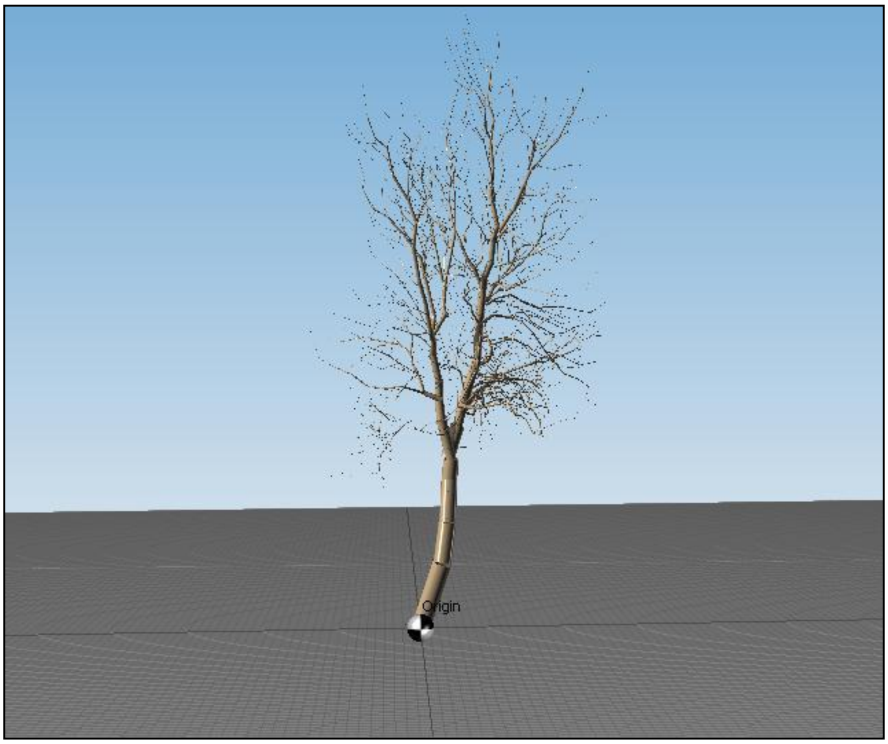


Figure 9. 3D architecture of cork oak shoot system.

Table 3. Biomass (leaves, new branches, branches, trunk) and length (branches and trunk) of aerial system.

	Shoot system				
	Leaves	**New branches**	**Branches**	**Trunk**	**Total**
Biomass (kg)	5.97	2.53	19.20	33.38	**64.74**
Length (m)	-	-	1 606.66	3.82	**1 610.48**

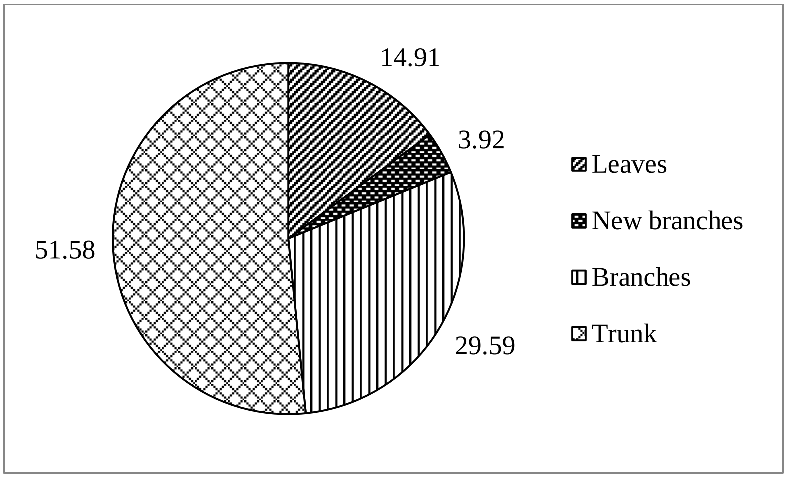


Figure 10. Aboveground biomass distribution of cork oak (%).

3.4.3 Cork oak root system architecture and morphology

In this work when it is referred the number of root segments, it should not be understood as the real number of roots. Meaning, as it was abovementioned, for an easier evaluation, the roots were cut and separated by diameter classes in laboratory and by so, there are cases that one single root can be separated into several root segments. Sanesi *et al.* (2013) defend that data collection on roots systems can be performed at different levels according to the aims of the research and can include topics such as: biomass measures (above and below-ground), dendrological parameters (length and diameter of roots at different sections) and architecture analysis. In our case the data collection was made with the purpose of architecture analysis but also biomass and length, measured through root axes, were evaluated.

Cork oak root system architecture show 2 subsystems, one at a superficial level (until 40cm depth) and another at a deeper level (around 1.20 m depth) (Figure 11) demonstrating that the root system of cork oak has the ability to explore the entire soil profile. Also Mètro and Sauvage (1957), Kurz-Besson *et al.* (2006) and recently, David *et al*. (2013) verified this dimorphic root system for cork oak. The connection between these two sub-systems is also composed of singular roots, usually from second or third order, that grow vertically in depth, called sinkers (Figure 12). This deep rooting strategy is an important feature for tree survival (Canadell *et al*., 1996), enabling the exploitation of deep soil and deep water tables (Cermak *et al*., 1980; Jackson *et al*., 1999) which are important for the maintenance of the transpiration of Mediterranean oaks during drought. David *et al.* (2013) mentioned that by accessing deep water sources, sinker roots may also contribute to the increase of whole-plant water transport efficiency through hydraulic redistribution. According to existent works (David *et al*., 2013; Kurz-Besson *et al*., 2006; Nadezhdina *et al.*, 2008) it is already proved that water in cork oak tree may move upwards (hydraulic lift), downwards (hydraulic descent or reverse hydraulic lift) or laterally. Hydraulic lift is the process of water movement from relative moist to dry soil layers using plant root system as a conduit (Caldwell *et al.*, 1998). This process allow that the water released from roots in the upper soil layers, when transpiration ceases, will be absorbed and used by the tree in the next day. Specifically, David *et al*. (2013) verified that hydraulic lift occurred at the end of summer from deep wet soil to superficial dry one and that hydraulic descent occurred in the wet periods following rain when the surface soil was wet. For instance, Kurz-Besson *et al.* (2006) had verified that in the warmer period of drought season, about 17 to 81% of the water lifted during night was used in the day after for transpiration. Pereira *et al.* (2004) stressed that hydraulic lift in cork oak may be also especially important on the onset of autumn rains. According to them, at this time of the year, herbaceous plant roots are not alive in the top soil to use the suddenly available nutrients and rain water is not plentiful enough yet to carry them deep into the soil.

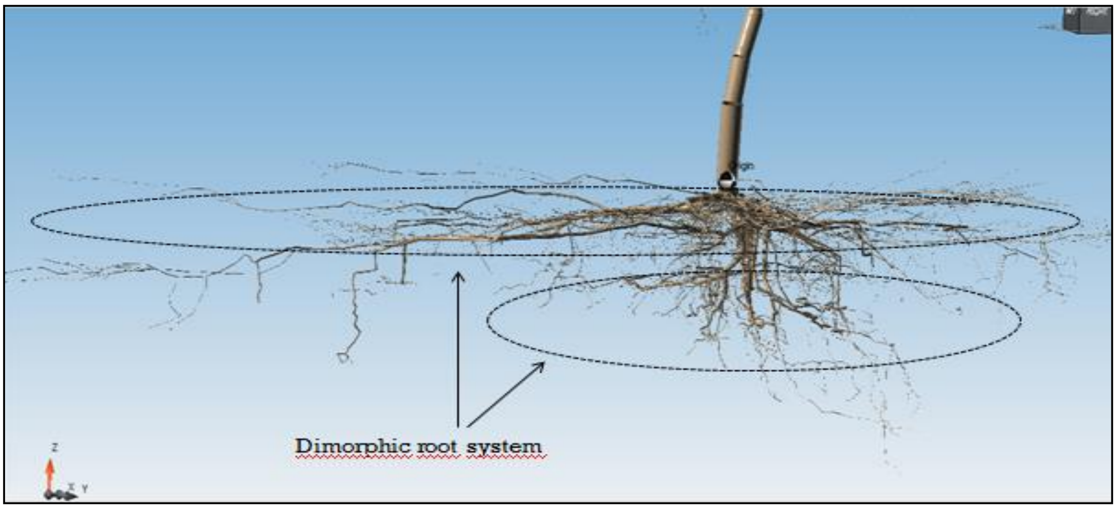


Figure 11. Cork oak dimorphic root system.

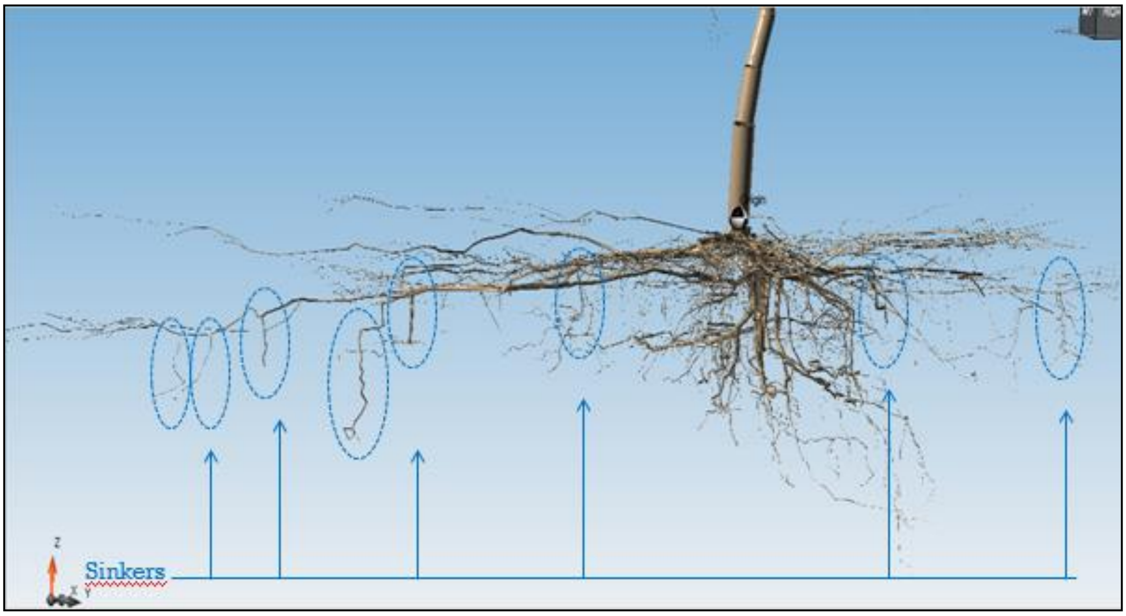
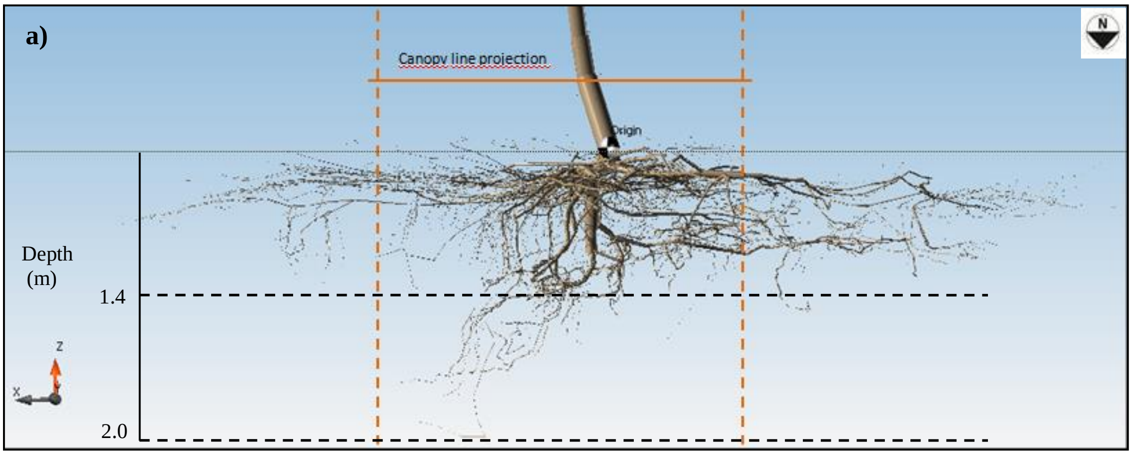


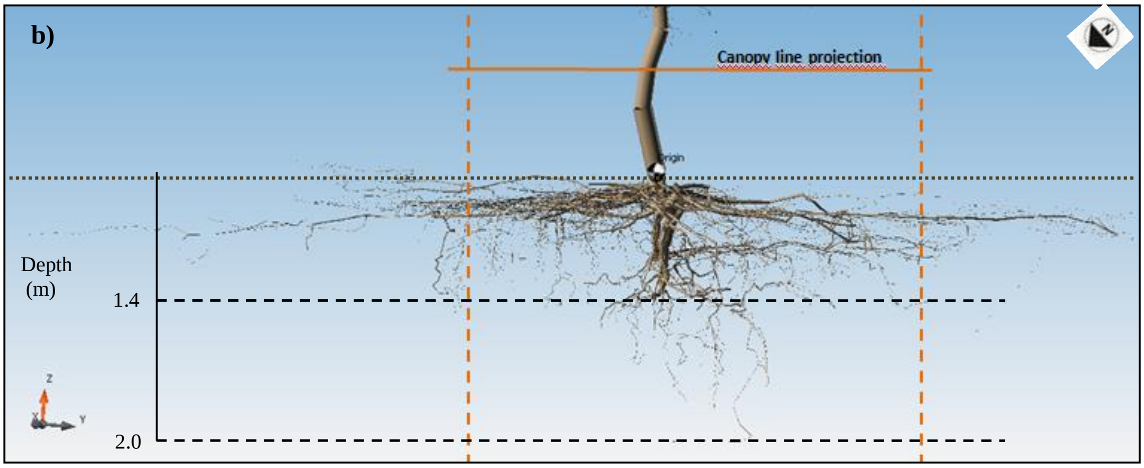
Figure 12. Presence of sinkers in cork oak root system.

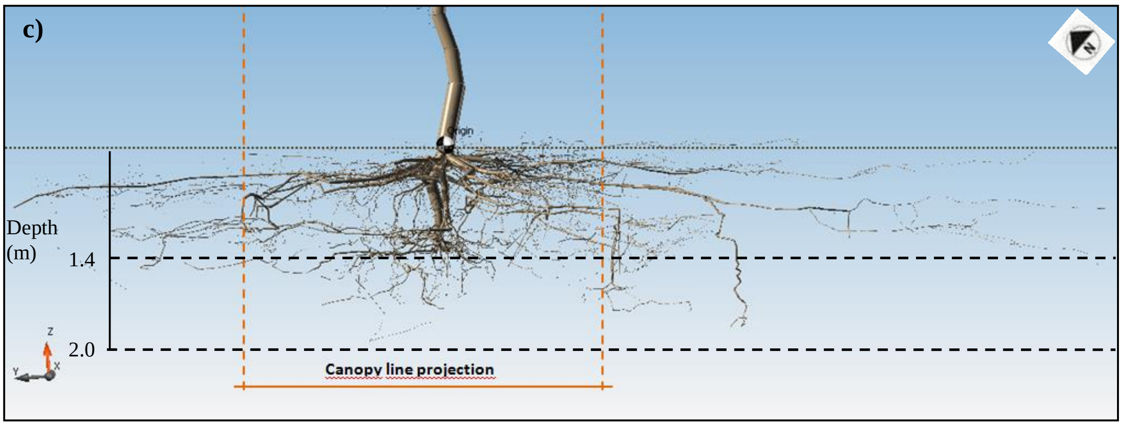
The maximum vertical root depth reached by cork oak root system was 2 m, although only few roots with very low diameters were verified below 1.4 m depth. This is in accordance with Serrasolses *et al.* (2009) when they mentioned that as the most oaks, cork oak is a deep-rooting species with a tap root depth of a meter or more. In this work we verified *in situ* that tap root growth vertically until 1.40 m and, probably due to soil characteristics it stopped the growth in vertical and continued horizontally, changing sometimes the anatomical form of the root (cylinder to smashed form) to allow the passing through zones with higher mechanical impedance (Figure 13 and Figure 14). Different views of cork oak architecture are presented in Figure 15 a, b, c, d, e.

Figure 13. Vertical tap root development.

Figure 14. Horizontal tap root development at 1.40 m depth.


a)
Canopy line projection
Origin
N
Depth
(m)
1.4
2.0
z
x


b)
Canopy line projection
Origin
N
Depth
(m)
1.4
2.0
z
y


c)
Origin
N
Depth
(m)
1.4
2.0
Canopy line projection
z
y
x

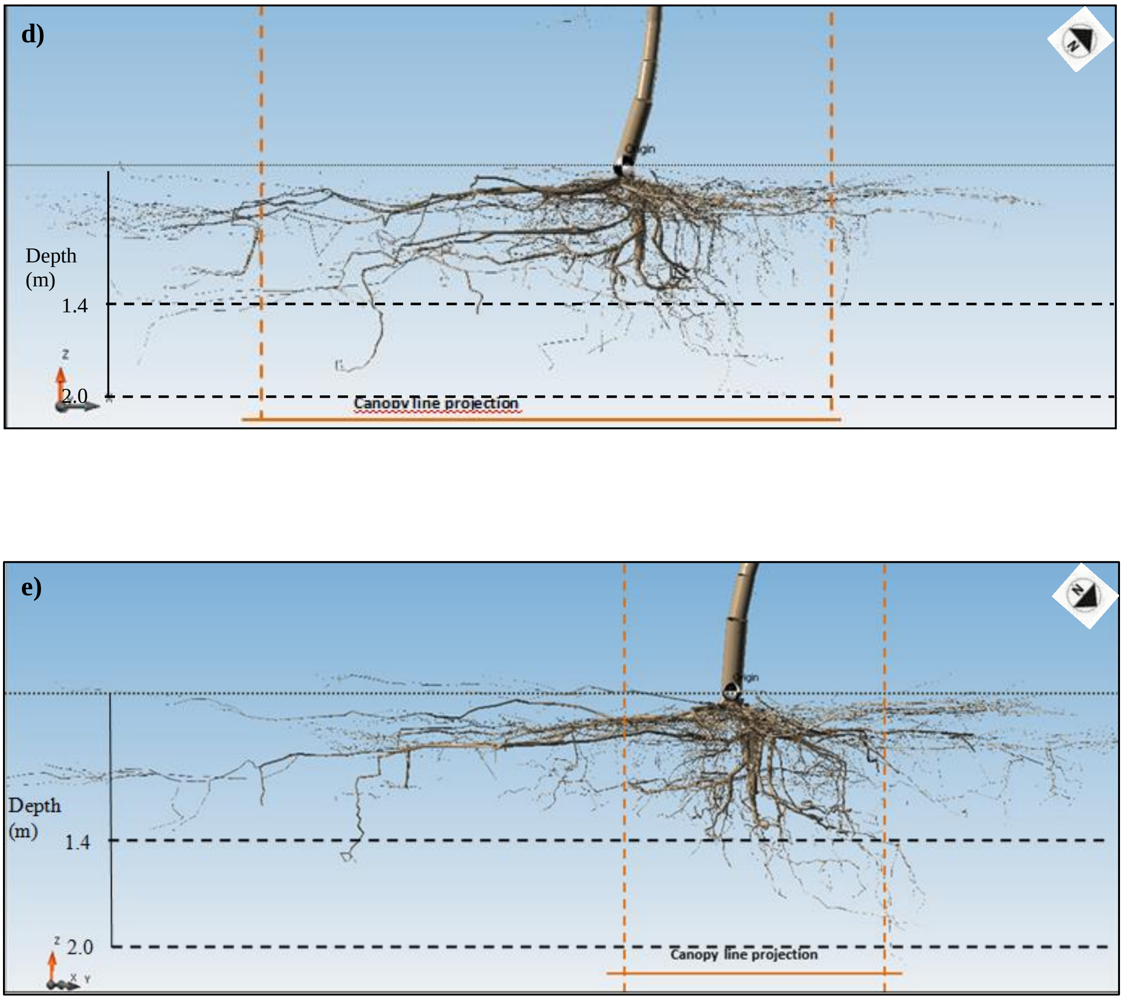


Figure 15. a), b), c), d), e) Different views of cork oak root system 3D architecture.

The higher percentage of roots is superficial and it's possible to verify that, for the majority of the directions (azimuths), they can spread away at least 2 times the canopy horizontal projection. The maximum root distance found had 4 times the horizontal line canopy projection (root code R6R14R2R12) (Figure 16). The growth of this particular root from 4th order was mainly horizontally between the 0.05 and 0.35 m depth and it was oriented for the SE azimuth. By so we confirm what was already mentioned by other authors (David *et al.*, 2004; 2013; Moreno *et al.*, 2005). Although they had not accessed the entire root system of these trees, they defend that in water-limited environments, roots may extend far beyond the crown projection area. For other *Quercus* species, *Quercus* *petraea*, some authors (Cermák *et al*., 1981; 1986; Hruska *et al.*, 1999) verified an enlargement of a large oak root system in sandy loam soil as a response to drought.

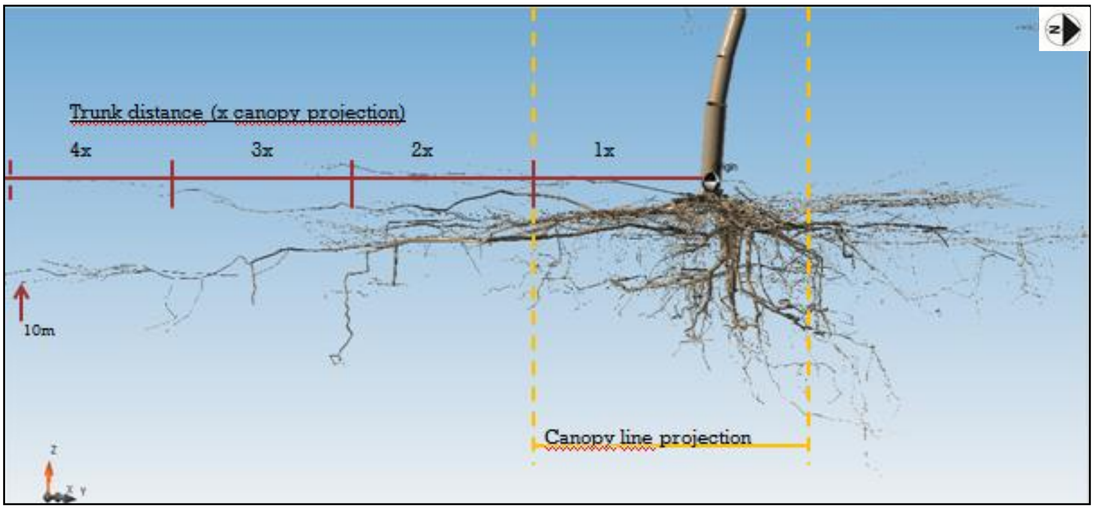


Figure 16. Maximum horizontal root distance observed.

Besides the abovementioned, pattern of root growth strategy is not simple to replicate. Each individual root has its own strategy of growth being misunderstood why and when it creates a new lateral root or why they develop in some specific direction or even, why they connect with others, exploring the same soil space. For this cork oak we verified some root grafts between coarse roots of this same tree (Figure 17) but no intergrowth was observed. During this work it was not possible to find a pattern that could allow us to try a modeling approach for its architecture.

Another interesting root behavior was found. During excavation process it was observed an injured root which had clearly suffered a cut. This cut probably was a result of the last soil disking operation (8 years ago). As it is possible to verify by the 3D representation (see Figure 18) the replacement root did not followed the same root growth direction as the original one. It seems that the tree as the ability to memorize the place where it was hurt and to never “send” again any root for the same place. This important factor was also observed in other study. According to Surovy *et al*. (unpublished data) in the sample of cork oak trees used in their previous study (Surový *et al*, 2011b) where they accessed partly of the root system and cut half of the root system the same trend was verified. The authors refer that after their study was complete, they closed the profiles with soil and the trees continue their growth. Three years later those profiles were open (excavated) again until the 20 cm depth and, for all the profiles, in the soil space previously occupied by the roots that were cut, no roots were found (Surový *et al*., personal information). This fact, concerning silvicultural management options, is very important hence it gives new reliable and valuable information about the effect of soil mobilization in depth.

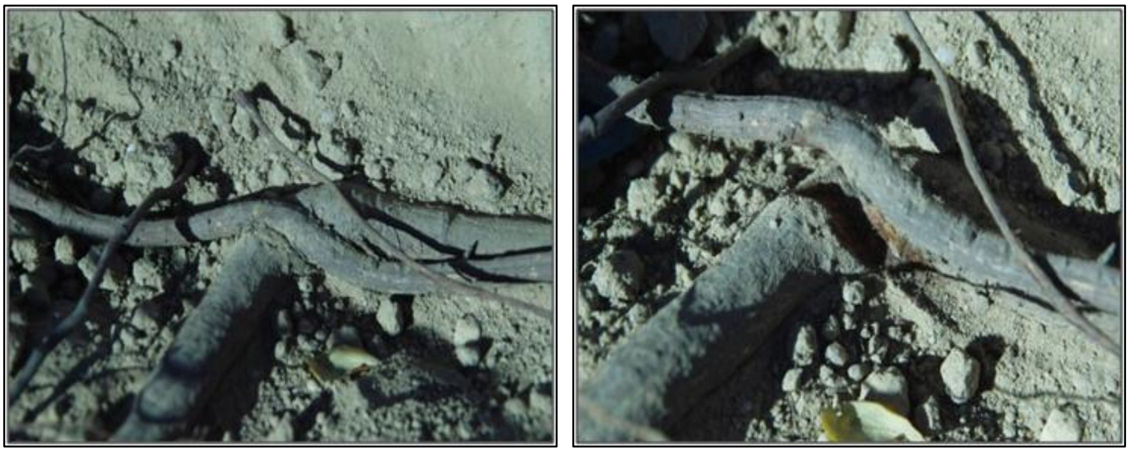


Figure 17. Root graft with no intergrowth, observed between 2 coarse roots from cork oak.

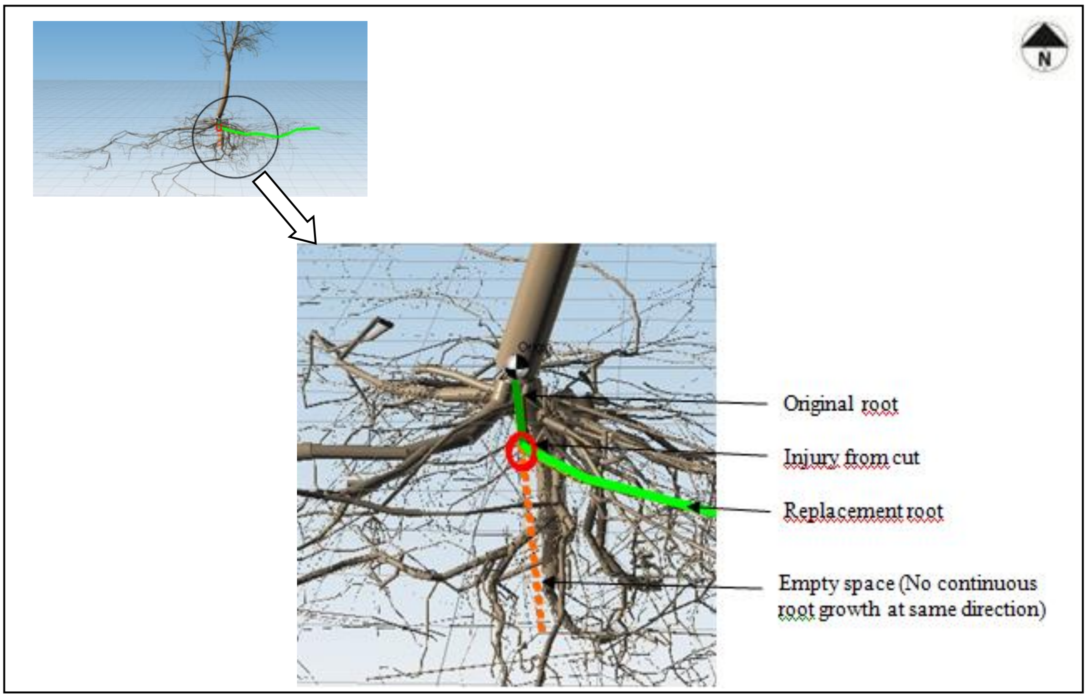


Figure 18. Example of root behavior after root cut injury.

In the revision made by Malamy (2005) about the intrinsic and environmental response pathways that regulate root system architecture, is highlighted the difficulty in understanding completely the architecture of the root. Options should be made taken into account the approach of study. According to Lynch (1995) studies of root distribution, typically, deal with root biomass and root length as a function of factors such as depth in soil, distance from the stem and, position between neighboring plants. In this study we focused on the distribution of two main parameters, root biomass and root length distribution through root categories, profile depth and azimuth orientation.

For the evaluation of root system a total of 1983 root segments were analyzed. For belowground system a total of 54.94 Kg of biomass and 1815.43 m length were obtained through laboratory analysis (Table 4). As referred in Material and Methods section, the root data were treated according to 2 categories, coarse roots (Ø>2 mm) and fine roots (Ø<2 mm). Results of biomass, expressed in terms of dry weight, demonstrate that coarse roots had 53 Kg and that fine roots represented only 3.2% of total biomass, due to their thickness. 753 meters were measured for coarse roots, and fine roots, as expected, presented a higher value, 1 062 meters representing 58% of total length.

Table 4. Biomass (Kg) and length (m) of cork oak root system categories.

	Root system		
	Coarse roots	**Fine roots**	**Total**
Biomass (kg)	53.15	1.79	54.94
Length (m)	753.32	1 062.11	1 815.43

From root system 3D architecture analysis, 25 roots from order 1 were observed. By their relevance in terms of structure 4 roots, including tap root (R22), are highlighted, R6, R8 e R21. The tap root, R22, presented a diameter in the origin (in the tree collar) of 29 cm, 14.6 Kg of biomass and a total length of 4.8 m, measured through root axes. Vertically, in depth, tap root achieved 1.40 m. When this main root reached the bedrock, due to the high bulk density (1.76 g cm^{-3}), it continued the growth horizontally whit a lot of curves (Figure 19). Root labeled as R6 (Figure 20) presented a diameter in the origin of 7.66 cm and was the longer root observed in the root system with 7 meters. It was the second with major weight in terms of biomass (2.7 Kg) and lateral roots originated from R6 growth until order 7. For the set of roots having the origin in R6 a result of 208 m length and 9.9 Kg of biomass was obtained. Other main root observed in terms of structure was the R8 (Figure 21). This root, represented in Figure 24, had 15 cm of diameter in the origin and it extends through 12.6 m measured in the root axes. A total of 5.9 Kg was obtained for this root. Lateral roots originated from R8 were verified until the 5th order. Another relevant root in terms of structure was observed through 3D architecture, R21 (Figure 22). This root with a diameter on the origin of 6.5 cm extended through 3.4 m and weighted 0.7 Kg.

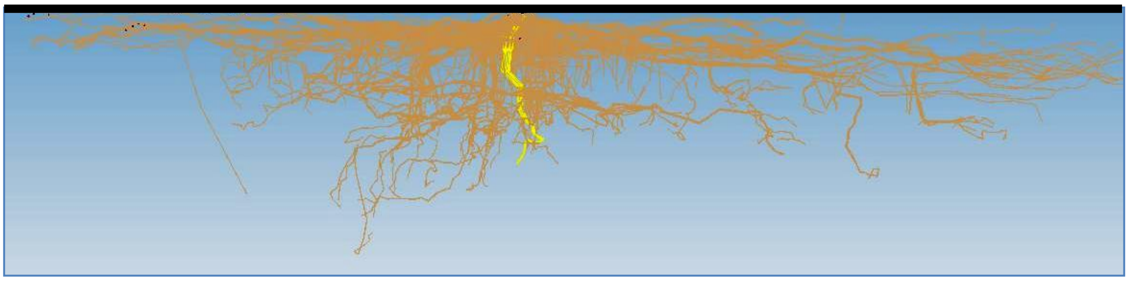


Figure 19. Tap root (R22).

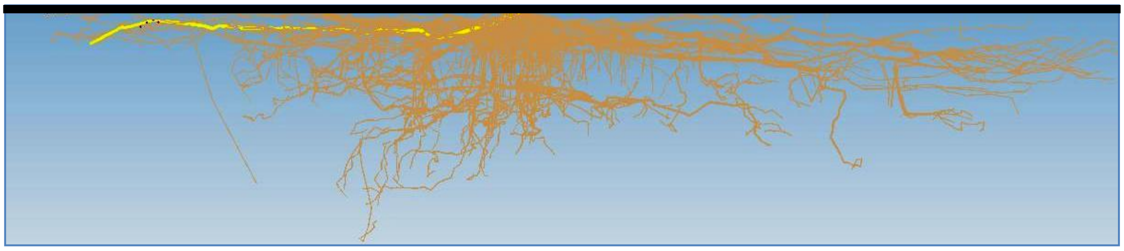


Figure 20. Root R6.

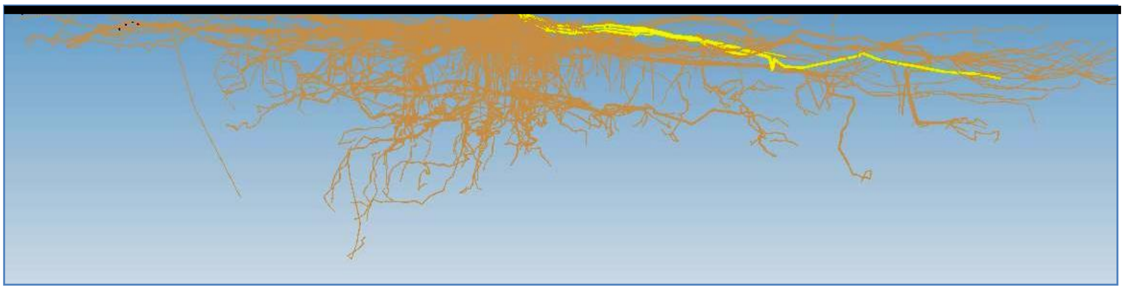


Figure 21. Root R8.

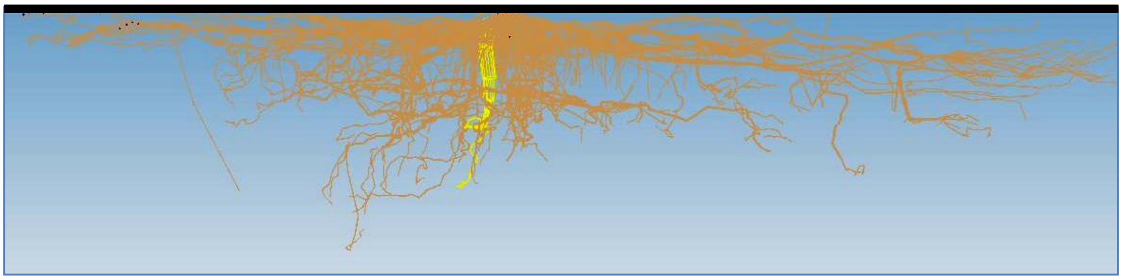


Figure 22. Root R21.

3.4.4 Depth distribution and azimuths distribution of roots

3D representation of roots shows that majority of roots were formed at the upper layers. During its growth they spread overall the soil profile, some of them growing mostly horizontally (Figure 23) and others grow smoothly in depth, occupying the entire profile.

Figure 23. Root horizontal growth near the surface.

As already mentioned, the horizontal distance achieved by roots could reached until 4 times the canopy horizontal line projection, although in depth the maximum root vertical depth verified was of 2 m. However, the main structure of root system (central root system) developed only until 1.40 m corresponding to the deepest water table level. Below this depth only few roots could penetrate in this layer that corresponds to the bedrock of the soil. However, David *et al.* (2013) for a cork oak growing under a well-drained deep Haplic Arenossoil soil observed that the deepest roots were at 4.5 m depth. In the present study, below 0.95 m soil presented a higher bulk density value and clay aggregations (see Table 2) which helps to verify that is a strong and compacted layer; inhibiting the majority of roots to penetrate. This evidence of soil compaction effect on root growth lead this research to a complementary study where it was evaluate the effect of soil compaction depth on cork oak seedlings growth (see Chapter 4). In the referred study it was simulated soil compaction layers at different depths (30 and 60 cm), in a greenhouse experiment. Through the results obtained it was possible to verify that already at first cork oak seedlings growing stages, for a soil bulk density of 1.73 g cm^{-3}, tap root length was negatively affected by this soil related factor. Yet, it was verified that compacted layers promote a decrease on total tree biomass. For that reason, in the present study, it can be though that probably the investment that the tree needed to produce new roots and promote the growth below 1.40 m was high. Meaning, if the roots were mainly growing until the 1.40 m, it can be presumed that until this depth the soil conditions and water and nutrients availability were enough to not compromise the growth of the tree. A 20 to 20 cm depth evaluation was made and results from 3D digitizing for volume (RV_{3D}) and length (RL_{3D}) are presented in Figures 24 and 25, respectively.

As expected by the observations made in the field, root volume decreased with depth. The highest percentage of root volume (37%) was found in the first 20 cm. This was expected and is in accordance with what was observed in the field due to soil profile characteristics from horizon A (0-18 cm depth) which present the better conditions for plant root development specially in radial growth (lower values of fine root fineness (0.55 cm cm^{-3}), see Figure 33). This horizon presented a higher nutrient availability, less mechanical impedance (soil bulk density of 1.62 g cm^{-3}) and was the layer that first benefits from the infiltrated water coming from the surface (water availability). This was the 2nd more representative layer (21%) for root length. As it was observed during the field survey longer roots segments were found between 20 and 40 cm. Results of 29.17 meters and 0.92 cm cm^{-3} of root fineness were obtained demonstrating that probably in this layer the tree invested more energy in root elongation than in radial growth. Probably this investment was made with the main purpose of anchoring and supporting functions (structural). 30% of root volume was observed in this layer being the second more representative layer for this parameter. A relevant decrease of 59% in root volume and of 47% in root length was observed between this layer and 40 to 60 cm depth. Probably due to less availability of water and nutrients, root development expressed through root volume and root length parameters, between 60 cm and 100 cm depth layers, was less observed comparing to top layers (Figures 23 and 24).

Until 1m depth a continuous decrease of 32.5% and 10.5% was observed in root volume. Root length decreased in orders of 48% and 28% for 60-80 cm and 80-100 cm depth, respectively. It is important to refer that at these layers an investment tendency on root radial growth was observed, justified by a decrease in root fineness parameter (Figure 26). These results allow assuming that at these depths structural root functions are reinforced probably with the main purpose of tree fixation and anchorage (larger cross sectional areas). Below 100 cm depth and due to soil horizon conditions (C horizon) a significant minor presence of roots was observed. Also horizontal root distribution was greatly diminished, possible to verify by the results of Table 7. This fact is justified on one side by the minor water and nutrients availability and on the other side, by the physical soil constrains. Soil profile analysis (Table 3) shows that below 98 cm depth soil texture became more clayed which creates more difficulties either to root radial and/or elongation growth. The soil pores are smaller and by so, the tree needs to invest more energy in root soil exploration in this layer. Yet, taking into account that tap root vertical growth stopped at 140 cm, below this depth a significant increase in root fineness was observed, meaning that only thinner roots were present (values between 4.8 cm cm^{-3} (at 140-160cm) and 7 cm cm^{-3} (at 180-200 cm)), representing only 0.15% (160-180 cm) and 0.1% (180-200 cm) of total root volume achieved.

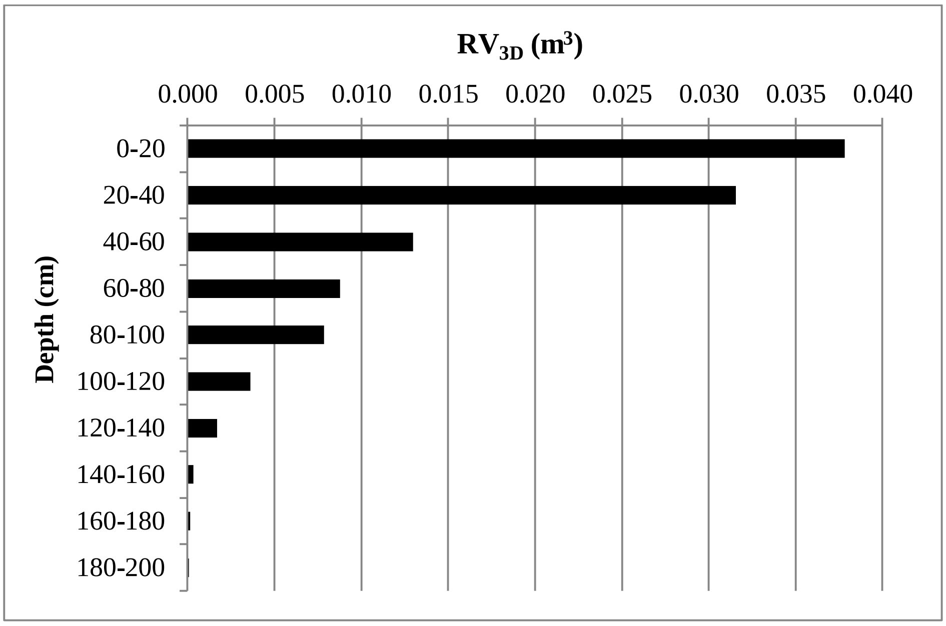


Figure 24. Root volume distribution in depth (m), obtained through 3D digitizing.

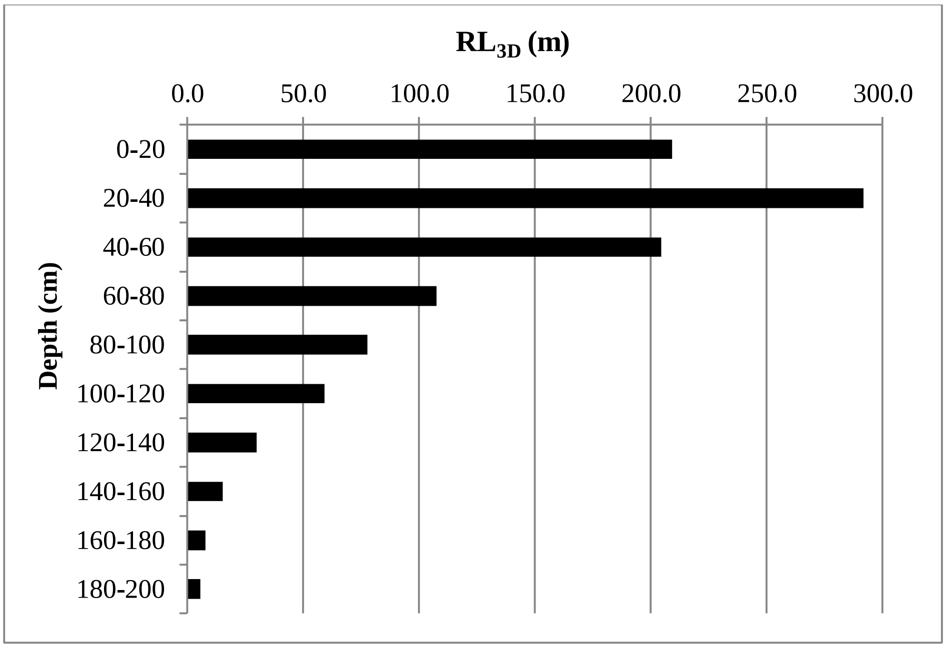


Figure 25. Root length distribution in depth (m), obtained through 3D digitizing**.**

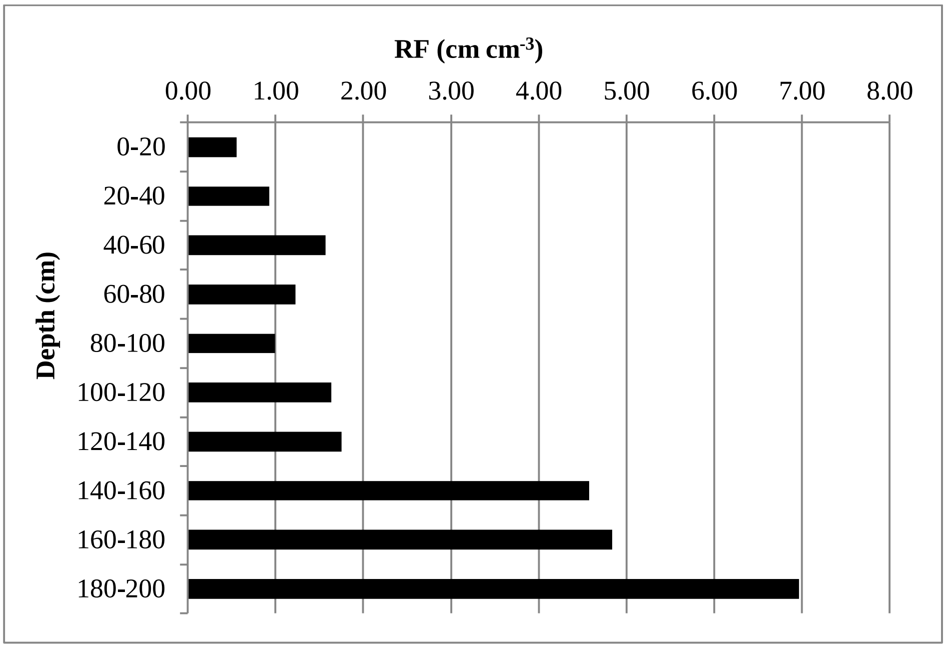


Figure 26. Root fineness (cm cm^{-3}) through depth layers.

Cork oak root spatial distribution showed a higher root soil exploitation in the NE and SE azimuths (Figure 27), verified by the higher values of root length (RL_{3D}) evaluated in this section. Results of 211, 241 and 201m were obtained respectivelly for North, East and South sections (Figure 28a). However, root volume (RV_{3D}) did not present the same pattern distribution, which is confirmed by the results presented in Figure 28b. Roots with higher diameters and consequently, with higher cross sectional areas are distributed majority in the North and East sections, representing 39% (0.04m^3) and 25% (0.015m^3) of total RV_{3D}. This is also confirmed by the lower values of root fineness (RF_{3D}) obtained for these azimuths (Figure 28c). Lower RF values indicate less length per root volume meaning that we are in the presence of shorter roots but probably thicker. Besides in South section root segments presented higher lengths, they probably have smaller diameters and consequently less volume. These results are in accordance with what was observed in the field. The roots exploited the soil where the competition between neighbor root trees was less intensive and for the opposite side of a field road, avoiding a physical soil barrier of higher soil mechanical impedance.

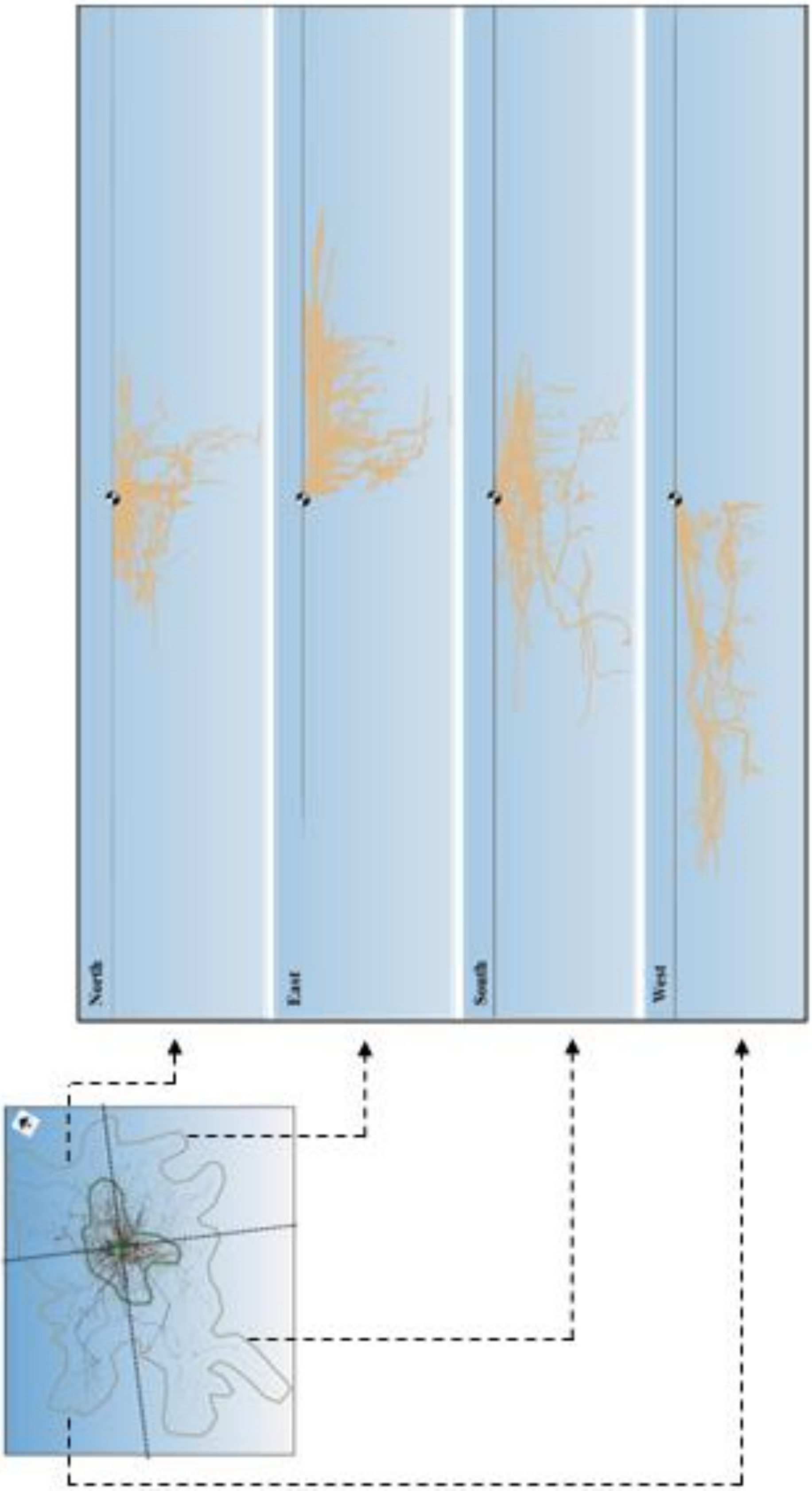


Figure 27. Spatial distribution by azimuths of roots.

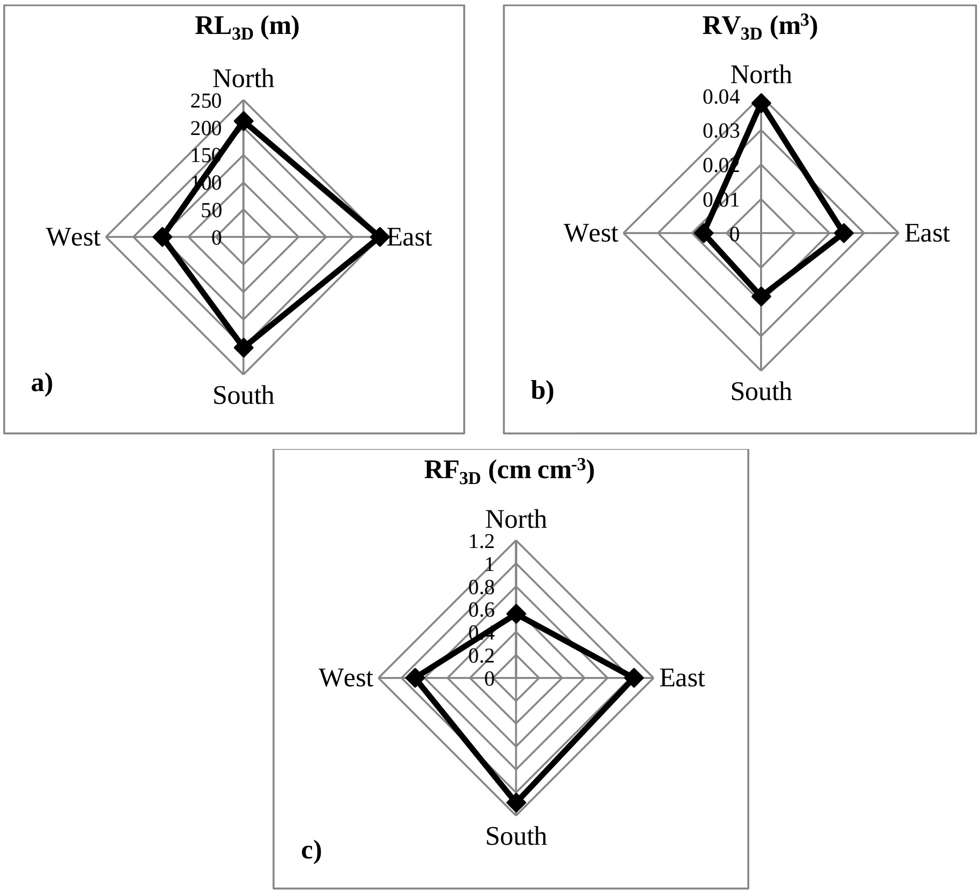


Figure 28. a) Root Length (RL$_{3D}$), b) Root Volume (VL$_{3D}$) and c) Root Fineness (cm cm^{-3}) through azimuths, evaluated from 3D digitizing.

3.4.5 Parameters evaluation through tree branching order distribution

Branching pattern is a useful indicator to understand plant functioning (Heuret *et al.* 2003) and gives information for a future modeling approach of tree structure. Evaluation of several parameters (e.g., biomass, length, areas, etc) by branching order was made for shoot (branches + new branches + leaves) and root system of the studied tree. Root:shoot ratio (R:S) indicates the tree strategy for biomass allocation to above and belowground compartments. Values lower than 1 indicates that the tree invested more in the shoot production. For 1st order (which we included also the trunk and tap root structures) an equal investment seems to had occurred in both systems (R:S of 1.002). Structures belonging to this order correspond mainly to the initial stage of plant life and by so was expected an equilibrated biomass allocation for new structures constructions to allow the growth and development of the tree. From 2nd to 6th orders cork oak invested always more in the shoot system. This indicates that roots were able to guarantee the tree functioning allowing the tree to reinforce the production of aerial structures Among these orders a continuous decrease was observed (Figure 29), expect for order 4. A result of 0.57 was obtained presenting a slightly increase due to 3rd order. Only for 7th order was observed a greater tree investment in root production compared with shoot production.

Figure 29. Root shoot biomass ratio per branching order.

3.4.5.1 Shoot system

The trend of shoot biomass allocation per branching order shows a decrease of biomass with the increase of orders, in the compartments evaluated (branches, new branches and leaves). Order 1 (which includes the trunk) represents 53% of total aboveground biomass, order 2 18.2%, order 3 18.1% and order 4 ≃8% (Figure 30). The last orders (5th, 6th and 7th) have a very small representativeness in terms of aboveground biomass allocation. As stated before, the trunk presented the highest value, having a significant weight in the first order biomass, making it the most representative order for this parameter (Figure 31). For branches biomass, first order represents 41%, 2nd order 51%, 3th order 35% and a representativeness of 13% for order 4. The remaining orders only represent 3.8% of the branches total biomass. In comparison with the other aerial compartments, from 4th order onwards, a pattern of higher biomass allocation is observed for leaves and new branches (Figure 31).

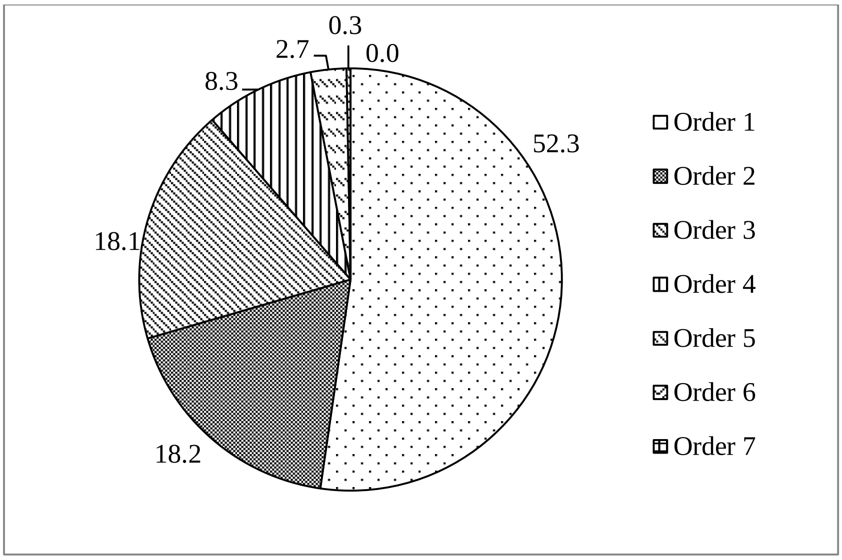


Figure 30. Shoot biomass allocation per branching order (%).

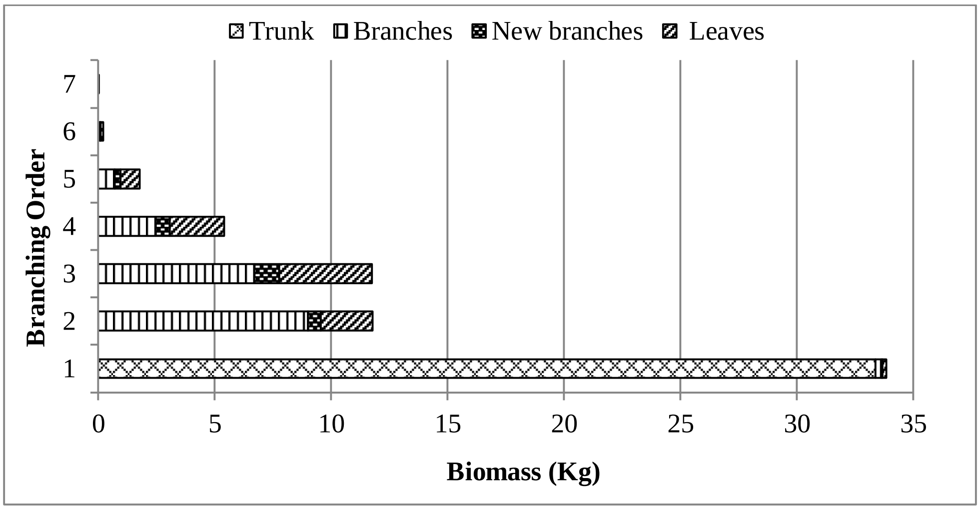


Figure 31. Cork oak aboveground biomass partition, per branching order**.**

The newer structures of the tree (leaves and new branches) presented the same distribution pattern in terms of biomass per bifurcation order. The most representative orders evaluated for leaves and new branches biomass, are 3rd, 4th and 2nd, respectively. Values of 3.99 Kg, 2.32 Kg and 2.25 Kg were obtained (Figure 32). Expectedly taking into account that the last formed structures have less weight in terms of dry weight because of their recent formation, lower values of biomass were obtained for these orders (6th and 7th), with distributions below 3%.

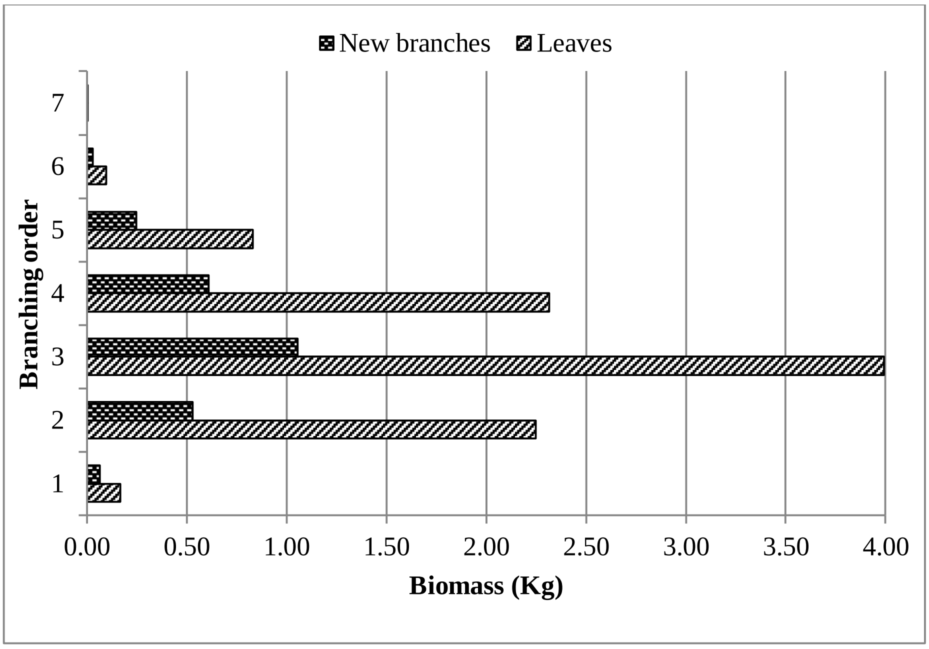


Figure 32. New branches and leaves biomass, per branching order.

To evaluate total leaf area (LA), important parameter which indicates the strategy of the tree to dryness conditions and consequently their photosynthesis performance, a model was tested between LA and LB (leaf biomass) (see Material and Methods section). Results of model (linear regression model) presented a coefficient of determination of 0.92. Through this estimation a total LA of 42.8 m^2 was obtained for the cork oak. Analysis of leaf area distribution per branching order follows the same trend as the biomass of leaves (Figure 33) with the 3rd order being the most representative with 18.2 m^2, followed by 4th order with 10.4 m^2 and 2nd order with 9.6 m^2. As expected, the orders with less biomass and leaf area were the 1st order, where only one new branch exists in the top of the tree with a small set of leaves and, the 7th order where only few wood segments (new branches) were observed and consequently only few sets of leaves were found in this order.

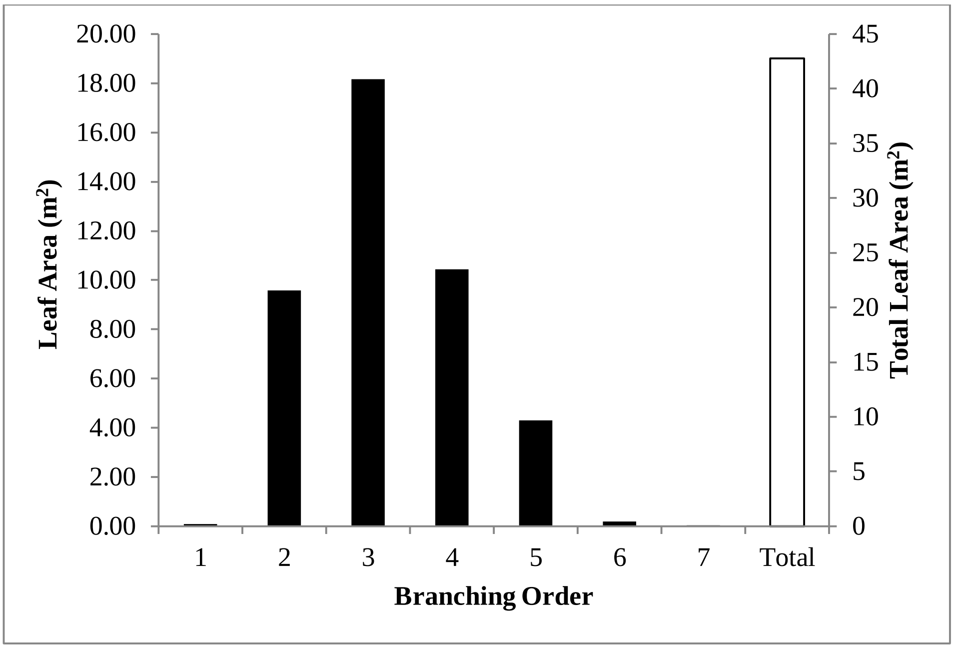


Figure 33. Total cork oak leaf area (m^2) and leaf area per branching order.

3.4.5.2 Root system

Branching order is identified through the location of roots formation according to the parental root. Several authors used this method justifying that is an easy method to categorize roots (Danjon & Reubens, 2008; Doussan *et al.*, 2003; Pagès, 1999). For this cork oak root system 7 orders were observed. For a better understanding it was decided to present the main results on the follow way: first, the results evaluated for coarse roots (structural system) and then the results obtained for fine root structures.

3.4.5.2.1 Coarse roots

As already mentioned, tap root, included in coarse roots category had a diameter in origin (collar) of 29 cm (Figure 34). For the other roots belonging to coarse roots it was verified a range of diameter from a mean maximum of 5.27±0.72 cm (roots from order 1) to a mean minimum diameter of 0.30±0.00 cm (corresponding mainly to roots from order 7). The maximum value was observed in root R8 within 15cm of diameter in origin. When mean diameters values are compared, 2nd order present a decrease of 80%, compared to order 1. The 2nd order diameter range goes through a maximum of 8.23 cm to a minimum of 0.30cm. For order 3, 3.80 cm was the maximum value observed. However the mean value evaluated for this order was 0.69±0.40 cm. Order 4 presented a mean diameter value of 0.57±0.03 cm. Unexpectedly an increase of 14% was observed between order 5 (0.44±0.01 cm) and order 6 (0.50±0.09 cm). This can be due to the fact that only 6 roots were classified as order 6 and one of them has a mean diameter of 0.6 cm which will affect positively the mean value of the order.

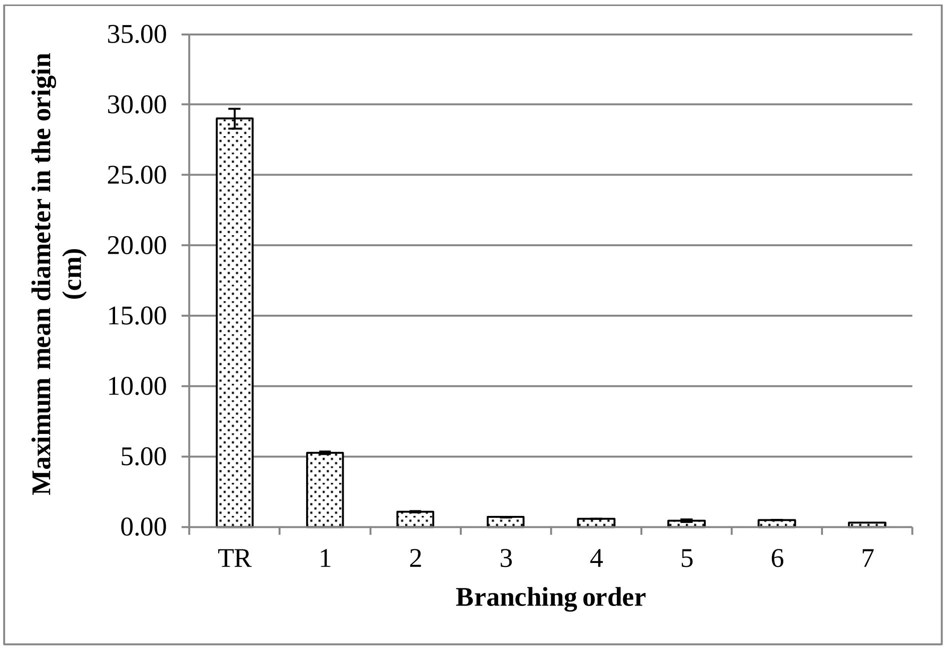


Figure 34. Maximum mean diameter verify for coarse roots, by branching order.

As expected, for biomass parameter (Figure 35a and b) first order is the most representative order presenting 36% of the total coarse root biomass with 19.3 Kg. Tap root by itself represents 27% (14.6 Kg). The pattern of biomass distribution shows that biomass decrease as branching order increases. This was expected hence the first roots formed (older ones) spend more energy in diameter growth (stretching) than in length (elongation). This elongation performance is leaved to the new roots than continuously are formed. From order 1 to 2 a decrease of 45% was verified and from 2nd to 3rd order the decrease was of 44%. Although order 3 present the higher result in terms of length (Figure 36a and b) and specific root area (Figure 37) the set of roots belonging to this branching order are thinner, and by so the biomass is lower than the previous orders. For this order a total of 3 Kg was obtained, representing 11% of the total. Order 5 represent only 5.7% of the total biomass, where 0.5 Kg were obtained. The last orders 6 and 7, with a low representativeness in relation to the total obtained, presented only 0.026 Kg of dry weight (Figure 34b). Lengths of 2nd and 4th orders were elevated, with 184.8 and 167.6 m, respectively, representing 24.5 and 22% of the total coarse root length. Specific root area (SRA) (Figure 37) showed the same pattern, which allows us to confirm that these values were mainly improved by the presence of longer roots than on largest diameters. Order 7 (last order being formed) for all the parameters evaluated, presented the lower values mainly to their almost inexistence in the root system (2 roots). For instance, only 90 cm were registered for this order. Tap root presented a total of 4.8 m, representing only 0.9% of total length (Figure 35a and b). Summarizing the values for the entire cork oak root system a result of 1281 m^2 Kg^{-1} of SRA was obtained. This fact reinforces the already demonstrated high capability that cork oak roots showed on the exploitation of this 2 m soil depth profile.

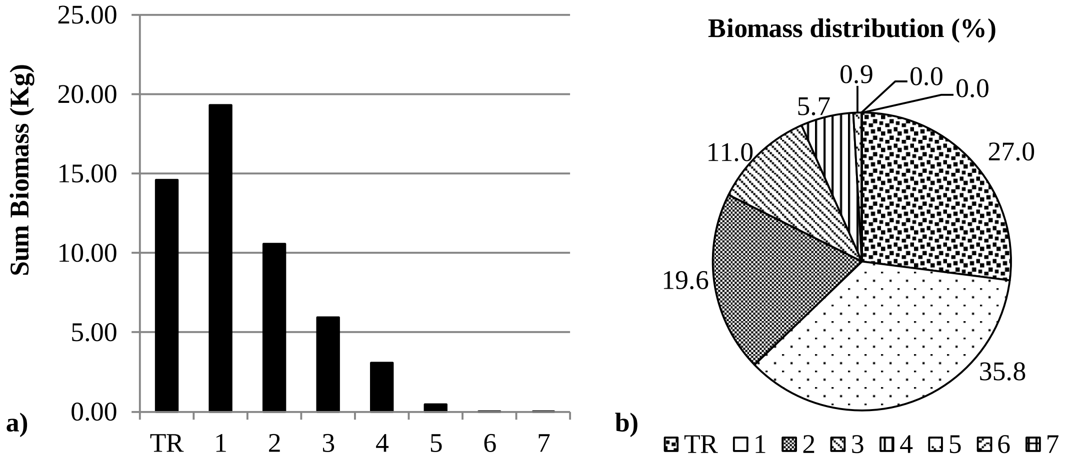


Figure 35. a) Sum of biomass (Kg) and b) biomass distribution (%), obtained by branching order, for coarse roots (diameter>0.2 cm). TR: tap root, 1: Order 1, 2: Order 2, 3: Order 3, 4: Order 4, 5: Order 5, 6: Order 6, 7: Order 7.

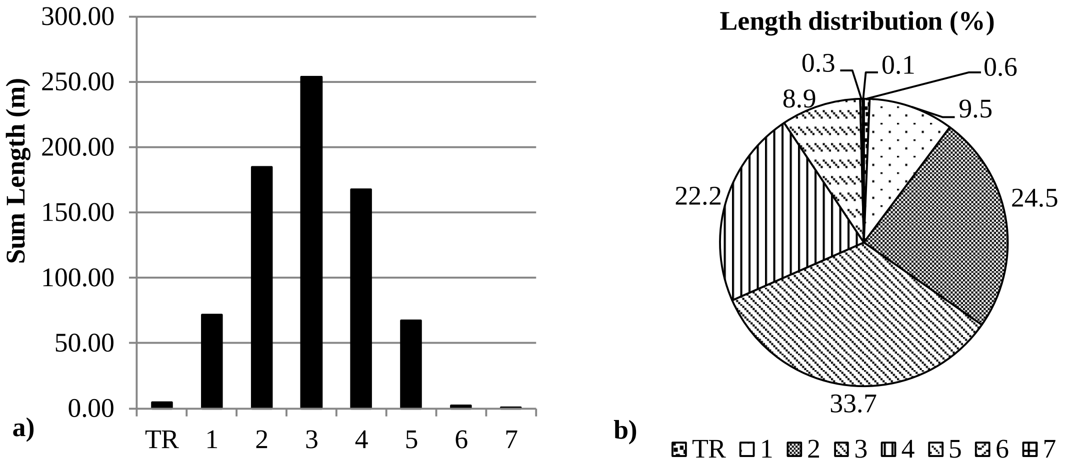


Figure 36. a) Sum of length (m) and b) Length distribution (%), by branching order, for coarse roots (diameter>0.2 cm). TR: tap root, 1: Order 1, 2: Order 2, 3: Order 3, 4: Order 4, 5: Order 5, 6: Order 6, 7: Order 7.

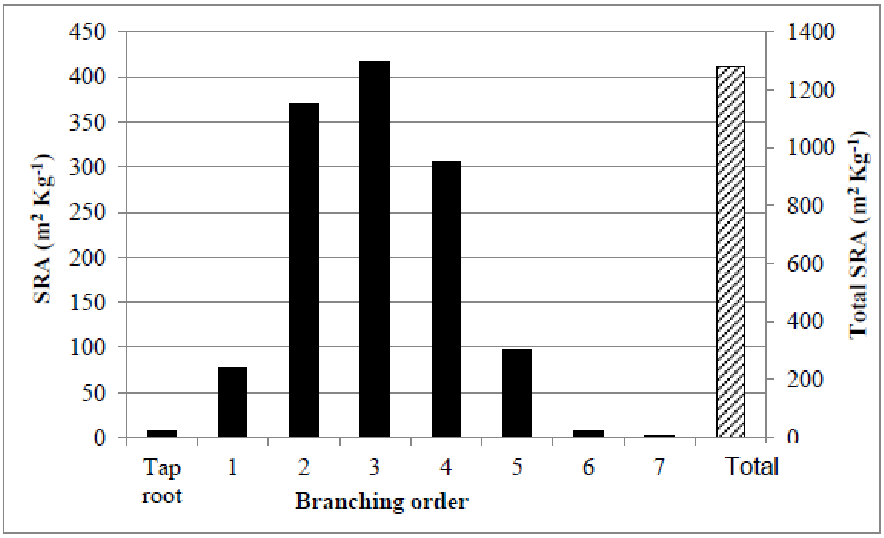


Figure 37. Total specific coarse root surface area (SRA; m^2 Kg^{-1}) and per branching order.

3.4.5.2.2 Fine roots

Fine root production is especially sensitive to the amount of new biomass needed for mechanical support of the crown because fine roots are the distal end of the carbohydrate source-sink network. For the studied cork oak a total of 1 062 m of fine roots were measured in the entire root system. 823 fine roots were analyzed where 1.79 Kg of biomass was registered. As we are dealing with fine roots category, no relevant changes in diameter occurs. However through the changes of morphological parameters (specific root length (SRL), specific root surface area (SRA)) it is possible to evaluate the tree ability to significantly influence the extent of the occupied soil bulk and subsequently to modify some physical features in the rhizosphere (Kucbel *et al.*, 2011). In our study the evaluation of these parameters was made for each branching order. Also total biomass per order was weighted (FRB). Concerning to branching order, as it was verify for CRL, 3rd order was the most representative order for all the fine root parameters evaluated (Figure 38a, b and c) meaning that most soil was exploited by roots of this order. For FRB, SRL and SRA results of 0.61 Kg, 25888 cm g^{-1} and 15954 cm^2 g^{-1} were obtained, respectively. Also fine roots of 4th and 2nd order played a significant role on water and nutrient absorption for tree maintenance, regarding the high distribution observed on SRL and SRA parameters (Figure 38b and c). It is important to mention that almost 7 m^2 of fine root surface area were in contact with soil in the entire cork oak root system.

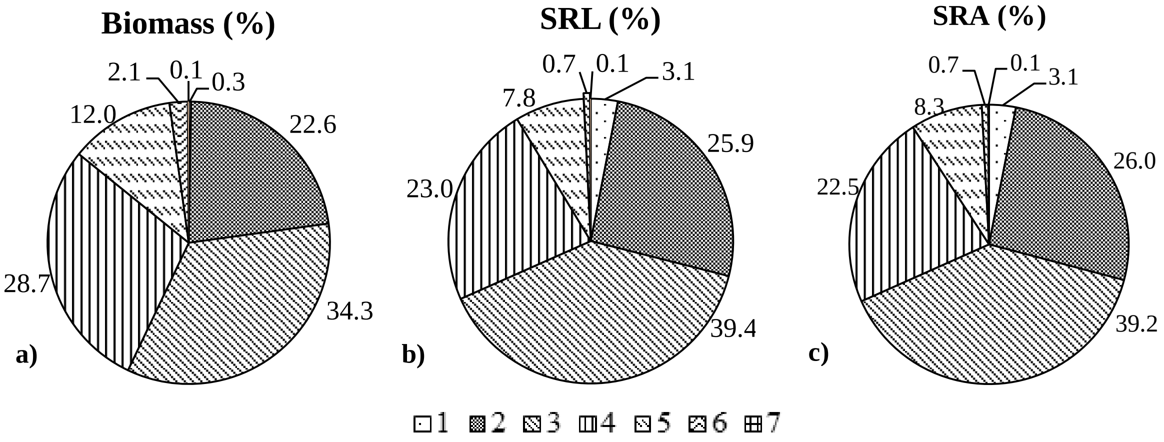


Figure 38. Sum of fine roots distribution of a) biomass (g), b) Specific root length (SRL; cm g^{-1}) and c) Specific root area (SRA; cm^2 g^{-1}). 1: Order 1, 2: Order 2, 3: Order 3, 4: Order 4, 5: Order 5, 6: Order 6, 7: Order 7.

3.5 Conclusions

This intensive study about cork oak architecture and morphology evaluation brings new highlights for all the research areas that focused on this typical Mediterranean tree species. A brand new possibility to observe how the entire root system architecture is distributed on the soil and how it is sensitive to surface practices mainly in silviculture practices is reinforced with this study. The dimorphic root system of cork oak already described by other authors was verified. 3D root system architecture showed the respective 2 subsystems, one at a superficial level until 40 cm depth and another at a deeper level around 1.20 m depth. This fact indicates that the root system of cork oak has the ability to explore the entire soil profile. A relative high quantity of sinkers distributed all over the soil profile was also found. These roots are originated from parental roots present on the first 20 cm depth and far away from the main central root system. Their strictly vertical growth until lower depths layers, to achieve water from deepest water tables, was observed. Specifically, a larger superficial root system that spread at least two times the horizontal canopy projection at the first 40 cm depth, reinforce the already defended soil mobilization limitation in depth, justified by the loss of the majority of root volume that are at the first 20cm depth. This study allows to focus on a possible direct relation between soil mobilization techniques and root system damaging (superficial roots) probably contributing to the cork oak decline and mortality verified nowadays in Mediterranean oak woodlands. We hope that this research together with other relevant studies about hydraulic root distribution can be used as an indicator for forestry/agriculture management options. For this Cambissoil soil we observed that although biomass allocation is distributed almost similarity in shoot and root systems, the aerial compartment presented a slightly higher biomass value.

References

Amato, M., & Pardo, A. (1994). Root length and biomass losses during sample preparation with different screen mesh sizes. *Plant and Soil*, 161, 299-303.

Asaah, E.K., Tchoundjeu, T.N., Wanduku, T.N, Van Damme, P. (2010). Understanding structural roots system of 5-year-old Afri-cam plum tree (D. edulis) of seed and vegetative origins (G.Don) H. J. Lam.*Trees Structure and Function*, 24, 789-796.

Atkinson, D. (2000). Root characteristics: why and what to measure. In Smit, A.L., Bengough, A.G., Engels, C., van Noordwijk, M., Pellerin, S., van de Geijnk, S.C. (Eds.). *Root Methods: A handbook*. (Pp:2–32) Springer-Verlag. Berlin. Heidelberg. Germany.

Barthelemy, D., & Caraglio, Y. (2007). Plant Architecture: a dynamic, multilevel and comprehensive approach to plant form, structure and ontogeny. *Annals of Botany*, 99(3), 375-407 .

Box, J.E. (1996). Modern methods for root investigations. In Waisel, Y., Eshel, A., Kafkafi, U. (Eds.) *Plant Roots: The Hidden Half*. (Pp:193–237). Marcel Dekker. New York.

Breda, N., Granier, A., Barataud, F., Moyne, C. (1995). Soil water dynamics in an oak stand. I. Soil moisture, water potentials and water uptake by roots. *Plant and Soil*, 172, 17-27.

Caldwell, M.M., Dawson, T.E., Richards, J.H. (1998). Hydraulic lift: consequences of water efflux from the roots of plants. *Oecologia*, 113, 151–161.

Canadell, J., Jackson, R.B., Ehleringer, J.R., Mooney, H.A., Sala, O.E., Schulze, E.D. (1996). Maximum rooting depth of vegetation types at the global scale. *Oecologia*, 108, 583-595.

Cermak, J., Huzulak, J., Penka, M. (1980). Water potential and sap flow rate in adult trees with moist and dry soil as used for assessment of the root system depth. *Biol. Plant*, 22, 34–41.

Cermak, J., Ulrich, R., Stanik, Z., Koller, J., Aubrecht, C. (2006). Electrical measurement of tree root absorbing surfaces by the earth impedance method: 2. Verification based on allometric relationships and root severing experiments. *Tree Physiology*, 26, 1113–1121.

Collet, C., Löf, M., Pagès, L. (2006). Root system development of oak seedlings analysed using an architectural model. Effects of competition with grass. *Plant and Soil*, 279(1-2), 367–383.

Danjon, F., Sinoquet, H., Drexhage, M. (1999). Characterisation of structural tree root architecture using 3d digitising and AMAPmod software. *Plant and Soil*, 211, 241-258.

Danjon, F., & Reubens, B. (2008). Assessing and analyzing 3D architecture of woody root systems, a review of methods and applications in tree and soil stability, resource acquisition and allocation. *Plant and Soil*, 303, 1-34.

David, T., Ferreira, M.I., Cohen, S., Pereira, J.S., David, J.S. (2004). Constraints on transpiration from an evergreen oak tree in southern Portugal. *Agricultural and Forest Meteorology*, 122, 193–205.

David, T.S., Pinto, C.A., Nadezhdina, N., Kurz-Besson, C., Henriques, M.O., Quilhó, T., Cermak, J., Chaves, M.M., Pereira, J.S., David, J.S. (2013). Root functioning, tree water use and hydraulic redistribution in Quercus suber trees: A modeling approach based on root sap flow. *Forest Ecology and Management*, 307, 136–146.

Dinis, C., Surovy, P., Ribeiro, N. (2011). Comparision of two methods to assess the root architecture as a potencial factor influencing the diversity of a stand. In: *Metody inventarizace a hodnoceni biodiversity stromove slozky - sbornik seminare.* Ceska Republica: Ceska Zemidilska Universita v Prague. Pp: 57-64.

Donès, N., Adam, B., Sinoquet, H. (2006). *PiafDigit – software to drive a Polhemus Fastrak 3 SPACE 3D digitiser and for the acquisition of plant architecture. Version 1.0.* UMR PIAF INRA-UBP, Clermont-Ferrand. 15 pp.

Doussan, C., Pagès, L., Vercambre, G. (1998). Modeling of the hydraulic architecture of root systems: an integrated approach to water absorption – Model description. *Annals of Botany*, 81, 213-223.

Doussan, C., Pagès, L., Pierret, A. (2003). Soil exploration and resource acquisition by plant roots: an architectural and modelling point of view. *Agronomie*, 23, 419–431.

Dupuy, L., Fourcaud, T., Stokes, A. (2007). A numerical investigation into the influence of soil type and root architecture on tree anchorage. *Plant and Soil*, 278, 119–134.

Dupuy, L., Gregory, P., Bengough, A. (2010). Root growth models: towards a new generation of continuous approaches. *Journal of Experimental Botany*, 61, 2131-2143.

Eissenstat, D.M., & Yanai, R. (1997). Ecology of Root Lifespan. *Advances in Ecological Research*, 27, 1-62.

FAO (2006). *World reference base for soil resources*. Food and Agriculture. Organization of the United Nations, Rome. ISBN: 92-5-105511-4

Fitter, A.H., & Strickland, T.R. (1991). Architectural analysis of plant root systems II. Influence of nutrient supply in architecture in contrasting plant species. *New Pythologist*, 118, 275-382.

Godin, C. (2000). Representing and encoding plant architecture: A review. *Annals of Forest Science*, 57, 413–438.

Godin, C., & Sinoquet, H. (2005). Functional–structural plant modeling. *New Phytologist*, 166(3), 705-708.

Heuret, P., Guedon, Y., Guerard, N., Barthelemy, D. (2003). Analysis of branching pattern in plantations of young red oak trees (*Quercus rubra* L., Fagaceae). *Annals of Botany*, 91, 479-792.

Hodge, A. (2009). Root decisions. *Plant, Cell & Environment*, 32, 628–640.

Hruska, J., Cermak, J., Sustek, S. (1999). Mapping tree root systemswith ground-penetrating radar. *Tree Physiologist*, 19, 125-130.

Jackson, R.B., Moore, L.A., Hoffmann, W.A., Pockman, W.T., Linder, C.R. (1999). *Ecosystem rooting depth determined with caves and DNA*. *PNAS*, 96, 11387–11392.

Kalliokoski, T., Nygren, P., Sievänen, R. (2008). Coarse root architecture of three boreal tree species growing in mixed stands. *Silva Fennica*, 42(2), 189-210.

Kalliokoski, T., Sievanen, R., Nygren, P. (2010). Tree roots as self-similar branching structures: axis differentiation and segment tapering in coarse roots of three boreal forest tree species.*Trees*, 24, 219-236.

Kucbel, S., Jaloviar, P., Spisak, J. (2011). Quantity, vertical distribution and morphology of fine roots in Norway spruce stands with different stem density. *Plant Root*, 5, 46-55. doi:10.3117/plantroot.5.46

Kummerov, J. (1981). Structure of roots and root systems. In Di Castri, F., Goodall, G.W., Spetch, R.L. (Eds) *Ecosystems of the world 11. Mediterranean-Type shrublands*. (Pp: 269-288). Elsevier, New York.

Kurz-Besson, C., Otieno, D., Lobo do Vale, R., Siegwolf, R., Schmidt, M., Herd, A., Nogueira, C., David, T.S., David, J.S., Tenhunen, J., Pereira, J.S., Chaves, M. (2006). Hydraulic lift in cork oak trees in a savannah-type Mediterranean ecosystem and its contribution to the local water balance. *Plant and Soil*, 282, 361–378.

Lacambra, L.C.J., Andray, A.B., Francés, F.S. (2010). Influence of the soil water holding capacity on the potential distribution of forest species. A case study: the potential distribution of cork oak (*Quercus suber* L.) in central-western Spain. *European Journal of Forest Resarch*, 129, 111–117.

Lintunen, A., & Kalliokoski, T. (2010). The effect of tree architecture on conduit diameter and frequency from small distal roots to branch tips in Betula pendula, Picea abies and Pinus sylvestris. *Tree Physiology*, 30, 1433–1447.

Lintunen, A. (2013). *Crown architecture and its role in species interactions in mixed boreal forests.* Dissertationes Forestales. 165. Pp:55 Available at: http://dx.doi.org/10.14214/df.165

Lynch, J. (1995). Root architecture and plant productivity. *Plant Physiology*, 109, 7–13.

Malamy, J.E. (2005). Intrinsic and environmental response pathways that regulate root system architecture. *Plant, Cell & Environment*, 28, 67–77.

MAMAOT (2012). *Plano de Gestão das Bacias Hidrográficas integradas na Região Hidrográfica 6, VOLUME I- Relatório Parte 2- Caracterização e Diagnóstico*; *Edição de Fev 2012*, Ministério da agricultura, mar, ambiente e ordenamento do Território, ARH Administração da Região Hidrográfica do Alentejo.

Mètro, A., & Sauvage, C.H. (1957). Observações sobre o raizame do sobreiro em Mamora (Marrocos ocidental). *Annales de la Recherche Forestière au Maroc*

Montero, G. (1988). *Modelos para cuantificar la producción de corcho de los alcornocales (Quercus suber L.) en función de la calidad de estación y de los tratamientos selvícolas.* PhD thesis, INIA, Ministerio de Agricultura, Pesca y Alimentación. Madrid.

Mulia, R., Dupraz, C., van Noordwijk, M. (2010). Reconciling root plasticity and architectural ground rules in tree root growth models with voxel automata. *Plant and Soil*, 337, 77-92.

Nadezhdina, N., Ferreira, M.I., Silva, R., Pacheco, C.A. (2008). Seasonal variation of water uptake of a Quercus suber tree in Central Portugal. *Plant and Soil*, 305(1-2), 105-119.

Nicoll, B., Berthier, S., Achim, A., Gouskou, K., Danjon, F., van Beek, L. (2006). The architecture of Picea sitchensis structural root systems on horizontal and sloping terrain. *Trees*, 20, 701-712.

Oliveira, G., Correia, A.O., Martins-Loução, M.A., Catarino, F.M. (1992). Water relations of cork-oak (*Quercus suber* L.) under natural conditions. *Quercus ilex* L. ecosystems: function, dynamics and management. *Advances in vegetation science*, 13, 199-208.

Ostonen, I., Lõhmus, K., Lasn, R. (1999). The role of soil conditions in fine root ecomorphology in Norway spruce (Picea abies (L.) Karst.). *Plant and Soil*, 208, 283–292.

Ostonen, I., Lohmus, K., Helmisaari, H.-S., Truu, J., Meel, S. (2007). Fine root morphological adaptations in Scots pine, Norway spruce and silver birch along a latitudinal gradient in boreal forests. *Tree Physiology*, 27, 1627-1634.

Otieno, D.O., Kurz-Besson, C., Liu, J., Schmidt, M.W.T., Vale-Lobo, R., David, T.S., Siegwolf, R., Pereira, J.S., Tenhunen, J.D. (2006). Seasonal variations in soil and plant water status in a *Quercus suber* stand: roots as determinants of tree productivity and survival in the Mediterranean-type ecosystem. *Plant and Soil*, 283, 121–137.

Pagès, L. (1999). Root system architecture: from its representation to the study of its elaboration. *Agronomie*, 19, 295–304.

Pagès, L., Asseng, S., Pellerin, S., Diggle, A. (2000). Modelling root system growth and architecture. In Smit, A.L., Bengough, A.G., Engels, C., van Noordwijk, M., Pellerin, S., van de Geijnk, S.C. (Eds.). *Root Methods: A handbook*. (Pp:113-145). Springer-verlag.

Pàges, L., Vercambre, G., Drouet, J., Lecompte, F., Collet, C., Le Bolt, J. (2004). Root type: a generic model to depict and analyse the root system architecture. *Plant and Soil*, 258(1-2), 103-119.

Pereira, J., Silva, T., Correia, A. (2004). Florestas e alterações climáticas – o sequestro de carbono nas florestas. *Ingenium*, 84, 62-63.

Pregitzer, K.S., DeForest, J.L., Burton, A.J., Allen, M.F., Ruess, R.W., Hendrick, R.L. (2002). Fine root architecture of nine North American trees. *Ecological Monographs*, 72, 293-309.

Rambal, S. (1984). Water balance and pattern of root water uptake in Quercus coccifera L. evergreen shrub. *Oecologia*, 62, 18-25.

Ryel, R., Leffler, A., Peek, M., Ivans, P., Caldwell, M. (2003). Water conservation in Artemisia tridentata through redistribution of precipitation. *Oecologia*, 141, 335-345.

Sanesi, G., Lafortezza, R., Colangelo, G., Marziliano, P.A., Davies, C. (2013). Root system investigation in sclerophyllous vegetation: an overview. *Italian Journal of Agronomy*, 8, e17.

Serrasolses, I., Pérez-Devesa, M., Vilagrosa, A., Pausas, J.G., Sauras, T., Cortina, J., Vallejo, V.R. (2009). Soil properties constraining cork oak distribution. In

Aronson, J., Pereira, J.S., Pausas, J.G. (Eds.) *Cork oak woodlands on the hedge*. (Pp:91-101). Island Press.

Silva, J.S., Rego, F.C., Martins-Loução, M.A. (2002). Root distribution of Mediterranean woody plants: a modelling approach. *Plant Biosystems*, 137, 63-72.

Sinoquet, H., & Rivet, P. (1997). Measurement and visualisation of the architecture of an adult tree based on a three-dimensional digitising device. *Trees – Structure and Function*, 11, 265–270.

Sinoquet, H., Rivet, P., Godin, C. (1997). Assessment of the three-dimensional architecture of walnut trees using digitising. *Silva Fennica*, 31(3), 265-273.

Sonohat, G., Sinoquet, H., Kulandaivelu, V., Combes, D., Lescourret, F. (2006). Three-dimensional reconstruction of partially 3D-digitized peach tree canopies. *Tree Physiology*, 26, 337–351.

Stokes, A., Fourcaud, T., Hruska, J., Cermak, J., Nadezdhina, N., Nadyezdhin, V., Praus, L. (2002). An evaluation of different methods to investigate root system architecture of urban trees in situ: I. ground-penetrating radar. *Journal of Arboriculture*, 28, 2-10.

Surový, P., Ribeiro, N.A., Pereira, J.S. (2011a). Observations on 3-dimensional crown growth of Stone pine. *Agroforestry Systems*, 82, 105 – 110.

Surový, P., Ribeiro, N., Brasil, F., Pereira, J.S., Oliveira, M.R.O. (2011b). Method for evaluation of coarse cork oak root system by means of digital imaging. *Agroforestry Systems*, 82(2), 111-119.

Surový, P., Yoshimoto, A., Ribeiro, N.A. (2012). Comparison of pruning regimes for Stone pine (Pinus pinea L.) using a Functional-Structural Plant Model. *FORMATH SAPPORO 2011, Japan*, 11, 27-43.

Tennant, D. (1975). A Test of a Modified Line Intersect Method of Estimating Root Length. *Journal of Ecology*, 63(3), 995-1001.

Tobin, B., Cermak, J., Chiatante, D., Danjon, F., Di Orio, A., Dupuy, L. (2007). Towards developmental modelling of tree root systems. *Plant Byosystem*, 141(3), 481-501.

Vaz, M. (2005). *Balanço do carbono e transpiração num montado alentejano de sobro e azinho.* Dissertação para a obtenção do grau de Doutor. Universidade de Évora. Évora.

Zhang, B.-G., De Reffye, P., Liu, L., Kang, M.-Z., Li, B.-G. (2003). Analysis and modeling the root system architecture of winter wheat seedlings. *International Symposium on Plant Growth Modeling.*

CHAPTER 4

THE EFFECT OF SOIL COMPACTION AT DIFFERENT DEPTHS ON CORK OAK SEEDLINGS GROWTH

Dinis, C., Surový, P., Ribeiro, N.A., Oliveira, M.R.G. (2014). The Effect of Soil Compaction at Different Depths on Cork Oak Seedling Growth. *Accepted for publication (DOI :10.1007/s11056-014-9458-0.)*

4.1 Abstract

Soil compaction promoted either by inadequate management (pressure of livestock and machinery) or by soil natural conditions (podzolisation) can influence the growth of cork oak seedlings. We hypothesized that compaction could be related with the lack of natural regeneration and decline on cork oak stands. In this paper, we evaluated the response of cork oak seedlings growth in terms of area and biomass production for above and belowground parts at different compaction depths tested for a sandy-loam soil. This study was done in a greenhouse, with germinated seedlings. Three treatments were applied. One no-compaction treatment (control, C0) and two with a soil compacted layer at 60 cm (C1) and 30 cm depth (C2). The level of compacted layer was 1.37 MPa of mechanical resistance. Results show that tap root length is negatively affected by compaction at 60 cm and 30 cm depth. Below and aboveground biomass are affected by compaction at 30cm depth. In addition, the leaf area results demonstrate that compaction is a sensitive factor for this parameter. In this 1-year stage, plants spend more energy in roots production. Due to soil formation and bad management of cork oak stands, soil compaction at depth could be a cause for the observed lack of natural regeneration, affecting the growth at earlier stages and probably for the decline of cork oak populations.

Key-words: cork oak seedlings, soil compaction, above and belowground biomass

4.2 Introduction

The actual area of the cork oak woodlands (*Montado*) in Portugal is approximately 715,922 ha, of which 601,906 ha are distributed in the southern region of Alentejo (AFN, 2010),which accounts for 22% of the total forest area in the country and 33% of the total world area of cork oak distribution. Cork oak (*Quercus suber* L.) is a typical tree species present in Mediterranean agro-silvo-pastoral systems - *Montado*. The ecological and economic value of this species is already well documented (see Costa *et al.*, 2010; David *et al.*, 2007; Pinheiro *et al.*, 2008; Pereira & Tomé, 2004; Ribeiro *et al.*, 2006; 2010).

In the last few decades, a decline in the cork oak density and population has been documented in the literature (David *et al.* 1992; Ribeiro & Surový, 2008) as a response to the probable inadequate management applied in these areas during long periods of time, along with other factors. Human disturbances, including tree thinning and soil tillage to keep open areas for livestock (Gouveia & Freitas, 2008), make natural regeneration difficult and also promote soil compaction (Kozlowski, 1999). Ribeiro & Surový (2008) clearly observed indications of the soil depth limitations on the intensity of mortality in a national study, especially when combined with the slope factor. However, according to the FAO (2006), the common types of soils where these Mediterranean stands develop are Podzols, Luvisols, Leptosols, Cambisols and Regosols soils. In the case of Podzols soils, the main process in the formation is podzolization. This complex process, in which organic material and soluble minerals (commonly iron and aluminum) are leached from the A and E horizons to the B horizon (spodic horizon), can sometimes lead to a dense (compacted) layer in the profile (FAO, 2006), known in Portugal as “*surraipa*”. Fortunately, it is possible to break these formations with common low impact silviculture practices used both in establishing and maintaining the stands. Specifically, ripper subsoiling is advised for this purpose, where a ripper (with different depths) is coupled to a high power tractor. In accordance with Pagliai *et al.* (2004), this alternative tillage system promotes a more open and homogeneous soil structure, allowing better water movement. Because of these specific soil conditions, the cork oak growth and, specifically, the ability of their roots to reach deep layers to receive water and nutrients can be compromised.

Soil compaction is often responsible for the poor performance or failure of the establishment of trees (Sinnett *et al.*, 2008). The compaction term is understood as the compression of unsaturated soil, especially affecting the larger soil pores (Kristoffersen & Riley, 2005). Compaction typically alters the soil structure and hydrology by breaking down soil aggregates, decreasing soil porosity, aeration and infiltration capacity and by increasing soil strength, water runoff and soil erosion. All of these factors could lead to physiological dysfunctions in plants, mainly influencing the normal and healthy growth of roots and promoting a decreased supply of physiological growth requirements at meristematic sites; this will make mature trees more vulnerable to wind-throw. In addition, the quantity of oxygen in the rhizosphere on compacted soils can be limiting for regular metabolic processes (Queiroz-Voltan *et al.*, 2000), stopping the detritus food chain, eliminating the diversity of living material and roots and favoring the emergence of “pests” that attack organisms and roots that are unable to defend themselves (Coder, 2007). This will affect the entire functionality of the trees.

As soils become increasingly compacted, the respiration of the roots shifts towards an anaerobic state. Compaction stops the respiration processes that are responsible for all tree functions. For instance, Kozlowski (1999) notes that the photosynthesis rate of plants growing in very compacted soil decreases because of both stomatal and non-stomatal inhibition. During growth, the roots use the soil water and nutrient uptake for structural support. Roots grow by following interconnected pores that occur between soil aggregates and through voids created by decomposing roots and animal burrows (Coder, 2007). According to Hakansson *et al*. (1998), in compacted soils, the lower development of the root system results in a minor soil volume that could be explored by the roots, influencing water and nutrient absorption. However, Benghough & Mullins (1990) show that the decrease in root development in compacted soils occurs because of the minor cellular elongation rate, which is a consequence of the decrease in the meristematic cellular division rate.

As a strategy against compaction, a tree initially promotes tap root thickening and the production of more lateral roots with various diameters. Then, if the lateral roots are thin enough to pass through the compacted soil pores, these specific roots continue to grow, while the tap root growth is restricted. If the soil pore is too small for the lateral roots, lateral root growth stops and another site of the subsoil is explored (Russel, 1997 *in* Coder, 1999). In some cases, roots can also enlarge the smaller pores by squeezing soil material aside (Kristoffersen & Riley, 2005). When the root-impeding layers are near the surface, they will slow the downward root growth (Bennie, 1991; Ehlers *et al.*, 1983 *in* Ganatsas & Spanos, 2005).

Previous studies show that plant growth is, in general, negatively affected by soil compaction (Bassett *et al.*, 2005; Kozlowski, 1999). On the other hand, some other studies conducted under a low range of compaction show a positive effect of this factor on plants (Alameda & Villar, 2009; Tubeileh *et al.*, 2003). In the case of oak species, Laliberte *et al.* (2008) found that the long-term survival and growth of trees is largely dependent on first-year establishment. Severe soil compaction adversely influences the regeneration of forest stands by inhibiting seed germination and the growth of seedlings and by inducing seedling mortality (Kozlowski, 1999). In greenhouses or in the field, roots show difficulties penetrating in compacted soil layers, promoting a higher root development on the less compacted upper or lower soil layers as a compensation procedure (Beulter & Centurion, 2004).

With this study, we wanted to evaluate the behavior of cork oak seedling growth, specifically, the behavior of their root system under conditions of compacted layers at different depths. Studies on plant growth during this seedling stage are crucial because of the plants’ higher vulnerability to environmental constraints (Silvertown & Charlesworth, 2001). The way resources affect the plant at this stage are fundamental for understanding tree recruitment patterns (Villar *et al.*, 2004; Tsakaldimi *et al.*, 2005; Gomez-Aparicio *et al.*, 2008), which largely influence forest composition and dynamics. The starting hypothesis for this work was that soil compaction will be a negative factor for cork oak seedling growth in *Montado* because it limits tap root growth at a certain depth, causing a decrease in biomass production at the aboveground part. The other hypothesis was that fine roots will not have the same strength to penetrate a soil compacted layer and, consequently, will not explore the layers under compaction in the same way, in terms of distribution, as on the non-compacted layers.

4.3 Material and Methods

4.3.1 Design of the experiment

The research was carried out in a greenhouse at the Mitra campus of the University of Évora, a site close to Évora in southern Portugal. The acorns of cork oak were collected from a single, isolated tree. The acorns were washed with water, and the ones that showed signs of infection (verified by a water fluctuation process and visual analysis) were discharged. One hundred and fifty acorns were artificially germinated in humid cotton beds in recipients closed with film. The film was bored to allow respiration. The acorns were irrigated once a day and stayed in a room under 23ºC. One week after germination, acorns that had a minimum root length of 1 cm were selected. We selected 45 samples and attempted to choose as many similar weights and root lengths as possible to avoid different levels of seed mass factors, which could influence the results. According to the statistical analysis, no significant differences were obtained between treatments for weight (P-value = 0.9255), length (P-value = 0.872) and radicle length (P-value = 0.717).

One large and homogenized soil sample was collected from a depth of 10 to 30 cm of the E horizon of a Podzol soil profile, avoiding the spodic horizon (FAO, 2006). The soil was collected in the Canha region, in South Portugal. This soil was passed through a 5-mm mesh sieve to separate it from bigger aggregates, dust and residues. Three samples from this original soil were collected for chemical and textural analysis. According to the International Granulometric Scale (Attenberg), the soil used in the experiment was a sandy-loam soil. The percentage of pebbles was 2 %, and for the fine earth fraction, the percentages of sand, silt and clay were 89.5%, 4.9% and 5.6%, respectively. The pH[H_2O] was 6.66. The results for organic carbon and organic matter were 0.34 and 0.58%, respectively.

To simulate the compaction of soil inside PVC tubes (inside diameter of 10.5 cm and height of 97 cm), we used a metallic weight of 2kg made specifically for this specific diameter tube. The experiment was designed for three treatments, C0 (Control) "No compaction", C1"Compaction at 60cm" and C2 "Compaction at 30cm" (Figure 1). For compaction at a depth of 60cm, (C1) loose soil was introduced until it reached a tube height of 35cm. A metallic weight was dropped 10 times, and more loose soil was introduced in the tube until it reached the top. For C2, the tubes were filled with loose soil until it reached a tube height of 65cm, a metallic weight was dropped 10 times and the tubes were filled again with loose soil until the top. Through an evaluation of the bulk density, we obtained results of 1.66 g cm^{-3} for the non-compacted soil (treatment C0), with a penetrometer resistance of approximately 0.01 MPa, and 1.73 g cm^{-3} of bulk density for compacted layers of the soil (presented in C1 and C2 treatments), with a penetrometer resistance of 1.37 MPa. The low differences in the bulk densities between non-compacted and compacted soil can be related to the process of compaction that only had an effect on a thin layer of a few centimeters and that was difficult to sample by the method referred (missing the compacted layer).

Fifteen acorns were selected for each treatment, planted individually in each tube and completely under the soil surface, and irrigated with 200ml of water. The samples were arranged randomly in a greenhouse (25°C day / 10°C night temperature and 50% air humidity). The plants were subjected to 100% of the natural radiation inside the greenhouse (from an average of 275.5 Wm^{-2} for spring/summer seasons to an average of 138.5 Wm^{-2} for autumn and winter) (www.cge.uevora.pt). The seedling growth was observed for 1 year (the time period that tap roots of the control treatment (no compaction) needed to reach the end of the tubes); during this period, irrigation was provided manually with 100 ml of water and was repeated every 48 hours. No fertilization or pesticide products were used in this experiment.

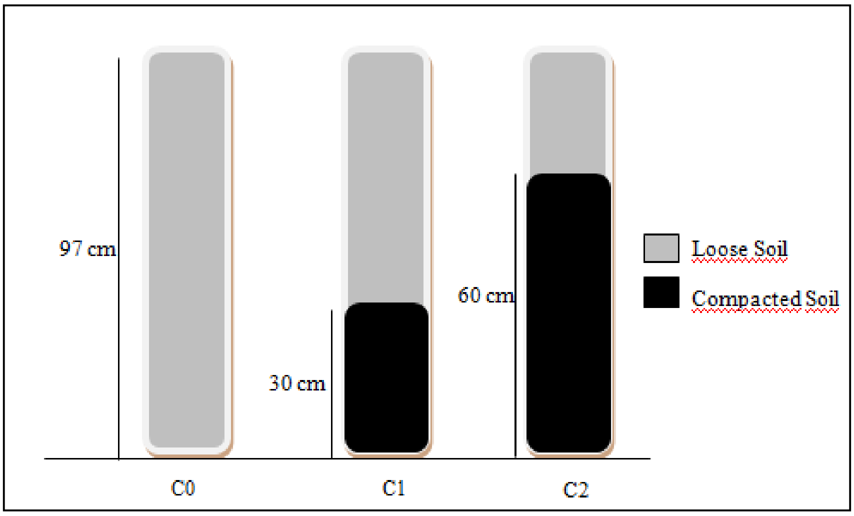
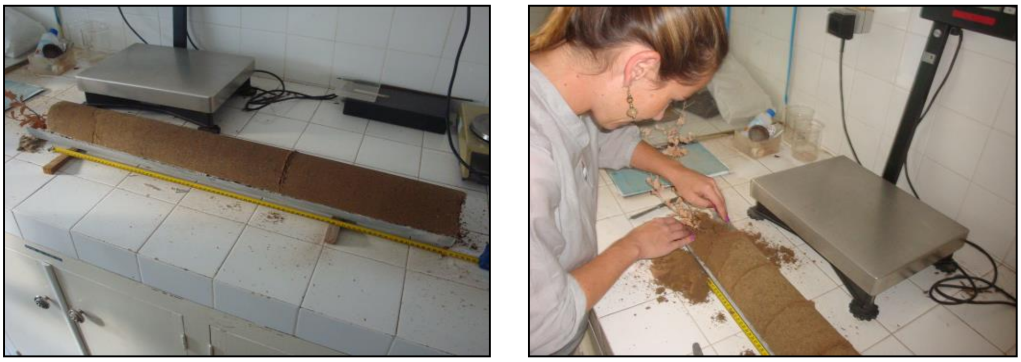


Figure 1. Schematic view of the experiment.

4.3.2 Data collection

To evaluate the soil strength applied for this experiment, we used a penetrometer (Penetrologger, Eijkelkamp, Agrisearch Equipment) equipped with a conical steel probe (cone top angle 60° with a base area of $1cm^2$). During the destruction process, 4 compacted soil samples (2 for each treatment, between a 60-70cm depth for C1 and between a 30-40cm depth for C2) and 2 non-compacted soil samples (between a 30-50cm depth) were collected randomly for bulk density analysis. For this purpose, we used cylindrical metallic samplers (with a diameter of 5 cm and a width of 3 cm). By evaluating the bulk density, we obtained results of 1.66 g cm^{-3} for the non-compacted soil (treatment C0), with a penetrometer resistance of approximately 0.01 MPa, and 1.73 g cm^{-3} of bulk density for compacted layers of the soil (presented in C1 and C2 treatments), with a penetrometer resistance of 1.37 MPa. The low differences in the bulk densities between non-compacted and compacted soil can be related to the process of compaction that only had an effect on a thin layer of a few centimeters and that was difficult to sample by the method referred (missing the compacted layer).

After the bulk densities analysis, the fine roots that were collected by the cylindrical samplers were integrated with the others from the original sample for biomass, length, area and volume analysis. For each sample, the leaves, branches and stem were separated for an analysis of the aboveground part and the tap root and fine roots for an analysis of the belowground part (Figure 2).



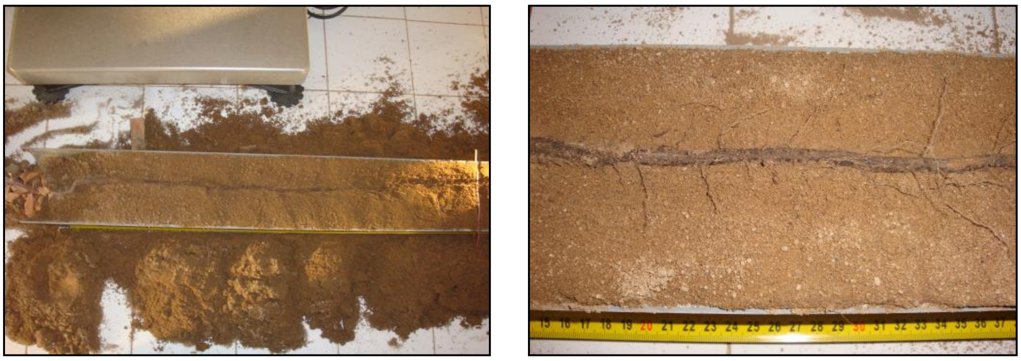


Figure 2. Destruction process resulting in the separation of leaves, branches and stem, tap root and fine roots.

The height, length and area of the branches and stem were measured. For the fresh leaves, the number of leaves and leaf area were also evaluated using scanned images (with 254 dpi resolution) and ImageJ software. The stem and branches were dried at 103ºC and the leaves at 75ºC for 48 hours, and the dry mass was found, thus obtaining the biomass of the components. The belowground part was divided into segments of 10 cm depths, in which the fine roots (diameter less than 2mm) and coarse roots (diameter higher than 2 mm) were separated, which, for all cases, corresponded to the tap root structure. The fine root area, for each 10cm of depth, was measured through scanned images with a 400 dpi resolution and ImageJ. For both the tap root and fine roots, dry weights were taken after 48 hours of drying in an oven at 103ºC, thus obtaining the respective biomass. The variables collected were stem height (SH) and biomass (SB); branch length (BL) and biomass (BrB); leaf area (LA), biomass (LB) and leaves number (LN); aboveground biomass (AB) and area (AA); tap root length (TRL), biomass (TRB) and number (TRN); fine roots biomass (FRB) and length (FRL); and belowground biomass (BB) and area (BA).

4.3.3 Data analysis

The soil was analyzed in the laboratory for texture using the SediGraph 5100 equipment; the organic carbon and organic matter were evaluated using the Leico Carbon Analyser (SC-144DR); humidity, pH[H_2O](1:2) and bulk density. The soil bulk density was calculated as the ratio of soil dry mass to soil volume (g cm^{-3}).

From the data obtained, the following parameters were determined and analyzed: specific leaf area (SLA), as the ratio between the leaf area, and leaf biomass (cm^2 g^{-1}); specific root length (SRL), as the ratio between the fine root length and fine root biomass (cm g^{-1}); total biomass (TB), as the sum of all tree components' biomass (g); shoot:root ratio (S:R), as the ratio between the aboveground and belowground biomass; fine roots belowground biomass ratio (FRB:BB); fine root length leaf area ratio (FRL:LA) (cm cm^{-2}); and fine roots length total biomass ratio (FRL:TB) (cm g^{-1}).

For the statistical analysis, we used the *SPSS software* (version 20.0, SPSS Inc., Chicago, IL). Because of non-normality (Shapiro-Wilk) and non-homocedasticity (Levene's test), we applied the Kruskal-Wallis test for K independent samples to verify the statistically significant differences among treatments.

4.4 Results

4.4.1 Effect of compaction on growth and allocation

Soil compaction had a clear negative influence on every evaluated variable (Table 1). The stem and branch biomass decreased by 35 and 55%, respectively. The leaf biomass was also negatively affected. The total aboveground biomass produced in compacted soils was 33% lower than the one produced in non-compacted soil. The leaf area and aboveground area were also affected by this factor and decreased by almost 30%. The difference between the C1 and C2 treatments for these parameters was small and statistically insignificant. However, the degree of this influence should be studied more thoroughly.

Table 1 – Aboveground part evaluation of cork oak seedlings developed under different depths of soil compaction.

	Variable	Compaction treatments			*H*
		0cm (C0)	**60cm (C1)**	**30cm (C2)**	
Biomass	SB (g)	9.51±0.98a	6.04±1.04ab	6.18±0.60b	7.123*
	BrB (g)	6.26±0.54a	4.68±0.89ab	3.42±0.56b	9.357**
	LB (g)	9.32±0.74a	6.53±0.98ab	6.96±0.49b	9.654**
	AB (g)	**25.10±0.74a**	**17.25±2.63ab**	**16.56±1.06b**	**13.397****
Area	LA (cm^2)	717.50±65.36a	493.70±71.08ab	509.62±38.93b	7.800*
	AA (cm^2)	802.15±68.98a	553.47±79.68ab	575.02±42.46b	8.342*
	SLA ($cm^2 g^{-1}$)	76.33±2.00	77.10±2.32	72.75±1.30	2.055
	SH (cm)	75.14± 3.54	66.30± 6.86	68.77±3.14	2.333
	BL (cm)	273.33±33.27	168.65±27.07	180.78±24.22	0.067
	LN	379.07±44.21	260.90±36.91	300.54±36.86	3.175

Mean±SE. *n*=45. SB, stem biomass (g), BrB, branches biomass, LB, leaves biomass, AB, aboveground biomass, LA, leaves area, SLA, specific leaf area, AA, aboveground area, SH, steam height, BL, branches length, LN, number of leaves. *H-values* for Kruskal-Wallis test. * Significant at 0.05 level, **at 0.01 level. Means with different letters are significantly different ($P<0.05$).

The results of belowground biomass production (Table 2) were similar to those in aboveground biomass production. The belowground biomass lost more than 40% of the potential growth compared to non-compacted soil. The tap root area was reduced by 42%. Compaction at 60cm (C1) had a negative impact on the length per unit of mass (specific root length, SRL) response.

Compaction significantly reduced the total biomass (TB) by 36 and 39% for C1 and C2, respectively (Table 3). In spite of not being statistically significant between treatments, the results obtained for the shoot:root ratio (S:R) (Table 3) demonstrated that, during earlier stages, cork oak seedlings allocate more energy to belowground plant tissue compared with the aboveground organ production (Figure 1). However, for the fine roots belowground biomass ratio (FRB:BB), it was possible to verify the significant effect of compaction at 60cm (C1) compared with the no compaction treatment.

Table 2 – Belowground part evaluation of cork oaks seedlings developed under different depths of soil compaction.

	Variable	**Compaction treatments**			***H***
		0cm (C0)	**60cm (C1)**	**30cm (C2)**	
Biomass	TRB (g)	39.86±3.33a	20.93±3.57b	21.67±1.30b	17.011**
	FRB (g)	2.94±0.52	3.03±0.55	2.93±0.44	0.044
	BB (g)	**42.80±3.33a**	**23.96±3.74b**	**24.60±1.56b**	**18.295****
Area	TRA (cm^2)	65.68±7.58a	48.49±7.15ab	38.24±2.48b	6.532*
	FRA (cm^2)	191.46±22.95	156.70±27.27	175.00±23.48	1.541
	BA (cm^2)	257.14±28.31	205.19±31.65	213.24±24.56	1.954
Length	TRL (cm)	93.07±0.75a	63.80±1.39b	43.46±1.15c	32.985**
	FRL (cm)	202.89±24.35	163.58±26.27	185.42±24.90	1.541
	SRL (cm g^{-1})	76.30±4.80a	57.04±4.33b	66.80±3.68ab	6.828**
	TRN	2.86±0.61	2.10±0.31	1.85±0.15	0.643

Mean ±SE. *n*=45. TRB, tap root biomass, FRB, fine root biomass, BB, belowground biomass, TRA, tap root area, FRA, fine root area, BA, belowground area, TRL, tap root length, FRL, fine root length, SRL, specific root length, TRN, number of tap roots. *H-values* for Kruskal-Wallis test. * Significant at 0.05 level, **at 0.01 level. Means with different letters are significantly different (*P*<0.05).

Table 3 – Evaluation of plant functionality variables.

Variable	***Compaction treatments***			***H***
	0cm (C0)	**60cm (C1)**	**30cm (C2)**	
TB (g)	67.90±4.87a	43.18±5.87b	41.16±2.45b	17.605**
S:R	0.65±0.07	0.74±0.09	0.68±0.04	4.488
FRB:BB	0.08±0.15a	0.15±0.34b	0.12±0.01ab	6.866*
FRL:LA (cm cm^{-2})	0.29±0.03	0.35±0.42	0.36±0.34	2.322
FRL:TB (cm g^{-1})	3.08±0.35	4.49±0.77	4.38±0.44	5.235

Mean ±SE. *n*=45. TB, total biomass, S:R, shoot:root biomass ratio (no units), FRL:LA, fine root length leaf area ratio, FRL:TB, fine root length total biomass ratio. *H-values* for Kruskal-Wallis test. * Significant at 0.05 level, **at 0.01 level. Means with different letters are significantly different (*P*<0.05).

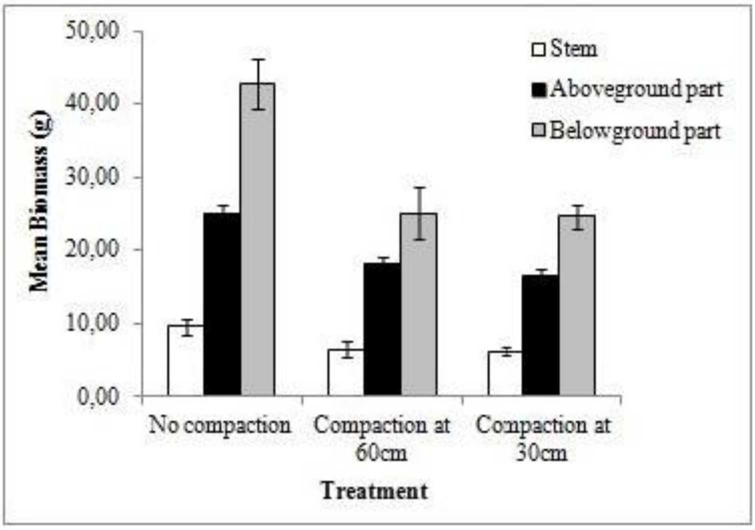


Figure 1 – Effects of the treatments on the stem, aboveground and belowground biomass (g) of cork oak seedlings. Mean biomass ± SE.

4.4.2 Depth distribution of the fine roots

The distribution of fine roots through the profile depth was clearly influenced by compaction (Figure 2). Figure 2 shows a decrease in fine roots below the compacted layers of the respective treatment, as we hypothesized. For non-compacted treatment, we verified that the seedling strategy was to produce and spread fine roots for all of the soil interval layers. Higher values of the fine root biomass were observed in the deepest layers (80-90cm and 90-93cm), representing 19 and 16% of the total biomass evaluated for this treatment, respectively. For compaction at a depth of 60cm (C1), higher values, each representing 17.2% of the total biomass evaluated, were observed on 50-60 and 60-70cm layers. In compaction at 30cm, higher values were observed in the 10-20cm layer, where 22.4% of the belowground biomass occurred.

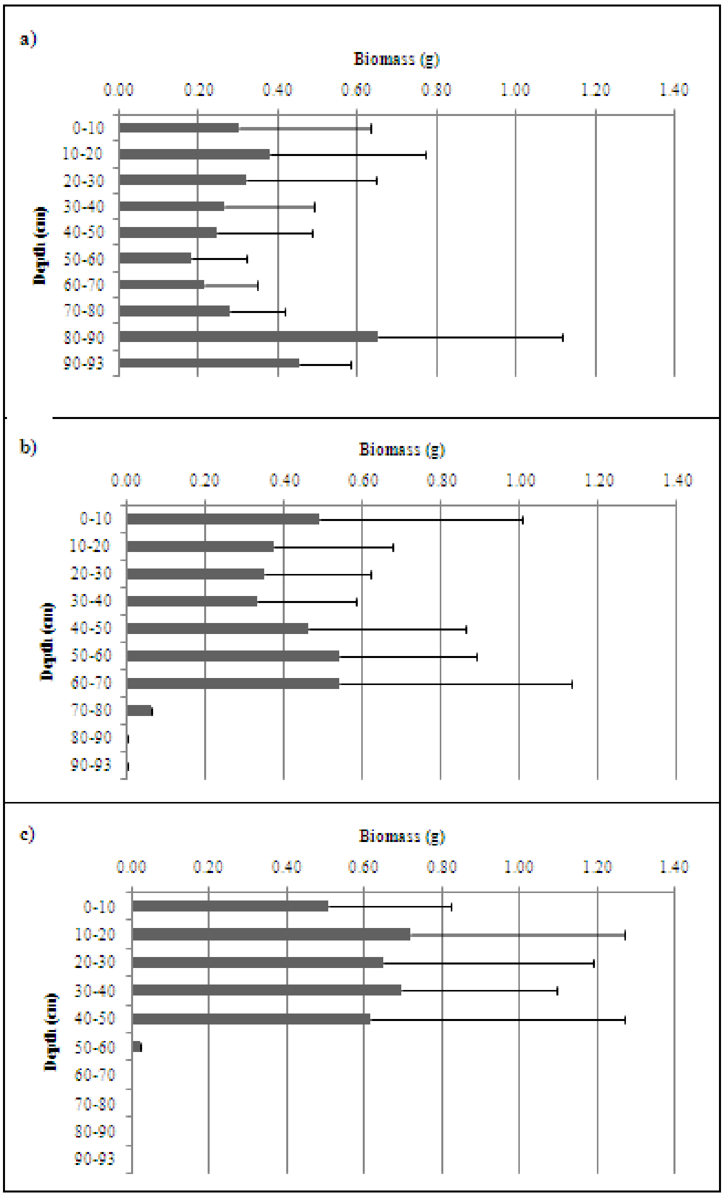


Figure 2 – Distribution of the fine roots biomass (g) in depth (cm). Treatments: a) C0; b) C1; and c) C2. Mean biomass ± SE.

4.5 Discussion

Cork oak seedling growth was evaluated in this work. Souch *et al*. (2004) state that this can be the most sensitive stage because the young roots, of slight thickness, have to colonize the soil and have to overcome the soil resistance. In this work, to diminish the possible noise in the experimental results, promoted by variations in seedlings, we decided to germinate acorns from one single tree and with similar length and weight. As far as we know, there has not been any study on these compaction levels for cork oak stands in Portugal; however, there are some studies available in Spain. Soil compaction levels from 0.9 to 3.4 MPa were found by Perez-Ramos *et al*. (2010) in a *Quercus* forest in SW Spain and from 0.14 to 4.2 MPa by Quero *et al*. (2008) in a Mediterranean forest in Granada (SW Spain). Alameda *et al*. (2009) evaluated *Quercus* species under a range of 0.14 to 1.16 MPa. As we hypothesized, the tap root length of cork oak seedlings are constrained by soil compaction (Table 2). As observed here for *Quercus suber*, a reduction in the rooting depth in compacted soils has also been reported by Perez-Ramos *et al*. (2010). The same growth behavior was also verified for other *Quercus* species [(*Q. ilex* (Cubera *et al*., 2009) and *Q. pyrenaica* seedlings (Bejarano *et al*., 2010)]. Whalley *et al*. (1995) showed that root growth in many plants is restricted above a soil penetration resistance of 2 MPa. Bejarano *et al*. (2010) found that the length of the main root in seedlings grown in a soil compacted to approximately 3 MPa was approximately 50% smaller than in less compacted soil. In our case, 1.37 MPa was the soil mechanical resistance limit that stopped vertical growth. According to Cubera *et al*. (2012), cork oak will develop deeper root systems in the absence of root impedance, probably as would other oak species.

For total seedling root system evaluation, in terms of biomass, we confirmed that compaction has a negative effect (Table 2). This is in line with the results of Chirino *et al*. (2008), specifically for a depth of 30cm. Our findings are also similar to those of Cubera *et al*. (2009), who reported reduced root development and, consequently, reduced aboveground plant growth. With our study, it was also possible to confirm that the strategy of the fine root distribution in the depth due to compaction is to decrease the volume of soil exploited per unit biomass (lower SRL), promoting the construction of thicker roots or roots with more tissue density. Alameda & Villar (2012) also observed this strategy in their work. As in the results for tap root evaluation, the decrease of fine roots below 10cm of the compacted layers was noticeable (Figure 2). Arvidsson (1999) showed that decreased small pore space can be positive by facilitating root-soil contact, thus promoting better water and nutrient absorption. In our study, we verified that seedlings established the same amount of fine roots, but only where the production costs can be balanced with the benefits, increasing access to water and nutrients. Our second hypothesis was also confirmed; fine roots have more difficulty penetrating small pore spaces when presented in compacted soils with a mechanical resistance of 1.37MPa.

For aboveground plant tissue, our results (Table 1) show that the stem biomass, branches biomass, leaf area, leaf biomass, aboveground area and biomass of the seedlings subjected to soil compaction at a depth of 30 cm significantly decreased when compared with non-compacted soils. Perez-Ramos *et al*. (2010) also observed an exponential reduction of the total leaf area for *Quercus canariensis*. Because of that effect, photosynthesis can be compromised. Despite that, for some variables calculated for plant allocation, the results show that no significant differences were found (Table 3). It was possible to evaluate that soil compaction at different depths had a negative effect on the total tree biomass, and the fine roots belowground biomass ratio was affected by this soil factor. As the fine root length per unit of the leaf area presented no significant differences, we can probably assume that, at least for this experiment, the water and nutrient requirements for the development of seedling structures must have been met despite the reduced root length observed, similar to findings reported by Bejarano *et al*. (2010). The results of this study are not statistically significant between treatments, probably because of the short experiment time, and demonstrate that for biomass allocation, cork oak seedlings invest more energy in roots formation, than with aboveground plant tissues, in this stage. This is consistent with findings by Chirino *et al*. (2008) when they referred that one of the main strategies of this species is to develop a deep tap root during the early stages of plant development. Yet, we can also assume that compaction effect, at seedling stage, will compromise the adult tree stabilization, in sandy loam soils, limiting the tap root fixation at major depths. Lloret *et al*. (1999) *in* Alameda *et al.* (2009) referred that this effect will also determine that, in situations of water deficit (such as Mediterranean case), plants with a lower root development may suffer drought more severely and, therefore, and it could seriously limit seedling survival.

Perez-Ramos *et al*. (2010) in their work, defended that acorn mass is responsible for most of the growth and morphological variables during the first year and hence, soil factors did not play an important role in seedling growth during this stage. However, our results demonstrate that for the same acorn mass (no significant mean differences were observed between treatments) cork oak seedling growth is affected by soil compaction. This reinforces our thesis of relating soil compaction with the lack of natural regeneration in

Mediterranean typical soil types (especially Podzols soils) as a reduced length of tap root in earlier stages of growth. Therefore, it will compromise the mature cork oaks survival by limiting not only their ability to reach water in dry periods, but also to remain erect and anchored to the substrate. By so, the practice of silviculture should be based on a sufficient knowledge about the response of each species to different environmental conditions (Cardillo & Bernal, 2006). As far as cork oak stands are concerned, the possibility to break the compacted layers will allow the trees to spread their root systems through the entire profile depth, as it is reported by Surový *et al.* (2011). Soil tillage practices, specifically the ripper subsoiling is advised for this purpose hence improves the soil pore system, preventing soil structural degradation and soil losses, as results of Pagliai *et al.* (2004) demonstrate. This effect will, consequently, promote a major root distribution on profile depth, as a consequence of compaction soil break and an increase of available water for plants (Pagliai *et al.*, 2004). More studies should be taken to reinforce the importance of the tillage management on cork oak seedlings and mature trees. Moreover, studies about tree root systems morphology, behavior and dependent factors are of huge emergence because it is necessary to understand and justify the better choice and less damageable management of *Montado*, promoting the maintenance of multifunctionality.

4.6 Conclusions

We found that compaction at different depths with a mechanical resistance of 1.37 MPa, limits the tap root growth of cork oak seedlings. Seedling root biomass, aboveground biomass and total seedling biomass are negatively affected by this factor. The effects of soil compaction also influence the distribution of fine roots at the profile depth, where the absence of these structures was verified below the compaction layer.

References

AFN (2010). *Inventário Florestal Nacional 2005-2006*. Autoridade Florestal Nacional. Lisboa, Portugal. ISBN: 978-972-8097-76-9

Alameda, D., & Villar, R. (2009). Moderate soil compaction: Implications on growth and architecture in seedlings of 17 woody plant species. *Soil and Tillage Research*, 103, 325–331. doi:0.1016/j.still.2008.10.029

Alameda, D., & Villar, R. (2012). Linking root traits to plant physiology and growth in Fraxinus angustifolia Vahl. seedlings under soil compaction conditions. *Environmental and Experimental Botany*, 79, 49- 57.

Arvidsson, J. (1999). Nutrient uptake and growth of barley as affected by soil compaction. *Plant and Soil*, 208, 9–19.

Bassett, I.E., Simcock, R.C., Mitchell, N.D. (2005). Consequences of soil compaction for seedling establishment: implications for natural regeneration and restoration. *Austral Ecology*, 30, 827–833. doi: 10.1111/j.1442-9993.2005.01525.x

Bejanaro, M.D., Villar, R., Murillo, A.M., Quero, J.L. (2010). Effects of soil compaction and light on growth of Quercus pyrenaica Willd. (Fagaceae) seedlings. *Soil and Tillage Research.*, 110, 108-114. doi: 10.1016/j.still.2010.07.008

Benghough, A.G., & Mullins, C.E. (1990). Mechanical impedance to root growth: a review of experimental techniques and root growth responses. *Journal of Soil Science*, (41)3, 341-358. doi:10.1111/j.1365-2389.1990.tb00070.x

Beulter, N.A., & Centurion, J.F. (2004). Compactação do solo no desenvolvimento radicular e na produtividade da soja. *Pesquisa Agropecuaria Brasileira*, (39)6, 581-588. doi:10.1590/S0100-204X2004000600010

Cardillo, E., & Bernal, C.J. (2006). Morphological response and growth of cork oak (Quercus suber L.) seedlings at different shade levels. *For. Ecol. Manag.*, 222, 296–301. doi:10.1016/j.foreco.2005.10.026

Chirino, E., Vilagrosa, A., Hernandez, E.I., Matos, A., Vallejo, V.R. (2008). Effects of a deep container on morpho-functional characteristics and root colonization in Quercus suber L. seedlings for reforestation in Mediterranean climate. *Forest Ecology and Management*, 256, 779–785. doi:10.1016/j.foreco.2008.05.035

Coder, K.D. (1999). *Tree Root Growth Control Series: Root Growth Requirements and Limitations*. University of Georgia. Cooperative extension service forest resources publication, FOR98-9.

Coder, K.D. (2007). *Soil Compaction Stress & Trees: Symptoms, Measures, Treatments.* Warnell School Outreach Monograph,WSFNR07-9.

Costa, J.L., Silva, A.L.L., Scheidt, G.N., Lemus, E.A.E., Soccol, C.R. (2010). Estabelecimento in vitro de sementes de pinhão manso (Jatrophacurcas L.) - Euphorbiaceae. *Caderno de Pesquisa Série Biologia*, 22, 5-12.

Cubera, E., Moreno, G., Solla, A., Madeira, M. (2009). Quercus ilex root growth in response to heterogeneous conditions of soil bulk density and soil NH_4-N content. *Soil and Tillage Research*, 103, 16-22. doi: 10.1016/j.still.2008.09.002

Cubera, E., Moreno, G., Solla, A., Madeira, M. (2012). Root system of Quercus suber L. seedlings in response to herbaceous competition and different watering and fertilization regimes. *Agroforestry Systems*, 85, 205-214. doi: 10.1007/s10457-012-9492-x

David, T.S., Henriques, M.O., Kurz-Besson, C., Nunes, J., Valente, F., Vaz, M., Pereira, J.S., Siegwolf, R., Chaves, M.M., Gazarini, L.C., David, J.S. (2007). Water-use strategies in two co-occurring Mediterranean evergreen oaks: surviving the summer drought. *Tree Physiology*, 27, 793–803. doi: 10.1093/treephys/27.6.793

David, T.S., Cabral, M.T., Sardinha, R.M.A. (1992). *A mortalidade dos sobreiros e a seca*. Finisterra, XXVII, 17-24.

FAO (2006). *World reference base for soil resources*. Food and Agriculture. Organization of the United Nations, Rome. ISBN: 92-5-105511-4

Ganatsas, P. & Spanos, I. (2005). Root system asymmetry of Mediterranean pines. *Plant Soil*, 278, 75–83. doi: 10.1007/s11104-005-1092-3

Gomez-Aparicio, L., Valladares, F., Zamora, R. (2006). Differential light responses of Mediterranean tree saplings: linking ecophysiology with regeneration niche in four co-occurring species. *Tree Physiology*, 26, 947–958. doi: 10.1093 /treephys/26.7.947

Gouveia, A.C., & Freitas, H. (2008). Intraspecific competition and water use efficiency in Quercus suber: evidence of an optimum tree density? *Trees-Strucure and Function*, (22)4, 521–530. doi:10.1007/s00468-008-0212-0

Hakansson, I., Stenberg, M., Rydberg, T. (1998). Long term experiments with different depths of mouldboard plough in Sweden. *Soil and Tillage Research*, 46, 209-223. doi:10.1016/S0167-1987(98)00099-3

Kozlowski, T.T. (1999). Soil compaction and growth of woody plants. *Scandinavian Journal of Forest Research*, 14, 596–619. doi:10.1080/02827589908540825

Kristoffersen, A.O., & Riley, H. (2005). Effects of soil compaction and moisture regime on the root and shoot growth and phosphorus uptake of barley plants growing on soils with varying phosphorus status. *Nutrient Cycling in Agroecosystems*, 72, 135–146. doi:10.1007/s10705-005-0240-8

Laliberte, E., Cogliastro, A., Bouchard, A. (2008). Spatiotemporal patterns in seedling emergence and early growth of two oak species direct-seeded on abandoned pastureland. *Annals of Forest Science*, 65(4), 407. doi:10.1051/forest:2008019

Pagliai, M., Vignozzi, N., Pellegrini, S. (2004). Soil structure and the effect of management practices. *Soil and Tillage Research*, 79, 131–143.

Pereira, H., & Tomé, M. (2004). Non-wood products: cork oak. In Burley, J., Evans, J., Youngquist, J.A. (Eds.) *Encyclopedia of forest sciences*. (Pp: 613-620). Elsevier, Oxford. ISBN: 978-0-12-145160-8

Pinheiro, A.C., Ribeiro, N.A., Surový, P., Ferreira, A.G. (2008). Economic implications of different cork oak forest management systems. *Int. J. Sustain. Soc.*, (1)2, 149-157. doi: 10.1504/IJSSOC.2008.022571

Queiroz-Voltan, R.B., Nogeuria, S.S.S., Miranda, M.A.C. (2000). Aspectos da estrutura da raíz e do desenvolvimento de plantas de soja em solos compactados. *Pesquisa Agropecuaria. Brasileira*, 35, 929-938. doi: 10.1590/S0100-204X20 00000 500010

Ribeiro, N.A., Surový, P., Pinheiro, A. (2010). Adaptive management on sustainability of cork oak woodlands. In Manos, B., Paparrizos, K., Matsatsinis, N., Papathanasiou, J. (Eds.) *Decision support systems in agriculture, food and the environment: trends, applications and advances.* (Pp: 437-449). IGI Global, Hershey. ISBN:978-1-61520-881-4

Ribeiro, N.A., & Surový, P. (2008). *Inventário nacional de mortalidade de sobreiro na fotografia aérea digital de 2004/2006*. ISBN:978-989-8132-01-7

Ribeiro, N.A., Surový, P., Oliveira, A.C. (2006). Modeling cork oak production in Portugal. In Hasenauer, H. (Ed.) *Sustainable forest management: growth models for Europe.* (Pp: 285–313). Springer-Verlag, Berlin. doi:10.1007/3-540-31304-4_18

Silvertown, J., & Charlesworth, D. (2001). *Introduction to plant population biology*. Blackwell Science.

Sinnett, D., Morgan, G., Williaws, M., Hutchings, T.R. (2008). Soil penetration resistance and tree root development. *Soil Use Management*, 24, 273-280. doi:10.1111/j.1475-2743.2008.00164.x

Souch, C.A., Martin, P.J., Stephens, W., Spoor, G. (2004). Effects of soil compaction and mechanical damage at harvest on growth and biomass production of short rotation coppice willow. *Plant and Soil*, 263, 173-182. doi:10.1023/B:PLSO. 0000047734.91437.26

Surový, P., Ribeiro, N., Brasil, F., Pereira, J.S., Oliveira, M.R.O. (2011). Method for evaluation of coarse cork oak root system by means of digital imaging. *Agroforestry Syst*ems, 82(2), 111-119.

Tsakaldimi, M., Zagas, T., Tsitsoni, T., Ganatsas, P. (2005). Root morphology, stem growth and field performance of seedlings of two Mediterranean evergreen oak species raised in different container types. *Plant and Soil*, 278, 85–93. Doi: 10.1007/s11104-005-2580-1

Tubeileh, A., Groleau-Renaud, V., Plantureux, S., Gucker, A. (2003). Effect of soil compaction on photosynthesis and carbon partitioning within a maize–soil system. *Soil Till. Res.*, 71, 151–161. doi: 10.1016/S0167-1987(03)00061-8

Villar-Salvador, P., Planelles, R., Enriquez, E., Penuelas, J. (2004). Nursery cultivation regimes, plant functional attributes, and field performance relationships in the Mediterranean oak Quercus ilex L. *Forest Ecology and Management*, 196, 257–266. doi:10.1016/j.foreco.2004.02.061

[on-line] www.cge.uevora.pt. Accessed 8 November 2012

CHAPTER 5

CORK OAK SEEDLINGS GROWTH UNDER DIFFERENT SOIL CONDITIONS FROM FERTILISERS, MYCORRHIZAL FUNGI AND AMINOACIDS APPLICATION

Dinis, C., Surový, P., Ribeiro, N.A., Machado, R., Oliveira, M.R.G. (2014). Cork Oak Seedlings Growth under Different Soil Conditions from Fertilisers, Mycorrhizal Fungi and Aminoacids Application. *Submitted to Agroforestry Systems journal*

5.1 Abstract

Regeneration process deals with some constrains related with the livestock management, pasture rotations, dependence of a sequence of favorable climatic years which can lead to a continuous delay in the initiation of the regeneration process which results in the late replacement of the young trees cohort. The main purpose of this study was to promote an increase of cork oak seedling growth in order to decrease the time required for regeneration and also to contribute to avoid the effect of post-transplant stress on cork oak. With this objective a study was carried out on a greenhouse, where the effect of fertilisation, mycorrhizal fungi inoculation and aminoacids supply were tested. Results show that cork oak seedling capability to growth, expressed as total seedling dry weight, is positively affected by treatments, except when only fertilisation was applied. We verified that cork oak seedlings inoculated with mycorrhizal fungi presented better results in terms of aerial structures growth. Any of the treatments was suitable to contribute positively for tap root and total belowground dry weight accumulation. Only fine roots structures were sensitive to treatments effects. It was verified that both inoculated and non-inoculated seedlings subjected to fertilisation were capable to invest largely on the production of these structures (33 and 30% respectively). To reinforce the cork oak seedlings growth, probably the equilibrium between fertilisers and mycorrhizal fungi inoculation is the better option to enhance the cork oak regeneration process. The balance between shoot and root systems growth is guaranteed, fertilisation mainly for root system and mycorrhizal fungi inoculation for shoot growth. This equilibrium probably will also promote a major efficiency on survival strategy of trees in post-stress plantation.

Keywords: Cork oak seedlings, regeneration, fertilisation, mycorrhizal fungi, aminoacids

5.2 Introduction

Cork oak (*Quercus suber* L.) tree is one of the main species of Mediterranean ecosystem woodlands – *Montado*, with a life time around 150 years. This specie has a high economical and environmental value. Its economic value relies on cork production which is renewable every 9 years. The most typical characteristic of the Portuguese *Montado* is its savanna-like physiognomy, spread throughout a large scale mosaic, with different densities, of cork oak (*Quercus suber* L.) and holm oak (*Quercus rotundifolia* Lam.) trees (Pinto-Correia *et al.*, 2011). These typical stands are usually open areas with trees growing on an isolated way or associated with shrubs and/or livestock. Being the woodland system based on trees, it sustainability (continuous crown cover in time) is strongly associated with the natural or artificial trees regeneration (Ribeiro *et al*., 2010). Ribeiro *et al.* (2006; 2010; 2012) refer that the system resilience is based on specific stand structure and densities that are applied, with new trees to compensate natural rates of mortality, allowing the maintenance of a stable crown cover. A crown cover between 30-70% (slope dependent) is fundamental to the woodland ecological sustainability, enhancing the multifunctionality of the system, promoting a protective effect on soil, preventing the erosion risk and improving the water and nutrient cycles (Ribeiro *et al.*, 2004). In the last decades it has been observed an increase on cork oak mortality and, at the same time, a lack of natural regeneration (Ribeiro & Surový, 2008), which is one of the major demands to revitalize the *Montado*. Regeneration process deals with some constrains related with the livestock management and pasture rotations but is also strongly dependent of a sequence of favorable climatic years (at least 10 years). The need of a grazing area to support the existing livestock leads to a continuous delay in the initiation of the regeneration process which results in the late replacement of the young trees cohort.

In the Mediterranean environment, which is characterized by seasonal droughts, the water availability is also a limiting factor that is of key importance in the regeneration of oaks and other woody species (Aranda *et al.*, 2007; Gakis *et al*., 2004). Hence, for this purpose it is necessary to understand the strategy of root growth according to the soil environmental matrix. In result of the difficulty to access root system of mature trees the studies have been relying in experiments with seedlings and young trees (Jonsson *et al*., 2001; Gogorcena *et al*., 2001; Rached-Kanouni *et al*., 2012). The seedling stage is an important and usually critical phase in the regeneration of woody species under natural conditions since the effect of environmental stress is very high at this stage. Seedlings establishment can be reinforced by some external and internal factors such as light protection, soil water capacity and strategy to drought tolerance, which will be reflected in tree biomass partitioning.

Seedling fertilization is refereed as being relevant in Mediterranean areas influencing seedling resistance to drought (Singh & Sale, 2000; Trubat *et al.*, 2006) by the increase of N and P availability (Sabaté & Gracia, 1994; Sardans *et al.*, 2004). Trubat *et al.* (2010) results show that above and belowground cork oak biomass accumulations are reduced by low nutrient availability. Aminoacids are fundamental ingredients, influencing directly or indirectly the physiological activities of the plant (protein synthesis, plant growth, photosynthesis, nutrients absorption). Under drought conditions they help the plant to sustain cellular functions and adjust the osmotic process (Khattab *et al.,* 2012). When incorporated into the soil contribute to improve the soil microflora thereby facilitating the assimilation of nutrients.

The need to evaluate the success of seedling establishment and also tree transplanting has led the research to complementary studies about the role of mycorrhizal symbiosis. Ectomycorrhizal (ECM) fungal species and fungi networks are mediators between soil processes and plant community, by enhancing nutrient uptake, drought tolerance, and pathogen resistance of their hosts, thus influencing seedling establishment, plant diversity, and vegetation community dynamics (Azul *et al.*, 2010; Futai *et al.*, 2008). Smith and Read (1997) point out that mycorrhizal symbiosis is essential for oak trees because it promotes water and nutrients uptake under natural conditions in result of a higher absorption surface area. According to Taylor and Alexander (2005) the ECM communities contain a high diversity of fungal tax, which are associated with a variety of strategies that contribute to the functioning and stability of the forest ecosystem. The Mediterranean oaks and specifically cork oak have been shown to be associated with a wide range of ectomycorrhizal fungi (ECM) (Ortega and Lorite, 2007). By so the importance of ECM symbiosis in cork oak seedlings establishment should be taken into account (Mousain *et al.*, 2009; Aronson *et al.*, 2009). Garbaye and Guehl (1997) also refer that mycorrhizal fungi are more efficient than roots in extracting water at very low soil water potential. Under the Mediterranean climatic conditions this will probably contribute to a major regularity of water absorption during the summer drought season.

These facts lead to the present study, which focuses on the increase of seedling growth in order to decrease the time required for regeneration. With this objective a study was carried out where the effect of fertilization, induction of mycorrhizal fungi and aminoacids application were tested on performance of above and belowground systems of the cork oak seedlings. The results of this study will contribute choosing the better treatment that should be applied to reduce the time for seedlings regeneration process and also contribute to avoid the effect of post-transplant stress of cork oak.

5.3 Material and Methods

5.3.1 Plant material and growing conditions

The present study was carried out in a pot experiment in a greenhouse at Mitra's campus from University of Évora, in South Portugal. Cork oaks seedlings with one year old were transplanted into individual 30cm height x 27cm Ø plastic containers, filled with a Cambissol/Podzol soil collected in the 10-30cm layer. The soil was sieved through a 5mm mesh sieve and a bulk density of 1.66g cm^{-3} was then achieved. The soil characteristics are described in Table 1.

Table 1. Soil characteristics.

Organic matter (%)	**pH [H_2O]**	**NO_3 (mg Kg^{-1})**	**K_2O (mg Kg^{-1})**	**P_2O_5 (mg Kg^{-1})**
0.58	4.86	38	34	16

The temperature inside the greenhouse ranged from 31ºC and 9ºC and air humidity was 50%. The plants were subjected to global radiation inside the greenhouse ($\simeq$275.5 Wm^{-2} for spring/summer and $\simeq$138.5 Wm^{-2} for autumn and winter seasons) (www.cge.uevora.pt).

5.3.2 Experimental design

Five treatments, including control, were arranged in a randomized complete block design with four replications. Treatments applied were: control (C); fertilization (F); fertilization + mycorrhizal fungi (FM); fertilization + aminoacids (FA); and fertilization + aminoacids + mycorrhizal fungi (FAM). Considering that there were 9 seedlings per treatment and replication, a total of 180 seedlings were used in this study.

Prior to transplanting each container was fertilized with 30 mg N, 10 mg NO_3^-, 20 mg NH_4^+, 60 mg P_2O_5, 125 mg K_2O, 12.5 mg MgO, 0.15 mg B, 0.05 mg Cu, 0.1 mg Mn and 0.15 mg Zn. For treatments with fertilization were also applied 8.3 mg N, 3.7 mg P_2O_5, 16.0 mg K_2O, 8.1 mg CaO, 4 mg MgO, 7.8 mg SO_3^-, $0.5\text{x}10^{-2}$ mg B, $0.4\text{x}10^{-2}$ mg Cu, $0.1\text{x}10^{-2}$ mg Fe, $2\text{x}10^{-2}$ mg Mn and $0.1\text{x}10^{-2}$ mg Zn through irrigation water, at each application. For FM and FAM treatments a commercial mixture of mycorrhizal fungi (ECTOVIT by Symbiom Ltd (www.symbiom.com)) was applied, according instructions, during the transplanting process. The mixture is compound by 4 strains of mycorrhizal fungi on a liquid medium and 2 strains of mycorrhizal fungi on a peat-based carrier with ingredients supporting the development of mycorrhiza (humates, ground materials, extracts from sea organisms), naturally degradable granules of a water-retaining gel. The ECM species are *Cenococcum geophilum*, *Hebeloma sinapizans*, *H. crustiliforme*, *Pisolithus tinctorius*, *Amanita rubescens* and *Tricholoma acerbum*.

FA and FAM seedlings were also subjected at each irrigation to a supply of 0.19 g of aminoacids and 0.47 g of vegetable organic matter dissolved in the water. The control treatment (C) was only subject to irrigation. Irrigation consisted of 0.5 L of water applied per container at each 10 days along the 18 months of the experiment.

5.3.3 Data collection

After 18 months all seedlings were handled under laboratory conditions for data collection. For each seedling, height (H) was registered. Each of the 180 seedlings was subjected to aerial components separation (leaves, branches and stem) and each component was labelled and preserved in a cold environment (5ºC). The root systems were carefully washed out of soil and fine roots (Ø <2 mm) were removed from tap root. In order to get the remaining fine roots, the entire soil volume of each container was sieved through a sieve of 1mm mesh and fine roots were manually collected and stored in a water and alcohol solution at 5ºC.

For each sample (individual seedling), fresh leaves, branches + stems and tap roots were scanned using a HP Scanjet 4850 scanner. For the analysis of scanned images the ImageJ software was used to calculate the superficial areas occupied by these structures, meaning leaf area (LA), wood area (WA) and tap root area (TRA). After the scanning process, branches + stems and tap roots were dried at 103º and leaves at 75ºC during 48 hours, and dry weight was obtained as leaf dry weight (LDw), wood dry weight (WDw) and tap root dry weight (TRDw). The seedlings growth was also analyzed as aboveground dry weight (ADw), aboveground area (AA), belowground dry weight (BDw) and belowground area (BA) and total seedling dry weight (SDw).

Fresh fine roots were spread on a water-filled transparent plastic tray and scanned with a transmitting light scanner (EPSON Expression 10000XL 3.4). The images were analyzed with WinRhizo Reg 2009.Total fine root length (FRL) and fine root areas (FRA) were obtained. After image analysis fine roots were dried at 103ºC during 48 hours, for dry weight (FRDw).

Additionally, to evaluate the biomass allocation and seedlings growth the following parameters were calculated:

$$Root\colon shoot\ ratio\ = \frac{Belowground\ dry\ weight}{Aboveground\ dry\ weight}\ \text{(adimensional)}$$

$$Weight\ of\ fine\ roots\ = \frac{Fine\ root\ dry\ weight}{Belowground\ dry\ weight} \times 100\ \ (\%)$$

$$Specific\ Root\ Length (SRL)\ = \frac{Fine\ root\ length}{Fine\ root\ dry\ weight}\ \ (\mathrm{cm\ g^{-1}})$$

$$Root\ Length\ Density\ (RLD)\ = \frac{Fine\ root\ length}{Volume\ of\ soil}\ \ (\mathrm{cm\ cm^{-3}})$$

$$Specific\ Leaf\ Area\ (SLA)\ = \frac{Leaf\ area}{Leaf\ dry\ weight}\ \ (\mathrm{cm^2\ g^{-1}})$$

$$Fine\ root\ area{:}\ leaf\ area\ ratio\ = \frac{Fine\ root\ area}{Leaf\ area}\ (\mathrm{adimensional})$$

$$Root\ length{:}\ seedling\ dry\ weight\ ratio\ (RLR) = \frac{Fine\ root\ length}{Total\ seedling\ dry\ weight}\ \ (\mathrm{cm\ g^{-1}})$$

$$Leaf\ area{:}\ seedling\ dry\ weight\ ratio\ (LAR) = \frac{Leaf\ area}{Total\ seedling\ dry\ weight}\ \ (\mathrm{cm^2\ g^{-1}})$$

5.3.4 Statistical analysis

Data were analyzed using SPSS software (version 20.0, SPSS Inc., Chicago, IL). Distribution was tested for normality by Kolmogorov-Smirnov criterion and homogeneity of variances tested by Levene's test. Significant differences between treatment means were tested using analysis of variance (one-way ANOVA). Means were separated at 5% level using Fisher's least significant difference (LSD) test.

5.4 Results

The effect of treatments on seedlings growth (seedlings height and dry weight) is shown on Table 2. Seedlings height and dry weight are only significantly affected when fertilisation is associated with mycorrhizal fungi (FM) and aminoacids application (FA), with a significant increase in seedling height of 54.53 cm and 56.54 cm/seedling, respectively. For total seedling dry weight the major increase (46%) was also obtained for treatment with fertilization+aminoacids+mycorrhizal fungi (FAM).

Mycorrhizal fungi treatments (FM and FAM) also increased leaf and wood dry weight. The highest values were observed for FMA seedlings, with increases of 108% for aboveground, 81% for leaves and 146% for wood. Although the highest growth expressed through dry weight for aboveground structures is verified on treatments subjected to mycorrhizal inoculation, it is not directly related to a higher root dry weight. For belowground dry weight and tap root dry weight (Table 2) no significant effect from treatments compared to control was verified. However it was observed a significant positive effect of FM and FA treatments in fine root dry weight (9.44 g/seedling). When the percentage of fine roots on the entire root system (Figure1) was analyzed it was verified that treatments F and FM present the highest percentages (30% and 33% respectively).

For growth evaluation expressed through area the effect of treatments are shown on Table 3. Treatments where mycorrhizal fungi were present increased significantly the area of aboveground (AA) and leaves (LA) in relation to control and fertilisation. The treatment where mycorrhizal fungi were associated with aminoacids (FAM) presented the best results for these parameters. Increases of 75% in aboveground area, 71% in leaf area and 149% in wood area were verified (Table 3). Root system area (BA) and fine roots surface area (FRA) were positively affected by all treatments. However tap root area was only affected by fertilisation and fertilisation with aminoacids. The highest increases for tap root, fine root and belowground areas were verified in FA (34%, 44.3% and 44.1%, respectively).

Table 2. Effect of treatment on seedlings height and dry weight (leaf, wood, aboveground, tap root, fine root, belowground and total seedling).

Treatment	**Height (cm)**	**Dry weight (g/seedling)**						
		Leaf (LD$_{w}$)	**Wood (WD$_{w}$)**	**Aboveground (AD$_{w}$)**	**Tap root (TRDw)**	**Fine root (FRD$_{w}$)**	**Belowground (BD$_{w}$)**	**Total Seedling (SD$_{w}$)**
C	44.58c	12.85cb	9.06d	21.91cb	26.28ab	7.02b	33.30ab	55.21c
F	47.90bc	14.79cb	10.94cd	25.72cb	27.28ab	9.08ab	35.35ab	61.07bc
FM	54.53ab	21.34a	18.22b	39.57a	22.94b	9.44a	31.75b	71.31ab
FA	56.54a	16.04c	12.66c	28.70c	30.16a	9.44a	39.60a	68.29b
FAM	51.72abc	23.27a	22.32a	45.59a	27.73ab	7.50ab	35.23ab	80.82a
F	*3.165**	*29.255***	*24.038***	*37.106***	*3.501***	*4.446***	*31.530***	*10.188***
P-value	*0.015*	*0.000*	*0.000*	*0.000*	*0.009*	*0.002*	*0.000*	*0.003*

Mean. n=180. *F-values* for ANOVA test. $^{ns.}$ No significant, * Significant at 0.05 level, **at 0.01 level. Means with different letters are significantly different (*P*<0.05).
C, control; F, fertilisation treatment; FM, fertilisation + mycorrhizal fungi treatment; FA, fertilisation+aminoacids treatment; FAM, fertilisation+aminoacids+mycorrhizal fungi treatment.

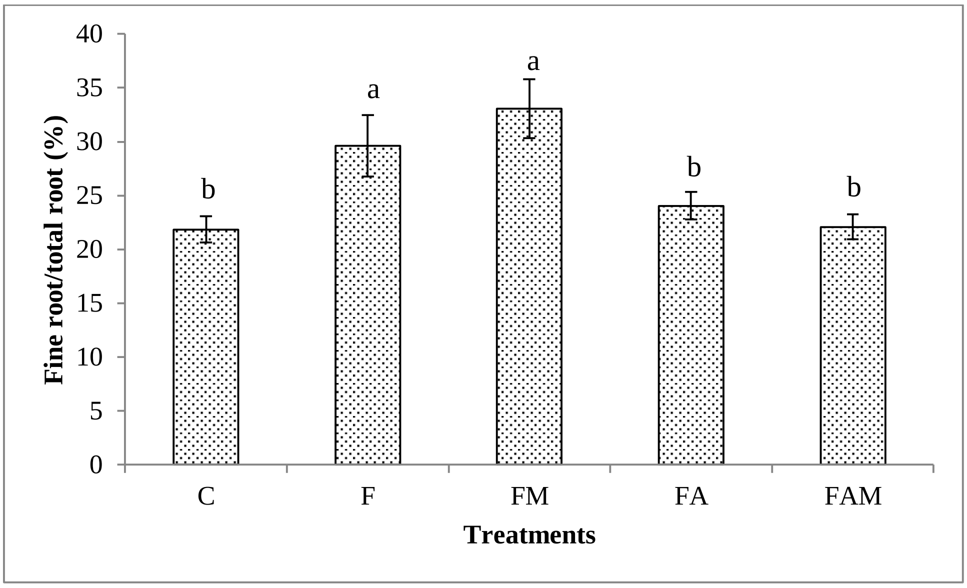


Figure 1. Effect of treatment on fine root dry weight to total root dry weight percentage. Means with different letters are significantly different (*P*=0.00). n=180. C, control; F, fertilisation; FM, fertilisation + mycorrhizal fungi; FA, fertilisation+aminoacids; FAM, fertilisaation+aminoacids+mycorrhizal fungi.

Fine root length (FRL) (Table 3) and specific root length (SRL) (Table 4) are significantly affected by F, FM and FA treatments. Fertilisation treatment (F) presented the highest values for the abovementioned parameters. Increases of 58% for FRL and 37% for SRL were obtained. Concerning to fine root volume which is also directly related to fine root functions, mainly to water and nutrient transport capacity, it was verified that all treatments affected positively this parameter (Table 3) with mycorrhizal fungi treatment presenting the highest increase (42%). For specific leaf area parameter none of the treatments promoted significant effects.

The root:shoot dry weight ratio was also affected by all treatments (Figure 2). Yet, it is verified that seedlings grown within associations of mycorrhizal fungi (FM) and mycorrhizal fungi+aminoacids (FAM) allocate less biomass to roots ($p<0.001$). These results allow assuming that production of aerial structures (leaves, branches and stems) is highly reinforced by the root symbiosis with mycorrhizae.

.

Table 3. Effect of treatment on seedlings area (tap root, fine roots, belowground, leaf, wood and aboveground).

Treatment	**Area (cm^2)**						**Fine root Length (FRL) (cm)**	**Fine Root Volume (FRV) (cm^3)**
	Leaf (LA)	**Wood (WA)**	**Aboveground (AA)**	**Tap root (TRA)**	**Fine root surface area (FRA)**	**Belowground (BA)**		
C	1102.40b	58.37d	1160.77b	48.54c	1704.57b	1753.12b	9537.53c	25.18b
F	1252.73b	66.42dc	1319.15b	61.07ab	2413.17a	2474.24a	15062.90a	32.02a
FM	1867.77a	117.42b	1985.19a	53.54bc	2364.53a	2418.07a	13057.25ab	35.82a
FA	1397.35b	80.20c	1477.55b	66.46a	2461.14a	2527.60a	13385.92a	35.42a
FAM	1886.32a	145.34a	2031.66a	55.20bc	2178.68a	2233.88a	10849.42bc	33.58a
F	*10.265***	*26.443***	*11.412***	*3.842***	*4.255***	*4.347***	*6.083***	*3.745***
P-value	*0.000*	*0.000*	*0.000*	*0.005*	*0.003*	*0.002*	*0.000*	*0.006*

Mean. N=180. *F-values* for ANOVA test. $^{ns.}$ No significant, ** Significant at 0.01 level. Means with different letters are significantly different (*P*<0.05). C, control; F, fertilisation; FM, fertilisation + mycorrhizal fungi; FA fertilisation+aminoacids; FAM, fertilisation+aminoacids+mycorrhizal fungi.

Table 4. Effect of treatment on parameters used to evaluate the seedlings growth (specific root length and specific leaf area).

Treatment	Specific Root Length (SRL) (cm g^{-1})	Specific Leaf Area (SLA) (cm^2 g^{-1})
C	1395.96c	84.89
F	1917.48a	84.14
FM	1474.16ab	86.00
FA	1514.97a	85.96
FAM	1486.24bc	78.82
F	*3.323**	*4.98$^{n.s.}$*
P-value	*0.012*	

Mean. n=180. *F-values* for ANOVA test. $^{ns.}$ No significant, * Significant at 0.05 level, ** Significant at 0.01 level. Means with different letters are significantly different (*P*<0.05). C, control; F, fertilisation; FM, fertilisation + mycorrhizal fungi; FA, fertilisation+aminoacids; FAM, fertilisation+aminoacids+mycorrhizal fungi.

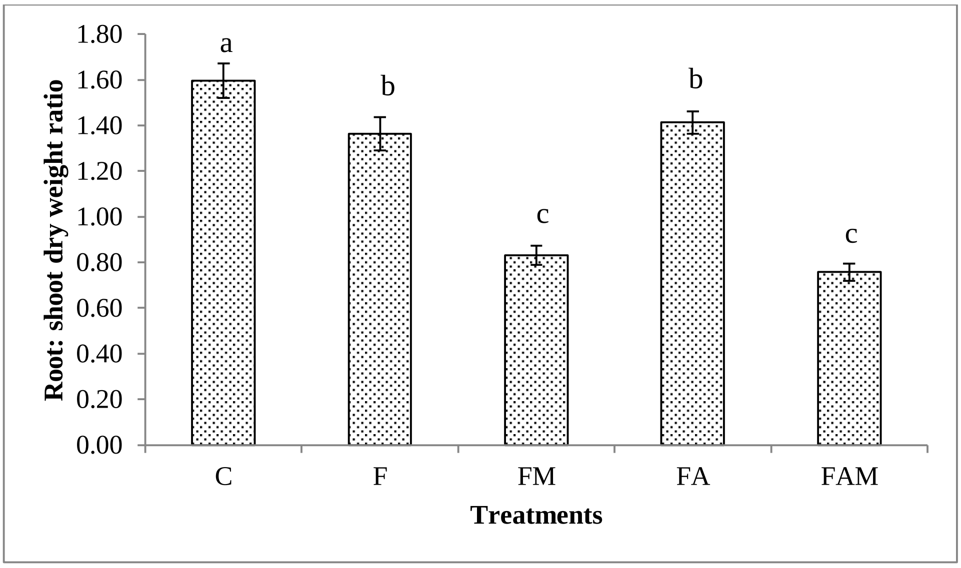


Figure 2. Effect of treatment on root:shoot dry weight ratio. Means with different letters are significantly different (*P*=0.00). n=180. C, control; F, fertilisation; FM, fertilisation + mycorrhizal fungi; FA, fertilisation+aminoacids; FAM, fertilisation+aminoacids+mycorrhizal fungi.

The data for the two parameters calculated to evaluate the capability of seedlings to acquire belowground and aboveground resources, RLR and LAR, are presented in Figures 3 and 4.

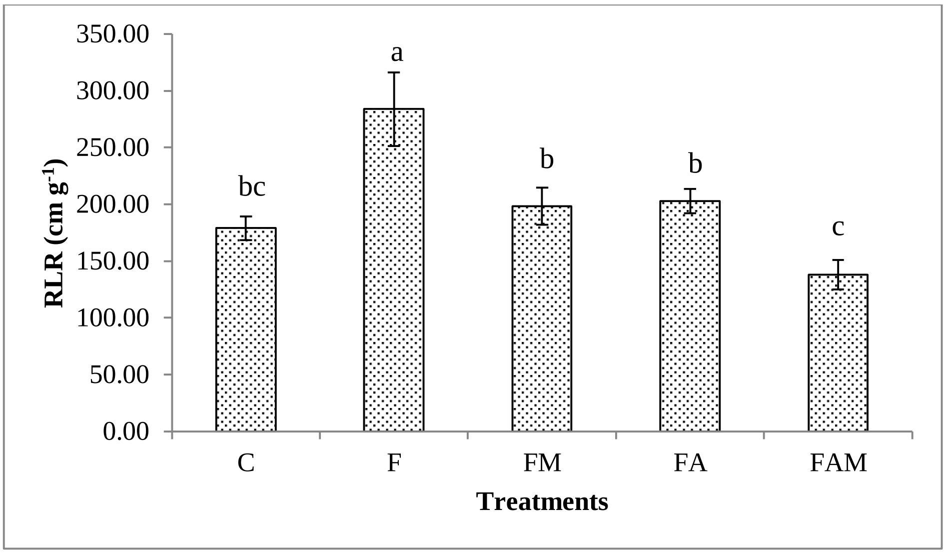


Figure 3. Fine root length per seedling biomass ratio (cm g^{-1}). Means with different letters are significantly different (*P*=0.000). n=180. C, control; F, fertilisation; FM, fertilisation + mycorrhizal fungi; FA, fertilisation+aminoacids; FAM, fertilisation+aminoacids+mycorrhizal fungi.

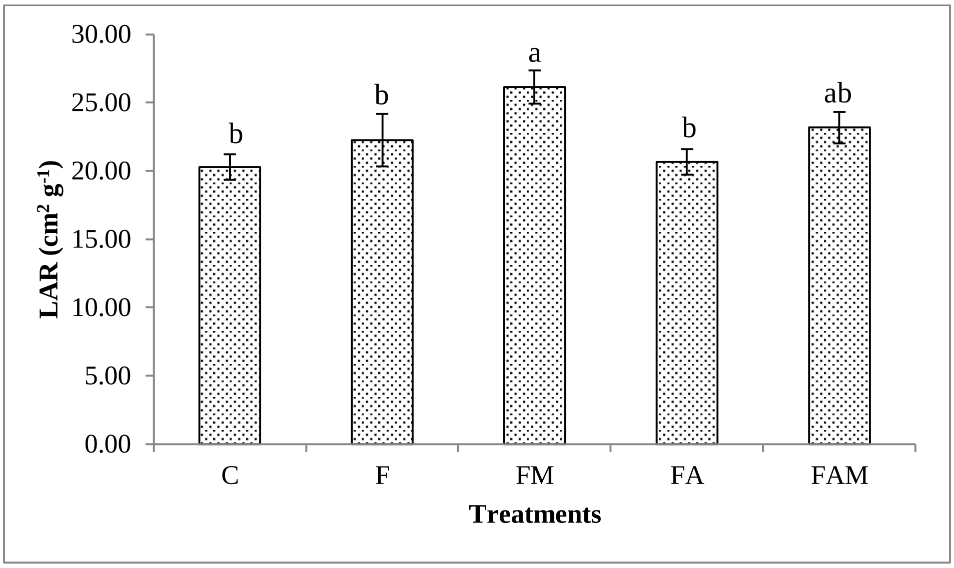


Figure 4. Leaf area per seedling biomass ratio (cm^2 g^{-1}). Means with different letters are significantly different (*P*=0.011). n=180. C, control; F, fertilisation; FM, fertilisation + mycorrhizal fungi; FA, fertilisation+aminoacids; FAM, fertilisation+aminoacids+mycorrhizal fungi.

Compared to control, fertilization treatment (F) is the only treatment affecting positively root length per seedling dry weight (RLR) (Figure 3). A mean value of ≃284 cm g^{-1}, reporting an increase of 59%, was verified. Although not significant, the treatment subjected to fertilisers, mycorrhizal fungi and aminoacids (FAM) presented a decrease of about 23% when compared to control. For leaf area per seedling dry weight (LAR) (Figure 4) only mycorrhizal fungi treatment (FM) affected positively this parameter.

Carbon investments expressed through exchange surfaces between leaf/atmosphere and soil/root interactions (FRA:LA ratio) (Figure 5) was only significantly affected by fertilization. Is important to point out that FMA treatment presented a decrease of 19% compared with control, probably due to the higher leaf production verified on this treatment.

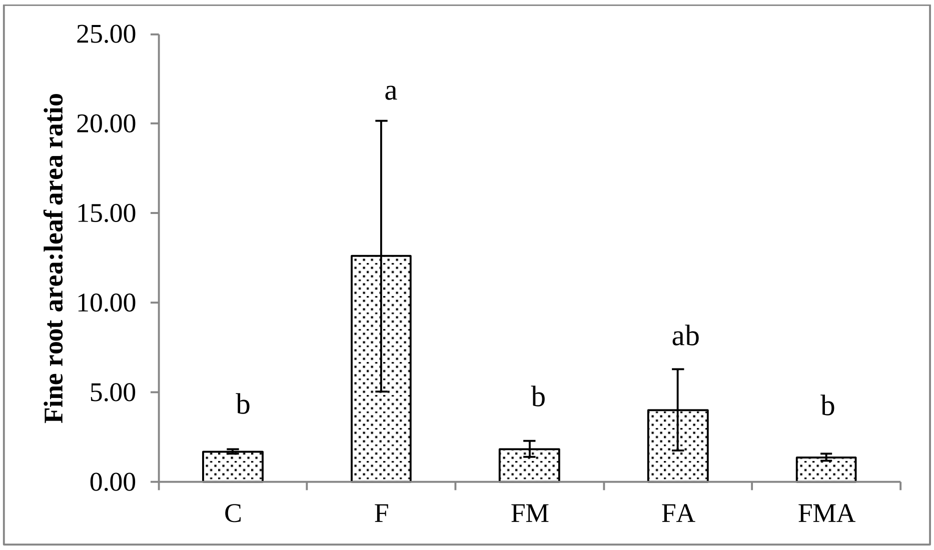


Figure 5. Fine root surface area leaf area ratio. Means with different letters are significantly different (P=0.000). n=180. C, control; F, fertilisation; FM, fertilisation + mycorrhizal fungi; FA, fertilisation+aminoacids; FAM, fertilisation+aminoacids+mycorrhizal fungi.

5.5 Discussion

For discussion, due to the fact that there is no extensive literature dealing with cork oak species, it was decided to make a more functional approach. This study shows that seedling capability to growth, expressed as total seedling dry weight (SD_w), is positively affected by treatments, except when only fertilisation was applied (F). The most effective treatment concerning to seedlings growth was composed by fertilisers, mycorrhizal fungi inoculation and aminoacids (FAM), justified by the 46% increase on SD_w. The other results of morphological parameters as leaves, wood and aboveground dry weight (Table 2) and area (Table 3) reinforce this positive effect of FMA treatment on 2 ½ years-old cork oak seedlings growth. The second higher production of aerial organs expressed, in terms of dry weight and area, was observed for the inoculated seedlings subjected to fertilisation (FM). As hypothesized, mycorrhizal inoculation promoted a higher growth of aerial structures of cork oak seedlings which is in agreement with Moussain *et al.* (2009) and Sebastiana *et al.* (2013) works. Moussain *et al.* (2009) observed significant aboveground biomass increments of 18 months-old cork oak seedlings inoculated with an ECM fungi; *Pisolithus arrhizus*. Through their study they also verified an increase in water use efficiency of these seedlings during the first two growing seasons. In the case of Sebastiana *et al.* (2013) they evaluated the effect of *Pisolithus tinctorius* inoculation on shoot systems of nursery cork oak seedlings. Again, the positive effect of mycorrhizal fungi innoculation in seedlings growth was verified mainly through a significant increase in leaf area and dry weight. Our results are also in accordance with results observed for other tree species (e.g., Diagnea *et al.*, 2013 for *Acacia mangnium* seedlings and Wu *et al.*, 2011 for peach seedlings). However in our study, data for aboveground evaluation showed no significant differences between inoculated and fertilised seedlings (FM) and inoculated and fertilised seedlings subjected to a supply of aminoacids (FAM). This indicates that the effect of aminoacids supply, to facilitate the nutrients assimilation, is not relevant for the growth rate increase of cork oak seedlings, at least until the 30 months-old.

Taking into account that all treatments applied in this study where subjected to a supply of nutrients (N, P, K) through fertilisation, the results obtained are in with the ones obtained Trubat *et al.* (2010). In their greenhouse experiment they subjected cork oak seedlings of 18-months to different supplies of N, P, K and evaluated the relationship between seedling morpho-functional traits and field performance of cork oak seedlings. They observed that aboveground accumulations were reduced by low nutrient availability, in cork oak seedlings. However, in our case we didn't verified any significant effect on biomass parameter (expressed through dry weight) of seedlings subjected only to fertilizers through irrigation water (F treatment) during the 18 months growing period.

The evaluation of cork oak seedlings root system area (BA), a morphological parameter which gives information about the quantity of root surface in contact with the soil, showed that all treatments increase significantly the root surface contact (values higher than 2200 cm^2) compared to control (1753 cm^2). Yet, the application of fertilisers plus aminoacids proves to be the more efficient treatment to increase significantly the root surface contact. Results of 34% for tap root and 44% for fine roots were verified on seedlings subjected to this treatment. Comparing results from above and belowground system areas we verified that after 30 months of growth, in all treatments including control, the amount of root system area is higher than shoot area. However, this study indicates that any of the treatments was suitable to contribute positively for tap root and total belowground dry weight accumulation. Only fine roots structures were sensitive to treatments effects. Significant differences in dry weights were observed when fertilisers and aminoacids (FA) and fertilisers with mycorrhizal fungi (FM) were present. Equal result was obtained for both treatments (9.44 g/seedling). However, through the analysis of mean fine root distribution per seedling (Figure 1), we verified that both inoculated and non-inoculated seedlings subjected to fertilisation (FM and F treatments, respectively) were capable to invest largely on the production of these structures (33 and 30% respectively). This is an important issue because despite fine root biomass contributes relatively little to total tree biomass, fine roots are major contributors to carbon inputs because of their rapid turnover (Kucbel *et al.*, 2011). In addition, for these important structures of root system we also verified that the abovementioned treatments and yet fertilisation plus aminoacids application (FA) promoted significant positive effects in fine root length (FRL) (Table 3) and in specific root length (SRL). SRL is considered a key factor to evaluate the amount of "harvesting" or absorptive tissue deployed per unit mass invested. This parameter is usually applied in prognoses of the capacity of root systems to changed nutrient availability in soils. The bigger the SRL the better is the adaptability of root systems to the changing environment (Zeleznik *et al.*, 2007). By so we can assume that the interactions between roots/soil interface and consequently, caption and absorption of nutrients are reinforced positively when F, FM and FA treatments are applied in cork oak seedlings. Specifically for fertilisation treatment we expected these results, hence this treatment only have the aid of one product and by so, needs to promote the growth in length of fine roots to allow a major capability to explore all the soil available, looking for available water and nutrients. It is noteworthy that the length of the fine roots in all treatments exceeded 9.5m, which shows that soil exploration capacity of cork oak seedlings during the first two growing seasons, is high. In contrast and although all treatments affected positively fine root volume non inoculated seedlings subjected to fertilisation presented the lower value when compared to control (32.02 cm^3). This allows us to assume that besides application of fertilisers on cork oak seedlings promote longer roots (higher FRL) they are thinner than the ones from other treatments.

According to Eissenstat and Yanai (1997) the root length is assumed to be proportional to resource acquisition (benefit) and the root dry weight to be proportional to construction and maintenance (cost). Struve *et al.* (2009) refer that generally higher fine root production present a slower growth. We disagree with the authors hence in our study inoculated seedlings subjected to fertilisation (FM) present also a significant growth of the upper system (9.44 g/seedling for FRD_w and 39.57 g/seedling for AD_w on FM, compared to 7.02 g/seedling for FRD_w and 21.91g/seedling for ADw on control). Instead, our results are partly in accordance with Mohammadi *et al.* (2011). They defend that mycorrhizal fungi frequently stimulate plants to reduce root biomass while simultaneously expanding nutrient uptake capacity by extending far beyond fine root surfaces. Although not significantly for mean total root system dry weight of seedlings subjected to FM treatment we observed a decrease when compared to control (Table 3) but when the evaluation of fine roots was made a significant increase was observed. However for the inoculated seedlings subjected to fertilisation and aminoacids (FAM) a slightly increase was observed on root biomass, expressed through dry weight. In contrast, Diagnea *et al.* (2013) for 4 months-old *Acacia mangnium* seedlings observed that mycorrhizal fungi enhance root biomass. In our study we verified that root biomass after 30 months-old cork oak seedlings weren't affected by none of the treatments applied. More studies should focus on this subject to verify the influence of ECM inoculation on cork oak root seedlings by itself.

The relative allocation of resources to roots or shoots has been considered a key factor in plant strategies regarding water and it is very important for seedling performance and survival in the field (South, 2000; Kostopoulou *et al.*, 2011). In our study root:shoot dry weight ratio (R:S) is enhanced by all the treatments tested (Figure 2). Expectedly, lower values of R:S, were observed for inoculated seedlings (FM and FAM). This allows us to assume that with these two treatments cork oak seedlings, during the first two growing seasons are able to transpire more water than the ones subjected to other treatments. Unlike us, Scagel & Linderman (1998) for douglas fir and lodgepole pine seedlings at the end of the first growing season and Jonsson *et al.* (2001) for *Pinus sylvestris*, found that inoculation with mycorrhizal fungi had little effect on this ratio. Also an increase in soil fertility is commonly associated with a reduction in the root:shoot ratio (Harris, 1992), which we confirm with our results comparing seedlings from control (1.60±0.08) with other treatments applied (1.36±0.07 for F, 1.41±0.05 for FA, 0.83±0.04 for FM and 0.76±0.04 for FAM) (Figure 2). However, in this work no differences between fertilisation (F) and fertilisation plus aminoacids addiction (FA) were found. For a future field establishment of these plants we believe that fertilized inoculated seedlings will be better adapted to a faster growth and survival. Villar-Salvador *et al*. (2004) observed that during the first two growing seasons the field performance of holm oak (*Quercus ilex*) seedlings, previously fertilized during 10 months on nursery, was higher on seedlings with larger shoots and lower R:S. They verified that plants with these attributes presented lower mortality and grew faster in the field than those with smaller shoots and high R:S. As in our case, the lower R:S was due to an increase in shoot growth but not to a reduction in the biomass allocated to roots. This response has also been observed in other Q*uercus* species suggesting that these species have a conservative pattern of root mass in response to variations in mineral nutrients (Villar-Salvador *et al*., 2004). Specific leaf area (SLA) being a measure of leaf thickness, is used to evaluate the drought resistance of the plants. An elevated SLA indicates a better adaptability to dryness environments. In a previous study Makita *et al.* (2012) investigated how colonization by different ectomycorrhizal fungal species affects the physiology and morphology of other *Quercus* specie (*Quercus serrata*) seedlings. They observed a positive effect of ECM on specific leaf area (SLA) in their 9-month-old *Q. serrata* seedlings inoculated with *Pisolithus tinctorius, Scleroderma citrinum, Laccaria amethystea,* and *Astraeus hygrometricus.* . We cannot confirm the same results for cork oak seedlings. After 30 months of growth, any effect on

SLA was observed in our inoculated seedlings (Table 4). However when leaf area is analyzed per total seedling dry weight (LAR) (Figure 4) we verified that fertilised inoculated treatment (FM) enhanced significantly the capacity of 30 months-old cork oak seedlings to acquire resources from the atmosphere. This is in conformity with results obtained by Merouani *et al.* (2005). In their work they also subjected cork oak seedlings to the effect of mycorrhizal fungi inoculation (with *Pisolithus tinctorius*) and fertilised them once a week, during 6 weeks, with 50±7.4 mL/seedling of NPK solution. After 18 months growth they also observed that SLA wasn't affected by this treatment. But, such as us, they observed a positive effect of this treatment on LAR parameter. Similar LAR results were obtained between our 30 months-old seedlings (26.13 cm^2 g^{-1}) and their 18 months-old seedlings (29.2 cm^2 g^{-1}). On the other side, the enhancement capacity of cork oak seedlings to acquire resources from soil (RLR) is only sensitive to fertilisation through irrigation (RLR, Figure 3). Non-inoculated and fertilised seedlings (from F treatment) presented the better result with a mean value of 283.97 cm^2 g^{-1}. It is important to refer that although not significantly mean differences were obtained, a decrease on RLR was observed for FAM when compared to control. Yet, with this work we verified that during the 30 months-old growing period only non-inoculated and fertilised cork oak seedlings (from F treatment) presented a growth strategy more focused on root functioning (water and nutrients absorption) than on photosynthesis function (leaf atmosphere exchanges). This assumption is made based on the results of the carbon investments balance of cork oak seedlings, expressed through the evaluation of exchanges surfaces between leaf/atmosphere and soil/root interactions (Figure 5). Significant increase compared to control was observed when root leaf area ratio was calculated. Cork oak seedlings of control presented a ratio of 1.71±0.13 and for non-inoculated and fertilised seedlings a mean value of 12.62±7.56 was observed. However, when focusing on our main objective, from our understanding it is of the outmost importance to maintain equilibrium between shoot and root system growth preserving the success of future growth stages (juvenile and mature) in the field. Then, a similar carbon investment in both compartments of the seedling must be guaranteed. Through the results (Figure 5), we assume that mycorrhizal fungi inoculation with fertilisation is the treatment that better can promote this balance (mean value of 1.85±0.45 for this ratio).

5.6 Conclusions

Conclusively, this work shows:

1) Mycorrhizal fungi inoculation with fertilisation and mycorrhizal fungi inoculation with fertilisation plus aminoacids are the treatments which are capable to enhance greatly shoot production of cork oak seedlings. Mycorrhizal fungi are probably responsible for this effect (major aboveground biomass allocation in comparison to belowground). Mycorrhizal fungi improved roots efficiency in extracting water and nutrients for aboveground maintenance;

2) Seedlings subject only to fertilisation or to fertilisation with aminoacids present slower growths in terms of total seedling biomass. Most energy is spending in root system construction, verified by the higher specific root length observed. Without the symbiosis with mycorrhizae, cork oak seedlings need to focus more intensively on root growth, to allow an efficient supply of water and nutrients for shoot system construction and development;

3) To reinforce the cork oak seedlings growth probably, the equilibrium between fertilisers and mycorrhizal fungi inoculation (FM treatment) is the better option to enhance the cork oak regeneration process. The balance between shoot and root systems growth is guaranteed, fertilisation mainly for root system and mycorrhizal fungi inoculation for shoot growth. This equilibrium probably will also promote a major efficiency on survival strategy of trees in post-stress plantation.

4) If no treatment is applied, the seedlings would promote root growth, until at least $2^{1/2}$ years-old.

Although the results presented in this study bring new highlights about the advantage of induction treatments to promote an increase in the rate of cork oak seedlings growth, this research lead to some open questions that have to be subject of future works. With this study, it is not clear if inoculation with mycorrhizal fungi by itself allows a rate of success in seedlings growth. Future work should test the effect of products separately.

References

Aranda, I., Pardos, M., Puertolas, J., Jimenez, M.D., Pardos, J.A. (2007). Water-use efficiency in cork oak (Quercus suber) is modified by the interaction of water and light availabilities. *Tree Physiology*, 27, 671-677.

Aronson, J., Pereira, J.S., Pausas, J.G. (2009). *Cork oak woodlands on the edge: conservation, adaptive management, and restoration*. Society for Ecological Restoration International and Island Press. 315 pp. ISBN:13: 978-1-59726-479-2

Azul, A.M., Sousa, J.P., Agerer, R., Martin, M.P., Freitas, H. (2010). Land use practices and ectomycorrhizal fungal communities from oak woodlands dominated by Quercus suber L. considering drought scenarios. *Mycorrhiza*, 20, 73-88. doi:10.1007/s00572-009-0261-2

Diagnea, N., Thioulousec, J., Sanguind, H., Prind, Y., Krasova-Wadea, T., Syllaa, S., Galiana, A., Baudoine, E., Neyra, M., Svistoonofff, S., Lebrune, M., Duponnois, R. (2013). Ectomycorrhizal diversity enhances growth and nitrogen fixation of Acacia mangium seedlings. *Soil Biology & Biochemistry*, 57, 468- 476.

Gakis, S., Mantzanas, K., Alifragis, D., Papanastasis, V.P., Papaioannou, A., Seilopoulos, D., Platis, P. (2004). Effects of understorey vegetation on tree establishment and growth in a silvopastoral system in northern Greece, *Agroforestry Systems*, 60, 149–157. doi: 10.1023/B:AGFO.0000013275.30617ad

Garbaye, J., & Guehl, J.M. (1997). The role of ectomycorrhizas in water use by forest trees. *Revue Forestiere Francaise*, 49, 110–120. doi: 10.5367/000000000101293068

Gogorcena, Y., Molias, N., Larbi, A., Abadia, J., Abadia, A. (2001). Characterization of the responses of cork oak (Quercus suber) to iron deficiency. *Tree Physiology*, 21, 1335-1340.

Eissenstat, D.M., & Yanai, R. (1997). Ecology of Root Lifespan. *Advances in Ecological Research*, 27, 1-62.

Futai, K., Taniguchi, T., Kataoka, R. (2008). Ectomycorrhizae and their importance in forest ecosystems. In Siddiqui, Z.A., Akhtar, M.S., Futai, K. (Eds.) *Mycorrhizae: Sustainable Agriculture and Forestry*. (Pp:241–285). doi: 10.1007/978-1-4020-8770-7_11

Harris, R.W. (1992). Root:shoot ratios. *Journal of Arboriculture*, 18(1), 39-42.

Jonsson, L.M., Nilsson, M.C., Wardle, D.A., Zackrisson, O. (2001). Context dependent effects of ectomycorrhizal species richness on tree seedling productivity. *Oikos*, 93, 353-364.

Khattab, M.M., Shaban, A.E., El-Shrief, A.H., Mohamed, A.S. (2012). Effect of Humic Acid and Aminoacids on Pomegranate Trees under Deficit Irrigation. I: Growth, Flowering and Fruiting. *Journal of Horticultural Science & Ornamental Plants*, 4(3), 253-259.

Kostopoulou, P., Radoglou, K., Dini Papanastasi, O., Adamidou, C. (2011). Effect of mini-plug container depth on root and shoot growth of four forest tree species during early developmental stages. *Turkish Journal of Agriculture and Forestry*, 35, 379-390. doi:10.3906/tar-1104-11

Kucbel, S., Jaloviar, P., Spisak, J. (2011). Quantity, vertical distribution and morphology of fine roots in Norway spruce stands with different stem density. *Plant Root*, 5, 46-55. doi:10.3117/plantroot.5.46

Makita, N., Hirano, Y., Yamanaka, T., Yoshimura, K., Kosugi, Y. (2012). Ectomycorrhizal-fungal colonization induces physio-morphological changes in Quercus serrata leaves and roots. *Journal of Plant Nutrition and Soil Science*, 175(6), 900–906.

Merouani, H., Costa e Silva, F., Sampaio, T., Lourenço, M.J., Faria, C., Pereira, J.S., Almeida, M.H. (2005). Efeito da Idade e da Fertilização na Qualidade das Plantas do Sobreiro (Quercus suber L.) em Viveiro. *Available at: https://www.repository.utl.pt/bitstream/10400.5/690/1/Congress5-H.Almeida-efeito.pdf*

Mohammadi, K., Khalesro, S., Sohrabi, Y., Heidari, G. (2011). A Review: Beneficial Effects of the Mycorrhizal Fungi for Plant Growth. *Journal of Applied Environmental and Biological Sciences*, 1(9), 310-319.

Mossain, D., Bouckim, H., Richard, F. (2009). Mycorrhizal symbiosis and its role in seedling response to drought. In Aronson, J., Pereira, J.S., Pausas, J.G. (Eds.) *Cork oak woodlands on the edge: ecology, adaptive management and restoration*. (Pp:81-87). Society for ecological restoration international.

Ortega, A., & Lorite, J. (2007). Macrofungi diversity in cork-oak and holm-oak forests in Andalusia (southern spain); an efficient parameter for establishing priorities for its evaluation and conservation. *Central European journal of Biology CEJB*, 2(2), 276-296. doi:10.2478/s11535-007-0015-0

Pinto-Correia, T., Ribeiro, N., Sá-Sousa, P. (2011). Introducing the montado, the cork and holm oak agroforestry system of Southern Portugal. *Agroforestry Systems*, 82, 99-104. doi:10.1007/s10457-011-9388-1

Rached-Kanouni, M., Alatou, D., Soulaiman, S. (2012). Responses of cork oak seedlings to short-term exposures to low temperatures. *American Journal of Scientific Research*, 59, 28-41. ISSN:1450-223X

Ribeiro, N., Dias, S., Surovy, P., Gonçalves, A.C., Ferreira, A.G., Oliveira, A.C. (2004). The importance of crown cover on the sustainability of cork oak stands: a simulation approach. *Advances in Geoecology*, 37, 275-267.

Ribeiro, N., Surovy, P., Oliveira, A.C. (2006). Modeling cork oak production in Portuga. In Hasenauer, H. (Ed.) *Sustainable forest management. Growth models for Europe*. (Pp: 285-313). Springer-Verlag Berlin Heidelberg.

Ribeiro, N.A., & Surový, P. (2008). *Inventário nacional de mortalidade de sobreiro na fotografia aérea digital de 2004/2006.* ISBN:978-989-8132-01-7

Ribeiro, N., Surovy, P., Pinheiro, C. (2010). Adaptative management and sustainability of cork oak woodlands. In Manos, B. (Ed.) *Decision Support Systems in Agriculture, Food and the Environment – Trends, applications and advances*. Information science reference. (Pp: 437-449). doi: 10.4018/978-1-61520-881-4.ch020

Ribeiro, N.A., Surovy, P., Yoshimoto, A. (2012). Optimal regeneration regime under contínuos crown cover requirements in cork oak woodlands. In Konoshima, M., Yoshimoto, A. (Eds.) *Formath vol.11 – Formath Sapporo 2011. Forest resource management and mathematical modeling.* (Pp: 83:102). Japan society of forest planning press.

Sabaté, S., & Gracia, C.A. (1994). Canopy nutrient content of a Quercus ilex L. forest: fertilization and irrigation effects. *Forest Ecoogy and Management*, 68, 31–37.

Sardans, J., Rodà, F., Peñuelas, J. (2004). Phosphorus limitation and competitive capacities of Pinus halepensis and Quercus ilex subsp. rotundifolia on different soils. *Plant Ecology,* 174, 307–319.

Scagel, C.F., & Linderman, R.G. (1998). Influence of ectomycorrhizal fungal inoculation on growth and root IAA concentrations of transplanted conifers. *Tree Physiology*, 18, 739:747.

Sebatiana, M., Pereira, V.T., Alcantara, A., Pais, M.S., Silva, A.B. (2013). Ectomycorrhizal inoculation with Pisolithus tinctorius increases the performance of Quercus suber L. (cork oak) nursery and field seedlings. *New Forests,* 44(6), 937-949.

Singh, D.K., Sale, P.W.G. (2000). Growth and potential conductivity of white clover roots in dry soil with increasing phosphorus supply and defoliation frequency. *Agronomy Journal*, 92, 868–874.

Smith, S.E., & Read, D.J. (1997). *Mycorrhizal Symbiosis*. 2nd ed. Academic Press, London. 605 pp. ISBN:0-12-652840-3

South, D.B. (2000). *Planting morphological improved pine seedlings to increase survival and growth.* Forestry and Wildlife Series No. 1. Alabama Agricultural Experiment Station. Auburn University, Alabama.

Struve, D.K., Ferrini, F., Fini, A., Pennati, L. (2009). Relative Growth and Water Use of Seedlings from Three Italian Quercus Species. *Arboriculture & Urban Forestry,* 35(3), 113–121.

Taylor, A.F.S., & Alexander, I. (2005). The ectomycorrhizal symbiosis: life in the real world. *Mycologist,* 19, 102–112.

Trubat, R., Cortina, J., Vilagrosa, A. (2006). Plant morphology and root hydraulics are altered by nutrient deficiency in Pistacia lentiscus L. *Trees*, 20, 334–339.

Trubat, R., Cortina, J., Vilagrosa, A. (2010). Nursery fertilization affects seedling traits but not field performance in Quercus suber L. *Journal of Arid Environments,* 74, 491–497.

Villar-Salvador, P., Planelles, R., Enriquez, E., Penuelas Rubira, J. (2004). Nursery cultivation regimes, plant functional attributes, and field performance relationships in the Mediterranean oak Quercus ilex L. *Forest Ecology and Management,* 196, 257–266.

Wu, Q.S., Li, G.H., Zou, Y.N. (2011). Roles of arbuscular mycorrhizal fungi on growth and nutrient acquisition of peach (prunus persica l. batsch) seedlings. *Journal of Animal & Plant Sciences*, 21(4), 746-750.

Zelenik, P.Z., Hrenko, M., Then, C., Koch, N., Grebenc, T., Levanic, T., Kraigher, H. (2007). CASIROZ: Root parameters and types of ectomycorrhiza of young beech plants exposed to different ozone and light regimes. *Plant Biology*, 9, 298–308.

CHAPTER 6

GENERAL DISCUSSION AND CONCLUSIONS

The complexity of the *Montado* production system management leads to a large set of solutions, derived mainly from empirical knowledge application, which has been absent by scientific based research findings. These knowledge gaps involve, as referred earlier, the cork oak root system. The present whole tree integrated research approach permits a better understanding of the production system vulnerabilities that can result in management modification proposals that will be useful for the managers in a near future. The cork oak root system is essential not only in the functioning of the natural cycles of water and carbon, but also as a structural component of landscape dynamic.

The main conclusions from the research studies are:

Regarding the comparison of two methods to assess the root architecture as a potential factor influencing the diversity of a stand it can be concluded

Knowing that root excavation is essential for the validation of non-invasive methods to access the root systems and for the collection of essential information for 2D and 3D root structure representation, in Chapter 3 two different methods were tested to access entire tree root systems. Two tree species, stone pine (*Pinus pinea*) and cork oak (*Quercus suber*), growing in the same soil type (Cambissoil soil (Vt)), were subjected to these methods. Tree root systems excavation is a technique that requires an effort in terms of time and costs, since all methods used for roots separation from soil have to be done very carefully. The methods tested were root system excavation by profile washing with water on the pine stone, and root system excavation by high pressure air jet on cork oak. Results showed that in the case of a soil with a sandy texture the most suitable excavation method is the excavation by high pressure air jet. Besides being logistically easier to install and apply, it is faster and less costly. In the case of soils presenting clayey characteristics both methods proved to be inadequate to excavate the entire root system of large trees. In this case, the manual option could be the best choice to accomplish better results.

Regarding the morphological evaluation of cork oak root system the conclusions are:

One of the main highlights obtained with the intensive study presented on Chapter 3, where the morphological evaluation of cork oak root system in a Cambissoil soil is studied, was the possibility to represent the entire root system at a 3dimensional scale. In a 1.40 m depth soil profile a dimorphic root system was observed and a relative high quantity of sinkers distributed all over the soil profile was observed. One root subsystem at a superficial level until 40 cm depth and another at a deeper level around 1.20 m depth were observed, indicating that cork oak root system has the ability to explore the entire soil profile. The root system spread far away from horizontal canopy projection, for example a 4th order root was found at 10 m distance from the tree trunk. Vertically, tap root reached 1.40 m depth, and at this depth continued its growth horizontally, probably due to soil bulk density (1.76 g m^{-3}) at that depth. Only few small roots were found between 1.40 and 2 m depth. The major distribution of root volume was verified at the first 20 cm depth and some superficial coarse roots with elevated diameters (eg., R8 with 15 cm Ø) were formed at only 8 cm depth. Through the *in situ* evaluation and through 3D representation it was proved that the pattern of root growth strategy is complex to replicate. It was found that each individual root has its own strategy of growth, without an understanding of why and when a new lateral root is created or why they develop in some specific direction or even, why they connect with others, exploring the same soil space. Still, the results obtained show that tree biomass allocation is distributed almost similarity in aerial and root systems, and that, concerning to length and biomass variables, 3rd order was the most representative branching order for shoot and root systems. The possibility to integrate these results with the soil management practices and consequently with the root system damaging, can bring new concerns about their relation with the observed cork oak decline and mortality verified in *Montado*.

Concerning to the effects of soil compaction depth on cork oak seedlings growth it can be concluded:

In the Mediterranean basin, where the water is scarce, being available only in deeper soil horizons trees that do not have deep roots are at a disadvantage during the summer season.

Specifically, in the case of seedlings or young trees, growing in Mediterranean areas, these facts can be crucial if the plants are unable to develop a deep root before the summer period, assuming that the better the root system develops at seedling stage the better will be the performance of the tree at its adult stage. A study was developed in a greenhouse, where the effects of soil compaction at different depths in the growth of cork oak seedlings were evaluated. The selected soil was a Podzol soil. The results of this study, presented in Chapter 4, led to the conclusion that the length of tap root and total root biomass (coarse and fine roots) are negatively affected by soil compaction in depth. The absence of fine roots (responsible for water and nutrients uptake) in the compacted soil layers was also observed. Results showed that soil mechanical impedance at 30 cm depth decreased leaf area, when compared to non-compacted soils. In conclusion, it can be stated that soil compaction in the initial growth stage disables the root penetration to greater depths compromising the stabilization of the tree in adulthood stage.

Regarding to cork oak seedlings growth under different soil conditions, from fertilization, mycorrhizal fungi and aminoacids application, the main conclusions are:

One of the main facts observed in cork oak stands, beside the observed decline of mature trees, is the low success rate of natural and artificial regeneration. In order to minimize the time required for the establishment of cork oak seedlings in the field, promoting a faster but equilibrated growth, a study was conducted in a greenhouse where several treatments were tested. The results of this study, presented in Chapter 4, show that seedlings` growth was not increased when only fertilization was added. The treatment that presented the better results in terms of growth, measured through several variables and parameters, was the one where fertilizers, aminoacids and mycorrhizal fungi inoculation were applied. This treatment substantially promoted the growth of the seedlings shoot, concerning total biomass and in terms of leaf area. Despite this the root growth was not affected by this treatment. Therefore, it is though that the imbalance between the components of the plant (shoot and root) in adulthood may not be beneficial, especially concerning tree anchorage and support, due to tensile forces increase between canopy and roots, as the tree grows. Consequently, the best treatment to be induced is the one that comprises fertilizers application and inoculation with mycorrhizal fungi. It was observed that seedlings subjected to this treatment presented a more equilibrated growth between shoot and root components.

Integration of this research results on the *Montado* dynamics

Relating the results obtained in this thesis (Chapter 2, 3, 4 and 5) with the existing knowledge about *Montado* decline factors (Chapter 1) some important considerations need to be highlighted:

- The effects of soil disking involves an high risk of damage to the structure of cork oak root system, since the superficial coarse roots with high diameters start to develop approximately at 8 cm depth;
- Results seem to indicate that the effect of root cuts by disking is permanent. Replacement roots may be formed but they will not occupy the same space as the original roots;
- Roots subjected to cuts, while not completely healed, will function as input focus of pathogens, which may jeopardize the entire functionality of the tree, leading to its death;
- Being the root structural dynamics affected, the maintenance of root system functionality will also be affected;
- Soil compaction caused by excessive heavy machinery and excessive livestock can affect the natural regeneration of cork oaks, inhibiting tap root growth in depth, at initial stage. This fact can latter prevent the tree access to deep groundwater sources, which are important water reservoirs during dry summers in the Mediterranean region.

It is expected that the research developed in the present thesis, can provide an essential tool for the future forest planning and management and for the natural and artificial regeneration processes in cork oak stands, ensuring the maintenance of typical *Montado* landscape.

CHAPTER 7

FINAL REMARKS

As mentioned throughout this thesis and taking into account the limitations of research focusing on root systems (expensive and time consuming work, little information about methodologies and comparison of results, among others), it can be assumed that the results obtained represent a valuable contribution to the knowledge of the structure and functioning of *Montado* ecosystem.

Studies focusing on cork oak belowground systems are very scarce and usually only one section of the root system is studied. From the results obtained for that small root portion an extrapolation is usually made for the entire root system. Instead, the research described in this work presents real values allowing less errors occur in further works especially in the cork oak root dynamics modeling approach.

As the present study was developed according with a long term multidisciplinary research strategy, combining the already available scientific knowledge, it is possible to propose the following technical solutions for sustainable management on cork oak stands for Cambissoil soils:

- Given that superficial coarse roots occur at the first 8 cm depth, a bandwidth management of up to 5 cm depth should be followed to minimize the risk of structural root damage. This can be attained with the use of shrub cutter instead of disking. When soil disking is used the working depth should also be limited to 5 cm.

- To improve natural regeneration survival rates, and if the shrub layer development compromises the fire risk management, a shrub cutter should be used. This management option will not damage permanently the young cork plantlets (that can re-sprout if root system is not damaged) and is beneficial to organic matter accumulation (at surface and in depth), fertility and soil water infiltration and storage.

- Due to the livestock feeding process permanent pastures or forage cultures are often installed under tree cover. In these situations no tillage seeding techniques should be used. For natural/artificial regeneration management, grazing pressure should be adequate to soil vulnerability conditions and the new cork oak trees should be protected.

- For natural pasture management, shrub control should be done with shrub cutter, or alternatively with soil disking of up to 5 cm, combined (if necessary) with soil fertilization to enhance the herbaceous layer development. The grazing pressure should be set to the available pasture production to increase system sustainability and avoid soil degradation.

- In areas where the cork oak stand regeneration is the main short term objective, care should be taken until the plants achieve an appropriate size/height. In those areas the use of space by animals must be controlled. The optimal grazing management over large areas can be done with a good planning of grazing zones rotation, with cattle exclusion according tree regeneration process in each area.
- When the stand is in a Podzol soil where often the existence of a *surraipa* layer (spodic B horizon resulting from the Podzolization process) causes a physical barrier to root penetration due to soil compaction) is advised, before any planting, transplanting and/or seeding intervention of young oaks, to perform a deep mobilization with the riper. This procedure will avoid soil compression promoting the increase of soil porosity and will contribute to a large-scale penetration of roots in depth, ensuring a more balanced functionality of the trees along their growth and development.

As it might be expected, future advances in the knowledge of this subject should focus primarily on the different types of soils where *Montado* ecosystem occurs, through the Mediterranean basin. It is recognized that the roots behavioral pattern formation and distribution varies according the soil type where it grow, not neglecting the influence of biophysical aspects and forestry management.

The potential advance in the modeling of the dynamics of cork oak root systems through a model that could relate the shoot and root systems, including the time variable, would certainly bring new perspectives and, consequently, new lines of research in terms of structure and functionality of trees. This would reinforce the knowledge of the root systems role within the ecosystem. Future steps should also focus on the scale level of work, trying to extrapolate the results to the stand level. This would allow a better understanding of the influence and role of these underground systems in landscape dynamics, especially on the water and carbon balances.

In an area where the available information is so scarce, it would be rewarding if this thesis could serve as a basis or reference point for future advances in the integrated study about the multifunctionality of this ecosystem.