INSTITUTO SUPERIOR DE AGRONOMIA
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MANAGEMENT PRACTICES AND SOIL QUALITY PATTERNS IN EVERGREEN OAK WOODLANDS (*MONTADO*)

Ana Raquel Martinho da Silva Felizardo Rodrigues

SCIENTIFIC ADVISORS:
Doctor Manuel Armando Valeriano Madeira
Doctor Maria Manuela Costa Neves Figueiredo
Doctor David Paulo Fangueiro

Thesis presented to obtain the Doctor Degree in
Forestry Engineering and Natural Resources

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ULISBOA UNIVERSIDADE DE LISBOA

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ABSTRACT

The sustainability of evergreen oak woodlands (*montado*) in Portugal is currently threatened in large areas, mainly due to limited tree recruitment constrains and soil degradation. It is therefore urgent to develop sustainable management options which enhance *montado* productivity, ensuring their long-term viability and all ecosystem services. The present study aimed specifically to evaluate trends in soil quality changes, associated with management practices currently followed in *montado*. Different *montado* areas, corresponding to different soil types and land use histories, with different management options, including sowed and natural pasture systems, and different stocking rates and grazer species, were considered; also, the specific influence of the tree cover on the soil characteristics was investigated. The soil quality of study areas was assessed by evaluating physical, chemical and biochemical soil properties. Carbon and the main nutrient fluxes were also evaluated, assessing GHG emissions and nutrient leaching. Results enabled the assessment of the benefits associated with the establishment of improved pastures, namely in soil hydrological conditions, nutrient availability and soil organic matter status, which was particularly enhanced in areas under the tree cover influence. Nevertheless, factors associated with the soil type (texture) and livestock management (stocking rate) might have strong influence on the extent and nature of such benefits. Pasture management influence over soil carbon and nutrient fluxes were found negligible, despite disturbance may occur. Scattered trees, in the *montado*, promote the creation of islands of improved physical conditions and soil fertility, where the organic carbon accumulation is strongly enhanced. Trees undoubtedly improve soil quality, enhance the soil resistance to face degradation threats, and contribute to carbon sequestration. This potential should be taken into account for policy and management decisions, at both local and regional scales.

Keywords: nitrogen; organic C; pasture systems; *Quercus* sp.; soil fertility.

RESUMO

A sustentabilidade dos montados está sob ameaça em vastas áreas do País, principalmente devido a restrições na regeneração das árvores e a condições de degradação do solo. Torna-se, assim, urgente desenvolver opções de gestão sustentáveis, que permitam reforçar a produtividade, garantir a viabilidade a longo prazo e assegurar os serviços do ecossistema do montado. O presente estudo foi desenvolvido com o objectivo de identificar os padrões de alteração da qualidade dos solos, decorrentes de práticas de gestão do montado actualmente em uso em Portugal. Foram consideradas diferentes áreas, com diferentes tipos de solo, historiais de uso e opções de gestão, incluíndo pastagens semeadas e naturais, diferentes animais e intensidades de pastoreio; investigou-se ainda a influência específica do coberto arbóreo. A qualidade dos solos foi avaliada através da determinação de propriedades físicas, químicas e bioquímicas. Fluxos de carbono e nutrientes foram também avaliados, através das emissões de GHG e dos nutrientes lixiviados. Os resultados permitiram observar benefícios associados à instalação de pastagens melhoradas no que respeita a caracterisiticas hidrológicas, à disponibilidade de nutrientes e ao teor de matéria orgânica do solo, que foram particularmente acrescidos nas áreas sob a influência das árvores. Porém, factores associados ao tipo de solo (textura) e à gestão do pastoreio (carga animal) influenciaram fortemente a extensão e natureza desses efeitos. Os efeitos da gestão da pastagem sob os fluxos de carbono e nutrientes do solo foram insignificantes, embora perturbações possam ocorrer. As árvores conduzem à criação de zonas onde as condições físicas e a fertilidade do solo são fortemente melhoradas, relativamente àquelas fora da sua influência. As árvores contribuem, assim, para melhorar a qualidade do solo, aumentar a resistência aos riscos de degradação e reforçar o sequestro de carbono. Tal potencial deve ser considerado nas decisões políticas e de gestão, tanto a nível local como regional.

Palavras-chave: azoto; carbono orgânico; fertilidade do solo; pastagens; *Quercus* sp.

RESUMO ALARGADO

As áreas de montado são parte importante do património social, económico e ambiental em Portugal. O montado é o sistema agroflorestal dominante na Península Ibérica (e o maior *cluster* agroflorestal da União europeia), ocupando mais de 3 milhões de hectares. Este sistema consiste na combinação de baixa densidade de árvores, tipicamente o sobreiro (*Quercus suber* L.) e a azinheira (*Q. ilex* L.), com culturas agrícolas, pastagens permanentes ou espécies arbustivas no sob coberto. Porém, a sustentabilidade destes sistemas multifuncionais encontra-se ameaçada, mormente no que respeita à degradação do solo e às dificuldades em assegurar uma eficiente regeneração do coberto arbóreo. Estas ameaças advêm principalmente de um longo historial de modificações do sistema de uso e poderão ser consideravelmente agravadas pelas alterações climáticas, previstas e já patentes na região Mediterrânica. Neste contexto, os modelos de gestão constituem ferramentas importantes para a prevenção e reversão dos eventuais processos de degradação dos sistemas de montado.

O principal objectivo do presente estudo consistiu em avaliar os actuais sistemas de gestão do montado quanto à qualidade do solo, bem como a sua potencial influência sobre os serviços mais relevantes dos respectivos ecossistemas. Pretende-se contribuir para uma melhor compreensão das alterações das funções destes solos, face às recentes modificações de gestão, o que permitirá estabelecer algumas bases de recomendação visando a sustentabilidade futura do sistema montado. Considerando que todos os factores associados à gestão, assim como as características inerentes ao ambiente físico, contribuem para a resposta do sistema às mudanças de gestão, foram desenvolvidos quatro estudos, incluíndo cinco áreas de montado representativas em Portugal.

No primeiro estudo, na *Herdade da Machoqueira do Grou*, compararam-se indicadores da qualidade do solo num montado de sobro com elevada densidade (mais de 100 árvores por hectare), com sistemas de gestão do subcoberto

contrastantes: o primeiro com coberto arbustivo e sem pastoreio e o segundo com pastoreio extensivo de bovinos (0,1 vacas ha^{-1} ano^{-1}), onde havia sido semeada uma pastagem melhorada (com elevada proporção de leguminosas) há cinco anos. Os resultados mostraram o potencial destas pastagens na melhoria da fertilidade do solo, com aumento da disponibilidade de azoto (N) e fósforo (P), mas não confirmaram o seu efeitoem potenciar o sequestro de carbono (C) no solo, a curto prazo. Esta diferença associa-se aos efeitos da remoção das espécies arbustivas, aquando da instalação da pastagem e da subsequente gestão do pastoreio. Concluiu-se que o coberto arbustivo salvaguarda o C orgânico do solo e potencia o renovo da cobertura arbórea, o mesmo não acontecendo com a gerstão associada à pastagem melhorada.

O segundo estudo visou a comparação dos efeitos, a longo prazo, da gestão de montados com pastagens melhoradas e naturais, sobre as características físicas, químicas e bioquímicas do solo. Consideraram-se duas áreas com solos de diferente classe textural e zonas sob e fora da influência das copas das árvores. Na *Herdade dos Esquerdos*, com solos de textura franco-arenosa, estudou-se uma pastagem melhorada com cerca de 35 anos, pastoreada por ovelhas (5 a 8 animais ha^{-1} ano^{-1}; 0,5 a 0,8 LU ha^{-1} ano^{-1}), comparada com uma pastagem natural adjacente, sujeita a pastoreio extensivo (< 1 ovelha ha^{-1} ano^{-1}) e apresentando algum grau de cobertura por espécies arbustivas. Na *Herdade do Olival*, onde os solos apresentam textura franca a franco-limosa, consideraram-se igualmente uma pastagem melhorada com 16 anos e uma pastagem natural, ambas usadas para pastoreio de gado bovino ao longo de todo o ano, com uma carga animal de cerca de 0,7 vacas por hectare (0.6 LU ha^{-1} ano^{-1}). Os resultados revelaram o efeito positivo das pastagens semeadas, relativamente às naturais, sobre o teor de C orgânico e a fertilidade do solo, sendo os efeitos ampliados nas áreas sob a influência das árvores. Porém, foram evidenciados os efeitos negativos da elevada carga animal sobre as características físicas do solo, particularmente no caso de textura franca a franco-limosa. Decorrendo do intenso pisoteio animal, o estado de compactação do solo determinou a redução da porosidade e da condutividade hidráulica, possíveis constrangimentos à penetração das raízes e ao arejamento, sem que se verificasse acréscimo da água útil do solo. Em tal caso, a instalação de pastagem melhorada não foi suficiente para contrariar os efeitos negativos da intensificação do pastoreio.

Em duas áreas de montado com historial de gestão contrastante e em que ocorrem solos com diferentes características -*Tapada Real de Vila Viçosa* e *Herdade da Mitra* - desenvolveu-se um terceiro estudo com o intuito de tipificar a variação espacial das características do solo sob a influência de árvores isoladas, desenvolvendo ainda um sistema de quantificação do contributo de cada elemento arbóreo para a acumulação de C orgânico no solo até 20 cm de profundidade. Amostraram-se os solos em torno de árvores representativas, considerando diferentes distâncias desde o tronco da árvore e relativas à projecção vertical da copa, desde 0,33 e até 2,0 vezes o raio (R) desta. Em ambos os locais, os resultados confirmaram o efeito positivo das árvores na acumulação de matéria orgânica e de nutrientes no solo numa área considerável que vai além da projecção vertical da copa. A variação da acumulação de carbono orgânico no solo, em função da distância ao tronco da árvore, foi explicada por um modelo exponencial negativo, para qualquer distância (r) dada por $2R \geq r > 0$. Quantificada a contribuição da árvore isolada para a acumulação de C orgânico no solo, concluiu-se que a sua influência ao nível da paisagem pode atingir cerca de 3 kg C m^{-2}, considerando uma média de 50 árvores por hectare.

Um estudo sobre o efeito do sistema de gestão da pastagem nos fluxos de C e de nutrientes do solo, foi desenvolvido num sistema lisimétrico em que se utilizaram blocos não perturbados de solo, provenientes da *Herdade dos Esquerdos* e da *Herdade do Olival*. Na primeira foram consideradas duas pastagens melhoradas, uma com 37 anos e outra da mesma idade, mas que fora recentemente resemeada (sementeira directa), bem como uma pastagem natural. Na segunda consideraram-se solos de uma pastagem melhorada (18 anos) e outra natural, e ainda solos de uma área com coberto arbustivo espontâneo, onde o pastoreio é ocasional. Em qualquer dos casos, apenas se estudaram as áreas fora da influência das árvores. Durante 15 meses, foram periodicamente determinados os fluxos dos principais gases de estufa - metano (CH_4), óxido nitroso (N_2O) e dióxido de carbono (CO_2) -, sendo também consideradas as perdas de C orgânico e de nutrientes do solo por lixiviação (C orgânico dissolvido, N-NO_3^-, N-NH_4^+, N total, P, K, Ca, Mg e Na), bem como a extracção dos principais nutrientes (N, P, K, Ca, Mg e Mn) pela biomassa herbácea. Os resultados foram maioritariamente influenciados pelas condições de humidade e temperatura, mas também pela textura e porosidade do solo. Os solos de textura média apresentaram maiores fluxos e emissões acumuladas de CH_4, mas não significativamente diferentes dos de textura mais grosseira. Este efeito resultou em maiores transferências de C para a atmosfera, por unidade inicial de C orgânico no solo, comparativamente às estimadas para os solos de textura mais grosseira. As pastagens melhoradas sem perturbação mostraram emissões acumuladas de CO_2 mais elevadas, associadas aos teores mais elevados de matéria orgânica do solo. Para além deste efeito, observaram-se ainda picos de emissão de N_2O com as primeiras chuvas, na pastagem melhorada mais antiga, que resultaram num maior valor potencial de aquecimento global (GWP). As mais elevadas quantidades de P lixiviado ocorreram nos solos de pastagem melhorada, realçando a necessidade de rever as práticas de aplicação contínua de fertilizantes fosfatados nestas áreas. A renovação da pastagem melhorada teve como consequências o aumento da produtividade das herbáceas, da actividade microbiana do solo, e também da lixiviação de C orgânico e NO_3^-, o que terá resultou numa redução das perdas gasosoas de N_2O e CO_2, promovendo assim o reequilíbrio dos fluxos de N e C do solo.

As pastagens melhoradas surgem como alternativa viável para o aumento da produtividade, com efeitos benéficos também sobre os teores de matéria orgânica e a fertilidade dos solos. Contudo, este potencial poderá ser grandemente modificado por outras variáveis, nomeadamente o tipo de solo e a gestão do pastoreio. Torna-se também evidente o grande contributo das árvores para a melhoria das funções do solo, sendo que o seu potencial para sequestrar C ao nível da paisagem poderá exceder em muito o das práticas actualmente subsidiadas. Uma gestão sustentável dos sistemas de montado deverá ser sempre adequada às condições locais, havendo necessidade de políticas que promovam a acumulação de C, previnam a degradação do solo e garantam a regeneração do coberto arbóreo. Para efeitos de monitorização, o teor de C orgânico e a massa volúmica aparente destinguem-se como os mais robustos indicadores de alterações na qualidade dos solos de montado, associadas a mudanças na gestão. Tendo em conta os longos tempos de resposta dos parâmetros de qualidade do solo face às modificações de gestão, estudos de longo prazo serão fundamentais para clarificar estes padrões e fundamentar decisões futuras. O estabelecimento de parcelas de controlo e a avaliação da rentabilidade económica associada às diferentes opções de gestão serão fundamentais para uma visão mais detalhada sobre o ponto de vista da sustentabilidade dos sistemas de montado.

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INTRODUCTION

INTRODUCTION

Montado, a valuable agroforestry system

Agroforestry systems, which combine agriculture and forest productions in the same land unit, are receiving a renewed interest for their socioeconomic value and potential environmental services (Alavalapati et al., 2004; Rigueiro-Rodríguez et al., 2009). Indeed, these systems have been suggested among the most promising options for carbon (C) sequestration on agricultural lands (Kumar and Nair, 2011; Lorenz and Lal, 2014), and can help landowners and society to address many other issues on rural areas, such as economic diversification (Campos et al., 2001), biodiversity and water quality (Schoeneberger, 2009; Torralba et al., 2016), and soil erosion control (Cardinael et al., 2015).

Evergreen oak woodlands, called *montado* in Portugal and *dehesa* in Spain, are ancient agroforestry systems, traditional of the Iberian Peninsula, that are formed by the combination of scattered oak trees - mainly *Quercus suber* L. and *Q. rotundifolia* Lam. - with variable understorey land use systems, such as agricultural crops, pastures and/or shrubs (Joffre et al., 1999; Moreno and Pulido, 2009; Pinto-Correia et al., 2013). Their extend has been estimated to be above 3 million hectares (Eichhorn et al., 2006), coinciding with one of the largest European agroforestry cluster areas (den Herder et al., 2017).

In Portugal, according to the last forest inventories, cork and holm oak trees are mainly found in low tree density stands that account for approximately 1.07 million hectares, which is about 12% of the country continental area and represents about 34% of the national forest area (ICNF, 2013; Pinto-Correia et al., 2013).

The *montado* values have been widely recognized, not only for the combination of agriculture and forest productions, but also for their vital environmental functions, and crucial social and cultural services (Campos et al., 2001; Moreno and Pulido, 2009). Although they have been acknowledged as important habitats requiring conservation, by the NATURA 2000 network (Habitats Directive, 1992), large *montado* areas are currently facing multiple sustainability threats, mainly due to soil degradation (and desertification) and lack of tree natural regeneration (Belo et al., 2014; Campos et al., 2008).

Current threats to *montado* sustainability

Current and foreseen climate changes in the Mediterranean region, more precisely higher temperature and increasing drought frequency (IPCC, 2014a), may strongly increase the *montado* vulnerability. A decrease of productivity (Costa et al., 2016; Jongen et al., 2011), enhancement of tree decline (Duque-Lazo et al., 2018), soil organic matter losses (Lozano-García et al., 2017), nutrient cycling modifications (Delgado-Baquerizo et al., 2014), and changes in the system functioning (e.g. Caldeira et al., 2015; Correia et al., 2012; Costa-e-Silva et al., 2015) are the main issues reported in recent studies. Also, most *montado* areas occur over degraded soils, presenting low fertility and low organic matter status (Pulido-Fernández et al., 2013; Ruiz-Sinoga et al., 2012; Rodeghiero et al., 2011), which limit both productivity and soil resistance to degradation. Additionally, large areas present generalized tree decline (Costa et al., 2009; Kim et al., 2017) and absence of tree recruitment (Plieninger et al., 2003; Pulido and Díaz, 2005), which seriously compromises the *montado* long-term viability.

Throughout their long existence, *montados* have been shaped by human activities, and social and economic factors have driven most of their management history (Bugalho et al., 2011; Joffre et al., 1999). Although several management patterns are being linked to the current state of threatened sustainability, the key for *montado* future viability may also be based on proper management practices (Costa et al., 2014; Moreno and Pulido, 2009).

In Portugal, until the first half of the XX century, *montado* areas were mostly used for cereal crops rotations, with extensive grazing by pig and sheep herds during fallow. A period of selective intensification and abandonment, according to the site productivity and mechanization potential, has followed, mainly as a consequence of agriculture technological developments (e.g. mechanization, chemical fertilization), and the EU common agricultural policies applications (e.g. set aside, synergetic areas) (Belo et al., 2014).

Currently, *montado* management is mostly driven by subsidy policies, as stake holders are often constrained to choose between system intensification or its abandonment (Belo et al., 2014; Plieninger and Wilbrand, 2001). For instance, grazing intensification, and cattle production in particular, became an appealing option (IFAP, 2016). In fact, a considerable increase of the permanent pasture area and livestock units occurred in the last decades (GPP, 2018; INE, 2018, 2016). However, the increasing grazing pressure may aggravate tree regeneration constrains (López-Sánchez et al., 2014) and soil degradation risks (Eldridge et al., 2017; Ordóñez et al., 2018; Pulido et al., 2018). Hence, a deeper understanding on the interactions between grazers and the complexity of the *montado* system is needed since only limited studies attend such issues.

Sowing selected mixtures of herbaceous species with high legume proportions (called improved pastures), has been considered a crucial strategy for sustainable livestock production (Lüscher et al., 2014). In Portugal, improved pasture sowing in *montado* areas is being recommended, aiming to address both the increasing livestock nutritional requirements, and the reversing of soil degradation patterns (Belo et al., 2014; Crespo, 2006). The sowed pasture area showed a notorious increase in the last decades (INE, 2016), which was associated with the Portuguese Carbon Fund financial support for improved pasture establishment, accounting for their potential for carbon sequestration enhancement (APA, 2017; Terraprima, 2018a). Although improved pasture establishment and management has been found to boost productivity (Aguiar et al., 2011; Franca et al., Hernández-Estebán et al., 2018;), increase atmospheric N_2 fixation (Carranca et al., 2015), and enhance soil organic C accumulation and upgrade the soil nutrient status and nitrogen availability (Gómez-Rey et al., 2012; Hernández-Estebán et al., 2018; Rodrigues et al., 2015), their long-term effects on the *montado* system functioning and sustainability is still poorly understood.

Some Portuguese *montados* areas are managed with emphasis on the tree productivity, mostly where the main goal is the production of cork of high quality (Costa and Oliveira, 2015). In such a case, if grazing intensity is low or even absent, the natural understorey vegetation tends to be dominated by naturally occurring shrubs in a similar way to abandoned *montado* areas that are often invaded. Since shrub encroachment may reduce nutrient and water availability (Caldeira et al., 2015), and excessive biomass accumulation can increase the wild fire risk, the Portuguese government guidelines indicate that the understorey vegetation in cork oak stands should not exceed one-meter height (IFAP, 2017). Although a financial incentive is provided by the Portuguese government, for practices that ensure minimum soil disturbance (Terraprima, 2018b), the most common shrub growth control is practiced by mechanical soil perturbation (typically by harrowing).

Several recent studies have linked the shrub cover to important *montado* ecosystem services, such as biodiversity conservation (Moreno and Pulido, 2009; Tárrega et al., 2009), carbon sequestration (Correia et al., 2014), soil quality enhancement (Gómez-Rey et al., 2013; Moreno and Obrador, 2007; Simões et al., 2009) and tree recruitment facilitation (Dias et al., 2016; Simões et al., 2016). In this context, the effects of shrub control, following land use intensification (like improved pasture installation or grazing intensification), is a topic deserving further investigation, regarding *montado* ecosystem functions and sustainability.

***Montado* ecossystem services**

As for other agroforestry systems (e.g. Baah-Acheamfour et al., 2014; Belsky et al., 1989; Galicia and García-Oliva, 2008; Harvey et al., 2011; Wilson and Lemon, 2004), many *montado* valuable services are acknowledged as oak tree-related, either by their direct productions (e.g. cork, acorns, firewood) or their indirect benefits for the system functioning (Dahlgren et al., 2003; Gallardo, 2003; Moreno and Obrador, 2007). In savannah-like ecosystems, scattered trees act as key ecosystem components, as they capture, distribute and facilitate cycling of nutrients and water (Rhoades, 1997). As a result, beneath the trees it is common to observe improved soil fertility (Gallardo, 2003), organic matter accumulation (Simón et al., 2013), physical conditions improvement (Dubbert et al., 2014) and pasture composition quality (Cubera et al., 2009); however, the pasture productivity may be strongly variable (Cubera et al., 2009; Dubbert et al., 2014; López-Carrasco et al., 2015).

Notwithstanding, tree presence in the *montado* is being mostly overlooked by current management practices and policies, even though there are clear evidences of a generalized high tree and stand age (Plieninger et al., 2003), lack of natural regeneration (Heydari et al., 2017; Kim et al., 2017) and alarming numbers of tree decline (Costa et al., 2009; Duque-Lazo et al., 2018). Hence, to ensure oak woodlands future viability, information on the tree contribution for ecosystem functions and related services must be clarified, in order to identify adequate protective measures at both local and landscape levels.

Increasing anthropogenic greenhouse gases (GHG) emissions to the atmosphere - mainly carbon dioxide (CO_2), methane (CH_4) and nitrous oxide (N_2O) - have been recognized as the main drivers of global climate changes, so efforts are being made globally to counterbalance and overcome their effects (IPCC, 2014b). Soil is not only the largest terrestrial sink of carbon and nitrogen (1500 Pg of C and 136 Pg of N, in the upper 1 m; Batjes, 1996), but is also responsible for maintaining and regulating all biogeochemical cycles. Therefore, soil is both a vulnerable and potentially valuable component, regarding climate change mitigation and adaptation strategies. For that reason, much attention is being given to the soil potential to sequester C (e.g. Lal et al., 2015; Minasny et al., 2017), for which silvopastural systems were identified as one of the most promising land management option (Kim et al., 2016).

It should be emphasized, that the potential to sequester C in soil by management or land use changes, is mostly associated with soil organic C accumulation, that is soil organic matter. Given the complex and dynamic nature of the soil organic matter (Janzen, 2006; Lehmann and Kleber, 2015), the effects of soil C sequestration may not be strictly evaluated as a simple transfer of C from the atmosphere to the soil. Indeed, several constrains should be taken into account when evaluating the potential of a specific management practice for soil C sequestration enhancement: first, the amount of C that soil can store is finite; second, the process can be rapidly reversed with minor changes in current conditions; and third, when soil C sequestration is intended to mitigate GHG emissions, all relevant gases species must be accounted, to ensure an effective global warming potential (GWP) decrease (Powlson et al., 2011). In this sense, it is now commonly accepted that studying fluxes, instead of stocks, is a more informative and reproducible approach (Bispo et al., 2017; Lehmann and Kleber, 2015).

While scarce information is available in Mediterranean ecosystem for soil C and N fluxes (Castaldi et al., 2006; Luyssaert et al., 2007; Oertel et al., 2016; Schulze et al., 2009), available estimates for the *montado* system indicate that their annual C intake may reach values close to those found in central Europe forests, but the wide variability of results appears associated with stand structure, climatic events, management history and current practices (Pinto-Correia et al., 2013). Most studies related to GHG emissions in *montado* have focussed on C emissions (e.g. Correia et al., 2012), namely CO_2 and CH_4 but few or no information is available regarding nitrous oxide fluxes (Shvaleva et al., 2015), despite its significantly higher GWP, relative to CH_4 (IPCC, 2014c).

Some management practices of *montado* imply the application of mineral fertilizers to soil, namely P fertilizers. A consequence is the increase of soil P status and potential risk of P runoff or leaching with potential impact on water quality. Such issue is relevant in Alentejo since water used for irrigation is mainly captured in small ponds filled with water from montado area. Furthermore, it is crucial to assess that management practices efficient to increase C sequestration in soil or with any positive impact on montado do not lead to other nutrient losses as nitrate leaching.

Monitoring soil quality and assessing *montado* sustainability

Given the threats which mine its sustainability, the *montado* future is undoubtedly entangled in the functions of its soils. Thus, monitoring soil disturbance, along management or environmental changes, becomes crucial to efficiently predict and reverse possible degradation patterns. By measuring relevant physical, chemical and biochemical properties, soil processes can be assessed and interpreted, enabling to infer a perception of soil quality. As defined by Doran and Safley (1997), soil quality is its ability to function within the ecosystems and land use limits, while sustaining biological productivity, promoting air and water quality and maintaining plant, animal and human health. Indeed, this concept is imbedded by the observer interests, which brings permanent discussion into the choice of the best set of soil parameters to reflect a particular ecosystem service (e.g. Askari and Holden, 2015; Knoepp et al., 2000; Sánchez-Navarro et al., 2015). In this context, identifying simple, reproducible and efficient soil quality indicators for *montados*, would constitute a valuable monitorization tool, in the pursue for its sustainable management.

In this context, it was hypothesized that recently introduced changes (e.g. improved pasture establishment, grazing intensification, shrub removal or tree decline, among others) may interact with all *montado* components, including soil quality status. Additionally, the extend and direction of these interactions are dependent on all other site-specific and management factors.

The overall objective of the present thesis was to evaluate the effects of some current management practices on relevant soil functions and assess whether and how they can influence *montado* expected services. Specifically, it was aimed to: i) obtain a better understanding on changes of *montado* soil functions associated with management; and 2) provide information to base proper management options regarding its major economic and environmental services achievement. Additionally, results will be useful to establish guidelines for monitoring soil quality in *montados*, at both land unit and policy-maker levels, as a tool to assure their sustainable management.

Four studies were developed in five evergreen oak woodland farms, representative of the main Portuguese *montado* areas and current management systems:

i) A first study is presented in Chapter 1 entitled “Do improved pastures enhance soil quality of cork oak woodlands in the Alentejo region (Portugal)?” At the *Herdade da Machoqueira do Grou*, soil physical, chemical and biochemical indicators were determined under a 5-year old improved pasture and an adjacent natural understory, both under a dense cork oak woodland;

ii) The Chapter 2 deals with a second study entitled “How are current management systems affecting soil quality in evergreen oak woodlands (*montados*)?”

    An assessment of soil physical, chemical and biochemical properties was conducted at two farms (*Herdade dos Esquerdos* and *Herdade do Olival*), with different soil texture and grazing management, under improved and natural pastures, and considering tree-covered and open areas;

iii) A third study assessing the "Spatial variation of soil characteristics and soil carbon stock as affected by single trees in evergreen oak woodlands (*montados*)" is discussed on Chapter 3.

A sampling design was established to account for soil properties spatial variation around cork and holm oak scattered trees, at *Herdade da Mitra* and *Tapada Real de Vila Viçosa* farms, special attention being given to the tree contribution to soil organic carbon accumulation.

iv) Finally, in Chapter 4, the "Influence of pasture management on nutrient fluxes in evergreen oak woodland (*montado)* soils" is presented.

A lysimetric experiment was assembled with open grassland undisturbed soil blocks, from two study sites presenting different soil texture (*Herdade dos Esquerdos* and *Herdade do Olival*), considering three pasture management systems in each (natural, improved and occasionally grazed). Data collection included initial soil properties, herbaceous biomass production and nutrient content, and greenhouse gases emissions and soil leachates along a 15-month period.

Each of the current Thesis chapters, corresponding to each of the studies developed, are presented in article publication format, the first being already published in the journal *Agroforestry Systems*.

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CHAPTER 1

Do improved pastures enhance soil quality of cork oak woodlands in the Alentejo region (Portugal)?

Do improved pastures enhance soil quality of cork oak woodlands in the Alentejo region (Portugal)?

ABSTRACT

Portuguese forest sustainability is currently threatened by forecasted climate changes and inappropriate management practices. Specifically, large cork oak woodland areas (*montados*) are subjected to soil degradation and tree recruitment impeachment. A study was developed to compare soil properties in cork oak woodlands with improved pastures (IP) grazed by cattle and natural understorey management (NU) without grazing. The IP system did not lead to soil organic C concentration increase, soil organic C stock being 0.7 kg m^{-2} lower in the upper 30 cm soil layer, compared to the NU system. Under the IP management, soil N content was 39.7 g m^{-2} higher up to 30 cm depth, and N mineralization potential was increased by 50% in the 10 cm top soil layer. Soil bulk density and C mineralization potential were similar in both systems. Sowing legume-rich pastures can result in an immediate soil quality improvement, especially regarding N availability, although grazing may hamper tree recruitment. Managing the natural understorey appears suitable for soil organic C maintenance, and also allows tree recruitment, while soil N availability limitation could be overcome by fertilizer applications.

Keywords: bulk density; nitrogen; management system; organic carbon; soil fertility.

INTRODUCTION

Evergreen oak woodlands (*montado* in Portugal, *dehesa* in Spain) are the most widely spread agroforestry systems in the Iberian Peninsula, occupying more than 3 million hectares (Eichhorn et al. 2006). Oak trees (mainly *Quercus ilex* and *Q. suber* L.) are intercropped with agriculture, pastures or natural shrubs, forming complex and highly variable landscapes. Cork oak woodlands are especially important in Portugal, for their role on the supply of raw material for the cork industry. However, their sustainability is now being questioned, particularly owing to soil degradation, productivity decline and lack of tree natural regeneration (Bugalho et al. 2011; Costa et al. 2014). In this context, the future of *montado* is dependent on management decisions that promote soil restoration and tree recruitment, thus ensuring the system long-term viability (Escribano et al. 2018).

Recent *montado* history is marked by land use intensification, as landowners have followed EU subsidies in an attempt to increase the system profitability (Belo et al. 2014). In the last decades, some areas were converted to permanent pastures and grazing pressure was increased by replacement of traditional pig and sheep herds for cattle (GPP 2018). Sowing improved pastures - selected mixtures of legume and grass species - has become an interesting alternative for enhancing pasture yield and improving soil functions in oak woodlands (Carranca et al., 2015; Gómez-Rey et al., 2012; Hernández-Esteban et al., 2018). Their potential for soil organic carbon (C) accumulation has been reported (Teixeira et al. 2015), and since 2009 the Portuguese Carbon Fund granted financial support for this management option, and therefore an increase in national sown pasture area occurred (APA 2017). Nevertheless, information regarding the effect of improved pastures establishment on *montado* agroecosystems is scarce (Gómez-Rey et al. 2012; Hernandéz-Esteban et al. 2018; Rodrigues et al. 2015).

Grazing can enhance soil nutrient cycling and accumulation (Bilotta et al. 2007), but excessive animal trampling can also damage soil structure by compaction. Also, animal excessive feeding might limit the growth of trees and shrubs, which may seriously threaten tree recruitment and drive losses of soil fertility (Dahlgren et al. 1997; López-Sánchez et al. 2014).

Montado areas are often colonized by naturally occurring shrub species, which can enhance standing biomass (Correia et al. 2014), increase soil organic C sequestration and fertility (Gómez-Rey et al. 2013), while soil N changes may depend on shrub species (Moreno and Obrador 2007; Simões et al. 2009). Also, shrub cover has been associated with successful tree recruitment (Dias et al. 2016; Simões et al. 2016), while warranting feed diversity for grazers and increasing natural biodiversity conservation potential (Moreno and Pulido 2009). As the excessive accumulation of shrub biomass may increase fire risks and compete with trees for water and nutrients (Caldeira et al. 2015), periodical shrub cutting is recommended, and practices ensuring minimum soil disturbance are financially supported by the Portuguese government (APA 2017).

In the light of global climate change scenarios forecasted for the Mediterranean region (IPCC 2015), management systems that ensure *montado* resilience and long-term sustainability should be developed. Such systems must improve soil functions, which can be assessed by measuring physical, chemical and biochemical soil properties - the so called soil quality indicators (Pulido et al. 2017). Understanding how different management systems are affecting soil properties of cork oak woodlands is essential to ensure permanent tree recruitment and cork productivity, that is, to address their long-term sustainability.

In this context, soil physical, chemical and biological properties were evaluated, regarding accumulation and mineralization of soil organic C and N, and soil fertility development. For that, two representative cork oak woodland areas were examined, one with natural understorey and absence of grazing and another with an improved pasture grazed by cattle. The authors hypothesized that improved pasture management would result in soil organic C build-up and higher soil nutrient availability. Therefore, results will provide useful information for land managers and policy-makers endeavouring proper management systems aiming the sustainability of cork oak woodlands.

MATERIALS AND METHODS

Study area

The study was conducted at *Herdade da Machoqueira do Grou*, Coruche county, Southern Portugal (39°08'18.29''N, 8°19'57.68''W), in a pure cork oak (*Quercus suber* L.) stand representative of the largest cork oak woodland area in Portugal. The climate is Mediterranean, with hot and dry summers and mild wet winters. Mean annual rainfall (1980-2002) is 685 mm, and mean annual air temperature (1960-1989) is 15.2 °C (SNIRH 2017). Landscape is made of Pliocenic and Mio-Pliocenic formations (Zbyszewski 1953), topography being mostly gently undulating (slope gradient: 6-8%; SROA 1965). Soils are developed on sandstones, and classified as Dystric Arenosols associated with Dystric Regosols (IUSS Working Group WRB 2015); they are coarse textured (clay less than 60 g kg^{-1}), strongly to moderately acidic, with low nutrient status.

The pure cork oak stand was installed in 1965, with approximate density of 177 trees per hectare, and canopy cover reaching 30 to 60%. In 1992, it was divided in two areas (estates) with different management regimes: one area was converted into a permanent natural pasture for extensive cattle grazing, while the other was kept ungrazed with its natural understorey vegetation, comprising an herbaceous layer and shrubs, mainly *Cistus* sp., *Lavandula stoechas* L. and *Ulex* sp.

In August 2009, the grazed area was tilled with a disking harrow followed by mechanical spreading of 500 kg ha^{-1} of dolomitic limestone (20% MgO, 65% CaO) and 500 kg ha^{-1} of phosphate fertilizer (18% P_2O_5, 10% CaO, 27% SO_3). A pasture mixture (IP; *Trifolium* spp., *Ornithopus* spp. and *Biserrula pelecinus* L.) was then broadcast seeded, and a roller was used to level soil surface and warrant seed cover. The area has been ever since grazed by about 1.4 cows per hectare for one month every year (0.1 LU ha^{-1} $year^{-1}$), at the end of spring, which ensures the control of natural shrub species.

In the natural understorey vegetation area (NU), management is exclusively oriented for cork production, and no fertilizers were applied. The shrub growth is controlled with a rotary mower every 4 to 6 years, the last control being carried out in February 2014. Seedling protection is ensured by adjusting the cutting height to its maximum distance to the soil surface, and sapling damage is prevented by postponing the control of shrub patches where they occur.

Vegetation measurements

Standing herbaceous vegetation biomass and mass soil floor litter layer have been evaluated since 2009 in late spring (end of May). A total of 14 points, seven in each experimental area, were marked along four south-north transects (spaced 50 m). Two 0.4×0.4 m samples were randomly taken around each point. Samples of herbaceous vegetation and soil surface-litter were dried (24 hours at 65 °C) and weighed. The three main botanical groups in herbaceous biomass - grasses, legumes and forbs - were treated separately. In 2011, biomass and coverage of the main shrub species (*Cistus salviifolius* L., *C. crispus* L. and *Ulex airensis* Esp. Santo, Cubas, Lousã, C. Pardo & J. C. Costa) were quantified in the NU area. Aboveground biomass of shrubs was collected inside four 30 m^2 randomly selected plots, samples being oven dried and weighed (Correia et al. 2014). Tree litter fall (leaves, branches, flowers and fruits) was collected monthly, from 2011 to 2016, in 16 littertraps (0.5 m^2) distributed in two transects in the NU experimental site. Given the similar tree density, age and dendrometric traits at NU and IP, estimates were considered representative of both study systems.

Soil sampling

Sampling took place between 2014 and 2017 in IP and NU adjacent areas. The two areas were comparable in terms of soil fertility until 1992, as they were subjected to similar management and located on the same geology, soil type and topography. To alleviate possible pseudo replication problems (Stamps and Linit 1999), in each management system an area of 200×100 m was delimitated, 15 m from the boundary, and within it 20 sampling plots (20×20 m) were established, considering a grid of 5×5 m cells in each (Figure 1). Given the high tree density in study areas, sampling plots were randomly selected.

Five sampling plots were randomly selected in each study area for soil bulk density assessment. In each plot, three grid cells were randomly selected, and undisturbed soil samples were collected in their centre at 0-10, 10-20 and 20-30 cm depth, by carving metal cylinders (ca. 368.8 cm^3) into each soil layer. Soil cores were trimmed to the exact cylinder volume and transferred to plastic bags.

In each system, disturbed soil samples were collected for soil fertility assessment in six randomly selected plots. Samples were taken with an auger in the centre of five grid cells (randomly selected) from each sampling plot, at 0-10, 10-20 and 20-30 cm depth. A total of 30 samples were taken per each soil depth and study area. Soil was sampled up to 30 cm soil depth, in conformity with international standards for soil organic C stock calculation (FAO 2017).

For assessing soil organic C and N mineralization, six sampling plots were randomly selected per management system. In the centre of four randomly selected cell grids at each sampling plot, soil cores were taken with an auger from the top 10 cm layer; samples were combined two by two, resulting in two composite samples per plot, and a total of 12 composite samples for each study system.

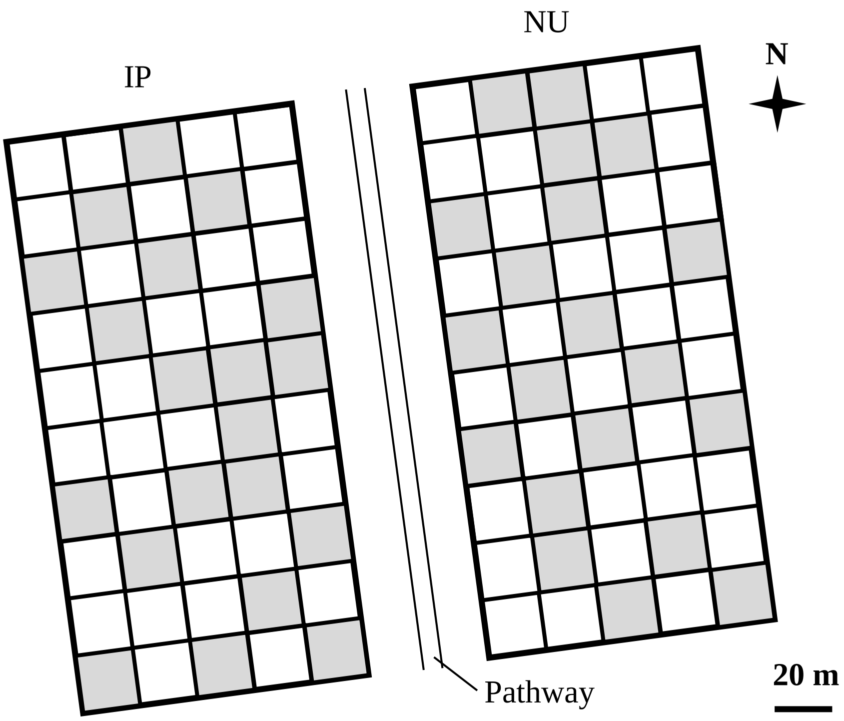

Figure 1 - Sampling plot distribution at the improved pasture (IP) and natural understory (NU) systems in *Herdade da Machoqueira do Grou*.

Laboratory procedures

Soil bulk density

Samples were oven-dried at 40 °C for a week, and then 10 g subsamples were placed overnight at 105 °C to allow dry weight calculations. Soil bulk density was determined as the ratio between sample dry weight and cylinder volume (Blake and Hartge 1986).

Soil fertility

Samples were air dried and then passed through a 2 mm sieve. Analyses were carried out on the <2 mm soil fraction. Soil pH was determined with a potentiometer (Metrohm 632) in soil suspensions in distilled water and 1 M KCl (1:2.5) after 1 h of intermittent shaking. Soil total organic C concentration was determined by the potassium dichromate oxidation following De Leenheer and Van Hove (1958). Particulate C fraction was separated by wet sieving soil samples at 53 μm, respective organic C concentration being determined as above. Total N was determined by the Kjeldahl procedure, using a Kjeltec digestion and distillation apparatus and a separated automated titration device. C and N stocks were calculated using soil bulk density and rock fragments correction (Poeplau et al. 2017). Non-acid exchangeable cations (Ca^{2+}, Mg^{2+}, Na^{+}, K^{+}) were extracted with 1 M ammonium acetate solution (pH 7 adjusted) and determined by atomic absorption spectroscopy (AAS). Exchangeable Al^{3+} was extracted with 1 M KCl solution (Barnhisel and Bertsch 1982) and determined by AAS. Extractable K and P were evaluated by the Egnér-Riehm (1958) test and determined by AAS and UV-visible spectroscopy, respectively. Hot water soluble C and N contents, as indicators of soil microorganism preferential substrates and product availability (Haynes 2000), were determined by suspending soil in distilled water (1:5) at approximately 85 °C for one hour (Khanna et al. 2001). Total dissolved organic C and N were determined in the resultant water extracts by using an automated segmented flow analyser (Houba et al. 1994).

Soil biochemical indicators

Samples were sieved and the <2 mm soil fraction was kept refrigerated (about 4 °C) in closed plastic bags to keep field moisture and restrain biologic activity. Six subsamples were used in the fumigation-extraction procedure (Vance et al. 1987): three replicates were immediately extracted with 50 mL of 0.5 M K_2SO_4 solution, while remaining three were first chloroform fumigated for 24h. Additionally, soil subsamples (50 g) were rewetted at approximately 60% of their water field capacity and placed in hermetic glass containers with 0.5 M NaOH solution. Containers were incubated in the dark at 25 °C for 120 days. Trapping solutions were changed at days 1, 2, 3, 4, 7, 15, 28, 56 and 119, and dissolved CO_2 has been precipitated with 0.5 M Ba_2Cl, the excess NaOH being titrated with

0.5 M HCl. Soil C potential mineralization was assessed by calculating the total amount of respired CO_2-C per initial soil organic C unit. Metabolic coefficient (qCO_2) was calculated as the CO_2-C respired at the seventh day of incubation per microbial biomass C unit. Approximately 1000 g soil samples (n=12) were incubated in the dark at 25 °C and 60% water field capacity inside plastic bags, for 16 weeks. Soil subsamples were taken (days 0, 7, 14, 28, 42, 56, 70, 84, 98 and 112) and extracted with a 2 M KCl solution. Extracts were used for NO_3^--N and NH_4^+-N determination in an automated segmented flow analyser (Houba et al. 1994). Net N mineralization rates were calculated as final net mineralized N per initial total N.

Statistical analyses

Differences between the two study systems (IP and NU), for the determined soil properties at each depth were assessed by Student's t-tests whenever population's normal distribution (Shapiro-Wilk test) and homogeneity of variances (Levene's test) were proven or achieved by logarithmic or arcsin transformations (only for 0-10 cm depth hot water soluble C proportion). For non-parametric variables, the Kruskal-Wallis test was used. Statistical analyses were conducted in R software (R Core Team 2014).

RESULTS

Vegetation

Standing biomass of herbaceous vegetation in the IP system was about 1.6 times higher than that in the NU (Figure 2). Legumes were the least represented botanical family for both systems, although their biomass was, on average, three times higher in the IP (0.12 Mg ha^{-1}) than in NU (0.04 Mg ha^{-1}) (data not shown). Grasses were the predominant botanical group, representing about 42 and 46% of total herbs in the IP and NU, respectively. An average of 3.9 Mg ha^{-1} year^{-1} tree litter fall was estimated. The mass of the soil surface litter was similar in both systems. The aboveground biomass of three-year old shrubs in the NU accounted for 1.59 Mg ha^{-1}.

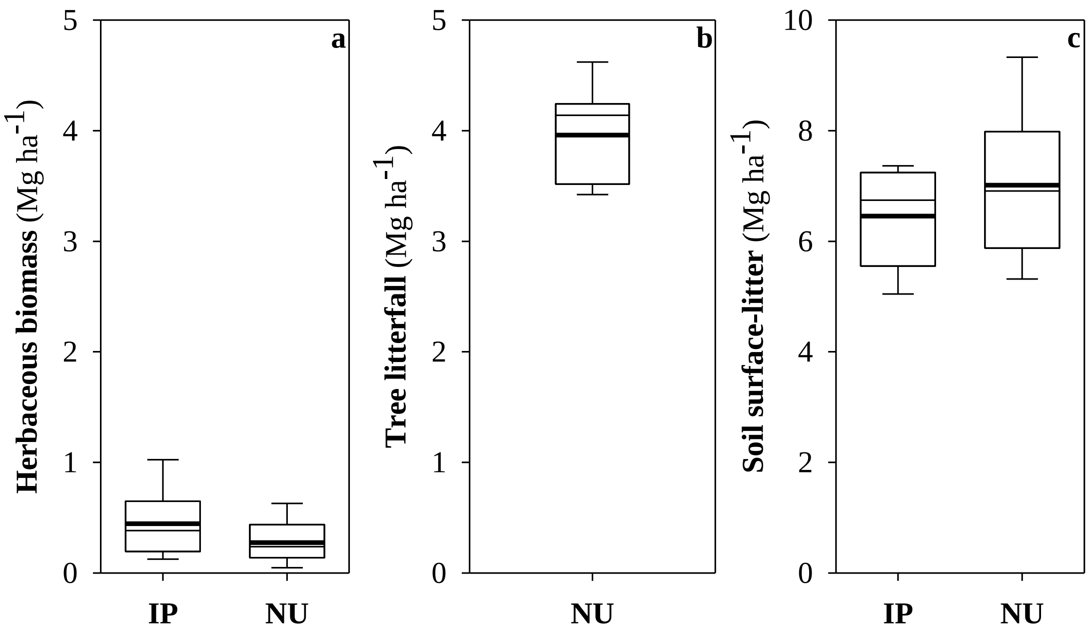

Figure 2 - Boxplot statistics for (a) herbaceous biomass and (c) soil surface-litter in late spring, during the 2009 - 2017 period (except 2010), for improved pasture (IP) and natural understorey (NU) systems; and (b) yearly tree litter fall for the 2011 - 2016 period in the NU. Mean values are represented with thick lines.

Soil bulk density, organic C and total N

Soil bulk density values did not show significant differences between the study systems at any depth (Table 1). Soil organic C concentrations and amounts did not differ between management systems up to 30 cm soil depth. Total N concentrations in the 0-10 and 10-20 cm soil layers were significantly higher in the IP (0.91 and 0.41 g kg^{-1}, respectively) than in NU (0.65 and 0.30 g kg^{-1}, respectively). A similar trend was observed for the total N amount, with the IP soil containing about 1.4 times that of NU, in the 0-10 cm soil layer. Soil C:N ratio was significantly lower in the IP system, up to 30 cm depth.

Table 1 - Soil bulk density, soil organic C and N concentration and amounts in the 5-year old improved pasture (IP) and natural understorey (NU). Mean ± standard error (n=30) and statistical significance: n.s.-p≥0,05, *-p<0,05, **-p<0,01, ***-p<0,001.

Depth	System	Bulk density	Organic C	Total N	C:N	Organic C	Total N
cm		g cm^{-3}	g kg^{-1}			kg m^{-2}	g m^{-2}
0-10	**IP**	1.18±0.02	17.5±1.07	0.91±0.05	19.5±1.23	1.6±0.10	84.8±5.1
	NU	1.18±0.03	21.4±2.43	0.65±0.06	32.6±1.32	1.85±0.14	61.9±6.1
		n.s.	n.s.	***	***	n.s.	***
10-20	**IP**	1.27±0.03	7.5±0.39	0.41±0.02	18.9±0.87	0.7±0.04	41.0±2.4
	NU	1.26±0.03	9.0±0.66	0.30±0.02	30.0±1.51	0.9±0.07	30.6±1.8
		n.s.	n.s.	***	***	n.s.	***
20-30	**IP**	1.34±0.02	5.1±0.45	0.30±0.02	17.3±0.82	0.5±0.05	31.0±2.5
	NU	1.31±0.01	6.0±0.42	0.24±0.02	27.3±1.91	0.6±0.04	24.6±1.8
		n.s.	n.s.	*	***	n.s.	*

Particulate organic matter and hot water soluble C and N

Particulate organic matter C concentration and relative proportion to total organic C did not differ significantly between NU and IP (Table 2). Hot water soluble C concentration in the 0-10 and 10-20 cm soil layers was significantly higher in the IP (0.63 and 0.34 g kg^{-1}, respectively) than NU (0.48 and 0.24 g kg^{-1}, respectively). HWS-C proportion of total organic C in the 0-10 and 10-20 cm soil layers was also significantly higher in the IP than NU (3.8 and 4.6%, 2.4 and 2.8%, respectively).

Hot water soluble N concentration followed the trend of total N, being higher under IP than NU soils, up to 20 cm depth. HWS-N relative proportion to soil total N was not different between management systems.

Carbon mineralization

After 28 days of incubation, soil samples from the IP released significantly more CO_2-C than those from NU, but between the 56 and 120th day this tendency was reversed (Figure 3). Mean total respired CO_2-C was significantly lower for IP (523.4 mg kg^{-1}) than NU (676.2 mg kg^{-1}) soils.

Table 2 - Soil particulate (POM-C) and hot water soluble C (HWS-C) and N (HWS-N) concentrations and corresponding percentage in relation to total soil organic C (POM-C/C, HWS-C/C) and N (HWS-N/N) in the 5-year old improved pasture (IP) and natural understorey (NU). Mean ± standard error (n=30) and statistical significance: n.s.-p≥0,05, *- p<0,05, **- p<0,01, ***- p<0,001.

Depth	System	POM-C	HWS-C	HWS-N	POM-C/C	HWS-C/C	HWS-N/N
cm			g kg^{-1}			%	
0-10	**IP**	8.2±0.80	0.63±0.04	0.08±0.00	44.9±2.08	3.8±0.21	9.6±0.53
	NU	9.6±1.51	0.48±0.06	0.05±0.01	41.6±2.99	2.4±0.21	8.2±0.65
		n.s.	*	***	n.s.	**	n.s.
10-20	**IP**	2.2±0.24	0.34±0.03	0.03±0.00	27.7±1.73	4.6±0.23	8.6±0.49
	NU	2.2±0.25	0.24±0.02	0.02±0.00	25.1±2.18	2.8±0.16	7.7±0.53
		n.s.	***	***	n.s.	***	n.s.
20-30	**IP**	1.3±0.15	0.23±0.03	0.02±0.00	25.5±1.23	4.5±0.29	7.0±0.53
	NU	1.6±0.18	0.22±0.03	0.02±0.00	26.8±2.76	4.3±0.79	9.6±1.18
		n.s.	n.s.	n.s.	n.s.	*	n.s.

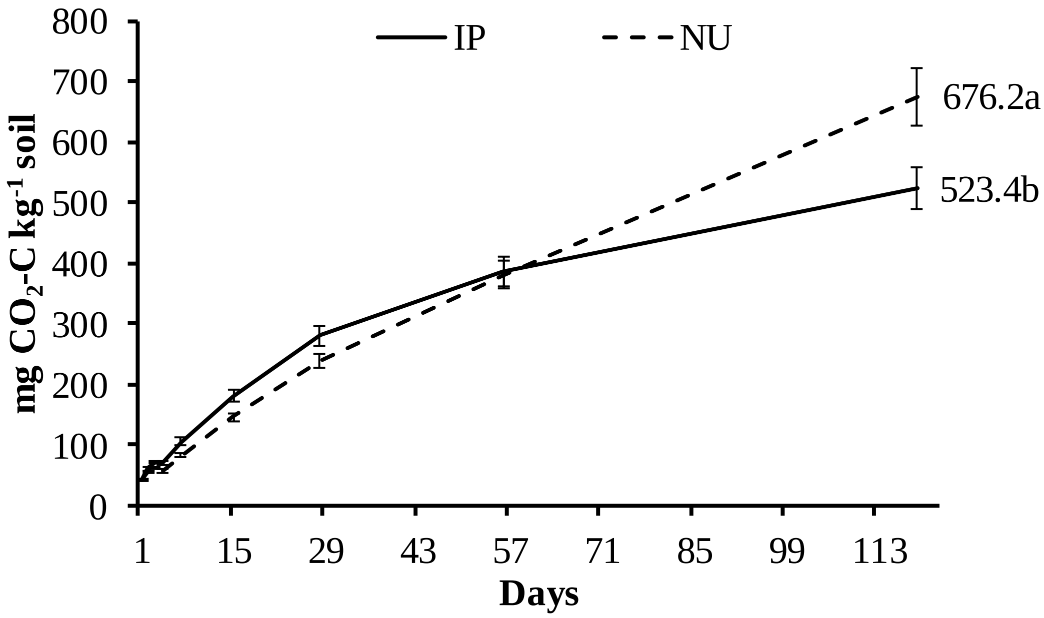

Figure 3 - Accumulated CO_2-C released by soils from the 5-year old improved pasture (IP) and the natural understorey (NU) systems during an incubation period of 120 days. Values at the end of each line are mean mineralized C (n=12); different superscripted letters indicate significant differences between management systems (p<0.05).

N mineralization

Soil from IP showed initial higher mineral N ($NH_4^+$and NO_3^-) concentrations than that from NU (Table 3). Net N mineralization and respective rates were also higher in IP (88.3 mg kg^{-1} soil; and 69.6 mg g N) than NU soils (52.9 mg kg^{-1}soil; and 45.5 mg g^{-1}N).

Table 3 - Initial soil mineral N concentrations, net mineralized nitrate- and ammonium-N, and mineralized N per unit of soil N (MN/N), after 112 days of aerobic incubation, in soils from the 5-year old improved pasture (IP) and the natural understorey (NU). Mean ± standard error (n=12) and statistical significance: n.s.-p≥0,05, *-p<0,05, **-p<0,01, ***-p<0,001.

	Initial mineral N			**Net mineralized N**			
System	**NH_4^+-N**	**NO_3^--N**	**(NH_4^+ + NO_3^-)-N**	**NO_3^--N**	**NH_4^+-N**	**N**	**MN/N**
	mg kg^{-1}			mg kg^{-1}			mg g^{-1}
IP	6.6±0.43	0.97±0.05	7.6±0.44	93.7±5.82	-5.4±0.43	88.3±5.94	69.6±2.71
NU	3.9±0.23	0.65±0.02	4.5±0.24	17.7±5.18	45.2±11.9	52.9±10.1	45.5±6.67
	***	***	***	***	***	**	**

In IP, net nitrification prevailed over net ammonification, the latter being negative from day 28 until the end of the incubation (Figure 4). In NU, net ammonification largely prevailed throughout the incubation period, and net nitrification was significantly lower than in IP. At the end of incubation, net NH_4^+-N concentration was 8.4 times greater in soils from NU than in those from IP system.

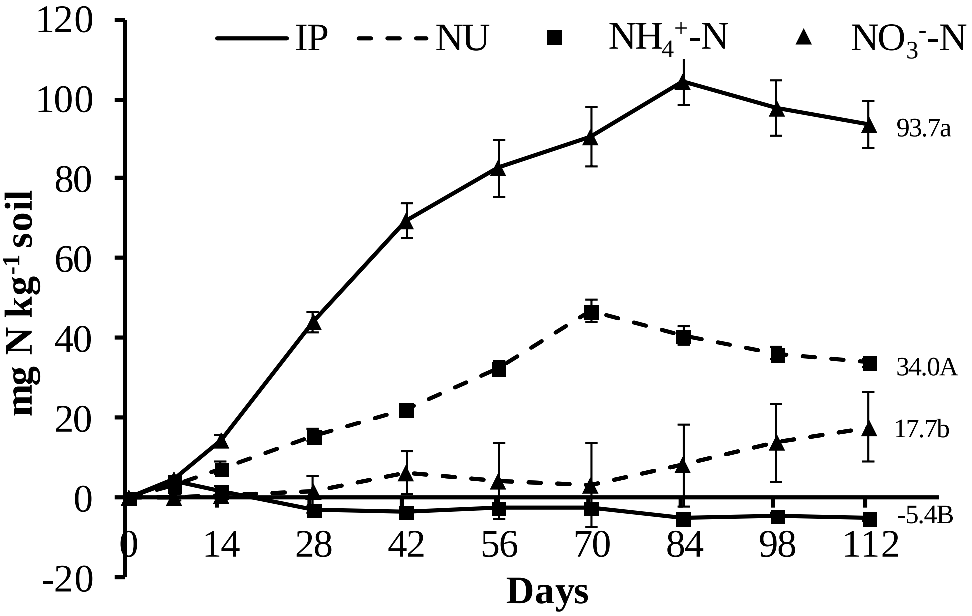

Figure 4 - Evolution of net NH_4^+-N and net NO_3^--N concentrations in the 5-year old improved pasture (IP) and natural understorey (NU) soils, along 16-week aerobic incubation. Values at the end of each line are mean net mineralized N (n=12); different superscript letters (uppercase for NH_4^+-N; lowercase for NO_3^--N) indicate significant differences between management systems (p<0.05).

Microbial C and N

Soil C mineralization, microbial C and N biomass and respective proportions relative to soil organic C and total N did not show significant differences between the study systems (Table 4). Soils from the NU system showed significantly higher microbial C:N ratio (6.9) and lower metabolic coefficient (2.1 mg g^{-1} h^{-1}) than in those from the IP (5.4, and 3.0 mg g^{-1} h^{-1}, respectively).

Table 4 - Mineralized C per unit of soil organic C (MC/C), microbial biomass C and N contents (C mic, N mic) and corresponding percentages in relation to total organic C (C mic/C) and N (N mic/N), microbial C:N ratio and metabolic coefficient (qCO_2) in the 5-year old improved pasture (IP) and natural understorey (NU). Mean ± standard error (n=12) and statistical significance: n.s.-p≥0,05, *-p<0,05, **-p<0,01, ***-p<0,001.

System	MC/C	C mic	N mic	C mic/C[a]	N mic/N[b]	C:N mic	qCO_2
	mg g^{-1}	mg kg^{-1}		%			mg g^{-1} h^{-1}
IP	26.0±1.17	172.9±13.6	31.9±2.54	7.6±0.03	25.0±0.07	5.4±0.10	3.0±0.23
NU	30.1±2.36	177.5±12.1	26.1±2.34	7.0±0.02	24.0±0.10	6.9±0.25	2.1±0.13
	n.s.	n.s.	n.s.	n.s.	n.s.	***	**

Soil fertility

Soil pH in water was significantly higher in the IP system up to 10 cm depth whereas the same was observed for pH in KCl for all soil layers (Table 5). Extractable P concentration in the IP soil was significantly higher, up to 20 cm depth, than in NU, while no significant differences were observed regarding extractable K. IP soils showed higher concentrations of exchangeable Ca^{2+} and Mg^{2+} up to10 and 20 cm depth, respectively. Exchangeable Al^{3+}concentration in the 0-10 cm soil layer was significantly lower in IP than in NU soils.

Table 5 - Soil pH, extractable P and K, non-acid exchangeable cations (Ca2+, Mg2+, Na+, K+) and extractable Al3+concentrations in the 5-year old improved pasture (IP) and natural understorey (NU). Mean ± standard error (n=30) and statistical significance: n.s.-p≥0,05, *-p<0,05, **-p<0,01, ***-p<0,001.

Depth	**System**	**pH**		**P**	**K**	**Ca^{2+}**	**Mg^{2+}**	**Na^{+}**	**K^{+}**	**Al^{3+}**
cm		**H_2O**	**KCl**	mg kg^{-1}		cmol$_c$ kg^{-1}				
0-10	**IP**	5.68±0.07	4.50±0.08	6.8±0.7	41.9±3.2	1.87±0.20	0.43±0.05	0.03±0.00	0.10±0.01	0.14±0.05
	NU	5.23±0.12	3.80±0.07	3.3±0.3	50.9±4.9	0.69±0.06	0.22±0.02	0.05±0.01	0.10±0.01	0.44±0.04
		**	***	***	n.s.	***	***	*	n.s.	***
10-20	**IP**	5.51±0.05	4.23±0.04	6.0±1.0	29.4±2.0	0.45±0.05	0.17±0.02	0.02±0.00	0.08±0.01	0.48±0.06
	NU	5.33±0.09	3.99±0.04	2.1±0.2	34.2±2.5	0.30±0.03	0.10±0.01	0.04±0.00	0.07±0.01	0.63±0.04
		n.s.	***	***	n.s.	n.s.	***	*	n.s.	n.s.
20-30	**IP**	5.66±0.05	4.43±0.03	3.1±0.5	29.7±2.2	0.56±0.13	0.17±0.03	0.03±0.00	0.08±0.01	0.48±0.06
	NU	5.49±0.07	4.17±0.06	1.9±0.2	34.5±2.9	0.31±0.04	0.09±0.01	0.04±0.01	0.07±0.01	0.59±0.04
		n.s.	***	n.s.	n.s.	n.s.	n.s.	n.s.	*	n.s.

DISCUSSION

High tree density at the study area led us to assume homogeneous tree cover for sampling, so our results are more likely to characterize an oak forest stand than a typical open oak woodland (<80 trees ha^{-1}; Moreno and Pulido 2009). It should not be overlooked that results from the improved pasture are influenced by the overlapping effects of cattle grazing and fertilizer application, being not possible to disentangle the specific effect of pasture sowing.

Soil bulk density in the study areas ranged between 1.18 and 1.34 g cm^{-3}, in the upper 0-10 and 20-30 cm soil layers, respectively. These values are similar to those reported by Gómez-Rey *et al.* (2012), for long-term natural and improved pastures grazed by sheep, under Mediterranean climate and sandy loam textured soils. Although improved pastures are commonly associated with surface soil porosity enhancement (Haynes and Williams 1993), such a trend was not observed in our study, probably due to the low soil organic C content. Also, the low stocking rate practiced, with cattle permanence being short and occurring only in late spring, may explain why soil porosity showed no significant modifications in the grazed pasture, as compared to the natural understorey, suggesting the absence of soil compaction risks in the former. In fact, values up to 30 cm depth were below 1.5 g cm^{-3}, the threshold above which root development might be constrained for similar textured soils (Weil and Brady 2017).

Although some studies report that pasture sowing can strongly enhance soil organic carbon accumulation at a short-term (e.g. Teixeira et al. 2015), in the present study a decrease of 0.7 kg organic C m^{-2} (though not statistically different) was estimated for the improved pasture system, compared with the natural understorey upper 30 cm soil layer. This trend may be explained, on one hand, by shrub elimination in the grazed area, which reduces soil organic residue inputs (Simões et al. 2009) and, on the other hand, by soil disturbance at pasture installation (disk harrowing) that might expose physically protected organic substrates, thus enabling their mineralization (Six et al. 2000). Our results are in accordance with those reported by Gómez-Rey et al. (2012) who observed negligible increases in the soil organic C stock up to 20 cm depth (0.18 and 0.84 kg m^{-2}, in open and tree covered areas, respectively) in a 26 year old improved pasture, as compared to a natural pasture with shrub control every six years.

Soil organic C stock in the natural understory management was similar to those reported for chestnut orchards growing on loamy soils (Borges et al. 2017), and to those obtained beneath tree crowns in sandy-loam soils under oak woodlands (Gómez-Rey et al. 2012), in Mediterranean conditions. This result highlights the important role of shrubs in the overall system C sequestration in our study site, which was estimated around 17% of total system C annual uptake (Correia et al. 2014). It should also be emphasized that structural heterogeneity introduced by shrubs is linked to several valuable ecosystem services, such as biodiversity conservation and tree recruitment facilitation (Dias et al. 2016; Simões et al. 2016).

Improved pasture system led to a strong decrease of soil C:N ratio (from 33 to 20, in the upper 10 cm layer). Such change suggests important modifications in soil organic matter quality and dynamics, especially regarding N mineralization patterns (Weil and Brady 2017). In fact, the substitution of shrubs with high C:N ratio residues (60 to 80; Simões et al. 2009) for a homogeneous legume-rich herbaceous cover, with considerably lower C:N ratio (25 to 30; Carranca et al. 2015), contributed to change soil organic matter quality. As a consequence, the establishment of improved pasture clearly enhanced soil nitrification potential, and soil N availability, which is in agreement with the trends reported by Gómez-Rey et al. (2012) for an older improved pasture.

Although soil N mineralization potential was increased by almost 50% (from 46 to 70 mg g^{-1}), it was not accompanied by soil pH increase, indicating that the initial lime and fertilizer applications might have balanced possible soil acidification effects (Haynes and Williams 1993). Regarding possible soil N losses, it should be emphasised that our N mineralization estimates, from sieved soil samples and under laboratory-controlled conditions, are certainly above the *in situ* mineralization amounts. Actually, soil N mineralization potentials in evergreen oak woodlands strongly decrease in the presence of herbaceous and tree decomposing roots (Gómez-Rey et al. 2011), and significant reduction of nitrate leaching is associated with oak root nitrate uptake (Nunes 2004). The high tree density at our study site may ensure low risk of nitrogen losses.

The establishment of improved pastures did not lead to marked changes on the soil C cycle as soil C mineralization rates and microbial biomass C and N were similar to that occurring in the natural understory system. However, higher metabolic coefficient (qCO_2) and lower microbial biomass C:N ratio indicate that the microbial population of the improved pasture soil may be less efficient in C metabolism than that from the natural vegetation area (Anderson and Domsch 1990). Improved pasture establishment enhanced the soil biochemical cycles, enabling the development of a soil microbial population with higher C consumption per unit of microbial biomass C, as compared to the natural understorey, following trends reported by Gómez-Rey et al. (2012) and Rosenzweig et al. (2016).

Besides similar soil organic C and POM-C proportions between the study systems, higher HWS-C in the improved pasture soil suggests enhanced soil microbial activity (Iqbal et al. 2010). This result is in agreement with Rodrigues et al. (2015) observations, in a 35-year old improved pasture from a Southern Portugal oak woodland, where HWS-C was more than doubled, along with a soil organic C increase, compared to an adjacent natural pasture. This trend, found only 5 years after pasture sowing and associated with a small decrease of soil organic C, highlights the efficiency of HWS-C as a soil organic matter indicator for monitoring changes of management and land use (Kalbitz et al. 2000).

Soil fertility was undoubtedly favoured by pasture sowing, mostly associated with significant N accumulation, up to 20 cm depth, but also as a consequence of fertilizer and lime inputs. Higher concentrations of extractable P and exchangeable Ca^{2+} and Mg^{2+}, agree with results reported for older improved pastures in oak woodlands, where chemical fertilizers were periodically applied (Gómez-Rey et al. 2012).

Increasing in non-acid cation concentrations contributed to a soil exchange complex with lower Al^{3+} saturation degree (from 29 to 5% in 0-10 cm layer) and, therefore, to change soil reaction from strongly to moderately acidic, which may also enhance nutrient availability (Weil and Brady 2017). These changes indicate a higher cation retention capacity, as the effective soil cation exchange capacity (sum of non-acid cations plus exchangeable Al^{3+}) in the 0-10 cm improved pasture soil layer reached 2.57 $cmol_c\ kg^{-1}$, while it was only 1.50 $cmol_c\ kg^{-1}$ in NU. It is noteworthy that only a few years after pasture sowing, most of the studied soil properties were in accordance with results from older improved pastures in open oak woodlands (Gómez-Rey et al. 2012; Rodrigues et al. 2015). This confirms the potential of sowing legume-rich mixtures for soil N enrichment and a fast soil quality improvement in cork oak woodlands, but they can be associated with soil organic C losses, with negative consequences for soil functions. Meanwhile, improved pasture sowing is commonly projected for grazing (intensification system), which hampers tree recruitment and threaten the long-term viability of cork oak woodlands, unless shelters are effectively used for seedlings protection. In contrast, tree recruitment is mostly facilitated under the natural understorey management, which allow shrub growth and saplings protective measures, avoid soil organic carbon losses and assure long-term cork production. As under this system the soil is N limited, occasional applications of fertilizers may be useful to improve tree nutritional status and soil organic matter quality.

CONCLUSIONS

Improved pastures extensively grazed by cattle do not necessarily lead to higher soil organic C stock in cork oak woodlands, as compared to ungrazed systems with natural shrub understory. In contrast, pasture management can promote soil quality, namely by enhancing soil organic matter quality and fertility, without detriment of soil physical conditions. Management aiming sustainable cork oak woodlands should be based on practices that effectively promote tree regeneration, such as those followed in the natural understory vegetation system, in which occasional application of fertilizers might be a promising option for improvement of soil quality and tree nutrition status. Long-term studies are needed regarding cork production and quality in study systems, for economic and environmental evaluation of cork woodlands.

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CHAPTER 2

How are current management systems affecting soil quality in evergreen oak woodlands (*montados*)?

How are current management systems affecting soil quality in evergreen oak woodlands (*montados*)?

ABSTRACT

Sustainability of Portuguese evergreen oak woodlands (*montados, dehesas* in Spain) is currently threatened by climate changes and inadequate management practices, which have led to soil degradation and tree recruitment impeachment in many areas. For assessment of the role of sowing improved pastures as a management option to reverse soil degradation and increase productivity in *montado* systems, a study was developed to assess their long-term management effects over soil properties. Improved and natural pastures soils were evaluated in two farms, with different soil types and grazing management systems, also considering the effect of tree cover. Soil bulk density, water retention, aggregates stability, saturated hydraulic conductivity, organic C and N concentrations, accumulation and mineralization, and soil fertility were measured. As compared with natural pastures, long-term improved pasture management has mostly decreased soil bulk density (and increased soil porosity), enhanced organic C accumulation and improved soil fertility, which occurred in open areas and even more markedly beneath tree crowns. Despite this, improved pasture establishment appeared not sufficient to assure adequate soil physical properties under higher stocking rates, mainly due to excessive soil compaction. Moreover, none of the study management systems considers measures to reverse tree regeneration failure, which poses critical issues on these systems future viability. Concerning *montad*o long-term sustainable management, these recent changes effects over the system economic and environmental performances, must also be evaluated.

Keywords: improved pasture, natural pasture, organic matter, physical status, soil fertility.

INTRODUCTION

Agroforestry systems are receiving a renewed interest, not only for their integrated approach to forest and agriculture productivity (Rigueiro-Rodríguez et al., 2009), but also for their potential to provide environmental services, such as carbon (C) sequestration (Kim et al., 2016; Kumar and Nair, 2011), and biodiversity conservation (Lemaire et al., 2014; Torralba et al., 2016). In the Iberian Peninsula, evergreen oak woodlands - *montado* in Portugal and *dehesa* in Spain - are the most important agroforestry system, where they occupy more than 3 million hectares (Eichhorn et al., 2006). Within these woodlands, scattered oak trees, mainly *Quercus suber* L. and *Q. ilex* L., are intercropped with agricultural crops, pastures and/or shrubs (Belo et al., 2009). Such multipurpose systems are a complex mosaic of biotic and abiotic conditions, with their multi-layer vegetation stratus crossing the landscapes diversity (Moreno et al., 2007; Moreno and Pulido, 2009).

Presently, large *montado* areas are facing several sustainability threats, including soil degradation, low tree and understorey productivities, lack of tree natural regeneration, and tree mortality (Moreno and Pulido, 2009; Plieninger et al., 2003; Pulido and Díaz, 2005; Ruiz Sinoga et al., 2012). Furthermore, land degradation (desertification) and the foreseen climate changes for the Mediterranean region, urge the need to implement adaptation and mitigation strategies (Duque-Lazo et al., 2018; IPCC, 2014; Lozano-García et al., 2017). Therefore, *montado* systems require human activity to sustain biodiversity and ecosystem services (Bugalho et al., 2011).

Recent studies addressing *montado* sustainable management have mainly focused on strategies for tree recruitment facilitation (Carmona et al., 2013; Dias et al., 2016; López-Sánchez et al., 2016; Pérez-Devesa et al., 2008; Ramírez and Díaz, 2008; Simões et al., 2016), pasture productivity enhancement (Cubera et al., 2009; Hernández-Esteban et al., 2018; Rivest et al., 2011), biodiversity conservation (Bugalho et al., 2011; Tárrega et al., 2009), and potential for carbon (C) sequestration (Alías et al., 2015; Correia et al., 2014; Oubrahim et al., 2016). However, contribution of soil functions for *montado* expected services are often overlooked and involved processes remain poorly understood, under current environmental and management changes.

Legume-based grassland systems constitute a pillar for sustainable and competitive livestock production (Lüscher et al., 2014), as a result of both N_2 symbiotic fixation and the efficiency of acquired N into biomass (Nyfeler et al., 2011). Following the need to make *montado* a more competitive system, sowing improved pastures (that is, a mixture of selected species with high proportion of legumes) has recently raised interest among Portuguese stakeholders, as an option to increase pasture productivity and reverse soil degradation (Crespo, 2006; Belo et al., 2014). In fact, accompanying an increase in national area of permanent pastures (GPP, 2018), improved pastures has recently increased, since it became a financially supported practice by the Portuguese Carbon Fund (APA, 2017). A great attention has been given to practices related to improved pastures establishment and persistence (Aguiar et al., 2006; Franca et al., 2016; Hernández-Estebán et al., 2018), their role on N_2 fixation from the atmosphere (Carranca et al., 2015), the soil nutritional status and productivity (Hernández-Estebán et al., 2018), the enhancement of soil organic carbon accumulation (Gómez-Rey et al., 2012; Hernández-Estebán et al., 2018), and soil nitrogen availability (Gómez-Rey et al., 2012). The effects of oak tree cover on such processes was also taken into account in most studies (Carranca et al., 2015; Gómez-Rey et al., 2012; Hernández-Estebán et al., 2018).

Higher productivity of improved pastures can support higher stocking rates, either by grazing density or by substitution of the traditional sheep and pig herds by cattle (Belo et al., 2009; Pinto-Correia et al., 2013). In fact, recent *montado* history is marked by land use intensification, as landowners often follow EU subsidies in an attempt to increase the system profitability (GPP, 2018; Belo et al., 2014). Such strong management changes are mostly resulting in overgrazing, which is now held responsible for failure of oak tree regeneration (López-Sánchez et al., 2014), significant biodiversity losses (Bugalho et al., 2011; Dias et al., 2016), and soil degradation (Pulido et al., 2018). Yet, grazing intensification has been associated with contrasting response in changes of soil properties (Abdalla et al., 2018), highlighting the need to integrate other factors, such as tree density, soil type and management system and specific practices (Pulido et al., 2017; Uribe et al., 2015).

Additionally, both grazing intensification and pasture sowing are generally associated with naturally occurring shrubs removal, a *montado* component that is recognized for their great potential for C sequestration (Correia et al., 2014), enhancement of soil fertility and C and N dynamics, especially under tree canopy cover (Gómez-Rey et al., 2013; Simões et al., 2009), and tree recruitment facilitation (Dias et al., 2016; Simões et al., 2016).

In this context, information on recent management changes long-term effects on the overall *montado* sustainability is still scarce, and wider approaches, including soil types and management systems diversity, are lacking. Also, in order to enable soil quality changes monitorization at farm level, indicator systems are needed.

A study was developed to obtain: (i) deeper understanding on modifications in soil properties associated with improved pasture establishment under different site conditions and management systems; and (ii) base information to develop guidelines for improvement of soil status and sustainable management of the *montado* system. It was hypothesized that the establishment of improved pastures enhances soil organic matter accumulation, fertility and resistance to degradation, and that changes on soil properties are dependent on site-specific ecological conditions and management options, such as soil type, tree cover, stocking rate and grazer species. Two representative *montado* farms, with different soil types, grazing animals and stocking rates, were evaluated for soil physical, chemical and biochemical properties, considering both natural and long-term improved pasture areas, and oak tree cover influence. Results of the current study are valuable to identify whether and how current management practices are affecting *montado* sustainability, aiding in the choice of alternatives to enhance its services. Results also provide information on the most relevant indicators to base monitoring systems of soil quality status in *montado* systems.

MATERIALS AND METHODS

Study areas

The study was conducted in two *montado* farms (mostly *Quercus suber* L. with few *Q. ilex* L.) both located in the Alto Alentejo region (Portugal): *Herdade dos Esquerdos* (HE; 39°07’-39°08’ N, 7°29’-7°30’ W; Vaiamonte) and *Herdade do Olival* (HO; 38°51’-38°52’ N, 7°32’-7’°33’ W; Mamporcão). In both farms, oak trees cover is about 35% of surface area, corresponding to 30-40 trees ha^{-1}. The landscape is gentle undulating (SROA, 1976; SROA, 1972), and climate is of Mediterranean type, with hot and dry summer and mild and wet winter. In the HE, the mean annual temperature is 15 °C, varying from 8.4 °C (January) to 23.5 °C (August), and the mean annual rainfall is 620 mm (INMG, 1991). In the HO, the mean annual rainfall is 670 mm, and the mean monthly temperature varies from 10 to 25 °C (Ferreira, 1970). Soils in the HE are developed over gneisses and show sandy loam texture (about110 g kg^{-1} clay, 140 g kg^{-1} silt), being classified as leptic *Regosols* associated with *Leptosols* with dystric characteristics (IUSS Working Group WRB, 2015). In the HO, soils are developed on granitic bedrock and are classified as endoleptic dystric *Cambisols* and endoleptic haplic *Luvisols* (IUSS Working Group WRB, 2015), showing loam texture (ca.160 g kg^{-1} clay, 210 g kg^{-1} silt.

Management systems

At the HE farm, two management systems were identified: 1) an improved pasture (IP) sowed in 1979 and grazed by 5 to 8 sheep per hectare every year (0.5 to 0.8 LU ha^{-1} year^{-1}); and 2) a natural pasture (NP) grazed by less than 1 sheep ha^{-1} year^{-1} (ca. 0.1 LU ha^{-1} year^{-1}). Sheep grazing in both pastures occurs intermittently throughout the year. The IP seed mixture included mainly *Trifolium* spp., *Ornithopus* spp. and *Lolium* spp., with 300 kg ha^{-1} of natural rock phosphate (26.5% P_2O_5, 35% CaO, 3.2% SO_3 and 0.8% MgO) being applied every two years. In the NP, naturally occurring shrubs (mostly *Quercus coccifera* L., *Cistus* spp. and *Crataegus monogyna* Jacq.) are controlled every four to six years by disk harrowing. Natural herbaceous vegetation consists mainly of *Chamaemelum mixtum* (L.) All., *Leontodon taraxacoides* (Vill.) Mérat, *Trifolium* spp., *Ornithopus* spp. and *Biserrula pelecinus* L. (FCT, 2014).

At the HO farm, two different management systems were also identified: 1) an improved pasture (IP) sowed in 1998, which is grazed by 0.7 cows per hectare and year (0.7 LU ha^{-1} $year^{-1}$); and 2) a natural pasture (NP) grazed by the same cattle herd at similar stocking rate. Both areas are grazed alternately throughout the year. In the IP area, 350 kg ha^{-1} of calcium phosphate fertilizer (18% P_2O_5, 10% CaO and 27% SO_3) were applied every two years. Dominant herbaceous species in natural pasture areas include *Agrostis castellana* Boiss. et Reut., *Chamaemelum nobile* (L.) All., *Vulpia geniculata* (L.) Link, *Lolium rigidum* Gaudin and *Carduus tenuiflorus* Curtis (FCT, 2014).

Soil sampling

In 2011, three 100×100 m plots were delimitated in each management system area, within each study farm, in order to alleviate possible pseudo replication problems (Stamps and Linit 1999). Each plot was then divided in four 50×50 m sub-plots and a circular 1256 m^2 area (40 m radius) was delimited in their centre (DGF, 2001). Two of the resulting circles, in each plot, were randomly selected and two representative trees (similar crown and breast height diameters) were marked in each selected circle, resulting in 12 trees for each management system, from each farm.

Soil samples (disturbed and undisturbed) from studied management systems at each farm (HE and HO) were collected between 2014 and 2017 in two positions relative to tree canopy: (i) beneath tree canopy (BC), at approximately 50% of crown radius projection, and (ii) in the open areas (OA), at least twice the crown radius away from the tree trunk, considering that oak canopies influence over soil properties can reach that distance (Simón et al., 2013). In each case, samplings were carried out according to the four major cardinal points direction, that is, four samples were taken for each position relative to tree. Soil samples were taken from the 10 cm top soil layer, because former studies indicated that soil changes mostly occurred in this layer (Gómez-Rey et al. 2012; Nunes, 2004).

Undisturbed samples

For bulk density measurements, undisturbed soil samples were collected around five randomly selected trees from the set established for each management system, at each farm. Four cylinders were carved into the soil, according to each cardinal direction, and samples were trimmed exactly to the cylinder volume (ca. 590 cm^3). A set of 20 soil samples was taken in each management system and position relative to tree canopy.

For measurements of soil water content at different matric potentials, undisturbed soil samples were collected by using metallic rings (ca. 59 cm^3) around six trees in each management system, samples being trimmed to the exact container volume. Twenty-four samples were taken for each management system and position relatively to tree canopy and were kept refrigerated (about 4 °C) until laboratory measurements.

For saturated hydraulic conductivity measurements, 100 cm^3 cylinders were used to sample the soil around five randomly selected trees. As a result, for each management system and position relatively to tree, 20 cylinders were collected. Cylinders were kept refrigerated (about 4 °C) until laboratory measurements.

Disturbed samples

Four disturbed soil samples were collected by excavation around 5 randomly selected trees, in each management system. Samples were air dried and used for aggregate size distribution and stability determinations.

Soil samples were taken with an auger around each of the 12 selected trees in each management system. Samples from each position relatively to tree were combined to form one composite sample, resulting in 12 samples of each management system and position relatively to tree. Samples were air-dried and passed through a 2 mm sieve.

For biochemical measurements, soil samples were collected by using an auger around the 12 selected trees, and 12 composite samples were obtained as described above. These composite samples were randomly paired, and therefore six samples were obtained for each management system and position relatively to tree. Samples were immediately sieved at 2 mm and refrigerated (about 4 °C) in closed plastic bags, to keep field moist content and avoid further soil microbial activity.

Laboratory procedures

Soil bulk density, water retention and saturated hydraulic conductivity

Soil bulk density was determined by the ratio of dry weigh of undisturbed soil cores (oven dried at 105 °C) and the cylinder volume (Blake and Hartge, 1986).

Soil water content at different matric potentials was determined by placing six samples randomly selected (from each management system and position relatively to tree) over each specific pressure-plate or pressure-membrane inside the pressure apparatus, where they were allowed to saturate with distilled water for at least 24 hours. The pressure was then adjusted to -5, -10, -33 and -1500 kPa, and kept continuously until no water leaked from the pan. Soil water contents were calculated by sample weight difference before and after drying at 105 °C (Richards, 1965).

For saturated hydraulic conductivity determination (Ks), undisturbed soil samples were placed in a tray and slowly saturated by raising the water level until water was visible at the soil surface (minimum 24 hours). Saturated samples were placed in a laboratory permeameter (Eijkelkamp Soil & Water, 2017), and the constant head method was applied (Reynolds and Elrick, 2002a). When the water level raised less than 2 cm a day, the falling head method was used (Reynolds and Elrick, 2002b).

Total soil porosity was calculated using the determined bulk density and soil particles density (2.65 g cm^{-3}), as described by Danielson and Sutherland (1986). Air filled pore volume was estimated as the difference between total pore volume and water filled volume, measured at -10 kPa.

Dispersion ratio, aggregate size distribution and stability

Particle-size fractions and dispersion ratio were determined in the <2 mm soil fraction from the air-dried disturbed samples. Two sets of subsamples (ca. 20 g) were taken: one was first treated with H_2O_2 solution and heated, and 20 mL of dispersing solution (containing $(NaPO_3)_6$ and Na_2CO_3) were added; while the other was simply dispersed in distilled water. Coarse sand was obtained by sieving and fine sand by washing/decanting, while silt and clay fractions were determined by pipetting, as described by Póvoas and Barral (1992). Dispersion ratios were calculated for the <0.02 and <0.002 mm particle size classes, as the ratio of the proportion obtained by water dispersion to that obtained with the dispersing solution.

Aggregate size fractions and water stable aggregates proportion were determined as described by Kemper and Rosenau (1986). Bulk air-dried soil samples (approximately 300 g) were separated by gentle sieving over 5, 2, and 1 mm sieves, in sequence, each retained fractions being then weighed. Approximately 4 g of the obtained 1 to 2 mm aggregates class were slowly saturated with water vapor and placed in a wet sieving equipment (0.25 mm) to be submerged and raised from distilled water for 3 minutes (35 cycles per minute), the dispersed material being collected. Retained aggregates were disrupted by using 100 mL of dispersing solution, collecting the dispersed material that passed through the sieve. Both dispersed soil fractions (by water or dispersing solution) were oven dried (105 °C) and weighed. Water stable aggregates proportion was calculated, as the ratio between the weight of the fraction collected after adding the dispersing solution, by the sum of that with the weight of the fraction that passed through the sieve during the 3-minute water dispersion procedure.

Soil biochemical properties

For microbial biomass C and N determination the fumigation-extraction procedure (Vance et al., 1987) was applied to the field-moist (<2 mm) soil samples, using six replicates with 10 g. Three replicates were immediately extracted with 50 mL of 0.5 M K_2SO_4 solution, while another three were firstly placed in the dark inside a vacuumed desiccator with ca. 50 mL of chloroform and 20 g of NaOH for 24 h. Contents of C and N in the extracts were determined by using an automated segmented flow analyser (Houba et al. 1994).

Soil respiration was measured by placing 50 g of soil (rewetted at approximately 60% of their water field capacity), in hermetic glass containers with a CO_2 trap solution (30 mL of 0.5 M NaOH), in the dark at 25 °C for 120 days. Trapping solutions were changed at days 1, 2, 3, 4, 7, 15, 28, 56 and 119, and excess of NaOH was titrated with a 0.5 M HCl solution, after dissolved CO_2 has been precipitated by a 0.5 M Ba_2Cl solution (García et al., 2003). Mineralized C per unit of soil initial organic C (MC/C) was calculated by dividing the total amount of respired CO_2-C along the incubation period, by the initial soil organic C content.

The metabolic coefficient was calculated as the ratio between the CO_2-C respired per day at the seventh day of incubation, and the initial soil microbial biomass C. Field-moist soil samples (ca. 1000 g) were rewetted at 60% water field capacity and incubated at 25 °C for 16 weeks. To follow N mineralization patterns, 10 g subsamples were taken periodically (days 0, 7, 14, 28, 42, 56, 70, 84, 98 and 112), adding 50 mL of 2M KCl solution (Keeney and Nelson, 1982). Extracts were used for NO_3^--N and NH_4^+-N determination in an automated segmented flow analyser (Houba et al. 1994). Net N mineralization was calculated by the difference of the determined initial to each date mineral N contents. Mineralized N per unit of soil N (MN/N), was obtained by dividing the final net mineralized N by the initial soil total N.

Soil chemical properties

Soil chemical properties were determined in the air-dried soil samples (< 2 mm). Total soil organic C (SOC) was determined by the potassium dichromate oxidation procedure (De Leenheer and Van Hove, 1958), and the particulate C fraction by using the material obtained after wet sieving of 50 g of soil with a 53 µm sieve. Coarse fragments proportion and soil bulk density were taken into account to calculate soil C accumulation in the 0-10 cm soil layer (Poeplau et al., 2017). Hot water soluble C was determined by using a suspension of 10 g of soil in 50 mL of water, at approximately 85 °C, for one hour (Khanna et al., 2001), and dissolved organic C was determined in an automated segmented flow analyser. Total N was determined by the Kjeldhal procedure. Non-acid exchangeable cations (Ca^{2+}, Mg^{2+}, Na^+, K^+) were extracted by percolating 5 g of soil samples with ammonium acetate at pH 7 and measured by atomic absorption spectrophotometry (AAS). Soil reaction was determined in distilled water or 1 M KCl suspensions (soil solution ratio: 1:2.5), using a potentiometer. Extractable K and P by the Egnér-Riehm (1958) test were obtained by shaking 5 g of soil with a solution of ammonium lactate and acetic acid for two hours, and determined by AAS and UV-visible spectroscopy, respectively.

Regarding the “4 per 1000” initiative - “Soils for Food Security and Climate” - (http:4p1000.org), with the objective to increase soil organic C accumulations globally by 0.4 percent per year, the absolute annual rate of change in SOC (kg C ha^{-1} yr^{-1}) and the relative rate (that is, to the control value of the SOC, ‰yr^{-1})

were calculated from the SOC accumulated under improved and natural pasture (synchronic approach) and the study period (Corbeels et al., 2018). Although SOC storage rates calculated by the diachronic approach lead to more accurate results (Costa Junior et al., 2013), since no data on soil organic C were available for the initial conditions, that is, prior to management changes, the synchronic approach was used.

Statistical analyses

All studied variables were separately analysed for *Herdade dos Esquerdos* and *Herdade do Olival*. When the population normal distribution (Shapiro-Wilk test) and homogeneity of variances (Levene's test) could be assumed, with or without data transformations (e.g. logarithm, square root), an analysis of variance (ANOVA) was conducted to test for differences between management system, tree canopy cover and respective interaction effects. If these conditions were not attainable, an aligned rank transformation (ART) was performed before submitting the data to the ANOVA. When needed, Tukey's or Waerden's (non-parametric) tests were used for means comparison. Due to the high variability of hydraulic conductivity results, they are presented as boxplots and histograms to enable data analyses and interpretation. All data analysis was carried out in the R environment (R Core Team, 2014), including adequate packages such as 'agricolae' (De Mendiburu, 2009), 'car' (Fox and Weisberg, 2011) and 'ARTool' (Kay and Wobbrock, 2016; Wobbrock et al., 2011).

RESULTS

Bulk density

At both HE and HO, soil bulk density was significantly lower beneath tree crown than in the open, and significantly lower in the IP than in NP pasture, but no significant interactions were observed between pastures and tree position (Table 1). At the HO, bulk density values in the IP and NP (1.55 and 1,62 g cm^{-3}, respectively) were higher than those measured in similar pastures (1.27 and 1.42 g cm^{-3}, respectively), at HE. Accordingly, values of total soil porosity were lower in the former (0.42 and 0.39 cm^3 cm^{-3}, respectively) than in the latter (0.52 and 0.46 cm^3 cm^{-3}).

Dispersion ratio, aggregate size distribution and stability

Soils at HE showed significantly higher proportion of smaller aggregates (1-2 mm) in the NP than in IP pasture, and beneath tree crown than in the open; the 2-5 mm aggregate fraction was also significantly higher in NP than in IP pasture (Table 1). At the HE, the proportion of the >5 mm aggregate fraction was significantly higher in the IP than in the NP, and significantly higher in the open than beneath tree crowns.

At HO, aggregate size distribution did not show significant differences between managements or position relative to trees (Table 1).

Soils under tree canopy, at HO, showed significantly higher proportion of water stable aggregates (1-2 mm fraction) than those in the open, whereas at HE no significant differences were observed (Table 1). The percentage of water stable aggregates was similar at both HE (93.4-96.5%) and HO (93.6-97.1%).

At HE, soils under the IP showed significantly lower dispersion ratios of particles lower than 0.02 and 0.002 mm (36 and 6%, respectively), as compared to those under the NP (42 and 11%, respectively), and significant interactions were observed between pastures and tree position. In contrast, at HO, dispersion ratio for soil particles < 0.02 mm was significantly higher in the IP than in the NP, and in the open than beneath tree crowns. The interaction between pasture management and position relative to tree was significant for the dispersion ration of particles smaller than 0.002 mm in HO soils.

Table 1 - Soil bulk density (BD), aggregate size distribution (> 1 mm), water stable 1-2 mm aggregates (WSA) and dispersion ratio (under 0.02 and 0.002 mm), in the 0-10 cm soil layer under improved (IP) and natural (NP) pastures, beneath canopy (BC) and in the open (OA), at *Herdade dos Esquerdos* and *Herdade do Olival*. Values are means with standard deviations in brackets (n=20, except dispersion ratio with n=12); different letters in the same column indicate significant differences within factor or interaction levels (p<0.05) by the Tukey test.

Systems	BD	Aggregate size distribution			WSA	Dispersion ratio	
		1 - 2 mm	2 - 5 mm	> 5 mm		< 0.02 mm	< 0.002 mm
	g cm^{-3}	%					
				HERDADE DOS ESQUERDOS			
IP	**1.27^{b}** (0.12)	**9.2^{b}** (3.3)	**22.2^{b}** (6.5)	**60.0^{a}** (12.6)	**94.9^{a}** (4.9)	**0.36^{b}** (0.12)	**0.06^{b}** (0.04)
NP	**1.42^{a}** (0.17)	**11.5^{a}** (4.2)	**25.4^{a}** (6.0	**52.2^{b}** (14.2)	**96.1^{a}** (2.5)	**0.42^{a}** (0.08)	**0.11^{a}** (0.06)
BC	**1.28^{b}** (0.18)	**11.3^{a}** (3.4)	**24.7^{a}** (4.5)	**52.1^{b}** (11.8)	**95.0^{a}** (5.1)	**0.37^{a}** (0.12)	**0.08^{a}** (0.04)
OA	**1.42^{a}** (0.10)	**9.3^{b}** (4.2)	**22.9^{a}** (7.8)	**60.1^{a}** (14.9)	**96.2^{a}** (1.8)	**0.41^{a}** (0.08)	**0.09^{a}** (0.07)
IP×BC	**1.18^{a}** (0.08)	**10.8^{a}** (2.7)	**24.3^{a}** (3.9)	**54.0^{a}** (9.9)	**93.4^{a}** (6.3)	**0.28^{b}** (0.09)	**0.08^{ab}** (0.04)
NP×BC	**1.36^{a}** (0.21)	**11.8^{a}** (4.1)	**25.0^{a}** (7.1)	**50.3^{a}** (13.5)	**96.3^{a}** (3.2)	**0.45^{a}** (0.08)	**0.08^{b}** (0.04)
IP×OA	**1.36^{a}** (0.06)	**7.5^{a}** (3.0)	**20.0^{a}** (7.8)	**65.9^{a}** (12.5)	**96.5^{a}** (2.2)	**0.44^{a}** (0.07)	**0.05^{b}** (0.03)
NP×OA	**1.48^{a}** (0.09)	**11.2^{a}** (4.5)	**25.9^{a}** (6.9)	**54.2^{a}** (15.1)	**96.0^{a}** (1.5)	**0.38^{a}** (0.07)	**0.14^{a}** (0.07)
				HERDADE DO OLIVAL			
IP	**1.55^{b}** (0.09)	**12.9^{a}** (4.8)	**21.6^{a}** (5.6)	**44.0^{a}** (19.8)	**95.4^{a}** (4.2)	**0.53^{a}** (0.07)	**0,13^{a}** (0.04)
NP	**1.62^{a}** (0.14)	**14.2^{a}** (4.1)	**21.8^{a}** (5.4)	**38.7^{a}** (18.4)	**94.8^{a}** (4.7)	**0.47^{b}** (0.07)	**0.13^{a}** (0.04)
BC	**1.50^{b}** (0.10)	**13.1^{a}** (4.3)	**21.5^{a}** (5.2)	**43.0^{a}** (18.9)	**96.5^{a}** (3.0)	**0.47^{b}** (0.06)	**0.12^{a}** (0.04)
OA	**1.67^{a}** (0. 09)	**14.1^{a}** (4.7)	**22.0^{a}** (5.8)	**39.8^{a}** (14.5)	**93.7^{b}** (5.2)	**0.53^{a}** (0.09)	**0.14^{a}** (0.04)
IP×BC	**1.48^{a}** (0.07)	**12.3^{a}** (3.9)	**23.3^{a}** (4.3)	**46.3^{a}** (16.5)	**97.1^{a}** (2.2)	**0.48** (0.06)	**0.11^{b}** (0.03)
NP×BC	**1.52^{a}** (0.12)	**13.8^{a}** (4.6)	**19.6^{a}** (5.6)	**39.6^{a}** (21.1)	**95.9^{a}** (3.6)	**0.46** (0.07)	**0.14^{ab}** (0.05)
IP×OA	**1.62^{a}** (0.06)	**13.6^{a}** (5.6)	**19.9^{a}** (6.4)	**41.6^{a}** (22.9)	**93.6^{a}** (5.0)	**0.58** (0.05)	**0.16^{a}** (0.05)
NP×OA	**1.72^{a}** (0.09)	**14.6^{a}** (3.6)	**24.1^{a}** (4.4)	**37.8^{a}** (15.9)	**93.8^{a}** (5.6)	**0.48** (0.08)	**0.12^{ab}** (0.03)

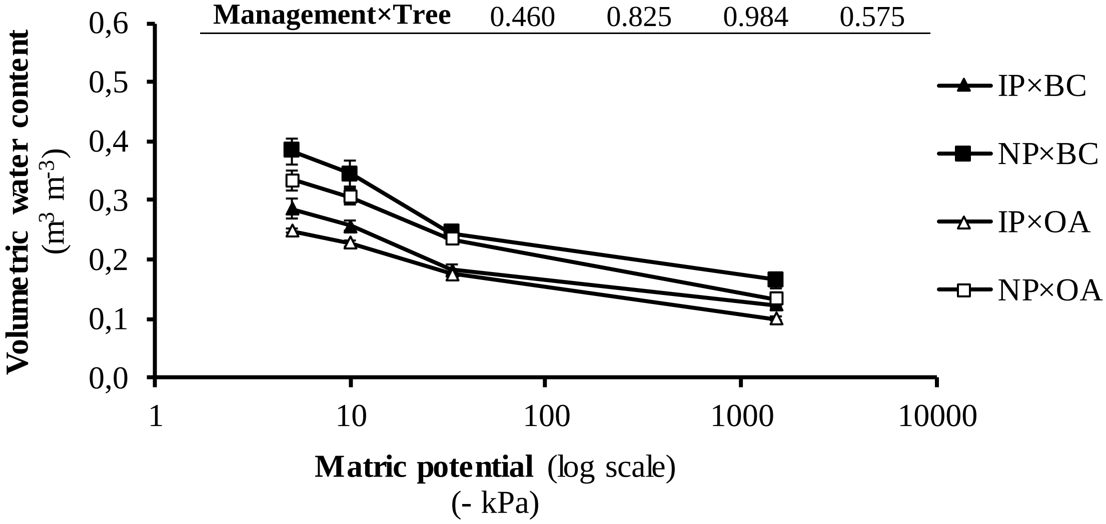
Water content and hydraulic conductivity

Soil water retention at the considered matric potentials was significantly lower in the IP than in the NP, at HE (Figure 1), while tree cover has significantly increased soil water retention at -5, -10 and -1500 kPa. At HO, soil water retention was significantly higher beneath tree canopy than in the open, and in the NP than in the IP, for high soil water potential (-5 kPa); interactions between management and tree position were only significant at low soil water potential -1500 kPa.

Data from water retention curves indicates that the difference between mean soil water contents at -10 kPa and at -1500 kPa, that is, approximately the soil available water content, was higher at HE (0.13 and 0.18 m^3 m^{-3}) than at HO (0.08 to 0.14 cm^3 cm^{-3}, in open and tree covered soils, respectively.

HE

ψ (kPa)	-5	-10	-33	-1500
	***p*-value**			
Management	<0.001	<0.001	<0.001	<0.001
Tree	0.011	0.018	0.536	0.003
Management×Tree	0.460	0.825	0.984	0.575



HO

ψ (kPa)	-5	-10	-33	-1500
	***p*-value**			
Management	0.013	0.304	0.054	0.659
Tree	<0.001	<0.001	<0.001	<0.001
Management×Tree	0.121	0.534	0.870	0.004

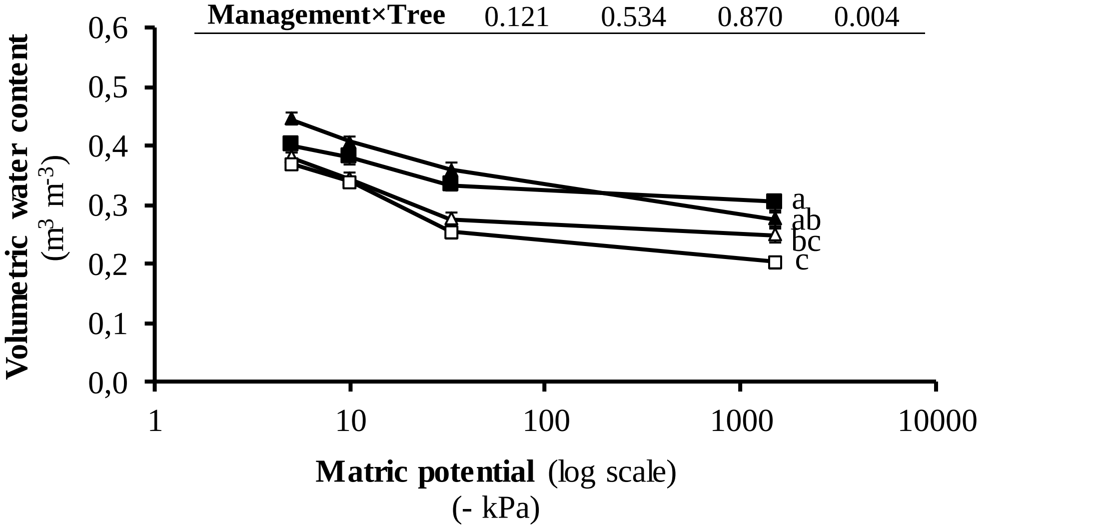


Figure 1 - Soil volumetric water contents at different matric potentials (ψ) in the 0-10 cm soil layer, at *Herdade dos Esquerdos* (HE) and *Herdade do Olival* (HO). Points are means, bars are standard errors, and *p*-values result from two-way ANOVA (n=6) with pasture management (IP - improved; NP - natural) and position relative to tree (BC - beneath canopy; OA - open areas) as independent variables; different letters for the same matric potential indicate significant differences between the interaction levels (p<0.05) by the Tukey test.

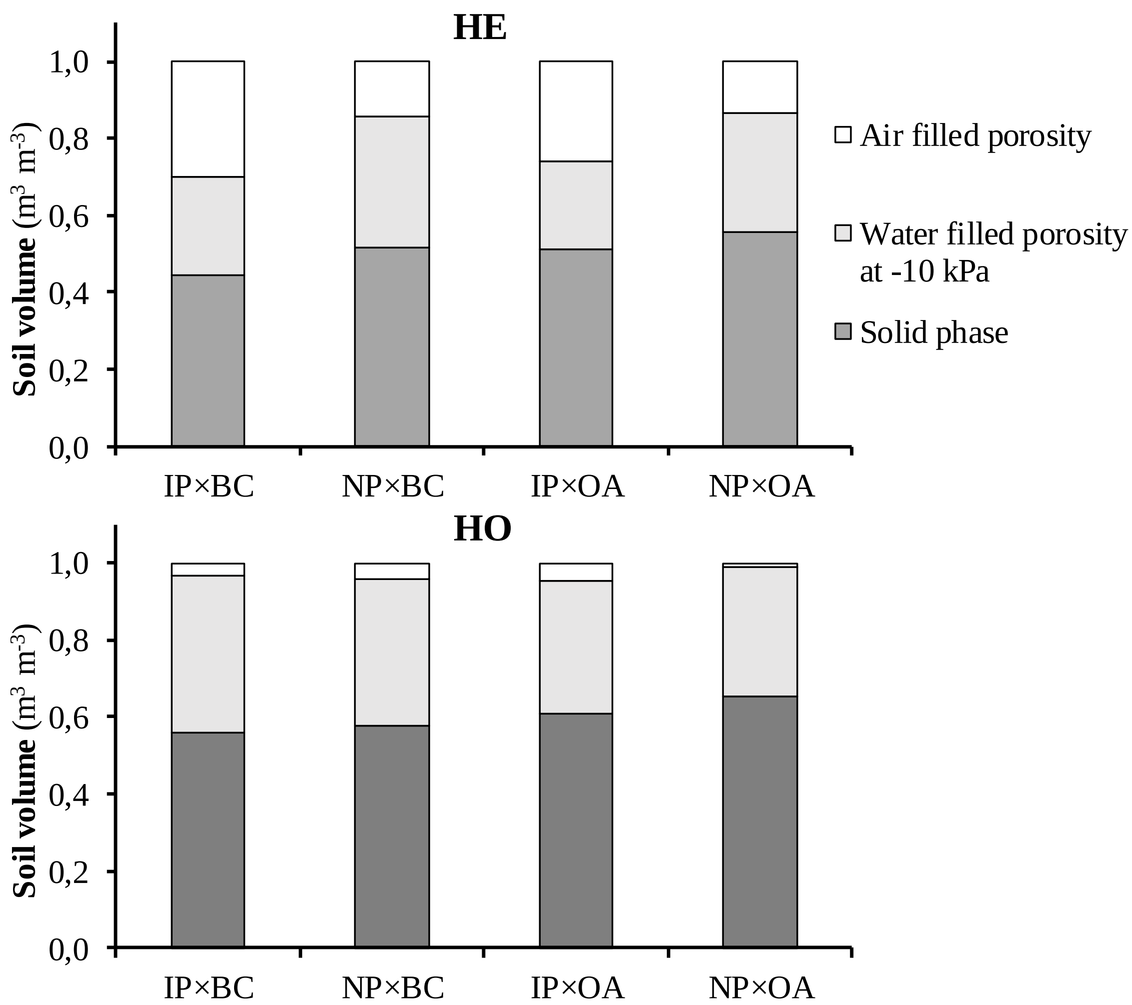


Figure 2 - Estimated mean air filled porosity (m^3 m^{-3}) for the 0-10 cm soil layer under improved (IP) and natural (NP) pastures, beneath canopy (BC) and open areas (OA), at *Herdade dos Esquerdos* (HE) and *Herdade do Olival* (HO).

At HE, estimated air filled porosity at field capacity (at -10 kPa soil water potential) beneath tree crowns was 0.14 and 0.30 m^3 m^{-3} (in the NP and IP respectively), while in the open was 0.14 and 0.26 m^3 m^{-3} (in the IP and NP, respectively; Figure 2). Values at HO were much lower, up to 0.04 cm^3 cm^{-3} beneath tree crowns, and varying between 0.05 and 0.01 m^3 m^{-3} in the open, for the IP and NP, respectively. Air filled pores at -33 kPa were higher but corresponded only to 0.08-0.11 m^3 m^{-3} at HO (data not showed).

Management system and position relative to trees did not significantly influence mean values of soil saturated hydraulic conductivity, at both farms (data not showed).

At HE, soils under the IP had generally higher and less variable Ks values than those under the NP (Figures 3 and 4).

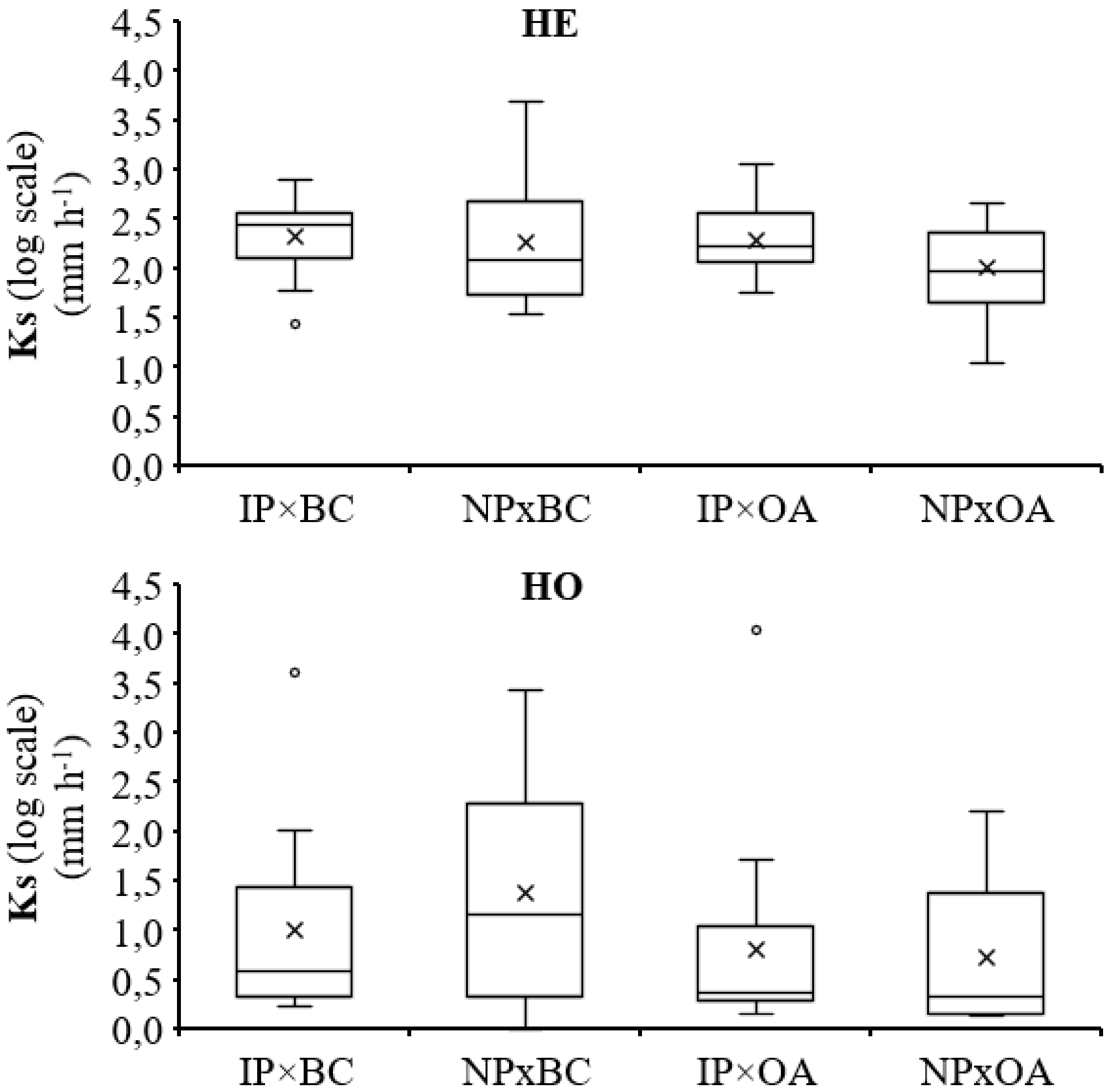


Figure 3 - Boxplots (n=20) for the logarithm of soil saturated hydraulic conductivity (Ks) measured in the 0-10 cm soil layer under improved (IP) and natural (NP) pastures, beneath canopy (BC) and in the open area (OA), at *Herdade dos Esquerdos* (HE) and *Herdade do Olival* (HO).

Saturated hydraulic conductivity in soils from HO showed wider ranges (from 0 to 11142 mm h^{-1}) than those from HE (10 to 5019 mm h^{-1}; Figure 3). In the HO open areas, IP soils presented more frequently Ks values between 1 and 10 mm h^{-1}, while in NP most values were below 1 mm h^{-1} (Figure 4). The highest variability of hydraulic conductivity results was found for the NP soil samples from under the trees, where zero hydraulic conductivity results contrasted with a greater proportion of values above 100 mm h^{-1}.

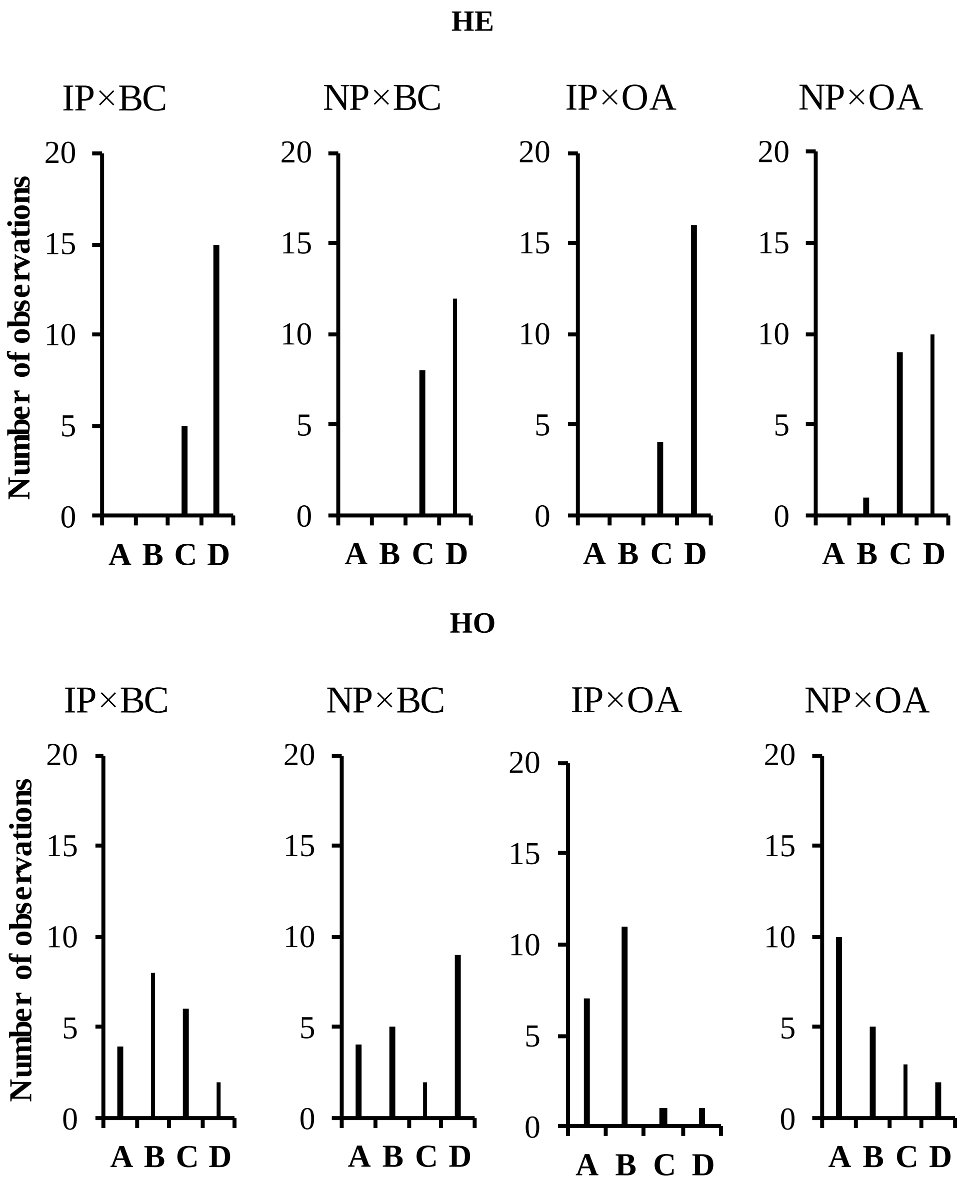


Figure 4 - Number of observations of saturated hydraulic conductivity values (Ks) within the 0-1 (A), 1-10 (B), 10-100 (C) and >100 (D) mm h^{-1} categories, measured in the 0-10 cm soil layer under improved (IP) and natural (NP) pastures, beneath canopy (BC) and in the open area (OA), at *Herdade dos Esquerdos* (HE) and *Herdade do Olival* (HO).

Soil organic C concentration and mineralization

At both HE and HO farms, soil organic C concentrations and organic C contents, up to 10 cm depth, were significantly higher in the IP than in the NP, and significant higher beneath tree crowns than in the open (Table 2). No significant interactions were observed between pastures and tree position. For the same pasture management and the same tree position, organic C concentrations and accumulation at the HE were higher than those observed at the HO.

Table 2 - Concentrations of organic C (C_{org}), organic C contents (C_c), particulate organic matter C (POM-C), hot water soluble C (HWS-C), mineral associated C (MA-C) and respective proportions of total organic C (POM-C/C, HWS-C/C, MA-C/C), in the 0-10 cm soil layer under improved (IP) and natural (NP) pastures, beneath canopy (BC) and in the open (OA), at *Herdade dos Esquerdos* and *Herdade do Olival*. Values are means with standard deviations in brackets (n=12); different letters in the same column indicate significant differences within factor or interaction levels (p<0.05) by the Tukey test.

Systems	C_{org}	C_c	POM-C	HWS-C	MA-C	POM-C/C	HWS-C/C	MA-C/C
	g kg^{-1}	kg m^{-2}	g kg^{-1}			%		
				HERDADE DOS ESQUERDOS				
IP	**27.1^a** (8.4)	**2.51^a** (0.72)	**12.8^a** (6.7)	**1.33^a** (0.6)	**14.3^a** (3.9)	**43.1^a** (9.2)	**4.6^a** (1.2)	**55.2^b** (12.7)
NP	**18.3^b** (6.5)	**1.94^b** (0.68)	**6.2^b** (2.5)	**0.59^b** (0.3)	**12.6^a** (4.9)	**35.7^b** (15.0)	**3.3^b** (1.2)	**66.7^a** (9.2)
BC	**28.4^a** (6.8)	**2.71^a** (0.56)	**12.4^a** (6.5)	**1.21^a** (0.7)	**16.0^a** (4.0)	**40.4^a** (10.9)	**3.9^a** (1.1)	**58.0^b** (14.3)
OA	**17.0^b** (6.3)	**1.74^b** (0.59)	**6.6^b** (3.6)	**0.72^b** (0.5)	**10.8^b** (3.2)	**38.5^a** (14.8)	**4.0^a** (1.6)	**63.9^a** (9.5)
IP×BC	**33.1^a** (5.9)	**2.98^a** (0.53)	**17.2^a** (5.8)	**1.63^a** (0.7)	**15.9^a** (4.6)	**48.1^a** (7.6)	**4.5^a** (1.2)	**48.6^b** (13.1)
NP×BC	**23.8^a** (3.6)	**2.44^a** (0.46)	**7.7^a** (2.0)	**0.79^a** (0.2)	**16.1^a** (3.6)	**32.6^b** (7.8)	**3.3^a** (0.7)	**67.4^a** (7.8)
IP×OA	**21.2^a** (5.8)	**2.04^a** (0.56)	**8.4^a** (4.0)	**1.03^a** (0.5)	**12.8^a** (2.3)	**38.1^{ab}** (8.1)	**4.8^a** (1.3)	**61.9^a** (8.1)
NP×OA	**12.9^a** (3.2)	**1.44^a** (0.46)	**4.8^a** (2.1)	**0.40^a** (0.2)	**8.7^b** (2.6)	**38.8^{ab}** (19.8)	**3.3^a** (1.6)	**66.0^a** (10.9)
				HERDADE DO OLIVAL				
IP	**18.7^a** (3.8)	**1.60^a** (0.38)	**7.45^a** (2.3)	**0.54^a** (0.2)	**11.2^a** (2.5)	**41.0^a** (4.7)	**2.95^b** (0.43)	**60.3^a** (7.8)
NP	**13.6^b** (5.2)	**1.32^b** (0.46)	**5.42^b** (3.4)	**0.46^b** (0.2)	**8.1^b** (2.7)	**35.6^b** (7.3)	**3.30^a** (0.69)	**61.1^a** (13.1)
BC	**19.6^a** (4.0)	**1.70^a** (0.37)	**8.24^a** (3.0)	**0.64^a** (0.2)	**11.3^a** (2.2)	**40.0^a** (6.5)	**3.27^a** (0.62)	**58.8^a** (8.7)
OA	**12.7^b** (3.8)	**1.23^b** (0.38)	**4.63^b** (1.8)	**0.36^b** (0.1)	**8.1^b** (2.9)	**36.7^a** (6.5)	**2.96^a** (0.54)	**62.6^a** (12.2)
IP×BC	**21.9^a** (2.1)	**1.79^a** (0.35)	**9.01^a** (1.8)	**0.69^a** (0.2)	**12.9^a** (1.0)	**40.9^a** (4.8)	**3.12^a** (0.44)	**59.0^a** (4.9)
NP×BC	**17.2^a** (4.3)	**1.60^a** (0.39)	**7.46^a** (3.8)	**0.59^a** (0.2)	**9.8^a** (2.0)	**39.0^a** (8.0)	**3.42^a** (0.75)	**58.5^a** (11.7)
IP×OA	**15.5^a** (2.0)	**1.42^a** (0.32)	**5.88^a** (1.6)	**0.39^a** (0.1)	**9.6^a** (2.6)	**41.0^a** (4.8)	**2.75^a** (0.34)	**61.5^a** (10.0)
NP×OA	**9.9^a** (2.8)	**1.04^a** (0.35)	**3.38^a** (1.0)	**0.32^a** (0.1)	**6.5^a** (2.3)	**32.3^a** (4.9)	**3.17^a** (0.63)	**63.8^a** (14.4)

Particulate organic matter carbon (POM-C) concentration followed the trend observed for total soil organic C concentration in both HE and HO (Table 2). As a fraction of total soil organic C, POM-C was significantly higher under IP than NP at HE, the interaction of management and tree position being also significant. In HO, the POM-C fraction was significantly higher beneath tree canopy than in the open, while no significant differences were associated to pasture management.

Hot water soluble carbon (HWS-C) concentration was significantly higher in the IP than in the NP pasture, and beneath tree crowns than in the open, for both farms. As a fraction of total soil organic C, HWS-C was significantly higher under the IP than the NP at HE, and an inverse trend was observed at HO.

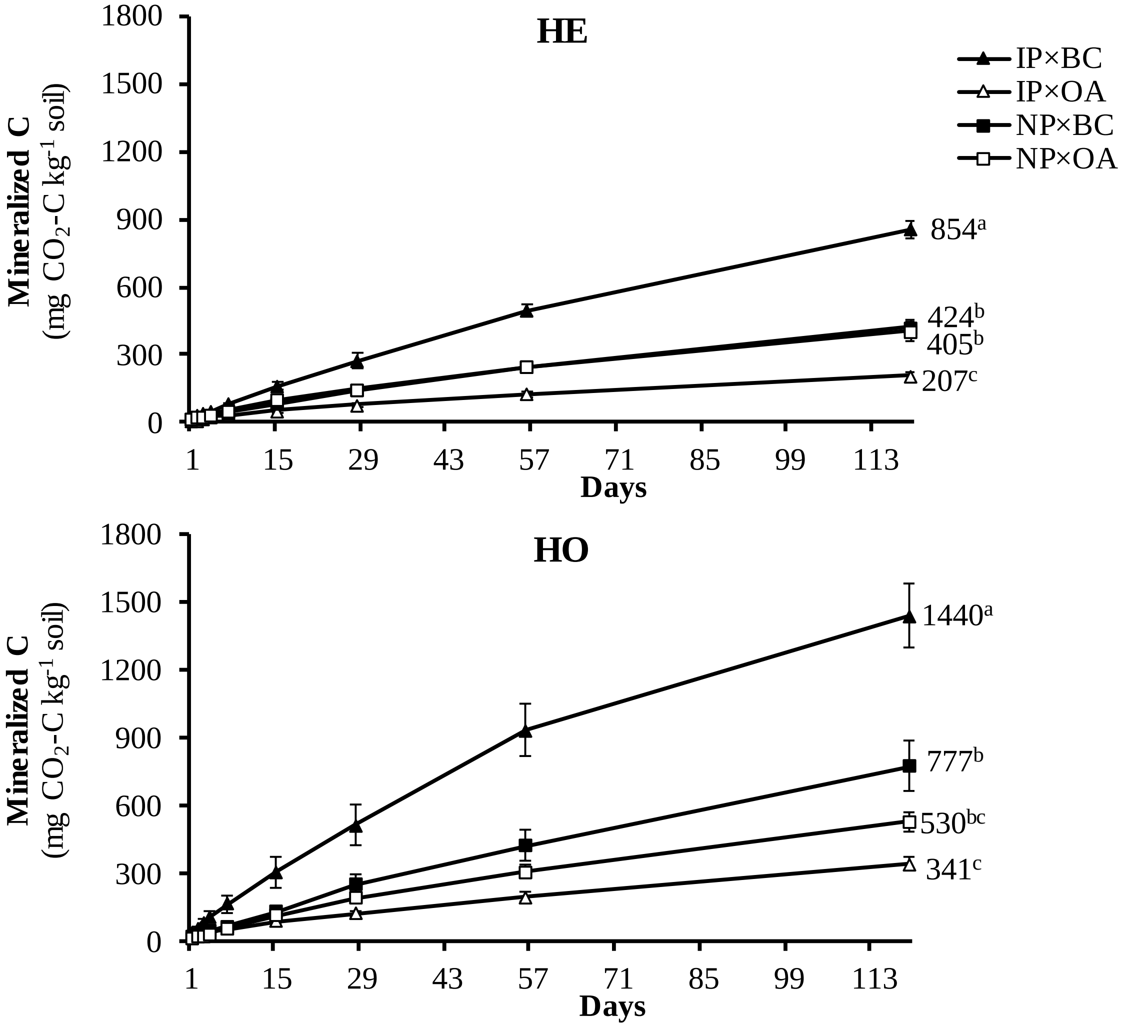


Figure 5 - Cumulative mineralised C along 120 days of laboratory incubation of the 0-10 cm soil layer under improved (IP) and natural (NP) pastures, beneath canopy (BC) and in the open area (OA), at *Herdade dos Esquerdos* (HE) and *Herdade do Olival* (HO). Points are means, bars are standard errors (n=6); different letters for the same day indicate significant differences between the interaction levels (p<0.05) by the Tukey test.

As observed for soil organic C concentrations, mineralized C throughout the incubation, both at HE and HO, was significantly higher in the IP than in the NP, and beneath tree crowns than in the open (Figure 5, Table 3). At HE, the mineralized C in the IP beneath tree crown doubled that of the NP, but an inverse trend was observed in the open; the mineralized C was significantly greater in soils beneath tree crowns than in the open, but differences were only statistically significant for the IP. At HO, the mineralized C in the IP beneath tree crown also doubled that of the NP, and that in the IP beneath tree crowns was about four times higher than in the open. Mineralized C in tree-covered areas was higher at HO than at HE for both IP and NP.

Significantly higher C mineralized per unit of C was observed, at HE, in soils beneath tree crown than in the open; also, it was significantly higher for the IP beneath trees than in the open. Values of C mineralized per unit of C were higher at HO than at HE (Table 3).

The concentration of organic microbial biomass C (C_{mic}), at HE, was significantly higher in the NP than in the IP, and beneath tree crowns than in the open; the C_{mic}, in the open was significantly higher in NP than in IP, and for IP was significantly higher beneath trees than in the open. At HO, the concentrations of C_{mic}, which were higher than at HE, were significantly higher beneath trees than in the open; the C_{mic} was significantly higher in the IP than in the NP, beneath trees, and significantly lower in the open; values for IP were significantly higher beneath trees than in the open.

The proportion of C_{mic} relative to total organic C, at both HE, was significantly higher in the NP than in the IP, and no significant interactions were observed between pastures and tree position. At HO, it was significantly higher in the NP than in the IP and in the open than beneath trees; this proportion, in the open was significantly higher in NP than in IP; and for the NP was significantly higher in the open than beneath trees.

No significant differences were observed for the metabolic coefficient (qCO_2) between pastures and tree positions, at both HE and HO. Among HE systems, the qCO_2 was significantly higher in the IP than in the NP, beneath tree canopies. At HO, values for the IP were significantly higher beneath canopy than in the open.

Table 3 - Concentration of organic microbial biomass C (C_{mic}) and N (N_{mic}), respective proportions of organic C (C_{mic}/C) and total N (N_{mic}/N), microbial C:N ratio (C_{mic}:N_{mic}), potential mineralizable C (C_{min}), metabolic coefficient (qCO_2) and mineralized C per unit of soil organic C (MC/C), in the 0-10 cm soil layer under improved (IP) and natural (NP) pastures, beneath canopy (BC) and in the open (OA), at *Herdade dos Esquerdos* and *Herdade do Olival*. Values are means with standard deviations in brackets (n=6); different letters in the same column indicate significant differences within factor or interaction levels (p<0.05) by the Tukey or Waerden tests.

Systems	C_{mic}	N_{mic}	C_{mic}:N_{mic}	C_{mic}/C	N_{mic}/N	qCO_2	C_{min}	MC/C
	mg kg^{-1}			%		mg CO_2-C g^{-1} C_{mic} h^{-1}	g CO_2-C kg^{-1}	mg CO_2-C g^{-1} C_{org}
				HERDADE DOS ESQUERDOS				
IP	**195.4**^{b} (103.6)	**22.2**^{b} (14.4)	**9.62**^{a} (1.64)	**0.74**^{b} (0.13)	**0.93**^{b} (0.31)	**1.26**^{a} (0.50)	**0.53**^{a} (0.35)	**15.4**^{a} (3.9)
NP	**240.1**^{a} (31.9)	**28.9**^{a} (4.8)	**8.37**^{b} (0.61)	**1.16**^{a} (0.23)	**1.74**^{a} (0.36)	**0.95**^{a} (0.49)	**0.41**^{b} (0.09)	**16.1**^{a} (2.6)
BC	**258.1**^{a} (58.0)	**30.8**^{a} (8.0)	**8.74**^{b} (0.79)	**0.89**^{a} (0.28)	**1.36**^{a} (0.39)	**1.17**^{a} (0.61)	**0.64**^{a} (0.24)	**17.2**^{a} (2.3)
OA	**177.4**^{b} (77.0)	**20.3**^{b} (11.5)	**9.52**^{a} (1.64)	**1.01**^{a} (0.29)	**1.31**^{a} (0.66)	**1.04**^{a} (0.40)	**0.31**^{b} (0.13)	**14.2**^{b} (3.4)
IP×BC	**284.3**^{a} (66.4)	**34.7**^{a} (9.0)	**8.27**^{b} (0.96)	**0.70**^{a} (0.13)	**1.11**^{bc} (0.28)	**1.59**^{a} (0.29)	**0.85**^{a} (0.10)	**18.1**^{a} (3.0)
NP×BC	**231.9**^{a} (36.6)	**26.9**^{a} (4.8)	**8.67**^{b} (0.60)	**1.09**^{a} (0.25)	**1.59**^{ab} (0.36)	**0.74**^{b} (0.55)	**0.42**^{b} (0.08)	**16.4**^{ab} (1.2)
IP×OA	**106.5**^{b} (14.9)	**9.7**^{b} (1.1)	**11.0**^{a} (0.80)	**0.79**^{a} (0.14)	**0.73**^{c} (0.20)	**0.93**^{ab} (0.45)	**0.21**^{c} (0.05)	**12.6**^{b} (2.6)
NP×OA	**248.3**^{a} (27.1)	**30.9**^{a} (4.4)	**8.07**^{b} (0.50)	**1.24**^{a} (0.20)	**1.90**^{a} (0.30)	**1.16**^{ab} (0.35)	**0.40**^{b} (0.10)	**15.9**^{ab} (3.6)
				HERDADE DO OLIVAL				
IP	**385.9**^{a} (177.7)	**46.1**^{a} (24.5)	**8.86**^{b} (1.35)	**1.09**^{b} (0.13)	**1.45**^{b} (0.24)	**1.06**^{a} (0.61)	**0.89**^{a} (0.62)	**19.1**^{a} (5.3)
NP	**379.2**^{a} (96.1)	**39.1**^{a} (11.1)	**9.78**^{a} (0.61)	**1.42**^{a} (0.41)	**1.79**^{a} (0.37)	**0.95**^{a} (0.43)	**0.65**^{b} (0.24)	**20.5**^{a} (4.1)
BC	**469.2**^{a} (126.2)	**55.1**^{a} (18.0)	**8.75**^{b} (1.02)	**1.16**^{b} (0.24)	**1.59**^{a} (0.29)	**1.18**^{a} (0.66)	**1.11**^{a} (0.46)	**20.9**^{a} (5.3)
OA	**295.8**^{b} (91.3)	**30.1**^{b} (9.0)	**9.89**^{a} (0.95)	**1.46**^{a} (0.32)	**1.65**^{a} (0.41)	**0.83**^{a} (0.25)	**0.44**^{b} (0.13)	**18.7**^{a} (4.0)
IP×BC	**550.3**^{a} (61.9)	**69.0**^{a} (6.0)	**7.98**^{a} (0.58)	**1.02**^{b} (0.15)	**1.62**^{b} (0.15)	**1.47**^{a} (0.61)	**1.44**^{a} (0.35)	**21.6**^{a} (5.1)
NP×BC	**388.2**^{b} (124.2)	**41.3**^{b} (14.6)	**9.52**^{a} (0.73)	**1.10**^{b} (0.32)	**1.56**^{bc} (0.39)	**0.88**^{ab} (0.62)	**0.78**^{b} (0.28)	**20.2**^{a} (5.8)
IP×OA	**221.5**^{c} (28.5)	**23.2**^{c} (4.6)	**9.74**^{a} (1.35)	**1.17**^{b} (0.07)	**1.28**^{c} (0.19)	**0.65**^{b} (0.23)	**0.34**^{c} (0.07)	**16.6**^{a} (4.7)
NP×OA	**370.1**^{b} (65.3)	**36.9**^{b} (6.7)	**10.0**^{a} (0.35)	**1.75**^{a} (0.14)	**2.01**^{a} (0.15)	**1.01**^{ab} (0.13)	**0.53**^{bc} (0.10)	**20.8**^{a} (1.5)

Soil N concentration and mineralization

Soil total N concentrations and accumulation, at HE, were significantly higher in the IP than in the NP, and under tree crowns than in the open, but no significant interaction was observed between pastures and tree position. Similar trend was observed for total N concentrations at HO (Table 4).

The C:N ratio, at both HE and HO, was significantly higher under tree crowns than in the open, and no significant differences were observed between pastures.

The concentration of organic microbial biomass N (N_{mic}), at HE, was significantly higher in the NP than in the IP, and beneath tree crowns than in the open (Table 3); the interaction pasture x tree position showed that, in the open, N_{mic} was significantly higher in the NP than in the IP; and for the IP it was significantly higher beneath trees than in the open. At HO, N_{mic} was significantly higher beneath trees than in the open; the interaction indicates that beneath trees it was significantly higher in the IP than in the NP, but in the open it was significantly higher in the latter than in the former; also, for the IP, it was significantly higher beneath trees than in the open.

The proportion of N_{mic} relative to total N, at both HE and HO, was significantly higher in the NP than in the IP (Table 3). At HE, the difference between pastures was stronger in the open (2.6 times) than beneath trees (1.4 times), difference being narrow at HO.

The C_{mic}:N_{mic} ratio (Table 3), at both HE and HO was significantly higher in the open than beneath trees; at the HE it was higher in the IP than in the NP, but at HO the inverse occurred. At HE, in the open, it was significantly higher in the IP than in the NP, and for the IP it was significantly lower beneath tree crowns than in the open.

At HE, net ammonification was negligible for the IP and the NP in both tree positions (Table 4). In contrast, at the HO net ammonification was observed throughout the incubation period, being significantly higher in the IP than in the NP, and beneath tree crown than in the open (Figure 6). Net ammonification values in both IP and NP beneath tree crown were much higher (90.3 and 82.9 mg N kg^{-1} soil, respectively) than those observed in the open (29.8 and 1.82, mg N kg^{-1} soil, respectively). Differences between pastures were much higher in the open (16.3 times) than beneath trees (1.1 times).

Net nitrification N, at HE, was significantly higher in the NP than in the IP, and beneath tree crowns than in the open (Table 4). Beneath trees it was significantly higher in the IP than in the NP, but in open areas an opposite trend was observed; for the IP, it was significantly higher beneath trees than in the open.

Table 4 - Total N concentration, N content (N_c), C:N ratio, initial and net mineralized N ($N\text{-}NH_4^+$, $N\text{-}NO_3^-$), and N mineralized N per unit of soil N (MN/N), in the 0-10 cm soil layer under improved (IP) and natural (NP) pastures, beneath canopy (BC) and in the open (OA), at *Herdade dos Esquerdos* and *Herdade do Olival*. Values are means with standard deviations in brackets (n=6); different letters in the same column indicate significant differences within factor or interaction levels (p<0.05) by the Tukey or Waerden tests.

Systems	Total N	N_c	C:N	Initial mineral N			Net mineralized N			MN/N
				NH_4^+-N	NO_3^--N	(NH_4^+ +NO_3^-)-N	NH_4^+-N	NO_3^--N	(NH_4^+ +NO_3^-)-N	
	g kg^{-1}	g m^2		mg N kg^{-1}						mg g^{-1}
					HERDADE DOS ESQUERDOS					
IP	**2.14**^{a} (0.6)	**197.9**^{a} (54.7)	**12.9**^{a} (1.2)	**9.80**^{a} (9.9)	**8.62**^{a} (12.1)	**18.42**^{a} (21.2)	**-2.48**^{a} (8.8)	**121.0**^{b} (43.8)	**118.5**^{b} (42.4)	**59.4**^{b} (12.9)
NP	**1.42**^{b} (0.5)	**150.1**^{b} (48.7)	**12.8**^{a} (0.9)	**4.73**^{b} (0.4)	**1.67**^{b} (1.6)	**6.40**^{b} (1.7)	**2.92**^{a} (4.6)	**133.1**^{a} (12.5)	**136.0**^{a} (15.5)	**81.6**^{a} (8.2)
BC	**2.18**^{a} (0.5)	**207.5**^{a} (45.3)	**13.3**^{a} (1.0)	**10.03**^{a} (9.8)	**9.34**^{a} (11.7)	**19.37**^{a} (20.6)	**-2.08**^{a} (9.0)	**146.4**^{a} (20.0)	**144.4**^{a} (20.9)	**65.7**^{b} (18.7)
OA	**1.38**^{b} (0.5)	**140.5**^{b} (46.5)	**12.4**^{b} (0.8)	**4.51**^{b} (0.2)	**0.95**^{b} (0.8)	**5.45**^{b} (0.8)	**2.52**^{a} (4.5)	**107.6**^{b} (30.5)	**110.2**^{b} (33.5)	**75.3**^{a} (10.3)
IP×BC	**2.54**^{a} (0.5)	**228.9**^{a} (47.7)	**13.6**^{a} (1.0)	**15.17**^{a} (12.2)	**16.03**^{a} (13.8)	**31.20**^{a} (24.5)	**-5.39**^{a} (12.2)	**161.2**^{a} (11.2)	**155.8**^{a} (19.9)	**51.8**^{a} (14.4)
NP×BC	**1.82**^{a} (0.2)	**186.1**^{a} (31.8)	**13.1**^{a} (1.0)	**4.88**^{b} (0.6)	**2.66**^{b} (1.7)	**7.54**^{b} (1.8)	**1.24**^{a} (1.2)	**131.7**^{b} (15.3)	**133.0**^{a} (16.0)	**79.7**^{a} (9.6)
IP×OA	**1.74**^{a} (0.5)	**166.9**^{a} 43.4)	**12.2**^{a} (0.9)	**4.43**^{b} (0.2)	**1.21**^{b} (1.0)	**5.64**^{b} (1.1)	**0.44**^{a} (0.1)	**80.9**^{c} (15.0)	**81.3**^{b} (15.0)	**67.0**^{a} (4.7)
NP×OA	**1.03**^{a} (0.2)	**114.1**^{a} (33.4)	**12.5**^{a} (0.8)	**4.58**^{b} (0.1)	**0.69**^{b} (0.4)	**5.26**^{b} (0.3)	**4.60**^{a} (5.9)	**134.4**^{b} (10.2)	**139.0**^{a} (15.8)	**83.6**^{a} (6.8)
					HERDADE DO OLIVAL					
IP	**1.49**^{a} (0.2)	**127.5**^{a} (20.6)	**12.6**^{a} (2.5)	**6.55**^{a} (3.3)	**2.13**^{a} (2.0)	**8.68**^{a} (5.0)	**60.1**^{a} (34.5)	**138.3**^{a} (39.6)	**194.2**^{a} (69.2)	**69.9**^{b} (12.2)
NP	**1.18**^{b} (0.4)	**116.9**^{a} (42.0)	**11.5**^{a} (2.5)	**4.56**^{b} (0.3)	**0.44**^{b} (1.1)	**5.00**^{b} (1.1)	**42.4**^{b} (43.5)	**130.6**^{a} (17.1)	**172.5**^{a} (43.1)	**81.9**^{a} (10.1)
BC	**1.49**^{a} (0.4)	**130.5**^{a} (38.9)	**13.4**^{a} (2.4)	**6.56**^{a} (3.3)	**1.94**^{a} (2.3)	**8.50**^{a} (5.2)	**86.6**^{a} (14.5)	**152.4**^{a} (27.2)	**231.1**^{a} (36.3)	**73.0**^{a} (15.8)
OA	**1.18**^{b} (0.3)	**113.9**^{a} (24.4)	**10.7**^{b} (1.9)	**4.55**^{b} (0.3)	**0.63**^{b} (0.7)	**5.19**^{b} (0.8)	**15.8**^{b} (17.6)	**116.4**^{b} (21.1)	**135.6**^{b} (24.6)	**78.8**^{a} (8.0)
IP×BC	**1.58**^{a} (0.2)	**129.2**^{a} (24.6)	**14.0**^{a} (2.5)	**8.51**^{a} (3.8)	**3.12**^{a} (2.4)	**11.63**^{a} (5.8)	**90.3**^{a} (14.6)	**173.6**^{a} (7.6)	**255.6**^{a} (24.4)	**61.9**^{b} (9.6)
NP×BC	**1.39**^{a} (0.4)	**131.8**^{a} (50.6)	**12.8**^{a} (2.3)	**4.60**^{b} (0.1)	**0.76**^{a} (1.5)	**5.36**^{b} (1.5)	**82.9**^{a} (14.8)	**131.2**^{b} (22.2)	**206.7**^{b} (29.6)	**84.1**^{a} (12.7)
IP×OA	**1.39**^{a} (0.1)	**125.8**^{a} (16.7)	**11.2**^{a} (1.6)	**4.59**^{b} (0.2)	**1,14**^{a} (0.7)	**5.73**^{b} (0.8)	**29.8**^{a} (14.6)	**102.9**^{c} (19.8)	**132.7**^{c} (29.8)	**77.9**^{a} (9.1)
NP×OA	**0.97**^{a} (0.2)	**102.0**^{a} (25.5)	**10.1**^{a} (2.1)	**4.52**^{c} (0.4)	**0.13**^{a} (0.3)	**4.65**^{c} (0.5)	**1.82**^{b} (1.4)	**130.0**^{b} (12.2)	**138.4**^{c} (20.5)	**79.7**^{a} (7.4)

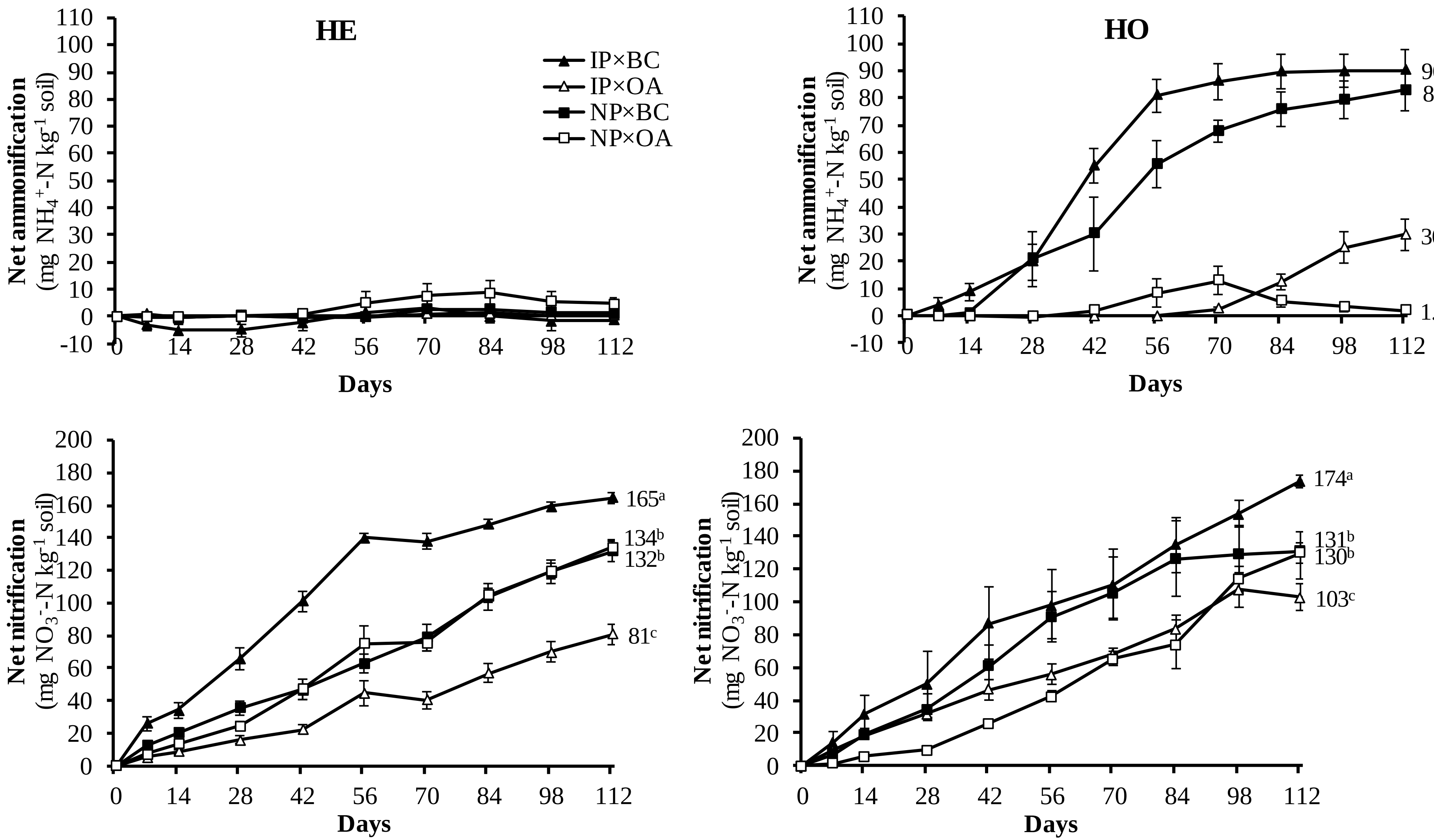


Figure 6 - Net ammonification and nitrification along 112 days of laboratory incubation of the 0-10 cm soil layer under improved (IP) and natural (NP) pastures, beneath tree canopy (BC) and in the open (OA), at *Herdade dos Esquerdos* (HE) and *Herdade do Olival* (HO). Points are means, bars are standard errors (n=6); different letters for the same day indicate significant differences between the interaction levels (p<0.05) by the Tukey test.

The lowest net nitrification was observed for the IP in the open, and at the end of the incubation period it was nearly a half (80.1 mg NO_3^--N kg^{-1} soil) of that registered from other HE soils (131 to 161 mg kg^{-1}).

At HO, net nitrification N was significantly higher beneath tree crowns than in the open, but no significant differences were observed between pastures (Table 4); interactions followed the trend described for HE. Net nitrification was significantly lower in soil with improved pasture in the open (102.9 mg NO_3^--N kg^{-1}) than in other soils (130.0-173 mg NO_3^--N kg^{-1}), while the highest value was observed in the IP beneath tree crowns (173 mg NO_3^--N kg^{-1}; Figure 6).

Net mineralized N (NH_4^+ +NO_3^-), at both HE and HO, followed the pattern exhibited by the net nitrification N (Table 4). At HE, values in soils with IP, in the open, showed the lowest values (81.3 mg mineralized N kg^{-1} soil), which were significantly lower than in the other studied HE systems (133.0-155.8 mg mineralized N kg^{-1} soil). In the HE open areas, values in the IP (132.7 mg mineralized N kg^{-1} soil) and in the NP (138.4 mg mineralized N kg^{-1} soil) were significantly higher than beneath tree crowns (respectively, 255.6 and 206.7 mg mineralized N kg^{-1} soil).

Net mineralized N per unit of initial N, at HE, was significantly higher in the NP than in the IP (81,6 and 59.4 mg mineralized N g^{-1} initial N**)**, and in the open than beneath (75.3 and 65.7 mg mineralized N g^{-1} initial N, respectively) tree crowns (Table 4); no interactions were observed between pastures and tree position. Values, at HO, were in the same range and were significantly lower in the IP (69.9 mg mineralized N g^{-1} initial N) than in the NP (81.9 mg mineralized N g^{-1} initial N). Net mineralized N per unit of initial N had only distinguished the IP beneath trees (61.9 mg mineralized N g^{-1} initial N) which was significantly lower than in the NP (84.1 mg mineralized N g^{-1} initial N), and significantly lower than in the open (77.9 mg mineralized N g^{-1} initial N).

Soil fertility

At HE, no significant differences were observed for pH values in water between pastures and between tree positions, while determinations in the KCl were significantly higher in the IP than the NP, and beneath tree crowns than in the open (Table 5). At HO, pH values in water were significantly higher in the NP than in the IP, and in the open than beneath tree canopy, but no interactions were observed between pastures and tree position (Table 5); in the open, values were significantly higher in the NP than in the IP, and values for NP were significantly higher in the open than beneath trees.

At both HE and HO, soil extractable P concentration was significantly higher in the IP than in the NP, and beneath tree crown than in the open, but no interactions between pastures and tree position were observed (Table 5).
At the HE soils, concentration of extractable K was significantly lower under the IP than in the NP, and higher beneath trees than in the open (Table 5). At the HO, it was significantly higher beneath trees than in the open, but beneath tree crown the concentration was significantly higher in the IP than in the NP, and within IP was significantly higher beneath trees than in the open.
Concentrations of exchangeable Ca^{2+}, at HE, were significantly higher in the IP than in the NP, and beneath tree crown than in the open, but no interactions were observed (Table 5). Similar trend was observed for Mg^{2+} concentrations between pastures, at HO, and tree position, at HE. Concentrations of K^{+}, at HE, were significantly lower in the IP than in the NP, and significantly higher beneath tree crown than in the open. At HO, concentrations of K^{+} were significantly higher beneath tree crowns than in the open, and in the open were significantly higher in the NP than in the IP; for the IP, exchangeable K^{+} concentrations were significantly higher beneath tree crowns than in the open.

Table 5 - Soil pH (in H_2O and KCl), extractable P and K, and exchangeable non-acid cations (Ca^{2+}, Mg^{2+}, Na^{+}, K^{+}) in the 0-10 cm soil layer under improved (IP) and natural (NP) pastures, beneath canopy (BC) and in the open (OA), at *Herdade dos Esquerdos* and *Herdade do Olival*. Values are means with standard deviations in brackets (n=12); different letters in the same column indicate significant differences within factor or interaction levels (p<0.05) by the Tukey test.

Systems	pH		Extractable		Exchangeable			
	H_2O	KCl	P	K	Ca^{2+}	Mg^{2+}	Na^{+}	K^{+}
			mg kg^{-1}		$cmol_c$ kg^{-1}			
HERDADE DOS ESQUERDOS								
IP	**5.62**^{a} (0.26)	**4.54**^{a} (0.27)	**62.9**^{a} (27.2)	**150.7**^{b} (102.6)	**4.68**^{a} (1.60)	**0.99**^{a} (0.65)	**0.14**^{b} (0.06)	**0.41**^{b} (0.25)
NP	**5.61**^{a} (0.21)	**4.39**^{b} (0.28)	**6.4**^{b} (3.6)	**207.5**^{a} (72.1)	**2.87**^{b} (1.02)	**0.74**^{a} (0.27)	**0.20**^{a} (0.02)	**0.53**^{a} (0.17)
BC	**5.66**^{a} (0.23)	**4.62**^{a} (0.25)	**41.7**^{a} (39.5)	**231.6**^{a} (93.9)	**4.58**^{a} (1.54)	**1.12**^{a} (0.55)	**0.17**^{a} (0.05)	**0.61**^{a} (0.18)
OA	**5.57**^{a} (0.23)	**4.31**^{b} (0.23)	**27.6**^{b} (27.5)	**126.5**^{b} (53.1)	**2.97**^{b} (1.26)	**0.60**^{b} (0.29)	**0.17**^{a} (0.05)	**0.33**^{b} (0.15)
IP×BC	**5.67**^{a} (0.25)	**4.68**^{a} (0.26)	**74.9**^{a} (29.0)	**204.7**^{a} (116.6)	**5.58**^{a} (1.45)	**1.37**^{a} (0.68)	**0.14**^{a} (0.06)	**0.57**^{a} (0.22)
NP×BC	**5.66**^{a} (0.21)	**4.56**^{a} (0.24)	**8.4**^{a} (3.5)	**258.5**^{a} (57.5)	**3.58**^{a} (0.83)	**0.87**^{a} (0.22)	**0.20**^{a} (0.02)	**0.65**^{a} (0.12)
IP×OA	**5.57**^{a} (0.27)	**4.40**^{a} (0.21)	**50.9**^{a} (20.0)	**96.6**^{a} (45.3)	**3.79**^{a} (1.23)	**0.60**^{a} (0.33)	**0.14**^{a} (0.06)	**0.24**^{a} (0.14)
NP×OA	**5.56**^{a} (0.20)	**4.22**^{a} (0.22)	**4.4**^{a} (2.4)	**156.4**^{a} (43.7)	**2.16**^{a} (0.61)	**0.60**^{a} (0.25)	**0.20**^{a} (0.02)	**0.41**^{a} (0.11)
HERDADE DO OLIVAL								
IP	**5.65**^{b} (0.23)	**4.34**^{a} (0.28)	**25.0**^{a} (10.6)	**138.3**^{a} (67.2)	**4.28**^{a} (0.94)	**1.40**^{b} (0.35)	**0.14**^{b} (0.02)	**0.35**^{a} (0.19)
NP	**5.80**^{a} (0.41)	**4.17**^{b} (0.39)	**2.4**^{b} (1.9)	**118.4**^{a} (42.5)	**3.98**^{a} (1.18)	**3.02**^{a} (0.95)	**0.22**^{a} (0.04)	**0.37**^{a} (0.13)
BC	**5.52**^{b} (0.28)	**4.13**^{b} (0.43)	**16.2**^{a} (15.5)	**159.6**^{a} (58.9)	**3.98**^{a} (1.11)	**2.20**^{a} (0.87)	**0.18**^{a} (0.06)	**0.45**^{a} (0.15)
OA	**5.93**^{a} (0.27)	**4.38**^{a} (0.15)	**10.8**^{b} (11.3)	**95.2**^{b} (27.7)	**4.28**^{a} (1.03)	**2.22**^{a} (1.27)	**0.18**^{a} (0.04)	**0.26**^{b} (0.10)
IP×BC	**5.59**^{bc} (0.27)	**4.33**^{bc} (0.37)	**30.8**^{a} (8.5)	**187.0**^{a} (57.4)	**4.47**^{a} (0.64)	**1.56**^{b} (0.27)	**0.14**^{a} (0.02)	**0.49**^{a} (0.16)
NP×BC	**5.46**^{c} (0.28)	**3.93**^{c} (0.40)	**2.8**^{a} (2.2)	**132.2**^{b} (48.1)	**3.50**^{a} (1.28)	**2.84**^{a} (0.80)	**0.23**^{a} (0.05)	**0.41**^{ab} (0.14)
IP×OA	**5.71**^{b} (0.18)	**4.34**^{b} (0.14)	**19.6**^{a} (9.7)	**85.0**^{c} (18.2)	**4.08**^{a} (1.17)	**1.25**^{b} (0.36)	**0.15**^{a} (0.02)	**0.20**^{c} (0.05)
NP×OA	**6.14**^{a} (0.14)	**4.42**^{a} (0.15)	**2.0**^{a} (1.5)	**104.6**^{bc} (32.2)	**4.47**^{a} (0.88)	**3.19**^{a} (1.08)	**0.21**^{a} (0.03)	**0.33**^{b} (0.10)

DISCUSSION

Soil physical status

Soils from the study pastures showed a wide range of bulk density values (1.18 to 1.72 g cm^{-3}), and the range at *Herdade dos Esquerdos* (1.18-1.48 g cm^{-3}) was substantially different from that observed at *Herdade do Olival* (1.48-1.72 g cm^{-3}). Results obtained at *Herdade dos Esquerdos* indicate that a 35-year period of improved pasture management can lead to decreasing soil bulk density, relative to natural pasture, both beneath trees and in open areas, following trends reported by Gómez-Rey et al. (2012) for a 26-year old improved pasture at a similar site. Such a trend may be mostly associated with the observed increase in soil organic C concentration, which are indicative of higher soil organic matter contents and related soil biological activity (Rabot et al., 2018). It is noteworthy that, despite the increase in stocking rate under the improved pasture, soil bulk density was lower (1.18-1.36 g cm^{-3}) than that in the natural pasture (1.36-1.48 g cm^{-3}), values being typical of non-compacted sandy loam textured soils (below 1.60 g cm^{-3}), for which no constrains to plant roots growth are expected (Weil and Brady, 2017). It is therefore clear, that the increase in sheep grazing density practiced in the improved pasture system, may not only present deleterious effects on soil physical conditions (e.g. total and aeration porosity), but also contribute to an improvement of the soil physical status, as compared with the extensively grazed natural pasture system.

However, results of the present study also indicate that the establishment of improved pastures does not necessarily ensures soil adequate physical conditions. Indeed, at *Herdade do Olival*, where pastures are grazed by cattle, soil bulk density values were also lower under improved (1.48 and 1.62, respectively beneath trees and in the open) than natural pasture (1.52 and 1.72 g cm^{-3}, respectively beneath trees and in the open), but those were mostly above the threshold for non-compacted soils with loam texture (1.5 g cm^{-3}; Weil and Brady, 2017). These high soil bulk density values may correspond to a strong decrease of total soil porosity (and aeration porosity), suggesting that, despite some soil structural preservation may have resulted from the 16-year old improved pasture management, it was not sufficiently effective to avoid soil physical degradation associated with cattle presence (Pulido et al., 2018). Soil structural deformation processes such as compaction, pugging and poaching, can result from the treading action of livestock on soil surface (Billota et al., 2007). Cattle hooves are known to exert greater forces onto the soil surface, creating much higher static pressures than those of sheep (Greenwood & Mackenzie, 2001; Billota et al., 2007). Additionally, cattle permanence throughout the whole year, including the wet season, when soil moisture is high, may further enhance soil physical damaging under these systems (Pulido et al. 2017; Billota et al. 2017).

As values of soil bulk density observed in the *Herdade do Olival* loam textures soils are within the “very high” soil degradation category (>1.6 g cm^{-3}), reported by Pulido et al. (2017) for similar Mediterranean rangelands occurring mainly over sandy-loam textured soils, and considering the present study stocking rate of 0.7 LU ha^{-1} $year^{-1}$, as compared to the 1 LU ha^{-1} $year^{-1}$ threshold found by those authors, soil texture seems to be conditioning the present study systems susceptibility to the physical degradation effects of cattle treading (Weil and Brady, 2017; Pulido et al. 2017).

Values of soil bulk density in the improved pasture, at *Herdade dos Esquerdos*, indicate more favourable total soil porosity conditions, particularly higher aeration porosity (air filled pores at soil water potential of -10 kPa), which has doubled that of the natural pasture system. Accordingly, even though soil saturated hydraulic conductivity in both pastures was consistently above 10 mm h^{-1}, a higher proportion of values higher than 100 mm h^{-1} was obtained for the improved pasture. These data suggest that the improved pasture management, and associated higher sheep grazing intensity, did not led to restrictions regarding soil water movement through the soil surface layer, following a pattern reported for non-compacted soils (Brevik & Fenton, 2012).

Nevertheless, negative effects over soil porosity, attributed to soil compaction by grazers treading, were observed in both natural and improved pastures at *Herdade do Olival*. While total soil porosity was lower in this farm (0.35-0.44 m^3 m^{-3}) than in the *Herdade dos Esquerdos* (0.44-0.55 m^3 m^{-3}), this effect was even more evident on the estimated air filled porosity (at water potential of -10 kPa), suggesting that, under *Herdade do Olival* conditions, soil aeration porosity is nearly negligible, as reported by Greacen & Sands (1980), and by Sharrow (2007) for compacted soils. This trend fully agrees with the overall low saturated hydraulic conductivity observed for these soils, with many observations under 1 mm h^{-1}, following reports by Brevik & Fenton (2012) and Greenwood & Mackenzie (2001), on heavily compacted soils. As such a hydrological behaviour suggests a decrease in soil infiltration rates, increased risks of surface runoff must also be considered (Billota et al., 2007; Pulido et al., 2017). Also, under these unfavourable conditions for soil drainage, soil water saturation and restriction to soil aeration during the wet season may become a problem, with negative effects on pasture productivity (Pulido et al., 2018), tree vitality (Costa et al., 2008; Hernández-Lambraño et al., 2018), soil nutrient cycling and potential soil greenhouse gas emissions (Oenema et al., 1997; Oertel et al., 2016).

The uneven spatial distribution pattern by animal treading strongly reflects in the high spatial variability observed for values of bulk density and saturated hydraulic conductivity, which is in line with trends reported for soils affected by grazing by Billota et al. (2007) and Sharrow (2007). Yet, in a parallel determination at the *Herdade do Olival*, in visually identified high pugging sites, soil bulk density was similar (1.74 g cm^{-3}; data not showed) to the mean value obtained for the natural pasture open areas with the current study sampling design, suggesting the presented data may broadly reflect these pastures soil physical conditions.

Variations in soil bulk density affected other soil properties, such as water retention and water availability. In *Herdade dos Esquerdos*, it is noteworthy that lower water retention capacity and water availability (difference between water contents at -10 and -1500 kPa), associated with higher proportion of hydraulic conductivity values above 100 mm h^{-1}, was exhibited by soils in the 35-year old improved pasture, compared to those in the natural pasture. This trend is in accordance with data reported by Greacen & Sands (1980) and Ordóñez et al. (2018), who found soil water holding capacity was increased by soil compaction, because of the occurrence of smaller pores able to retain water (Sharrow, 2007). In this sense, improved pastures long-term management does not necessarily lead to higher soil water retention or availability, but mostly results in better soil hydrological and aeration conditions.

Notwithstanding, data obtained at *Herdade do Olival* did not agree with the increase of water holding capacity reported by Greenwood & Mckenzie (2001) for compacted soils. Despite the observed differences in soil bulk density indicate different degrees of soil compaction, between natural and improved pasture, these soils water holding capacity was similar, both under tree crowns and in the open. Moreover, when compared to the *Herdade dos Esquerdos* sandy loam soils, *Herdade do Olival* loamy soils showed lower water availability, suggesting that the excessive compaction in the former, may not lead to increasing water holding capacity (Zhang et al. 2017), and can, in addition, lead to an increased water retention in smaller pores, and therefore a reduction of roots access to water. In fact, low soil porosity, due to the development of excessive soil compaction in *Herdade do Olival*, was associated with a very small air filled porosity (0.01-0.05 m^3 m^{-3}), at field capacity conditions (-10 kPa). As these values are much lower than the 0.1 m^3 m^{-3} threshold assumed to be critical for plant growth (Chen et al., 2014; Fashi et al., 2017), such compacted and low water availability soil conditions suggest that the near soil surface physical conditions are strongly restrictive for potential root growth (Weil and Brady, 2017; Leão et al., 2006). Furthermore, water resources use efficiency can be reduced, raising critical issues under these Mediterranean environments (Turner, 2004).

Overall, changes in soil physical conditions (e.g. soil bulk density and saturated hydraulic conductivity) caused by the establishment of improved pastures, compared to the respective natural systems, may be mostly related to herbaceous biomass production increase (both above and belowground), as their organic inputs and associated biological activity can promote soil structure development (Bronick and Lal, 2005; Oades, 1984). Yet, no consistent changes were revealed regarding soil aggregation development or stability enhancement, suggesting the period of pasture establishment and the magnitude of changes were not sufficient to express effects on the study soils structural organization. As our results suggest that soil bulk density and related characteristics, such as saturated hydraulic conductivity, may be useful indicators for assessment of soil physical quality changes, using visual methodologies, such as the one developed by Ball et al. (2007), could also be of great usefulness for these pasture systems monitorization purposes.

Soil aggregates (1-2 mm) stability in water was high (above 93%) for both farms and pasture management systems. Accordingly, relatively low water dispersion ratios of clay particles (0.05-0.16) were determined, which may reflect these soils nearly undisturbed management (no tillage or occasional tillage), across all study management systems (Bronick and Lal, 2005; Totsche et al., 2018). Nevertheless, further studies are needed for a deeper understanding on soil microaggregation temporal and spatial dynamics, as highlighted by Totsche et al. (2018).

Data obtained in both farms highlight the recognized role of oak trees on the improvement of soil physical conditions in pasture systems, as they contributed to decrease soil bulk density and increase soil porosity, following trends reported by Belsky et al. (1989), Dahlgren et al. (1997, 2003) and Rhoades (1997). Such trends may be mostly a consequence of tree organic residue inputs, via litterfall and root litter (Escudero et al., 1985; Sá et al., 2005), and soil surface protection by the accumulation of a soil litter layer (Nunes, 2001; Fisher and Binkley, 2000), leading to higher concentration of soil organic C and stronger soil biological activity (Spohn, 2015; Waldrop and Firestone, 2006). In this sense, maintenance of oak trees at the *montado* landscape level can be a management tool to reconcile increasing grazing intensity with soil quality improvement and resilience to degradation. However, the results of the current study indicate that the role of trees in promoting soil physical quality and reversing soil physical degradation patterns, is strongly dependent on the management systems. Indeed, under the more intensive grazing conditions at *Herdade do Olival*, the presence of oak trees in both improved and natural pastures was not enough to achieve adequate soil physical conditions (such as soil aeration, water infiltration and drainage) and did not ensure suitable rooting environment, which in turn may harm both pasture and tree vitality and productivity (Pulido et al., 2018).

Soil organic C accumulation and mineralization

Soil organic C concentrations at *Herdade dos Esquerdos* and at *Herdade do Olival* varied from 13 to 36 and 10 to 27 g C kg^{-1} respectively, which are within those reported by Pulido-Fernández et al. (2013) for Mediterranean rangelands, similar to those reported by Gómez-Rey at al. (2012) for improved and natural pastures under similar systems, but higher than those reported by Rodeghiero et al. (2011) for the top soil of certain *montado* areas.

Sowing improved pastures generally leads to increments in soil organic C and N in oak woodlands (Hernández-Esteban et al., 2018), but their accumulation must be strictly referred to the alternative management (Soussana et al., 2004; Powlson et al., 2011), that is, the comparable natural pasture systems. Indeed, the 35-year old improved pasture at *Herdade dos Esquerdos* showed 1.6 and 1.4 times increase in soil organic C concentration, in open areas and beneath tree crowns, respectively, relative to the natural pasture. The amount of soil organic C up to 10 cm depth was increased by 42% (0.60 kg m^{-2}) in the open, and by 22% (0.54 kg m^{-2}) beneath tree crowns, following the pattern reported by Gómez-Rey et al. (2012) for a 26-year old improved pasture in a similar site, and results reported by Teixeira et al. (2011) for treeless 5-year old improved pastures, under similar Mediterranean climate. The annual rate of organic C accumulation, relative to the natural pasture (about 0.017 and 0.015 kg m^{-2}, in the open and beneath tree crowns, respectively), was much lower than that reported by Conant et al (2001) for incorporation of legumes in grasslands (0.075 kg m^{-2}), but follow results reported by Hernández-Estebán et al. (2018) for a chronosequence of improved pastures established under evergreen oak woodlands. Such a low rate of annual soil organic C accumulation may be associated with the stabilization of organic C accumulation along the 35-year period, differences in stocking rates, and the presence of native shrubs in the natural pasture, which are also recognized to enhance soil organic C status in oak woodlands (Simões et al., 2009). At *Herdade do Olival*, the amount of soil organic C in the improved pasture, relative to the natural pasture, was increased by 36% (0.38 kg m^{-2}) in the open, and 12% (0.19 kg m^{-2}) beneath trees, representing a similar annual rate of organic C accumulation (about 0.024 and 0.012 kg m^{-2}, respectively in the open and beneath tree crowns) to that observed at *Herdade dos Esquerdos*, despite the differences in the establishment period, soil type and grazing management.

The observed soil organic C accumulation following the establishment of improved pastures in the study farms can be mostly associated with their greater above- and below-ground biomass productivity (Hernández–Estebán et al., 2018; Gómez-Rey et al. 2012). In fact, at *Herdade dos Esquerdos*, annual above ground biomass of improved pasture open areas was estimated to be about the double (476 g dry matter m^{-2}) of that in the comparable natural pasture (234 g dry matter m^{-2}; unpublished); whereas, at *Herdade do Olival*, herbaceous biomass production in the improved pasture was almost thrice of that in the natural pasture (413 and 148 g dry matter m^{-2}, respectively; unpublished).

Differences in pasture biomass production are also, at least partially, in accordance with the higher soil organic C concentrations in tree-covered areas. Beneath *Herdade dos Esquerdos* tree canopies, despite similar mean annual herbaceous biomass production in improved and natural pastures (288 and 245 g dry matter m^{-2}, respectively; unpublished), soil organic C was increased in about 22% (0.54 kg m^{-2}) in the former, compared to the latter. Under the *Herdade do Olival* trees, the increase in accumulated soil organic C due to improved pasture management was only of 12% (0.19 kg m^{-2}) but pasture productivity was about 60% higher (263 *versus* 166 g dry matter m^{-2} for improved and natural pastures respectively; unpublished). This result is in line with the differences in soil type and grazing management, as in the improved pasture from *Herdade dos Esquerdos*, both vegetation biomass production and soil organic C accumulation seem to have reached a new stability level, while in the *Herdade do Olival* improved pasture, although herbaceous biomass productivity is considerably higher than that in the comparable natural pasture, soil organic C accumulation appears lower than that in *Herdade dos Esquerdos*.

Considering the 4 per 1000 goal (Minasny et al., 2017; http://4p1000.org), results of the present study indicate that the increment of soil organic C storage in the open areas, as a result the of improved pasture establishment, was 11 and 23‰ for the 35-year and the 16-year old pastures, respectively, and beneath tree crowns the accumulation increment was 6.1 and 7.5‰, for the same systems. The fact that such rates are considerably above the 4‰ goal, is in agreement with the trends reported by Corbeels et al (2017) for African agroforestry systems. Higher carbon storage increment in the soil of the younger pasture (at *Herdade do Olival*) might be related to the system lower organic C status, while the much lower increment in the soil beneath tree crowns might be a result of the long-term input of tree litterfall, and thus of the higher initial soil organic C status. These results undoubtedly indicate that improved pasture establishment is an opportunity in Mediterranean environments to sequester organic carbon (Hernández-Esteban et al. 2018) and, therefore, to fulfil global commitments regarding climate change mitigation. Such great potential for soil organic C storage in the *montado* system, may occur especially in areas with strong depletion of soil organic C, where practices to restore soil C are needed. Yet, as the efficiency of such practices will depend on the specific ecological and socio-economic conditions and may be driven by farm income increase, due to crop productivity enhancement (Corbeels et al. 2017), the relatively long period of time needed for the present study observed benefits to take place (1.6 and 3.5 decades, for HO and HE, respectively) must be considered and addressed at both policy and managers levels.

Tree canopy cover has increased soil organic C content in 0.94 (56%) and 1.0 kg m^{-2} (69%) in the improved and natural pasture, respectively, relative to the open areas at *Herdade dos Esquerdos*. At *Herdade do Olival*, tree-related increments were of 0.37 and 0.56 kg m^{-2} (26 and 54%). These results suggest that the effect of trees themselves is dependent on factors other than the management system, in agreement with the widely acknowledged role of trees in the soil organic C enhancement of silvopastoral systems, compared with native pastures alone (e.g., Dahlgren et al., 1997; Gómez-Rey et al., 2011; Moreno et al., 2007; Howlett et al., 2011), and of other agroforestry systems (e.g. Cardinael et al., 2015; Cardinael et al., 2017; Pardon et al., 2017;). It must be emphasized that the contribution of scattered oak trees to the current study *montados* soil organic C build-up, may exceed the increments obtained by the long-term management of improved pastures, even more so if considering their extended influence far beyond the crowns projection (Simón et al., 2013). Also, as trees play an important role in the C cycle and on the distribution of organic C (Howlett et al., 2011, Nunes; 2001), tree density should be approached as a valuable management tool towards soil organic C sequestration goals. Also, as Portuguese policies on C sequestration are currently attributing incentives to the single decision of sowing pastures (APA, 2017), regardless of any other management or site-specific factors, the specific tree role in the *montado* system deserves further consideration and discussion (Rhoades, 1997).

The proportions of soil particulate organic matter (POM) at *Herdade dos Esquerdos* (32-48%) and *Herdade do Olival* (32-41%) are within those reported for shrub encroached oak woodlands (28-45%; Gómez-Rey et al., 2013) and dense oak woodlands (42-45%. Rodrigues at al., 2019), but are higher than those in agroforestry systems with arable crops (22-30%; Borges et al., 2013), and lower than those in degraded soils excluded from grazing (62-63%, Simões et al., 2009).

It is noteworthy that the major registered changes of organic C concentrations in the study soils, were mostly explained by those observed in the POM fraction. In fact, in soils at *Herdade dos Esquerdos*, the POM-C concentration increases, due to either improved pasture or tree cover (about doubled), was stronger than that observed for the total soil organic C (about 1.5 and 1.7 times, respectively). For instance, beneath the trees, the concentration of organic C allocated in the POM fraction in the improved pasture was 2.2 times higher than in the comparable natural pasture, whereas the difference for the total organic C was only 1.4 times; also, the concentration in improved pastures beneath trees was twice of that observed for the same pasture in the open, but the concentration of total organic C was only about 1.6 times higher. Such differences were much more visible when the soil mineral associated carbon (MA-C) was considered (that is, the difference between the concentration of total soil organic C and the concentration of POM-C). In fact, in the first case (improved *versus* natural pasture, beneath tree crowns) the concentration of MAC was of the same magnitude, while in the second (beneath trees *versus* open, in improved pasture) it was 1.2 times higher, following results reported by Nogueira et al. (2016). This trend suggests that, at the *Herdade dos Esquerdos*, the enhancement of POM accumulation is being favoured by the improved pasture and tree cover interaction, as suggested by the higher POM-C proportion of total organic C. Such interaction, between tree cover and improved pasture, was reported by Gómez-Rey at al. (2012) and Rodrigues et al. (2015) in studies developed in similar sites, and may be related to the high inputs of tree and pasture litter, combined with the soil stability conditions (absence of tillage). Indeed, Six et al. (2000) have found that the initially faster soil microbial transformation of fresh POM residues can be rapidly slowed down by the macroaggregates formation and POM particles enclosure. The chemically active POM residues (frequently designated as labile organic matter) are then physically protected from microbial activity and can be considered stabilized, as long as such structural units are not disrupted (e.g. by soil tillage), a process which enhances the potential of soil C sequestration (Six et al., 2000; Six et al., 2002). Such trend of POM accumulation was not observed for improved pasture in the open areas, which may be explained by the lower residue inputs. This fact suggests a comparably lower potential of long-term improved pastures to enhance soil organic C sequestration in the treeless areas, which is in agreement with Hoosbeeck et al. (2018) observations, in tropical silvopasture systems.

At *Herdade do Olival*, soil organic C increments partition between the POM and MA fractions was not markedly altered by the pasture system or the tree cover. In fact, the increments in total soil organic C concentration were of similar magnitude for both POM-C and MA-C concentrations, whether considering sowed pasture (1.4 times higher) or tree cover effects (1.5 times higher). Such a trend may be related to the sowed pasture age and respective floristic composition. Indeed, sown species adaptation and persistence may vary with a large number of factors, but their coverage is most likely to be reduced with time (Hernández-Esteban et al., 2018; Carranca et al., 2015). Accordingly, a comparatively higher species diversity has been generally found at the current study younger improved pasture at *Herdade do Olival*, than at the older one, at *Herdade dos Esquerdos* (FCT, 2014).

The hot water soluble C (HWS-C) has been considered as a very sensitive indicator regarding effects of soil management or land use changes (Rovira and Vallejo, 2007; Ghani et al., 2003). In fact, at *Herdade dos Esquerdos*, the concentration of this fraction in the improved pasture was 2.3 times higher than in the natural pasture, whereas the incremental ratios for the POM-C and the total organic C were about 2.0 and 1.5, respectively. Furthermore, the proportion of HWS-C, relative to total organic C, was about 1.4 five times higher in the improved than in the natural pasture, regardless of tree position.

Although an active microbial community is commonly associated with higher HWS-C proportions (Iqbal et al., 2010; Marschner and Bredow, 2002), a lower proportion of microbial C (1.5 times), similar C mineralization per carbon unit (C_{min}/C), and reduced N cycling (discussed below) were observed under the improved pasture in open area, as compared to the natural pasture. This trend suggests the existence of strong differences between the study pastures, regarding soil organic matter turnover processes, possibly related to differences in soil microbial community composition. Although similar metabolic coefficients have been calculated (at the 7th day of laboratory incubation), it is noteworthy that the initial microbial biomass C to N ratio was higher for the improved pasture in the open areas, probably as a result of relatively low soil microbial biomass N concentration and proportions. Such a result may be explained by vegetation cover changes associated with the improved pasture establishment, as several authors have reported a clear relationship between vegetation type and soil microbial community composition (e.g. Rosenzweig et al., 2016; Garcia-Franco et al., 2015; Waldrop & Firestone, 2006). As natural occurring shrubs have been found to influence soil organic matter contents and dynamics under *montado* systems (Rodrigues et al. 2019; Gómez-Rey et al., 2013; Simões et al., 2009), their removal due to improved pasture installation and management (including grazing intensity) may have contributed to change microbial communities in the respective soils.

Soil N accumulation and mineralization

For both *Herdade dos Esquerdos* and *Herdade do Olival*, the introduction of higher proportion of legume species by pasture sowing has enhanced the soil atmospheric nitrogen (N) fixation potential through legume roots and *Rhizobium* bacteria symbiosis, thus promoting soil N accumulation (Carranca et al., 2015; Haynes and Williams, 1993). This has led to higher accumulation of N in soils of the improved pastures than those measured in natural pastures, especially at the *Herdade dos Esquerdos*. It is noteworthy that the C:N ratio in soils under the improved pastures is of the same magnitude of that in soils of the comparable natural pastures (12-14 at *Herdade dos Esquerdos*, 10-14 at *Herdade do Olival*), indicating negligible differences regarding the quality of organic substrates in the soil, which is probably reflecting the effect of the long period elapsed from pasture sowing, along which the reduction of the legumes proportion is expected (Carranca et al., 2015; Hernández-Esteban et al., 2018). Therefore, at long term, soils under improved pastures might accumulate organic C and N at similar rates as those under natural pastures, although their cycling may differ.

Despite of the observed similarities in soil organic substrates, the net mineralized N, under laboratory incubation conditions, was lower in the improved than in the natural pastures, especially at *Herdade dos Esquerdos*; moreover, the net mineralized N per unit of soil N was lower in soils of the improved than the natural pastures, at both farms. These trends are not in agreement with those reported by Rodrigues et al. (2019) for a 5-year old improved pasture growing under dense cork oak woodlands, in which a strong decrease of the C:N ratio and a higher potentially mineralized N per unit of soil N were observed. They also disagree with results reported by Gómez-Rey et al. (2012), who observed a potential enhancement of soil N mineralization for long-term improved pastures (both under and beyond oak canopies), which showed no differences in the soil C:N ratio. The contrasting trend observed in the current study may be related with changes in the composition of soil microbial communities, associated with the improved pasture management.

For instance, at the *Herdade dos Esquerdos*, although similar soil C:N ratios were determined in improved and natural pastures soils, in open areas, the homogeneous herbaceous cover of the improved pasture has been replenishing soil with considerably lower C:N ratio organic substrates (25-40; Carranca et al., 2015; Gómez-Rey et al., 2011), as compared to those from shrubs occurring at the natural pasture (60-80; Simões et al., 2009). Additionally, the higher stocking rates practiced at the improved pasture, compared to those at the natural pasture, could also have determined differences in soil organic substrates composition, due to higher animal depositions (Haynes and Williams, 1993). As a similar trend was observed at Herdade do Olival, and in both farms underneath the trees - soil similar C:N ratios, but lower net N mineralization per unit of initial N in improved than natural pastures - a transversal effect of improved pasture long-term management becomes evident, which is in accordance with some studies on the effect of vegetation cover over soil microbial communities composition (e.g. Garcia-Franco et al., 2015; Rosenzweig et al., 2016; Waldrop and Firestone, 2006), and on grazing intensification over soil biochemical functioning (e.g. Oenema et al., 1997; Uribe et al., 2015). However, these mechanisms could not be fully understood by the current study design, so further studies are needed to clarify the processes involved in the observed soil functions modifications.

Remarkable N mineralization patterns differences were observed between the study farms. In fact, at *Herdade do Olival*, especially beneath tree crowns, ammonium accumulation was observed, while at the *Herdade dos Esquerdos* net nitrification has prevailed. Such a difference may be mostly associated with different grazers (and stocking rates), as at *Herdade do Olival* cattle may produce larger urine patches, where urea concentrations are high, which is firstly mineralized into ammonium (Oenema et al., 1997). Animal preferential activity in shaded areas (Haynes and Williams, 1993) may explain why this trend is more pronounced underneath tree crowns. Although ammonium maintenance is not common under soil aerobic conditions, the observations of the present study may be related to the relatively short incubation period (16 weeks), which may have not been enough to promote N substrates complete oxidation. For example, Goméz-Rey et al. (2010) results from 35-weeks long laboratory incubations, with soils from eucalypt plantation, have showed that the initial net ammonification could be decreased in time, as net nitrification develops. Moreover, differences in soil microbial communities' composition and functioning may also determine such strong modifications to N mineralization, as nitrifying bacteria are usually weaker competitors for ammonia, compared with heterotrophic species or plants roots (Verhagen et al., 1995). In the light of the present study results, further studies are needed to clarify the observed N mineralization patterns.

It is noteworthy that the study soils from areas beneath tree canopy showed generally higher potential for mineral N availability than those from open pastures. The fact that the net mineralized N per unit of soil N was, on the contrary, lower under the trees, suggests that such mineral N availability enhancement may be mostly reflecting the higher organic substrates (and N) in these soils, due to tree litterfall, in line with Gómez-Rey et al. (2012) and Shvaleva et al. (2014) results, under similar *montado* systems. This result could raise environmental concerns on N losses, particularly considering the fact that most of the mineral N enhancement was by NO_3^--N, which can be easily moved down the soil profile and cause environmental contamination (Di and Cameron, 2002). Notwithstanding, as oak tree root uptake has been reported to greatly reduce nitrate leaching (Nunes, 2004), study soils *in situ* nitrate losses are probably very low.

Soil fertility

Results of the current study show that the establishment of improved pasture in both farms has undoubtedly influenced soil fertility. Indeed, the concentration of soil extractable P at *Herdade dos Esquerdos* changed, according to the Portuguese scale of soil P availability (LQARS, 2006), from the level “very low” (<25 mg P_2O_5 kg^{-1} soil) in the natural pasture system, to the level “high” (100-200 mg P_2O_5 kg^{-1} soil) in the improved pasture system; while at *Herdade do Olival* it has changed from the level “very low”, to the levels “low” (25-50 mg P_2O_5 kg^{-1} soil) and “medium” (50-100 mg P_2O_5 kg^{-1} soil), in the open and beneath tree crowns, respectively. This trend fully agrees with results reported by Gómez-Rey *et al*. (2012) for a 26-year old improved pasture under similar ecological conditions. Such remarkable soil P availability enhancement is a consequence of the continued phosphate fertilizer applications followed in the current improved pasture management systems. Differences in the changes degree between farms may be mostly related to the time elapsed from the installation of improved pastures, as the annual increment of soil extractable P estimated at the *Herdade dos Esquerdo*s (about 1.3 and 1.9 mg P kg^{-1} in the open and beneath tree crowns, respectively) is close to that at the *Herdade do Olival* (1.2 and 2.0 mg P kg^{-1} soil, respectively). As the soil in the 35-year old improved pasture is close to the threshold for optimum pasture development under Mediterranean climate conditions (Serrano et al., 2011), at such high levels of soil extractable P saturation eventual losses by leaching and run-off should not be disregarded, considering the management of improved pastures in a long-term perspective (Horta and Torrent, 2010). Moreover, the tree effect on the increment of soil available P is noticed both in natural and improved pasture systems, for both study farms.

A decrease in soil extractable K (and exchangeable K or soil available K) was observed in the 35-year old improved pasture, established at *Herdade dos Esquerdos*, both in the open and beneath tree crowns (about 1.7 and 1.5 mg kg^{-1} year^{-1} in the open and beneath tree crowns, respectively). Overall, the soil extractable K decrease in the improved pasture, relative to the natural pasture, was about 38% in the open and 21% beneath tree crowns. Similar trend occurred, at a lower extent, in the 16-year old improved pasture open areas (decrease of 1.2 mg kg^{-1} year^{-1}, 18,7% loss), relative to the natural pasture, at *Herdade do Olival*. The lower decrease in extractable potassium observed in the open16-year old pasture, compared to the older pasture at *Herdade dos Esquerdos*, may be associated with pasture age, but also with the lower soil organic matter (and nitrogen) accumulation and the lower soil drainage (as expressed by hydraulic conductivity), observed in *Herdade do Olival* soils, which may all contribute to reduce potassium losses by leaching (Alfaro et al., 2003).

Since no potassium fertilizers were applied following pasture installation, the decrease in soils extractable potassium under improved pasture, may be explained by the increments of pasture dry mass and animal production (and stocking rate), resulting in higher potassium uptake by pasture, as reported by Alfaro et al. (2003). Such losses of available potassium in the soil may be the cause for the previously reported lower potassium concentration in cork and holm oak foliage at the improved pastures, compared to natural ones, at the present study areas (FCT, 2014). Furthermore, our data suggest that soil potassium availability in open areas, under the present study long-term improved pasture management systems, can fall below the threshold reported as the optimum for pasture development (125-150 mg kg^{-1}) under similar climate conditions (Serrano et al., 2014). Therefore, potassium fertilizer application should be reviewed, for the maintenance of adequate soil K status in long-term improved pastures, especially when they are installed in naturally poor soils, where this nutrient availability must be considered a limiting factor.

Beneath tree crowns, improved pasture long-term management resulted in lower potassium losses at the *Herdade dos Esquerdos*, and even a small increase at the *Herdade do Olival*, which is undoubtedly associated with the role of trees on the potassium cycling, as well as different pasture ages.

Results of the present study also suggest that the concentration of exchangeable Ca^{2+} (as well as the sum of non-acid cations) in older improved pastures at *Herdade dos Esquerdos*, follow the pattern exhibited by the extractable P. In fact, the exchangeable Ca^{2+} concentration increased by 2.00 and 1.63 $cmol_c$ kg^{-1}, respectively, beneath tree crowns and in the open, whereas the increment of the sum of non-acid exchangeable cations was 2.36 and 1.40 $cmol_c$ kg^{-1}. The increase of exchangeable Ca^{2+} observed in the improved pasture may be mostly associated with the current fertilizer application, as the fertilizer applied is responsible for a Ca input of about 75 kg every two years. The negligible changes noticed in the 16-year old pasture might be mostly related to the shorter period

elapsed from its installation and to the lower input of calcium by fertilizer application (only about 25 kg every two years).
In the *Herdade dos Esquerdos*, the increment of the sum of non-acid cations under the improved pasture suggests an increase in retained cations, that is, in soil effective cation exchange capacity. As the soil pH values were similar (5.6-5.7) and in the same range in the natural and improved pastures (both beneath tree crowns and in the open), such an increase might be mostly attributed to the increment observed in soil organic matter concentration, following results reported by Gallardo (2003) and Moreno et al. (2007). Although some studies report that grazed pastures can be associated with soil pH decrease and unbalance (e.g. Dorrough et al., 2007; Haynes and Williams, 1993), the phosphate fertilizer application in the older improved pasture at *Herdade dos Esquerdos* may have been able to counterbalanced the potential acidification associated with soil organic matter accumulation. Nevertheless, fertilizer and lime application adjustments might be considered necessary to overcome eventual soil degradation patterns following livestock intensification (Weil and Brady, 2017).
It is also clear that the presence of oak trees in both improved and natural pasture systems contributes to the accumulation of non-acid cations (especially Ca^{2+}), as reported for several agroforestry systems (Dahlgren et al., 2003; Moreno et al., 2007; Pardon et al., 2017). Therefore, the observed absence of changes in these soils reaction suggests that the effect of organic matter accumulation, and potentially higher amounts of mineralized N, suggests an efficiently balanced by the non-acid cations increment beneath tree crowns.

Overall, results of the current study suggest that main soil fertility changes following long-term improved pastures, namely soil N, P and Ca availability enhancement, are mostly attributed to fertilizer inputs and organic matter concentration increments. However, such improvements may depend on pasture age, management system (fertilizer application, grazers species and density) and soil characteristics.

CONCLUSIONS

Current *montado* management systems are changing soil functions, urging the need to evaluate their thin balance between economical profitability, environmental services and future sustainability.

Sowing and maintaining improved pastures may contribute positively for soil physical status, fertility, and organic matter accumulation and stabilization, that is, for overall soil quality enhancement. Nevertheless, other factors, including soil texture, grazing management, fertilizer application and tree cover, can affect the rate and extent of such benefits. Additionally, current improved pastures management guidelines are not adapted to the singularities of *montado* systems, so tree recruitment, as the base for their long-term sustainability, is generally overlooked. Finer texture soils are particularly prone to soil physical degradation, following grazing intensification and related trampling effects. Tree cover is crucial to soil functions and resilience, namely structure stability, soil organic matter accumulation and stabilization, and nutrient cycling. All relevant changes in *montado* soil functions were expressed by or associated to soil compaction and organic matter status, suggesting soil quality monitorization across management and land use changes can be achieved by relatively common indicators, namely, soil organic C and/or related active fractions proportion shifts (e.g. HWS-C, POM-C), and bulk density modifications and/or categorical indicators of soil compaction, such as stocking rates and visual evidences of animal treading.

According to the presented results, it is suggested that: i) long-term management of improved pastures can be a promising strategy for soil fertility, structure and organic matter accumulation enhancement in *montado*; ii) tree cover maintenance and additional regeneration practices must be a priority, at research, policy and farm management levels; and iii) all site-specific and management factors and interactions (e.g. soil texture, fertilizer inputs, grazer species, stocking rate, tree cover) need further consideration, whenever evaluating the potential of any management or land use option for soil quality improvement, in *montado* systems.

Future studies are needed to assess soil quality influence on these systems economic and environmental performances under current management practices, in order to identify strategies that will ensure *montado* long-term sustainability.

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CHAPTER 3

Spatial variation pattern of soil characteristics and soil organic carbon accumulation as affected by single trees in Mediterranean oak woodlands

Spatial variation pattern of soil characteristics and soil organic carbon accumulation as affected by single trees in Mediterranean oak woodlands

ABSTRACT

Scattered isolated trees in Mediterranean woodlands are recognized to provide ecosystem services like shade, fodder, fruits, wood, cork, climate change mitigation and biodiversity. Effects of scattered oak trees (*Quercus ilex* L. and *Quercus suber* L.) on soil physical and chemical properties were assessed in two grazer Mediterranean woodlands (*montado*), located in the Alentejo region, Southern Portugal. Special attention was given to the spatial variation of organic soil C and nutrient concentrations according to the distance to the trees (from the trunk onwards to the open), and to the soil organic C accumulation as influenced by a single tree, up to 20 cm soil depth. Results show that scattered isolated oak trees create islands of enhanced soil fertility and quality through organic matter accumulation and nutrient cycling. Compared to the open areas, soils beneath the canopy of oak trees showed lower bulk density, and greater concentrations of organic C, nitrogen, extractable P and K, and exchangeable Ca^{2+}, Mg^{2+} and K^{+}, especially in the upper soil layer (0-5 cm). These concentrations strongly decreased from the tree trunk to the open. Scattered oak trees in the *montado* system (e.g. 50 trees ha^{-1}) can lead, in area basis, to the accrual of large amounts of organic C (0.59 to 1.48 kg C m^{-2}); the accrual is much higher if the carbon in tree biomass is taken into account (1.75 to 2.38 kg C m^{-2}). Our results indicate that oak trees in the *montado* system are paramount to enhance carbon sequestration and soil fertility, and their removal or loss should be avoided. Results of this study also emphasize the importance of scattered trees on soil quality and resistance to degradation. The challenge for agroforesters is to determine under what conditions the positive effect of trees will accumulate simultaneously with current management systems.

Key words: Montado, *Quercus ilex* L. subsp. *rotundifolia* Lam.; *Quercus suber*, soil spatial variation, soil fertility.

INTRODUCTION

Mediterranean-type evergreen oak woodlands, mainly constituted by *Quercus ilex* L. (holm oak) and *Quercus suber* L. (cork oak), occupy about 1 068 x10^3 ha (ICNF, 2013), which corresponds to about 12 % of the Continental Portugal area, and 34 % of the forestry area; they are mostly located in the Alentejo Region (Southern Portugal). Although both species often coexist, the *Q. ilex* mainly occurs in the eastern inland drier regions, whereas the *Q. suber* predominates in the wetter western coastal areas (David, 2000). Anthropogenic factors have shaped these oak woodlands into savannah-type ecosystems or landscape designated by *montado* (*dehesa*, in Spain), which are characterized by the presence of trees intermittently distributed without a regular pattern within a continuous grass matrix, and having grasslands/pastures, crops and fallows as understory (Elena-Rosselló et al., 1887; Joffre et al., 1999; Carreiras et al., 2006; Simón et al., 2013; Pulido et al., 2017). The *montados* are multipurpose systems mainly managed to feed livestock (pastures, acorns), and for cork and firewood extraction (Joffre et al., 1999; Moreno and Pulido, 2009). The net effect of scattered trees on grass production can be negative, neutral or positive and change with tree age or size and density (Scholes & Archer, 1997). Although *montado* shows strong temporal variability (between and within years) driven by the presence of livestock, pastures, shrubs and/or crop-fallow cycles, the constant presence of the tree layer provides stability and is crucial for the ecosystem functions (Joffre et al., 1999; Costa et al., 2014).

In savanna ecosystems, scattered trees alter inputs to the soil system by increasing of wet and dry deposition, and affect the morphology, chemical and physical conditions of the soil, as a result of the amount and characteristics of above- and belowground residue inputs (Rhoades, 1997). Trees drive litterfall inputs, livestock manure and rainfall distribution, soil temperature, and, consequently, a shift in soil biological activity (Young, 1997; Waldrop and Firestone, 2006). Higher concentrations of soil organic C, N and non-acid cations, and higher values of cation exchange capacity, have been observed for soils under the influence of scattered tree canopies, in different climate zones (e.g. Barth, 1980; Ryan & McGarity, 1983; Young, 1997). More developed soil profiles and improved water regimes have also been observed in ecosystems under scattered tree canopies (Dahlgren et al., 1997). Moreover, Zinke (1962) pointed out that individual scattered trees have an influence proportional to their crown area projected onto the soil surface.

Scattered trees in *montado* systems may alter chemical, physical and biological soil properties by their impact on energy and nutrient fluxes (Gallardo et al., 2000). As reported for agro-ecosystems in the Mediterranean area of California (Dahlgren et al., 1997; 2003; Jackson et al., 1990), soils from *montado* systems may show a positive differentiation in their characteristics as a result of the presence of oak trees. Indeed, several studies have shown that scattered trees in oak woodland systems lead to positive effects on physical soil properties (Joffre and Rambal, 1988), soil chemical characteristics (Joffre et al. 1999; Gallardo, 2003; Moreno and Obrador, 2007; Gómez-Rey et al., 2012), soil fertility (Moreno et al., 2007) and soil organic matter quantity and quality (Escudero et al*.,* 1985; Rovira & Vallejo, 2007; Howllet et al., 2011). They also enhance the nitrogen turnover and microbial biomass N, and inorganic N availability, under their canopy relatively to soils occurring in open areas (Gallardo et al., 2000; Gallardo, 2003), and facilitate the use of water resources (Cubera and Moreno, 2007). Also, some studies indicate that the effect of scattered trees on such soil characteristics are dependent on the soil management and land use (Gómez-Rey et al., 2012; Hernández-Esteban et al., 2018).

The spatial variations on soil characteristics driven by scattered oak trees in the *montado* may lead to variations in competitive abilities, a mechanism allowing the local coexistence of plant species (Reynolds et al., 1997). Scattered trees can likely modify soil properties improving their habitat conditions, by generating positive feedback loops between plant and soil compartments (Ehrenfeld et al. 2005). Consequently, knowledge on the heterogeneity of soil characteristics will be paramount to understand the dynamics of specific populations and processes within the *montado* ecosystem. Such information can be used to predict the potential habitat distribution for a given species and to promote conservation and restoration efforts, in view of predicted climate (Miranda et al., 2002; IPPC, 2014) or land use changes.

Agroforestry systems have a key role in organic C sequestration (Garrity et al., 2006), and are recognized as a valuable integrated approach for sustainable land use, aside from their contribution to climate change adaptation and mitigation (Lorenz and Lal, 2014). Agroforestry systems, such as *montado*, are an appealing option for sequestering C on agricultural lands and help landowners and society to address many other issues facing these lands, such as economic diversification, biodiversity and water quality (Peichl et al., 2006; Schoeneberger, 2009), and erosion control (Cardinael et al., 2015). Despite of the potential agroforestry systems to increase soil organic C accumulation, quantitative estimates are yet scarce (Kim et al., 2016), especially for temperate (Cardinael et al 2015) or Mediterranean (Howlett et al 2011) agroforestry systems. The extent to which the tree layer influences the soil organic C accumulation in such ecosystem is also still poorly understood (Simón et al., 2013; Howllet et al., 2007). Although trees affect the spatial distribution of organic matter inputs to the soil, sampling protocols have not always taken this impact into account (Simón et al., 2013). The distribution of the organic C accumulation close and away from scattered trees was seldom considered, some authors reported higher SOC stocks under the tree canopy than at 5 m from the tree (Howlett et al., 2011) or with the age of the trees (Bambrick et al. 2010) or that the spatial distribution of soil organic C accumulation to 20 cm depth was not explained by the distance to the tree (Upson and Burguess, 2013). Although the *montado* ecosystem offer a unique opportunity to quantify single-tree effects on soil resources and understory vegetation, and on the potential of organic C accumulation, no information is yet available at such scale. Considering tree density (Lorenz and Lal, 2014), such approach may be of great usefulness to estimate soil organic C accumulation at landscape level.

The vast extent and great economic and ecological importance of the *montado*, and the concerns about their long-term sustainability (Costa et al., 2014), raises the relevance of studying the functioning of this agroecosystem oak trees, and their influence on ecosystem nutrient cycling in a way to understand how management practices affect their overall long-term sustainability. Several studies were developed in Portugal regarding the *montado* system, in relation to the tree nutrient cycling (Nunes, 2004; Sá et al., 2005), the soil nutrient content and availability (Nunes 2004; Nunes et al., 1999, Nunes et al., 2001), the precipitation interception and transpiration (David, 2000; David et al., 2006), the herbaceous production and nutrient concentration (Sá, 2001; Cubera et al. 2009), the herbaceous residues decomposition (Sá et al., 2004), and the soil C accumulation according to pasture management (Gómez-Rey et al., 2012).

However, some uncertainty subsists regarding the role of trees on soil physical and chemical characteristics, nutrient availability, and especially on soil organic C accumulation spatial variability.

In this context, it is paramount to assess the effect of scattered trees on the pattern and scale of soil heterogeneity on a spatial basis in the *montado* ecosystem (tree canopy *versus* open grassland). Therefore, a study was developed to evaluate whether scattered oak trees in two representative grazing woodlands affect: (i) the mass of soil litter layers and the soil bulk density; (ii) the spatial distribution of organic C and nutrient concentrations; and (iii) the accumulation of soil organic carbon associated to a single tree. It was hypothesized that organic C and nutrient concentrations vary with the distance to the tree, and that the evaluation of the accumulated C associated with the scattered trees is a tool for assessing soil organic C stock in oak woodland landscapes. Results would provide deeper understanding on the soil patches found beneath tree canopies, and on their role in ecosystem functions, especially organic C accumulation. Also, they will constitute a support for the development of management strategies aiming land use sustainability, and for policies associated with the optimization of ecosystem services, especially the mitigation and adaptation to climate change for fulfilment of international commitments.

MATERIALS AND METHODS

Study sites

The study was carried out in Southern Portugal (Alentejo region) during 2014, at the *Centro de Estudos e Experimentação da Mitra* (Herdade da Mitra), University of Évora (HM; 38º32'N, 8º01'W, 243 m a.s.l.), and at the *Tapada Real de Vila Viçosa* (TR; 38 47´N, 8°19'57.68''W), an enclosed estate with an area of 800 ha, located at the Vila Viçosa county. Both sites are under climate of Mediterranean type, with hot and dry summers and mild wet winters. Mean annual rainfall is 665 - 685 mm, mainly concentrated from autumn to early spring (90%), in less than 75 days of rain per year (INMG, 1991). Mean annual air temperature is about 15.4ºC, and the mean monthly temperature ranges from 8.6ºC in January to 23.1 ºC in August. The mean air relative humidity is about 70%. Both sites show mature oak trees, with approximately even-aged tree distribution, and are representative of the large evergreen oak woodland areas in Portugal.

The landscape at HM is gently undulating and the slope ranges from 3 to 8%. The geological substratum consists of granites and gneisses (Carvalhosa et al., 1969), and soils are mostly Eutric Leptosols (IUSS Working Group WRB, 2015), with sandy-loam texture. They are typically strongly to moderately acidic, and with low nutrient status. The vegetation consists of a native pasture with scattered trees of *Q. suber* L and *Q. ilex* L. subsp. *rotundifolia* Lam.; the oak stocking ranges from 35 to 45 trees ha^{-1}, with an average canopy coverage of 21% (David, 2000). The study area was formerly used for cereal crops and fallow including sheep grazing; during the last decades it has been grazed by goats in an extensive regime, with two passages a day of the flock through the area. Common annual grass species are *Vulpia bromoides* (L.) S.F. Gray, *Bromus rigidum* Gaudin, *Hordeum murinum* L. and *Briza maxima* L.; major forbs include *Rumex bucephalophorus* L., *Silene gallica* L., *Geranium purpureum* Vill., *Tolpis barbata* (L.) Gaertner, *Tuberaria guttata* L. Fourr.; and major legume species are *Ornithopus compressus* L., *Ornithopus pinnatus* (Miller) Druce. The understorey is currently invaded by shrubs, mainly *Cistus salviifolius* L.

The landscape at TR is made of metamorphic formations from the Silurian (Gonçalves, 1969; 1972), corresponding to schists**,** mostly with vertical stratification, associated with felsitic metavolcanites. The topography is gently undulating to undulating (slope gradient: 6-8%; SROA, 1964). Soils are classified mainly as Dystric Epileptic Regosols and Dystric Leptosols (IUSS Working Group WRB, 2015), and their texture is mostly silty-loam (Atterberg scale), clay content varying between 171 and 194 g kg^{-1}, and that of silt between 400 and 410 g kg^{-1}. Soils are mostly strongly to moderately acidic, with low nutrient status. The estate is an oak woodland with a dominant tree cover of holm oak (*Q. ilex* L. subsp. *rotundifolia* Lam.) and cork oak (*Q. suber* L.), the tree density varying between 50 and 60 trees ha^{-1}. The understorey is dominated by grasses (*Avena sativa* L., *Avena barbata* Pott ex Link, *Bromus* spp., *Agrostis* spp.) and forbs (*Chamaemelum mixtum* (L) All. and *Coleostephus myconis* (L.) Rchb. f.), associated with low abundance of legume species (*Trifolium arvense* L., *Trifolium angustifolium* L., *Trifolium campestris* Schreb. in Sturm, and *Ornithopus compressus* L.); the understory shrub vegetation is dominated by the gum cistus (*Cistus landanifer* L.) associated with *Cistus salvifolius* L., *Daphne gnidium* L. and blackberry (*Rubus* spp.), and is mostly controlled by grazing. Until 1994, the estate was used for small hunting game species and never was used for cereal crops (as occurred in the neighborhood), with occasional raising of pig herds; therefore, no fertilizers were applied. Ungulates herbivorous such as red deer (*Cervus elaphus*) and fallow deer (*Dama dama*) were introduced in 1994, at a density of approximately 0.35 red deer and of 0.1 fallow deer per hectare (about 0.2 LU ha^{-1}), a density that is uncommon in the Iberian Peninsula hunting estates, maintained by a limited culling policy and supplementary feeding in years of lower food availability (Lecomte et al. 2016). Afterwards, triticale was occasionally cropped, and soil has tilled (disc harrowing) every 4-6 years for shrub growth control. Nowadays, the oak stand is exclusively oriented for browsing by herbivore ungulates production (and marginally for cork production).

Samplings

Five isolated holm oak trees, at HM, or cork oak trees, at TR, with similar crown diameter (about 12 m), and circumference at breast height or perimeter at the first bifurcation, were randomly selected for major sampling procedures. At HM, the mean perimeter of randomly selected trees at first bifurcation was 1.67 m (range: 1.35-2.05 m), and the mean tree crown radius was 5.9 m (range: 5.8-6.2 m). At TR, the mean diameter at breast height of randomly selected trees was

0.47 m (range: 0.37-0.53 m), and the mean tree crown radius was 5.6 m (range: 5.5-6.5 m).

Soil samples from each site were collected between 2014 (HM) and 2015 (TR). All samplings, around each tree and in each site, were carried out in four transects according to the four cardinal points direction. In each transect, samples were taken at different positions relative to the tree canopy projection radius (R): 0.33R, 0.66R, R, 1.33R and 2R. Therefore, the positions 0.33R and 0.66R were beneath tree crowns; the position R was in the edge of the tree crown; and the position 2R (twice the tree crown radius) was considered as a reference (open grassland), as followed in other studies (Gómez-Rey et al., 2012; see also Chapter 2). The sampling design was adopted to account for short range soil spatial variability (Belsky et al., 1989), and was based in studies which reported that tree canopies in agroforestry systems influence soil properties beyond their crowns (Rhoades, 1997), and that such influence in *dehesas* occurs to a distance which can be twice the crown radius of oak trees (Simón et al., 2013).

For assessment of the surface litter layer mass (only at HM), samples were collected in March 2014 (before the litterfall peak) around each tree at the aforementioned different positions prior to soil sampling, by using a 0.5 x 0.5 m square wooden frame.

Soil sampling was carried out up to 20 cm depth because the soils at both sites can show a depth less than 30 cm, and former studies indicated that changes in soil characteristics mostly occurred within this soil depths (Gómez-Rey et al. 2012; Nunes, 2004).

Undisturbed soil samples for bulk density determinations were taken around each randomly selected trees, at each cardinal direction transect and position relative to the tree crown radius, at 0-5, 5-10 and 10-20 cm soil depth. Metallic cylinders were carved into the soil, and samples were trimmed exactly to the cylinder volume (ca. 590 cm^3).

Following the same sampling scheme, disturbed soil samples were taken with an auger, at 0-5, 5-10 and 10-20 cm depth. Samples from the same tree and same position relatively to tree crown radius, collected according to the four cardinal directions transects, were to form one composite sample per tree and crown radius position. Samples were air-dried at room temperatures to a constant weight and passed through a 2 mm sieve. The fraction that did not pass the 2 mm sieve (coarse fragments) was separated, dried for 24 hours (75ºc), weighed and then discarded; the respective weigh was used to convert data derived from the 2 mm sieved fraction back to field conditions.

Laboratory procedures

Litter layer residues were oven-dried at 80ºC for 48 hours, to obtain the respective dry weight, and afterwards were ground in a centrifugal grinder with a 0.5 mm sieve. The concentration of N (Kjeldahl) in the residues was determined by using a Kjeltec Auto 1030 Analyser distillation system, while that of the organic C was determined by the potassium dichromate oxidation procedure by De Leenheer and Van Hove, (1958). Mineral elements (K, Mg, Ca and P) were solubilised in a CEM Microwave Digestion System (MDS-2000 model). For this purpose, 0.5 g of the material was placed in LDV (Line Digestion Vessel) tubes, which were in contact with 10 mL of HNO_3 with a 65% concentration. The resulting solution was evaporated in *Fourneau* cups, and the residue was made soluble by addition of 10 mL of 3 M HCl. Calcium, Mg and K were measured by atomic-absorption spectrometry (AAS), while P was determined colorimetrically (Murphy & Riley, 1962).

Cores for bulk density determination were dried at 105 ºC, in an oven, to constant weight. Soil bulk density was determined by the ratio of dry weigh of undisturbed soil cores (oven dried at 105 °C) and the cylinder volume (Blake and Hartge, 1986).

After being air dried and sieved (< 2 mm), disturbed soil samples for chemical characterization were oven-dried at 40ºC for 48 hours. Particle size analysis was carried out by using the pipette method, as described by Póvoas and Barral (1992). Soil concentrations of N and organic C were determined as abovementioned. The organic C concentration corresponding to the particulate organic matter fraction was determined in the material obtained after wet sieving of 50 g of soil in a 53 µm sieve. Non-acid exchangeable cations (Ca^{2+}, Mg^{2+}, Na^{+}, K^{+}) were extracted by percolating 5 g of soil samples with 1 M ammonium acetate adjusted at pH 7, and measured by AAS. Soil pH was measured in distilled water and 1 M KCl suspensions (soil to solution ratio of 1:2.5) using a potentiometer. Exchangeable Al^{3+} was extracted with 1 M KCl solution (Barnhisel and Bertsch 1982) and determined by AAS. Extractable K and P by the Egnér-Riehm (1958) test, were obtained by shaking 5 g of sample with a solution of ammonium lactate and acetic acid for two hours, and measured by AAS and UV-visible spectroscopy, respectively.

Soil organic C accumulation

The amount of organic C accumulated in the soil at each position relative to tree crown radius, for each soil layer up to 20 cm depth, was calculated taking into account the corresponding concentration of organic C measured in the <2 mm soil fraction, soil bulk density, and the proportion of coarse fragments (>2 mm fraction) (Poeplau et al., 2017).

For the evaluation of the soil organic C accumulation in the area influenced by each isolated oak tree, it was assumed that its distribution can be adjusted to a negative exponential function of the type:

$$C = be^{-ar} \qquad [1]$$

Where *C* is the soil organic C accumulation (kg m^{-2}), determined at any distance *r* (m) from the tree trunk, *b* is the base and *a* is the rate parameter. Thus, soil organic C accumulation data was adjusted to this model for each soil layer in each study site.

Considering that, in *montado* systems, the oak tree influence over soil properties is known to extend up to two times the tree crown projection radius (*R*) (Simón et al., 2013), the soil organic carbon amount (in kg) in a circular area around a single tree (C_{tree}), for any $r \leq 2R$, can be calculated as:

$$C_{tree}(r) = \int_0^r 2\pi x b e^{-ax}\, dx = 2\pi b \int_0^r x e^{-ax}\, dx = -\frac{2\pi b}{a}\left[\left(x + \frac{1}{a}\right) e^{-ax}\right]_o^r =$$

$$= -\frac{2\pi b}{a}\left[\left(r + \frac{1}{a}\right) e^{-ar} - \frac{1}{a}\right] = -\frac{2\pi b}{a^2}\left[(ar+1)e^{-ar} - 1\right] =$$

$$= \frac{2\pi b}{a^2}\left[1 - (ar+1)e^{-ar}\right] \;\;, \quad r \leq 2R \qquad [2]$$

Similarly, the amount of soil organic carbon in any tree-less circular area (C_{open}), as defined by its radius *r* (m), can be estimated by the base soil organic carbon accumulation (c, kg m^{-2}), that is, the soil organic carbon accumulation value determined at any point beyond 2R, as follows:

$$C_{open}(r) = \int_0^r 2\pi x \, c \, dx = 2\pi c \left[\frac{x^2}{2}\right]_0^r = c\pi r^2 \qquad [3]$$

Solving expressions [2] and [3] for $r = 2R$ - the first using the parameters obtained with expression [1] after adjusting the model to the study observations (at 0.33R, 0.66R, R, 1.33R and 2.0R); and the second considering c as the mean value of soil organic carbon accumulation at 2.0R sampling point -, a single tree contribution to soil organic C accrual, for each study site and soil layer, was estimated by difference, as follows:

$$C_{accrual} = C_{tree}(2R) - C_{open}(2R) \qquad [4]$$

Statistical analyses

For each study site and each soil layer, soil surface litter layer and determined soil properties were considered as independent variables, and analyses of variance (ANOVA; α=0.05) were performed to test differences between sampling distances to tree trunk. When sample normal distribution (Shapiro-Wilk test) and homogeneity of variances (Levene's test) could not be accepted, even with data transformations (e.g. logarithm, square root), a non-parametric Kruskal-Wallis (α=0.05) procedure was performed. If significant differences between points averages were assumable, the Tukey or Waerden (non-parametric) tests (α=0.05) were used for means separation. All statistical analyses were done in the R environment (R Core Team, 2014), including adequate packages, such as 'car' (Fox and Weisberg, 2011) and 'agricolae' (De Mendiburu, 2009).

RESULTS

Soil texture

Soils at HM showed coarser texture (sandy-loam) than soils at TR (silty-loam). However, soils at both sites did not show significant differences in concentrations of particle-size fractions between different distances to trees (Table 1). Similar trend was observed for soil depth (data not shown).

Table 1 - Concentrations (g kg^{-1}; mean ± standard deviation; n=5) of coarse sand (CS), fine sand (FS); silt (SL) and clay (CL) particles in soil at different points relative to tree crown radius (R), at *Herdade da Mitra* (HM) and *Tapada Real de Vila Viçosa* (TR).

	HM					TR				
	0.33R	**0.66R**	**1R**	**1.33R**	**2R**	**0.33R**	**0.66R**	**1R**	**1.33R**	**2R**
CS	378±33	393±40	385±34	376±42	394±59	162±7	160±8	166±7	162±7	162±16
FS	451±22	443±34	449±30	449±34	435±49	239±9	249±14	248±14	247±24	239±17
SL	83±8	79±9	79±14	83±5	82±10	409±9	407±10	405±20	409±15	411±11
CL	88±8	86±8	87±13	92±8	89±14	189±10	183±7	182±17	182±19	188±24

Soil litter layer mass

The soil litter layer mass at HM (Table 2), significantly decreased from areas close to the tree trunk (270.4 g m^{-2}) to the canopy vertical projection limit (127.9 g m^{-2}), and from that to open areas (34.1 g m^{-2}). The mass of the litter layer, at the vertical canopy projection limit, was less than the half of that observed in the area closest to the tree trunk.

Table 2 - Means (± standard errors; n=20) of litter layer mass (LLM) and amounts (g m^{-2}) of C, N, P, K, Ca and Mg in the litter layer measured at different points relative to tree crown radius (R) in the *Herdade da Mitra* study site. Values followed by the same letter are not statistically different by the Tukey test (*p* <0.05).

R	LLM	C	N	P	K	Ca	Mg
0.33R	270,4^{a}±30,0	112,3	3,52^{a}±0.76	0.21^{a}±0.05	0.58^{a}±0.23	5.27^{a}±1.14	0.65^{a}±0.15
0.66R	241,2^{a}±36,0	103,8	2,77^{a}±0.58	0.15^{a}±0.03	0.57^{a}±0.09	4.33^{a}±0.73	0.49^{ab}±0.08
1R	127,9^{b}±19,0	49,8	1,72^{b}±0.12	0.10^{b}±0.01	0.36^{a}±0.04	2.45^{b}±0.20	0.37^{b}±0.05
1.33R	68,3^{c}±12,0	28,0	0,90^{c}±0.18	0.05^{c}±0.01	0.23^{b}±0.06	1.15^{c}±0.25	0.18^{c}±0.06
2R	34,1^{d}±9,0	13,6	0,46^{c}±0.10	0.03^{c}±0.01	0.09^{b}±0.03	0.58^{c}±0.13	0.09^{c}±0.02

The amounts of org C and nutrients accumulated in the litter layers followed approximately the trend observed for the litter layer mass (Table 2). The amounts decreased 9-10 times from the zone closer the tree trunk to the open, for C, N, P and Ca; differences for K and Mg were smaller (6-7 times). The C/N ratio (29-36) did not vary with the distance to tree trunk.

Bulk density

Soil bulk density, in the 0-5 cm layer, was the lowest in the areas close to the tree trunk (1.14 and 1.22 g cm^{-3} in the HM and TP, respectively), and significantly increased towards the open (Figure 1); values at 0,33 and 0.66R were significantly lower (p<0.01) than those measured beyond tree canopy. At both sites, bulk density increased with soil depth, but values observed in the 5-10 cm layer followed the trend found in the 0-5 cm layer. Values in the 10-20 cm soil layer, at HM, were not significantly affected by the distance to tree trunk, whereas at TR significantly increased towards the open.

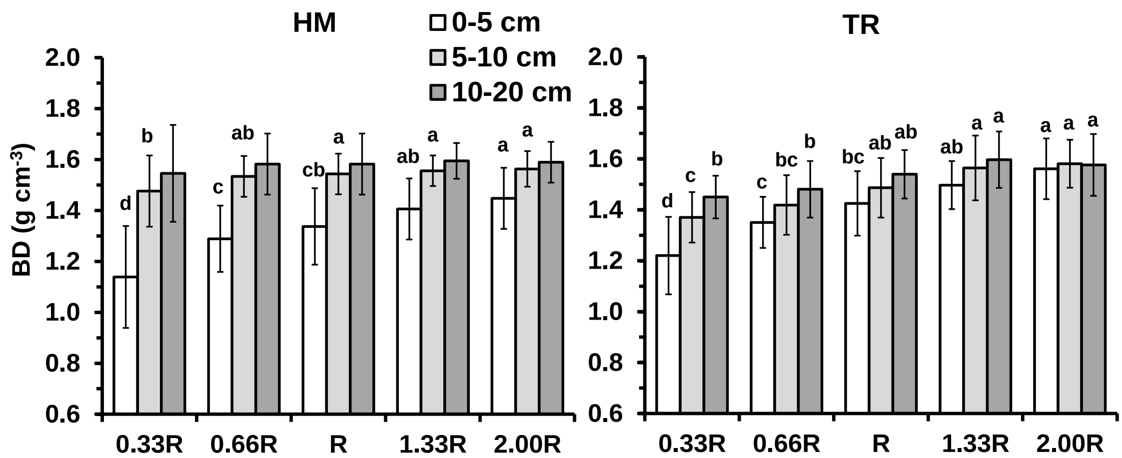

Figure 1 - Bulk density (BD) in the 0-5, 5-10 and 10-20 cm soil layers, at different points relative to tree crown radius (R), at *Herdade da Mitra* (HM) and *Tapada Real de Vila Viçosa* (TR). Bars are mean ± standard deviation (n=20). Different letters in the same depth correspond to significant differences by the Tukey test (p< 0.05).

Organic C and N concentrations

Concentrations of soil organic C (SOC) were much higher at TR than at HM for the different soil layers (Figure 2). In the 0-5 cm soil layer of both sites they were the highest in the position closer to the tree trunk and were about 2.4 times higher than in the open (2R). Organic C concentrations were significantly higher (p<0.05) underneath the tree crown than in the open.

The concentrations of total N (Figure 2) significantly decreased with the increasing of the distance from the tree trunk, following the pattern exhibited by those of SOC. This trend was observed up to 10 cm depth at HM, and up to 20 cm depth at TR. In the zone closer to the tree trunk at both sites, the N concentrations in the 0-5 cm soil layer doubled those observed in the open, difference being narrower than those observed for SOC.

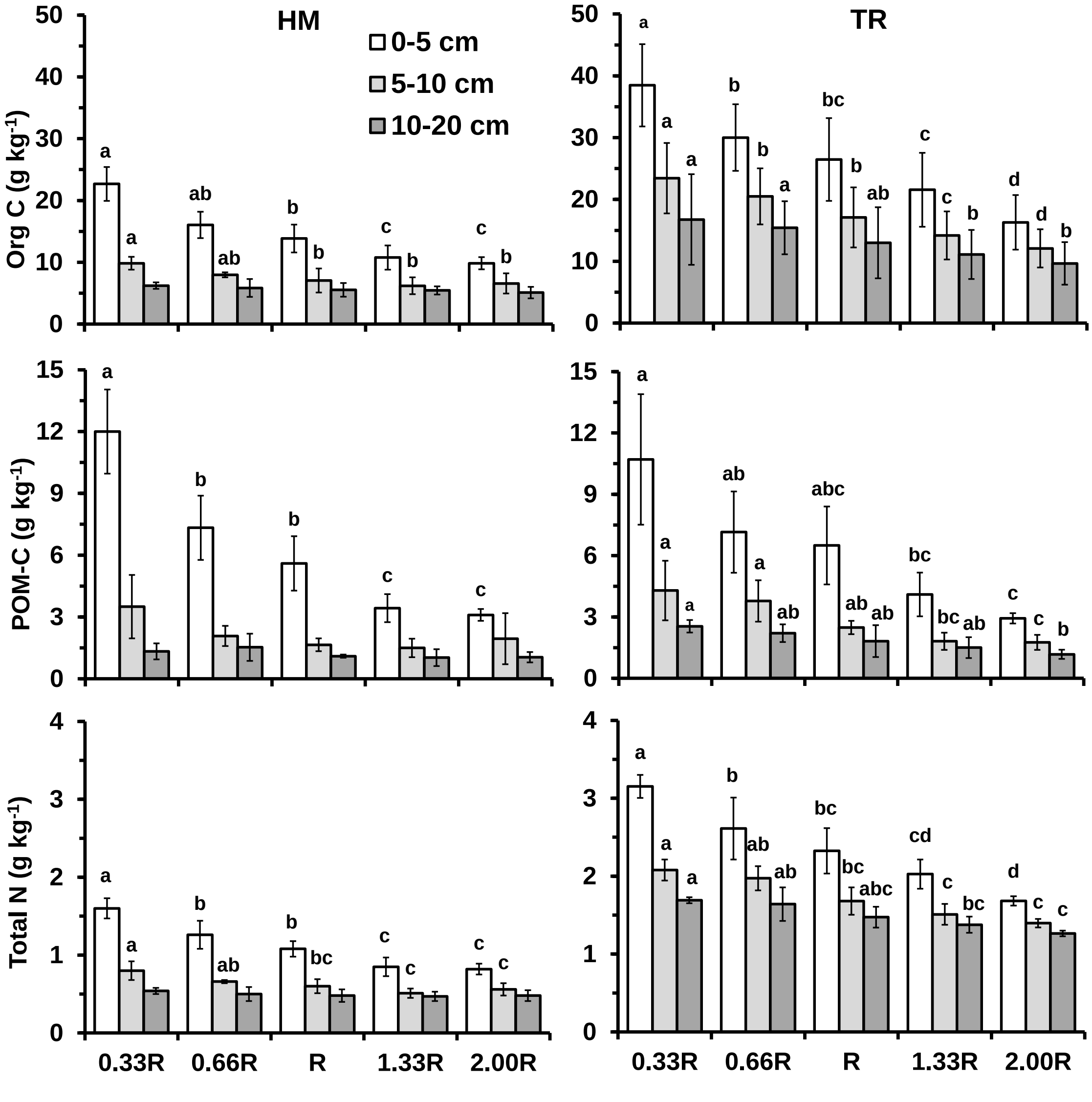

Figure 2 - Concentrations of organic C (org C), nitrogen and particulate organic C (POM-C) in the 0-5, 5-10 and 10-20 cm soil layers, at different points relative to tree crown radius (R), at *Herdade da Mitra* (HM) and *Tapada Real de Vila Viçosa* (TR). Bars are mean ± standard deviation (n=5; except for org C, with n=20). Different letters in the same depth correspond to significant differences by the Tukey test ($p< 0.05$).

The soil C:N ratio showed a narrow range and was higher at HM (14.2-12.0) than at TR (12.2-9.7), values decreasing with soil depth (Table 3). At HM, the values of the C:N ratio did not show significant changes with the distance to the tree trunk. In contrast, values at TR significantly decreased towards the open for all soil depths, as occurred for the SOC and N concentrations.

Despite the differences in SOC concentrations, the concentration of organic C corresponding to the particulate organic matter (POM-C) were of the same magnitude in both study sites (Figure 2), and followed the trend exhibited by the SOC concentrations according to the distance to the tree trunk. The proportion of POM-C relatively to the SOC was much higher, in all soil layers, at HM than at TR (Table 3); for instance, the proportion in the 0-5 cm layer was 52.9-31.5 and 27.8-18.0% for the former and the latter, respectively. Differences in POM-C concentrations corresponding to the different positions relative to tree trunk were larger than those observed for the SOC, as the concentrations close to the tree trunk in the 0-5 cm layer were about 4.0 and 3.7 times higher than in the open, respectively at HM and TR.

Table 3 - Means (± standard errors; n=5) of C:N ratio and proportion of organic C corresponding to the particulate organic fraction (POM-C/SOC, %) in the 0-5, 5-10 and 10-20 cm soil layers, at different points relative to tree crown radius (R), at *Herdade da Mitra* (HM) and *Tapada Real de Vila Viçosa* (TR). Values followed by the same letter are not statistically different by the Tukey test (*p* <0.05).

Depth	**HM**					**TR**				
(cm)	**0.33R**	**0.66R**	**1R**	**1.33R**	**2R**	**0.33R**	**0.66R**	**R**	**1.33R**	**2R**
						C:N				
0-5	14.6 ±0.36	13. 9 ±0.90	13.9 ±0.55	13.6 ±1.21	13.6 ±1.35	12.20^{a} ±0.80	11.56^{ab} ±0.59	11.35^{ab} ±0.42	10.65^{bc} ±0.25	9.69^{c} ±0.51
5-10	12.2 ±1.36	12.1 ±0.26	13.4 ±1.52	12.7 ±0.98	13.4 ±1.06	11.33^{a} ±1.13	10.36^{ab} ±0.50	10.18^{abc} ±0.25	9.40^{bc} ±0.43	8.65^{c} ±0.39
10-20	11.8 ±0.57	11.6 ±0.83	12.1 ±1.42	11.2 ±0.69	11.7 ±2.75	9.91^{a} ±0.65	9.40^{ab} ±0.03	8.79^{abc} ±0.63	8.07^{bc} ±0.73	7.65^{c} ±0.51
						POM-C/SOC				
0-5	52.9^{a} ±7.38	$45. 7^{a}$ ±7.17	40.4^{b} ±10.38	31.8^{c} ±2.69	31.5^{c} ±2.10	27.8 ±6.99	23.8 ±6.84	24.6 ±3.44	19.0 ±5.06	18.0 ±1.99
5-10	35.5 ±12.97	26.1 ±7.48	23.4 ±5.24	24.2 ±2.61	29.6 ±11.96	18.3 ±6.74	18.5 ±2.84	14.5 ±1.32	12.8 ±2.71	14.6 ±2.77
10-20	21.4 ±5.14	26.2 ±14.15	19.8 ±4.63	18.9 ±6.25	20.6 ±4.44	15.2 ±2.32	14.3 ±1.86	14.0 ±4.87	13.5 ±3.25	12.2 ±2.91

Exchangeable non-acid cations and extractable Al

The sum of the concentrations of exchangeable non-acid cations (SB) was higher at TR than at HM, and was the highest in the 0-5 cm soil layer beneath the tree canopy, in the zone closest the tree trunk (Figure 3). At both sites, values of SB significantly decreased with the distance to the tree trunk, following the trend observed for the SOC and N concentrations. Values determined close the tree trunk in bot HM and TR were about twice those determined in the open.

In both HM and TR, the predominant exchangeable non-acid cation was the exchangeable Ca^{2+}. Its concentration variation with the distance to the tree trunk was similar to that observed for the SOC and SB (Figure 3). Concentrations close the tree trunk in the 0-5 cm soil depth were about 2.2 and 2.5 times higher than those measured in the open, respectively at HM and TR. Concentrations of exchangeable Ca^{2+}, at TR, significantly decreased towards the open, up to 20 cm depth, while at HM such trend only was observed for the 0-5 cm soil layer. Similar trends were observed for the concentrations of exchangeable Mg^{2+} and K^{+}, and of extractable K (data shown).

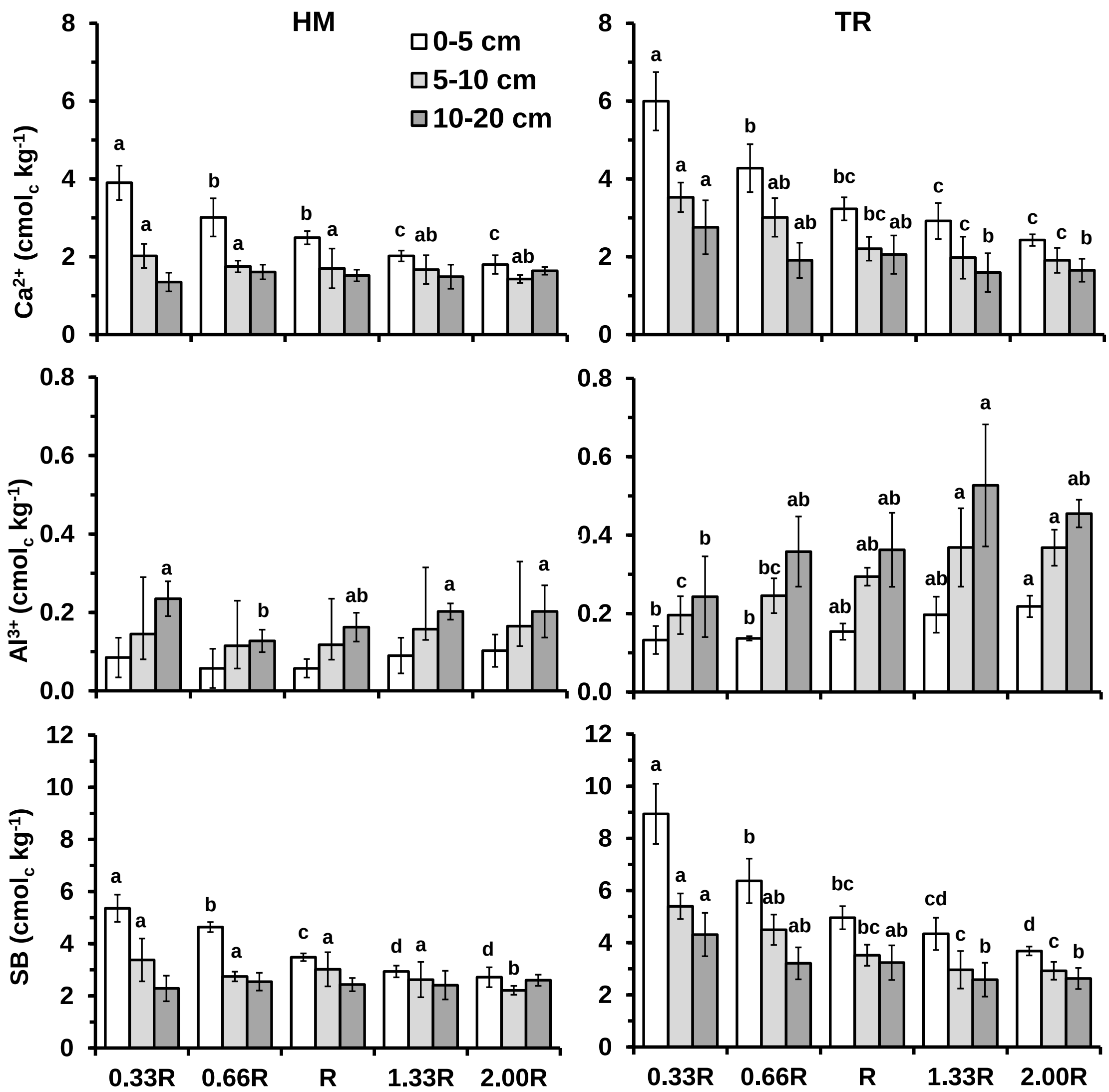

Figure 3 - Concentrations of exchangeable Ca^{2+}, exchangeable Al^{3+}, and sum of non-acid cations (Ca^{2+}, Mg^{2+}, K^{+}, Na^{+}) in the 0-5, 5-10 and 10-20 cm soil layers, at different points relative to tree crown radius (R), at *Herdade da Mitra* (HM) and *Tapada Real de Vila Viçosa* (TR). Bars are mean ± standard deviation (n=5). Different letters in the same depth correspond to significant differences by the Tukey test (p< 0.05).

Concentrations of exchangeable Al^{3+} were much lower than those corresponding to the exchangeable Ca^{2+} and to the sum of the non-acid cations (Figure 3). At both HM and TR, exchangeable Al^{3+} concentrations were the lowest in the 0-5 cm soil layer, and the highest in the 10-20 cm soil layer. At TR, inversely to other characteristics of the soil exchangeable complex, the concentrations of exchangeable Al^{3+} significantly increased from the tree trunk towards the open, in soil layers up to 20 cm depth. At HM, concentrations were much lower and did not show a definite trend with the distance to the tree trunk.

Effective cation exchange capacity (ECEC)

Values of the ECEC, as for the exchangeable Ca^{2+} and the SB, were greater at TR than at HM (Table 4) and decreased with the soil depth, especially in the zones close to the tree trunk. These values followed the spatial trend exhibited by the concentrations of organic C, significantly decreasing from the tree trunk towards the open. At TR, this trend was observed up to 20 cm depth, while at HM only occurred in the 0-5 cm soil layer. Values of the ECEC determined in the 0-5 cm soil layer close to the tree trunk were about 1.9 and 2.3 times higher than those measured in the open, respectively at HM and TR.

Soil pH

Soil pH (in water), at HM, was in general higher in the 0-5 cm soil layer independently of the distance from tree trunk (Table 4) and increased with the distance to the tree trunk; only in the 0-5 cm soil layer the differences were significantly different. At TP, pH values were slightly higher than at HM and also decreased from the 0-5 to the 10-20 cm layer. Negligible differences were observed according to the distance to the tree trunk.

Extractable phosphorous

Extractable P concentrations were higher at HM than at TR (Table 4), in all study soil layers. Concentrations in both sites significantly decreased from positions close to the tree trunk, to those in the open, following the trend observed for the SOC. In the 0-5 cm soil layer, the decreasing was about two and four times, respectively at the HM and TR.

Table 4 - Means (± standard errors; n=5) of the effective cation exchange capacity (ECEC), soil pH (in H_2O), and extractable P (P_{ext}) concentrations in the 0-5, 5-10 and 10-20 cm soil layers, at different points relative to tree crown radius (R), at *Herdade da Mitra* (HM) and *Tapada Real de Vila Viçosa* (TR). Values followed by the same letter are not statistically different by the Tukey (p <0.05).

Depth	**HM**					**TR**				
(cm)	**0.33R**	**0.66R**	**1R**	**1.33R**	**2R**	**0.33R**	**0.66**	**1R**	**1.33R**	**2R**
					ECEC (cmol$_c$ kg^{-1})					
0-5	5.45^{a} ±0.49	4.41^{b} ±0.21	3.54^{c} ±0.17	3.04^{d} ±0.23	2.81^{d} ±0.34	9.07^{a} ±0.99	6.51^{b} ±0.74	5.11^{bc} ±0.38	4.54^{c} ±0.49	3.90^{c} ±0.15
5-10	3.53^{a} ±0.80	2.86^{a} ±0.19	2.84^{a} ±0.67	2.78^{a} ±0.70	2.38^{b} ±0.19	5.59^{a} ±0.38	4.72^{ab} ±0.49	3.81^{bc} ±0.33	3.33^{c} ±0.53	3.29^{c} ±0.26
10-20	2.53^{a} ±0.51	2.69^{a} ±0.37	2.59^{a} ±0.28	2.62^{a} ±0.57	2.80^{a} ±0.25	4.55^{a} ±0.62	3.57^{ab} ±0.46	3.60^{ab} ±0.50	3.11^{b} ±0.41	3.08^{b} ±0.34
					pH (H_2O)					
0-5	5.07^{a} ±0.09	5.12^{a} ±0.14	5.30^{b} ±0.13	5.33^{b} ±0.06	5.30^{b} ±0.06	5.68 ±0.48	5.59 ±0.42	5.53 ±0.37	5.51 ±0.39	5.56 ±0.37
5-10	4.88 ±0.15	5.03 ±0.16	5.07 ±0.09	5.15 ±0.06	5.12 ±0.10	5.53 ±0.50	5.43 ±0.49	5.41 ±0.33	5.35 ±0.41	5.38 ±0.38
10-20	4.93 ±0.05	4.99 ±0.09	5.06 ±0.10	5.12 ±0.11	5.11 ±0.08	5.50 ±0.47	5.40 ±0.41	5.43 ±0.36	5.41 ±0.45	5.41 ±0.38
					P_{ext} (mg kg^{-1})					
0-5	11.5^{a} ±0.6	10.1^{a} ±1.3	6.8^{b} ±1.3	6.4^{b} ±1.0	5.1^{c} ±0.5	7.7^{a} ±1.4	5.3^{ab} ±1.1	4.7^{abc} ±3.0	3.3^{bc} ±1.0	1.8^{c} ±0.6
5-10	5.2 ±1.9	4.1 ±0.3	4.5 ±1.2	3.3 ±0.6	3.4 ±0.5	3.1^{a} ±1.2	2.5^{ab} ±1.2	2.3^{ab} ±0.6	0.9^{b} ±1.1	1.2^{ab} ±0.3
10-20	4.0 ±0.9	3.9 ±1.5	4.9 ±1.2	3.9 ±1.1	4.3 ±1.8	1.9 ±1.3	2.2 ±1.1	1.4 ±0.4	1.4 ±0.3	1.1 ±0.9

Accumulation of organic C

The logarithm transformed linear regressions of soil organic C content (C) as a function of the distance from tree trunk, relative to tree crown radius (R), for each study site and for each considered soil layer, are shown in the Figure 4, and the estimated model adjustment parameters are presented in the Table 5.

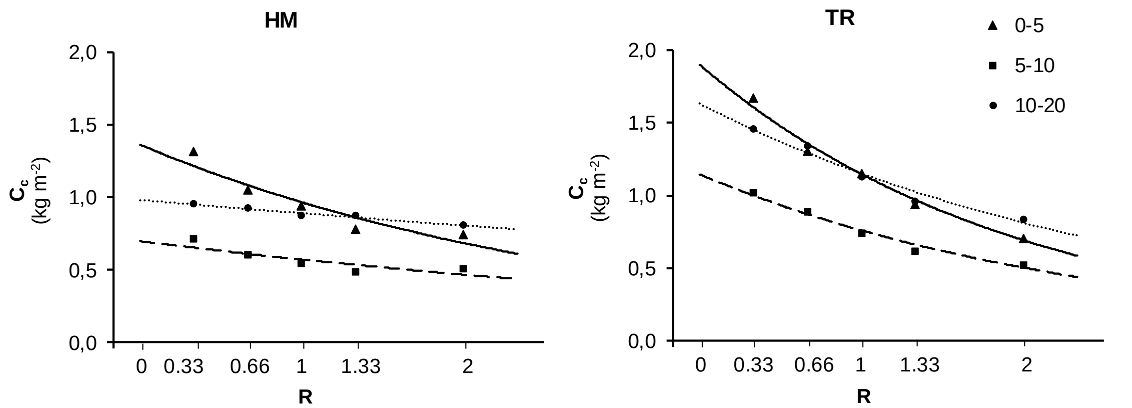

Figure 4 - Logarithm transformed linear regression models for the soil organic C accumulation (C_c) as a function of the distance to the tree trunk (R), in the 0-5, 5-10 and 10-20 cm soil depth layers, at the *Tapada Real de Vila Viçosa* (TR) and *Herdade da Mitra* (HM).

It is notable that the regression models are significant for each of the considered soil layers ($p<0.001$) at TR (Table 5). Similar trend was observed at HM, but the adjustment was only significant for the 0-5 and 10-20 cm soil layers.

Table 5 - Estimated parameters values and standard errors of the logarithm transformed linear regression of the soil organic C accumulation (C_c) for any distance relative to the tree crown radius (R), for each studied soil layer (0-5, 5-10 and 10-20 cm depth) at *Tapada Real de Vila Viçosa* (TR) and Herdade da Mitra (HM).

Depth (cm)	**log(b)**	**Std. Error**	***b***	***a***	**Std. Error**	R^2_{adj}	***p*-value**
				HM			
0-5	0.310	0.083	1.363	0.344	0.068	0.859	0.015
5-10	-0.359	0.085	0.698	0.200	0.070	0.639	0.066
10-20	-0.017	0.011	0.984	0.099	0.009	0.967	0.002
				TR			
0-5	0.644	0.033	1.905	0.509	0.025	0.988	<0.001
5-10	0.139	0.043	1.149	0.418	0.033	0.969	<0.001
10-20	0.499	0.045	1.646	0.366	0.035	0.957	<0.001

In both TR and HM, the highest amounts of accumulated soil organic C were estimated for the circular area between 0.66R and 1R points (Figure 5): 74 and 29 kg, respectively at TR and HM (Figure 5). The soil organic C accumulation in this area due to the tree cover represented 26.5%, at TR, and 29%, at HM, of the total amount accumulated around each tree. The lowest contribution for the soil organic C accumulation in the area considered influenced by trees was estimated for the area closer to the tree trunk (up to 0.33R), which added only about 10% of the total soil organic C accumulated in the tree-influenced area, at both study sites.

It is notable that high amounts of soil organic C, associated with the single tree influence, were also estimated beyond tree crowns (115 and 36 kg, respectively at TR and HM), which corresponded to 41 and 38% of the total accumulated around each tree (Figure 5).

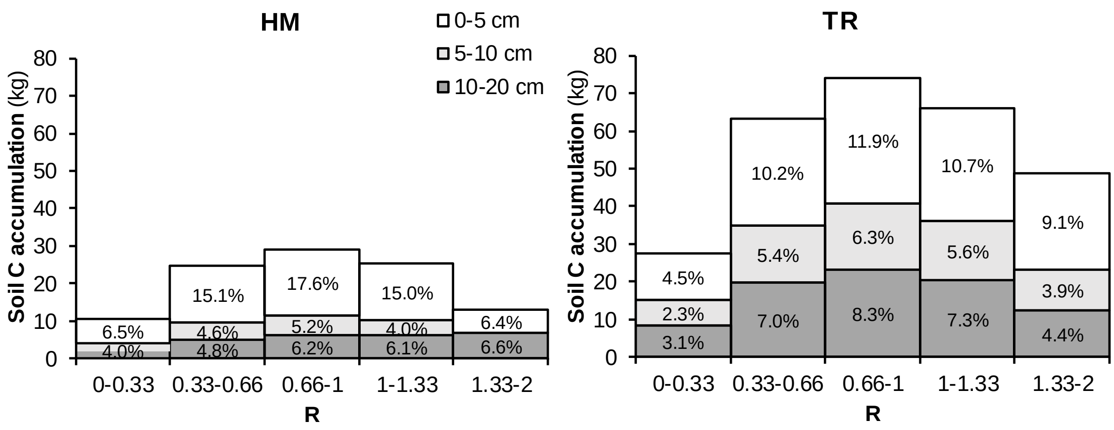

Figure 5 - Estimated soil organic C accumulated in each study soil layers (0-5, 5-10 and 10-20 cm), as influenced by a single tree, for the 0-0.33, 0.33-0.66, 0.66-1, 1-1.33 and 1.33-2 circular areas, defined relatively to the tree crown radius (R), at *Tapada Real de Vila Viçosa* (TR) and *Herdade da Mitra* (HM) sites.

The soil organic C accumulation in the area considered affected by scattered trees (up to 2R) was much higher at TR than at HM (respectively 280 and 101 kg; Figure 6). The proportion of the organic C accumulated in the 0-5 cm soil layer, at TR, was higher (46%) than that estimated in the 5-10 and 10-20 cm layers (24 and 30%, respectively); the difference was stronger at HM, as the proportion accumulated in the 0-5 cm soil layer reach 60% of the total, whereas in others was only 14 and 26% (5-10 and 10-20 cm soil layers, respectively).

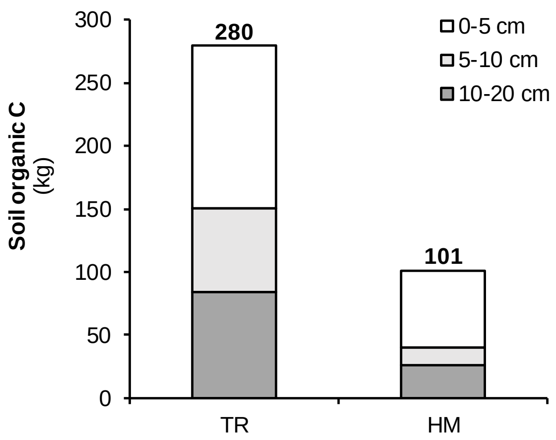

Figure 6 - Estimated soil organic C accumulation in the area affected by a single tree - from the tree trunk up to two times the tree crown radius -, in the 0-5, 5-10 and 10-20 cm soil layers, at the *Tapada Real de Vila Viçosa* (TR) and the *Herdade da Mitra* (HM).

DISCUSSION

Litter layer mass

The litter layer mass measured at *Herdade da Mitra* is of the same magnitude of that reported for an agroforestry system with wide spaced ash trees (*Fraxinus angustifolia* L.) in North-eastern Portugal (Pereira et al., 2004), and is within the range reported in *dehesas* by Escudero et al. (1985) for the litter layer mass accumulated beneath *Quercus rotundifolia* and *Q. pyrenaica*, situated to the West of the Province of Salamanca (Spain); also, it is close to that measured by Lecomte et al. (2018) in an oak woodland grazed by ungulates. Meanwhile, it is much lower than that observed for an ungrazed cork oak woodland with higher oak tree density (about 170 trees/ha) (Rodrigues et al., 2019; chapter 1). Naturally, the litter layer mass measured in the present study is much lower than that reported for forest plantations in Portugal (Madeira and Ribeiro 1995; Madeira et al., 1995).

At *Herdade da Mitra*, the litter layer mass shows a strong spatial variability as it decreases from tree trunk onwards, which is in accordance with the spatial variability observed for the amount of litterfall in a similar site (Sá, 2001), which was considerably greater at 2 m than at 4 m from the tree trunk. Also, it agrees with observations reported by Pereira et al (2004) for wide spaced ash trees, in which the amount of litterfall close to (0.5 m) the tree trunk was 2-3 times higher than that measured in the limit of the tree crown vertical projection, and by Escudero et al. (1985) for scattered *Quercus* spp. trees, in which the litterfall measured at 0.5 m from the tree trunk was almost twice the amount at the edge of the tree crown

It is noteworthy that high amounts of litterfall (0.43-0.51 kg m^{-2} $year^{-1}$; leaf litter 0.14-0.32 kg m^{-2} $year^{-1}$) were estimated for the same study area holm and cork oak trees, at a tree crown area basis (Sá et al., 2005), but the mass of the soil litter layer accumulated underneath tree canopies was relatively small. This trend is unexpected taking into account the *Q. rotundifolia* leaf litter decomposition rate (k= -0.48 $year^{-1}$) determined in a long-term litterbag experiment in the study site by Sá et al. (2005), which is much lower than the calculated turnover rate (0.73 $year^{-1}$; *sensu* Olson,1963). Indeed, the average annual litterfall was 4.7 Mg DM ha^{-1} $year^{-1}$ (Sá et al., 2005) and the necromass on the soil surface before the litterfall peak was only 1,7 Mg DM ha^{-1} year^{-1}. The difference between the expected and the actual litter layer accumulation suggests a considerable high organic residues turnover rate, which may be related to the activity (consumption and transference of new litterfall) of grazing and other herbivorous animals (and possibly of meso and macrofauna), as reported by Escudero et al. (1985) for *dehesa* systems, and by Simões et al. (2009) for shrub encroached *montados*. Lecomte et al. (2018) results also support this trend, as the amount of the litter layer mass on grazed areas was about half of that measured in those in which the grazing was prevented. Meanwhile, it should be also emphasized that the low litter layer mass beneath isolated tree crowns may also be related to the distribution of litterfall beyond the tree crown projection (Pereira et al., 2004) and to the removal of litter components other than leaf litter.

Despite the low average mass (about 31 kg) and spatial distribution of the litter layer accumulated around scattered single trees, it may cause a positive feedback (Ehrenfeld et al., 2005) on the tree-soil system, given its effect on soil temperature (Rhoades, 1997), soil protection from erosional forces and improvement of water infiltration rates, nutrient supply and biological activity (Cadish and Giller, 1997; Fisher and Binkeley, 2000; Hoosbeek et al., 2018).

Organic C concentration and accumulation

The presence of oak trees in agroforestry systems is a decisive factor to increase the concentration of organic C in the soil (Lorenz and Lal, 2014). This trend, commonly found in tropical regions (Albrecht and Kandji, 2003; Hoosbeek et al., 2018; Somarriba et al., 2013; Takimoto et al., 2008, 2009), undoubtedly occurs in the study *montado* sites, following results reported by Dahlgren et al. (1997; 2003) for California oak woodlands, by Gallardo (2003), Cubera and Moreno (2007), Gerardo and Obrador (2007) and Howlett et al. (2011) for *dehesas* in Spain, and by Cardinael et al. (2015) and Pardon et al. (2017) for alley cropping agroforestry in agroforestry sites (silvoarable and silvopastoral) in sub-humid Mediterranean and non-Mediterranean climate. Also, our results are in fully agreement with those reported by Gómez-Rey et al. (2013) for similar Mediterranean oak woodlands with shrub encroachment, and by Gómez-Rey et al. (2011; 2012) for Mediterranean oak woodlands with natural and improved pastures. Moreover, the results of the present are in accordance with those reported by Simões et al. (2009) for soil properties under Mediterranean shrub cover as compared to those observed in the open areas.

The increment of the organic C concentration beneath tree canopy observed in both sites mostly occurred in the 0-5 cm soil depth layer, suggesting that it is mostly in relation with the amounts of organic residues annually deposited on the soil surface by litterfall (Escudero et al. 1985), and with the root litter produced in such layer (Moreno et al., 2007). This trend agrees with the strong relationship between the organic C concentration in the top soil layer and the amount of the organic litter layer mass (r=0.9496; p<0.0134) observed at *Herdade da Mitra*. Also, this trend is in accordance with the fact that the increment of the organic C concentration in the top soil layer associated with the particulate organic matter fraction (that is, the fraction in soil particles higher than 50 µm), in relation to that in the 5-10 cm layer (3.4 and 2.5 times, at HM and TR, respectively), was higher than the increment of the total organic C (2.3 and 1.7 times, respectively). Moreover, the concentration of the POM-C at *Herdade da Mitra* was strongly correlated with the organic layer mass (r=0.9493, p<0.0136). The similar concentration of POM-C in both sites also suggests the effect of residues associated with the litterfall. Hence, it is assumed that the influence of the herbaceous understory mass (above- and belowground) on the differences of soil organic matter concentration, observed under tree crowns and in the open, may be negligible at this study site, as the amounts of such understory biomass were lower underneath trees than in the open, as reported by Sá (2001) at *Herdade da Mitra*.

Despite the similar variation pattern according to the position relatively to trees, the concentrations of the soil organic C, at *Herdade da Mitra*, were much lower than those observed at *Tapada Real*. This difference could be related to the soil finer texture (silty-loam) in the latter as compared with the former (sandy-loam), which may determine strong differences on the potential soil organic carbon saturation (*sensu* Hassinck, 1997). In spite of the higher proportion of the POM-C observed at *Herdade da Mitra*, differences in soil organic C concentrations in the 5-10 and 10-20 cm soil layers, according to the distance to tree, were negligible, while at *Tapada Real* these were observed up to 20 cm depth. Similar trend was observed for differences in concentrations of the mineral-associated C (that is, the difference between the soil organic C concentration and that POM-C;

Bayer et al., 2004), which increased 2.1, 1.9 and 1.7 times in the 0-5, 5-10 and 10-20 cm soil layers at *Tapada Real*, while at *Herdade da Mitra*, the increment was only 1.5, 1.4 and 1.1 times. These differences are unexpected, as trees had similar size at both study sites, and might be explained by the disturbances associated with the contrasting management histories (Plieninger et al., 2003), which were stronger at *Herdade da Mitra* than at *Tapada Real*.

The continuous decreasing of the soil organic C accumulation (up to 20 cm depth) observed from the zones closest the tree trunk to those in the open, is similar to the trend obtained for the variation of organic C concentrations. This pattern highlights the strong influence of oak trees, in the *montado* system, on the spatial variability of the soil organic C accumulation. This result is in accordance with the greater soil carbon storage measured underneath tree canopies in the *dehesa* cork oak silvopasture of central-west Spain (Howllet at al., 2011), and with the spatial variability of the organic C pool, measured up to 5 cm depth, in a holm oak *dehesa* (Simón et al.; 2013). Indeed, these authors stated that the association between trees and soil organic C levels occurs beyond the tree crown, in a distance equal to the double of the tree crown projection radius.

The organic C accumulation estimated up to 20 cm depth in the open (2.08 and 2.05 kg C m^{-2} at TR and HM, respectively), are smaller than those reported, for the same soil depth and in similar sites, by Gómez-Rey et al. (2012) in a natural pasture (3.0 kg C m^{-2}) and a 35-year old improved pasture (3.18 kg C m^{-2}), and by Rodrigues et al (2019; see chapter 1), for a 5-year old improved pasture (2.30 kg C m^{-2}) and an oak woodland with natural understory (2.75 kg C m^{-2}), where tree density was higher (177 trees/ha) than those in study sites. Our results emphasize that, for a deep understanding on the organic C accumulation in the *montado* system, it is indispensable to take into account the soil type, the land use type, soil management, tree density and the site history.

Data of the current study indicate that scattered single trees in the *montado* can largely contribute for the level of organic C storage. In fact, at *Tapada Real*, an increment of about 280 kg of soil C was accumulated up to 20 cm depth in the area influenced by each tree, as compared with the open. If the stand tree density is considered (at least 50 trees per hectare), the accrual of accumulated organic

C per hectare can reach about 14 000 kg, which corresponds to 1.4 kg C m^{-2}. Similar estimation for the *Herdade da Mitra* indicates an accrual of about 0.51 kg C m^{-2}; if the estimated organic C accumulated in the soil litter layer around each tree (about 15 kg) is taken into account, the accrual should be about 1.48 and 0.59 kg C m^{-2}. It is noteworthy that this level of organic C accumulation is, for example, much higher than that reported by Gómez-Rey et al. (2012) for a 26-year old open improved pasture (only 0.18 kg C m^{-2}), as compared with a natural pasture. These results undoubtedly indicate a greater potential of the scattered trees, in the *montado*, for the enhancement of soil C sequestration.

For an estimation at the system level, the role of trees on the organic C accumulation should be considered. If the biomass of trees is taken into account, considering data reported by Lecomte et al. (2018) for the *Tapada Real* site, we estimate that the average amount of C in a single cork oak tree is about 350 kg, and that in a single holm oak is about 476 kg (for trees with similar dendrometric features as those selected in the present study). Therefore, in a *montado* with at least 50 trees per hectare, the amount of C accumulated in the trees can reach 17 500 and 23 800 kg per hectare, which corresponds to 1.75 and 2.38 kg C m^{-2}, for cork and holm oak stands, respectively. Schematically, this means that the accumulation of organic C (biomass plus soil accrual), in the *montado* at TR, due to cork oak trees is approximately 3.2 kg C m^{-2}, whereas that in the *montado* at HM can reach 2.9 kg C m^{-2} (see Figure 7).

In short, the amounts of organic C associated with scattered trees can be much higher than those estimated for open soils (2.08 and 2.05 kg C m^{-2}, respectively at TR and HM), assumed as treeless pasture areas.

Notwithstanding, it must be noted that the current study was developed in sites where mature oak trees with an even-aged distribution occurred. In such a case, the aforementioned results suggest that they can be a useful tool for the evaluation of the organic C accumulation in the *montado* at landscape level, but deeper studies are necessary for a broader implementation of such approach. In fact, sites with uneven aged tree distribution may show a much wider spatial variability, including that related to the residual effect of dead trees.

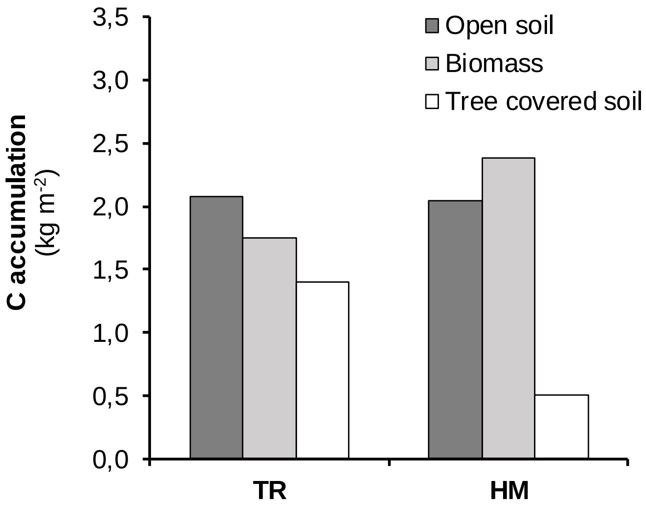

Figure 7 - Estimated C accumulation in the open soil, and accruals associated with oak trees, for a tree density of 50 trees ha^{-1}, at the Tapada Real de Vila Viçosa (TR) and the Herdade da Mitra (HM).

Soil physical conditions

Bulk density is a very useful indicator for assessing soil quality and degradation (Weil and Brady, 2017; Pulido et al., 2017). Lower values of soil bulk density observed in the current study underneath tree crowns than in the open grassland are in agreement with results and trends reported by Belsky et al. (1989) and Belsky et al. (1993) for a semiarid savannah and low and high rainfall savannas, by Dahlgren et al. (1997, 2003) for California oak woodlands, by Cardinael et al. (2015) for alley cropping agroforestry in a Mediterranean context, and by Gómez-Rey at al. (2012) for oak woodlands with improved and natural pastures, or with those reported in the Chapter 2 of the present thesis.

The lowering soil bulk density in the upper soil layer beneath the tree crowns is most likely associated with the higher soil organic matter contents (and organic residues inputs), which coupled with increased mixing of soil biota and soil fauna/flora activity may enhance soil structure, resulting in increased total porosity (Dahlgren et al., 1997, 2003). Although the accumulation of organic matter itself contributes to decrease the soil bulk density (Weil and Brady, 2017), differences between soil bulk density observed underneath tree crowns and in the open, may be mostly explained by the increasing of soil porosity (and eventually macroporosity). Indeed, the soil porosity in the 0-5 cm layer, at both sites, increases from about 0.42 and 0.41 cm^3 cm^{-3}, in the open grassland, to about 0.57 and 0.54 cm^3 cm^{-3} (at HM and TR sites, respectively) in the zones

closer the tree trunk, following the spatial trends observed for the increasing of the litter layer mass and for the organic C concentration in the soil mineral layers under canopy. Such a trend may be a result of higher amounts (flux of organic debris) of decomposing residues (both litterfall and root litter) and the associated stronger biological activity, resulting in dead root channels and biopores, as explained by Weil and Brady (2017). This improvement of soil porosity may enhance the saturated soil hydraulic conductivity and soil aeration, which in turn, promotes an improved environment for soil organisms (Fisher and Binkley, 2000).
It is noteworthy that the values of bulk density observed up to 10 cm depth in the open at *Tapada Real* (1.56 and 1.58 g cm^{-3}) and at *Herdade da Mitra* (1.50 and 1.54 g cm^{-3}) are of the same magnitude, but are higher than those reported by Gómez-Rey at al. (2012) for natural (1.40 g cm^{-3}) and improved (1.36 g cm^{-3}) pastures growing in soils with sandy loam texture. Such a difference suggests the diagnosis of soil compaction, a documented process associated with the stocking rate increasing (Billota et al., 2007; Pulido et al., 2017), especially at the TR site. In fact, the soil at this site, due to its finer texture (silty loam), may be much more susceptible to compaction (Weil and Brady, 2017) than that at *Herdade da Mitra*. Indeed, the measured bulk density at *Tapada Real* is above the critical value (about 1.5 g cm^{-3}) which is reported to cause strong reductions on aeration porosity and soil drainage, and, therefore, restrictions in rooting and water resource use by plants (Weil and Brady, 2017; Leão et al. 2006). Despite these features observed in the open, scattered trees in both sites tend to reverse the soil compaction tendency (that is, increasing porosity, by reducing bulk density), creating patches of more favourable soil physical conditions, in which soil degradation is avoided or alleviated.
Therefore, results of the present study highlight that scattered trees in the *montado* system play an important role in the landscape by improving soil conditions which enhances soil aeration and biological processes, and facilitates plant rooting, and soil water infiltration and drainage, which decreases rainfall losses by run-off and allows quicker water recharge of deeper layers, leading to an improvement of water conditions under tree crowns as reported by Joffre & Rambal (1988) for similar systems.

Soil fertility

Soils underneath tree crowns of both study sites are more fertile than soils from the surrounding open grassland, following trends reported for other oak woodlands occurring in California (Dahlgren et al., 2003) and in the Iberian Peninsula (Gallardo, 2003; Moreno et al., 2007; Moreno and Obrador, 2007). Also, they follow trends which have been reported for several agroforestry systems in tropical regions (Weltzin and Coughenour, 1990; Isichei and Muoghalu, 1992; Belsky et al., 1993), and for agroforestry systems occurring in Belgian (Pardon et al., 2017). Moreover, results of the present study follow those reported for nutrient availability in soils beneath Mediterranean shrubs, as compared with those in the open (Simões et al., 2009).

In both study sites, the different soil fertility indices (e.g. organic matter concentration, extractable P, exchangeable Ca^{2+} and Mg^{2+}) were similarly more elevated underneath trees than in the open. This alteration regarding the soil environment surrounding the scattered trees is reported to primarily occur through the addition of organic matter residues and nutrient cycling (Dahlgren et al., 1997). Although the exact source of the nutrient enrichment of soils beneath trees in both study sites was not specifically investigated, it was probably, in part, associated with the nutrient redistribution by tree litterfall (and via their decomposition), because trees transfer nutrients from the surrounding surface and deep soil layers to their canopy, and then drop them in their sorounding soil surface, through leaf and stem litter (Escudero et al., 1985; Moreno and Obrador, 2007). The expansion of the oak tree roots to large distances beyond the edge of the canopy (David, 2000; Moreno et al., 2005) may also decisively contribute to translocate nutrients from the open grassland, thus enhancing nutrient concentrations differences between open and tree-covered soils. Then, the greater concentration of exchangeable non-acid cations in the soil beneath tree crowns is a consequence of the nutrient cycling by oak trees, which selectively replenishes the Ca^{2+}, Mg^{2+}, and K^{+} concentrations while Na^{+}, a non-essential plant nutrient, is not accumulated beneath the tree canopy. In fact, the leaf litterfall of cork ok and holm oak is known to return high amounts of these nutrients to the soil (Escudero et al., 1985; Andivia et al., 2010), and the average flux of Ca, Mg, and K to the soils beneath these oak canopies, measured at the *Herdade da Mitra* site, was about 21, 5 and 9 kg ha^{-1} yr^{-1}, respectively (Sá, 2001).

The amount of nutrients transported by throughfall and stemflow will also affect the difference of nutrient availability in soil under and outside tree canopy, as reported in studies of several authors (Wolfe *et al*., 1987; Kretinin, 1993; Seiler & Matzner, 1995). Indeed, at *Herdade da Mitra*, the measured returns of deposition and canopy throughfall reach about 21, 7 and 38 kg ha^{-1} yr^{-1} respectively for Ca, Mg and K (Nunes et al., 2001; Nunes, 2004).

A further effect of scattered trees on nutrient cycling occurs through canopy processes, which increases transpiration and rainfall interception (David, 2000; David et al., 2006; Cubera and Moreno, 2007), thus reducing the water available for leaching in the soils beneath the tree crowns. In short, it means that much of such flux represents nutrients that would have been lost from the soil profile in the absence of oak trees (Dahlgren et al., 1997). Other factors may also contribute to the soil nutrient enrichment underneath oak trees. For instance, shading up by grazers may also result in some transport of nutrients from the open grassland to soils beneath tree crowns as they may preferably defecate beneath the oak canopy (Dahlgren et al., 1997).

Results of the current study also indicate that the enrichment of the soil in nutrients beneath tree crowns shows a wide variability from the tree trunk to the edge of the canopy. Such a variability may be partly associated with the aforementioned variability related to the litterfall distribution beneath tree crowns (Sá et al. 2005; Escudero et al., 1985). Furthermore, the soil nutrient spatial variation might be also dependent on the spatial variability of the nutrient fluxes related to the throughfall and stemflow**,** which at *Herdade da Mitra* showed a wide variability with the distance to the tree trunk, reaching the highest values close the trunk and the lowest at the edge of the crown (Nunes et al., 2001; Nunes, 2004), following trends reported by Wolfe *et al*. (1987) and Seiler & Matzner (1995) for other ecosystems. It should be emphasized that the redistribution of nutrients in the soil close the tree trunk, is strongly influenced at a local level by the stemflow, which corresponds to a high devolution in a relatively small area effectively influenced by it (Voight, 1960). This trend, observed by Nunes et al. (2001) and Nunes (2004) at *Herdade da Mitra*, can assume relevance in the nutrient availability in the soil closer to the tree trunk, especially regarding K. Indeed, the variability observed for the characteristics of the top soil layer with distance to the tree trunk, is in fully agreement with the distribution of nutrients by the precipitation solutions and litterfall spatial distribution, as pointed by Zinke (1962).

It is noteworthy that soil fertility in the open was overall more pronounced at *Tapada Real* than at *Herdade da Mitra*, which may be associated with differences in soil texture (silty loam in the former and sandy loam in the latter) and soil organic matter concentrations (higher in the former than in the latter). Despite such soil differences, the enhancement of soil fertility beneath tree crowns in both sites showed a similar trend, mostly associated with the accumulation of soil organic matter. This accumulation leads to a higher concentration of nitrogen and higher capacity to retain cations, which is supported by the fact that the effective cation exchange capacity close the tree trunk was 1.9 and 2.3 times (at TP and HM, respectively) higher than that estimated for the open areas, and by the strong correlation between the soil organic carbon concentration and the effective cation exchange capacity (see Annex I). That is, the increment of soil fertility beneath tree crowns is strongly associated with the carbon cycle and driven by the soil organic matter accumulation.

Despite of the stronger accumulation of organic matter in the 0-5 cm soil layer, pH values in this layer were higher than in the other layers, which may be associated with the retention of non-acid cations (and decreasing aluminium) in the top soil layer. However, it is noteworthy that the saturation degree at the soil pH in both sites was similar in all soil depths (97-98%).

CONCLUSIONS

The present study undoubtedly shows that scattered cork and holm oak trees in the *montado* system create gradients of soil physical and chemical properties, from the tree trunk into open areas, up to distances that can be twice the tree crown radius. This trend evidences the important role of the oak trees in the capture, devolution and retention of nutrients in the top soil layers. Soils underneath oak tree canopies are more fertile and show higher quality than soils from the surrounding open pastures. The current study also shows that soil fertility indices (e.g. organic matter, extractable P, K and Ca) were similarly elevated in the canopy zone of both study sites as compared to outside the canopy. Also, the transference of great amounts of organic residues to the soil surface under tree canopy leads to soil protection and a considerable improvement of soil physical conditions (e.g. lower soil bulk density and higher soil porosity) which favour soil aeration and drainage. However, physical conditions and nutrient levels may be dependent on soil type, land and soil management and tree age. Results suggest that oak trees are an important component of the ecosystem that serve a valuable role in the retention of nutrients and organic carbon which in turn will contribute to the long-term ecosystem sustainability.

Trees in the *montado* are responsible for huge amounts of organic C accumulation at landscape scale, a high proportion being sequestered into the soil. Consequently, the decline of *montado* and associated loss of oak trees, may lead to considerable organic C and nutrient losses from the ecosystem. In these circumstances, management practices and policies should be developed to support tree regeneration rates that can maintain or increase tree cover in oak woodland areas. Such a strategy is crucial to enhance organic carbon sequestration, aiming the accomplishment of international commitments as well as the improvement of soil resistance to face degradation risks.

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ANNEX I

Pearson correlation coefficients (*r*) between relevant soil properties in the 0-5 cm soil layer at Tapada Real de Vila Viçosa

r						pH		Extractable		Exchangeable							
		C	**POM-C**	**N**	**C:N**	**H_2O**	**KCl**	**P**	**K**	**Ca^{2+}**	**Mg^{2+}**	**Na^{+}**	**K^{+}**	**Sum**	**Al^{3+}**	**ECEC**	**Dist.**
	BD	-0,966	-0,804	-0,964	-0,751	-0,333	-0,841	-0,915	-0,867	-0,937	-0,932	-0,232	-0,848	-0,941	0,666	-0,940	0.888
	C		0,891	0,975	0,838	0,236	0,818	0,922	0,858	0,935	0,914	0,276	0,837	0,934	-0,648	0,934	-0.920
	POM-C			0,845	0,773	-0,042	0,550	0,802	0,673	0,758	0,747	0,462	0,622	0,758	-0,390	0,761	-0.801
	N				0,706	0,257	0,828	0,925	0,852	0,934	0,909	0,272	0,860	0,935	-0,640	0,934	-0.894
	C:N					0,146	0,634	0,710	0,678	0,708	0,691	0,199	0,603	0,704	-0,615	0,701	-0.838
pH	**H_2O**						0,582	0,094	0,388	0,465	0,512	-0,396	0,361	0,465	-0,641	0,457	-0.291
	KCl							0,732	0,865	0,920	0,882	0,007	0,888	0,916	-0,805	0,912	-0.760
Ext.	**P**								0,784	0,836	0,809	0,242	0,827	0,840	-0,551	0,840	-790
	K									0,905	0,893	0,323	0,966	0,921	-0,676	0,919	-0.824
Exchangeable	**Ca^{2+}**										0,981	0,189	0,893	0,998	-0,678	0,998	-0.853
	Mg^{2+}											0,199	0,861	0,987	-0,669	0,987	-0.846
	Na^{+}												0,209	0,212	0,128	0,218	-0.337
	K^{+}													0,906	-0,651	0,905	-0.760
	Sum														-0,677	0,999	-0.857
	Al^{3+}															-0,665	0.719
	ECEC																0.338

Pearson correlation coefficients (*r*) between relevant soil properties in the 5-10 cm soil layer at Tapada Real de Vila Viçosa

	r					pH		Extractable		Exchangeable							
		C	**POM-C**	**N**	**C:N**	**H_2O**	**KCl**	**P**	**K**	**Ca^{2+}**	**Mg^{2+}**	**Na^+**	**K^+**	**Sum**	**Al^{3+}**	**ECEC**	**Dist.**
	BD	-0,948	-0,777	-0,906	-0,801	-0,399	-0,732	-0,789	-0,813	-0,824	-0,822	-0,573	-0,809	-0,851	0,661	-0,861	0.852
	C		0,808	0,940	0,867	0,501	0,817	0,814	0,849	0,906	0,882	0,586	0,858	0,927	-0,777	0,933	-0.915
	POM-C			0,854	0,750	0,162	0,568	0,526	0,599	0,744	0,743	0,671	0,541	0,762	-0,574	0,773	-0.744
	N				0,651	0,442	0,779	0,772	0,703	0,882	0,846	0,665	0,734	0,896	-0,766	0,900	-0.873
	C:N					0,450	0,669	0,679	0,874	0,717	0,715	0,392	0,822	0,745	-0,192	-0,080	-0.808
pH	**H_2O**						0,809	0,456	0,593	0,704	0,675	0,234	0,658	0,710	-0,864	0,689	-0.428
	KCl							0,745	0,805	0,928	0,807	0,390	0,878	0,918	-0,933	0,908	-0.683
Ext.	**P**								0,702	0,746	0,711	0,312	0,839	0,762	-0,640	0,767	-0.634
	K									0,799	0,806	0,434	0,895	0,831	-0,760	0,831	-0.807
Exchangeable	**Ca^{2+}**										0,893	0,482	0,838	0,990	-0,910	0,989	-0.776
	Mg^{2+}											0,578	0,812	0,945	-0,831	0,947	-0.803
	Na^+												0,274	0,533	-0,507	0,530	-0.709
	K^+													0,861	-0,774	0,861	-0.713
	Sum														-0,909	0,999	-0.812
	Al^{3+}															-0,893	0.703
	ECEC																-0.815

Pearson correlation coefficients (*r*) between relevant soil properties in the 10-20 cm soil layer at Tapada Real de Vila Viçosa

	r					pH		Extractable		Exchangeable							
		C	**POM-C**	**N**	**C:N**	**H_2O**	**KCl**	**P**	**K**	**Ca^{2+}**	**Mg^{2+}**	**Na^{+}**	**K^{+}**	**Sum**	**Al^{3+}**	**ECEC**	**Dist.**
	BD	-0,801	-0,804	-0,744	-0,726	-0,067	-0,409	-0,350	-0,640	-0,531	-0,699	-0,746	-0,553	-0,615	0,474	-0,628	0.707
	C		0,845	0,934	0,911	0,121	0,540	0,381	0,560	0,593	0,748	0,684	0,576	0,671	-0,532	0,683	-0.866
	POM-C			0,763	0,803	-0,125	0,323	0,118	0,455	0,419	0,568	0,648	0,413	0,490	-0,260	0,524	-0.741
	N				0,707	0,339	0,655	0,494	0,570	0,673	0,805	0,545	0,623	0,741	-0,617	0,748	-0.811
	C:N					-0,146	0,316	0,216	0,469	0,392	0,535	0,733	0,429	0,466	-0,333	0,481	-0.805
pH	**H_2O**						0,825	0,566	0,459	0,726	0,514	-0,151	0,665	0,680	-0,771	0,646	-0.189
	KCl							0,659	0,629	0,931	0,731	0,166	0,878	0,905	-0,866	0,891	-0.511
Ext.	**P**								0,511	0,595	0,442	0,065	0,672	0,575	-0,550	0,567	-0.400
	K									0,651	0,657	0,411	0,703	0,685	-0,622	0,682	-0.755
Exchangeable	**Ca^{2+}**										0,845	0,287	0,837	0,987	-0,879	0,985	-0.552
	Mg^{2+}											0,493	0,721	0,918	-0,836	0,912	-0.716
	Na^{+}												0,292	0,383	-0,390	0,372	-0.685
	K^{+}													0,845	-0,794	0,835	-0.537
	Sum														-0,900	0,996	-0.632
	Al^{3+}															-0,858	0.543
	ECEC																-0.634

Pearson correlation coefficients (*r*) between relevant soil properties in the 0-5 cm soil layer at Herdade da Mitra

	r					pH		Extractable		Exchangeable							
		C	POM-C	N	C:N	H_2O	KCl	P	K	Ca^{2+}	Mg^{2+}	Na^{+}	K^{+}	Sum	Al^{3+}	ECEC	Dist.
	BD	-0.943	-0.900	-0.951	-0.695	-0.047	-0.712	-0.894	-0.808	-0.923	-0.344	-0.337	-0.814	-0.879	0.272	-0.877	0.897
	C		0.937	0.983	0.787	-0.086	0.632	0.847	0.892	0.933	0.409	0.516	0.810	0.910	-0.132	0.913	-0.8871
	POM-C			0.936	0.687	-0.119	0.584	0.870	0.848	0.894	0.438	0.438	0.765	0.885	-0.028	0.892	-0.892
	N				0.669	0.027	0.720	0.906	0.917	0.951	0.376	0.482	0.868	0.917	-0.227	0.917	-0.904
	C:N					-0.483	0.199	0.449	0.595	0.613	0.461	0.461	0.423	0.653	0.225	0.699	-0.642
pH	H_2O						0.596	0.109	0.129	0.203	-0.327	-0.486	0.351	0.068	-0.902	0.033	-0.041
	KCl							0.707	0.727	0.808	0.171	0.109	0.876	0.737	-0.708	0.716	-0.697
Ext.	P								0.836	0.908	0.491	0.371	0.839	0.918	-0.294	0.915	-0.911
	K									0.853	0.317	0.381	0.913	0.824	-0.240	0.823	-0.871
Exchangeable	Ca^{2+}										0.373	0.358	0.905	0.954	-0.376	0.948	-0.911
	Mg^{2+}											0.300	0.305	0.633	0.173	0.646	-0.547
	Na^{+}												0.268	0.403	0.324	0.420	-0.273
	K^{+}													0.866	-0.482	0.854	-0.874
	Sum														-0.262	0.999	-0.938
	Al^{3+}															-0.225	0.229
	ECEC																-0.938

Pearson correlation coefficients (*r*) between relevant soil properties in the 5-10 cm soil layer at Herdade da Mitra

	r					pH		Extractable		Exchangeable							
		C	**POM-C**	**N**	**C:N**	**H_2O**	**KCl**	**P**	**K**	**Ca^{2+}**	**Mg^{2+}**	**Na^+**	**K^+**	**Sum**	**Al^{3+}**	**ECEC**	**Dist.**
	BD	-0.782	-0.671	-0.880	-0.167	0.140	-0.353	-0.558	-0.701	-0.430	-0.496	0.194	-0.668	-0.504	0.300	-0.504	0.632
	C		0.739	0.864	0.623	-0.215	0.254	0.385	0.725	0.544	0.534	0.075	0.632	0.612	-0.043	0.612	-0.666
	POM-C			0.843	0.260	-0.234	0.051	0.487	0.538	0.432	0.729	0.176	0.517	0.635	0.115	0.647	-0.479
	N				0.151	-0.134	0.331	0.578	0.762	0.622	0.663	0.095	0.773	0.729	-0.189	0.719	-0.723
	C:N					-0.177	0.004	-0.103	0.228	0.118	0.075	0.022	0.035	0.106	0.203	0.122	-0.155
pH	**H_2O**						0.764	0.316	0.241	0.047	-0.112	-0.191	0.236	-0.014	-0.788	-0.074	0.074
	KCl							0.556	0.618	0.193	-0.017	-0.209	0.575	0.140	-0.876	0.074	-0.456
Ext.	**P**								0.364	0.363	0.530	0.030	0.414	0.489	-0.481	0.455	-0.528
	K									0.569	0.341	0.046	0.907	0.556	-0.429	0.527	-0.714
Exchangeable	**Ca^{2+}**										0.642	0.325	0.714	0.936	-0.188	0.927	-0.499
	Mg^{2+}											0.112	0.368	0.868	0.022	0.875	-0.452
	Na^+												0.099	0.278	0.460	0.315	-0.148
	K^+													0.660	-0.435	0.631	-0.626
	Sum														-0.114	0.997	-0.550
	Al^{3+}															-0.039	0.245
	ECEC																-0.535

Pearson correlation coefficients (r) between relevant soil properties in the 10-20 cm soil layer at Herdade da Mitra

	r					pH		Extractable		Exchangeable							
		C	**POM-C**	**N**	**C:N**	**H_2O**	**KCl**	**P**	**K**	**Ca^{2+}**	**Mg^{2+}**	**Na^{+}**	**K^{+}**	**Sum**	**Al^{3+}**	**ECEC**	**Dist.**
	BD	-0.252	-0.094	-0.388	0.048	0.648	0.017	-0.321	-0.285	0.121	-0.120	0.039	-0.240	-0.004	-0.044	-0.010	0.319
	C		0.261	0.783	0.654	-0.310	0.187	0.331	0.473	-0.130	-0.091	0.130	0.358	-0.075	0.065	-0.063	-0.402
	POM-C			0.180	0.179	0.030	0.338	-0.201	0.193	-0.174	-0.234	0.215	0.175	-0.178	-0.066	-0.181	-0.430
	N				0.045	-0.421	0.190	0.366	0.750	-0.127	-0.107	0.135	0.678	-0.047	0.165	-0.022	-0.351
	C:N					0.015	0.045	0.083	-0.139	-0.056	-0.013	0.047	-0.244	-0.061	-0.092	-0.072	-0.194
pH	**H_2O**						0.460	-0.127	-0.181	0.208	-0.215	-0.242	-0.108	0.005	-0.613	-0.081	0.372
	KCl							0.334	0.271	-0.217	-0.568	-0.441	0.325	-0.367	-0.828	-0.471	-0.274
Ext.	**P**								0.104	-0.237	-0.310	0.012	0.068	-0.269	-0.389	-0.315	0.061
	K									-0.054	-0.064	-0.010	0.977	0.038	0.015	0.039	-0.560
Exchangeable	**Ca^{2+}**										0.822	0.225	0.000	0.963	0.019	0.932	0.320
	Mg^{2+}											0.262	-0.053	0.933	0.342	0.949	0.139
	Na^{+}												-0.095	0.285	0.394	0.331	-0.043
	K^{+}													0.074	-0.031	0.069	-0.507
	Sum														0.177	0.990	0.197
	Al^{3+}															0.312	0.027
	ECEC																0.194

CHAPTER 4

Influence of pasture management on carbon and nutrient fluxes in evergreen oak woodland (*montado*) soils

Influence of pasture management on carbon and nutrient fluxes in evergreen oak woodland (*montado*) soils

ABSTRACT

Montado (*dehesa* in Spain) is the largest agroforestry system in the Iberia Peninsula, characterized by the combination of scattered evergreen oak trees with agriculture, pasture or shrubs understorey. Despite its acknowledged values, most *montado* areas are now threatened by lack of proper management and climate changes, highlighting the need for guidelines that can ensure the continuity of their economic and environmental services, as a base for their long-term sustainability. In order to identify possible effects of some current management practices on soil carbon and nutrient fluxes, a lysimetric study was performed. Undisturbed soil blocks from two *montado* farms were considered, representing different soil types (textures), long-term improved, recently renewed and natural pastures, under different stocking rates and grazer species. Soils greenhouse gases (GHG) fluxes (CH_4, N_2O, CO_2), nutrient leaching and N, P and C soils fluxes were monitored along a 15-month period, under even climatic conditions. Significant variations of GHG fluxes between management practices were observed, namely differences in hourly emissions were largely explained by sampling date and soil texture. Long-term improved pasture management have showed generally higher accumulated CO_2 emissions, prominent initial N_2O fluxes and enhanced P leaching. Pasture renewal was associated with higher soil microbial activity and increasing nitrate and cations leaching. Soils with finer texture showed higher potential to transfer C to the atmosphere, mainly due to higher CH_4 emissions. Our study highlighted the major role of abiotic factors over open grassland soils C and nutrient fluxes, but further studies are still needed to address climate factors and eventual global changes effects over these soils functions.

Keywords: grassland; greenhouse gases; nitrogen; organic carbon; phosphorus; soil leachates.

INTRODUCTION

Driven by the increase in anthropogenic greenhouse gases (GHG) emissions - particularly carbon dioxide (CO_2), nitrous oxide (N_2O) and methane (CH_4) - climate changes are already affecting the Mediterranean region, with higher temperatures, and increasing drought frequency (IPCC, 2014a). Soil is the largest terrestrial sink of carbon (C) and nitrogen (N) (Batjes, 1996) and is a key component of ecosystems, maintaining and regulating all biogeochemical cycles. Adequate soil management practices are being studied and suggested worldwide as mitigation strategies to minimize impacts of climate change (Bispo et al., 2017; Minasny et al., 2017; Stockmann et al., 2013). Agroforestry systems, the combination of trees with intercropped agricultural productions, are among the most promising land use options to sequester C from the atmosphere, in both soil and biomass (Kim et al., 2016; Kumar and Nair, 2011).

Evergreen oak woodlands (*montado* in Portugal, *dehesa* in Spain) are the most important agroforestry system in the Iberian Peninsula, where it occupies more than three million hectares (Eichhorn et al., 2006). Shaped by human needs along centuries, these multipurpose agroecosystems combine scattered oak trees - mainly *Quercus suber* L. and *Q. ilex* L. - with diverse understorey land uses, such as pastures, agricultural crops and/or shrubs (Belo et al., 2009; Moreno and Pulido, 2009). Despite their widely recognized social, environmental and economic values, crucial sustainability issues have been related to recent management trends (Bugalho et al., 2011; Escribano et al., 2018). Soil degradation and tree decline appear as a direct consequence of shifts in land use, namely intensification or abandonment (Costa et al., 2010; Hernández-Lambraño et al., 2018; Pulido-Fernández et al., 2013). A generalized decrease of tree natural regeneration has also been associated with grazing intensity and timing (Carmona et al., 2013; López-Sánchez et al., 2014; Plieninger, 2007). Furthermore, as climate changes become more evident in the Mediterranean region (IPCC, 2014a), *montado* adaptation and resilience is being questioned (Duque-Lazo et al., 2018; Lozano-García et al., 2017). In the last decades, Portuguese *montado* management has been mainly driven by subsidy policies, which are currently favouring silvo-pastoral systems, particularly those including cattle (IFAP, 2016). Indeed, national statistics show that, over the last decades, permanent pasture area strongly increased and traditional sheep and pig herds have been replaced by cattle, (GPP, 2018). A recent European survey estimates that livestock production under woodlands is the major agroforestry system in Portugal, occupying about 799.1 thousand hectares (den Herder et al., 2017).

In this context, sowing improved pastures - selected species mixtures, with high proportions of legumes - has become attractive to meet the higher livestock nutritional requirements (Hernández-Esteban et al., 2018). Furthermore, some studies reported positive impacts of improved pasture sowing on soil quality restoration, mainly due to organic carbon sequestration and nitrogen availability enhancement (Crespo, 2006; Teixeira et al., 2011). A financial support granted since 2009 by the Portuguese Carbon Fund has also stimulated the increase of national sowed pasture area (APA, 2017).

Some recent studies suggest potential positive effects of pasture sowing on organic C and N pools of *montados* top soil (Gómez-Rey et al., 2012; Hernández-Esteban et al., 2018). But the rate and extent of these soil quality improvements seem to rely on other site-specific and management factors, while tree regeneration problems remain overlooked (Rodrigues et al., 2019; see Chapter 2). Moreover, simple soil organic C stock increment does not ensure an effective C sequestration, since information on the net transfer of C from the atmosphere to the soil, as well as other soil GHG fluxes balances, is still lacking

The increase of soil organic matter may lead to significant modifications of the nitrogen dynamics, namely nitrification, with potential increases of nitrate leaching (Di and Cameron, 2002; Trolove et al., 2019). Also, since improved pasture management usually implies mineral phosphate (P) applications, soil high P saturation levels may lead to P runoff or leaching (Horta and Torrent, 2010).

In this context, a study was developed to assess soil C and N fluxes from open pastures under *montado* systems, as affected by different soil type and pasture management. By assembling a lysimetric experiment, with minimally disturbed soil blocks from six differently managed pastures, we were able to compare soil initial physical and biochemical status, CO_2, N_2O and CH_4 fluxes, and top soil nutrient leaching along a 15-month period. It was hypothesized that, under similar meteorological conditions, pasture and livestock management, along with soil type (texture), would influence the soil biogeochemical cycles and associated C and N fluxes. Our main objectives were to discuss how recent management modifications influence soil functions in *montado*, and to suggest adequate practices to overcome major sustainability challenges, namely, soil degradation, tree regeneration and overall system resilience in the face of climate change.

MATERIALS AND METHODS

Study systems

Two *montado* farms located in the Alto Alentejo region (Portugal) were considered: *Herdade dos Esquerdos* (**HE**; 39°07'-39°08' N, 7°29'-7°30' W - Vaiamonte) and *Herdade do Olival* (**HO**; 38°51'-38°52' N, 7°32'-7'°33' W - Mamporcão). Both farms have a tree density of 30 to 40 oak trees ha^{-1} (mostly *Quercus suber* L. with fewer *Q. ilex* L.), with approximately 35% ground cover. Topography is generally gentle undulated, and climate is typically Mediterranean, with hot and dry summer and mild and wet winter. Mean monthly temperature varies from 8 to 25 °C, and mean annual rainfall is between 620 and 670 mm (Ferreira, 1970; INMG, 1991). Soils in the HE are developed over gneisses, have sandy loam texture and are classified as *Leptic Regosols* associated with *Leptosols* with dystric characteristics (IUSS Working Group WRB, 2015). In the HO, soils are developed on granitic bedrock and are classified as *Eutric Luvisols* (IUSS Working Group WRB, 2015), showing loam to clay loam texture.

Three pasture management systems were considered in HE:

1) a 37 years old improved pasture (**IP**), grazed by 5 to 8 sheep per hectare every year (0.5 to 0.8 LU ha^{-1} $year^{-1}$); improved pasture seed mixture included mainly *Trifolium* spp., *Ornithopus* spp. and *Lolium* spp., and application of 300 kg ha^{-1} of natural rock phosphate (26.5% P_2O_5, 35% CaO, 3.2% SO_3 and 0.8% MgO) is carried out every two years.
2) a recently renewed improved pasture (**IPr**), managed as the IP and grazed by the same sheep herd at similar annual stocking rate; Pasture renewal was carried out by direct drilling (maximum 5 cm depth), approximately six months before the present study soil sampling.
3) a natural pasture that is occasionally grazed (**OG)** by less than one sheep per hectare (< 0.1 LU ha^{-1} $year^{-1}$). Herbaceous vegetation at the occasionally grazed area is mainly composed by *Chamaemelum mixtum* (L.) All., *Leontodon taraxacoides* (Vill.) Mérat, *Trifolium* spp., *Ornithopus* spp. and *Biserrula pelecinus* L. (FCT, 2014).

Three management systems were also selected in the HO farm:

1) an improved pasture (**IP**) established 18 years ago and grazed by cattle (0.7 LU ha^{-1} $year^{-1}$); Improved pasture species mixture included *Trifolium* spp., *Ornithopus* spp. and *Lolium* spp. 350 kg ha^{-1} of calcium phosphate fertilizer (18% P, 10% Ca and 27% S) are applied every two years
2) an adjacent natural pasture grazed by the same cattle herd (**NP**) at similar annual stocking rate; Dominant herbaceous species include *Agrostis castellana* Boiss. et Reut., *Chamaemelum nobile* (L.) All., *Vulpia geniculata* (L.) Link, *Lolium rigidum* Gaudin and *Carduus tenuiflorus* Curtis (FCT, 2014).
3) a control area with natural herbaceous vegetation and shrub encroachment, that is occasionally grazed (**OG**) by less than one sheep or pig per hectare (< 0.1 LU ha^{-1} $year^{-1}$). Dominant herbaceous species are the same as those present in NP. Natural occurring shrubs are mainly *Cistus* spp. and *Quercus coccifera* L., and their control is made when needed, at approximately 6 to 8 years intervals, by soil harrowing.

Experimental design

A lysimetric experiment was conducted from July 25th 2016 to October 4th 2017, at *Tapada da Ajuda*, in Lisbon. The climate is Mediterranean, with mean annual precipitation of 774 mm, 85% of which occurring from October to April, and mean annual temperature of 17.4 °C (IPMA, 2018a). Accumulated rainfall, and mean soil (10 cm depth) and air temperatures were registered daily by a nearby automatic meteorological station (IPMA, 2018b), being presented in Figure 1.

Thirty wooden lysimeters (three-layer spruce plywood, coated with synthetic melamine resin) were built to contain soil blocks with 0.203 m^2 surface area and up to 25 cm depth (boxes outer dimensions 50×50×28 cm). The bottom was isolated with a plastic film (PE, 200 µm), topped by a gravel layer (ca. 5 cm), a filtering geotextile fabric layer (Terram 2000, Fiberweb Geosynthetics Ltd.) and a sand layer (ca. 3 cm). A draining tube (PVC, 16 mm) was carved to one bottom corner of each lysimeter in a 45° downward angle, and the diagonally opposite corner was slightly elevated (ca. 1 cm) to ensure that all infiltrating water will flow through the tube and into a collecting bottle (ca. 2 L).

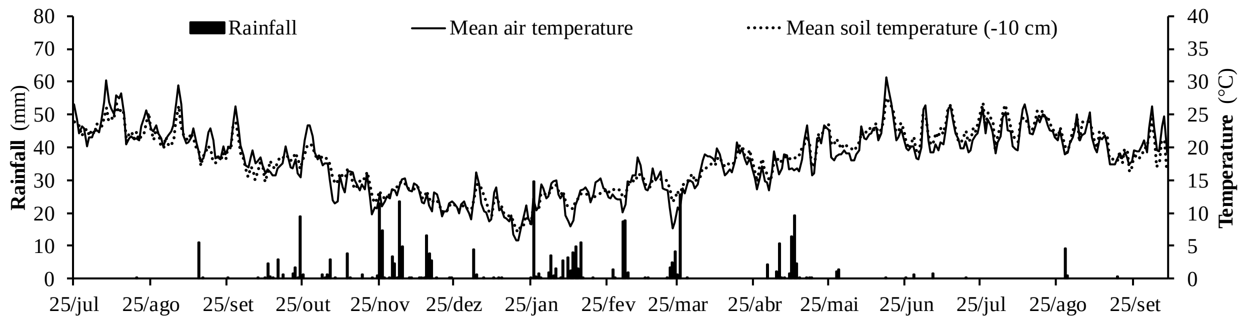

Figure 1 - Daily meteorological parameters registered by the *Tapada da Ajuda* automatic station between 25-07-2016 and 04-10-2017.

In May 2016, a 100×100 m plot was randomly chosen in each study area, and a 10×10 m grid was considered inside it. Ensuring absence of trees and the maximum possible distance from trees canopies, one sub-plot was randomly selected, and a 5×1 m rectangle was delimited inside it. A 0.5 m depth trench was dig around the rectangle to facilitate undisturbed soil blocks collection. Five 0.40×0.40×0.20 m soil blocks were collected in each sampling area. For that, a wooden frame (0.45×0.45×0.25 m) was carved into the soil profile, cutting the bottom by running a stainless-steel plate through a proper gutter system attached to the frame. The soil blocks were lifted and transferred to the lysimeters by carefully removing the bottom plate of the collecting frame placed upon them.

The lysimeters were then transported to the experiment location at *Tapada da Ajuda*, where four out of the five from each study system where randomly chosen for greenhouse gases emissions measurements, while in the fifth lysimeter, an access tube was installed for a soil moisture probe (PR2, Delta-T Devices). Experimental layout was assembled in June 2016 and lysimeters were kept undisturbed for a month before any data collection.

Samplings and measurements

Greenhouse gases fluxes

Gases fluxes from soil to the atmosphere were measured using a closed dynamic manual chamber technique (Oertel et al., 2016) along 14 months, from July 25th 2016 to October 4th 2017. The chambers (23 cm width and 24 cm height) were inserted into the soil to a depth of 8 cm at the beginning of the experiment and remained there until the end of the experiment. One chamber was installed per lysimeter, adding two litters of distilled water to facilitate installation. For measurement of gas emissions, the chambers were covered with a gas-tight cap equipped with a Teflon tube, which allowed air sampling in the headspace atmosphere using a syringe. Air samples (30 mL) were taken 30 (T1) and 60 (T2) min after closure and transferred to vials (20 mL) sealed with PTFE–silicon septa. Four surrounding air replicates were also collected, two before T1 and two after T2, to represent T0 chambers conditions (Chadwick et al., 2014). Gases concentrations were measured by gas chromatography (GC) using a GC-2014 (Shimadzu, Japan), with an electron capture ^{63}Ni detector (ECD), a thermal conductivity detector (TCD) and a flame ionization detector (FID), for N_2O, CO_2 and CH_4 determinations, respectively. The N_2O, CO_2 and CH_4 flux rates were calculated from the slope of the temporal change of the concentration within the chamber, and corrected for daily mean air temperature, chamber volume and the surface area of the chamber. A total of 71 sampling dates were considered, with a maximum interval of 15 days in periods without precipitation, and in at least three consecutive days following any rain event.

Cumulative emissions were estimated by multiplying the average flux between consecutive measurements, by the respective time interval. To consider the treatment effects on greenhouse gas emissions as a whole, N_2O and CH_4 emissions were converted to CO_2 equivalents using conversion factors of 298 and 25 for N_2O and CH_4, respectively for the global warming potential (GWP) at 100-year time scale (IPCC, 2014b).

Leachate solutions

Throughout the study period, leachate collecting bottles were checked after each rain event. Leachates volumes were measured, and a subsample was taken (ca. 300 ml) and kept refrigerated in 25 dates. Leachate samples were filtered (0.45 µm) and a 15 mL subsample was immediately frozen. Total N and dissolved organic C were determined in a elemental analyser (Skalar FORMAC Combustion TOC/N) using a chemiluminescence detector and near-infrared spectroscopy, respectively. Determinations of NO_3^--N and NH_4^+-N were made in a segmented flow autoanalyzer, using α-naphthylamine and sulphanilamide method, after reduction with Cd, and the modified Berthelot method, respectively. Remaining filtered leachates solutions were kept refrigerated (4 °C), pH was measured, concentrations of Ca, Mg, Na and K were determined by atomic absorption spectroscopy (AAS; Aanalyst 300, Perkin Elmer) and P by the molybdate blue method.

Herbaceous biomass

Shortly after the first significant rain events, in December 2016, the IPr system lysimeters were abundantly encroached by *Urtica dioica* L., a specie known to opportunistically develop in high inorganic N and P availability conditions (Taylor, 2009). A selective cutting was performed, removing only *U. dioica* L. individuals. Aboveground herbaceous biomass was cut in all lysimeters at the beginning of spring 2017, ensuring that soil was left covered by ca. 5 cm high vegetation layer. All samples were oven dried at 65 °C, weighed and mechanically grounded (0.5 mm). Total N was determined by Kjeldahl digestion. Mineral elements were determined after ashing at 450 °C for 6 hours with HNO_3 solution and measured by colorimetry for P and AAS for Ca, Mg, K and Mn.

Soil moisture and properties

Soil moisture was registered at each GHG sampling date (71 times), throughout the lysimetric experimental period. A PR2 profile probe with a HH2 moisture meter reading unit (Delta-T Devices) was used.

By the time soil blocks were collected from each studied pasture system, a set of 12 soil samples was also taken with an auger from the 0-10 cm soil layer, within the 10×10 m sampling plot. Samples from each management system were randomly paired to obtain six replicates per management system, which were immediately sieved (<2 mm) and field-moist subsamples were taken for microbial biomass C and N estimation by the fumigation-extraction procedure (Vance et al., 1987). Remaining soil was air dried and used to determine total N by Kjeldahl digestion, total and particulate (> 53 µm by wet sieving) organic C (De Leenheer and Van Hove, 1958), and extractable P (Egnér Riehm, 1958), measured by colorimetry.

Six replicates of undisturbed soil samples were also collected in field conditions, along with soil blocks collection. By carving metallic cylinders (ca. 590 cm^3) into the upper 10 cm soil layer, undisturbed soil cores were carefully trimmed to the cylinders volume and oven dried (105 °C) until constant weigh. Soil bulk density was calculated dividing the soil cores dry weigh by the cylinders volume (Blake and Hartge, 1965).

Statistical analysis

Soil properties, leachates pH and nutrient contents, vegetation biomass and nutrient concentrations, were considered as independent variables and analyses of variance (ANOVA; α=0.05) were performed to test differences between management systems, separately for each farm (IP, IPr and OG for *Herdade dos Esquerdos*; IP, NP and OG for *Herdade do Olival*). If samples normal distribution (Shapiro-Wilk test) and homogeneity of variances (Levene ‘s test) could not be accepted, even with data transformations (e.g. logarithm, square root), Kruskal-Wallis test (α=0.05) procedure was performed. When significant differences between management systems averages were assumable, Tukey’s or Waerden’s (non-parametric) tests (α=0.05) were used to discriminate differences between management systems.

Greenhouse gases fluxes determinations were also analysed for significant differences between management system, sampling date, and lysimeter box, expressed by the following mixed effects model:

$$G_{lpd} = \mu + P_{lp} + D_{ld} + (PD)_{lpd} + \delta_{lp} + \epsilon_{lpd}$$

Where G represents each GHG gas flux mean, µ is the population mean, P is the pasture management effect with $p = 1, \ldots, 3$ levels (IP, IPr and OG at *Herdade dos Esquerdos*; IP, NP and OG at *Herdade do Olival*), D is the sampling date effect, with $d = 1, \ldots, 71$ levels, δ is the lysimeter associated error, with $l = 1, \ldots, 4$ subjects, and the errors are assumed as $\epsilon \sim N(0, \sigma)$. ANOVA procedures for non-parametric longitudinal data were applied, for each GHG gas and farm, and when significant differences were found between management system, mean separation was achieved by pair comparison.

Since accumulated GHG emissions, respective global warming potential and relative proportions of emitted C and N, to the initial soil organic C and total N contents did not show any significant differences between management systems averages, within each farm, an ANOVA procedure (α=0.05) was used to test for differences between all management systems, as a nested factor of their respective farm (HE/IP, HE/IPr, HE/NG, HO/IP, HO/NP and HO/OG). Whenever significant differences were found for the interaction, Tukey's test (α=0.05) was used for means separation.

All data analysis were conducted in the R environment (R Core Team, 2014), using adequate packages such as 'car' (Fox and Weisberg, 2011), 'agricolae' (De Mendiburu, 2009) and 'nparLD' (Noguchi et al., 2012).

RESULTS

Soil properties and moisture content

Soil moisture content along the experimental period (Figure 2) agrees with the precipitation pattern presented in Figure 1. Soils from the HO farm showed higher soil water content along the rain season (October 2016 to January 2017), compared to the HE soils, but no differences were observable for lower frequency rain events.

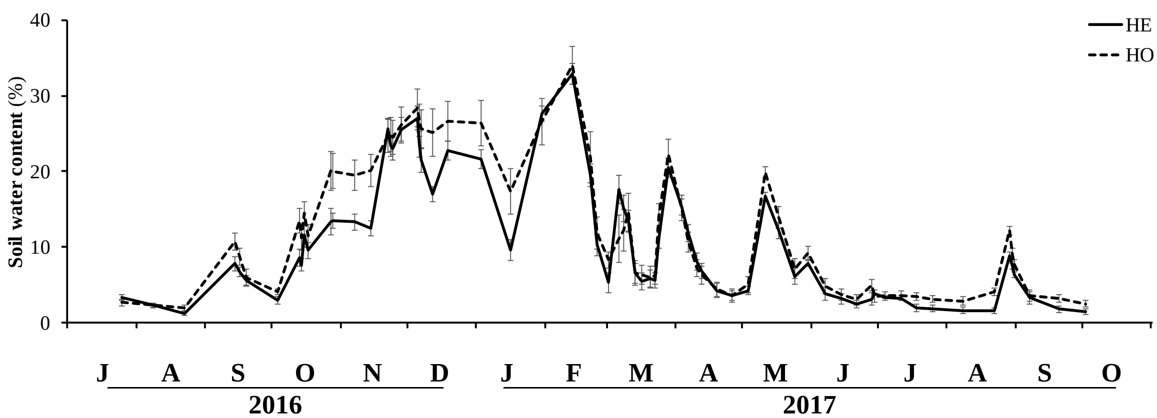

Figure 2 - Soil water content (v/v) measured in the lysimeter soil blocks from *Herdade dos Esquerdos* (HE) and *Herdade do Olival* (HO) systems along the experimental period (July 25th 2016 to October 4th 2017). Line points are means and error bars are standard errors (n=9).

Soils from the HE improved pastures showed significantly lower bulk density, and higher organic C and total N concentrations and accumulation, as compared to the no occasionally grazed area (Table 1). Extractable phosphorus concentration was also lower for the occasionally grazed soil than for those with sowed pasture.

In the HO farm, soil bulk density was higher for the natural than the improved pasture (Table 1). Occasionally grazed soil showed significantly lower bulk density than both cattle grazed systems. Significantly higher total N concentrations were determined in cattle grazed pasture soils (IP and NP), compared to the occasionally grazed system. Soil extractable P was significantly higher in the improved compared to natural pasture, while the occasionally grazed soil showed the lowest extractable P value, compared to both cattle grazed pastures.

Table 1 - Bulk density (BD), organic C (C_{org}), total N (N_{total}) and particulate organic matter C (POM-C) concentrations, POM fraction of organic C (POM-C/C), and accumulation of organic C (C_c) and N (N_c), in the 0-10 cm depth soil layer of the study farms improved (IP), renewed (IPr), natural (NP) and occasionally grazed (OG) pasture systems. Values are means and standard deviations in brackets (n=6), different letters indicate significant differences between management systems by the Tukey test (p<0.05).

System	BD	C_{org}	N_{total}	P	C:N	POM-C/C	C_c	N_c
	g cm^{-3}	g kg^{-1}		mg kg^{-1}		%	kg m^{-2}	
				HERDADE DOS ESQUERDOS				
IPr	**1.21** ^{b} (0.06)	**25.6** ^{a} (6.0)	**2.70** ^{a} (0.90)	**179.7** ^{a} (37.5)	**11.58** ^{a} (7.99)	**36.4** ^{a} (6.7)	**2.16** ^{a} (0.50)	**0.228** ^{a} (0.076)
IP	**1.31** ^{b} (0.13)	**26.8** ^{a} (4.5)	**2.36** ^{a} (0.51)	**211.0** ^{a} (95.4)	**11.49** ^{a} (0.96)	**39.2** ^{a} (14.4)	**2.47** ^{a} (0.42)	**0.217** ^{a} (0.047)
OG	**1.54** ^{a} (0.04)	**14.2** ^{b} (0.5)	**1.10** ^{b} (0.02)	**19.3** ^{b} (2.7)	**12.89** ^{a} (0.70)	**26.9** ^{a} (4.1)	**1.57** ^{b} (0.06)	**0.122** ^{b} (0.003)
				HERDADE DO OLIVAL				
IP	**1.62** ^{b} (0.05)	**14.5** ^{a} (7.4)	**1.43** ^{a} (0.58)	**29.2** ^{a} (16.5)	**10.19** ^{a} (0.84)	**35.6** ^{a} (25.3)	**1.16** ^{a} (0.59)	**0.114** ^{ab} (0.047)
NP	**1.72** ^{a} (0.09)	**14.6** ^{a} (4.6)	**1.41** ^{a} (0.28)	**4.3** ^{b} (1.6)	**10.34** ^{a} (1.65)	**22.9** ^{a} (9.4)	**1.45** ^{a} (0.45)	**0.137** ^{a} (0.028)
OG	**1.57** ^{c} (0.10)	**9.1** ^{a} (0.9)	**0.79** ^{b} (0.06)	**1.5** ^{c} (1.0)	**11.55** ^{a} (0.65)	**32.8** ^{a} (12.4)	**0.85** ^{a} (0.08)	**0.074** ^{b} (0.006)

In the HE systems, soil microbial biomass C and N concentrations and relative proportions of organic C and total N were significantly higher in renewed improved pasture, compared to both the occasionally grazed and the older improved pasture areas (Table 2). Soil microbial biomass C and N concentrations were also significantly higher in the older improved pasture, compared to the occasional grazing area at the HE farm.

Table 2 - Soil microbial biomass C (C_{mic}) and N (N_{mic}), respective fractions of organic C and N (C_{mic}/C and N_{mic}/N), and microbial biomass C:N ratio (C_{mic}/N_{mic}) in the 0-10 cm depth soil layer of of the study farms improved (IP), renewed (IPr), natural (NP) and occasionally grazed (OG) pasture systems. Values are means and standard deviations in brackets (n=6), different letters indicate significant differences between management systems by the Tukey test (p<0.05).

System	C_{mic}	N_{mic}	C_{mic}/C	N_{mic}/N	C_{mic}/N_{mic}
	mg kg^{-1}		%		
			HERDADE DOS ESQUERDOS		
IPr	**201.5**^{a} (16.5)	**35.86**^{a} (18.92)	**0.73**^{a} (0.06)	**1.33**^{a} (0.70)	**7.30**^{a} (4.83)
IP	**99.59**^{b} (22.73)	**10.67**^{b} (4.33)	**0.48**^{b} (0.11)	**0.45**^{b} (0.18)	**9.93**^{a} (2.23)
OG	**50.74**^{c} (6.03)	**5.07**^{c} (0.23)	**0.35**^{b} (0.04)	**0.46**^{b} (0.02)	**10.0**^{a} (1.21)
			HERDADE DO OLIVAL		
IP	**318.4**^{a} (19.12)	**38.15**^{a} (3.22)	**1.76**^{a} (0.11)	**2.95** ^{a} (0.25)	**8.38**^{b} (0.80)
NP	**240.1**^{ab} (46.24)	**25.90**^{b} (3.67)	**1.86**^{a} (0.36)	**1.84**^{b} (0.26)	**9.22**^{b} (0.55)
OG	**205.7**^{b} (29.14)	**14.80**^{c} (0.77)	**1.61**^{a} (0.23)	**1.88**^{b} (0.10)	**13.94**^{a} (2.16)

Improved pasture soil from the HO showed significantly higher microbial biomass C concentration than the occasionally grazed one. Microbial biomass N concentrations were significantly different for all HO systems, the higher values being measured in improved pasture soil, followed by the natural pasture, while the occasionally grazed soil showed the lowest mean microbial N concentration. The ratio (microbial biomass N: soil total N) was significantly higher under the sowed pasture, compared to both natural pasture and occasionally grazed area at the HO farm. The microbial biomass C:N ratio was significantly higher in the occasionally grazed than in the both pasture soils (natural and improved) from the HO.

Herbaceous biomass production and nutrient concentrations

Herbaceous vegetation biomass productivity was significantly higher in soil blocks from improved pasture areas of both HE and HO (Table 3), pasture productivity in sowed areas being about 2 to 3 times greater than that in the occasionally grazed or natural pasture soils.

Significantly higher P and N concentrations in the improved pasture systems herbaceous biomass, compared to those in the natural pasture systems, were the most relevant differences regarding the herbaceous vegetation nutrient concentrations, in both studied farms. It is noteworthy that herbaceous biomass C:N ratios were similar in all study pastures, ranging between 25 and 35.

Table 3 - Aboveground herbaceous biomass dry matter (HB) and nutrient concentrations (N, P, K, Ca, Mg and Mn) in the lysimeters containing soil blocks from the study farms improved (IP), renewed (IPr), natural (NP) and occasionally grazed (OG) pasture systems. Values are means and standard deviations in brackets (n=6), different letters indicate significant differences between management systems by the Tukey test (*p*<0.05).

System	HB	N	P	K	Ca	Mg	Mn
	g m^{-2}	mg g^{-1}					
				HERDADE DOS ESQUERDOS			
IPr	**296.44**[a] (41.34)	**17.34**[a] (3.37)	**4.85**[a] (1.09)	**25.96**[a] (5.95)	**32.41**[a] (7.36)	**3.27**[a] (0.66)	**0.17**[a] (0.06)
IP	**242.38**[b] (24.53)	**14.61**[ab] (0.74)	**4.06**[a] (0.71)	**17.79**[b] (2.40)	**26.38**[ab] (7.16)	**2.66**[a] (0.34)	**0.27**[a] (0.12)
OG	**91.44**[c] (21.50)	**12.43**[b] (2.03)	**2.37**[b] (0.54)	**13.71**[b] (0.45)	**15.93**[b] (1.18)	**3.03**[a] (0.34)	**0.33**[a] (0.13)
				HERDADE DO OLIVAL			
IP	**147.81**[a] (38.85)	**15.89**[a] (3.41)	**2.27**[a] (0.67)	**15.73**[a] (1.95)	**15.06**[ab] (3.43)	**2.40**[ab] (0.23)	**0.45**[a] (0.27)
NP	**67.94**[b] (19.98)	**13.05**[a] (1.72)	**1.51**[b] (0.30)	**15.48**[a] (1.68)	**17.72**[a] (1.71)	**2.79**[a] (0.21)	**0.27**[a] (0.05)
OG	**46.74**[b] (14.07)	**15.27**[a] (2.16)	**1.38**[b] (0.34)	**16.00**[a] (0.48)	**11.58**[b] (2.63)	**2.00**[b] (0.37)	**0.40**[a] (0.39)

Leachate nutrient contents

Mean value of leached dissolved organic carbon was higher in the improved pasture than natural pasture or occasionally grazed systems from the HO (Table 4). Among the HE systems, the renewed improve pasture soils showed significantly higher dissolved organic C in leachates, compared to old improved pasture and occasionally grazed systems.

Nitrate leaching was higher in the renewed improved pasture than in the older improved pasture and occasional grazing soils from the HE; while leached ammonium was significantly lower in the occasionally grazed soils, compared to both improved pasture ones. Higher amounts of total dissolved N were leached from the recently renewed improved pasture soils, compared to the occasionally grazed or older improved pasture ones, from the HE. The natural pasture soils from the HO have lost higher amounts of nitrate through leaching, in comparison to improved pasture soils in the same farm, along the experimental period.

Table 4 - Cumulative dissolved organic C (DOC), soluble N (N_{sol}), ammonium (NH_4^+-N), nitrate (NO_3^--N), organic N (N_{org}), and proportions of mineral N per unit of initial soil N (N_{min}/N_{total}), and of DOC per unit of initial soil organic C (DOC/C_{org}), in the leachate solutions of the study farms improved (IP), renewed (IPr), natural (NP) and occasionally grazed (OG) pasture systems. Values are means and standard deviations in brackets (n=6), different letters indicate significant differences between management systems by Tukey's test (p<0.05).

System	DOC	N_{sol}	NH_4^+-N	NO_3^--N	N_{org}	N_{min}/N_{total}	DOC/C_{org}
	mg m^{-2}					%	
			Herdade dos Esquerdos				
IPr	**17.22^a** (3.16)	**2837.4^a** (690.6)	**21.7^a** (3.9)	**1820.2^a** (428.0)	**995.7^a** (266.1)	**0.81^a** (0.19)	**0.80^a** (0.15)
IP	**7.33^b** (2.98)	**614.9^b** (261.7)	**16.8^a** (2.9)	**119.1^b** (59.9)	**479.1^b** (202.1)	**0.26^b** (0.39)	**0.30^b** (0.12)
OG	**2.71^b** (6.22)	**419.6^b** (113.8)	**8.1^b** (2.2)	**212.2^b** (68.2)	**199.3^b** (46.9)	**0.18^b** (0.06)	**0.17^b** (0.04)
			Herdade do Olival				
IP	**4.13^a** (0.83)	**366.0^a** (71.9)	**13.7^a** (4.4)	**95.9^b** (30.4)	**256.5^a** (50.1)	**0.10^b** (0.02)	**0.35^a** (0.07)
NP	**2.08^b** (0.35)	**612.1^a** (109.5)	**20.6^a** (10.9)	**385.9^a** (110.5)	**205.6^a** (5.3)	**0.30^a** (0.08)	**0.14^b** (0.02)
OG	**1.70^b** (0.45)	**470.7^a** (202.4)	**25.2^a** (13.7)	**262.1^{ab}** (148.2)	**183.6^a** (73.5)	**0.39^a** (0.21)	**0.20^b** (0.05)

In the HE, average pH of leachates was significantly higher in the occasionally grazed soils, compared to the improved pasture ones (Table 5). In the HO, the natural pasture leachates showed significantly higher pH than the improved pasture and occasionally grazed soils.

Leached phosphorus amounts were higher in both sowed pasture soils from HE than in the occasionally grazed area. In the HO, soils from the improved pasture system have leached more P comparatively to natural pasture and occasionally grazed ones.

Potassium in soil leachates was higher in the renewed improved pasture, followed by the older improved pasture and, lastly, the occasionally grazed soils from the HE. Amounts of Ca, Mg and Na in soil leachates where higher in the recently renewed improved pasture from the HE, and in the natural pasture from the HO, when compared to their respective IP and OG systems.

Table 5 - Mean pH and cumulative nutrients (P, K, Ca, Mg and Na) in the leachate solutions of the soil blocks from the study farms improved (IP), renewed (IPr), natural (NP) and occasionally grazed (OG) pasture systems. Values are means and standard deviations in brackets (n=6), different letters indicate significant differences between management systems by Tukey's test (p<0.05).

System	pH	P	K	Ca	Mg	Na
		mg m^{-2}				
			HERDADE DOS ESQUERDOS			
IPr	**5.92^{ab}** (0.31)	**241.4^{a}** (103.7)	**3381.3^{a}** (1403.3)	**2601.7^{a}** (408.0)	**642.2^{a}** (102.6)	**1606.9^{a}** (356.7)
IP	**5.52^{b}** (0.29)	**168.6^{a}** (168.9)	**1121.1^{b}** (495.0)	**451.9^{b}** (180.3)	**166.3^{b}** (104.8)	**713.0^{b}** (341.1)
NG	**6.20^{a}** (0.03)	**16.4^{b}** (4.6)	**247.9^{c}** (30.9)	**482.9^{b}** (96.7)	**218.1^{b}** (53.6)	**771.3^{b}** (155.1)
			HERDADE DO OLIVAL			
IP	**6.26^{b}** (0.21)	**28.6^{a}** (4.9)	**317.2^{a}** (72.7)	**524.0^{b}** (150.7)	**168.2^{b}** (45.2)	**543.8^{b}** (101.2)
NP	**6.52^{a}** (0.09)	**13.0^{b}** (2.7)	**311.3^{a}** (68.7)	**746.3^{a}** (56.3)	**294.6^{a}** (42.6)	**884.0^{a}** (67.7)
OG	**6.15^{b}** (0.11)	**11.6^{b}** (5.1)	**205.3^{a}** (77.5)	**464.8^{b}** (109.4)	**180.6^{b}** (64.6)	**538.8^{b}** (117.6)

Soil greenhouse gases fluxes

Soil GHG fluxes evolution along the experimental period was similar for all study systems (Figures 3 and 4). Methane emissions have ranged from -0.041 to 0.113 mg m^{-2} h^{-1} and two significant peaks were observed at the end of August 2016 and February 2017. Nitrous oxide fluxes varied between -0.038 and 0.089 mg m^{-2} h^{-1} and several peaks were observed along the experiment, mainly following rain events. Carbon dioxide fluxes registered variations between -16.2 to 336.6 g m^{-2} h^{-1}.

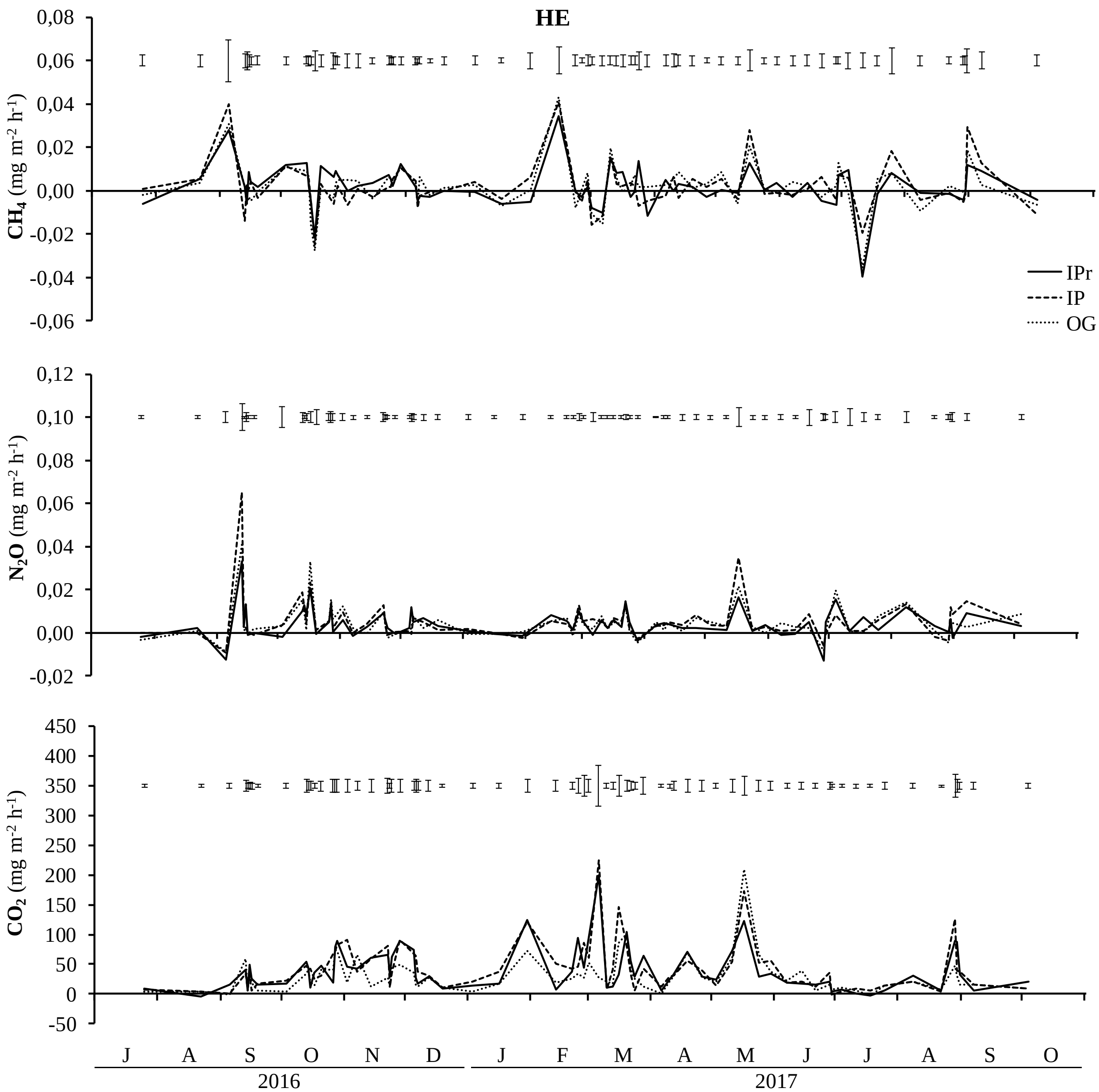

Figure 3 - Methane (CH_4), nitrous oxide (N_2O) and carbon dioxide (CO_2) fluxes from *Herdade dos Esquerdos* (HE) improved (IP), renewed (IPr) and occasionally grazed (OG) pasture soils, at each sampling date, from July 2016 to October 2017. Line points represent medians (n=4) and error bars represent global standard error for each farm (n=12).

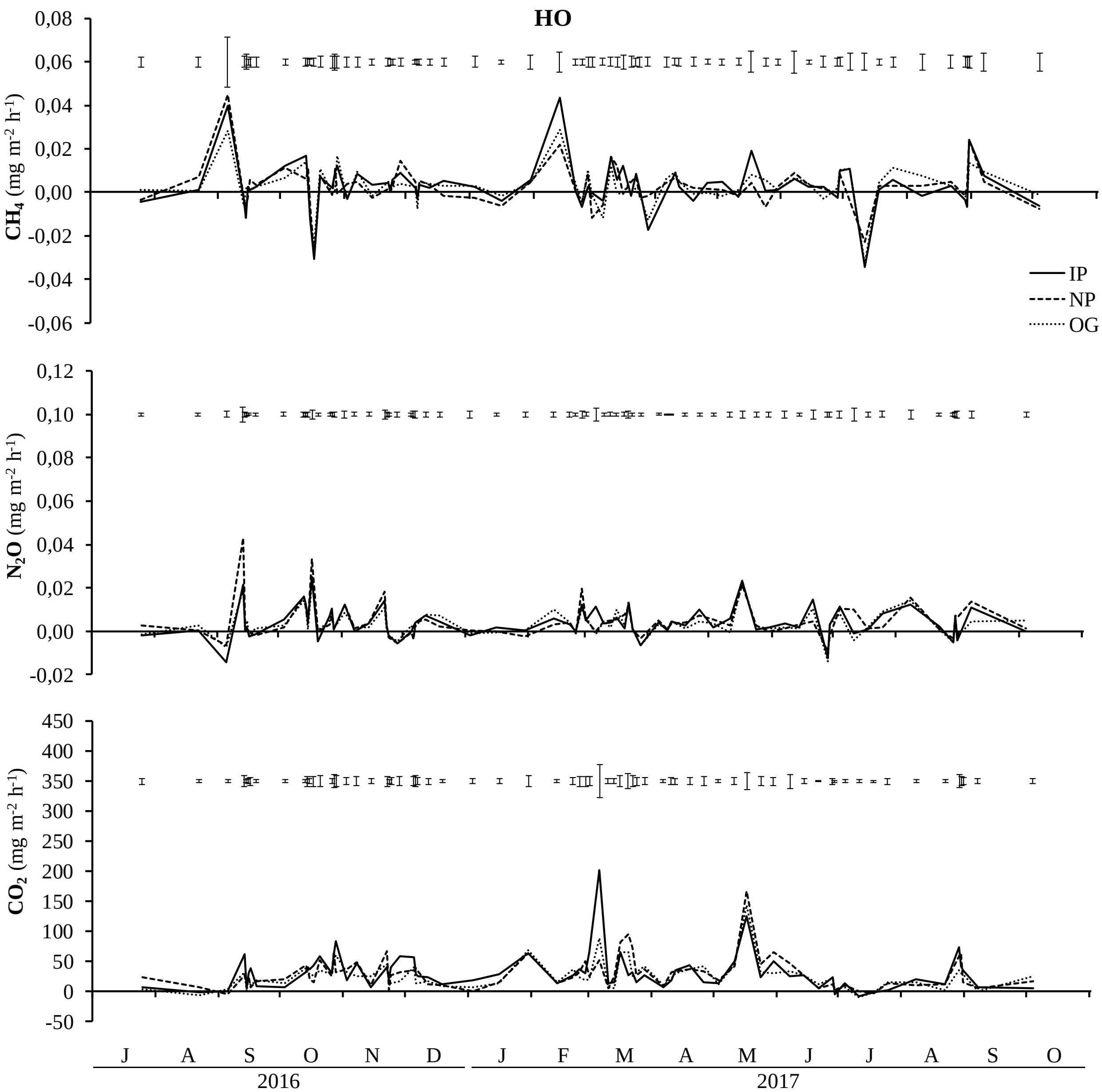

Figure 4 - Methane (CH_4), nitrous oxide (N_2O) and carbon dioxide (CO_2) fluxes from *Herdade do Olival* (HO) improved (IP), natural (NP) and occasionally grazed (OG) pasture soils, at each sampling date, from July 2016 to October 2017. Line points represent medians (n=4) and error bars represent global standard error for each farm (n=12).

Longitudinal data analysis revealed a strong influence of sampling date, as the main factor controlling soils GHG fluxes, in both HE and HO farms (Table 6). Soil CO_2 fluxes were significantly different among differently managed HE systems. Pair comparison of HE pasture systems has revealed that mean soil CO_2 fluxes were comparable for improved pasture systems, both renewed and old (p=0.245), while the mean soil CO_2 flux from the occasionally grazed system was significantly lower than the former two (p=0.036 for IPr; and p<0.001 for IP).

Table 6 - Statistic, degrees of freedom (dF) and *p*-values of the non-parametric factorial ANOVA (α=0.05) analysis of CH_4, N_2O and CO_2 soil fluxes (mg m^{-2} h^{-1}) longitudinal measurements (n=4), for HE and HO management systems.

GHG	Origins of variation	Statistic	dF	*p*-value
	HERDADE DOS ESQUERDOS			
CH_4	System	1.593	2.0	0.204
	Date	7.087	7.6	<0.001
	System×Date	0.887	8.1	0.528
N_2O	System	0.486	1.4	0.548
	Date	14.480	7.1	<0.001
	System×Date	1.392	7.7	0.197
CO_2	System	5.876	1.6	0.006
	Date	13.240	7.6	<0.001
	System×Date	1.354	8.2	0.210
	HERDADE DO OLIVAL			
CH_4	System	0.172	1.7	0.813
	Date	7.399	7.4	<0.001
	System×Date	0.834	8.0	0.573
N_2O	System	0.192	1.5	0.763
	Date	21.926	7.4	<0.001
	System×Date	1.426	8.1	0.179
CO_2	System	2.073	1.6	0.135
	Date	11.207	8.0	<0.001
	System×Date	1.029	8.5	0.412

Accumulated N_2O and CH_4 emissions did not differ between the study management systems and farms (Table 7). At the end of the experimental period, cumulative CO_2 emissions were significantly higher for the HE, compared to the HO soils, as both improved pasture areas (renewed and old) from the HE showed significantly higher accumulated CO_2 emissions than any HO management system. Proportions of initial organic C and total N, emitted as greenhouse gases throughout the experimental period, were significantly higher in the HE than in the HO soils (Table 7). Soil form the renewed pasture at HE, had the smallest N fraction emitted as N_2O, which was significantly lower than those from the improved pasture from HO (HO/IP) and both occasional grazing systems (HE/OG and HO/OG). Global warming potential was significantly higher in the HE soils, compared to those from HO, the older improved pasture showing greater annual GWP than the occasional grazing system from HO.

Table 7 - Soil accumulated methane (CH_4), nitrous oxide (N_2O) and carbon dioxide (CO_2) emissions, respective emitted proportions of initial soil organic C (C_{em}/C_{org}) and total N (N_{em}/N_{org}) and annual global warming potential (GWP), in *Herdade dos Esquerdos* (HE) and *Herdade do Olival* (HO) pasture management systems (IP - improved; IPr - improved renewed; NP natural; and OG - occasionally grazed). Values are means and standard deviations in brackets (n=4), different letters indicate significant differences between management systems (Tukey's test) with $p<0.05$.

System	CH_4	N_2O	CO_2	C_{em}/C_{org}	N_{em}/N_{total}	GWP
	mg m^{-2}			mg g^{-1}		g CO_2e m^{-2} year^{-1}
HE	**29.2^{a}** (10.8)	**42.7^{a}** (12.3)	**340.5^{a}** (73.1)	**45.3^{b}** (6.9)	**0.16^{b}** (0.06)	**296.3^{a}** (62.3)
HO	**36.6^{a}** (12.9)	**37.7^{a}** (5.6)	**256.3^{b}** (37.6)	**55.9^{a}** (8.8)	**0.21^{a}** (0.04)	**224.7^{b}** (32.3)
HE/IPr	**23.5^{a}** (14.6)	**35.6^{a}** (10.0)	**349.8^{a}** (83.3)	**44.2^{a}** (10.5)	**0.10^{b}** (0.03)	**302.2^{ab}** (72.3)
HE/IP	**36.3^{a}** (7.9)	**49.5^{a}** (17.1)	**394.8^{a}** (52.7)	**43.7^{a}** (5.8)	**0.15^{ab}** (0.05)	**343.6^{a}** (43.0)
HE/OG	**27.9^{a}** (6.1)	**43.1^{a}** (5.6)	**276.9^{ab}** (21.9)	**48.0^{a}** (3.8)	**0.22^{a}** (0.03)	**243.2^{ab}** (19.3)
HO/IP	**38.2^{a}** (13.5)	**36.3^{a}** (5.3)	**267.1^{b}** (28.7)	**62.5^{a}** (6.7)	**0.20^{a}** (0.03)	**233.4^{ab}** (25.0)
HO/NP	**33.0^{a}** (17.7)	**40.1^{a}** (2.4)	**269.1^{b}** (44.2)	**50.8^{a}** (8.4)	**0.19^{ab}** (0.01)	**236.0^{ab}** (37.2)
HO/OG	**38.7^{a}** (9.4)	**36.8^{a}** (8.3)	**232.6^{b}** (35.8)	**54.2^{a}** (8.3)	**0.23^{a}** (0.05)	**204.7^{b}** (31.9)

DISCUSSION

Soil organic C fluxes

The range of soils CO_2 fluxes observed here were similar to those reported by Shvaleva et al. (2011) and Correia et al. (2012) for oak woodlands, and by Shvaleva et al. (2014) for improved pastures under similar Mediterranean conditions. Also, study soils CH_4 fluxes were relatively small, with soil uptake during some periods, and ranges were similar to those reported for savannas and seasonally-dry ecosystems (Castaldi et al., 2006), and for similar pasture systems under oak woodlands (Shvaleva et al., 2015).

Accumulated CO_2 emissions were higher under *Herdade dos Esquerdos* than *Herdade do Olival* pasture soils, which can be, at least partially, explained by the differences in soil organic C contents, although soil textural classes may have also contributed for this trend. Indeed, in a study under pasture and agricultural soils, Lohila et al. (2003) have reported soil respiration to be mostly explained by soil C contents, while soils with coarser texture appeared with higher CO_2 emissions than those with clayey texture, with similar crop type.

Nevertheless, the accumulated C transfer per unit of soil organic C, from the soil to the atmosphere, as both CH_4-C and CO_2-C, presented a reverse trend, being higher for finer texture soils, at *Herdade do Olival*, compared to those with coarser texture at *Herdade dos Esquerdos*. As this appears to be mostly associated with differences in soil CH_4-C emissions, differences in soil texture and porosity may have been involved. Indeed, under the finer textured soils at *Herdade do Olival*, high soil bulk density values, compared to those at *Herdade dos Esquerdos*, may indicate reduced aeration porosity under field capacity conditions (see Chapter 2), suggesting that soil pores in the former may easily develop anaerobic sites, thus constraining methane oxidation, and enabling its accumulation and diffusion to the atmosphere (Oertel et al., 2016).

At *Herdade dos Esquerdos*, the improved pasture soils showed higher CO_2 fluxes throughout the experiment, under both renewed and long-term management, compared with the occasionally grazed soils, throughout the experimental period.

Availability of organic C substrates (additional 0.90 and 0.59 kg C m^{-2}, in IP and IPr, respectively) may be the main reason for such a behaviour, which is most likely related to the increase in herbaceous productivity (more than the double). Also, the higher soil C and N microbial biomass concentrations determined at these improved pastures, indicates a more abundant soil microbial community, which can result in higher respiration, compared to the occasionally grazed pasture. Additionally, soil bulk density lowering at the improved pasture sites, may indicate soil physical conditions improvement. In fact, soil organic matter accumulation and higher root densities development may have increased soil porosity (see Chapter 2) and enhanced soil aeration and oxygen availability. Milne and Haynes (2004) have also associated improved soil physical status and soil microbial biomass concentration increments with soil respiration enhancement, under permanent pastures compared with native vegetation or annual (tilled) pasture systems in South Africa, and suggested that both the accumulation of soil organic matter and soil structural development, would influence microbial communities composition and potentially benefit its functioning.

However, these trends were not fully verified at *Herdade do Olival* improved pasture, as compared with the similarly managed natural pasture. Although pasture productivity has showed a similar enhancement as that found at *Herdade dos Esquerdos* (nearly doubled), soil bulk density was significantly lowered, and microbial activity may have been slightly increased (increased leaching of dissolved organic C and slight higher microbial biomass C concentrations), soil organic C concentrations and accumulation were not significantly changed, and fluxes and accumulated CO_2-C and CH_4-C emissions were not changed. Hence, the compound effects of stocking rate intensification, shrub vegetation removal and pasture sowing becomes difficult to disentangle, highlighting the importance of all site-specific and management factors effects over soil organic matter dynamics following management changes (Abdalla et al., 2018; Whitehead et al., 2018).

Dissolved organic C leaching from the upper 20 cm soil layer was positively influenced by the improved pasture management, at both farms, which is in agreement with the increase in these soils microbial biomass contents and expected activity. The water soluble organic C fraction has been associated with soil microbes substrates and products, and is currently suggested as a good indicator of soil biochemical functioning (Iqbal et al., 2010; Marschner and Bredow, 2002). Also, organic C movement down the soil profile is an important process regarding its stabilization in deeper horizons (Kalbitz et al., 2000), which may favour the soil potential for C sequestration.

It is also noteworthy that higher soil microbial biomass C and dissolved organic C proportions, per unit of soil organic C, were observed for the renewed improved pasture, compared to the older improved one. This trend agrees with the expected effects of disturbances associated with pasture renewal. In one hand, soil mechanical disturbance can facilitate access of microbial communities to organic matter substrates (Six et al., 1998), thus enhancing microbial growth and consequent release of soluble organic substrates (Kalbitz et al., 2000). On the other hand, several studies support that vegetation cover modifications can change soil microbial communities composition and functioning (e.g. Waldrop and Firestone, 2006).

The relative proportions of soil C:N ratio, particulate organic matter fractions and C microbial biomass C, were not changed by the long-term improved pasture management, as compared to the respective natural pasture, at each study farms. Therefore, improved pasture may have not significantly changed these soils C cycling, agreeing with other studies on these pastures, under similar conditions (Gómez-Rey et al., 2012; Rodrigues et al., 2015; Rodrigues et al., 2019; see Chapter 2). Accordingly, estimated global soil organic C transfers to the atmosphere, per unit of soil organic C, were similar between the study pasture management systems, in each farm. Therefore, improved pasture management appears as a usefulness tool to enhance *montado* soils pasture productivity and C sequestration potential.

Results also suggest that pasture renewal by direct sowing procedures can be recommended to enable the introduced species persistence and consequent higher productivity (Hernández-Esteban et al., 2018), with negligible effects on the soil C balance. This is in agreement with Rutledge et al. (2017) observations on soils C balance after grassland renewal, where eventual soil C losses were easily reversed by the developing pasture C intake.

Soil N fluxes

The soils N_2O fluxes observed here were in the same range of those reported by Shvaleva et al. (2015) for pasture soils under evergreen oak woodland systems. The fact that, by the end of the experimental period, relative soil N_2O-N losses, per unit of soil N, were higher at *Herdade do Olival* than at *Herdade dos Esquerdos*, may be mostly related to differences in these pasture soils N availability (Oenema et al., 1997).

At *Herdade do Olival*, soils N_2O fluxes and accumulated emissions did not differ between pasture systems, although soil N accumulation under the 18-year old improved pasture (40 g N m^{-2}) was accompanied by enhancement of soil microbial biomass N concentration (2.5-fold increase) and proportion (1.6 times greater), compared with the occasionally grazed system. Such a trend suggests that improved pasture establishment under these *montado* soils may be a viable option to enhance soil N availability (Gómez-Rey et al., 2012) and pasture productivity (Hernández-Esteban et al., 2018), without relevant changes to its nitrous oxide emission patterns.

At *Herdade dos Esquerdos*, notorious higher N_2O fluxes were registered at the first rain events (September 13th, 2016 and September 7th, 2017) in the older improved pasture system, despite no differences were found between these and the occasionally grazed pasture soils accumulated nitrous oxide emissions, soil C:N ratio or microbial biomass C and N proportions. These peaks may be mainly associated with soil N accumulation (95 g N m^{-2} increase) along the 37 years of improved pasture management (Oertel et al., 2016). The fact that such a trend was not evident under the recently renewed improved pasture is probably explained by this pasture higher herbaceous productivity and respective N contents. Indeed, the newly sowed species growth have shown higher N intake, while their denser rooting system may have enhanced soil aeration, which may also favour N substrates complete oxidation (into NO_3^-), thus reducing denitrification patterns (Oenema et al., 1997; Trolove et al., 2019).

The fact that soil mineral N leaching was not enhanced by improved pasture long-term management at *Herdade dos Esquerdos*, and nitrate leaching was even reduced by the improved pasture at *Herdade do Olival*, compared to each farm ocassionnaly grazed systema, suggests that the environmental contamination problems associated with mineral N movements down the soil profile should be considered negligible following pasture long-term managment under these *montado* systems (Di and Cameron, 2002; Kušlienė et al., 2015). Nevertheless, pasture renewal has greatly enhanced mineral N leaching, with NO_3^--N amounts reaching values up to 18 kg ha^{-1}, which are in agreement with those reported by Trolove et al. (2019), following pasture renewal with soil tillage in New Zealand. Considering the high capacity of oak roots to uptake nitrate (Nunes, 2004), and that in *montado* systems these can extend far into open grassland areas (Moreno et al., 2005), such soil nitrate availability would probably be easily cycled by the trees under field conditions. Additionally, the fast development of opportunistic herbaceous specie, such as *Urtica dioica* L. found at the present study renewed pasture soils at the first rainy season, may also efficiently cycle part of the available inorganic N, thus preventing losses through leaching.

Due to the high global warming potential of N_2O (298 times than of CO_2; IPCC, 2014b), eventual emission peaks should be taken in consideration when assessing soil potential to mitigate GHG emissions effects. Therefore, despite their confirmed potential to enhance soil C sequestration, current study results suggest that the long-term establishment of improved pastures may unbalance the system N fluxes which can limit their potential as a mitigation strategy (Powlson et al., 2011).

Yet, pasture renewal, with minimum soil disturbance, appears efficient to onset these soils N cycling, shifting the direction of their high N availability from the atmosphere to the vegetation. Moreover, under lower soil N availability levels and finer texture soils, long-term improved pasture management appeared suitable to enhance the system productivity, without significant N fluxes changes.

Nutrient leaching

Continuous phosphate fertilizer applications in the improved pasture systems have determined a strong increase in soil extractable P, in both *Herdade dos Esquerdos* and *Herdade do Olival* farms. Similar trends have been reported for long-term improved pastures under *montado* systems (Gómez-Rey et al. 2012; see Chapter

2), which highlights the need to consider possible soil P losses by run-off and leaching (Horta and Torrent, 2010). Accordingly, the amounts of P leached from the current study improved pasture soil blocks were two- to twenty-times higher, than those leached by the respective natural pasture or occasionally grazed ones. Therefore, improved pastures phosphate applications should be reviewed, regarding their long-term management.

At *Herdade dos Esquerdos*, nitrate leaching enhancement under the renewed improved pasture system was accompanied by cations co-leaching, which may increase the risks of soil acidification (Weil and Brady, 2017). Although mineral phosphate applications are accompanied by Ca^{2+} additions at these areas, monitoring these soils pH, following such management intensification patterns appears crucial.

Despite the present study main goal was to identify possible effects of pasture management on soil C and N global fluxes, results showed that the influence of pasture management was small, compared to the major influence of abiotic conditions variability, such as soil water availability and temperature. Results interpretation was constrained by the limitations associated with disturbances at sampling, moving and maintaining soil blocks under similar controlled conditions. Indeed, as soils were moved from their natural field conditions and management influences, presented results correspond to the residual effect of the complex pastoral systems under evaluation. Additionally, it was not possible to disentangle the effects of individual management options, namely grazing and vegetation features, as they coexist and overlap asymmetrically in our experimental design, for which further studied are needed.

CONCLUSIONS

The results obtained in the current study, highlight the major role of abiotic factors over open grassland soils C and nutrient fluxes, further studies being needed to address climate factors and eventual global changes effects over these soils functions.

Improved pastures role on soils C sequestration potential enhancement appeared confirmed, mostly by soil organic matter accumulation. Nevertheless, as soils structural status may change their potential to oxidize CH_4, livestock intensification must be issued, especially for finer textured soils. Soil N storage is also achievable by these pastures long-term management, although changes in soil N cycling may strongly influence N_2O transfers to the atmosphere. Pasture renewal, by direct drilling, appeared as an effective practice to increase pasture productivity and enhance soil C and N cycling. Chemical fertilizers additions under long-term improved pastures should be reassessed, regarding potential soil P losses through leaching and runoff.

Highly variable and somewhat contrasting results have highlighted the need to render further consideration to all management aspects and soil characteristics, when attempting to evaluate changes in these systems C and N balances.

Future studies should also address tree cover influence and actual management conditions, as these largely influence organic inputs to the soil, thus controlling soil C and nutrient budgets.

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CONCLUSIONS

CONCLUSIONS

Results obtained in the current study have evidenced the complex and heterogeneous nature of Mediterranean evergreen oak woodlands, that can broadly be related to the diversity of the land use history, the present management systems, and the physical environment (e.g. soil type). Therefore, for interpreting their functions and resulting services, it is of utmost importance that this multiplicity of factors enmeshed in *montado* systems are taken in account.

Intensification management systems associated with livestock breeding and pasture sowing, can be a good option regarding soil organic C sequestration, soil physical status protection, soil fertility improvement and herbaceous productivity increments. The extent of these effects being dependent on the pasture age and all site-specific and management factors.

Meanwhile, the establishment of improved pasture mixtures by itself may not ensure adequate soil physical status, particularly under finer textured soils, where excessive compaction due to grazers trampling, may still compromise soil water availability and movement.

The stability of the tree cover is not by any means guaranteed by improved pasture establishment. To ensure proper tree recruitment the management of such pastures demands additional protective measures, for which proper research is still lacking.

The influence of pasture management over greenhouse gases emissions in *montado* soils, were found to be negligible, despite disturbance on soil nutrient fluxes may occur. Environmental services associated with climate change mitigation and maintaining groundwater quality, are still poorly understood and need further research, considering the extent and the socio-economical relevance of *montados*.

Results of the current study undoubtedly confirm the trees great potential to enhance C sequestration, improve soil quality and increase soil resistance to face degradation. Concerning threats associated with current management and climate changes, scattered trees in the *montado* play a crucial role in preventing and reversing degradation patterns. Management practices that ensure tree cover long-term conservation must be promoted, at both management and policy levels.

Monitoring soil quality changes in the *montado*, associated with management systems and land use modifications, can be a useful tool to adress sustainable management and environmental quality issues.

Among the current study methodology, soil organic C concentration appeared suitable to reflect the most relevant changes in soil organic matter global dynamics. Structural modifications associated with soil porosity and water availability conditions were more reliably inferred through soil bulk density measurements. Some straightforward categorical parameters, such as stocking rate, structure visual quality, and visual evidences of physical disturbance (e.g. soil compaction, soil erosion, soil bareness) may also be considered as prompt hazard indicators.

Relevant and highly sensitive information on soil functions can be obtained by using soil biochemical indicators. However, these indicators still suffer from lack of reproducibility and simplicity, while their interpretation is generally more complex, requiring high level expertise. Therefore, the inclusion of these indicators in routine soil monitoring systems appears not pertinent.

Additionally, the need for deep information on the diversity of soil characteristics at local level must also be emphasized.

Future research must address the economic performance of these management options, as the *montado* profitability is undoubtedly the main driver of stakeholder decisions. A deeper knowledge on the relationship between management practices, soil quality trends and resulting system sustainability should be based on long-term studies, for which the establishment of national and international reference areas would be of major significance.